JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
287

Irina Ovčarenko

The Role of Agroecosystems for Invasion of a Generalist Herbivore

JYVÄSKYLÄN YLIOPISTO

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 287

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212,
syyskuun 12. päivänä 2014 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2014

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JYVÄSKYLÄ 2014

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Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-5795-7
ISBN 978-951-39-5795-7 (PDF)

ISBN 978-951-39-5794-0 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2014

“Not all those who wander are lost.”

J.R.R. Tolkien

ABSTRACT

Ovčarenko, Irina
The role of agroecosystems for invasion of a generalist herbivore
Jyväskylä: University of Jyväskylä, 2014, 70 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 287)
ISBN 978-951-39-5794-0 (nid.)
ISBN 978-951-39-5795-7 (PDF)
Yhteenveto: Kasvihuoneviljelyn merkitys generalistikasvinsyöjän levittäytymisessä.
Diss.

Agricultural insect pests are among the first studied invasive species. By inhabiting crop plants they became mobile elements in global network of agroecosystems connected through plant trade. The greenhouse whitefly (*Trialeurodes vaporariorum*) is a widespread invasive pest. Since 1920 the species has managed to survive in Finland without any overwintering resting stage in the matrix of greenhouse habitats. To understand the factors affecting species persistence in boreal climate I analyzed the role of greenhouse habitats, crop cultivation practices and surrounding natural vegetation for naturalization of *T. vaporariorum*. I also compare the factors contributing to genetic structure of *T. vaporariorum* populations in boreal and Mediterranean regions and study the global genetic diversity of *T. vaporariorum* and its endosymbionts. Results indicated that greenhouse habitats can have an impact on pest persistence and naturalization in the boreal climate. Variation in the pest responses to insecticide treatments suggests pest persistence in the study area. Continuous cultivation of the same greenhouse crop species creates possibilities for host adaptation and maintains high propagule pressure with sources of fecund and persistent pest populations. Furthermore, natural species are preferred over the cultivated crops as host plants for whiteflies, which could facilitate pest dispersal into natural vegetation. However, greenhouse habitats can also create physical barriers to pest dispersal in both climate zones. This can be observed by significant genetic structure of greenhouse populations, which contrasted with low genetic diversity of the species and its endosymbionts on a global scale. My results suggest that greenhouse agroecosystems can extend the distribution of pests. However, crop cultivation practices also affect their ecology and contribute to reduction of genetic diversity, which in turn, can influence their long term persistence.

Keywords: Agroecosystems; host plants; insecticide resistance; microsatellite markers; naturalization; *Trialeurodes vaporariorum*; whitefly.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following five original papers, which will be referred to in the text by their Roman numerals I-V.

I Ovčarenko, I., Lindström, L., Saikkonen, K., Jauhiainen, L., Kaseva, J. & Vänninen, I. Greenhouses as a gateway for invasion of a generalist herbivore in the boreal ecosystem? Submitted manuscript.

II Ovcarenko, I., Clouet, C., Knott, E., Tsagkarakou, A. & Gauthier, N. 2013. Thirteen polymorphic microsatellite loci and PCR multiplexing in the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). *Molecular Ecology Resources* 13 (2): 341-343. http://tomato.bio.trinity.edu/manuscripts/13-2/mer-12-0390.pdf

III Ovčarenko, I., Kapantaidaki, D. E., Lindström, L., Gauthier, N., Tsagkarakou, A., Knott, K. E. & Vänninen, I. 2014. Agroecosystems shape the genetic structure of greenhouse whitefly (*Trialeurodes vaporariorum*) populations in Northern and Southern Europe. *BMC Evolutionary Biology* 14 (165): 1-17.

IV Ovčarenko, I., Lindström, L., Saikkonen, K. & Vänninen, I. 2014. Variation in mortality among populations is higher for pymetrozine, than for imidacloprid and spiromesifen in *Trialeurodes vaporariorum* in greenhouses in Finland. *Pest Management Science*. In Press. DOI: 10.1002/ps.3766.

V Kapantaidaki, D. E., Ovčarenko, I., Fytrou, N., Knott, K. E., Bourtzis, K. & Tsagkarakou, A. Low levels of mitochondrial DNA and symbiont diversity in the worldwide agricultural pest, the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). Submitted manuscript.

The table shows major contributions to the original manuscripts. Smaller contributions are stated in the acknowledgements of the original manuscripts and acknowledgement section of the thesis.

Study	**I**	**II**	**III**	**IV**	**V**
Original idea	IV, KS, LL, IO	NG, AT	IV, IO, AT	IV	AT, KB, IO, IV
Data collection	IO, IV	CC, NG, IO, EK	IO, DK, LL, IV	IO	DK, IO, AT, IV
Data analysis	IO, LJ, JK	CC, NG, IO, EK	IO, EK	IO	DK, AT
Writing of the manuscript	IO, LL, KS, IV	NG, IO, EK, AT	IO, EK, LL, IV,	IO, LL, IV, KS	DK, AT, IO, EK, KB

IO = Irina Ovčarenko, IV = Irene Vänninen, LL = Leena Lindström, EK = Emily Knott, KS = Kari Saikkonen, LJ = Lauri Jauhiainen, JK = Janne Kaseva, DK = Despoina Kapantaidaki, AT = Anastasia Tsagkarakou, KB = Kostas Bourtzis, NG = Nathalie Gauthier, CC = Cécile Clouet

1 INTRODUCTION

1.1 Agroecosystems-unique habitats of modern landscape

1.1.1 Natural and agricultural ecosystems

Agroecosystems consists of open and enclosed ecosystems, i.e. fields and greenhouses, designed to produce agriculture crops, such as ornamental plants and vegetables. Unlike natural ecosystems, these habitats are managed to maintain a limited number of crop plants and aim to promote only those insect species beneficial for crop yield. Detrimental insect species for crops are exterminated in agroeosystems through the use of chemical pesticides or biological control agents. Deliberately reduced biodiversity in agroecosystems increases their vulnerability to pests and diseases (Altieri and Nicholls 2004). Majority of agroecosystems, especially those practicing conventional agriculture, create a monoculture environment not typically encountered in nature. Intensification of agriculture and specialisation in harvesting single crop species has led to expansion of habitats with reduced biodiversity, which is suggested to be one of the major reasons for insect pest outbreaks (Altieri *et al.* 1984, Matson *et al.* 1997). Biodiversity and complexity of interactions among biota in agroecosystems is somewhat proportionate to the degree of agroecosystem isolation from the surrounding natural habitats. The degree of isolation is in turn dependent on agroecosystem design and management, and diversity of biota in the surrounding natural ecosystem.

Enclosed greenhouse environments are designed to improve crop management efficiency by maintaining stable climate conditions and reducing evaporation, the influx of insect pests from outside habitats and, subsequently, insecticide applications. Due to the isolation of greenhouses, indoor vegetation is relatively independent from succession in natural habitats. However, plant senescence and changes in the community structure of biota occur to some degree. Communities of insect pests, intentionally introduced beneficial insects and pollinators, as well as biota in soil substrates (if such substrates are used)

undergo changes following development of crop plants. Vegetation in greenhouses can be characterized by fast growth, high productivity and replacement with young plants by the end of crop season, which is independent from seasonal changes in natural ecosystems. Greenhouse agroecosystems are more productive than natural ecosystems, but they are dependent on human input of nutrients and water supply (Parrella 1999). Biomass, which serves as a source of nutrients and moisture in natural ecosystems, is always directed out of the agroecosystem at the time of harvest or senescence of plants. Thus, agroecosystems are dependent on human interference in many ways and are less resilient than natural ecosystems (Gliessman 1990).

1.1.2 Agroecosystems in Southern and Northern Europe

The ongoing improvements of greenhouse efficiency have resulted in modern commercial greenhouses with sophisticated climate and light control systems able to operate at any latitude year round (Pardossi *et al.* 2004, Moe *et al.* 2006). The distribution of plastic film covered structures, low technology greenhouses and tunnels increased rapidly with the advent of plastics in the 1960s, since the use of plastics decreased construction costs (Mugnozza 1995). Greenhouse production expanded quickly and currently there are more than two million hectares of protected agriculture worldwide (Pardossi *et al.* 2004). Plastic covered structures are less enclosed from the surrounding environment than are high technology greenhouses due to their simple construction design. Production in these agroecosystems is common in countries with warm climate, where such greenhouses have constantly open ventilation due to lack of climate control systems (Parrella 1999). In temperate climates, plastic covered structures are usually operating only seasonally and high technology greenhouses prevail. To protect from seasonal temperature fluctuation and snow, high technology greenhouses are covered with glass or double acrylic sheets and have climate and light control systems (Von Zabeltitz 1999). Thus, greenhouse cover may represent resilience of agroecosystem to surrounding environment. High technology enclosed greenhouses are protected from surrounding climate and biota, whereas low technology plastic covered greenhouses and tunnels are dependent on climate conditions and prone to insect income from outside habitats. However, distinction between high and low technology greenhouses is not always simple since technological advancement depends on financial capacity and crop management knowledge of agricultural crop producers. This results in a heterogeneous landscape of greenhouse agroecosystems around the world.

In the Mediterranean, medium and high technology greenhouses equipped with climate control systems are usually covered with glass, but these make up only 7% of protected horticulture in the region (Pardossi *et al.* 2004). Protected horticulture under plastic, however, is constantly expanding and often creating dense production clusters in the Mediterranean (Castilla *et al.* 2004). The predominance of these agroecosystems can be attributed to the lower construction costs of plastic structures than high technology greenhouses and

mild Mediterranean climate, which allows crop production in less enclosed agroecosystems (Mugnozza 1995, Pardossi *et al.* 2004). The cost of crop maintenance even in these simple structures has a lower environmental burden than does maintenance in the fields of the Mediterranean region in terms of the use of water, fertilizers and pesticides (Muñoz *et al.* 2008). Favorable climate conditions and a diversity of horticulture practices create opportunity for year-round production in the Mediterranean region. There, crop production in the plastic tunnels with uncontrolled climate terminates only during very hot periods and is compensated by production in fields. In winter, agroecosystems with uncontrolled climate conditions can produce crops requiring lower temperatures. Therefore, the crops in Mediterranean region undergo frequent changes during the year depending on the temperature fluctuations during the season and demands of the crop market. This contributes to variation in host plant availability for insect pests throughout the year.

Production in greenhouses at northern latitudes (e.g. Scandinavia and Finland) requires higher financial and energy input to get the same yield than does production in the fields of the Mediterranean region (Nordenström *et al.* 2010). For example, the area of protected horticulture in Finland reached 401 ha in 2012 (Eurostat 2014, TIKE and OSF 2014) compared to 5 300 ha in Greece and 63 300 in Spain in 2009 (Eurostat 2014). Contrary to diverse and flexible crop production in the Mediterranean region, the greenhouse crop producers in Scandinavia mostly specialize in cultivation of just a few vegetable crops, such as tomato and cucumber, or in the production of tree seedlings and ornamental plants. The harsh winter conditions, competition among producers and demands of the crop market in Northern Europe determine prevalence of greenhouses with climate control systems operating most of the year and specializing in a few crops.

Continuous year round production in high technology greenhouses expands and gradually replaces seasonal greenhouse horticulture under plastic cover in Northern Europe. Majority of protected horticulture crops are cultivated for more than seven months in Finland (70% of area under cover) (TIKE and OSF 2014). Contrary to Southern Europe, greenhouses with plastic cover at northern latitudes require heating even during seasonal production in summer. Thus, the area of all heated greenhouse structures in Finland (382 ha) is higher than that without controlled indoor climate conditions (28.3 ha) (TIKE and OSF 2014). Heated greenhouses are bringing novel habitat types to the boreal and Mediterranean landscapes. These habitats have potentially higher impact to dynamics of local and introduced insect populations in the North, than in warmer southern regions of Europe, where the climate difference between natural and greenhouse habitats is high only during the short and mild winter season.

1.1.3 Greenhouses as gateways for species invasion

Agricultural habitats are designed to provide agricultural services, but unintentionally, they also provide suitable habitats worldwide for a number of

exotic herbivore species of tropical origin (Parrella 1999, Hanafi 2005). Year-round maintained temperature, humidity and light conditions coupled with availability of abundant host plants and a lack of natural enemies in the monoculture environment facilitates greenhouse invasions. Common greenhouse pests are aphids, leafminers, whiteflies, thrips and mites, which cause high economical and biological impact in areas of introduction (van Driesche and Enkegaard 2002, Kiritani 2006). Alien arthropod invasions are responsible for billions of dollars in economic losses in Europe (Bacon *et al.* 2014). Transport of non-native plant species for commercial purposes is the main vector of invasive plants, insects and viruses (Drew *et al.* 2010). Agroecosystems serve as sources and recipients of invasive species by being a part of the global seedling trade (Reichard and White 2001, Drew *et al.* 2010). Thus, greenhouses can contribute to latitudinal range expansions of many insect pest species by creating a network of suitable habitats connected by seedling trade in an otherwise hostile environment (Bebber *et al.* 2013). Therefore, theoretically even tropical species can extend their distribution into the cold boreal climate despite severe winters. However, further dispersal may require development of cold tolerance or initiation of a dormancy period. Alternatively, greenhouses can create temperate habitats and refuges in the hot Mediterranean climate in summer for both local and introduced biota.

In addition to negative impact of accidentally introduced insect pests in agroecosystems, intentionally released commercial strains of beneficial insects sometimes can also negatively affect local biota. For example, *Harmonia axyridis* the Asian lady beetle introduced to North America to control aphid pests, dispersed worldwide competing with local ladybird beetles and becoming a pest on grapes with negative effects on wine quality (Koch and Tederson 2008). Deliberate global introductions of bumblebees *Bombus terrestris*, pollinators of greenhouse crops, have been justified by movement of populations within the species' cosmopolitan distribution range, i.e. by avoiding introductions of non indigenous species. However, the ecology and morphology of *B. terrestris* populations differ remarkably, and bumblebees escaping greenhouse environments sometimes hybridise and outcompete local populations, reducing biodiversity (Ings *et al.* 2005). As these examples show, physically isolated greenhouse environments are prone to leakage of both beneficial and pest species.

However, only a small fraction of escapees is able to establish permanent populations outside introduction sources and become naturalized (Williamson and Fitter 1996). The species are considered naturalized once they are able to maintain self-sustaining populations and are incorporated with resident biota, whereas they become invasive once they bring negative impact to local ecosystems (Richardson *et al.* 2000). The status of species introduced into greenhouse environments, but not established in the natural ecosystems is unclear, even though these introduced species may have been present in the region for decades, bringing along with them the high financial costs of prevention and control methods. These species could be characterized as

invasive species based on their non-native origin, high abundance, high dispersal rate and negative impact. However, such description usually refers to invasion in natural ecosystems. Therefore, the status of invasive species that are abundant and widespread but restricted to anthropogenic greenhouse habitats needs to be defined.

1.2 The process of invasion

1.2.1 From pests to aliens

Among the first studied invasive species were pests, which received attention from researchers primarily because of their negative impact to natural or agricultural ecosystems (Metcalf and Flint 1928, Brown and Beecham 1989, Vänninen *et al.* 2011). The non-native origin of such species, sometimes referred to as "exotic" or "alien" (Carlton 2003), was highlighted by Charles Elton, who is considered to be the founder of the field of Invasion Biology (Elton 1958). However, many other researchers before C. Elton addressed the issue of newly arriving species that bring negative impacts (see Chew 2011 for review). Initially the perception of invasive species closely resembled colonizers, emphasizing their movement and dispersal. This could be an influence from C. Elton's initial publication on the subject (Elton 1958). He perceived invasion as dispersal and an increase in numbers resulting in negative impact to humans and local ecosystems. The book was written in a style appealing to the masses. In light of the events of World War II, invasive species were compared to ecological explosions, i.e. "enormous increase in numbers of some kind of living organisms", which are out of control of the forces that previously restrained them. In contrast to outbreaks of local pests, a lack of restraining forces, such as predators, competitors or parasites, allow ecological explosions of invasive species because they are novel in the ecosystem. Non-native origin of invasive species is often highlighted by conservationists (Willis and Birks 2006). However, researchers have proposed avoiding emotional and biased descriptions of non-native taxa (Larson 2005, Larson and Kueffer 2013), and concentrate on their negative environmental effects rather than on their origin (Davis *et al.* 2011). For example, the majority of plants used in agriculture in North America are not native to the continent, but are not invasive and are beneficial for humans (Reichard and White 2001). However, a small proportion of introduced plants escapes from cultivation and become pests of natural areas.

1.2.2 Defining the invasion process

Non-native origin, dispersal and high abundance are major components of the invasion process (Colautti and MacIsaac 2004, Blackburn *et al.* 2011). Despite the long tradition, the terminology of invasive species is still an object of discussion

and it highlights various aspects of non-indigenous species (NIS) (Richardson *et al.* 2000, Davis 2009). To summarize, a species is considered invasive if it causes ecological or economic impact and produces a large number of offspring in self sustaining populations outside its native distribution range (Richardson *et al.* 2000, Lockwood *et al.* 2013). The confusion and debate over terminology has mainly arisen due to validity of invasive status of NIS going through different stages in the invasion process, which lead to the status of a successful invader. The invasion stages can be defined as "transported", "introduced", "established", "naturalized" and "dispersed" NIS (Lambrinos 2004, Henderson *et al.* 2006, Davis 2009). A discussion of the main characteristics distinguishing the "invasive" status from "naturalized" is still open (Richardson *et al.* 2000). Researchers have proposed several frameworks to describe the process of invasion. Some frameworks emphasize barriers preventing invasions (Richardson *et al.* 2000), others highlight invasion stages and the status of NIS (Williamson 1993, Williamson and Fitter 1996). Blackburn *et al.* (2011) combined these frameworks and described a "captivity/cultivation" barrier for invasion and several invasion stages of organisms restricted to such ecosystems. This barrier coupled with frameworks describing plant invasions and a few modifications, could adequately describe invasion of arthropods in agroecosystems. Here, I further define invasion stages occurring in a human mediated environment and combine several unifying frameworks of invasion process designed to describe the invasion of plants and other organisms (Colautti and MacIsaac 2004, Colautti *et al.* 2006, Catford *et al.* 2009, Blackburn *et al.* 2011). The proposed schematic of invasion process (Fig. 1) describes barriers preventing occurrence of invasion stages. Five classes of determinants may affect positively or negatively the probability of introduced biota to pass through each barrier.

1.2.3 Invasion in natural habitats and agroecosystems

Frameworks designed to describe the invasion process in natural ecosystems usually emphasize introduction followed by establishment and dispersal. Dispersal is associated with negative impact occurrence and a change in NIS status to "invasive" (Colautti and MacIsaac 2004, Blackburn *et al.* 2011). Such a scheme resembles the initial idea of C. Elton, who perceived NIS as colonizers. While introductions of NIS in agroecosystems may follow the same stepwise scheme, the widespread network of agroecosystems coupled with a strong NIS transportation vector, i.e. seedling trade, predisposes agroecosystems to multiple introduction events and the occurrence of NIS over large areas. Thus, contrary to invasion in natural ecosystems, where dispersal of NIS usually refers to colonization process by NIS, dispersal of NIS in agroecosystems is often human assisted. Therefore, widespread dispersal in agroecosystems should not always be considered an achievement of NIS in the process of invasion leading to the "invasive" status, as suggested by the above mentioned invasion frameworks. In turn, widespread NIS occurrence in agroecosystems could be considered as a result of separate multiple introductions at their own

stage of the invasion process. Nevertheless, widespread distribution of NIS indicates its invasion stage requiring urgent management actions (Fig. 1). High persistent local abundance of NIS causing negative impact should be enough to acquire the status of invasive species over the area of potential NIS dispersal. Acquisition of the "invasive" status regionally would help in the management of invasive species and in the prevention of further dispersal in the early stages of impact occurrence.

Another discrepancy of the invasion process between natural habitats and agroecosystems is achievement of "invasive" status only after establishment and naturalization. NIS in agroecosystems are not able to become naturalized due to anthropogenic nature of this habitat. This, however, does imply that NIS cannot become invasive in agroecosystems. NIS in agroecosystems have to pass through similar barriers of introduction and establishment as do NIS in natural ecosystems. The novelty of any encountered environment brings a challenge not only for establishment, but also for persistence of NIS, despite welcoming conditions in agroecosystems, such as reduced biodiversity and an optimized microclimate. The equivalent of naturalization in agroecosystems is persistence – the presence of abundant populations over several generations. At this stage, NIS may become invasive due to their high abundance and long term impact, as well as their high dispersive capacity. They may disperse continuously from a single source by-passing the naturalization barrier and developing into a widespread metapopulation within a network of agroecosystems. In the schematic of the invasion process (Fig. 1) this stage is represented by the captivity/cultivation barrier, corresponding to agroecosystems. Therefore, the negative impact of NIS should be prioritized over dispersal or naturalization processes for achievement of the "invasive" status.

Contrary to earlier proposed frameworks of the invasion process (Colautti and MacIsaac 2004, Colautti *et al.* 2006, Catford *et al.* 2009, Blackburn *et al.* 2011) the schematic proposed here is not finalized with achievement of the status of a successful invader. However, thorough understanding of invasion steps is needed for successful management regardless of the final status of the invader in the proposed scheme.

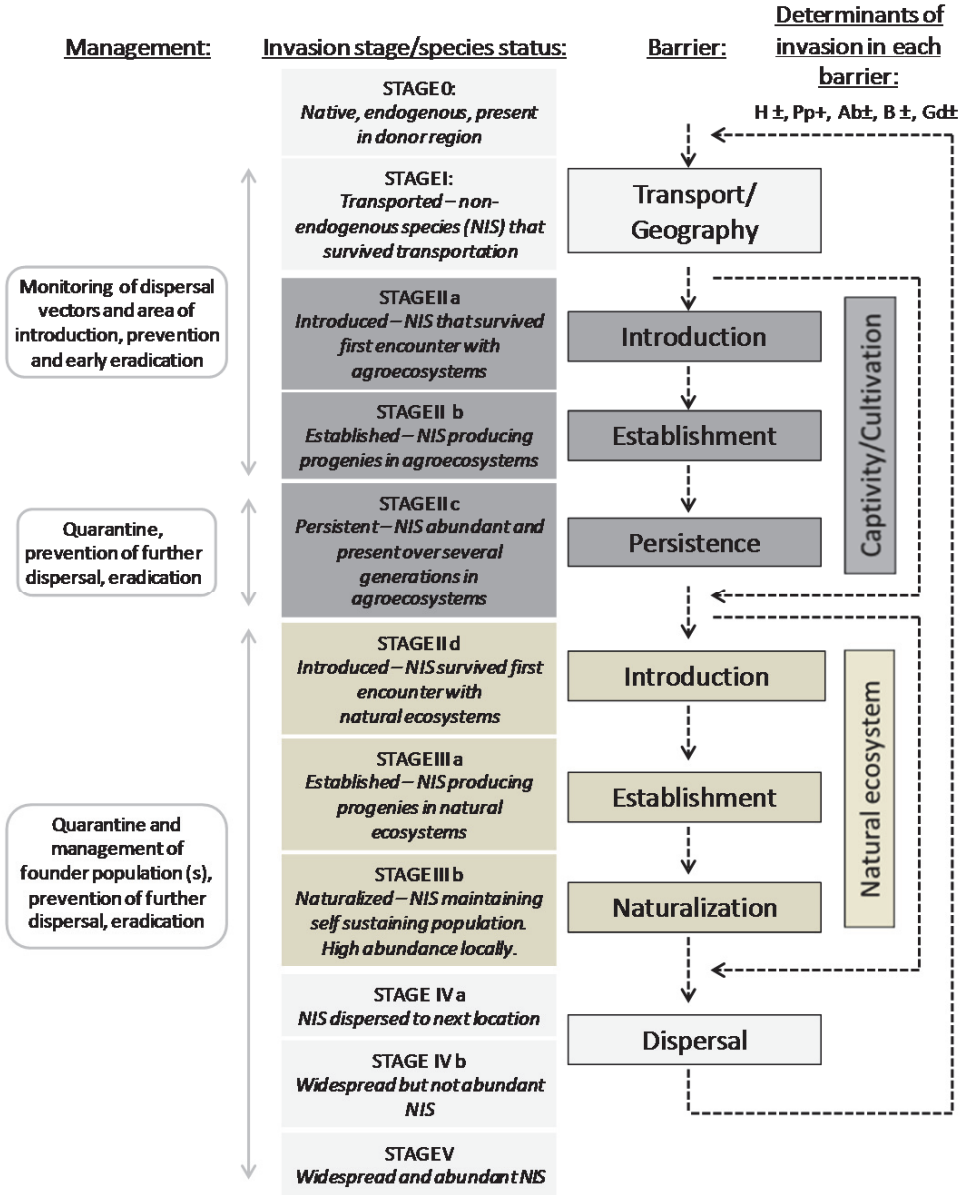

FIGURE 1 The scheme addressing the role of agroecosystems in the invasion process, modified from Colautti and MacIsaac (2004), Catford et al. (2009) and Blackburn et al. (2011). Stages occurring in "captivity/cultivation" correspond to those in agroecosystems. The invasive species status could be acquired at any stage of negative impact occurrence from non-endogenous species (NIS). The letters indicate classes of determinants affecting the probability to pass through each barrier: H-human activity, Pp-propagule pressure, Ab-abiotic characteristics and B-biotic characteristics and Gd-genetic diversity. Determinants may positively (+) or negatively (-) affect the number of propagules passing through each barrier.

1.3 Determinants of agricultural pests invasion

1.3.1 Human activity

Human activity may prevent or facilitate occurrence of NIS at any stage of invasion. Monitoring of the potential area of introduction, as well as the transport vectors is essential as a preventive measure against invasions in both natural and agricultural ecosystems. Human mediated environments, such as agroecosystems should receive considerable attention to pest monitoring and management, since they can serve as source and stepping stones for naturalization of NIS dispersing from persistent populations (Fig.1). Factors affecting invasion success in natural ecosystems (stage IId-IIIb, Fig. 1) include climate suitability, competition and predation-free niche availability. However, establishment of NIS in the greenhouse agroecosystems, (stage IIa-c, Fig. 1) characterized by stable climate conditions and low biodiversity, instead depends on human activities, such as local and regional NIS management. Thus, establishment of NIS populations in agroecosystems depends on an additional, socio-economic factor, which can be characterized by a ratio of NIS management knowledge, economic investment capabilities and benefit. Fast income oriented producers often prefer "silver-bullet" products promising quick NIS control (Lewis *et al.* 1997). However, slower methods of knowledge acquisition, taking into account surrounding ecosystem dynamics could be more efficient in the long term.

The modern trend of intensification of agriculture, i.e. to increase yields generated from the same area, promotes year-round crop cultivation (Matson *et al.* 1997). Production in year-round operating high technology greenhouses is more efficient than in plastic covered greenhouses or open fields, but requires higher investment (Pardossi *et al.* 2004, Muñoz *et al.* 2008). In addition, year-round cultivation may contribute to NIS persistence and frequent use of costly chemical NIS control methods. Thus, profits in relation to capital invested in development of high technology greenhouses could be granted only upon implementation of alternative NIS management practices, such as annual crop rotation and biodiversity maintenance to reduce the need for chemical control (Lewis *et al.* 1997). Greenhouse crops relying on biological control of NIS, the biotic components of agroecosystems, are characterized by maintenance of low abundance of NIS already from the beginning of their unintentional introductions (Knipling 1979, Pilkington *et al.* 2010). Agroecosystems relying on chemical NIS control are abiotically stable except for fluctuating chemical applications and can be characterized by stochastic events of high NIS abundance and mortality. Periods of high NIS abundance may lead to NIS persistence and dispersal into natural ecosystem by increasing propagule pressure. Survival in natural environments may require adaptation of NIS to novel hosts and subsequently result in changes in the genetic diversity of NIS. Thus, human activity can contribute also to each class of determinants of

invasion success: propagule pressure, abiotic characteristics of environment, biotic interactions, as well as genetic diversity of NIS.

1.3.2 Propagule pressure

High abundance, widespread dispersal and negative impact can be achieved at any stage of the invasion process in the case of high propagule pressure. Propagule pressure is the number of individuals able to propagate in the area of introduction, as well as the frequency of such events (Colautti *et al.* 2006, Simberloff 2009). Propagule pressure can be acquired from natural processes, such as high NIS productivity and dispersal capacity, or from human activities contributing to NIS transfer. Examples of human facilitated propagule pressure through increased numbers of NIS can be seen in the transport of large quantities of soil used as ballast in transcontinental ships, which led to the introduction of over 90% of exotic insect species in North America in the 19th century (Kiritani and Yamamura 2003). Once the ballast soil was replaced with ballast water, the turnover of aquatic invasive species increased considerably (Molnar *et al.* 2008), highlighting the high propagule pressure created by this invasion vector for aquatic as well as for terrestrial ecosystems. Another example of high propagule pressure facilitated by frequency of NIS introductions is production of ornamental plants in greenhouses. Seedlings are often received from multiple sources for production of ornamental plants, instead of cultivated locally as for vegetable production, thereby increasing the chances of transfer of novel exotic pests on seedlings (Thuiller *et al.* 2005, Dehnen-Schmutz *et al.* 2007, Kenis *et al.* 2007). Abundance of insects on the transported seedlings depends on the local pest management efficiency and level of isolation from the surrounding environment in the donor area, i.e. the level of greenhouse technology and investment into infrastructure. In the Mediterranean region propagule pressure may be high for several reasons. First, due to prevalence of simple plastic film covered constructions greenhouse agoecosystems there are less isolated from surrounding natural ecosystems than in Northern Europe. Second, due to warm climates greenhouses use ventilation, often in the form of open side vents without sufficient prevention of insect pest movement into or out of greenhouses (Parrella 1999). Thus, greenhouses producing seedlings in warm climates may have poor pest management leading to transmission of local insect pests on seedlings among greenhouses (Hanafi and Papasolomontos 1999). Spread of introduced exotic species in this region is fast and often detected only after the establishment of permanent populations, especially if the species is of tropical origin (Kenis *et al.* 2007).

In some cases propagule pressure has had the highest impact on invasion success when considered together with climate suitability and host availability in the area of introduction (Bacon *et al.* 2014). Expansion of less isolated, low technology greenhouses and structures under plastic cover in the Mediterranean basin is creating greater opportunities for establishment of invasive species and posing a threat to local ecosystems compared to high

technology greenhouses (Castilla *et al.* 2004). Establishment success of introduced herbivores in natural ecosystems can be also facilitated by constant propagule pressure from year-round producing commercial greenhouses (McGlynn 1999, Hanafi 2005, Franklin *et al.* 2010). Constant growth rate of area of protected horticulture, therefore, could facilitate establishment success of exotic species dispersing from greenhouses. Crop production clusters and surrounding habitats allowing for pest persistence year round, such as those in the Mediterranean region, are at a high risk of pest invasions and often experience difficulties with pest management (Hanafi and Papasolomontos 1999, van Lenteren 2000). Thus, human activity can play an important role in increasing propagule pressure, which can contribute to the achievement of each stage of invasion process (Fig. 1).

1.3.3 Abiotic characteristics of the environment and NIS responses

The tolerance of abiotic conditions is essential for acclimation and survival of invasive species taken into a transport vector (stage I, Fig. 1) or introduced into a novel environment (stage II a, Fig. 1). Adverse abiotic characteristics of the environment are recommended as prevention and control methods for unintentional transfer or management of NIS in advanced stages of invasion. For example, NIS transferred in the tanks of intercontinental ships could be exterminated by high marine water salinity (Ovcarenko *et al.* 2006) or insect pests in greenhouses could be eliminated by extremely high or low temperature during crop production breaks before planting new crop plants (van Lenteren 2000).

Abiotic conditions in natural ecosystems, such as seasonal changes in day length, light quality and temperature are key determinants of invasion success or failure for many NIS (Dingle 1974, Lehmann *et al.* 2012). Contrary to natural ecosystems, the initial environment encountered by introduced biota in greenhouse agroecosystems is more stable and benign. Greenhouse agroecosystems often have controlled climate conditions and photoperiod facilitating the best yield of target crops and also suitable for herbivores of tropical origin. The temperatures for high yield production of the most common crops in temperate latitudes, tomatoes and cucumbers, are 22˚C and 25˚C respectively (Grimstad and Frimanslund 1993, Uzun 2007). The temperatures can vary up to 8˚C in vertical gradient of the greenhouse creating suitable microclimate conditions for various insects (Kittas *et al.* 2003). However, the success of outdoor establishment of invasive species that flourish indoors depends on their physiological plasticity and tolerance, as well as on environmental similarities between donor and recipient regions (Thuiller *et al.* 2005). Such matching of climate conditions is often used for prediction of establishment success of introduced species (Baker *et al.* 2000). Most of NIS introduced in Europe originated in regions with benign climate, in Asia, Europe and South Africa (Thuiller *et al.* 2005, Kenis *et al.* 2007), making Mediterranean region a high risk area of NIS establishment. On the contrary, naturalization of NIS of tropical origin in the boreal region, which is characterized by cold winter

periods and long photoperiods in the summer, is less likely (van Lenteren 2000, Saikkonen *et al.* 2012). Furthermore, greenhouse habitats may decrease the risk of NIS establishment in natural habitats. Cold tolerance of introduced insects, which are able to overwinter only in thermally-buffered microhabitats, i.e. greenhouses, might be reduced or lost over time, as observed in leafminer (*Liriomyza sativae*) populations in China (Chen and Kang 2005). Some insects, such as spider mites, have lost their ability to react to diapause triggering environmental factors after long term indoor survival and, thus, their chances of establishment in natural ecosystems of temperate latitudes is reduced remarkably (van Lenteren 2000). Thus, abiotic conditions can have a positive impact on NIS persistence in agroecosystems and negative impact on NIS naturalization success.

The chances of NIS survival in the otherwise welcoming conditions of greenhouse agroecosystems, however, are reduced with chemical treatments, i.e. human activity. Propensity to develop resistance to insecticide treatments in the past could serve as an indicator of the invasive potential of an introduced species to agroecosystems (Morse and Hoddle 2006). For example, as a result of frequent insecticide use since 1970, a resistant genotype of western flower thrips *Frankliniella occidentalis* (Pergande) has spread through plant trade from greenhouses in western North America worldwide (Kirk and Terry 2003). Resistance to neonicotinoids contributed to establishment success of whitefly *Bemisia tabaci* in China by facilitating competitive advantage of resistant subspecies (Crowder *et al.* 2010, Rao *et al.* 2012). The frequency of exposure to pesticides often correlates with pest management problems in the region and high dispersal rates from chemically treated habitats (Denholm *et al.* 1998, van Lenteren 2000). However, if NIS has not the ability to escape from enclosed high technology greenhouses, they may develop resistance to pesticides under strong selection pressure of frequent applications leading to survival of only resistant individuals. At the end of crop season, when old crop plants are stored outdoors until recycled, dispersal of insects from these sources is active. Establishment chances in other agroecosystems exposed to chemical treatments are higher for resistant insect strains than for susceptible populations. Thus, human activity could facilitate invasion success by increasing invasion potential.

Pests can persist in chemically treated environments by employing various strategies. For example, some successful invaders of greenhouses are able to reproduce parthenogenetically thereby saving the time required to search for mates. Others employ a strategy to lay eggs on the underside of the leaf, avoiding contact with insecticides applied foliarly (e. g. whiteflies (Inbar and Gerling 2008)). These strategies have higher efficiency when combined with physiological and genetic mechanisms of insecticide resistance. Selection pressure of insecticide applications could lead to the survival of only resistant individuals due to several possible resistance mechanisms. For example, reduced sensitivity to insecticide can be caused by modification of insecticide target site, e.g. a single nucleotide mutation in a gene responsible for

detoxification of the insecticide, or due to tolerance of the stress causing factor, or even from phenotypic plasticity, without any changes in the genome (Nauen and Denholm 2005, Zhou *et al.* 2012). However, the development and maintenance of insecticide resistance through genetic and physiological mechanisms can be a costly strategy and could reduce fitness (McKenzie 2001).

1.3.4 Genetic diversity and phenotypic plasticity of NIS

Invasion success could be facilitated by a variable gene pool of the source population, broad tolerance and phenotypic plasticity (Lee 2002). Phenotypic plasticity is the ability of a single genotype to produce different phenotypes in response to changing environments (Bradshaw 1965, Zhou *et al.* 2012). Introduced NIS with high genetic and phenotypic diversity have higher chances of establishment in novel environments due to lower vulnerability to predators and diseases and higher adaptation potential to varying abiotic conditions (Forsman 2014). However, many invasive species has less genetic diversity in the invaded than in the native distribution range (Grapputo *et al.* 2005). This can be seen as paradoxical (Frankham 2005) as genetic diversity is often positively correlated with fitness (Reed and Frankham 2003).

On the other hand, genetic diversity may be less important for survival of asexually reproducing insects inhabiting greenhouses. Parthenogenetic reproduction may enable survival under strong selection pressure in human mediated environments by facilitating fixation of genes linked with insecticide resistance and lead to the predominance of homogeneous resistant populations (Dunley and Croft 1992, Denholm *et al.* 2007, Karunker *et al.* 2008). Low overall genetic diversity of resistant populations may be outweighed by beneficial resistance traits contributing to establishment and invasion success. Low genetic variability does not always reduce phenotypic plasticity. *Adelges cooleyi* in eastern North America has significantly reduced genetic and phenotypic variation, but despite this it is able to retain host phenotypes similar to those found in the potential donor region (Ahern *et al.* 2008). Furthermore, phenotypic plasticity in morphology, behaviour and life history traits that contribute to invasion success could persist in genetically homogeneous clone populations of asexually reproducing successful invaders (Gray *et al.* 1986, Gorur 2000). Phenotypic plasticity is even favoured over genetic polymorphism under short term, seasonal exposures to stochastic heterogeneous environments (Gorur 2000, Moczek 2010). Such temporal environments are commonly encountered by NIS populations dispersing from greenhouses to seasonal outdoor habitats in the boreal region. Elimination of mating barriers among these populations, e.g. during active dispersal at the end of crop season, may lead to the formation of super colonies (Cox 2004). Furthermore, it has been observed, that species introduced to new environments are particularly prone to rapid evolution (Cox 2004). Therefore, agroecosystems may serve as arenas for evolutionary change (Via 1990).

1.3.5 Biotic interactions

Biological interactions can already occur at the initial stages of invasion (stage II, Fig. 1) and could disrupt establishment of permanent populations of exotic species in the newly encountered environment. Although, agroecosystems exhibit reduced biodiversity aiding NIS establishment in these habitats (stage II b, Fig. 1), proceeding to further invasion stages is arduous. Establishment and naturalization outside monoculture agroecosystems (stage III a and b, Fig. 1) depend on the success of interactions with potential competitors and predators inhabiting diverse natural vegetation (Altieri and Nicholls 2004).

Indoor and outdoor vegetation is an important component of the biotic environment, which can induce phenotypic plasticity in introduced herbivores and provide arenas for pest interactions with local insect fauna. Indoor vegetation usually consists of only single or few crop species demanded by the crop market. This can create a selection pressure for insect herbivores to specialize in a few common crops, favouring dominance of a few host races within species (Drès and Mallet 2002, Carletto *et al.* 2009). Adaptation to commonly produced crops ensures host plant availability and invasion success, as in case with widely distributed Colorado potato beetle adapted to potato plants (Hsiao 1978, Grapputo *et al.* 2005). However, low host plant diversity, which is typical in commercial greenhouses, could decrease the ability of herbivorous NIS populations to utilize novel hosts in outdoor habitats. In addition, it has been observed that adaptation to some crop plants commonly grown in greenhouses reduces insect susceptibility to some insecticides (Castle *et al.* 2009, Xie *et al.* 2011). Thus, crop rotation practice reducing opportunities for NIS to adapt to host plants should be considered as important NIS management tool. Possible effects of outdoor vegetation on insect susceptibility to insecticides, however, remain unexplored.

Vegetation is the first element of natural ecosystem encountered by introduced herbivores attempting to establish local populations and eventually naturalize (stages III a and b respectively, Fig.1). The ability to adopt a change from the indoor monoculture environment to diverse natural vegetation highlights the higher invasive potential of generalist herbivores. Generalist species are able to adapt to various habitat changes and are rapidly expanding their ranges by replacing specialist species (Clavel *et al.* 2011).

Selection of suitable outdoor host plants is followed by interaction with predators and potential competitors sharing the same hosts. Natural fauna outdoors might be outcompeted by abundant pest populations generated in optimal conditions inside greenhouses. Greenhouse fauna dispersed to live on outdoor plants can induce changes in natural vegetation repelling other insects or have detrimental effects on their developmental parameters. For example *B. tabaci* nymphs can have plant-mediated negative influence on development speed of lepidopteran larvae (Inbar *et al.* 1999) or survival of other whitefly species (Zhang *et al.* 2013). Thus, local communities might be affected by plant-mediated competition among insect herbivores, when one species induces

changes in plant chemistry, nutrition, or morphology that are unsuitable to other insects. An example of such a case is competition between potato pests. Leafhoppers (*Empoasca fabae*) occurring earlier on potato modify the morphology of leaves and repel Colorado potato beetles (*Leprinotarsa decemlineata*) (Lynch *et al.* 2006). The interaction of natural biota with exotic pests escaping greenhouse environments is enhanced by pest transmission of plant viruses and insect bacteria. Bacterial endosymbionts can have a range of effects on the biology of their insect hosts by inducing changes in insecticide resistance and thermal tolerance, by providing nutritional supplements to their hosts, by increasing survival and fecundity or manipulating the reproduction of hosts (Brownlie and Johnson 2009, Saridaki and Bourtzis 2010, Mouton *et al.* 2012, Kikuchi *et al.* 2012). In addition, biotic interactions in natural habitats could be broadened by dispersal of other elements of indoor trophic network - commercially produced biological control agents, i.e. predators and parasites of pests.

These potential networks of community interactions differ in their complexity in different climate regions. In general, biodiversity in the Mediterranean basin is high, with potential for complex multi-trophic interactions between pests, beneficial insects and natural biota (Médail and Quezél 1999). Pest management employing only biological control agents in this region, especially in less isolated plastic covered greenhouses is less effective, whereas producers in the boreal region are able to efficiently control pests using beneficial insects targeting certain common pest species (van Lenteren 2000). The differences of pest restraining forces may result in different dynamics of local populations leading to a varying degree of pest dispersal in the region of introduction and complexity of connectivity among populations.

1.4 Metapopulation structure of agroecosystem pests

Metapopulation in classical terms considered an "assemblage of spatially delimited local populations that are coupled by some degree of migration" (Hanski 1999). The term was originally introduced by Levins (1969) to estimate the dynamics of pest subpopulations and find an effective pest management strategy (Levins 1969, Hanski and Gilpin 1991). Due to varying degree of connectivity of greenhouse pest populations moving through global seedling trade, we, perhaps, should consider perceiving pests in agroecosystems as part of a worldwide metapopulation.

According to Colautti and MacIsaac (2004) the final stages of invasion occur in widespread populations, which are potentially forming a metapopulation if some degree of connectivity is present among them. Dispersal can, however, already occur after introduced NIS individuals established a persistent population in a greenhouse agroecosystem (stage II c, Fig. 1) or become naturalized outdoors (stage III b, Fig. 1). After each dispersal period the NIS goes through similar stages in invasion process overcoming the

geography barrier, introduction, and so on (Fig. 1). This is in concordance with Davis (2009), who proposed that the stages of invasion can also be described as an ongoing series of cyclical iterations, summarized as two fundamental processes: dispersal and establishment, occurring on the individual, population and metapopulation levels.

Agroecosystems consisting of field and greenhouse habitats surrounded by weed biotopes form a network of resource patches fluctuating in space and time with crop rotation and seasonal changes of habitat availability. These dynamics of resources may be a major determinant for population structure of herbivorous insects (Bailly *et al.* 2004). Frequent changes of available host plants might favour generalist phenotypes and lead to homogenization of populations. Alternatively, long term availability and fragmentation of resources might limit dispersal facilitating local adaptation to available host plants and result in increased genetic structure. Thus, population structure might be determined not only by habitat availability but also by species plasticity (generalist or specialist species traits). Connectivity of populations also depends on the dispersal abilities of the insect. Actively flying species have an advantage over sessile non-flying insect stages or species. The flying insects have higher dispersal rates and gene flow among populations than do non-flying species. Thus, flying insects are potentially less vulnerable to stochastic mortality events from insecticide applications or genetic drift, than are more sedentary organisms.

Stochasticity for NIS in agroecosystems is created by eradication of crop plants at the end of the crop season, insecticide applications and/or release of predators or parasites as a mean of biological pest control. Frequent high mortality events reduce genetic diversity, but at the same time create vacant habitats. These empty habitat patches can be partially or completely recolonized by new pest populations, leading to changes in the overall heterozygosity of the metapopulation. The genetic diversity of populations in recolonized habitat is likely to be low in case of arrival from a single source – a propagule gene pool, and is likely to be higher if the new population is established from mixed sources – a migrant pool (Whitlock and McCauley 1990). Development of low genetic diversity among and within populations on wide geographical scales may occur as a result of selection pressure of insecticide applications leading to dominance of resistant genotypes. For example, insecticide selection pressure and monoculture cultivation resulted in low genetic diversity of the common insect pest, *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Zamoum *et al.* 2005). However, since the development of resistance may involve physiological changes and phenotypic plasticity there could be no effect on genetic diversity. The stochastic pest mortality events created by individual management of agroecosystems create a network of insect sinks and sources and contributes to ineffective pest management (Levins 1969, Hanski and Gilpin 1991).

The variation of resources in different climate regions may result in different metapopulation structure of the same species. In Northern Europe, isolation in winter is higher than it is in southern regions, since vegetation is

deciduous and dispersal among patches will be low under subzero temperatures. Northern climate facilitating isolation of populations might cause Wahlund effects by increasing population structure, but reducing genetic diversity within populations due to genetic drift in founder populations resulting in overall low heterozygosity (Hamilton 2009). Insect movement could be restricted at northern latitudes also due to a high proportion of enclosed high technology greenhouses and availability of fields and other less enclosed habitats for herbivores only in the short benign season. Higher connectivity of agriculture habitats in the Mediterranean region due to year-round availability of natural vegetation may facilitate homogenisation of herbivore populations. Shorter development time under higher temperatures of Southern climate may lead to higher insect abundances and formation of a larger gene pool with time. Analysis of connectivity of agricultural landscapes can be used to predict dispersal rates of invasive species in the area as well as design the regional pest management programmes (Margosian *et al.* 2009).

1.5 The case of Greenhouse Whitefly

Whiteflies (Hemiptera: Aleyrodidae) are phloem feeding insects. Two polyphagous invasive species feeding on herbaceous plants, *Trialeurodes vaporariorum* (Westwood, 1856) and *Bemisia tabaci* (Gennadius, 1889) are the whitefly species most often mentioned in the literature (Byrne and Bellows 1991). The first record of *T. vaporariorum* in Europe was made in 1856 in UK (Westwood 1856) and *B. tabaci* was described in 1889 in Greece (Oliveira *et al.* 2001). Recent findings indicate a Paleotropical origin of whiteflies (Aleyrodidae) (Boykin *et al.* 2013). However, *T. vaporariorum* in Europe potentially originated from South America, whereas the *B. tabaci* species complex originated in the sub-Saharan region of Africa (Boykin *et al.* 2013). Based on analysis of the mitochondtrial (mt) COI gene, *B. tabaci* is a complex of at least 24 morphologically indistinguishable, cryptic species (De Barro *et al.* 2011, Boykin *et al.* 2012). The most widespread and damaging members of this species complex are the Middle East-Asia Minor 1 species (MEAM1, commonly known as the B biotype) and the Mediterranean species (MED, commonly known as the Q biotype) (De Barro *et al.* 2011). In contrast, the few studies analyzing genetic diversity of *T. vaporariorum* demonstrate low polymorphism and absence of cryptic species (Malumphy *et al.* 2007, Roopa *et al.* 2012, Prijović *et al.* 2014). Although both *B. tabaci* and *T. vaporariorum* have been recorded in Europe at a similar time, more active spread was recorded in 1980 (Baufeld and Unger 1994). This could be attributed to an increase of production in year-round nurseries and large scale commercial greenhouses that provide habitats for these pests. Development of agriculture, however, has not contributed to speciation of *B. tabaci,* since major lineages diverged earlier, but were not noticed until the development of molecular markers (Boykin *et al.* 2013).

Nevertheless, human activity might have facilitated spread of whiteflies worldwide.

Both commonly studied whitefly species have many traits attributed to successful invaders. The economic impact of both *B. tabaci* and *T. vaporariorum* can be estimated through yield loss due to virus transmission and changes in physiology of plants. While feeding on the phloem of plants, whiteflies excrete honeydew that enables mold development and reduces photosynthesis (Inbar and Gerling 2008). The damage done by *T. vaporariorum* may reduce the yield of such common greenhouse crops as cucumber by up to 40% (HeungYong *et al.* 2009). However, the interest of researchers is often directed to *B. tabaci* due to much higher frequency of virus transmission that that in *T. vaporariorum* (Rosell *et al.* 1999). Nevertheless, resistance to commonly used insecticides is frequently described for both whitefly species (Gorman *et al.* 2001, Nauen and Denholm 2005, Longhurst *et al.* 2013). Resistance reduces species susceptibility to the main control force in the greenhouse environment and can lead to ecological explosions, as described in Elton (1958). Their parthenogenetic reproduction, when unfertilized diploid females produce haploid males, not only facilitates faster breeding in whiteflies compared to other insect pests, but also leads to the fixation of resistance related genes (Karunker *et al.* 2008). Natural control forces, such as predators, can be ineffective, since *B. tabaci* is known to avoid plants bearing predatory mites (Nomikou *et al.* 2003).

T. vaporariorum and *B. tabaci* are polyphagous insects, able to utilize diverse crop, ornamental and wild host plants. Polyphagy is expected to have enabled the formation of persistent widespread populations in areas with mild winters and year-round production, such as in Arizona and California (Castle *et al.* 2009). Small size of the adult body (1.5 mm) (Martin 1999) and hard to detect eggs allows whiteflies to remain unnoticed during initial stages of introduction and produce at least one generation of flying adults. Small body size, however, does not make them weak fliers, as they may sustain flight for at least 3h against wind current and disperse from 7 km in 12h (Byrne 1999) to 20 km (Berlinger *et al.* 1996). Occurrence on globally traded ornamental seedlings, the main vector of agricultural pests, results in their current, almost cosmopolitan distribution (Kenis *et al.* 2007).

Species with cosmopolitan distributions offer an opportunity to study the impact of environmental heterogeneity and climate variability on phenotypic plasticity within the same species. Such studies allow determination of traits leading to successful worldwide dispersal and help us to predict invasions of other species. Due to frequent transmission of harmful crop plant viruses and high genetic variability of *B. tabaci*, this species has been an important object of research almost worldwide (Inbar and Gerling 2008). However, the research on *B. tabaci* at northern latitudes is limited, because here the species is still under quarantine, it occurs mostly on imported ornamental plants and it faces complete eradication upon encounter (Vänninen *et al.* 2011). The increasing frequency of such events (EVIRA 2008), however, calls for exploration of pathways and vectors of introduction, as well as local dispersal potential of *B.*

tabaci at northern latitudes. Such studies would help to prepare management guidelines upon *B. tabaci* dispersal to production units in the area.

The greenhouse whitefly, *T. vaporariorum* is a common pest occurring in sympatry with *B. tabaci* almost worldwide. It can serve as model organism for the inference of *B. tabaci* establishment and dispersal potential at northern latitudes. The cold winters currently prevent naturalization of both species outdoors, since neither of them has a diapause resting stage or are freeze tolerant. However, *T. vaporariorum* has been able to persist in Finland since 1920 (Linnaniemi 1921). The distribution of the species in winter is limited to greenhouses with climate control system. The dispersal among populations may occur from spring to late autumn, when diverse outdoor habitats may provide host plants for the species. Although *T. vaporariorum* is known for its polyphagy and morphological phenotypic plasticity in response to host plants (Neal and Bentz 1999), a prolonged period of host experience sometimes leads to specialization on the host and formation of host races (Roditakis 1990, Lei *et al.* 1998, Bezerra 2004). Thus, the ability of this species to utilize host plants other than greenhouse crops in the boreal climate zone is uncertain. The stage of invasion of this herbivore at northern latitudes remains unclear. The species has passed through initial stages of the invasion process in agroecosystems (stage II, Fig. 1) and dispersal among populations (stages IV-V, Fig. 1) and may be forming a widespread metapopulation with varying levels of abundance and degree of connectivity in Finland. However, the degree of naturalization in boreal ecosystem remains unexplored. Furthermore, genetic diversity of *T. vaporariorum* populations and the degree of connectivity regionally or on a global scale have been analyzed only rarely.

1.6 Aims of the thesis

The main objectives of the thesis were to investigate the role of greenhouse agroecosystems, crop management practices and surrounding natural habitats to naturalization and persistence of a generalist herbivore, *T. vaporariorum*.

In the first part of the thesis (study I) I examined the extent of *T. vaporariorum* naturalization in the boreal ecosystem, since it has occurred in the area for almost 100 years in protected horticulture. Because monoculture habitats allow for year-round insect populations, create possibilities for host adaptation and could lead to the development of host races over time (Altieri *et al.* 1984, Altieri and Nicholls 2004), I first studied if *T. vaporariorum* still has the ability to reproduce on seasonal diverse natural vegetation (study I). Then, using the knowledge gained in the host plant survey, I wanted to evaluate if preconditioning on a greenhouse crop species might increase fecundity or facilitate dispersal of *T. vaporariorum* to novel hosts. In the host choice experiment, I analyzed the ability of adults preconditioned on three common crop plant species to utilize novel hosts and reproductive suitability of the hosts chosen for oviposition (study I).

Relatively isolated greenhouse environments can reduce gene flow and increase genetic structure of populations (Franklin *et al.* 2010). In addition, single crop cultivation can select for the prevalence of homogeneous host race (Drès and Mallet 2002). I further examined the role of greenhouse crops and enclosed greenhouse environments on the genetic structure of populations by developing species specific microsatellite markers for *T. vaporariorum* (study II) and analyzing genetic diversity and population genetic structure of the pest in Southern and Northern Europe (study III). In this study (III), I aimed to compare the role of climate, horticulture practices and diversity of agroecosystems in the different climatic zones on the population genetic structure of this insect of tropical origin.

To determine if occurrence in greenhouse environments under chemical control resulted in reduced insecticide susceptibility and contributed to insect persistence in the area, I measured the response of *T. vaporariorum* to insecticides. Populations of *T. vaporariorum* inhabiting greenhouses with year-round tomato and cucumber crops, and varying insecticide application histories were studied (study IV).

The worldwide distribution of insects dispersing through global seedling trade and inhabiting habitats with varying climate conditions in heterogeneous landscapes may result in surprisingly homogeneous populations due to monoculture cultivation and stochastic mortality events in agroecosystems (Navajas *et al.* 1998). In the last study I analyzed genetic diversity of *T. vaporariorum* from different locations worldwide by examining mitochondrial DNA polymorphism and endosymbiont diversity (study V).

To summarize, in my thesis I asked four general questions:

1. What is the role of host plant experience to invasive potential of a generalist herbivore? (studies I, III)
2. How do agroecosystems shape the genetic population structure of a greenhouse pest in Northern and Southern Europe? (studies II, III)
3. Is there variation in physiological responses to insecticide treatments of populations with low genetic diversity distributed over a small spatial scale? (studies III, IV)
4. What is the genetic diversity of the cosmopolitan greenhouse pest? (study V)

2 MATERIAL AND METHODS

2.1 Insect sampling (studies II-V)

Each sampled population is described in Table 1 and its location is indicated in Fig. 2. Majority of *T. vaporariorum* populations from greenhouses in Finland and Greece (studies II, III, IV) were sampled within a single greenhouse room containing a single host plant (except HR, ML a, NL and MA2, study III) from different greenhouse crop producers and various locations. In Finland *T. vaporariorum* was sampled from greenhouse crops in springs of 2010-2012 before the start of active whitefly dispersal in the region. To describe insect persistence and temporal differences among locations (study III), ten of these greenhouses were sampled repeatedly in 2010 and 2011. All 18 greenhouses sampled in Finland (study II, III, V) were high-technology glass or double acrylic sheet covered greenhouses (except NR3 greenhouse covered with plastic sheets) with climate control system producing tomato and/or cucumber crops year round. In Greece, insects were sampled during various periods in 2004-2011 and from various crops (studies II, III, V) growing in eight fields and eight greenhouses of varying technology levels (study III). Whiteflies were collected using a mouth aspirator and immediately preserved in 90% ethanol.

Samples collected for global phylogeography study (study V) comprised whiteflies sampled from 38 locations, various crop producing greenhouses or laboratory cultures (Table 1, Fig 2). Whiteflies (studies II, V) were shipped by post in Ethanol or dry after preservation in Ethanol due to postal restrictions. All samples were preserved either at -80°C or in 90% Ethanol until being sexed and used for genetic analyses.

For insecticide bioassays (study IV) 100 adults from each sampled location (Fig. 2) were aspirated and transported in plexi glass cages to greenhouse compartments in MTT Agrifood Research (Jokionen) to obtain adequate insect numbers for bioassays (Fig. 3). Pupae of the susceptible reference population (REF, Table 1, Fig. 2) were received from Rothamsted Research (Harpenden, Hertfordshire, United Kingdom).

TABLE 1 Description of *T. vaporariorum* populations analyzed in this thesis. Acronyms G, F and lab indicate populations collected from greenhouse, field and laboratory cultivations, respectively.

Geographical information			**Host plant**		**Collection**		**Study**
Country/Region	Locality	Sample codes	Species	Family	Crop production system	Date	
Finland/ Ostrobothnia	Böle	BL	Tomato	Solanaceae	G	May-2012	IV
	Härkmeri	HR	Cucumber Tomato	Cucurbitaceae Solanaceae	G	May-2010 Apr-2011	III
	Korsnäs	KR	Cucumber	Cucurbitaceae	G	May-2010 Apr-2011	III
	Malax	ML	Cucumber Tomato Tomato	Cucurbitaceae Solanaceae Solanaceae	G	May-2010 Apr-2011 May-2012	III III IV
	Närpes	NR 1	Tomato	Solanaceae	G	May-2010 Apr-2011	III
		NR 2	Cucumber	Cucurbitaceae	G	May-2010	III
		NR 3	Cherry tomato Tomato	Solanaceae	G	May-2010 Apr-2011	III
	Pjelax	PJ 1	Tomato	Solanaceae	G	May-2010 Apr-2011 May-2012	I, III III, IV IV
		PJ 2	Tomato	Solanaceae	G	May-2010	III
		PJ 3	Tomato	Solanaceae	G	May-2010 Apr-2011	III
		PJ 4	Tomato	Solanaceae	G	Apr-2011 May-2012	III, IV IV
		PJ 5	Tomato	Solanaceae	G	Apr-2011 May-2012	III, IV IV

	Pörtom	PR, Finland, FI_PO	Tomato	Solanaceae	G	May-2010 Apr-2011 May-2012	I, III II, III, IV, V IV
	Töjby	TJ 1, FI_TO	Cucumber	Cucurbitaceae	G	May-2010 Apr-2011	I, III III, IV, V
		TJ 2	Cucumber	Cucurbitaceae	G	May-2010 Apr-2011	III
	Övermark	OV	Tomato	Solanaceae	G	Apr-2011	III
Uusimaa	Lohja	Finland, LH	Cucumber	Cucurbitaceae	G	Apr-2011	II, III
	Nilsiä	NL	Cucumber	Cucurbitaceae	G	Jul-2012	III
Greece/ West Peloponnese	Kourtesi	WP 1, GR_PY	Cucumber	Cucurbitaceae	F	Jun-2004	III, V
	Filiatra	WP 2, GR_FI	Zuccini	Cucurbitaceae	F	Jul-2004	III, V
	Elea	WP 3	Eggplant	Solanaceae	F	Aug-2011	III
	Prasidaki	WP 4	Bean	Fabaceae	F	Aug-2011	III
	Anemochori	WP 5	Tomato	Solanaceae	F	Sep-2011	III
	Terpsithea	WP 6	Bean	Fabaceae	F	Sep-2011	III
	Andravida	WP 7	Marrow	Cucurbitaceae	F	Sep-2011	III
North Peloponnese	Aigio	NP	Rose	Rosaceae	G	Aug-2011	III
West Greece	Agrinio	WG, GR_AG	Tomato	Solanaceae	G	Jun-2011	III, V
East Peloponnese	Navplion	EP, GR_NA	Bean	Fabaceae	F	Oct-2011	III, V
Attica	Athens	AT, GR_AT	Eggplant	Solanaceae	G	Apr-2005	III, V
Island of Crete	Fodele	Greece CR 1, GR_FO	Rose	Rosaceae	G	Mar-2011 Mar-2010	II III, V
	Sissi	CR 2, GR_SI	Rose	Rosaceae	G	Apr-2011	III, V
	Ierapetra	GR_IE	Zuccini	Cucurbitaceae	G	Jun-2002	V
	Malades	CR 3	Datura	Solanaceae	G	Apr-2011	III
Island of Rhodes	Madriko	GR_RH	Tomato	Solanaceae	G	Jul-2011	V
Island of Evia	Vasilika	GR_EV	Marrow	Cucurbitaceae	G	Jun-2008	V
Macedonia	Sidirokastro	Greece	Tomato	Solanaceae	G	May-2011	II
	Serres	MA 1, GR_SE	Tomato	Solanaceae	G	May-2011	III, V
	Drama	MA 2, GR_DR	Sweet pepper	Solanaceae	G	May-2011	III, V

France	Toulouges	France	Tomato, Cabbage	Solanaceae Brassicaceae	F	Oct-2007	II
	Alenya	FR_AL	Tomato	Solanaceae	G	Sep-2007	V
	Palau de Vivre	FR_PA	Zuccini	Cucurbitaceae	F	Jun-2007	V
	Montferrier	FR_MO	Tobacco	Solanaceae	G	Dec-2007	V
	Cabrera	FR_CA	Zuccini	Cucurbitaceae	-	Jul-2007	V
	Pernes	FR_PE	Eggplant, Tomato	Solanaceae	G	Aug-2008	V
Italy	Ragusa, Sicily	IT_SI	*Borago officinalis*	Boraginaceae	F	Oct-2011	V
Turkey	-	TR	Vegetables	-	-	2006	V
Israel	-	IL_Lab	Cucumber	Cucurbitaceae	lab	Apr-2009	V
Kenya	-	KE2	Rose	Rosaceae	F	Sep-2011	V
	-	KE1	Ornamentals	-	F	Sep-2011	V
USA	California	US_CA	*Physalis wrightii*, *Malva parriflora*	Solanaceae, Malvaceae	lab	1960	V
Equador	-	EC	Rose	Rosaceae	F	Sep-2011	V
Colombia	-	CO	*Solidago* sp.	Asteraceae	F	Oct-2011	V
Australia	-	AU_Lab	Tobacco	Solanaceae	lab	1992	V
	Virginia	AU_VI	Eggplant	Solanaceae	G	2011	V
Japan	Tsu-city	JP_TS	Tobacco	Solanaceae	lab	Jul-2004	V
China	Beijing	CN_BE	Tomato	Solanaceae	G	Oct-2011	V
Russia	Pushkin	RU_PU	Ornamental	-	G	Sep-2011	V
Norway	Arendal	NO_AR	Datura	Solanaceae	G	Apr-2008	V
UK	Albrighton	UK_AL	Rose	Rosaceae	-	2010	V
	Harpenden	REF, UK_Lab	Bean	Fabaceae	lab	1971	IV, V
Netherlands	Lienden	NL_LI	*Pelargonium radens*	Geraniaceae	F	Oct-2011	V
Germany	-	DE	Ornamentals	-	-	2008	V
	-	DE_Lab	Various hosts	-	lab	Jul-2011	V

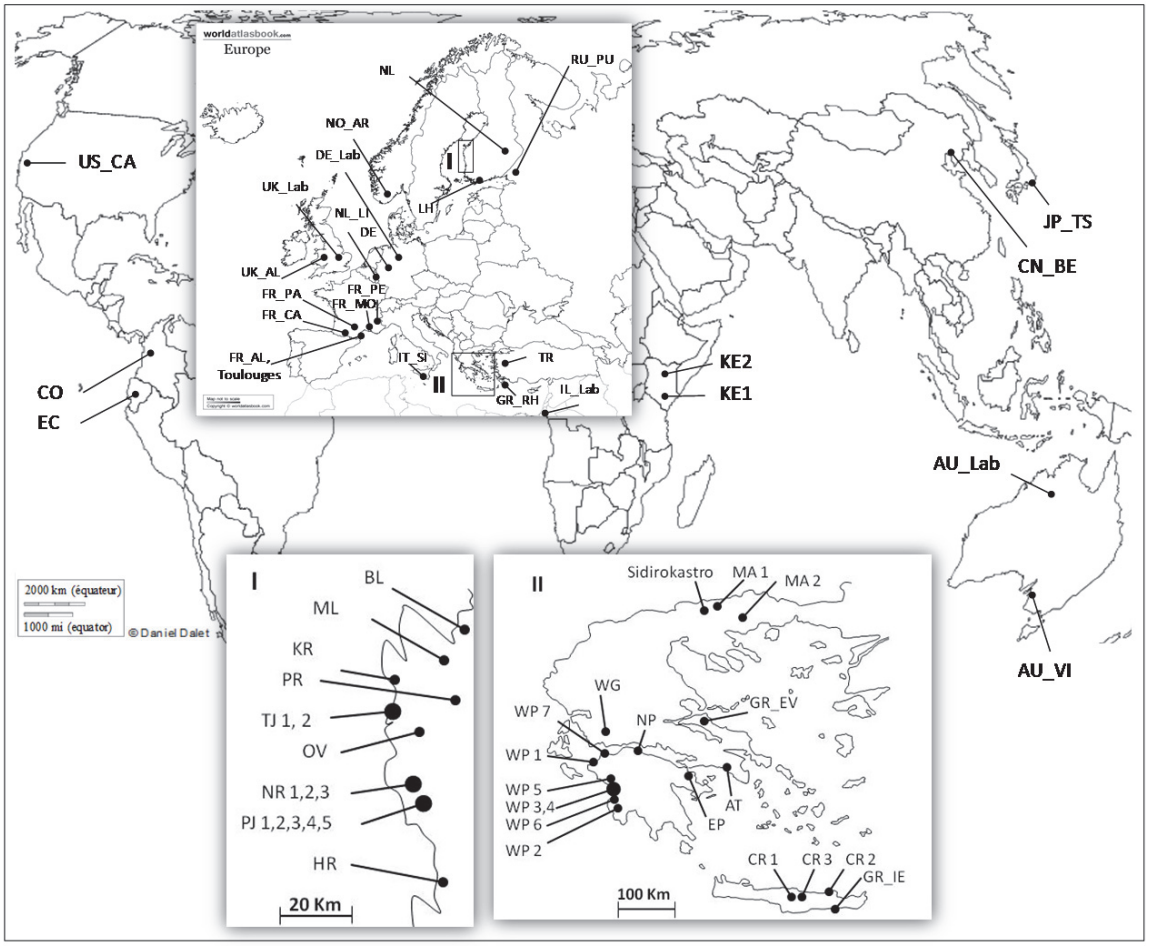

FIGURE 2 Geographical locations of *T. vaporariorum* populations analyzed in this thesis. Acronyms of populations and the specific studies in which they were used for are shown in Table 1.

2.2 Insect and plant rearing conditions (studies I, IV)

To test the effect of host plant experience on host choice (study I), three whitefly strains with different host experience were created. A population maintained on poinsettia since 2007 was divided in 2011 into three groups by transferring 100 mixed gender adults into separate plexi-glass cages with tomato (*Solanum lycopersicum* L. cv. Encore), cucumber (*Cucumis sativus* L. cv. Eminentia) and poinsettia (*Euphorbia pulcherrima* Willd.ex Koltz. cv. Allegra by Lazzeri®). These three *T. vaporariorum* strains were maintained (Fig. 3) in the greenhouses of MTT (Jokioinen) for one year before the start of the choice experiment in 2012.

Populations collected for insecticide bioassays (study IV) were maintained (Fig. 3) and tested on their original host plant to minimize mortality and lower fitness due to exposure to novel host plant species (Van Lenteren 1990). The only exception was made for the susceptible reference population (REF), which was reared on tomato for at least three generations before insecticide bioassays, although it has been reared on bean in UK. Three generations are considered the minimum period of adjustment on the new hosts for *T. vaporariorum* (Greenberg *et al.* 2009).

The crop plants for insect maintenance (studies I, IV) were grown from seeds (cucumber and tomato) or seedlings (poinsettia). The wild plant species: nettle (*Urtica dioica* L.), fireweed (*Epilobium angustifolium* L.), dandelion (*Taraxacum officinale* F.H. Wigg) and red clover (*Trifolium pratense* L.) were grown from seeds purchased from Herbiseed© (UK). All plants were grown in pots containing peat, watered daily and maintained in pest free greenhouse room prior to the experiments.

The greenhouse climatic settings for both insect and plant rearing were as follows: 20-24˚C and 16:8 h light:dark photoperiod maintained using high pressure sodium lamps (Philips, 200W).

FIGURE 3 Insect rearing on tomatoes in plexi glass cages (studies I and IV).

2.3 Host plant survey and choice experiment (study I)

Ability of *T. vaporariorum* to utilize diverse natural vegetation after exposure to monoculture in greenhouses (Fig. 4) was studied in host plant survey during 20-29 July, 2010 and host choice experiment during October - December, 2012. The survey was conducted around three infested greenhouses, cultivating tomato and cucumber year round (Table 1, Fig. 2). Plants within one meter distance from greenhouses were inspected for presence of eggs, nymphs, pupae and adults. The majority of the plants were identified to species level but vegetative or seedling stages were identified only to genus level (e.g. *Geranium* sp) (Mossberg and Stenberg 2003). Grasses were identified only as members of Poaceae, as no life stage of *T. vaporariorum* was found on them during the survey. Overall, the plots consisted of more than 50 plant species. Three 1m^2 vegetation plots were inspected from four sides (north, south, east and west) of the greenhouse. This resulted in 12 plots per greenhouse and 36 plots in total. I also estimated coverage of each plant species in the plot and Shannon-Wiener diversity index.

FIGURE 4 Monoculture greenhouse environment and diverse natural vegetation near greenhouses in Ostrabothnia, Finland.

Based on the host plant survey I designed a host choice experiment. Here *T. vaporariorum* females were first preconditioned on three common crop plants in Finland (tomato, cucumber or poinsettia) for three generations. Then the females' host preference was tested by allowing them to make a choice among seven offered plant species (Fig. 5). *T. vaporariorum* females (100 per stain/ repeat) were released in three separate greenhouse compartments (W: 2.8, L: 8.5, H: 6 m) and the procedure was repeated three times for each strain, randomizing location (greenhouse compartment) and arrangement of the plants. Feeding and oviposition preference were estimated by counting

whiteflies after 1h and 48h from release, respectively, following modified protocols of Van Lenteren and Noldus (1990) and Verschoor and Van Lenteren (1978). After 48h from release the adults were removed from the plants and fecundity was estimated by counting eggs. Host plant species suitability was estimated by counting pupal exuviae.

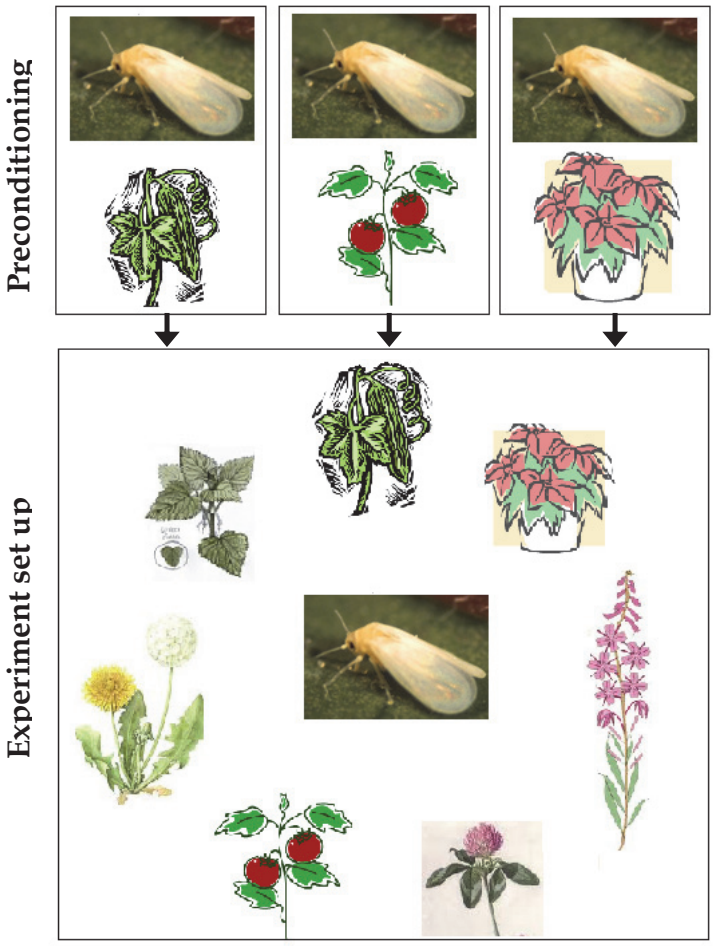

FIGURE 5 The scheme of host plant choice experiment. Greenhouse whitefly strains preconditioned on cucumber, tomato or poinsettia for one year were allowed to make a choice between seven host plants: dandelion, nettle, cucumber, poinsettia, fireweed, red clover and tomato. Females of each preconditioned whitefly strain were released into insect free greenhouse room with varying arrangements of plants. Releases were repeated three times for each strain.

2.4 Insecticide bioassays (study IV)

The susceptibility of different whitefly population was tested against three insecticides. These were selected based on their importance for whitefly management in the area. The greenhouse crop producers have observed a reduced efficacy of pymetrozine and imidacoprid in the study area but have not noticed such a decrease for spiromesifen (Vänninen pers. comm.). The susceptibility of whiteflies was estimated from 7 populations (Table 1) and compared to the reference strain (REF), which was maintained without exposure to insecticides since 1971. In the bioassays pymetrozine, imidacoprid and spiromesifen were applied as formulated products: Plenum WG-50 (Syngenta), Confidor WG-70 and Oberon SC-240 (Bayer CropScience) respectively. The products were diluted to required concentrations in distilled water containing 0.1g L-1 of non-ionic wetter Agral® (Syngenta). Control treatments contained water and Agral® only.

Pymetrozine and imidacloprid were tested using modified (Karatolos 2010, Cahill 1995) leaf dip bioassays (Fig. 6) using cut leaves. First, tomato leaf discs were dipped for 1 min in any of the five used insecticide solutions (five replicates per concentration) and air-dried for 20 min. Then, the disks were laid abaxial side up on 1% agar in petri dishes. The petri dishes lids were fully covered with thin breathable filter paper (Hygia™) to absorb excess moisture and to prevent the formation of static electricity. The edges of the petri dishes were covered with a rubber seal to prevent the leaf disc from drying due to contact with the filter paper. Twenty to thirty adult whiteflies of mixed gender and age were transferred to petri dishes containing leaf discs treated with insecticides. Closed petri dishes were sealed by wrapping sealing film (PARAFILM® M) around their edges to prevent whiteflies from escaping. To imitate natural leaf position (abaxial side down) dishes were inverted and transferred to a metal grid shelf (to allow ventilation) in an incubator (16h light: 8h dark photoperiod and 22-24˚C temperature).

Mortality was scored after 72h for imidacloprid and after 96h for pymetrozine. Whiteflies were considered dead if no movement was observed in response to touching them with a brush. The period of insect exposure to adulticides exceeded the period of insect survival without food, since 50% of whiteflies usually die from starvation within 35h in empty petri dishes (Nauen *et al.* 1998). Thus, live individuals found on pymetrozine treated leaf discs had fed on the treated leaf discs and showed resistance to this feeding inhibitor. Consequently, I expect the results were not affected by delayed mortality from starvation.

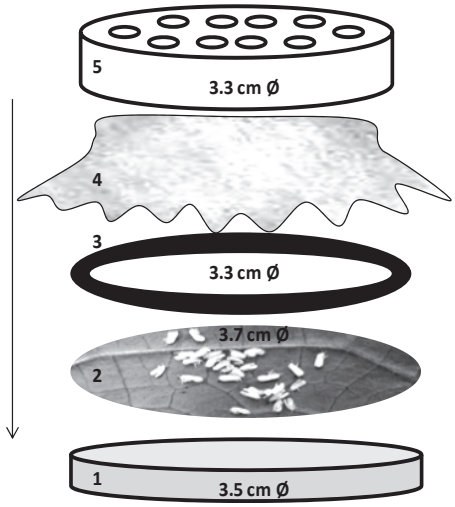

FIGURE 6 Schematic of the petri dish chambers used for pymetrozine and imidacloprid bioassays: 1 – petri dish containing 1% agar; 2 – leaf disc treated with insecticides and 30 immobilized adult whiteflies; 3 – rubber seal; 4 – thin breathable filter paper (Hygia™); 5 – perforated petri dish lid.

Spiromesifen was tested using a modified Liu (2004) method on leaves of living plants. First, synchronized by age cohorts of 2nd instar nymphs were produced by allowing females to lay eggs for 24h. For that purpose, during the same day one female and one male were released into 15 mini clip cages (1cm deep, 1cm in diameter, Fig. 7), attached to leaves of individually planted seedlings (three clip cages per seedling, one clip cage per leaf) and removed after 24h. Then, these seedlings were kept in separate insect-free greenhouses. After 12 days from egg-laying on cucumber and 15 days on tomato, the number of 2nd stage nymphs per leaf was counted. Nymphs on leaves were dipped in either one of four insecticide concentrations (each with three replicate plants), and allowed to hatch into adults. The mortality of nymphs due to spiromesifen was scored as the 1- abundance of hatched pupal cases after four weeks from nymph counting allowing for a maximum hatching rate (Yun *et al.* 2006).

FIGURE 7 The clip cage designed for whitefly mating and production of larval cohorts used for spiromisifen bioassays.

2.5 Genetic analyses (studies II, III, V)

2.5.1 Development and genotyping of microsatellite markers (study II, III)

Analysis of microsatellite loci can reveal recent processes, such as movement and relatedness of individuals, as well as recent colonisation events and dynamics within metapopulations (Sunnucks 2000). Among the whitefly species, such markers have been developed only for *B. tabaci*. Application of microsatellite markers developed for closely related species, i.e. cross species amplification, is a common practice (Primmer *et al.* 1996). Thus, 48 markers developed for *B. tabaci* (Barro and Scott 2003, Tsagkarakou and Roditakis 2003, Delatte *et al.* 2006, Tsagkarakou *et al.* 2007, Dalmon *et al.* 2008, Gauthier *et al.* 2008), were tested on *T. vaporariorum* females collected from Finland. However, the amplification success using these primers was poor and when amplification of the markers was successful, sequencing of the products did not reveal homologous microsatellite regions.

As a result, species specific primers were developed for *T. vaporariorum* (study II) using a biotin-enrichment protocol (Gauthier *et al.* 2008) to isolate novel microsatellite loci. I then optimized 36 selected loci using DNA of *T. vaporariorum* individuals from a laboratory strain (MTT Agrifood Research, Finland). Twenty eight of the loci were successfully amplified as scored from agarose gel electrophoresis. These loci were then screened for a preliminary assessment of polymorphism using 8 females from Finland, France and Greece. After testing, 13 loci gave high quality amplifications with non-ambiguous allelic patterns and apparent polymorphism. These loci were arranged in three multiplex Polymerase Chain Reactions (PCR), that minimized differences in annealing temperatures and maximized the range of amplification product sizes using Multiplex Manager v1.1 (Holleley and Geerts 2009). To characterise the 13 loci, 104 *T. vaporariorum* females were genotyped from natural populations collected from three geographically distant countries: 40 from two locations in Finland, 40 from two locations in Greece and 24 from the South of France (Table 1, study II).

These developed markers were used to study how agroecosystems shape genetic population structure (study III). 800 *T. vaporariorum* females from Finland and 474 from Greece (Table 1, study III). Since *T. vaporariorum* males are haploid and females are diploid, only females were used for all genetic analyses of *T. vaporariorum* in studies II and III. Genomic DNA was extracted from females according to the protocol described by Delatte *et al.* (2005) (study II) and Tsagkarakou *et al.* (2007) (study III).

2.5.2 Analysis of mitochondrial genes (study V)

The phylogeography of *T. vaporariorum* was analyzed using mitochondrial genes. These markers are located in relatively conserved regions of the mitochondrial genome and have slower mutation rate than nuclear

microsatellite markers. Thus, analysis of mitochondrial genes can help to understand evolutionary history, systematics and phylogeography on the species level (Sunnucks 2000). Mitochondrial DNA polymorphism of 471 females, from at least one specimen per population was analyzed from partial sequences of three genes: cytochrome c oxidase subunit I (COI), cytochrome b (CytB) and NADH dehydrogenase 5 (ND5). DNA extraction was performed according to the protocol described by Tsagkarakou *et al.* (2007).

2.5.3 Analysis of *T. vaporariorum* endosymbiont diversity (study V)

Endosymbiotic bacteria can affect genetic diversity and reflect evolutionary history and phylogeny of their insect hosts (Moran *et al.* 1993, Wernegreen 2002). Therefore, the presence of secondary bacterial symbionts in *T. vaporariorum* was analysed using 16S rRNA gene and primers specific for *Wolbachia*, *Rickettsia*, *Hamiltonella* and *Cardinium* and the 23S rRNA gene and primers specific for *Arsenophonus* and *Fritschea*. A total of 640 females (4–20 per population) (see Table 1) were examined for the presence of any of six above mentioned endosymbionts and 218 males (up to 10 per population) were analyzed for occurrence of Arsenophonus. Genetic diversity of the most commonly occurring endosymbiont may resemble phylogeny of its host (Mouton *et al.* 2012). Thus, Arsenophonus-positive individuals (1-2) were randomly chosen from each population and analysed using Multi Locus Sequence Typing (MLST). PCR and sequencing of three different genes of Arsenophonus (ftsK, yaeT and fbaA) was carried out following Mouton *et al.* (2012).

2.6 Data analysis

2.6.1 Statistical analyses (study I, IV)

Host preference in the survey was analyzed using mixed models (PROC GLIMMIX) and SAS® software version 9.3 (SAS Institute Inc 2012). The choices made by *T. vaporariorum* in the experiment were estimated by a mixed model procedure (PROC MIXED) using SAS® Enterprise Guide® software version 5.1 (SAS Institute Inc 2012).

The mortality data for insecticide analysis was first adjusted to take into account mortality in control treatments using Schneider-Orelli's method (Schneider-Orelli 1947). To obtain LC_{50} values and their 95% confidence limits probit regression analysis was performed in PASW Statistics v. 18 (SPSS Inc.). Differences in LC_{50} between insecticides were considered significant if their respective 95% confidence limits did not overlap. The level of insecticide resistance of each population was estimated as resistance ratio (RR) by dividing the LC_{50} value of the sampled populations by the LC_{50} of the susceptible population.

2.6.2 Analyses of genetic data (study II, III, V)

Levels of polymorphism, possible departure from Hardy-Weinberg expectations (HWE) and linkage disequilibrium among loci (LD) were checked using GENEPOP 4.0 (Rousset 2008). For multiple tests, statistical significance was adjusted using strict Bonferroni corrections. For each sample, mean observed (H_O) and expected heterozygosity (H_E), and mean number of alleles (N_A) per locus were calculated using GenAlEx v. 6.5 (Peakall and Smouse 2012). The presence of null alleles or/and scoring errors was estimated using MICRO-CHECKER (van Oosterhout *et al.* 2004) (studies II and III).

Additional analyses were performed for study III. Genetic distances between samples, both between and within countries, were calculated using pairwise estimates of F_{ST} in Arlequin 3.11 (Excoffier *et al.* 2005). Since there were no significant differences between Finland and Greece, all further analyses were done for each country separately. To estimate the percentage of genetic variation explained by various groups, analyses of molecular variance (AMOVA) were performed using Arlequin 3.11 (Excoffier *et al.* 2005). The following groups were tested: 1) country (Finland vs. Greece), 2) host plant species (cucumber vs. tomato) in samples from Finland only, 3) host plant botanical family (Cucurbitaceae vs. Solanaceae vs. Fabaceae vs. Rosaceae) in samples from Greece only, and 4) crop production system (greenhouse vs. field) in samples from Greece only. The relationship between genetic and geographical distance was analyzed in GenAlEx v. 6.5 (Peakall and Smouse 2012). Using default settings of the software GESTE v. 2.0 (Foll and Gaggiotti 2006), I estimated how different environmental variables might explain the genetic structure. Latitude, host plant species, cultivar, crop source and year of sampling were evaluated as explanatory variables of population structure in Finland. Latitude, longitude, four host plant families and crop production system (field or greenhouse) were evaluated as explanatory variables of population structure in Greece. Bayesian clustering analysis implemented in STRUCTURE v.2.3.4 (Pritchard *et al.* 2000) was used to infer the number of genetically distinct clusters (K) in each country using a model of no admixture, correlated allele frequencies and including the sampling location as a prior (Hubisz *et al.* 2009).

In study V, each unique allelic combination comprised a haplotype. Genetic diversity of mt DNA sequences was assessed using DnaSP 5.10 (Rozas *et al.* 2003). Due to low polymorphism of the sequences a concatenated dataset combining the three genes (*COI*, *cytb* and *ND5*) was used in the study. The concatenation approach increases accuracy of phylogenetic relationships (Gadagkar *et al.* 2005). Phylogenetic analyses were performed using the maximum-likelihood method (ML) implemented PhyML v.3.0 (Guindon and Gascuel 2003). The best-fit model of nucleotide substitution was HKY85 based on the Akaike Information Criterion (AIC) determined using jModeltest (Posada 2008). Since the analyzed genes (*fbaA*, *yaeT* and *ftsK*) of *Arsenophonus* from 38 samples were monomorphic, no further analyses were carried out for this data.

3 RESULTS AND DISCUSSION

3.1 Greenhouse agroecosystems increase the invasive potential of *T. vaporariorum* in Finland (studies I, III)

3.1.1 Experience of monoculture does not diminish the generalist abilities of a herbivore (studies I, III)

Exposure to a single host plant is shown to facilitate adaptation and formation of host races in plant-feeding insects (Drès and Mallet 2002). Therefore, I hypothesized that *T. vaporariorum* inhabiting monocultures in greenhouses will have reduced abilities as a generalist herbivore and have better performance on familiar crop plants than on diverse outdoor vegetation. Contrary to my hypothesis, *T. vaporariorum* was able to lay eggs and they developed into pupae on 12 wild plant species (study I). This is particularly interesting, as in Finland diversity of greenhouse vegetable crops is low and production is concentrated on the cultivation of two main plant species: tomato and cucumber (TIKE and OSF 2014). In addition, year-round production dominates the market (TIKE and OSF 2014). Such conditions possibly favored pest adaptation to greenhouse crop plants, as observed from association of genetic structure of *T. vaporariorum* populations with crop plants they inhabited (study III). This could indicate an achievement of some degree of adaptation to tomato and cucumber hosts in Finland. Nevertheless, results of host choice experiments indicated a preference for outdoor hosts over crop plants, which the insects had experienced for one year previously.

Common weeds, such as nettles (Urticaceae) and fireweeds (Onagraceae) were the most preferred hosts of *T. vaporariorum* in the survey (study I), whereas dandelion attracted whiteflies the most in the experiment. Common occurrence of these outdoor hosts in temperate latitudes creates an opportunity for naturalization of the greenhouse whitefly (stage IIIb, Fig. 1). *T. vaporariorum* disperses to outdoor vegetation in spring (study I), when old crop plants are stored outdoors until recycled (pers. obs.) (Fig. 8) and returns to the same

greenhouse in autumn (study III), when new seedlings are planted in greenhouses. The latter is indicated by persistence of similar genotypes in most of the locations between years (study III). In addition, outdoor vegetation could serve as temporal habitats and refuges from insecticide treatments facilitating local pest persistence (Carriere *et al.* 2012). *T. vaporariorum* was observed on the weeds around greenhouses in early spring (study I) allowing it to have up to four generations in this temporal habitat until vegetation decays in late autumn. This period could be sufficient for decrease of *T. vaporariorum* susceptibility to imidacloprid, as resistance has been observed to be reduced in three generations without insecticide pressure (Gorman *et al.* 2007).

FIGURE 8 The storage of old infested greenhouse crops close to natural vegetation near greenhouses in Ostrabothnia, Finland. Photos courtesy of Irene Vänninen.

3.1.2 Experience of crop plants may increase propagule pressure and invasive potential of *T. vaporariorum* (studies I, III)

Invasion of the greenhouse whitefly in Finland can be facilitated by its experience of common crop plants, such as tomato, cucumber and poinsettia. Results of choice experiments indicate that experience of less preferred crop plants, poinsettia and tomato, may contribute to the invasive potential of *T. vaporariorum* by increasin its dispersal to other plant species. However, the propagule pressure from less preferred crops is likely to be lower than from cucumber due to lower fecundity of *T. vaporariorum* on them. Figure 9 shows that whiteflies preconditioned on poinsettia were significantly less fecund on offered hosts than whiteflies preconditioned on cucumber. On the other hand, fecundity of tomato strain on offered hosts did not differ from that of cucumber strain statistically significantly (Fig. 9). The absence of this difference, however, could be a result of high variation of egg abundance among offered hosts. This variation is increasing with number of eggs laid and is the highest for cucumber strain.

Since cucumber plants are characterized by relatively large leaf areas compared to other hosts, cucumbers provide more host plant resources for *T. vaporariorum* than other crop species. As observed from experimental data, leaf area contributed significantly to the abundance of whiteflies on the plants. Large leaf areas, coupled with preference and high fecundity on cucumber hosts may lead to the formation of highly abundant pest populations. In addition, results indicated that whiteflies originating from cucumber were the most fecund on majority of offered hosts. Therefore, cucumber can serve as a crop plant facilitating establishment of greenhouse whitefly in natural habitats by increasing the propagule pressure of the pest.

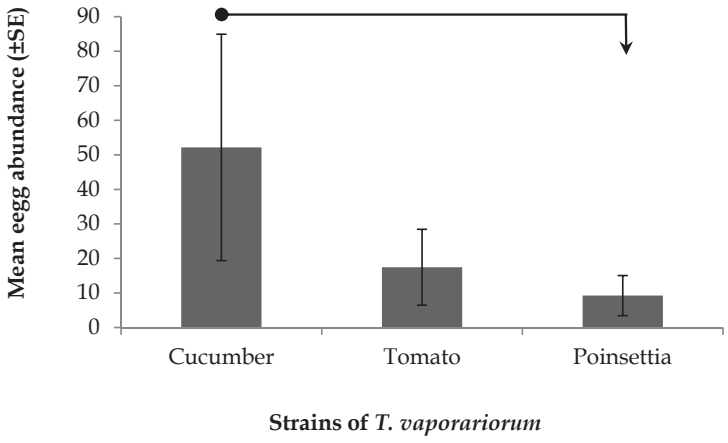

FIGURE 9 Average fecundity of *T. vaporariorum* strains on all offered hosts (study I). The arrow indicate the only significant ($p<0.05$) pairwise difference.

Furthermore, short development time (Yun *et al.* 2006) and high fecundity (study I) of *T. vaporariorum* on cucumber may lead to development of large *T. vaporariorum* population on cucumber crop. Genetic theory predicts a positive correlation between genetic variation and population size (Frankham 1996). However, whitefly populations on tomato were more genetically diverse than those on cucumber (study III). The potentially larger populations on tomato could be explained by the unequal sample sizes (Table 1) of this study or by local agricultural practices. Tomato cultivation is more common in Finland than cucumber (TIKE and OSF 2014) and our sampling efforts corresponded to this proportion. In addition, tomato crops are maintained for a longer part of the year leading to fewer genetic bottlenecks and creating possibility for development of larger populations of *T. vaporariorum* on tomato than on cucumber in Finland.

3.1.3 Persistence of *T. vaporariorum* in greenhouse agroecosystems provide constant propagule pressure to natural ecosystems (studies I, III)

The pest persistence in greenhouses (stage II c, Fig. 1) can create a constant propagule pressure to natural habitats. Persistence of the pest in greenhouses over two consecutive years was indicated by genetically similar pest populations in almost each sampled location in Finland (study III). Greenhouses not only provide propagule pressure to natural habitats, but also reduce the impact of environmental factors, such as wind for *T. vaporaroprum*. Host survey (study I) indicated that the highest occurrence of insect was on the side of greenhouse, which is sheltered from the prevailing wind. The role of greenhouses as shelter creating microhabitats outdoors was supported by observations of greenhouse crop producers. They noticed that season of naturally deciduous vegetation was prolonged for weeds growing around greenhouses. Furthermore, *T. vaporariorum* has been observed during winter on *Taraxacum sp.* plants near greenhouses (greenhouse crop producers obs.). Thus, naturalization of the pest could be facilitated by greenhouses, which allow gradual adaptation to natural habitat. Although, the chances of *T. vaporaroprum* establishment in natural habitats in Finland are low due to absence of overwintering stage and freeze intolerance (Stenseth 1983), it might be possible under prediction of warmer average winter temperatures (Peltonen-Sainio *et al.* 2009). Under this prediction, the season of naturally deciduous vegetation would be prolonged creating opportunity for overwintering and maintenance of self sustaining populations of *T. vaporariorum* in boreal environment.

The ability to maintain self sustaining populations, i.e. naturalization is considered to be an essential step for invasion (Richardson *et al.* 2000). It is suggested to be followed by dispersal and development of a widespread, abundant metapopulation, which finalize the invasion process according to Colautti and MacIsaac (2004). However, naturalization step seems to be omitted in the invasion process of Greenhouse Whitefly in Finland, where the species have formed a metapopulation in agroecosystems with varying degrees of connectivity among populations (stage IV-V, Fig. 1) (study III). Nevertheless, long term persistence of the pest in such a fragmented metapopulation is questionable. Reduced population size due to pest control leads to frequent bottlenecks, genetic drift and loss of genetic diversity. Genetic stochasticity can lead to population extinction due to loss of fitness (Palstra and Ruzzante 2008). Existence of abundant source populations having high propagule pressure, on the other hand, may facilitate long term maintenance of the metapopulation and gradual species naturalization (Simberloff 2009). Such sources could be seen in year-round cultivating greenhouse agroecosystems inhabited by *T. vaporaroprum* in Finland. However, monitoring of potential sources over longer period than two years is required to analyze dynamics of *T. vaporaroprum* metapopulation in Finland. The data collected during the two years of this study indicate persistence of populations but do not allow identifying sources or sinks.

3.2 *T. vaporaroprum* in greenhouse agroecosystems has high population structure in both climate regions (studies II, III)

Physical isolation of greenhouse habitats from the surrounding environment increases cost efficiency of crop production in terms of reduced use of fertilizers and insecticides (Muñoz *et al.* 2008). However, the isolating effect of greenhouses can also increase fragmentation of pest populations inhabiting greenhouse agroecosystems. Results of this thesis show that greenhouses contributed to the limited gene flow among populations in both boreal (Finland) and Mediterranean (Greece) climate regions. Isolation by distance, which may indicate gradual insect dispersal and connectivity among populations, was significantly lower among greenhouses than among fields in Greece (fields/greenhouses: R_{XY} = 0.791/-0.219, R^2 = 0.625/0.048, P = 0.005/0.113). Isolation by distance in Finland was significant only at the scale of about 10 km, whereas in Greece isolation by distance persisted for areas covering 100 km in diameter. In Finland, where only greenhouses were sampled, populations exhibited higher levels of fragmentation and genetic structure than in Greece, where both fields and greenhouses were sampled (Finland -0.006 < F_{ST} < 0.533; Greece -0.007 < F_{ST} < 0.164). Thus, higher connectivity among populations in the South is associated with presence of agricultural field ecosystems, which are occupied by *T. vaporariorum* year round.

Connectivity among populations was possibly also affected by different climate conditions influencing survival and flight activity of *T. vaporariorum.* As observed during preleminary examination of preferred vegetation at various distances from potential invasion sources in Finland (study I), whiteflies were concentrated in the habitats closest to greenhouses. Restricted dispersal of *T. vaporarioprum* at further distances from the greenhouses could be associated with lower temperatures in natural habitats than in indoor climate conditions. The lowest temperature at which *T. vaporariorum* initiate flight is 16-17˚C (Liu *et al.* 1994). Dispersal of whiteflies in the Mediterranean, where temperature facilitates flight activity during most of the year, potentially covers larger areas than it does in the boreal region. Warm climate prolonging availability of natural vegetation possibly resulted in *T. vaporarioprum* naturalization (stage III b, Fig. 1) and high dispersal rate (stage V, Fig. 1) in the Mediterranean region.

Gene flow among populations may have contributed to significantly (P = 0.001) higher genetic diversity in terms of allelic richness (3.234 vs. 2.498) and heterozygosity (H_O/H_E = 0.451/0.496 vs. H_O/H_E = 0.350/0.385) in Greece than in Finland, respectively (study III). In addition, high genetic diversity of the pest in the Mediterranean region might be related to higher diversity of natural and agricultural vegetation in this area. It has been predicted that herbivore populations experiencing a diverse host plant community will retain a greater reservoir of genetic variability than herbivore populations on single host plant species (Agrawal et al. 2006, Bernays 2001, Provenza et al. 2003). Diversity and

frequent changes of crop plants in Greece (Tsagkarakou pers. comm.), which potentially contributed to diminished host effect for population structure of *T. vaporariorum* in this region, have not reduced the overall genetic diversity of the pest.

3.3 Low genetic diversity of populations did not reduce variation in responses to insecticide treatments in a small spatial scale (studies III, IV)

Enclosed greenhouse agroecosystems can create reproductive isolation of insect pest populations increasing chances of insecticide resistance development. Resistance to insecticides is favored by natural selection and can quickly spread in isolated populations due to restricted possibilities for mating with susceptible individuals. Higher insecticide resistance for greenhouse populations than for those inhabiting fields was described for many insect pests (Parrella 1999). In this thesis, isolation of greenhouse populations was indicated by their high genetic structure (study III). In addition, insecticide resistance may be induced by persistence of abundant pest population. Persistence of *T. vaporariorum* over the years was observed in study III and may increase frequency of exposure to insecticides of a single insect population, depending on producer-level differences in chemical use (study IV). High abundances of *T. vaporariorum*, as can be interpreted from high genetic diversity of whiteflies on tomato (study III), may increase insecticide application rate and facilitate development of tolerance or resistance.

Despite high genetic structure of greenhouse populations, dispersal among greenhouse habitats occurs to some extent. Genetic homogeneity of *T. vaporariorum* populations in Pjelax village in Finland indicated frequent exchange of whiteflies among greenhouses located in the area of at least 1 km radius (study III). However, most of these populations differed significantly by their responses to pymetrozine and imidacloprid treatments and were more resistant than the susceptible reference population (study IV).

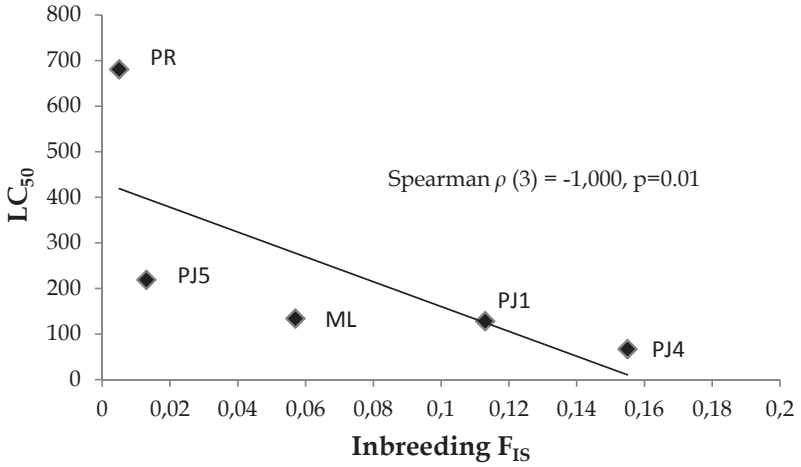

FIGURE 10 The relationship between inbreeding and resistance to pymetrozine among populations analyzed for susceptibility to pymetrozine (study IV) and genetic diversity (study III). Populations PJ 1-5 do not differ by pairwise estimates of F_{ST}, but are different by their susceptibility to pymetrozine.

Genetic diversity and fitness in resistant population may be reduced due to prevalence of resistant genotype and fitness costs involved in insecticide resistance maintenance (Gazave *et al.* 2001, Foster *et al.* 2002). This would predict positive correlation between resistance and inbreeding (loss of genetic diversity). However, the level of inbreeding and pymetrozine resistance were negatively correlated (Fig. 10). Thus, it seems that decreasing genetic diversity possibly contributes to lower fitness and increases susceptibility to insecticide. However, this hypothesis needs to be confirmed with experimental data testing a larger number of populations and comparing genetic diversity of neutral genetic markers, such as microsatellites and of genes under selection pressure.

Furthermore, most of the occurred variation in susceptibility to pymetrozine was below recommended concentration (except PR population) and thus, represented physiological tolerance, rather than high resistance commonly associated with changes in the genome. Varying responses indicate absence of single resistant genotype with uniform resistance level. Susceptibility levels among populations also were positively correlated with treatment frequencies among locations (i.e. greenhouse crop producers) (Spearman ρ (3) = 0.949, p=0.051). Thus, genetically homogeneous populations may differ in their level of inbreeding and their tolerance of insecticides due to varying selection pressure posed by individual pest management. Reduced susceptibility to insecticide treatment might be an explanation for insect persistence in the area.

3.4 Low genetic diversity did not prevent invasion of cosmopolitan greenhouse pest (studies I-V)

Analysis of genetic diversity on a species level using mitochondrial markers revealed low polymorphism of *T. vaporariorum* worldwide (study V). The combined data of three genes resulted in two major clades of global *T. vaporariorum* metapopulation. Both clades were characterized by low nucleotide diversity (Pi=0.0002 and Pi=0.00004 of clade 1 and 2 respectively). The diversity of each of three mitochondrial genes among populations collected from various locations worldwide (Table 1, Fig. 2) was very low. Nucleotide diversity among three genes varied from 0.00015 to 0.00069 and haplotype diversity varied from 0.079 to 0.493. Low genetic diversity was also supported by the presence of monomorphic populations of the endosymbiotic bacteria, *Arsenophonus*. Furthermore, these findings were supported by Prijovic et al (2014) (Fig. 11), who studied sequences of COI gene from sampled populations of *T. vaporariorum* and available sequences from GenBank. The most frequently occurring clades corresponded to clades detected in study V. Correspondence of clades from both studies was detected by aligning COI sequences available in GenBank with COI sequences from study V. Low genetic diversity may indicate recent dispersal of the pest, whereas presence of two clades may represent different invasion sources of *T. vaporariorum*. Extensive sampling is needed in South America or southwest of USA, which are potential sites of origins of the European populations (Russell 1948).

Alternatively, low diversity of mitochondrial genes and prevalence of *Arsenophonus* in majority of analyzed whitefly specimens may indicate sympatric evolution of whitefly and its endosymbiont and a selective sweep created by this endosymbiont on its host (Rokas *et al*. 2001). Whether these results represent low genetic diversity of *T. vaporariorum* or correspond to phylogeny of its endosymbiont instead, need to be defined with additional analyses of nuclear genes of *T. vaporariorum*. However, homogeneity in nuclear genes of some *T. vaporariorum* populations was already reported earlier (Roopa *et al*. 2012), confirming low genetic variation of the host, *T. vaporariorum*.

It is interesting that a global pest can have such low genetic diversity. Although, no recent exchanges of genetic material were detected between Finland and Greece (study III), *T. vaporariorum* from these regions belongs to the same mitochondrial clade (study IV, Fig. 11). Low genetic diversity of the species, however, has not affected invasive characteristics of *T. vaporariorum* (studies I, III and IV). The species was able to adapt to various host plants in agroecosystems and natural habitats (studies I and III). Greenhouse whitefly also showed varying responses to insecticide treatments (study IV).

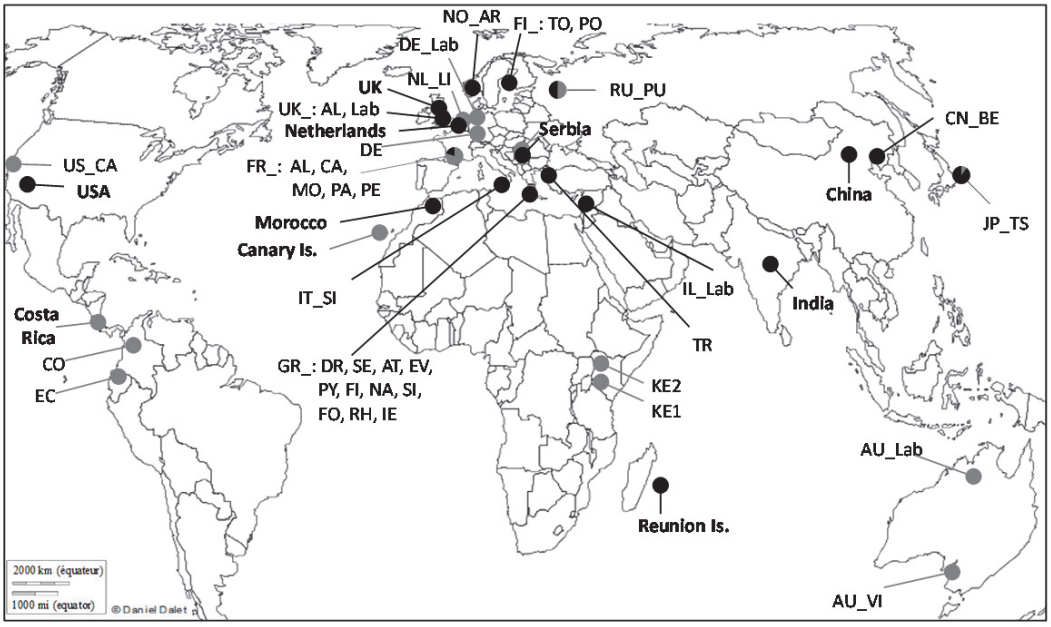

FIGURE 11 The worldwide distribution of two *T. vaporariorum* mitochondrial clades indicated by black and grey circles. Results from phylogenetic analysis of concatenated data of COI, CytB and ND5 genes (study V). Acronyms of names of populations are explained in Table 1. Additional data of COI gene sequences from Prijovic et al. (2014) is indicated by full names of origin of populations. Correspondence of clades among both studies was detected by aligning sequences from GenBank used by et al. (2014) with data from study V.

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4 CONCLUSIONS AND PRACTICAL IMPLICATIONS

Low species genetic diversity (study V) has not reduced adaptation, naturalization and invasion potential of a generalist herbivore, *T. vaporariorum*. Furthermore, greenhouse agroecosystems might have facilitated naturalization of invasive species, since experience of monoculture greenhouse environment did not select against polyphagy (study I). Furthermore, constant propagule pressure posed by year round operating greenhouses may have increased persistence of the pest (study III). By allowing frequent experience of natural vegetation surrounding greenhouses (Fig. 4) and by creating indoor overwintering habitats, greenhouse agroecosystems have increased naturalization possibilities for an introduced herbivore of tropical origin.

Cultivation of the same crop plants over an extended period of time has created host adaptation possibilities for *T. vaporariorum* in Northern Europe. Genetic structure of whitefly populations in Finland indicated initial signs of tomato and cucumber host race development (study III). Formation of host races may increase pest abundance and persistence in the area leading to a higher extent of crop damage. Greenhouse whitefly preconditioned on cucumber can be more fecund on other crops, such as tomato, than whiteflies preconditioned on tomato (study I), as indicated by high fecundity of cucumber strain on all offered hosts. Thus, the practice of using cucumber as a "trap crop" (i.e. a crop plant used to attract and exterminate pests) in greenhouses growing tomato should be avoided, unless effective pest extermination is applied. "Trap crops" should not be treated with insecticides having repellent qualities, such as imidacloprid, which might facilitate insect dispersal. Dispersal from crops treated with different insecticides and viability of the insects after these treatments need to be studied to assure effective "trap crop" usage. Furthermore, dispersal among crop species can be also induced by cultivating less and more preferred crops in the same greenhouse compartment. *T. vaporariorum* preconditioned on poinsettia for one crop growing season may have a high tendency for dispersal to other plant species (study I). Thus, it is recommended that poinsettia is not grown in the same greenhouse room with other crop species.

Stochastic pest mortality events in greenhouse environment leading to formation of genetic bottlenecks increased genetic structure among populations in both climate regions (study III). Inability to avoid insecticide exposure in physically isolated greenhouses may either lead to effective insect control or development of insecticide resistance depending on persistence and abundance of the pest, as well as insecticide treatment frequency. Selection pressure of insecticide treatments may lead to the dominance of a single resistant genotype. However, low local genetic diversity of whitefly populations within their potential dispersal limits was not an indicator of resistant genotype dominance (studies III, IV). High variation in resistance levels to pymetrozine among populations in Finland reflected variation in the usage of this compound among individual greenhouse crop producers (study IV). Therefore, pest management is recommended initially on individual greenhouse crop producer level, especially in a dense production cluster, where pest dispersal among greenhouses and exchange of resistant individuals are high.

Regional pest management in a dense greenhouse cluster could reduce persistence of a pest metapopulation by synchronizing crop cultivation practices. Different numbers of crop rotations during the year can result in varying number of pest generations produced in the same location and exposed to insecticides. Unsynchronized crop removal between production units or companies can create a possibility for dispersal of populations from removed crop plant in one unit/company to recently planted crop plants in the neighbouring units/companies or to surrounding natural ecosystems (Fig. 8). Natural vegetation around greenhouses could serve as refugia for the pest during its extermination in greenhouses and should be eliminated in a distance of at least 5m from greenhouses. The differences in monitoring practices between companies can result in unsynchronized releases of parasitoids used for biological control leading to ineffective pest management and the need to use insecticides (Pinto-Zevallos and Vänninen 2013). Thus, for successful long term pest control individual IPM programmes should be complemented with regional crop and pest management strategy, especially in dense production clusters.

Although results of this thesis are species specific, observed patterns of crop cultivation practices indicate a possibility for invasion of other whitefly species, *B. tabaci* in Finland. The species is currently under quarantine in the country. However, establishment of permanent populations in agroecosystems (stage II b-c, Fig. 1) can be facilitated by the propagule pressure from increasing frequency of pest introductions by ornamental plants importers (EVIRA 2008). Based on results of homogeneity and persistence of *T. vaporarioum* populations, establishment of *B. tabaci* should be anticipated especially in dense clusters of producers cultivating same crops year round. This corresponds to results of model estimating potential dispersal rate of *B. tabaci* in Finland, which highlight greenhouse clusters as the areas of fast spread of the pest (Heikkilä et al. unpubl.). To reduce pest dispersal potential to other greenhouse agroecosystems, synchrony in pest monitoring, exterminations and in crop rotations among crop producers is advised.

Acknowledgements

This thesis is a result of tight collaboration among four supervisors, colleagues in Finland and abroad and most of all luck.

I would like to start by express my gratitude to supervisors. I have not experienced that much attention and dedication to the student work. I appreciate your time and opportunity to feel important. The main person whom I would like to thank for generating the thesis idea and creating this project is Irene Vänninen. Together with Kari Saikonen, they create fresh breezes carrying innovative research ideas to MTT. I would like to thank Leena Lindström, for welcoming a shy student to the University of Jyväskylä from the first day of arrival. I appreciate her support and sense of humour that helped on a daily basis. I cannot be more thankful to Emily Knott, who saved the project at its most critical stage and has been open minded to new research subjects and areas.

I am grateful to Finnish system of education, BIOINT graduate school and Johanna Mappes. The links created between lost students from all over the world and curious researchers in Finland allow students to follow their carrier paths and generate fruitful research projects. I hope this practice will be adopted in other countries and universities as well.

This thesis would not be done in time without a help from technicians at MTT Agrifood Research, Jokioinen (Finland); colleagues at Plant Protection Institute of Heraklion (Greece) and the Center for Biology and Management of Populations (France). I appreciate the help from Outi Järvinen and other team members at MTT. I am grateful to Anastasia Tsagkarakou for this fruitful collaboration, Despoina Kapantaidaki for hard work and impressive driving on the narrow streets of Heraklion, and Natasa Fytrou for hospitality in Greece. The thesis would not be able to advance as fast without the help from Nathalie Gauthier (France). I am grateful to her for appearance just in time in this project.

Many funding sources made this research possible and are listed in acknowledgement sections of related publications.

I would like to thank the members of my Thesis Support Group, the critical duo, Jelmer Elzinga and Craig Primmer for bringing a third party perspective and helping to arrange the priorities in long list of tasks of a PhD student. I am grateful to Craig for provision of expertise and facilities at the rocky start of this PhD project.

For initiation of my career as a researcher I would like to thank Sergej Olenin, Asta Audzijonyte and colleagues at Coastal Research and Planning Institute (Lithuania). I am grateful to Sergej for investment and faith in young generation and encouragement from the first days at the Klaipeda University. I am lucky I got to work with such an enthusiastic supervisor as Asta, who contributed remarkably to my development as a scientist.

Last but not least, I would like to thank my family. I am grateful for the support of my partner Lina and sacrifices she made moving along with her crazy researcher to the uncertainty, which I think work out pretty well

eventually. I appreciate the understanding of my sister and brother, who were growing up without me due to my career choices. And finally, I am most of all thankful to my mother who pushed me to pursue a university degree. I wish you were still here to see that your mission was accomplished to the advanced level.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Kasvihuoneviljelyn merkitys generalistikasvinsyöjän levittäytymisessä

Kansainvälinen kasvikauppa yhdistää maapallon agroekosysteemit suureksi verkostoksi, jossa kasveja syövät lajit liikkuvat paikasta toiseen. Vieraslajien uusille levinneisyysalueille kotoutumisen dynamiikkaa eli invaasioprosessia on tarkasteltu yleensä luonnon ekosysteemien näkökulmasta. Tällöin vieraslaji määritellään invasiiviseksi, kun laji on kotoutunut uuden levinneisyysalueensa luontoon ja sen populaatiot leviävät ilman ihmisen apua ja voivat aiheuttaa ekologista tai taloudellista haittaa. Agroekosysteemeissä elävien vieraslajien kotoutumisdynamiikan tarkasteluun kyseinen luontoon kotoutumista painottava invaasioprosessi ei sellaisenaan sovellu. Vieraslajit voivat elää pitkään pelkästään agroekosysteemeissä kotoutumatta luonnonhabitaatteihin. Agroekosysteemeissä ihminen vaikuttaa toiminnallaan oleellisesti tekijöihin, joilla on merkitystä nvaasioprosessin etenemiselle: elinympäristön abioottisiin ja bioottisiin ominaisuuksiin sekä vieraslajin leviäinpaineeseen ja lajinsisäiseen geneettiseen diversiteettiin.

Kasvihuonetuotannon monokulttuurit ovat erityisen alttiita vieraslajisten kasvintuhoojien kotoutumiselle. Kasvihuoneissa vieraslajit voivat lisääntyä ja säilyä uudella levinneisyysalueellaan, vaikka ilmasto ei mahdollistaisi lajin pysyvää esiintymistä ulkona agroekosysteemeissä tai luonnonhabitaateissa. Kasvihuoneissa vieraslajisia kasvintuhoojia toki torjutaan, mutta torjunnan epäonnistuessa kasvihuoneista voi tulla vieraslajin runsaan esiintymisen takia leviämislähteitä, jotka ajan myötä edistävät lajin leviämistä luonnonympäristöönkin. Kasvihuoneissa vallitsevat abioottiset olot kuten korkeat lämpötilat voivat toisaalta hidastaa vieraslajin sopeutumista luonnonekosysteemeihin, joissa eläminen vaatisi kylmyydensietokykyä. Saman vieraslajin metapopulaatiorakenne ja geneettiset ominaisuudet voivat muotoutua erilaisiksi eri ilmastovyöhykkeillä sijaitsevissa kasvihuoneissa. Erot johtuvat geneettisen ajautumisen vaihtelevista todennäköisyyksistä eriasteisesti isoloituneissa kasvihuonehabitaateissa. Eroja voi aiheutua myös siitä, miten tehokkaasti ihminen vaikuttaa vieraslajin elinympäristön abiottisiin ja bioottisiin ominaisuuksiin tuotantoympäristössä.

Ansarijauhiainen (*Trialeurodes vaporariorum*) on laajalle levinnyt moniruokainen kasvintuhooja, joka on kotoisin ilmeisesti Etelä- tai Väli-Amerikasta. Boreaalisella vyöhykkeellä laji esiintyy pysyvästi vain kasvihuoneissa. Niissä ympärivuotinen tuotanto mahdollistaa lajin metapopulaation säilymisen luontaisen levinneisyysalueen ulkopuolella. Tämän tutkimuksen tavoitteena oli saada tietoa tekijöistä, jotka vaikuttavat erityisesti boreaalisen ilmastovyöhykkeen oloissa ansarijauhiaisen pitkäkestoiseen esiintymiseen, luontoon kotoutumisen mahdollisuuksiin ja lajinsisäiseen geneettiseen diversiteettiin, jolla on merkitystä luontoon kotoutumiselle. Tutkimuksen teoreettisena viitekehyksenä käytettiin vieraslajien invaasioprosessia. Sitä muokattiin olemassa oleviin malleihin verrattuna niin, että invasiivisuus määrittyy agroekosysteemeille aiheutuvan

taloudellisen haitan kautta riippumatta siitä, missä invaasioprosessin vaiheessa haitta ilmenee.

Tutkimuksen soveltavana tavoitteena oli ymmärtää ansarijauhiaisen leviämistä kasvihuonehabitaattien välillä tutkimalla lajin populaatiogeneettistä rakennetta kahdella maantieteellisellä alueella: Pohjanmaan kasvihuonekeskittymässä Suomessa ja Välimeren alueella Kreikassa. Paikallisen leviämisen ymmärtäminen auttaa suunnittelemaan lajin torjunta-aineresistenssin hallintaa tuotantokeskittymissä, joissa resistentit yksilöt voivat siirtyä kasvihuoneesta toiseen. Koska vieraslajista voi tulla avomaakasvien kasvintuhooja kesäisin lajin siirtyessä kasvihuoneiden ulkopuolelle, oltiin kiinnostuneita myös siitä, miten ansarijauhiainen hyödyntää kasvihuoneiden ulkopuolella olevaa kasvillisuutta lisääntymisessään. Elinolot kasvihuoneissa voivat vaikuttaa siihen, mitä isäntäkasveja ansarijauhiainen suosii kasvihuoneiden ulkopuolella ja miten tehokkaasti se pystyy niillä lisääntymään. Kasvihuoneiden lähiympäristön ruderaatti- ja luonnonkasvit ovat myös vieraslajin potentiaalisen luontoon kotoutumisen mahdollistava ensimmäinen askel.

Mikrosatelliittimarkkereihin perustuvat populaatiogeneettiset analyysit osoittivat, että viljelykasvilaji selitti merkitsevän osan ympärivuotisilta tomaatti- ja kurkkuviljelmiltä kerättyjen jauhiaisten geneettisten ominaisuuksien vaihtelusta. Ansarijauhiaiset joko sopeutuvat isäntäkasvilajinsa ominaisuuksiin tai viljelyskasvien tuotanto-olosuhteet ja –käytännöt muutoin vaikuttavat kasvihuoneissa elävien jauhiaispopulaatioiden geneettisiin ominaisuuksiin noin 8-9 kk:n pituisen viljelykierron aikana. Kontrolloiduissa kasvihuonekokeissa tomaatilla tai joulutähdellä vuoden eläneiden jauhiaispopulaatioiden naaraat valitsivat munintapaikakseen mieluummin muita kasvilajajeja kuin sen, millä ne olivat ennen koetta eläneet. Valintakokeessa kurkun osoitettiin tuottavan erityisen suuren lisääntymispotentiaalin omaavia jauhiaisia, jotka siirtyivät halukkaasti munimaan sekä kurkulle että tietyille ruderaatti- ja luonnonkasveille. Kokeessa käytetyt ruderaatti- ja luonnonkasvit olivat samoja, joita esiintyy yleisesti kasvihuoneiden ympäristössä ja joilla ansarijauhiaisten myös osoitettiin kasvihuoneiden ulkopuolella elävän. Jauhiaiset suosivat lisääntymispaikkoinaan etenkin maitohorsmaa ja nokkosta, mutta lisääntyivät kasvihuoneiden ympärillä 12 muullakin kasvilajilla. Samojen harvojen kasvilajien jatkuva viljely kasvihuoneissa luo ansarijauhiaisille mahdollisuuksia isäntäkasvilajikohtaiseen sopeutumiseen, mutta tutkimustulosten mukaan se ei estä jauhiaisia käyttämästä myös muita kasvilajeja lisääntymisresursseinaan sen voidessa valita eri isäntäkasvilajien välillä. Sekä tomaatti- että kurkkukasvihuoneet voivat olla merkittäviä jauhiaisten leviämislähteitä boreaalisella vyöhykkeellä.

Mikrosatelliittimarkkereihin perustuva populaatiogeneettinen tutkimus osoitti, että ansarijauhiaisten geneettinen diversiteetti on Suomen ympärivuotisissa vihanneskasvihuoneissa alhaisempi kuin Kreetan ja manner-Kreikan kasvihuone- tai avomaapopulaatioissa. Suomen kasvihuoneissa elävät jauhiaispopulaatiot ovat eristyneempiä kuin Välimeren ilmastoalueella, jossa ne voivat elää ympäri vuoden myös avomaalla. Silti kasvihuoneissa elävien jauhiaispopulaatioiden geneettinen muuntelu oli Kreikassakin suurempi kuin avomaapopu-

laatioiden. Kasvihuoneet rajoittavat jauhiaisten liikkumista ja geenivirtaa populaatioiden välillä kummallakin tutkitulla ilmastoalueella. Tilastollinen analyysi osoitti, että Pohjanmaan kasvihuoneista kerätyt jauhiaispopulaatioiden geneettisen ominaisuudet olivat samankaltaisimmat läpimitaltaan noin 10 km:n suuruisella alueella, kun taas Kreikassa samankaltaisuus ulottui noin 100 km etäisyydelle. Suomessa samasta ympärivuotisesta kasvihuoneesta kerättyjen jauhiaisten geneettiset ominaisuudet eivät poikenneet perättäisinä vuosina toisistaan merkitsevästi kuin yhdessä tapauksessa kymmenestä.

Tulosten perusteella pääteltiin, että jauhiaiset ovat Suomen oloissa selvästi paikkauskollisempia kuin Välimeren alueella. Tosin kylissä, joissa on paljon lähekkäisiä kasvihuoneita, paikkauskollisuus toteutuu mikrosatelliittianalyysin perusteella kylän eikä yksittäisen kasvihuoneyrityksen tasolla. Tästä huolimatta jauhiaisten torjunta-aineresistenssissä pymetrotsiinia vastaan oli biotestien mukaan eroja samankin kylän eri kasvihuoneissa elävien populaatioiden välillä. Erot selittyivät tutkituissa kasvihuoneissa vuoden aikana tehtyjen torjunta-ainekäsittelyjen määrällä, mutta osittain myös populaatioiden sisäsiittoisuuden asteella, joka saattaa vaikuttaa torjunta-aineen sietokyvyn kehittymisalttiuteen. Kasvihuoneet voivat siis rajoittaa geenivirtaa resistenttien ja kemikaaleille herkkien populaatioiden välillä jopa saman kylän alueella, joten kasvihuoneyrityskohtainen resistenssinhallinta on mahdollista huolimatta kasvihuoneiden lähekkäisyydestä.

Ansarijauhiaisten geneettistä diversiteettiä globaalilla tasolla tutkittiin mitokondriogeeneistä kuudelta eri mantereelta kerätyistä populaatioista. Niistä analysoitiin Multi Locus Sequency Typing -menetelmällä myös kaikista ansarijauhiaispopulaatioista löytyneen *Arsenophonus*-bakteeriendosymbiontin geneettistä diversiteettiä. Tutkitut jauhiaispopulaatiot kuuluivat vain kahteen kliiniin eikä *Arsenophonus*-populaatioiden välillä ollut lainkaan polymorfisuutta. Kasvihuoneiden ansiosta ansarijauhiaisen levinneisyys on kosmopoliittinen, mutta agroekosysteemeissä eläminen pienentää lajinsisäistä geneettistä diversiteettiä.

Tutkimus tuotti ansarijauhiaisen ekologiasta ja populaatiogenetiikasta tietoa, jota voidaan hyödyntää suunniteltaessa ja toteutettaessa tämän kasvintuhoojan alueellista hallintaa kasvintuottajien yhteistyön avulla. Ansarijauhiaisen leviäminen kasvihuoneyritysten välillä ymmärretään nyt paremmin. Tieto siitä, että kasvihuoneyritysten jauhiaispopulaatiot ovat pääosin samoja vuodesta toiseen yritys- tai kylätasolla, ja että kemikaaliresistenssi vaihtelee yrityksestä toiseen samankin kylän sisällä, voi merkittävästi vaikuttaa kasvintuottajien näkemyksiin heidän kasvustoissaan esiintyvien jauhiaisten alkuperästä. Alkuperätiedolla voi olla merkitystä kasvintuottajien halukkuuteen neuvotella keskenään kylä- tai aluetason kasvinsuojelutoimenpiteistä, jotka ottavat paremmin huomioon yritysten keskinäisen riippuvuuden yhteisen kasvintuhoojan kautta. Tulokset ansarijauhiaisen isäntäkasvien käytöstä ja kasvihuonekasveilla elävien jauhiaisten taipumuksesta suosia muita isäntäkasveja ovat tärkeitä, koska ne havahduttavat jauhiaisten esiintymisen tarkkailuun myös avomaan viljelykasveilla kuten perunalla ja mansikalla. Kemikaaliresistenssiä koskevien tulosten perusteella voidaan tehdä hypoteeseja ja suunnitelmia alueellisen resistens-

siseurannan ja -hallinnan järjestämiseksi niiden torjunta-aineiden osalta, joille jauhiaisten sietokyvyn ei vielä tässä tutkimuksessa todettu alentuneen, mutta jotka ovat tärkeitä biologisen torjunnan satunnaisesti tarvittavina täydentäjinä. Ansarijauhiaisen leviämistä koskevien tulosten avulla voidaan tarkentaa etelänjauhiaisen leviämisen mallinnusta Suomessa ja suunnitella paremmin tämän karanteenituhoojan leviämisen esto- tai hidastamistoimenpiteitä siltä varalta, että se laji kotoutuu pysyvästi suomalaiseen kasvihuonetuotantoon.

REFERENCES

Agrawal A.A., Lau J.A. & Hambäck P.A. 2006. Community heterogeneity and the evolution of interactions between plants and insect herbivores. *Q. Rev. Biol.* 81: 349–376.

Ahern R.G., Hawthorne D.J. & Raupp M.J. 2008. Founder effects and phenotypic variation in Adelges cooleyi, an insect pest introduced to the eastern United States. *Biol. Invasions* 11: 959–971.

Altieri M.A., Letourneau D.K. & Risch S.J. 1984. Vegetation diversity and insect pest outbreaks. *CRC. Crit. Rev. Plant Sci.* 2: 131–169.

Altieri M.A. & Nicholls C.I. 2004. *Biodiversity and pest management in agroecosystems.* Altieri M.A. & Nicholls C.I. (eds.). Food Products Press, New York, NY.

Bacon S.J., Aebi A., Calanca P. & Bacher S. 2014. Quarantine arthropod invasions in Europe: the role of climate, hosts and propagule pressure Essl F. (ed.). *Divers. Distrib.* 20: 84–94.

Bailly X., Migeon A. & Navajas M. 2004. Analysis of microsatellite variation in the spider mite pest Tetranychus turkestani (Acari: Tetranychidae) reveals population genetic structure and raises questions about related ecological factors. *Biol. J. Linn. Soc.* 82: 69–78.

Baker R.H.., Sansford C.., Jarvis C.., Cannon R.J.., MacLeod A. & Walters K.F.. 2000. The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. *Agric. Ecosyst. Environ. Environ.* 82: 57–71.

Barro P. De & Scott K. 2003. Isolation and characterization of microsatellite loci in *Bemisia tabaci*. *Mol. Ecol. Notes* 3: 40–43.

Barro P.J. De, Liu S.-S., Boykin L.M. & Dinsdale A.B. 2011. *Bemisia tabaci*: a statement of species status. *Annu. Rev. Entomol.* 56: 1–19.

Baufeld P. & Unger J.G. 1994. New aspects on the significance of *Bemisia tabaci* (Gennadius). *Nachr. Dtsch. Pflanzenschutzd.* 46: 252–257.

Bebber D.P., Ramotowski M.A.T. & Gurr S.J. 2013. Crop pests and pathogens move polewards in a warming world. *Nat. Clim. Chang.* 3: 985–988.

Berlinger M.J., Lehmann-Sigura N. & Taylor R.A.J. 1996. Survival of *Bemisia tabaci* adults under different climatic conditions. *Entomol. Exp. Appl.* 80: 511–519.

Bernays E. 2001. Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annu. Rev. Entomol.* 46: 703–727.

Bezerra M. 2004. Does the presence of weeds affect *Bemisia tabaci* (Gennadius)(Hemiptera: Aleyrodidae) infestation on tomato plants in a semi-arid agro-ecosystem? *Neotrop. Entomol.* 33: 769–775.

Byrne D.N. 1999. Migration and dispersal by the sweet potato whitefly, *Bemisia tabaci*. *Agric. For. Meteorol.* 97: 8.

Byrne D.N. & Bellows T.S. 1991. Whitefly Biology. *Annu. Rev. Entomol.* 36: 431–457.

Blackburn T.M., Pyšek P., Bacher S., Carlton J.T., Duncan R.P., Jarošík V., Wilson J.R.U. & Richardson D.M. 2011. A proposed unified framework for biological invasions. *Trends Ecol. Evol.* 26: 333–339.

Boykin L.M., Armstrong K.F., Kubatko L. & Barro P. De. 2012. Species delimitation and global biosecurity. *Evol. Bioinform. Online* 8: 1–37.

Boykin L.M., Bell C.D., Evans G., Small I. & Barro P.J. De. 2013. Is agriculture driving the diversification of the *Bemisia tabaci* species complex (Hemiptera: Sternorrhyncha: Aleyrodidae)?: Dating, diversification and biogeographic evidence revealed. *BMC Evol. Biol.* 13: 228.

Bradshaw A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* 13: 115–155.

Brown J. & Beecham H.A. 1989. Crop pests and diseases. In: Mingay G.E. (ed.), *The agrarian history ofEngland and Wales, VI: 1750-1850*, Cambridge, pp. 311–313.

Brownlie J.C. & Johnson K.N. 2009. Symbiont-mediated protection in insect hosts. *Trends Microbiol.* 17: 348–354.

Carletto J., Lombaert E., Chavigny P., Brévault T., Lapchin L. & Vanlerberghe-Masutti F. 2009. Ecological specialization of the aphid *Aphis gossypii* Glover on cultivated host plants. *Mol. Ecol.* 18: 2198–2212.

Carlton J. 2003. *Invasive Species: Vectors And Management Strategies*. Island Press.

Carriere Y., Ellers-Kirk C., Hartfield K., Larocque G., Degain B., Dutilleul P., Dennehy T.J., Marsh S.E., Crowder D.W., Li X., Ellsworth P.C., Naranjo S.E., Palumbo J.C., Fournier A., Antilla L. & Tabashnik B.E. 2012. Large-scale, spatially-explicit test of the refuge strategy for delaying insecticide resistance. *Proc. Natl. Acad. Sci.* 109: 775–780.

Castilla N., Hernández J. & Abou-Hadid A.F. 2004. Strategic Crop and Greenhouse Management in Mild Winter Climate Areas. *Acta Hortic.* 633: 183–196.

Castle S.J., Prabhaker N., Henneberry T.J. & Toscano N.C. 2009. Host plant influence on susceptibility of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to insecticides. *Bull. Entomol. Res.* 99: 263–273.

Catford J. a., Jansson R. & Nilsson C. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Divers. Distrib.* 15: 22–40.

Chen B. & Kang L. 2005. Can greenhouses eliminate the development of cold resistance of the leafminers? *Oecologia* 144: 187–195.

Chew M. 2011. Invasion biology. Historical precedents. In: Simberloff D. & Rejmánek M. (eds.), *Encyclopedia of Biological Invasions*, University of California Press, Berkeley and Los Angeles, pp. 369–375.

Clavel J., Julliard R. & Devictor V. 2011. Worldwide decline of specialist species: toward a global functional homogenization? *Front. Ecol. Environ.* 9: 222–228.

Colautti R.I., Grigorovich I. a. & MacIsaac H.J. 2006. Propagule Pressure: A Null Model for Biological Invasions. *Biol. Invasions* 8: 1023–1037.

Colautti R.I. & MacIsaac H.J. 2004. A neutral terminology to define "invasive" species. *Divers. Distrib.* 10: 135–141.

Cox G.W. 2004. *Alien Species and Evolution: The Evolutionary Ecology of Exotic Plants, Animals, Microbes, and Interacting Native Species*. Island Press.

Crowder D.W., Horowitz A.R., Barro P.J. De, Liu S.-S., Showalter A.M., Kontsedalov S., Khasdan V., Shargal A., Liu J. & Carrière Y. 2010. Mating behaviour, life history and adaptation to insecticides determine species exclusion between whiteflies. *J. Anim. Ecol.* 79: 563–570.

Dalmon A., Halkett F., Granier M., Delatte H. & Peterschmitt M. 2008. Genetic structure of the invasive pest *Bemisia tabaci*: evidence of limited but persistent genetic differentiation in glasshouse populations. *Heredity* 100: 316–325.

Davis M.A. 2009. *Invasion Biology*. Oxford University Press.

Davis M.A., Chew M.K., Hobbs R.J., Lugo A.E., Ewel J.J., Vermeij G.J., Brown J.H., Rosenzweig M.L., Gardener M.R., Carroll S.P., Thompson K., Pickett S.T. a, Stromberg J.C., Tredici P. Del, Suding K.N., Ehrenfeld J.G., Grime J.P., Mascaro J. & Briggs J.C. 2011. Don't judge species on their origins. *Nature* 474: 153–154.

Dehnen-Schmutz K., Touza J., Perrings C. & Williamson M. 2007. A century of the ornamental plant trade and its impact on invasion success. *Divers. Distrib.* 13: 527–534.

Delatte H., David P., Granier M., Lett J.M., Goldbach R., Peterschmitt M. & Reynaud B. 2006. Microsatellites reveal extensive geographical, ecological and genetic contacts between invasive and indigenous whitefly biotypes in an insular environment. *Genet. Res.* 87: 109–124.

Delatte H., Reynaud B., Granier M., Thornary L., Lett J.M., Goldbach R. & Peterschmitt M. 2005. A new silverleaf-inducing biotype Ms of *Bemisia tabaci* (Hemiptera: Aleyrodidae) indigenous to the islands of the south-west Indian Ocean. *Bull. Entomol. Res.* 95: 29–35.

Denholm I., Cahill M., Dennehy T.J. & Horowitz A.R. 1998. Challenges with managing insecticide resistance in agricultural pests, exemplisfied by the whitefly *Bemisia tabaci*. *Philos Trans R Soc L. B Biol Sci.* 353: 1757–1767.

Denholm I., Gorman K. & Williamson M. 2007. Insecticide resistance in *Bemisia tabaci*: a global perspective. *J. Insect Sci.* 8: 16.

Dingle H. 1974. Diapause in a migrant insect, the milkweed bug *Oncopeltus fasciat*us (Dallas) (Hemiptera: Lygaeidae). *Oecologia* 17: 1–10.

Drès M. & Mallet J. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 357: 471–492.

Drew J., Anderson N. & Andow D. 2010. Conundrums of a complex vector for invasive species control: a detailed examination of the horticultural industry. *Biol. Invasions* 12: 2837–2851.

Driesche R. van & Enkegaard E. 2002. Invasive species as pests in greenhouses: forecasting, preventing and remediating future invasions. *IOBC/WPRS* 25: 277–280.

Dunley J.E. & Croft B.A. 1992. Dispersal and gene flow of pesticide resistance traits in phytoseiid and tetranychid mites. *Exp. Appl. Acarol.* 14: 313–325.

Elton C.S. 1958. *The Ecology of Invasions by Animals and Plants*. University of Chicago Press, Science, Chicago.

Eurostat. 2014. Crops products: areas and productions. Available at http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home

EVIRA. 2008. Tobacco Whitefly found in poinsettia cultivations. Available at http://www.evira.fi/portal//en/frontpage/frontpage+news/?bid=189

Excoffier L., Laval G. & Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1: 47–50.

Foll M. & Gaggiotti O. 2006. Identifying the environmental factors that determine the genetic structure of populations. *Genetics* 174: 875–891.

Forsman A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. *Proc. Natl. Acad. Sci. U. S. A.* 111: 302–307.

Foster S.P., Harrington R., Dewar A.M., Denholm I. & Devonshire A.L. 2002. Temporal and spatial dynamics of insecticide resistance in *Myzus persicae* (Hemiptera: Aphididae). *Pest Manag. Sci.* 58: 895–907.

Frankham R. 1996. Relationship of Genetic Variation to Population Size in Wildlife. *Conserv. Biol.* 10: 1500–1508.

Frankham R. 2005. Resolving the genetic paradox in invasive species. *Heredity* 94: 385.

Franklin M.T., Ritland C.E. & Myers J.H. 2010. Spatial and temporal changes in genetic structure of greenhouse and field populations of cabbage looper, *Trichoplusia ni*. *Mol. Ecol.* 19: 1122–1133.

Gadagkar S.R., Rosenberg M.S. & Kumar S. 2005. Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. *J. Exp. Zool. B. Mol. Dev. Evol.* 304: 64–74.

Gauthier N., Dalleau-Clouet C. & Bouvret M.-E. 2008. Twelve new polymorphic microsatellite loci and PCR multiplexing in the whitefly, *Bemisia tabaci*. *Mol. Ecol. Resour.* 8: 1004–1007.

Gazave E., Chevillon C., Lenormand T., Marquine M. & Raymond M. 2001. Dissecting the cost of insecticide resistance genes during the overwintering period of the mosquito Culex pipiens. *Heredity* 87: 441–448.

Gliessman S.R. 1990. The Agroecosystem Concept. In: Gliessman S.R. (ed.), *Agroecology. The Ecology of Sustainable Food Systems*, CRC Press, Taylor and Francis Group, New York, NY, pp. 23–32.

Gorman K., Devine G., Bennison J., Coussons P., Punchard N. & Denholm I. 2007. Report of resistance to the neonicotinoid insecticide imidacloprid in *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Pest Manag. Sci.* 63: 555–558.

Gorman K., Hewitt F., Denholm I. & Devine G.J. 2001. New developments in insecticide resistance in the glasshouse whitefly (*Trialeurodes vaporiorum*) and the two-spotted spider mite (*Tetranychus urticae*) in the UK. *Pest Manag. Sci.* 58: 123–130.

Gorur G. 2000. The role of phenotypic plasticity in host race formation and sympatric speciation in phytophagous insects, particularly in aphids. *Turkish J. Zool.* 24: 63–68.

Gray A.J., Mack R.N., Harper J.L., Usher M.B., Joysey K. & Kornberg H. 1986. Do Invading Species have Definable Genetic Characteristics? [and Discussion]. *Philos. Trans. R. Soc. B Biol. Sci.* 314: 655–674.

Grapputo A., Boman S., Lindström L., Lyytinen A. & Mappes J. 2005. The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Mol. Ecol.* 14: 4207–4219.

Greenberg S., Jones W. & Liu T. 2009. Tritrophic interactions among host plants, whiteflies, and parasitoids. *Southwest. Entomol.* 34: 431–445.

Grimstad S.O. & Frimanslund E. 1993. Effect of different day and night temperature regimes on greenhouse cucumber young plant production, flower bud formation and early yield. *Sci. Hortic.* 53: 191–204.

Guindon S. & Gascuel O. 2003. A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Syst. Biol.* 52: 696–704.

Hamilton M. 2009. *Population Genetics*. Wiley-Blackwell.

Hanafi A. 2005. Invasive pests and diseases: A challenge to IPM in greenhouse crops. *Phytoparasitica* 33: 423–426.

Hanafi A. & Papasolomontos A. 1999. Integrated production and protection under protected cultivation in the Mediterranean region. *Biotechnol. Adv.* 17: 183–203.

Hanski I. 1999. *Metapopulation Ecology* May R.M. & Harvey P.H. (eds.). Oxford University Press, Incorporated.

Hanski I. & Gilpin M. 1991. Metapopulation dynamics: brief history and conceptual domain. *Biol. J. Linn. Soc.* 42: 3–16.

Henderson S., Dawson T.P. & Whittaker R.J. 2006. Progress in invasive plants research. *Prog. Phys. Geogr.* 30: 25–46.

HeungYong J., HyeongHwan K., ChangYul Y., TaekJun K. & DongSoon K. 2009. A tentative economic injury level for greenhouse whitefly on cucumber plants in the protective cultivation. *Korean J. Hortic. Sci. Technol.* 27: 81–85.

Holleley C.E. & Geerts P.G. 2009. Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. *Biotechniques* 46: 511–517.

Hsiao T.H. 1978. Host plant adaptations among geographic populations of the Colorado potato beetle. *Entomol. Exp. Appl.* 24: 437–447.

Hubisz M.J., Falush D., Stephens M. & Pritchard J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Resour.* 9: 1322–1332.

Inbar M., Doostdar H. & Mayer R.T. 1999. Effects of Sessile Whitefly Nymphs (Homoptera: Aleyrodidae) on Leaf-Chewing Larvae (Lepidoptera: Noctuidae). *Environ. Entomol.* 28: 353–357.

Inbar M. & Gerling D. 2008. Plant-mediated interactions between whiteflies, herbivores, and natural enemies. *Annu. Rev. Entomol.* 53: 431–448.

Ings T.C., Schikora J. & Chittka L. 2005. Bumblebees, humble pollinators or assiduous invaders? A population comparison of foraging performance in Bombus terrestris. *Oecologia* 144: 508–516.

Karunker I., Benting J., Lueke B., Ponge T., Nauen R., Roditakis E., Vontas J., Gorman K., Denholm I. & Morin S. 2008. Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 38: 634–644.

Kenis M., Rabitsch W., Auger-Rozenberg M. & Roques a. 2007. How can alien species inventories and interception data help us prevent insect invasions? *Bull. Entomol. Res.* 97: 489–502.

Kikuchi Y., Hayatsu M., Hosokawa T., Nagayama A., Tago K. & Fukatsu T. 2012. Symbiont-mediated insecticide resistance. *Proc. Natl. Acad. Sci. U. S. A.* 109: 8618–8622.

Kiritani K. 2006. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. *Popul. Ecol.* 48: 5–12.

Kiritani K. & Yamamura K. 2003. Exotic insects and their pathways for invasion. In: Carlton J. (ed.), *Invasive Species: Vectors And Management Strategies*, Island Press, p. 536.

Kirk W.D.J. & Terry L.I. 2003. The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agric. For. Entomol.* 5: 301–310.

Kittas C., Bartzanas T. & Jaffrin a. 2003. Temperature Gradients in a Partially Shaded Large Greenhouse equipped with Evaporative Cooling Pads. *Biosyst. Eng.* 85: 87–94.

Knipling E.F. 1979. *The basic principles of insect population suppression and management.* U.S. Government Printing Office, p. 576.

Koch R.L. & Tederson G.L. 2008. Bad side of a good beetle: the North American experience with *Harmonia axyridis*. In: Roy H.E. & Wajnberg E. (eds.), *From Biological Control to Invasion: the Ladybird Harmonia axyridis as a Model Species*, Springer Netherlands, Dordrecht, pp. 23–35.

Lambrinos J. 2004. How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecology* 85: 2061–2070.

Larson B. 2005. The war of the roses: demilitarizing invasion biology. *Front. Ecol. Environ.* 3: 495–500.

Larson B.M.H. & Kueffer C. 2013. Managing invasive species amidst high uncertainty and novelty. *Trends Ecol. Evol.* 28: 255–256.

Lee C.E. 2002. Evolutionary genetics of invasive species. *Trends Ecol. Evol.* 17: 386–391.

Lehmann P., Lyytinen A., Sinisalo T. & Lindström L. 2012. Population dependent effects of photoperiod on diapause related physiological traits in an invasive beetle (*Leptinotarsa decemlineata*). *J. Insect Physiol.* 58: 1146–1158.

Lei H., Tjallingii W.F. & Lenteren J.C. van. 1998. Probing and feeding characteristics of the greenhouse whitefly in association with host-plant acceptance and whitefly strains. *Entomol. Exp. Appl.* 88: 73–80.

Lenteren J.C. van. 2000. A greenhouse without pesticides: fact or fantasy? *Crop Prot.* 19: 375–384.

Lenteren J.C. van & Noldus L.P.J.J. 1990. Whitefly plant relationships, behavioural and ecological aspects. In: *Whiteflies: their bionomics, pest status and management*, Intercept Ltd, Andover, pp. 47–49.

Levins R. 1969. Some Demographic and Genetic Consequences of Environmental Heterogeneity for Biological Control. *Bull. Entomol. Soc. Am.* 15: 237–240.

Lewis W.J., Lenteren J.C. van, Phatak S.C. & Tumlinson J.H. 1997. A total system approach to sustainable pest management. *Proc. Natl. Acad. Sci. U. S. A.* 94: 12243–12248.

Linnaniemi W.M. 1921. *Ästerochiton vaporiorum* (Westw.) Suomessa. *Medd. Af Soc. Pro Fauna Flora Fenn.* 7: 66–68.

Liu T.X., Oetting R.D. & Buntin G.D. 1994. Temperature and diel catches of Trialeurodes vaporariorum and *Bemisia tabaci* (Homoptera: Aleyrodidae) adults on sticky traps in the greenhouse. *J. Entomol. Sci.* 29: 222–230.

Lynch M.E., Kaplan I., Dively G.P. & Denno R.F. 2006. Host-Plant-Mediated Competition via Induced Resistance: Interactions between Pest Herbivores on Potatoes. *Ecol. Appl.* 16: 855–864.

Lockwood J.L., Hoopes M.F. & Marchetti M.P. 2013. *Invasion Ecology*. Wiley-Blackwell, p. 466.

Longhurst C., Babcock J.M., Denholm I., Gorman K., Thomas J.D. & Sparks T.C. 2013. Cross-resistance relationships of the sulfoximine insecticide sulfoxaflor with neonicotinoids and other insecticides in the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Pest Manag. Sci.* 69: 809–813.

Malumphy C., Suarez M. & Glover R. 2007. Morphological and molecular evidence supporting the validity of Trialeurodes lauri and T. ricini (Hemiptera: Sternorrhyncha: Aleyrodidae). *Eur. J. Entomol.* 104: 295–301.

Margosian M.L., Garrett K. a., Hutchinson J.M.S. & With K. a. 2009. Connectivity of the American Agricultural Landscape: Assessing the National Risk of Crop Pest and Disease Spread. *Bioscience* 59: 141–151.

Martin N. 1999. Whitefly: biology, identification and life cycle. *Crop Food Res. Broadsh.* 91: 1–8.

Matson P.A., Parton W.J., Power A.G. & Swift M.J. 1997. Agricultural Intensification and Ecosystem Properties. *Science* 277: 504–509.

McGlynn T. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. *J. Biogeogr.* 26: 535–548.

McKenzie J.A. 2001. Pesticide resistance. In: Fox C.W., Roff D.A. & Fairbairn D.J. (eds.), *Evolutionary Ecology*, Oxford University Press, pp. 347–360.

Médail F. & Quezél P. 1999. Biodiversity Hotspots in the Mediterranean Basin: Setting Global Conservation Priorities. *Conserv. Biol.* 13: 1510–1513.

Metcalf C.L. & Flint W.P. 1928. *Destructive and Useful Insects. Their Habits and Control*. New York & London, McGraw-Hill Pubg. Co. Ltd.

Moczek A.P. 2010. Phenotypic plasticity and diversity in insects. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365: 593–603.

Moe R., Grimstad S.O. & Gislerod H.R. 2006. The use of artificial light in year round production of greenhouse crops in Norway. *Acta Hortic.* 711: 35–42.

Molnar J.L., Gamboa R.L., Revenga C. & Spalding M.D. 2008. Assessing the global threat of invasive species to marine biodiversity. *Front. Ecol. Environ.* 6: 485–492.

Moran N.A., Munson M.A., Baumann P. & Ishikawa H. 1993. A Molecular Clock in Endosymbiotic Bacteria is Calibrated Using the Insect Hosts. *Proc. R. Soc. B Biol. Sci.* 253: 167–171.

Morse J.G. & Hoddle M.S. 2006. Invasion biology of thrips. *Annu. Rev. Entomol.* 51: 67–89.

Mossberg B. & Stenberg L. 2003. *Den nya nordiska floran*. Wahlström & Widstrand.

Mouton L., Thierry M., Henri H., Baudin R., Gnankine O., Reynaud B., Zchori-Fein E., Becker N., Fleury F. & Delatte H. 2012. Evidence of diversity and recombination in Arsenophonus symbionts of the *Bemisia tabaci* species complex. *BMC Microbiol.* 12: S10.

Mugnozza G.S. 1995. Sustainable greenhouse production in Mediterranean climate: a case study in Italy. *Prospett. E Propos. Mediterr. - Riv. Di Econ. Agric. E Ambient.* 4: 48–53.

Muñoz P., Antón A., Nuñez M., Paranjpe A., Ariño J., Castells X., Montero J.I. & Rieradevall J. 2008. Environmental impacts of greenhouse versus open-field tomato production in the Mediterranean region. *Acta Hortic.* 801: 1591–1596.

Nauen R. & Denholm I. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *J. Insect Biochem. Physiol.* 58: 200–215.

Nauen R., Koob B. & Elbert A. 1998. Antifeedant effects of sublethal dosages of imidacloprid on *Bemisia tabaci*. *Entomol. Exp. Appl.* 88: 287–293.

Navajas M., Lagnel J., Gutierrez J. & Boursot P. 1998. Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. *Heredity* 80: 742–752.

Neal J.W. & Bentz J.-A. 1999. Evidence for the Stage Inducing Phenotypic Plasticity in Pupae of the Polyphagous Whiteflies *Trialeurodes vaporariorum*

and *Bemisia argentifolii* (Homoptera: Aleyrodidae) and the raison d ′ e ˆ tre. *Ann. Entomol. Soc. Am.* 92: 774–787.

Nomikou M., Janssen A. & Sabelis M.W. 2003. Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. *Oecologia* 136: 484–488.

Nordenström E., Guest G. & Fröling M. 2010. LCA of local bio-chp fuelled greenhouses versus Mediterranean open field tomatoes for consumption in northern Scandinavia. In: *ECO-TECH´ 10, 22-24 November 2010, Kalmar, Sweden*.

Oliveira M., Henneberry T. & Anderson P. 2001. History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Prot.* 20: 709–723.

Ovcarenko I., Audzijonyte A. & Gasiunaite Z.A. 2006. Tolerance of *Paramysis lacustris* and *Limnomysis benedeni* (Crustacea, Mysida) to sudden salinity changes: implications for ballast water treatment. *Oceanologia* 48: 231–242.

Palstra F.P. & Ruzzante D.E. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol. Ecol.* 17: 3428–3447.

Pardossi A., Tognoni F. & Incrocci L. 2004. Mediterranean greenhouse technology. *Chron. Horticult.* 44: 28–34.

Parrella M.P. 1999. Arthropod fauna. In: Stanhill G. & Enoch H.Z. (eds.), *Ecosystems of the world. Greenhouse ecosystems*, Elsevier Science Publishers, pp. 213–250.

Peakall R. & Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* 28: 2537–2539.

Peltonen-Sainio P., Jauhiainen L., Hakala K. & Ojanen H. 2009. Climate change and prolongation of growing season: changes in regional potential for field crop production in Finland. *Agric. Food Sci.* 18: 171–190.

Pilkington L.J., Messelink G., Lenteren J.C. van & Mottee K. Le. 2010. "Protected Biological Control" – Biological pest management in the greenhouse industry. *Biol. Control* 52: 216–220.

Pinto-Zevallos D.M. & Vänninen I. 2013. Yellow sticky traps for decision-making in whitefly management: What has been achieved? *Crop Prot.* 47: 74–84.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25: 1253–1256.

Prijović M., Skaljac M., Drobnjaković T., Zanić K., Perić P., Marčić D. & Puizina J. 2014. Genetic variation of the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), among populations from Serbia and neighbouring countries, as inferred from COI sequence variability. *Bull. Entomol. Res.* 1: 1–10.

Primmer C.R., Møller A.P. & Ellegren H. 1996. A wide-range survey of cross-species microsatellite amplification in birds. *Mol. Ecol.* 5: 365–378.

Pritchard J.K., Stephens M. & Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Provenza F., Villalba J., Dziba L., Atwood S. & Banner R. 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. *Small Rumin. Res.* 49: 257–274.

Rao Q., Xu Y., Luo C., Zhang H., Jones C.M., Devine G.J., Gorman K. & Denholm I. 2012. Characterisation of Neonicotinoid and Pymetrozine Resistance in Strains of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from China. *J. Integr. Agric.* 11: 321–326.

Reed D.H. & Frankham R. 2003. Correlation between Fitness and Genetic Diversity. *Conserv. Biol.* 17: 230–237.

Reichard S.H. & White P. 2001. Horticulture as a Pathway of Invasive Plant Introductions in the United States. *Bioscience* 51: 103.

Richardson D.M., Pyšek P., Rejmánek M., Barbour M.G., Panetta F.D. & West C.J. 2000. Naturalization and invasion of alien plants: concepts and definitions. *Divers. Distrib.* 6: 93–107.

Roditakis N.E. 1990. Host plants of greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) in Crete. Attractiveness and impact on whitefly life stages. *Agric. Ecosyst. Environ.* 31: 217–224.

Rokas A., Atkinson R.J., Brown G.S., West S.A. & Stone G.N. 2001. Understanding patterns of genetic diversity in the oak gallwasp *Biorhiza pallida*: demographic history or a Wolbachia selective sweep? *Heredity* 87: 294–304.

Roopa H.K., Kumar N.K.K., Asokan R., Rebijith K.B., Mahmood R. & Verghese A. 2012. Phylogenetic Analysis of *Trialeurodes Spp.*(Hemiptera: Aleyrodidae) from India Based on Differences in Mitochondrial and Nuclear DNA. *Florida Entomol.* 95: 1086–1094.

Rosell R.C., Torres-Jerez I. & Brown J.K. 1999. Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, hemolymph, and honeydew. *Phytopathology* 89: 239–246.

Rozas J., Sanchez-DelBarrio J.C., Messeguer X. & Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496–2497.

Russell L.M. 1948. *The North American species of whiteflies of the genus Trialeurodes*. U.S.D.A. Miscellaneous publication, p. 85.

Saikkonen K., Taulavuori K., Hyvönen T., Gundel P.E., Hamilton C.E., Vänninen I., Nissinen A. & Helander M. 2012. Climate change-driven species' range shifts filtered by photoperiodism. *Nat. Clim. Chang.* 2: 239–242.

Saridaki A. & Bourtzis K. 2010. Wolbachia: more than just a bug in insects genitals. *Curr. Opin. Microbiol.* 13: 67–72.

SAS Institute Inc. 2012. SAS® Enterprise Guide®.

Schneider-Orelli O. 1947. *Entomologisches Praktikum: Einführung in die land- und forstwirtschaftliche Insektenkunde*. Sauerländer Aarau, Germany, p. 237.

Simberloff D. 2009. The Role of Propagule Pressure in Biological Invasions. *Annu. Rev. Ecol. Evol. Syst.* 40: 81–102.

SPSS Inc. PASW Statistics for Windows.

Stenseth C. 1983. Cold-hardiness in eggs of greenhouse whitefly (*Trialeurodes vaporiorum*). *IOBC-WPRS Bull.* 6: 84–86.

Sunnucks P. 2000. Efficient genetic markers for population biology. *Trends Ecol. Evol.* 15: 199–203.

Thuiller W., Richardson D., Pyšek P., Midgley G.F., Hughes G.O. & Rouget M. 2005. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Glob. Chang. Biol.* 11: 2234–2250.

TIKE & OSF. 2014. Official Statistics of Finland. Available at http://www.maataloustilastot.fi/en/horticultural-statistics

Tsagkarakou a. & Roditakis N. 2003. Isolation and characterization of microsatellite loci in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Mol. Ecol. Notes* 3: 196–198.

Tsagkarakou A., Tsigenopoulos C.S., Gorman K., Lagnel J. & Bedford I.D. 2007. Biotype status and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Greece: mitochondrial DNA and microsatellites. *Bull. Entomol. Res.* 97: 29–40.

Uzun S. 2007. The effect of temperature and mean cumulative daily light intensity on fruiting behavior of greenhouse-grown tomato. *J. Am. Soc. Hortic. Sci.* 132: 459–466.

Vänninen I., Worner S., Huusela-Veistola E., Tuovinen T., Nissinen A., Saikkonen K., Worner S. & Vänninen I, Worner S, Huusela-Veistola E, Tuovinen T, Nissinen A S.K. 2011. Recorded and potential alien invertebrate pests in Finnish agriculture and horticulture. *Agric. Food Sci.* 20: 96–114.

Verschoor P.J.G. & Lenteren J.C. van. 1978. Host-plant selection by the greenhouse whitefly Trialeurodes vaporiorum (Westwood) (Homoptera: Aleyrodidae). *Meded. Van Fac. Landbouwwet. Rijksuniv. Gent.* 43: 387–396.

Via S. 1990. Ecological genetics and host adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. *Annu. Rev. Entomol.* 35: 421–446.

Wernegreen J.J. 2002. Genome evolution in bacterial endosymbionts of insects. *Nat. Rev. Genet.* 3: 850–861.

Westwood J.O. 1856. The new Aleyrodes of the greenhouse. *"Gardeners" Chron.*: 852.

Whitlock M.C. & McCauley D.E. 1999. Indirect measures of gene flow and migration: FST≠ 1/(4Nm+ 1). *Heredity* 82: 117-125.

Williamson M. 1993. Invaders, weeds and the risk from genetically manipulated organisms. *Cell Mol. Life Sci.* 49: 219–224.

Williamson M.H. & Fitter A. 1996. The characters of successful invaders. *Biol. Conserv.* 78: 163–170.

Willis K.J. & Birks H.J.B. 2006. What is natural? The need for a long-term perspective in biodiversity conservation. *Science* 314: 1261–1265.

Xie W., Wang S., Wu Q., Feng Y., Pan H., Jiao X., Zhou L., Yang X., Fu W., Teng H., Xu B. & Zhang Y. 2011. Induction effects of host plants on insecticide

susceptibility and detoxification enzymes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manag. Sci.* 67: 87–93.

Yun G., Li-ning S. & Li-juan S. 2006. Life Table of Experimental Population of White House Fly *Trialeurodes vaporiorum* (Westwood). *J. Laiyang Agric. Coll. Sci.* 2.

Zabeltitz C. Von. 1999. Greenhouse structures. In: Stanhill G. & Enoch Z.H. (eds.), *Ecosystems of the world. Greenhouse ecosystems*, Elsevier Science Publishers, Amsterdam, pp. 17–70.

Zamoum T., Simon J.-C., Crochard D., Ballanger Y., Lapchin L., Vanlerberghe-Masutti F. & Guillemaud T. 2005. Does insecticide resistance alone account for the low genetic variability of asexually reproducing populations of the peach-potato aphid Myzus persicae? *Heredity* 94: 630–639.

Zhang G.-F., Lövei G.L., Hu M. & Wan F.-H. 2014. Asymmetric consequences of host plant occupation on the competition between the whiteflies *Bemisia tabaci* cryptic species MEAM1 and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Pest Manag. Sci.* In press.

Zhou S., Campbell T.G., Stone E. a, Mackay T.F.C. & Anholt R.R.H. 2012. Phenotypic plasticity of the Drosophila transcriptome. *PLoS Genet.* 8: e1002593.

ORIGINAL PAPERS

I

GREENHOUSES AS A GATEWAY FOR INVASION OF A GENERALIST HERBIVORE IN THE BOREAL ECOSYSTEM?

by

Irina Ovčarenko, Leena Lindström, Kari Saikkonen, Lauri Jauhiainen, Janne Kaseva & Irene Vänninen

Submitted manuscript

II

THIRTEEN POLYMORPHIC MICROSATELLITE LOCI AND PCR MULTIPLEXING IN THE GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM* WESTWOOD (HOMOPTERA: ALEYRODIDAE)

by

Irina Ovcarenko, Cécile Clouet, Emily Knott, Anastasia Tsagkarakou & Nathalie Gauthier 2013

Molecular Ecology Resources 13(2): 341-343.

III

AGROECOSYSTEMS SHAPE THE GENETIC STRUCTURE OF GREENHOUSE WHITEFLY (*TRIALEURODES VAPORARIORUM*) POPULATIONS IN NORTHERN AND SOUTHERN EUROPE

by

Irina Ovčarenko, Despoina Kapantaidaki, Leena Lindström, Nathalie Gauthier, Anastasia Tsagkarakou, Emily Knott & Irene Vänninen

BMC Evolutionary Biology, 14 (165): 1-17.

Ovčarenko et al. BMC Evolutionary Biology 2014, 14:165
http://www.biomedcentral.com/1471-2148/14/165

BMC Evolutionary Biology

RESEARCH ARTICLE Open Access

Agroecosystems shape population genetic structure of the greenhouse whitefly in Northern and Southern Europe

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Abstract

Background: To predict further invasions of pests it is important to understand what factors contribute to the genetic structure of their populations. Cosmopolitan pest species are ideal for studying how different agroecosystems affect population genetic structure within a species at different climatic extremes. We undertook the first population genetic study of the greenhouse whitefly (*Trialeurodes vaporariorum)*, a cosmopolitan invasive herbivore, and examined the genetic structure of this species in Northern and Southern Europe. In Finland, cold temperatures limit whiteflies to greenhouses and prevent them from overwintering in nature, and in Greece, milder temperatures allow whiteflies to inhabit both fields and greenhouses year round, providing a greater potential for connectivity among populations. Using nine microsatellite markers, we genotyped 1274 *T. vaporariorum* females collected from 18 greenhouses in Finland and eight greenhouses as well as eight fields in Greece.

Results: Populations from Finland were less diverse than those from Greece, suggesting that Greek populations are larger and subjected to fewer bottlenecks. Moreover, there was significant population genetic structure in both countries that was explained by different factors. Habitat (field vs. greenhouse) together with longitude explained genetic structure in Greece, whereas in Finland, genetic structure was explained by host plant species. Furthermore, there was no temporal genetic structure among populations in Finland, suggesting that year-round populations are able to persist in greenhouses.

Conclusions: Taken together our results show that greenhouse agroecosystems can limit gene flow among populations in both climate zones. Fragmented populations in greenhouses could allow for efficient pest management. However, pest persistence in both climate zones, coupled with increasing opportunities for naturalization in temperate latitudes due to climate change, highlight challenges for the management of cosmopolitan pests in Northern and Southern Europe.

Keywords: *Trialeurodes vaporariorum*, Pest management, Microsatellite markers, Climate zone, Host adaptation

Background

The dispersal of phytophagous insect pests can be enhanced by worldwide trade and human movement [1,2]. In addition, climate change facilitates movement of various taxa polewards [3,4]. Following introduction to new habitats, the establishment of insect pest populations can be favored by benign climates, as well as by monocultures in agroecosystems, i.e. agricultural fields and greenhouses [5-7]. Low genetic diversity of insect pest populations in newly occupied habitats suggests that even a single successful founder event is enough to establish populations [8,9]. However, further spread of introduced pests into natural ecosystems depends on the environment surrounding the initial introduction and on the origin of the introduced species [10,11]. For example, pests of tropical origin may be more likely to establish themselves in the Mediterranean than in the boreal climate zone [12,13]. At

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southern latitudes there are suitable climatic conditions and year-round availability of host plants, in both natural ecosystems and densely aggregated agroecosystems. In contrast, at northern latitudes, natural habitats are only seasonally available and greenhouses are often sparsely distributed. Thus, the extent of establishment and spread of invasive pests in the North might be more dependent on the distribution of agroecosystems, particularly greenhouses, than it is in the South.

Enclosed greenhouse environments are designed to reduce evaporation, pest entry [14] and loss of expensive biological pest control agents [15], to ensure efficient crop maintenance. Because greenhouses are relatively closed environments, pest populations in greenhouses might be generally more affected by insecticide applications and host plant changes than are pest populations in fields. These crop management practices can lead to reductions in population size and selection for resistant genotypes in the pests leading to increased homozygosity within and differentiation between pest populations [16]. Thus, populations of insects inhabiting greenhouses might show more genetic differentiation than those in fields. Indeed, populations of phytophagous pests inhabiting greenhouses often show population genetic structure, e.g. *Tetranychus urticae* Koch [17,18], although dispersal and gene flow can also be restricted among pest populations inhabiting fields, e.g. *Leptinotarsa decemlineata* Say [8].

The greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) is an invasive pest which was brought to Europe (UK) on Orchidaceae from Mexico in 1856 [19]. Soon after introduction, it spread to the European continent, and in 1920 it was recorded in greenhouses in Finland [20]. In Finland, it spread through transportation on plant seedlings above the Arctic Circle to Rovaniemi, bringing considerable damage to tomato and cucumber crops, as well as to ornamental plants [21,22]. *T. vaporariorum* was reported from the Mediterranean region only later, in 1963 [19]. The species was noticed in Greece (Crete) only in 1978, when it began to cause pest management problems due to its resistance to insecticides [23,24].

T. vaporariorum currently has an almost cosmopolitan distribution [25,26]. Its success can be attributed to the worldwide distribution of greenhouse habitats, polyphagy [27], its tolerance of higher or lower temperatures than its biological control agents [17,26], and its haplodiploid mode of reproduction [28]. However, the absence of an overwintering resting stage [29] potentially limits its spread to natural ecosystems. Since the development of *T. vaporariorum* ceases at 8.3°C [30], year-round populations might persist at southern latitudes, where some host plants are available during winter, but are not likely to persist at northern latitudes, where crop cultivation in fields is seasonal and wild host plants decay during winter.

To date, population genetic structure has been analyzed in only a few whitefly species, and little is known about population genetic structure in *T. vaporariorum*. The related *Bemisia tabaci* species complex, particularly Mediterranean *B. tabaci* (Med), is characterized by high genetic diversity and differentiation of populations, as indicated by both mitochondrial and microsatellite markers [31,32] (except in recently introduced populations in Taiwan and France [33,34]). Populations of *B. tabaci* (Med) in Greece separated by just a few kilometers show population genetic structure, possibly due to separate founder events or an older population history in this country [35]. Unlike the *B. tabaci* species complex, *T. vaporariorum* populations have low genetic diversity in mitochondrial genes [36,37]. Recent findings indicate that sequences of three mitochondrial genes and composition of endosymbiont communities from populations sampled from different continents show little variation (Kapantaidaki et al., unpubl.). Analysis of a few nuclear genes (allozymes) in *T. vaporariorum* populations from greenhouses in South Korea revealed their subdivision possibly due to restricted gene flow by natural geographic barriers [38]. However, studies of population genetic structure in *T. vaporariorum* with other, more polymorphic genetic markers, allowing description of more recent evolutionary processes, have not been performed until now. Recent findings of variation in phenotypic responses, particularly diverse responses to insecticide treatments among geographically close populations, suggest differentiation and low gene flow among invaded greenhouses [39].

To understand how insect pests respond to different environmental conditions, such as climate, habitat, and crop management practices, and the role of agroecosystems in shaping population genetic structure, comparative studies of population genetic diversity of pests in different climate zones are necessary. In this study we present the first extensive genetic data on population structure of the greenhouse whitefly. We compare the genetic structure of *T. vaporariorum* populations in Finland and in Greece, representing boreal and Mediterranean climate zones, and evaluate the influence of host plants and agricultural practices on the spatial and temporal population genetic structure of this invasive species. We hypothesize that *T. vaporariorum* populations in Northern Europe are more likely to be genetically differentiated than populations in Southern Europe, because this species is expected to be restricted to greenhouses in the North.

Methods

Sampling

In Finland we sampled commercial greenhouses that operate year-round and produce primarily tomato and cucumber crops of various cultivars (Table 1). Samples were collected from greenhouses belonging to different

growers. In total 18 greenhouses were sampled in spring of 2010–2012. Ten of these were sampled twice: in 2010 and in 2011. Sampling was concentrated in Ostrobothnia (16 greenhouses) but also included two distant locations in other parts of the country (Figure 1-I). Ostrobothnia was the focus of our study because in this area we could find multiple greenhouses with different management practices in terms of host plant species, their cultivars (Table 1) and the origin of seedlings. Two to five greenhouses belonging to different growers were sampled within Närpes, Töjby and Pjelax villages. The minimum and maximum distances between these villages were 9 and 32 km, respectively, measured as straight line distance between coordinates. The distances between greenhouses within villages ranged from 1.1 to 3.7 km in Närpes, 0.4 km in Töjby and from 0.28 to 0.9 km in Pjelax.

In Greece we sampled 16 agroecosystems in different seasons over several years: 2004–2011. These included eight greenhouses and eight fields growing various crop plants (Table 1) which were distributed throughout mainland Greece, the Peloponnese and the Island of Crete (Figure 1-II). Sampling was concentrated in the fields of West Peloponnese because open environments in this region cover the expected range (7–20 km) of the potential dispersal abilities of whiteflies (natural or by wind, as known from the *B. tabaci* species complex; [40,41]). In West Peloponnese, the minimum distance between samples ranged from 3.4 km (between WP3 and WP4) to 24.4 km (between WP3 and WP5), and the maximum distance between locations reached 100 km.

During sampling, both genders of whiteflies were collected using a mouth aspirator. The whiteflies were preserved in 90% Ethanol and stored at 4°C until they were sexed and used for genotyping. Since *T. vaporariorum* is a haplodiploid species, only adult females were chosen for genotyping.

DNA extraction and microsatellite genotyping

Total genomic DNA was extracted from each individual female as described in Tsagkarakou et al. [42]. Nine microsatellite markers (Table 2) out of the 13 characterized in Molecular Ecology Resources Primer Development Consortium et al. [43] were used to genotype 1274 *T. vaporariorum* females, 800 from Finland and 474 from Greece (Table 3). Four of the microsatellite markers described previously did not amplify consistently, and thus, were excluded from this study. Three multiplex amplification reactions were performed as described in Molecular Ecology Resources Primer Development Consortium et al. [43] with slight modifications. Diluted amplification products were separated on an ABI 3130xl genetic analyzer (Applied Biosystems). Allele sizes were scored against GeneScan™ 500 LIZ standard using GeneMapper® v 4.0 software (both Applied Biosystems) and were confirmed manually.

Data analysis

To analyze genetic distance between samples, both between and within countries, pairwise estimates of F_{ST} were calculated in Arlequin 3.11 [44]. The significance of the genetic distances at the 0.05 level was tested by permuting the individuals or genotypes between the samples 110 times and adjusting P values with strict Bonferroni correction. Since all pairwise F_{ST} between samples from Finland and Greece showed statistically significant differences, and due to the low probability of gene flow between the two distant countries, all further analyses were done for each country separately.

The samples taken in two consecutive years from the same greenhouse in Finland showed no genetic differentiation, except for one sampling location (TJ-2 a and TJ-2 b). Therefore, in most analyses we used a *combined* dataset, which pooled samples collected from the same greenhouse in 2010 and 2011, (except for TJ-2 a and TJ-2 b, which were considered as separate samples). For other analyses (specified below) we used a *separated* dataset, in which each sampling effort in Finland was considered a separate sample. Each sampling effort in Greece was considered a separate sample in all analyses, because none of the locations were sampled in consecutive years.

For each sample, mean observed (H_O) and expected heterozygosity (H_E), and mean number of alleles (N_A) per locus were calculated using GenAlEx v. 6.5 [45]. Observed and expected heterozygosities were also calculated for each locus over the total data from each country. Departure from Hardy-Weinberg expectations (HWE) was tested with 1000 permutations using a global test across loci or samples as implemented in GENEPOP v. 4.2 [46]. The test was performed using Fisher's method, testing hypotheses of heterozygote deficiency and heterozygote excess [47], and producing global p value estimates for each sample over all loci and for each locus over samples from Finland and Greece. Genotypic linkage disequilibrium for each pair of loci in the samples was tested using the log likelihood ratio statistic (G-test) as implemented in GENEPOP v. 4.2. For multiple tests, statistical significance was adjusted using strict Bonferroni corrections [48]. The samples were analyzed for potential scoring errors in all loci using MICRO-CHECKER v. 2.2.3 and the frequency of null alleles (f) was estimated [49].

To investigate the relationship between genetic and geographic distance, isolation by distance was analyzed in GenAlEx v. 6.5 [45]. Genetic distance was defined by pairwise linear $F_{ST,}$ (F_{ST}/(1- F_{ST})), and geographic distance was defined as pairwise distances generated from geographical coordinates expressed in decimal degrees. The correlation between the two data matrices was assessed using a Mantel test and its significance estimated by P values, the

Table 1 Description of the samples collected in Finland and Greece

Geographical information					Host plant			Collection	
			Geographical coordinates:						Date:
Country/Region	Locality	Sample code	Latitude	Longitude	Species	Cultivar	Family	Habitat	Month-year
Finland									
Ostrobothnia	Härkmeri	HR a	62.165219	21.467372	Cucumber [1]	Imea	Cucurbitaceae	G	May-10
		HR b			Tomato [1]	Espero	Solanaceae	G	Apr-11
	Korsnäs	KR a	62.778983	21.204792	Cucumber	Cadense R2	Cucurbitaceae	G	May-10
		KR b			Cucumber	Cadense R2	Cucurbitaceae	G	Apr-11
	Malax	ML a	62.938797	21.526186	Cucumber [1]	Diligare	Cucurbitaceae	G	May-10
		ML b			Tomato [1]	DRW	Solanaceae	G	Apr-11
	Närpes	NR 1a	62.476119	21.416114	Tomato	Encore	Solanaceae	G	May-10
		NR 1b			Tomato	Encore	Solanaceae	G	Apr-11
		NR 2	62.479328	21.395703	Cucumber	Imea	Cucurbitaceae	G	May-10
		NR 3a	62.467842	21.346608	Cherry tomato	Gonchita	Solanaceae	G	May-10
		NR 3b			Tomato	Gonchita	Solanaceae	G	Apr-11
	Pjelax	PJ 1a	62.393006	21.382206	Tomato	Encore	Solanaceae	G	May-10
		PJ 1b			Tomato	Encore	Solanaceae	G	Apr-11
		PJ 2	62.395511	21.381911	Tomato	Encore	Solanaceae	G	May-10
		PJ 3a	62.396372	21.382139	Tomato	Encore	Solanaceae	G	May-10
		PJ 3b			Tomato	Encore	Solanaceae	G	Apr-11
		PJ 4	62.397450	21.375103	Tomato	Dometica	Solanaceae	G	Apr-11
		PJ 5	62.389081	21.371075	Tomato	Dometica	Solanaceae	G	Apr-11
	Pörtom	PR a	62.710939	21.623539	Tomato	Encore	Solanaceae	G	May-10
		PR b			Tomato	Encore	Solanaceae	G	Apr-11
	Töjby	TJ 1a	62.664411	21.221228	Cucumber	Ventura	Cucurbitaceae	G	May-10
		TJ 1b			Cucumber	Logica	Cucurbitaceae	G	Apr-11
		TJ 2a	62.661847	21.226625	Cucumber	Annica	Cucurbitaceae	G	May-10
		TJ 2b			Cucumber	Annica	Cucurbitaceae	G	Apr-11
	Övermark	OV	62.611700	21.471772	Tomato	Several cultivars [4]	Solanaceae	G	Apr-11
Uusimaa	Lohja	LH	60.176453	23.981306	Cucumber	Imea	Cucurbitaceae	G	Apr-11
Northern Savonia	Nilsiä	NL	63.151436	27.987397	Cucumber [2]	Imea	Cucurbitaceae	G	Jul-12
Greece									
West Peloponnese	Kourtessi	WP 1	37.966667	21.330278	Cucumber	-	Cucurbitaceae	F	Jun-04
	Filiatra	WP 2	37.119983	21.584281	Zuccini	-	Cucurbitaceae	F	Jul-04
	Elea	WP 3	37.372628	21.688894	Eggplant	-	Solanaceae	F	Aug-11
	Prasidaki	WP 4	37.397167	21.711822	Bean	-	Fabaceae	F	Aug-11
	Anemochori	WP 5	37.588725	21.538794	Tomato	-	Solanaceae	F	Sep-11
	Terpsithea	WP 6	37.227417	21.628542	Bean	-	Fabaceae	F	Sep-11
	Andravida	WP 7	38.007222	21.395833	Marrow	-	Cucurbitaceae	F	Sep-11
North Peloponnese	Aigio	NP	38.216853	22.114178	Rose	-	Rosaceae	G	Aug-11
West Greece	Agrinio	WG	38.579722	21.418056	Tomato	-	Solanaceae	G	Jun-11
East Peloponnese	Nafplion	EP	37.745556	22.850278	Bean	-	Fabaceae	F	Oct-11
Attica	Athens	AT	37.983147	23.706583	Eggplant	-	Solanaceae	G	Apr-05

Table 1 Description of the samples collected in Finland and Greece *(Continued)*

Island of Crete	Fodele	CR 1	35.398228	24.963689	Rose	-	Rosaceae	G	Mar-10
	Sissi	CR 2	35.305961	25.535006	Rose	-	Rosaceae	G	Apr-11
	Malades	CR 3	35.268528	25.104956	Datura	-	Solanaceae	G	Apr-11
Macedonia	Serres	MA 1	41.225933	23.361469	Tomato	-	Solanaceae	G	May-11
	Drama	MA 2	41.124744	24.162803	Sweet pepper [3]	-	Solanaceae	G	May-11

Lower case letters adjacent to population codes indicate the same location sampled in 2010 and 2011.
G indicates samples collected from greenhouses, F – from fields.
[1]Cucumber and tomato were growing in the same greenhouse compartment.
[2]Cucumber and tomato were growing in different greenhouse compartments.
[3]Tomato and eggplant were growing in the same greenhouse compartment.
[4]Encore, Careza, Dometica, Dirk and Axxion cultivars grown in the same greenhouse compartment.

regression coefficient (R^2), and the mean correlation coefficient (R_{XY}) over 999 random permutations of linear F_{ST} values as implemented in GenAlex v.6.5. Isolation by distance was assessed with smaller subsets of the data as well as using the full datasets, to evaluate the influence of scale on the relationships.

Analyses of molecular variance (AMOVA) were performed using Arlequin 3.11 [44] to estimate and compare the percentage of genetic variation explained by different hierarchical groups (i.e. individual, sample, group of samples). Four analyses were constructed to test the following groups: 1) country (Finland vs. Greece), 2) host plant species (cucumber vs. tomato) in samples from Finland only, 3) host plant botanical family (Cucurbitaceae vs. Solanaceae vs. Fabaceae vs. Rosaceae) in samples from Greece only, and 4) habitat (greenhouse vs. field) in samples from Greece only. For analysis 2, samples HR, ML and NL were excluded since the whiteflies in these greenhouses might have been exposed to both cucumber and tomato grown in the same compartment or greenhouse. However, in analysis 3, sample MA 2 was not excluded because several hosts grown in the same compartment belonged to the same family (Solanaceae) (see Table 1).

To assess the level of genetic differentiation between groups defined above for AMOVA, we compared summary statistics calculated for the different groups: F_{IS} (inbreeding coefficient measuring heterozygote deficit within populations), F_{ST} (a measure of population structure and heterozygote deficit among populations), allelic richness (measure of the number of alleles independent

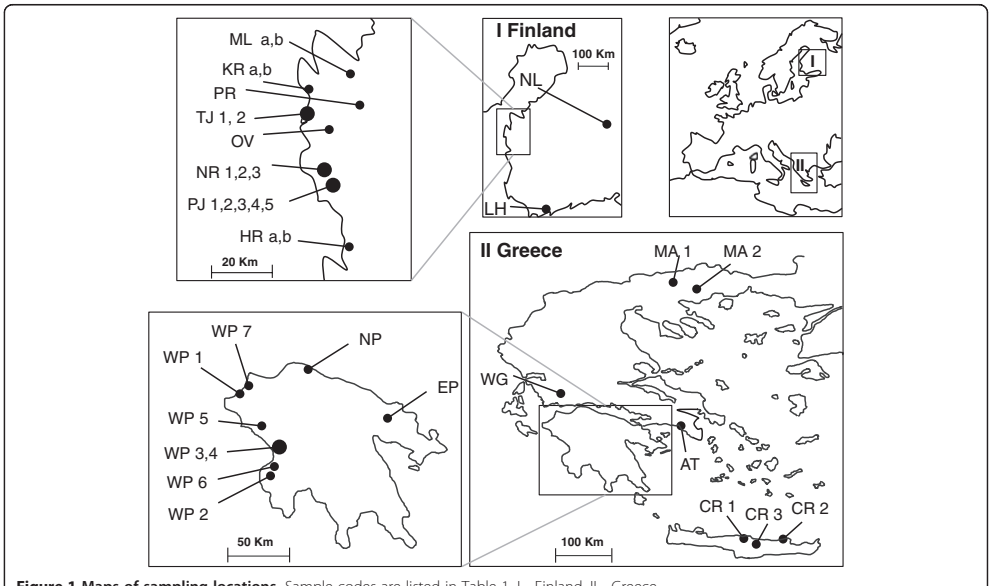

Figure 1 Maps of sampling locations. Sample codes are listed in Table 1. I - Finland, II - Greece.

Table 2 Characteristics of the nine polymorphic microsatellite loci analysed in *T. vaporariorum*

Locus (Genbank Accession no.)	Primer sequence (5′-3′) (F: [dye]-forward; R: reverse)	Repeat motif (cloned allele)	Size range (bp)	No. of alleles	H_O/H_E Finland	H_O/H_E Greece
Tvap-1-1C	F: [6-FAM]- GAGACTCCACGATGTCTGTC	$(GT)_6$ GG$(GT)_9$	195-215	3	0.469/0.499*	0.455/0.461
(GF112015)	R: TTCCCCTATCGTATGTTCAC					
Tvap-1-2	F: [VIC]- CTGTGAATCCCTCAGAAATC	$(GT)_6$	233-236	2	0.094/0.108	0.299/0.308
(GF112025)	R: TGACCTCTCTCAGGCTTTTA					
Tvap-3-1	F: [PET]- GAGATGGACAAACTACAACG	$(AC)_{15}$	228-230	2	**0.246/0.437***	**0.268/0.355***
(GF112016)	R: GATTGGATGTCGTGGTTG					
Tvap-3-2	F: [6-FAM]- GGAGGTCATTACTCATTTCG	$(AC)_6$	170-182	4	0.401/0.405	0.522/0.581
(GF112017)	R: CATAAATTTTCGGCTCACTC					
Tvap-3-3	F: [VIC]- CGCAAATCATACTTCCTTTC	$(CA)_5$	235-237	2	0.417/0.412	0.496/0.459
(GF112019)	R: AAATACAGGCGACTCATGTC					
Tvap-4-2	F: [NED]- GGTGGTATTGTGGCGTC	$(GA)_{29}$	298-314	7	0.446/0.468	**0.585/0.667**
(GF112027)	R: CTGCCTCTTATGACTCTTCC					
Tvap-1-4	F: [PET]- GATTTAGCCCAGTTCATTTG	$(TG)_5$	265-267	2	0.091/0.097	**0.137/0.179***
(GF112020)	R: CTTCAGTTGAGCTGCTGATG					
Tvap-1-5	F: [6-FAM]- CAGTTGTGGTAGTGTGGTG	$(TG)_{12}$	124-146	10	0.416/0.411	**0.703/0.757**
(GF112028)	R: CTCATCGGCTCATACATTC					
Tvap-2-2C	F: [VIC]- CTGAAAGTCTTATTAGAGCC	$(TC)_8$ GC $(TC)_{10}$	210-220	6	0.568/0.55	0.588/0.608
(GF112021)	R: CTAACTGATTCCATAGTCG					

No. of alleles indicates the maximum number of alleles found in this study.
H_O, observed heterozygosity; H_E, unbiased expected heterozygosity.
*indicate H_O/H_E values with potential presence of null alleles with frequency > 0.2.
H_O/H_E in bold indicate loci with significant deviations from Hardy–Weinberg equilibrium in terms of heterozygote deficiency after Bonferroni correction (no significant heterozygote excess was detected).
Heterozygosities, deviations from HWE and null allele frequencies were estimated over 800 females from 18 samples in Finland and 474 females from 16 samples in Greece.

of sample size), H_E (unbiased expected heterozygosity) and H_O (observed heterozygosity). FSTAT v. 2.9.3 [50] was used to calculate the average (over samples and loci; weighted by sample size) of the chosen statistics for each group and for their comparison. Statistical significance was assessed after 1000 permutations. As in the AMOVA groups, some samples were excluded because multiple hosts were grown in the same compartment or greenhouse (see above).

The relationship between environmental variables and genetic structure of the studied populations was estimated using default settings of the software GESTE v. 2.0 [51]. The software gives the highest posterior probability (*Pr*) to the model explaining genetic structure the best, evaluating environmental variables separately and in combination through a generalized linear model. In this analysis, the *separated* data set for the samples from Finland was used. Latitude, host plant species, cultivar, crop source and year of sampling were evaluated as explanatory variables of population structure in Finland. Latitude, longitude, four host plant families and habitat (field or greenhouse) were evaluated as explanatory variables of population structure in Greece.

Bayesian clustering analysis implemented in STRUCTURE v.2.3.4 [52] was used to infer the number of genetically distinct clusters (K) in each country using a model of no admixture, correlated allele frequencies and including the sampling location as a prior [53]. Initial analyses were performed both with admixture and no admixture models, but the later was selected since visualization of the results was more straightforward and no differences in the most likely number of clusters were observed for the two models. Analysis parameters included a burn-in period of 250,000 followed by 500,000 MCMC iterations. For each dataset, Finland and Greece, we tested K from 2 to 10, with ten replicate analyses per value of K. Subsets of each dataset were analyzed with the same settings. The most likely number of clusters in our samples was determined using the ΔK approach [54] as implemented in Structure Harvester v. 0.56.3 [55]. Results were visualized as bar plots by finding the optimal alignment of the ten replicate analyses of the "best" K in CLUMPP v. 1.1.2 [56] using the Greedy algorithm and 1000 random input orders, and then by creating graphics in Distruct v. 1.1 [57]. For Finland, the *combined* dataset was used first, and then two subsets of the data were created. In these subsets, the

Table 3 Genetic diversity estimated over the nine microsatellite loci for samples of *T. vaporariorum*

Country	Region	Locality	Sample code	*N*	N_A (±SE)	H_O/H_E
Finland	Ostrobothnia	Härkmeri	HR a,b	30 + 30	2.556(±0.294)	0.375/0.435*****
		Korsnäs	KR a,b	29 + 30	2.333(±0.373)	0.222/0.254*
		Malax	ML a,b	30 + 30	2.667(±0.236)	0.375/0.404
		Närpes	NR 1a,b	30 + 30	3.111(±0.423)	0.369/0.406
			NR 2	30	2.333(±0.167)	**0.256/0.341***
			NR 3a,b	30 + 30	2.889(±0.351)	0.308/0.375*
		Pjelax	PJ 1a,b	30 + 30	2.889(±0.389)	**0.375/0.429***
			PJ 2	30	2.667(±0.236)	0.422/0.422
			PJ 3a,b	30 + 30	3.111(±0.455)	0.396/0.429*
			PJ 4	30	2.667(±0.289)	0.359/0.417*
			PJ 5	30	2.667(±0.333)	0.407/0.406
		Pörtom	PR a,b	30 + 30	3.444 (±0.669)	0.434/0.484
		Töjby	TJ 1a,b	30 + 30	3.222 (±0.494)	0.570/0.556*
			TJ 2a	30	3.333 (±0.577)	0.465/0.517
			TJ 2b	30	3.667 (±0.707)	0.554/0.531*
		Övermark	OV	30	3.556 (±0.648)	0.511/0.543*
	Uusimaa	Lohja	LH	21	3.667 (±0.799)	0.541/0.529
	Northern Savonia	Nilsiä	NL	30	3.333 (±0.645)	0.448/0.512
Greece	West Peloponnese	Kourtessi	WP 1	30	3.222(±0.494)	0.422/0.450
		Filiatra	WP 2	29	3.333 (±0.553)	0.415/0.524
		Elea	WP 3	30	3.444 (±0.626)	0.459/0.540
		Prasidaki	WP 4	30	2.667 (±0.236)	0.409/0.457
		Anemochori	WP 5	30	3.444 (±0.603)	0.437/0.394
		Terpsithea	WP 6	30	3.222 (±0.494)	0.428/0.469*
		Andravida	WP 7	30	3.111 (±0.484)	0.441/0.419
	North Peloponnese	Aigio	NP	30	3.222 (±0.494)	0.277/0.396
	West Greece	Agrinio	WG	30	3.444 (±0.689)	**0.395/0.455**
	East Peloponnese	Navplion	EP	30	3.222(±0.494)	**0.422/0.450**
	Attica	Athens	AT	28	3.333 (±0.553)	0.415/0.524*
	Island of Crete	Fodele	CR 1	30	3.444 (±0.626)	0.459/0.540
		Sissi	CR 2	30	2.667 (±0.236)	0.409/0.457
		Malades	CR 3	30	3.444 (±0.603)	0.437/0.394*
	Macedonia	Serres	MA 1	27	3.222 (±0.494)	**0.428/0.469***
		Drama	MA 2	30	3.111 (±0.484)	0.441/0.419

For Finland the *combined* dataset, which pooled samples from consecutive years at the same location (except TJ 2) is described, since it was used in the majority of analyses. Lower case letters adjacent to population codes indicate the same location sampled in 2010 and 2011. N number of analyzed females, H_O observed and H_E expected heterozygosity and N_A mean number of alleles per population averaged over 9 loci. * indicate H_O/H_E values in samples with null allele frequency > 0.2. **H_O/H_E** in bold indicate loci with significant deviations from Hardy–Weinberg equilibrium in terms of heterozygote deficiency after Bonferroni correction (no significant heterozygote excess was detected).

samples were grouped by host plant species and samples HR a, b and ML a, b were separated since they had been collected from different hosts (see Table 1). Samples HR, ML and NL were included in both data subsets since these samples might have been exposed to several hosts grown in the same compartment or greenhouse. For Greece, all samples were first analyzed together, then data subsets were created grouping samples by habitat (field or greenhouse). The definition of the data subsets (by host plant species or habitat) was chosen after considering the results of initial analyses with the full data sets and our analysis with GESTE v. 2.0 [51].

Results

Genetic diversity of microsatellite loci and samples

Significant deviations from HWE through heterozygote deficiencies were detected at one locus (Tvap 3–1) and 4 loci (Tvap-4-2, Tvap-1-4, Tvap-1-5 and Tvap-3-1) in the Finnish and Greek samples, respectively (Table 2). At the sample level, a test of HWE across the nine microsatellite loci indicated significant heterozygote deficiency in two Finnish and three Greek samples (Table 3). There were no cases of significant heterozygote excess.

Three loci showed a null allele frequency > 0.2: Tvap-1-1C (2 samples), Tvap-3-1 (12 samples) and Tvap-1-4 (1 sample). For each of these loci, the frequency of null alleles within a sample varied: $f = 0.110$-0.258 (Tvap-1-1C), $f = 0.164$-0.401 (Tvap-3-1), and $f = 0.166$-0.238 (Tvap-1-4), and the average frequency of null alleles over the samples ranged from 0.119 to 0.250. No cases of large allele drop out were found. Even though null alleles are present, deviations from HWE could be also due to significant homozygosity in populations inhabiting the human-mediated environment (i.e. due to population bottlenecks and inbreeding), rather than due to significant genotyping errors.

Genotypic linkage disequilibrium tested for each pair of loci for each sample revealed a potential association between loci Tvap-1-1 and Tvap 3–1 in sample PJ 4. Since locus Tvap-3-1 was characterized by homozygote excess and had a high frequency of null alleles only in sample PJ 4 ($f = 0.401$), we suspect that the linkage disequilibrium indicated for this sample does not reflect a true association between the loci. Therefore, data from all nine loci were used in the analyses.

Differences between Finland and Greece

AMOVA indicated significant genetic structure between the two geographic areas (Table 4). The percentage of variation explained by country of origin, Finland vs. Greece, was higher than that among the samples within each country (9.90% and 6.87%, respectively; Table 4), indicating that overall genetic variation might be explained by these groups. *T. vaporariorum* from the two countries also differed significantly in their global observed and expected heterozygosities (Finland: $H_O/H_E = 0.350/0.385$ vs. Greece: $H_O/H_E = 0.451/0.496$), and in allelic richness (2.498 vs. 3.234 for Finland vs. Greece, respectively) (all $P = 0.001$). However, F statistics calculated for each country did not differ statistically (Finland/Greece; F_{IS}: 0.091/0.090, $P = 0.157$; F_{ST}: 0.093/0.055, $P = 0.976$). Nevertheless, the range of pairwise F_{ST} values between samples within countries was broader for Finland ($-0.006 < F_{ST} < 0.533$) than it was for Greece ($-0.007 < F_{ST} < 0.164$) (Tables 5A and B).

Population structure in Finland

Seventy nine percent of pairwise F_{ST} comparisons (121 of 154) between samples from Finland showed significant population differentiation (Table 5A). Some populations (HR, KR, PR, TJ 2b and LH) were differentiated from all other samples (Table 5A). However, one of the samples most distant from the Ostrobothnia region (NL) was not significantly different in pairwise F_{ST} from one of the Ostrobothnian samples (OV). For samples collected from different greenhouses at the same location (NR, PJ and TJ), there was no significant genetic structure, except for TJ: TJ1 was not differentiated from TJ 2a, but both of

Table 4 Distribution of the molecular variance between and within four groups of samples of *T. vaporariorum*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P* values
Between countries	1	275.947	0.221	9.900	F_{CT}: 0.099	0 ± 0
Among samples within countries	32	425.048	0.153	6.870	F_{SC}: 0.076	0 ± 0
Within samples	2514	4665.034	1.856	83.240	F_{ST}: 0.168	0 ± 0
Between host plant groups in Finland[1]	1	34.818	0.031	1.690	F_{CT}: 0.017	0.036 ± 0.006
Among samples within groups	13	195.663	0.157	8.590	F_{SC}: 0.087	0 ± 0
Within samples	1285	2111.236	1.643	89.720	F_{ST}: 0.103	0 ± 0
Among host plant groups in Greece[2]	3	32.540	0.006	0.250	F_{CT}: 0.002	0.266 ± 0.013
Among samples within groups	12	114.290	0.124	5.330	F_{SC}: 0.053	0 ± 0
Within samples	932	2045.532	2.195	94.420	F_{ST}: 0.056	0 ± 0
Between habitats in Greece	1	32.665	0.052	2.200	F_{CT}: 0.022	0.001 ± 0.001
Among samples within groups	14	114.165	0.010	4.290	F_{SC}: 0.044	0 ± 0
Within samples	932	2045.532	2.195	93.510	F_{ST}: 0.065	0 ± 0

[1]Between groups of samples collected from Cucurbitaceae, Solanaceae host plant families in Finland (samples HR, ML and NL are not included, see Methods for details).
[2]Among groups of samples collected from Cucurbitaceae, Solanaceae, Fabaceae and Rosaceae host plant families in Greece.

Table 5 Pairwise estimates of F_{ST} between samples in Finland (A) and Greece (B) over the nine microsatellite loci

A Finland

	HR	KR	ML	PJ 1	PJ 2	PJ 3	PJ 4	PJ 5	NR 1	NR 2	NR 3	PR	OV	TJ 1	TJ 2a	TJ 2b	LH	NL
HR	0.000																	
KR	0.207	0.000																
ML	0.025	0.181	0.000															
PJ 1	0.055	0.173	0.024	0.000														
PJ 2	0.052	0.222	0.027	**0.009**	0.000													
PJ 3	0.058	0.201	0.022	**0.015**	**0.003**	0.000												
PJ 4	0.064	0.216	**0.027**	**−0.004**	**−0.006**	**0.002**	0.000											
PJ 5	0.073	0.222	0.044	**0.004**	**0.001**	**0.018**	**−0.005**	0.000										
NR 1	0.050	0.204	**0.010**	**−0.004**	**0.017**	**0.012**	**0.006**	**0.013**	0.000									
NR 2	0.095	0.240	0.044	0.036	0.067	0.065	0.052	0.053	**0.023**	0.000								
NR 3	0.079	0.207	**0.024**	**0.029**	0.059	0.051	0.044	0.043	**0.010**	**0.001**	0.000							
PR	0.082	0.212	0.053	0.080	0.109	0.010	0.096	0.116	0.063	0.050	0.045	0.000						
OV	0.048	0.217	**0.015**	0.024	0.026	0.028	**0.023**	**0.033**	**0.024**	0.039	**0.027**	0.034	0.000					
TJ 1	0.113	0.311	0.064	0.077	0.104	0.094	0.088	0.087	0.050	**0.011**	0.021	0.056	0.044	0.000				
TJ 2a	0.085	0.263	0.035	0.051	0.070	0.053	0.058	0.061	0.025	**0.003**	**0.001**	0.033	**0.030**	**0.002**	0.000			
TJ 2b	0.248	0.533	0.236	0.197	0.225	0.215	0.212	0.206	0.193	0.183	0.211	0.232	0.198	0.129	0.185	0.000		
LH	0.131	0.293	0.115	0.063	0.073	0.102	0.057	0.055	0.090	0.083	0.104	0.156	0.078	0.119	0.123	0.196	0.000	
NL	0.060	0.327	0.031	0.066	0.089	0.045	0.086	0.111	0.034	0.109	0.077	0.050	**0.035**	0.096	0.061	0.294	0.227	0.000

Table 5 Pairwise estimates of F_{ST} between samples in Finland (A) and Greece (B) over the nine microsatellite loci *(Continued)*

B Greece																
	AT	**CR 1**	**CR 2**	**CR 3**	**WG**	**MA 1**	**MA 2**	**NP**	**WP 1**	**WP 2**	**WP3**	**WP4**	**WP5**	**WP6**	**WP7**	**EP**
AT	0.000															
CR 1	0.144	0.000														
CR 2	0.098	0.057	0.000													
CR 3	0.154	0.010	0.064	0.000												
WG	0.116	0.090	0.028	0.036	0.000											
MA 1	0.164	0.037	**0.034**	0.096	0.084	0.000										
MA 2	0.140	0.070	0.055	0.116	0.044	0.079	0.000									
NP	0.089	0.047	**0.007**	0.084	0.067	**0.015**	0.077	0.000								
WP 1	0.102	0.096	0.043	0.081	0.037	0.075	0.099	0.050	0.000							
WP 2	0.085	0.097	0.052	0.087	0.027	0.100	0.070	0.071	0.029	0.000						
WP 3	0.113	0.097	0.041	0.095	0.045	0.073	0.076	0.051	**0.012**	**0.011**	0.000					
WP 4	0.113	0.089	0.051	0.075	0.035	0.076	0.076	0.056	**0.006**	**0.012**	**−0.006**	0.000				
WP 5	0.078	0.099	0.042	0.077	**0.028**	0.079	0.078	0.043	**0.003**	**0.004**	**−0.004**	**−0.005**	0.000			
WP 6	0.087	0.066	**0.011**	0.056	**0.017**	0.047	0.051	0.026	**0.004**	**0.010**	**−0.001**	**−0.002**	**−0.004**	0.000		
WP 7	0.097	0.079	**0.021**	0.054	**0.023**	0.048	0.067	0.025	**0.002**	0.023	**0.004**	**0.002**	**−0.006**	**−0.007**	0.000	
EP	0.093	0.085	0.064	0.077	**0.018**	0.083	0.057	0.070	0.045	0.038	0.051	0.032	0.030	**0.029**	0.034	0.000

Most comparisons indicate significant difference after strict Bonferroni correction, those F_{ST} that are not significantly different are indicated in bold. For Finland, the *combined* dataset is shown.

these samples were differentiated from TJ 2b. Moreover, some of these samples were also not differentiated from other samples in a neighboring village (Table 5A).

There was no evidence for isolation by distance when all Finnish samples were analyzed together (R_{XY} = 0.259, R^2 = 0.067, P = 0.190), nor when samples outside Ostrobothnia were excluded from the dataset (R_{XY} = 0.138, R^2 = 0.019, P = 0.211). However, it was moderately strong and significant (R_{XY} = 0.480, R^2 = 0.231, P = 0.001), when only the core samples in Ostrobothnia were analyzed (outlying samples HR, ML and KR). The data was explained best when only NR, PJ and TJ samples were analyzed (R_{XY} = 0.618, R^2 = 0.382, P = 0.001) and at the smallest geographic scale, when including only NR and PJ samples (R_{XY} = 0.876, R^2 = 0.767, P = 0.007).

In AMOVA, the percentage of variation explained by host plant species (cucumber vs. tomato) was low (1.69%) but statistically significant (P = 0.036). The percentage of variation explained by the groups was lower than that among samples within each group (8.59%, Table 4). However, *T. vaporariorum* collected from cucumber and tomato hosts differed significantly in allelic richness (P = 0.005, 2.25 vs.2.61), heterozygosities (P = 0.002, H_O/H_E: 0.278/0.300 vs. 0.371/0.413), and genetic differentiation (P = 0.002, F_{ST}: 0.228 vs.0.043) (cucumber vs. tomato respectively). Overall, samples collected from cucumber exhibited less genetic diversity and a higher degree of genetic differentiation than samples collected from tomato.

Furthermore, host plant species was indicated to be the most important factor explaining population genetic structure according to our analysis with GESTE. The model with highest posterior probability (Pr = 0.81) was the model with host plant species. All other models including other environmental factors or their combination had very low probability values. For example, Pr = 0.0446 was determined for the model including crop origin in combination with host plant species and Pr = 0.0443 was determined for the model including crop origin in combination with cultivar, to mention a few.

The Bayesian analysis of population structure indicated that the 18 samples from Finland (Table 1) represent three main genetic clusters (K = 3; Figure 2A). The genetic clusters could be characterized by both host plant species and geographic location. Most samples were clearly assigned to one of the three clusters (assignment higher than 80%). However, for samples PR and

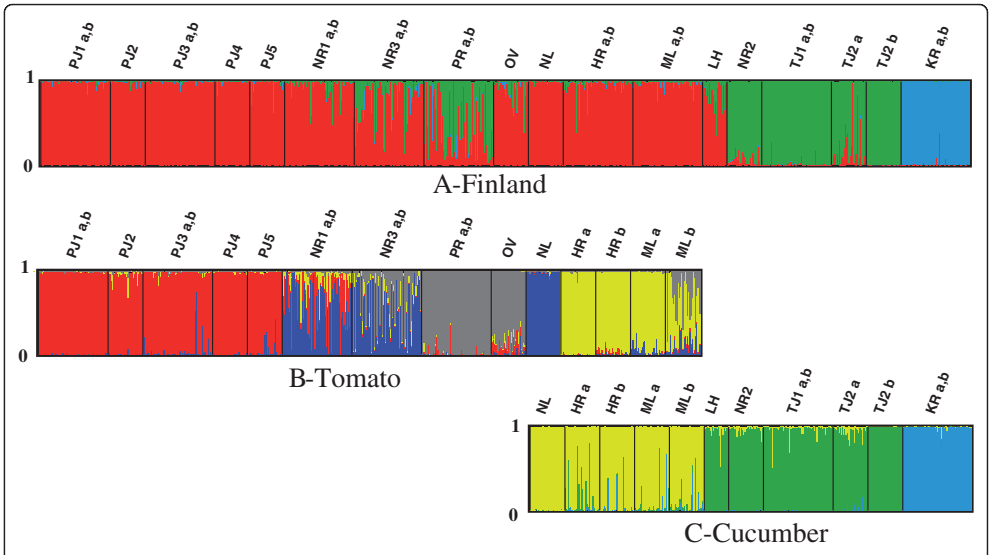

Figure 2 Genetic clusters of *T. vaporariorum* sampled in Finland delineated by Bayesian analysis implemented in STRUCTURE [52]. In each graph individuals are represented by vertical bars broken into K differently colored genetic clusters, with length proportional to assignment in each cluster. Probability of assignment of each individual is indicated from 0 to 1. Individuals are grouped by sample with the sample code listed at the bottom of the bar graph. **A**: Analysis of all 18 samples, 800 individuals, K = 3. **B**: Analysis of a subset of 14 samples collected from tomato, 570 individuals, K = 4. **C**: Analysis of a subset of 11 samples collected from cucumber, 380 individuals, K = 3. The *combined* dataset was used in the initial run **(A)**. Samples HR, ML and NL were included in analysis of both host plant groups **(B and C)** since these samples might have been exposed to several hosts grown in the same compartment or greenhouse. Samples HR a, b and ML a, b were separated in analyses **B** and **C** as they were exposed to different hosts.

NR3, the assignment was mixed between cluster 1 and 2 (PR: $K_1 = 0.453$ and $K_2 = 0.517$; NR3: $K_1 = 0.769$ and $K_2 = 0.195$). Majority of samples in K_1 were collected from tomato, with two exceptions: LH and NL were collected from cucumber and these samples were geographically distant from the other samples in this cluster. K_2 included four samples collected from cucumber (NR 2, TJ 1, TJ 2a and TJ 2b). Notably, NR 2 was assigned to a different cluster than the samples from other greenhouses in Närpes. K_3 corresponded to a single greenhouse growing cucumber (KR), which, being surrounded by forests was somewhat geographically isolated from other greenhouses in Ostrobothnia.

To resolve sub-clustering, further Bayesian analyses with STRUCTURE were conducted for samples collected from tomato and cucumber hosts separately (Figure 2B and C), since host plant species was the major component of genetic structure revealed in our initial analysis and our analysis with GESTE. Some samples were included in both subsequent runs (see Methods). The subset of samples from tomato (K_1 in the initial analysis) was characterized best by four sub-clusters ($K = 4$) (Figure 2B). Whiteflies collected from PJ formed sub-cluster 1 ($K_{1.1}$). NR 1 and NR 3 were not clearly resolved and partially formed sub-cluster 2 ($K_{1.2}$) with NL (NR 1a, b: $K_{1.2} = 0.584$ and $K_{1.1} = 0.287$; NR 3a, b: $K_{1.2} = 0.542$ and $K_{1.3} = 0.372$). PR and OV formed sub-cluster 3 ($K_{1.3}$), whereas HR and ML formed sub-cluster 4 ($K_{1.4}$). Assignment was not resolved well for ML b: $K_{1.4} = 0.543$ and $K_{1.3} = 0.297$. Additional analysis of an even smaller subset of samples (PJ 1-PJ 5) collected from greenhouses that were all growing tomato and located within the natural dispersal range expected for the whitefly, revealed complete genetic homogeneity (data not shown) and indicated high gene flow at this location. The subset of samples collected from cucumber (K_2 and K_3 in the initial analysis) was characterized best by three well-resolved sub-clusters ($K = 3$), all with 85-100% assignment (Figure 2C). HR, ML and NL formed sub-cluster 1 ($K_{2.1}$), whereas LH, NR 2, TJ 1, TJ 2a and TJ 2b formed sub-cluster 2 ($K_{2.2}$). KR alone formed sub-cluster 3 ($K_{3.1}$).

Population structure in Greece

Seventy five per cent of pairwise F_{ST} comparisons (91 out of 120) between samples from Greece showed significant population differentiation (Table 5B). Samples AT, CR 1, CR 3 and MA 2 differed significantly from all other samples (Table 5B). Significant differentiation among samples was absent only in some geographically close sites, in particular among the samples collected from fields in West Peloponnese (except WP 2 vs. WP 7). Some samples collected from greenhouses in West Greece and Peloponnese were not differentiated from the samples collected from fields: WG was not differentiated from WP 5–7 and EP, and WP 6 was not differentiated from EP. One sample from Crete (CR 2) was similar to some samples from West Peloponnese (WP 6 and WP 7), as well as the northern samples (MA 1 and NP), which were similar to each other as well (Table 5B).

There was evidence for isolation by distance in the Greek dataset ($R_{XY} = 0.436$, $R^2 = 0.190$, $P = 0.011$), and this relationship was stronger when the three samples from Crete were removed from the analysis ($R_{XY} = 0.579$, $R^2 = 0.335$, $P = 0.001$). There was also a strong relationship when only samples from Peloponnese were analyzed ($R_{XY} = 678$, $R^2 = 0.460$, $P = 0.001$). In Crete, however, isolation by distance was not detected ($R_{XY} = 0.963$, $R^2 = 0.927$, $P = 0.186$). Isolation by distance in the field samples was much greater than that between greenhouses ($R_{XY} = 0.791$/-0.219, $R^2 = 0.625$/0.048, $P = 0.005$/0.113; fields/greenhouses).

AMOVA indicated that genetic variation was not explained by host plant families, but there was a significant percentage of variation explained by habitat (fields vs. greenhouses), and among the samples within each of these groups (Table 4). Samples from fields (all in Peloponnese) displayed significantly greater genetic diversity and less population differentiation than those from greenhouses (allelic richness: 3.380 vs. 3.139, $P = 0.006$; Ho/H_E: 0.497/0.536 vs. 0.404/0.454, $P < 0.001$; F_{ST}: 0.012 vs. 0.078, $P = 0.003$; fields vs. greenhouses, respectively). Comparison of groups of samples differing in host plant family (Table 1) did not indicate differences in F statistics, heterozygosity or allelic richness (all $P > 0.05$) (data not shown).

Analysis with GESTE revealed that a model including habitat (field vs. greenhouse) had the highest posterior probability ($Pr = 0.407$) of explaining the genetic structure. The population structure in Greece was also partially explained by longitude ($Pr = 0.260$) followed by a model which combined the habitat and longitude factors ($Pr = 0.174$).

The Bayesian analysis of population structure in Greece indicated the presence of three major genetic clusters (Figure 3A). Samples from fields of West Peloponnese (WP 1–7) formed cluster 1 (K_1). Cluster 2 (K_2) consisted of samples from Crete and from two mainland locations (NP and MA 1), and the sample from West Greece (WG) had a mixed assignment (WG: $K_1 = 0.475$ and $K_2 = 0.397$). Samples AT and MA 2 formed cluster 3 (K_3). Mixed assignments were indicated for MA 2 ($K_3 = 0.599$ and $K_2 = 0.279$) and EP, which grouped either with the other samples from the Peloponnese (K_1), or with cluster 3 (K_3) (EP: $K_3 = 0.515$ and $K_1 = 0.461$).

Since habitat (field vs. greenhouse) was a major component of the genetic structure indicated in our initial analysis and our analysis with GESTE, we analyzed subsets of samples from greenhouses and fields separately in STRUCTURE to investigate possible sub-structuring (Figure 3B and C). Analysis of the subset sampled from

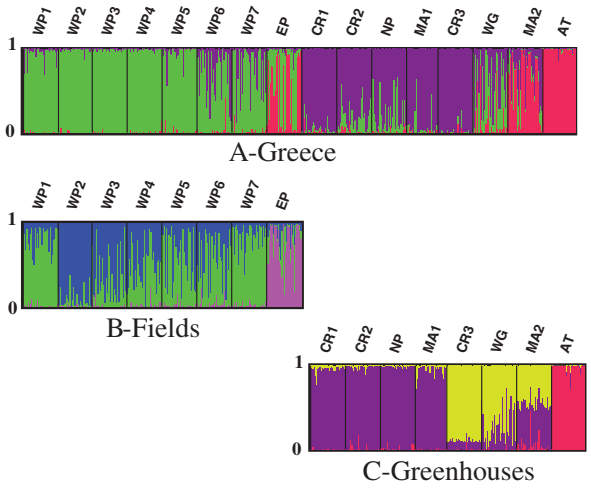

Figure 3 Genetic clusters of *T. vaporariorum* sampled in Greece assigned by Bayesian analysis implemented in STRUCTURE [52]. In each graph individuals are represented by vertical bars broken into *K* differently colored genetic clusters, with length proportional to assignment in each cluster. Probability of assignment of each individual is indicated from 0 to 1. Individuals are grouped by sample with the sample code listed at the bottom of the bar graph. **A**: Analysis of all 16 samples, 474 individuals, $K = 3$. **B**: Analysis of a subset of 8 samples collected from fields, 239 individuals, $K = 3$. **C**: Analysis of a subset of 8 samples collected from greenhouses, 235 individuals, $K = 3$.

fields (K_1 in the initial analysis) revealed the presence of three sub-clusters ($K = 3$). Majority of samples (WP 3-WP 6) were not well-resolved and assigned to both $K_{1.1}$ and $K_{1.2}$, however samples WP 1 and WP 7 had higher assignment probability to $K_{1.1}$ (0.80 for WP1, and 0.825 for WP7). Sub-cluster 2 ($K_{1.2}$) was best represented by WP 2 (with assignment probability of 0.90). Sub-cluster 3 ($K_{1.3}$) consisted of the sample from East Peloponnese (EP: $K_{1.3} = 0.754$). The subset of samples from greenhouses (K_2 and K_3 in the initial analysis) was characterized best by three well-resolved genetic sub-clusters ($K = 3$) (Figure 3C), with 71-98% assignment rate. Sub-cluster 1 ($K_{2.1}$) consisted of two Cretan samples (CR 1 and CR 2) and samples from Peloponnese and the mainland (NP, MA 1 and, in part, MA 2). CR 3, however, formed sub-cluster 2 ($K_{2.2}$) with WG and MA 2. MA 2 was only partially resolved and grouped both with $K_{2.1}$ (0.499) and $K_{2.2}$ (0.450). Thus, populations were differentiated even within a relatively short distance on the Island of Crete. AT formed a sub-cluster 3 ($K_{3.1}$).

Discussion

Agricultural ecosystems can serve as temporal oases and increase distribution of many beneficial and pest species [5,58]. The invasive pest greenhouse whitefly (*Trialeurodes vaporariorum)* has a cosmopolitan distribution [25,26]. In this study, we examined population genetic structure among samples from its Northern (Finland) and Southern (Greece) European distribution range. There was no evidence of gene flow between the different climate zones. Dispersal of *T. vaporariorum* appears to be limited, since we found significant spatial population genetic structure among samples in both countries. Samples from Finland were less diverse and showed greater genetic differentiation than samples from Greece, which could be explained by differences in agroecosystems found in the different climate zones. In Greece, habitat (field vs. greenhouse) explained population genetic structure, but in Finland, genetic structure was dictated by host plant species. Related whiteflies in the *B. tabaci* complex also show population genetic structure [59]. However, population differentiation of *B. tabaci* (Med) in Tunisia is not related to host plant species nor is it related to type of agroecosystem in Greece [35,60].

The role of agroecosystems

We hypothesized that greenhouses contribute to population genetic structure in *T. vaporariorum* by limiting dispersal and gene flow among populations, and that populations in the North are more genetically differentiated than populations in the South. In Finland, our samples of *T. vaporariorum* were limited to greenhouses (since in this country this species is not able to persist year-round in agricultural fields), whereas in Greece we sampled from both greenhouses and fields. The extent

of the area inhabited by homogeneous populations was approximately 1–10 km straight line distance in Finland and approximately 100 km in Greece, indicated in our analyses as samples with non-significant differences in pairwise F_{ST} (Finland: PJ 1–5, NR 1; Greece: WP 1–7), and shared cluster assignment in Bayesian analysis (Finland: PJ 1–5; Greece: WP 1–7). Whiteflies sampled at these spatial scales also showed significant isolation by distance. Although whiteflies are poor fliers, natural dispersal of up to 20 km has been observed previously for the related *B. tabaci* species [41]. Low pairwise F_{ST} values and significant isolation by distance even between remote populations in Greece suggest that the presence of agricultural fields and wild host plants year-round [61] enables greater connectivity among *T. vaporariorum* populations. Nevertheless, greenhouse agroecosystems increase genetic structure in both climate zones. Similarly, populations of whiteflies, *B. tabaci* (Med), and moths, *Trichoplusia ni,* inhabiting greenhouses in the United States and Canada, respectively, were characterized by higher genetic structure than those inhabiting fields [62,63].

Although frequent high mortality events occur in agroecosystems in both climate zones (via chemical and biological control methods), the high abundance of less enclosed/hermetic agroecosystems (fields, plastic tunnels and greenhouses with constantly open vents) and suitable climate in the South reduces effectiveness of pest management. On the other hand, isolation of populations in more enclosed agroecosystems, such as those in the North, can create an opportunity for the development of insecticide resistance through natural selection [39]. In open environments resistance genes might be less easily fixed, but they could spread over longer distances, as indicated by the higher connectivity of field populations in our study. Populations of insect pests inhabiting greenhouses are often characterized by higher insecticide resistance than those in the fields [58].

Agroecosystems, particularly greenhouses, could affect genetic structure of pests not only by placing limitations on pest dispersal, but also by limiting their population size. Crop management practices and frequent chemical insecticide exposure can cause population bottlenecks, leading to reduction in within-population genetic variation (increases in homozygosity), as well as increase in between-population genetic differentiation [18]. Higher genetic diversity (allelic richness and heterozygosities) of Greek *T. vaporariorum* populations indicated a larger gene pool and overall population size and possibly a lower frequency of bottlenecks in the South than in the North. This poses a threat to effective pest management in the Mediterranean region. However, we observed higher than expected homozygosity in samples from both countries (reflected by deviations from HWE; Table 3). Although deviations in HWE could reflect technical problems in genotyping, they could also result from population bottlenecks and inbreeding, and homozygote excess in insect populations inhabiting agroecosystems is not uncommon [59,64]. Nevertheless, samples deviating from HWE did not occur more frequently in the North than in the South, and in Greece, samples deviating from HWE were collected from fields as well as from greenhouses.

Despite the frequent stochastic events in agroecosystems that can reduce genetic diversity, our results indicated that *T. vaporariorum* is able to persist over years in the same greenhouses in Finland. Samples collected from the same greenhouses in 2010 and 2011 were not genetically differentiated from each other in all but one case. Prevalence of the pest year-round might eventually allow further spread into natural ecosystems. Pest persistence in agroecosystems can create propagule pressure to natural habitats and favor utilization of wild host plants that surround greenhouses and fields (Ovcarenko et al. unpubl.). Adaptation to crop species could also lead to the development of a preference for particular wild host plants with similar chemistry, as was observed for *Tetranychus urticae* [15,65].

The role of host plants

Occupation of agroecosystems and differences in their individual management in the two climate zones could potentially allow *T. vaporariorum* to specialize and adapt to particular host plant species or their cultivars. Although *T. vaporariorum* is a polyphagous insect, it is able to develop preference not only for certain plant species [66], but also for particular varieties or cultivars [67]. In Finland, major genetic clusters were characterized by the two common host plants (Figure 2). In Greece, however, host plant taxonomic family did not explain population structure. Absence of host associations in Greece may reflect the frequent alterations of host crops that are dictated by the market. On the other hand, the limited number of samples restricted to particular host plant species may have prevented detecting a possible association.

It is known that *T. vaporariorum* prefer cucumber hosts to tomato, and have higher fecundity and shorter development time on cucumber as well [28]. Thus, larger and more diverse populations would be expected on cucumber. On the contrary, our study indicated that Finnish samples from tomato were characterized by larger population size, with higher heterozygosity and allelic richness and lower values of F statistics than samples from cucumber. This might also be due to differences in individual management of agroecosystems. Cucumber crops are changed every three to four months, whereas tomato crops are maintained in greenhouses for nine to ten months, leading to more frequent reductions in pest population size in greenhouses growing cucumber than in those growing tomato.

The result might also be a sampling effect: there were more greenhouses growing only tomato than those growing only cucumber (nine and five, respectively) (Table 1). However, our sampling reflects the tendency in Finland for more common cultivation of tomato than cucumber [68].

Associations between particular host plant species and genetic structure of *T. vaporariorum* populations might alternatively reflect different introduction sources, as well as varying frequencies of repeated introductions [69]. When there is a single introduction source, e.g. infested plant material from a single supplier, even isolated populations experiencing no gene flow will not show genetic differentiation, since all populations will share the same alleles present in the source [70]. One sample in Finland (KR) that formed a distinct cluster in our Bayesian analysis was collected from a site that has been producing its own cucumber cultivar since 2005. Although such conditions could favor adaptation of *T. vaporariorum* to this cultivar, KR is also somewhat geographically isolated from other agroecosystems and is surrounded by forests, which potentially limits insect dispersal. Therefore, it is difficult to determine if its genetic differentiation is due to adaptation or isolation. Other samples from Finland collected from cucumber (except NL) clustered together (LH, NR 2 and TJ 1–2; Figure 2C). Such genetic structure could reflect either adaptation to the host, or a common vector or origin of *T. vaporariorum*. Although growing different crops, greenhouses PJ1-5, NR1-3, NL, HR, ML, LH and TJ2 had all obtained seedlings from the same producer, and some similarities between these samples could be seen in our analysis with STRUCTURE (Figure 2A). However, origin of seedlings did not explain local F_{ST} in our analysis with GESTE, whereas the model with host plant species explained the data best. In Greece, populations from greenhouses in Crete (CR 1 and CR 2) clustered with distant locations in the mainland (NP and MA 1; Figure 3A, C), suggesting that human-mediated transfer of whiteflies has occurred between these distant locations. Human-mediated transfer of *B. tabaci* between northern and southern regions of Greece has been noted previously [32]. Unfortunately, no information on the origin of seedlings in Greece was available for our analysis.

Limitations of the study

Our study compares pest populations living in different climate zones and subjected to different kinds of agroecosystems, so it may not be surprising that we have detected differences in population structure in the North and South. Moreover, our results might be affected by differences in the timing of sampling of *T. vaporariorum* in Finland and Greece, which matched periods of high insect abundance. In Finland maximum abundance peaks are in spring, whereas in Greece whiteflies are abundant year-round. Sampling *T. vaporariorum* in Finland in spring, when dispersal is low or non-existent might have facilitated detecting significant population structure. However, persistence of the populations over two years in the same greenhouses indicates that genetic structure is present despite the sampling period. Furthermore, no differences in population structure of *B. tabaci* in Greece were detected between late and early sampling periods from both field and greenhouse populations [35].

Conclusions

Greenhouse agroecosystems contribute to population genetic structure in *T. vaporariorum* by limiting gene flow among populations. Populations in Finland sampled from greenhouses are less diverse and more genetically differentiated than populations in Greece, collected from both greenhouses and fields. Within Greece, pest populations inhabiting greenhouse agroecosystems were more genetically differentiated than those inhabiting fields, and habitat (field vs. greenhouse) together with longitude explained population genetic structure. In contrast, host plant species (tomato vs. cucumber) explained population genetic structure in Finland. The differing influence of type of agroecosystem and potential host plant adaptation on population genetic structure of the pest in different climate zones highlights challenges for the management of a cosmopolitan invasive pest species.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IV, IO, AT were the authors of original idea. IO, LL and IV took part in sample collection in Finland, whereas DEK and AT collected samples from Greece. NG participated in microsatellite development. DEK extracted DNA of samples from Greece, whereas IO and KEK carried all further sample and data analysis steps of all samples. KEK, NG and AT advised on data analysis methods. IO drafted the manuscript. All authors participated in preparation of the manuscript, read and approved the final manuscript.

Acknowledgements

The Finnish Ministry of Education and Culture, Finnish Ministry of Agriculture and Forestry, BIOINT Doctoral Programme at the University of Jyväskylä and the Oiva Kuusisto Foundation provided financial support to I. Ovcarenko, and MTT and the Finnish Ministry of Agriculture and Forestry to I. Vänninen. The Academy of Finland provided financial support to L. Lindström (project 250248) and to K. E. Knott (project 258365). The Centre of Excellence in Biological Interactions Research provided laboratory materials for the research. The EU European Social Fund (ESF) and the Greek State, as well as the General Secretariat General for Research and Technology (SYNERGASIA 09SYN-22-617) provided financial support to D. E. Kapantaidaki and A. Tsagkarakou.

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Received: 14 April 2014 Accepted: 15 July 2014
Published: 29 July 2014

References

1. Hsieh C-H, Wang C-H, Ko C-C: **Evidence from molecular markers and population genetic analyses suggests recent invasions of the Western North Pacific region by biotypes B and Q of *Bemisia tabaci* (Gennadius).** *Environ Entomol* 2007, **36:**952–961.
2. Margaritopoulos JT, Kasprowicz L, Malloch GL, Fenton B: **Tracking the global dispersal of a cosmopolitan insect pest, the peach potato aphid.** *BMC Ecol* 2009, **9:**13.
3. Bebber DP, Ramotowski MAT, Gurr SJ: **Crop pests and pathogens move polewards in a warming world.** *Nat Clim Chang* 2013, **3:**985–988.
4. Hickling R, Roy DB, Hill JK, Fox R, Thomas CD: **The distributions of a wide range of taxonomic groups are expanding polewards.** *Glob Chang Biol* 2006, **12:**450–455.
5. Hanafi A: **Invasive pests and diseases: a challenge to IPM in greenhouse crops.** *Phytoparasitica* 2005, **33:**423–426.
6. Van Driesche R, Enkegaard E: **Invasive Species as Pests in Greenhouses: Forecasting, Preventing and Remediating Future Invasions.** *IOBC/WPRS Bull* 2002, **25**(1):277–280.
7. Altieri MA: **The ecological role of biodiversity in agroecosystems.** *Agric Ecosyst Environ* 1999, **74:**19–31.
8. Grapputo A, Boman S, Lindström L, Lyytinen A, Mappes J: **The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations.** *Mol Ecol* 2005, **14:**4207–4219.
9. Piiroinen S, Lindström L, Lyytinen A, Mappes J, Chen YH, Izzo V, Grapputo A: **Pre-invasion history and demography shape the genetic variation in the insecticide resistance-related acetylcholinesterase 2 gene in the invasive Colorado potato beetle.** *BMC Evol Biol* 2013, **13:**13.
10. Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ: **Naturalization and invasion of alien plants: concepts and definitions.** *Divers Distrib* 2000, **6:**93–107.
11. Loxdale H, Lushai G: **Slaves of the environment: the movement of herbivorous insects in relation to their ecology and genotype.** *Philos Trans R Soc Lond B Biol Sci* 1999, **354**(January):1479–1495.
12. Freeman J: **Problems of stored products entomology in Britain arising out of the import of tropical products.** *Ann Appl Biol* 1976, **84:**120–124.
13. Hill DS: *Pests of Crops in Warmer Climates and Their Control.* Dordrecht, The Netherlands: Springer; 2008.
14. Muñoz P, Antón A, Nuñez M, Paranjpe A, Ariño J, Castells X, Montero JI, Rieradevall J: **Environmental impacts of greenhouse versus open-field tomato production in the Mediterranean region.** *Acta Hort* 2008, **801:**1591–1596.
15. Perdikis D, Kapaxidi E, Papadoulis G: **Biological control of insect and mite pests in greenhouse Solanaceous crops.** *Eur J Plant Sci Biotech* 2008, **2:**125–144.
16. Hoffmann AA, Willi Y: **Detecting genetic responses to environmental change.** *Nat Rev Genet* 2008, **9:**421–432.
17. Tsagkarakou A, Navajas M: **Genetic differentiation in *Tetranychus urticae* (Acari: Tetranychidae) from greenhouses in France.** *Exp Appl Acarol* 1999, **23:**365–378.
18. Tsagkarakou A, Navajas M, Papaioannou-Souliotis P, Pasteur N: **Gene flow among *Tetranychus urticae* (Acari: Tetranychidae) populations in Greece.** *Mol Ecol* 1998, **7:**71–79.
19. Mound LA, Halsey SH: *Whitefly of the world. a systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data.* British Museum (Natural History): John Wiley and Sons; 1978.
20. Linnaniemi WM: ***Ästerochiton vaporiorum* (Westw.) Suomessa.** *Medd Soc Fauna Flora Fenn* 1921, **7:**66–68.
21. Hulden L: **The whiteflies and their parasites in Finland.** *Not Entomol* 1986, **66:**1–40.
22. Vappula NA: **Pests of cultivated plants in Finland.** *Acta Entomol Fenn* 1965, **19:**163–169.
23. Michelakis SE: **Problems in the application of biological control against *Trialeurodes vaporariorum* in unheated plastic glasshouses in Crete.** *Bull OEPP* 1986, **16:**423–427.
24. Pelekassis C: **A catalogue of the more important insects and other animals harmful to the agricultural crops of Greece during the last thirty-year period.** *Ann Inst Phytopathol Benaki* 1962, **5:**9–75.
25. Martin JH, Mifsud D, Rapisarda C: **The whiteflies (Hemiptera: Aleyrodidae) of Europe and the Mediterranean Basin.** *Bull Entomol Res* 2000, **90:**407–448.
26. Van Lenteren JC, Van Roermund H, Sütterlin S: **Biological Control of Greenhouse Whitefly (*Trialeurodes vaporariorum*) with the Parasitoid *Encarsia formosa*: How Does It Work?** *Biol Control* 1996, **6:**1–10.
27. Inbar M, Gerling D: **Plant-mediated interactions between whiteflies, herbivores, and natural enemies.** *Annu Rev Entomol* 2008, **53:**431–448.
28. Van Lenteren JC, Noldus LPJJ: **Whitefly Plant Relationships, Behavioral and Ecological Aspects.** In *Whiteflies: Their Bionomics, Pest Status and Management.* Edited by Gerling D. Andover, UK: Intercept Ltd; 1990:47–49.
29. Stenseth C: **Cold-hardiness in eggs of Greenhouse Whitefly (*Trialeurodes vaporiorum*).** *IOBC-WPRS Bull* 1983, **6:**84–86.
30. Osborne L: **Temperature-dependent development of greenhouse whitefly and its parasite, *Encarsia formosa*.** *Environ Entomol* 1982, **11:**483–485.
31. Boykin LM, Bell CD, Evans G, Small I, De Barro PJ: **Is agriculture driving the diversification of the *Bemisia tabaci* species complex (Hemiptera: Sternorrhyncha: Aleyrodidae)?: Dating, diversification and biogeographic evidence revealed.** *BMC Evol Biol* 2013, **13:**228.
32. Gauthier N, Clouet C, Perrakis A, Kapantaidaki D, Peterschmitt M, Tsagkarakou A: **Genetic structure of *Bemisia tabaci* Med populations from home range countries inferred by nuclear and cytoplasmic markers: impact on the distribution of the insecticide resistance genes.** *Pest Manag Sci* 2014, in press.
33. Hsieh C-H, Chiang Y-H, Ko C-C: **Population genetic structure of the newly invasive Q biotype of *Bemisia tabaci* in Taiwan.** *Entomol Exp Appl* 2011, **138:**263–271.
34. Dalmon A, Halkett F, Granier M, Delatte H, Peterschmitt M: **Genetic structure of the invasive pest *Bemisia tabaci*: evidence of limited but persistent genetic differentiation in glasshouse populations.** *Heredity* 2008, **100:**316–325.
35. Tsagkarakou A, Mouton L, Kristoffersen JB, Dokianakis E, Grispou M, Bourtzis K: **Population genetic structure and secondary endosymbionts of Q *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Greece.** *Bull Entomol Res* 2012, **102:**353–365.
36. Prijović M, Skaljac M, Drobnjaković T, Zanić K, Perić P, Marčić D, Puizina J: **Genetic variation of the Greenhouse Whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), among populations from Serbia and neighboring countries, as inferred from COI sequence variability.** *Bull Entomol Res* 2014, **1:**1–10.
37. Roopa HK, Kumar NKK, Asokan R, Rebijith KB, Mahmood R, Verghese A: **Phylogenetic Analysis of Trialeurodes Spp. (Hemiptera: Aleyrodidae) from India Based on Differences in Mitochondrial and Nuclear DNA.** *Fla Entomol* 2012, **95:**1086–1094.
38. Shin D, Mo H, Lee S-E, Park J-J, Cho K: **Elucidation of the genetic differences in *Trialeurodes vaporariorum* populations under vegetable greenhouse conditions by using the allozyme approach.** *Entomol Res* 2013, **43:**271–281.
39. Ovcarenko I, Lindström L, Saikkonen K, Vänninen I: **Variation in mortality among populations is higher for pymetrozine, than for imidacloprid and spiromesifen in *Trialeurodes vaporariorum* in greenhouses in Finland.** *Pest Manag Sci* 2014, in press.
40. Byrne DN, Bellows TS: **Whitefly biology.** *Annu Rev Entomol* 1991, **36:**431–457.
41. Berlinger MJ, Lehmann-Sigura N, Taylor RAJ: **Survival of *Bemisia tabaci* adults under different climatic conditions.** *Entomol Exp Appl* 1996, **80:**511–519.
42. Tsagkarakou A, Tsigenopoulos CS, Gorman K, Lagnel J, Bedford ID: **Biotype status and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Greece: mitochondrial DNA and microsatellites.** *Bull Entomol Res* 2007, **97:**29–40.
43. Molecular Ecology Resources Primer Development Consortium, Aksoy S, Almeida-Val VMF, Azevedo VCR, Baucom R, Bazaga P, Beheregaray LB, Bennetzen JL, Brassaloti RA, Burgess TI, Caccone A, Chang S-M, Ciampi AY, Ciancaleoni S, Clímaco GT, Clouet C, Coimbra MRM, Coutinho LL, Dantas HL, De Vega C, Echodu R, Enyaru J, Figueira A, Filho MAG, Foltz B, Fressigné L, Gadomski M, Gauthier N, Herrera CM, Hyseni C, *et al*: **Permanent genetic resources added to molecular ecology resources database 1 October 2012–30 November 2012.** *Mol Ecol Resour* 2013, **13:**341–343.
44. Excoffier L, Laval G, Schneider S: **Arlequin ver. 3.0: an integrated software package for population genetics data analysis.** *Evol Bioinform Online* 2005, **1:**47–50.

45. Peakall R, Smouse PE: **GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update.** *Bioinformatics* 2012, **28:**2537–2539.
46. Rousset F: **Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux.** *Mol Ecol Resour* 2008, **8:**103–106.
47. Raymond M, Rousset F: **An exact test for population differentiation.** *Evolution* 1995, **49:**1280–1283.
48. Rice WR: **Analyzing tables of statistical tests.** *Evolution* 1989, **43:**223–225.
49. Oosterhout C, Van Hutchinson WF, Wills DPM, Shipley P: **Micro-checker: software for identifying and correcting genotyping errors in microsatellite data.** *Mol Ecol Notes* 2004, **4:**535–538.
50. Goudet J: *Fstat 2.9.3: A Program to Estimate and Test Gene Diversities and Fixation Indices (Updated from Goudet 1995).* Lausanne: Switzerland; 2002.
51. Foll M, Gaggiotti O: **Identifying the environmental factors that determine the genetic structure of populations.** *Genetics* 2006, **174:**875–891.
52. Pritchard JK, Stephens M, Donnelly P: **Inference of population structure using multilocus genotype data.** *Genetics* 2000, **155:**945–959.
53. Hubisz MJ, Falush D, Stephens M, Pritchard JK: **Inferring weak population structure with the assistance of sample group information.** *Mol Ecol Resour* 2009, **9:**1322–1332.
54. Evanno G, Regnaut S, Goudet J: **Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study.** *Mol Ecol* 2005, **14:**2611–2620.
55. Earl DA, VonHoldt BM: **STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method.** *Conserv Genet Resour* 2011, **4:**359–361.
56. Jakobsson M, Rosenberg NA: **CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure.** *Bioinformatics* 2007, **23:**1801–1806.
57. Rosenberg NA: **Distruct: a program for the graphical display of population structure.** *Mol Ecol Notes* 2004, **4:**137–138.
58. Parrella MP: **Arthropod Fauna.** In *Ecosystems of the World. Greenhouse Ecosystems.* Edited by Stanhill G, Enoch HZ. Amsterdam; New York: Elsevier Science Publishers; 1999:213–250.
59. Delatte H, David P, Granier M, Lett JM, Goldbach R, Peterschmitt M, Reynaud B: **Microsatellites reveal extensive geographical, ecological and genetic contacts between invasive and indigenous whitefly biotypes in an insular environment.** *Genet Res* 2006, **87:**109–124.
60. Saleh D, Laarif A, Clouet C, Gauthier N: **Spatial and host-plant partitioning between coexisting *Bemisia tabaci* cryptic species in Tunisia.** *Popul Ecol* 2012, **54:**261–274.
61. Roditakis NE: **Host plants of greenhouse whitefly *Trialeurodes vaporariorum* westwood (Homoptera: Aleyrodidae) in Crete. Attractiveness and impact on whitefly life stages.** *Agric Ecosyst Environ* 1990, **31:**217–224.
62. Franklin MT, Ritland CE, Myers JH: **Spatial and temporal changes in genetic structure of greenhouse and field populations of cabbage looper, *Trichoplusia ni*.** *Mol Ecol* 2010, **19:**1122–1133.
63. Dickey AM, Osborne LS, Shatters RG, Hall PM, Mckenzie CL: **Population genetics of invasive *Bemisia tabaci* (Hemiptera: Aleyrodidae) cryptic species in the United States based on microsatellite markers.** *J Econ Entomol* 2013, **106:**1355–1364.
64. Simón B, Cenis JL, De La Rúa P: **Distribution patterns of the Q and B biotypes of *Bemisia tabaci* in the Mediterranean Basin based on microsatellite variation.** *Entomol Exp Appl* 2007, **124:**327–336.
65. Navajas M, Tsagkarakou A, Lagnel J, Perrot-Minnot MJ: **Genetic differentiation in *Tetranychus urticae* (Acari: Tetranychidae): polymorphism, host races or sibling species?** *Exp Appl Acarol* 2000, **24:**365–376.
66. Ma R-Y, Kong W-N, Hao L-J: **Host preference of Greenhouse Whitefly (*Trialeurodes vaporariorum*) for several horticultural plants in greenhouse.** *Entomol Knowl* 2005, **3:**016.
67. Thomas DC: **Host plant adaptation in the Glasshouse Whitefly.** *J Appl Entomol* 1993, **115:**405–415.
68. **Official Statistics of Finland.** [http://www.maataloustilastot.fi/en/horticultural-statistics]
69. Whitlock M: **Selection and Drift in Metapopulations.** In *Ecology, Genetics and Evolution of Metapopulations.* Edited by Gaggiotti O, Hanski I. Amsterdam: Elsevier Academic Press; 2004:153–173.
70. Sakai A, Allendorf F, Holt J: **The population biology of invasive species.** *Annu Rev Ecol Syst* 2001, **32:**305–332.

doi:10.1186/s12862-014-0165-4
Cite this article as: Ovčarenko *et al.*: Agroecosystems shape population genetic structure of the greenhouse whitefly in Northern and Southern Europe. *BMC Evolutionary Biology* 2014 **14**:165.

IV

VARIATION IN MORTALITY AMONG POPULATIONS IS HIGHER FOR PYMETROZINE, THAN FOR IMIDACLOPRID AND SPIROMESIFEN IN *TRIALEURODES VAPORARIORUM* IN GREENHOUSES IN FINLAND

by

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Pest Management Science, In press.
DOI: 10.1002/ps.3766

Pest Management Science. In Press. DOI: 10.1002/ps.3766

Variation in mortality among populations is higher for pymetrozine, than for imidacloprid and spiromesifen in *Trialeurodes vaporariorum* in greenhouses in Finland

Running title: Variation in insecticide susceptibility of *T. vaporariorum* populations in Finland

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Abstract

BACKGROUND: Insecticide resistance in *Trialeurodes vaporariorum* W. is unknown in the species' northern distribution range where it inhabits mainly commercial greenhouses. Resistance development in whiteflies feeding on year-round crops in greenhouses is possible due to use of chemical treatments to back up biocontrol. We tested the response levels to spiromesifen, pymetrozine and imidacloprid in whiteflies collected from seven greenhouses within a 35 km radius in Western Finland.

RESULTS: All except one (PR) population had LC_{50} values below the recommended concentrations for the tested compounds. However, some populations showed reduced susceptibility to pymetrozine in comparison to reference susceptible population. Resistance ratios to pymetrozine were highly variable (RR 0.5-39.7) even among closely-located greenhouses and higher than those for imidacloprid (RR 1.05-10.5) and spiromesifen (RR 0.8-11.5). LC_{50} values and application frequencies of pymetrozine correlated positively among the sampled populations.

CONCLUSION: High variation in resistance levels to pymetrozine among-populations within natural whitefly dispersal limits reflects variation in the usage of this compound among individual greenhouse crop producers. Thus, resistance management is recommended on individual greenhouse crop producer level, even in a dense production cluster.

Keywords: Finland; imidacloprid; pymetrozine; resistance; spiromesifen; *Trialeurodes vaporariorum*

1 INTRODUCTION

Insecticide resistance among insect populations differs depending on the treatment history and dispersal possibilities of resistant individuals in the area. [1] Crop production clusters with a high density of production units (i.e. fields or greenhouses), in combination with suitable surrounding habitats that promote pest dispersal year round, are likely at a higher risk of pest invasions and often experience difficulties with pest management. [2] These circumstances can lead to a continuous cycle of pesticide use. On the contrary, more scattered crop producers in climatic zones that are less conducive to pest dispersal between crop production units, e.g. in Northern Europe, may use pesticides rarely and only when biological control agents are not sufficient (Vänninen pers. obs.). Low usage of pesticide with the lack of gene flow among populations could mean that resistance development in pests at the northern limits of their distribution may be less likely. However, with the advance of year-round crop production even in the most remote locations, persisting populations of pests are likely to be exposed to pesticides more frequently, than in seasonal crops, increasing the possibilities for resistance development. Frequent pesticide exposure, in addition to limited dispersal among populations, could lead to variability in the susceptibility of pest populations in the northern latitudes to insecticides.

In this study we focus on the insecticide susceptibility of the greenhouse whitefly (*T. vaporariorum*), a cosmopolitan polyphagous pest feeding on various commercially important plants. [3] *T. vaporariorum* has been controlled with insecticides and it is known to have developed resistance to at least 22 insecticide compounds. [4] For example, resistance to imidacloprid, [5-8] pymetrozine [7] and insect growth regulators (buprofezin, [9] spiromesifen [10]) have been previously reported. Although biological control is the principal method of whitefly management in the greenhouses of Finland, insecticide treatments are needed to back-up biological control of whiteflies in year-round production units during pest outbreaks (Vänninen pers. obs.). Greenhouse crop producers in western Finland observed reduced efficacy of pymetrozine and imidacoprid after six years of use, but such a decrease was not noticed for spiromesifen. All three insecticides, however, are still used in integrated pest management (IPM) programs of whiteflies. Owing to the selectivity of pymetrozine to whiteflies and not to its biological control agents, [11] the greenhouse crop producers are interested in keeping it in their whitefly management programs.

The aim of this study was to measure variability of response to selected insecticides of *T. vaporariorum* populations inhabiting greenhouses with year-round tomato and cucumber crops. Three commonly used insecticides

were assayed against populations from a greenhouse cluster in Ostrobothnia, western Finland, which have varying insecticide application histories. This is the first scientific publication reporting differences in insecticide susceptibility of *T. vaporariorum* in Scandinavia.

2 MATERIALS AND METHODS

2.1 Insecticides

Three insecticides were selected for the study based on their importance for whitefly management. These products, their modes of action and commercialization history in Finland are described in Table 1. The highest concentration used in the assays correspond to manufacturer' recommended concentration (MRC) for foliar application on tomato and cucumber against whiteflies and other pests. In the bioassays all insecticides were applied as formulated products diluted to required concentrations in distilled water containing 0.1g L^{-1} of non-ionic wetter Agral® (Syngenta). Control treatments contained water and Agral® only.

2.2 Whitefly populations

To evaluate the importance of insecticide treatment history for whitefly resistance development on the individual greenhouse crop producer basis, whitefly populations (Fig. 1, Table 2) were sampled in spring before the start of active whitefly dispersal in the region. Each population was sampled from a different greenhouse crop producer and from multiple locations within a single greenhouse room containing single host plant species. Whiteflies used in spiromesifen assays were collected from five producers in 2011 and those used for pymetrozine and imidacloprid assays were collected from six producers in 2012 (Table 2, Fig. 1). All except one population (BL) had previous, varying treatment histories for all three compounds. Greenhouse crop producers were unwilling to disclose full insecticide treatment histories and only general results representing treatments of the three insecticides which were applied in the last 9 months (winter production) are presented. The frequencies of insecticide applications for PR and ML populations were not provided. The BL population had been controlled without insecticides using only biological control agents during the three preceding years. Equal number of individuals was sampled from each population: 100 adults were aspirated into separate plexi glass cages onto living host plants, and transported to research facilities (MTT, Jokionen), where stocks of whiteflies were reared for bioassays. As a susceptible reference we used a population from Rothamsted Research in Harpenden, Hertfordshire, United Kingdom (Table 2), which had been reared on bean *(Phaseolus vulgaris* L. cv. Canadian Wonder*)* without insecticides since 1971. Beginning

May, 2012 it was maintained on tomato (*Solanum lycopersicum* L. cv. Encore) for at least three generations before tests since this is the required minimum number of generations for adaptation of *T. vaporariorum* to a novel host. [12] All populations were reared in controlled light (16h light: 8h dark) and temperature (22-24˚C) conditions at MTT Agrifood Research, Jokioinen, Finland.

2.3 Test plants

All populations were reared and tested on the same host plant species from which they were initially collected (Table 2). This was done to minimize mortality and any potential lower fitness of the populations due to exposure to novel host plant species [13] and/or to minimize the impact of plant chemistry on susceptibility of the populations. [14] Tomato (*S. lycopersicum* L. cv. Encore) and cucumber (*Cucumis sativus* L. cv. Eminentia) seedlings for tests were grown at 20-22˚C, 16h light: 8h dark photoperiod.

2.4 Bioassays

The bioassays routinely used to determine insect resistance to insecticides were adjusted to make it work reliably and to be able to compare our results with other studies. Adulticides (pymetrozine and imidacloprid), insecticides designed to kill adult insects, were tested using modified [8, 15, 16] leaf dip bioassays (Fig. 2). Bioassays were performed using 3.7 cm diameter tomato leaf discs. The discs were dipped for 1 min in pesticide concentration, air-dried for 20 min, and then laid abaxial side up on 1% agar in petri dishes (0.5 cm deep, 3.5 cm in diameter; one disc per petri dish; Thermo Fisher Scientific Inc.). Five insecticide concentrations were tested (Fig. 3-4) with five replicates per concentration. Twenty-thirty adult whiteflies of mixed gender and age were immobilized on ice for up to 3min in 5 ml plastic pipette tips (serving as collector units of a mouth aspirator) that were covered with sealing film (PARAFILM® M). Immobilized whiteflies from the aspirators were transferred to petri dishes containing leaf discs treated with insecticides. The insides of perforated (~10 holes, 2mm in diameter) petri dishes lids (1 cm deep, 3.3 cm in diameter) were fully covered with thin breathable nonwoven milk filter paper (Hygia™) to absorb excess moisture and to prevent the formation of static electricity. We observed that static electricity captured whiteflies onto bare plastic and prevented their contact with the leaf disc unless the filter paper was used. The edges of the petri dishes were covered with a rubber seal (3.3 cm in diameter) to prevent the leaf disc from drying due to contact with the filter paper. Closed petri dishes were sealed by wrapping parafilm around the dish's edges to prevent whiteflies from escaping. To imitate natural leaf position (abaxial side down) dishes were inverted and transferred to

a metal grid shelf (to allow ventilation) in an incubator (16h light: 8h dark photoperiod and 22-24˚C temperature). Due to this inversion and to prevent the leaf disc from falling from the agar bed, the lid of the petri dish was designed to be smaller than the bottom part of the dish, which held the leaf disc. Mortality was scored after 72h for imidacloprid and after 96h for pymetrozine after partially minimizing whitefly activity in the petri dish by keeping them at +10˚C for 10 min. Whiteflies were considered dead if we observed no movement in response to touching them with a brush. The period of insect exposure to adulticides exceeded the period of insect survival without food, since 50% of whiteflies usually die from starvation within 35h in empty petri dishes [17]. Thus, we concluded that majority of alive individuals found on pymetrozine treated leaf discs had fed on the treated leaf discs and thus, showed resistance to this feeding inhibitor, and that the results were not affected by delayed mortality from starvation.

Resistance to the larvicide (spiromesifen), an insecticide designed to kill juvenile insect stages, was tested using a modified method [18, 19] on leaves of living plants. Leaves of seedlings were dipped in either one of four insecticide concentrations (Fig. 3) (each with three replicate plants). The test leaves had a known number of 2^{nd} instar nymphs that were allowed to develop and hatch into adults. To produce synchronized cohorts of 2^{nd} instar nymphs, whitefly adults were allowed to lay eggs for 24 h, producing progenies of similar age. For that purpose, 15 females and 15 males, immobilized as described above, were released into each mini clip cage (1cm deep, 1cm in diameter) attached to leaves of individually planted tomato seedlings (three clip cages per seedling, one cage per leaf). Clip cages with adults were removed from plants after 24h. The seedlings containing eggs were kept in separate insect-free greenhouses with 16h light: 8h dark photoperiod and 22-24˚C. After 12 days from egg-laying on cucumber and 15 days on tomato, the number of 2^{nd} stage nymphs per leaf was counted with the help of a 20x magnifying lens. Nymphs mortality from spiromesifen was scored as the abundance of hatched pupal cases after four weeks from nymph counting allowing for a maximum hatching rate. [20]

2.5 Statistical analysis

All data were corrected for mortality in control treatments using Schneider-Orelli's method [21] and analyzed with probit regression function in PASW Statistics v. 18 [22] individually for each compound and population to obtain LC_{50} values and their 95% confidence limits. Differences in LC_{50} between insecticides were considered significant if their respective 95% confidence limits did not overlap. Resistance levels were calculated as resistance ratios (RR) by dividing LC_{50} values of the sampled whitefly populations by the LC_{50} of the susceptible population. Spearman rank

correlation between population treatment frequency of each insecticide and the corresponding insecticide LC_{50} values was analyzed using PASW Statistics v. 18. [22]

3 RESULTS

Manufacturers' recommended concentrations (MRC) of imidacloprid and spiromesifen (Table 1) on average killed 98% and 94% of tested individuals, respectively, whereas the recommended dose of pymetrozine on average caused 63.5% mortality of tested individuals (Fig. 3-5). Susceptibility among the tested populations was significantly lower and varied more in pymetrozine treatments (RR: 0.46-39.72) than in imidacloprid (RR: 1.05-10.46) and spiromesifen (RR: 0.84-11.47) treatments (Fig. 3-5).

Greenhouse crop producers that had used chemical control applied imidacloprid and spiromesifen 0-3 times each and used pymetrozine 0-2 times, all during the 9 month crop season preceding sampling. The correlation between frequency of applications and LC_{50} values was not significant for imidacloprid and spiromesifen ($p > 0.05$), whereas it was significant for pymetrozine (Spearman $r = 0.949$, $n = 4$, $p = 0.051$).

Although the resistance ratios were not high in comparison to other studies [7, 23] (Table 4-3), populations PJ4, PR and PJ 1 were significantly less susceptible to imidacloprid than the reference population, with PJ 4 and PR populations showing the lowest mortality values and highest resistance ratios (Fig. 3). Two of the populations that had been controlled with imidacloprid during the two preceding sampling years (PJ 5 and ML) were susceptible to imidacloprid at the same level as the reference population (Fig. 3). The susceptibility to imidacloprid and pymetrozine of the population that was not exposed to insecticide treatments during the three years before sampling (BL) did not significantly differ from that of the reference population for either insecticide (Fig. 3-4). The maximum resistance ratios to pymetrozine were higher than those to imidacloprid. Populations PR, PJ 5, ML, PJ 1 and PJ 4 (in increasing order) were significantly less susceptible to pymetrozine than the reference population (Fig. 4). Only the population from cucumber (TJ 1) was significantly less susceptible to spiromesifen than the reference population (Fig. 5).

4 DISCUSSION

After 4-13 years of use in Finland imidacloprid and spiromesifen were still effective in all populations (LC_{50}<MRC), whereas pymetrozine had reduced efficacy and was marginally effective in one population

(LC_{50}>MRC) (Fig. 3-5). Furthermore, recommended concentrations of imidacloprid and spiromesifen induced up to 90% mortality in majority of studied populations (Fig. 3 and 5). The high susceptibility of Finnish whitefly populations suggests that compared to other regions resistance development in the northern Europe has been relatively slow as resistance to neonicotinoids has been recorded in other whitefly populations (in Israel and Arizona, USA) to occur after only 2-3 years of use. [24] However, there was variability among responses of populations to the tested compounds (Fig 3-5), which might reflect different treatment frequencies in the region.

The susceptibility of whiteflies to pymetrozine compared to imidacloprid was clearly reduced although both compounds have been used in greenhouses for a similar period of time (Table 1). Although cross resistance of pymetrozine and imidacloprid have been reported in other studies, [7, 8, 25] only one population in our study (PR) showed low susceptibility to both compounds. However, it is not clear whether this case represents cross resistance between pymetrozine and imidacloprid or several non overlapping modes of resistance for these compounds. [7, 7]

Over the years, the use of pymetrozine has been more prevalent in the study area due to its better compatibility with biological pest control. [11] Indeed there was higher variation among populations in the LC_{50} values for pymetrozine (Fig. 4) which could be explained either by limited dispersal among whiteflies or selection by insecticides. Since the BL population, which was the most susceptible to pymetrozine (Fig. 4), was located within the whiteflies' potential distribution radius of 7-20 km [26, 27] from other less susceptible populations (Fig. 1), it could be hat the spread of resistant individuals has been restricted in the sampled area. Limited dispersal might be also supported by observations of greenhouse crop producers of different *T. vaporariorum* susceptibility levels even among greenhouses managed by the same producer or in our data among the different populations in the same village (PJ 1, 4 and 5, Fig. 3-5). However, in another study (Ovcarenko et al., unpubl.) analysis of these populations using microsatellite markers indicated that PJ 1, 4 and 5 populations represent a single genetic population. Thus, the observed variation in susceptibility to pymetrozine described here might be due to behavioural plasticity of local populations that share the same genetic background and/or due to differential selection pressure on the genes responsible for resistance development. [8, 28] Although our sample size is small, we found a positive correlation between insecticide usage history and LC_{50} values for pymetrozine suggesting that variable use of insecticides might explain the higher variation in pymetrozine susceptibility. We do not suggest that two applications are enough to cause the higher tolerance as correlative data do not provide evidence for cause –effect and is just reflecting the trend of insecticide application likelihood in the past. This is also supported by the absence of exposure to

insecticides of the most susceptible population to all three compounds for at least three years. The observed results call for a need to monitor whitefly resistance at the level of individual greenhouse crop producers, even in a dense production cluster.

Only the population from cucumber had decreased susceptibility to spiromesifen (Fig. 5) either due to initial resistance development or to the host plant effect. [19, 14] Insects feeding on cucumber for a few generations are known to have relatively high levels of detoxification enzymes and therefore low susceptibility to insecticides. [29, 30] However we can only speculate if this is the reason for the observed lower susceptibility of the TJ 1 population to spiromesifen, because only one population from cucumber was tested.

The high susceptibility levels to imidacloprid and spiromesifen might be explained by the loss of resistant individuals during the 3 and 5 generations (respectively) when whiteflies were kept in rearing before our bioassays (Table 1). It is known that, due to fitness costs, resistance can decrease when insects are grown for several generations without insecticides. [8-10, 31, 32] However, the length of rearing period in the laboratory before bioassays is usually not reported in publications, [8-10, 31] and the rate of decrease in whitefly resistance is only known for imidacloprid . A decrease in *T. vaporariorum* susceptibility to imidacloprid was observed after three generations spend in rearing without selection pressure, whereas for *B. tabaci* only after 10 generations. [6, 33] Thus, it is unlikely that imidacloprid resistance in our populations disappeared completely during the three generations of rearing before bioassays. Furthermore, the same generation of whiteflies was tested using imidacloprid and pymetrozine and variation in susceptibility to pymetrozine among populations suggests that three generations is not enough to abolish resistance at least to this compound. Therefore, the observed variation among populations in susceptibility to the tested compounds most likely reflects the local insecticide application history rather than a reduction in susceptibility levels prior to testing.

Dispersal of the greenhouse whitefly in Finland is currently being analyzed with microsatellite markers, and preliminary results indicate relatively persistent populations in the same greenhouses over two consecutive years (Ovcarenko et al. unpubl). *T. vaporariorum* persistence highlights the need for diligent use of pest management strategies to avoid increased insecticide use, which could lead to resistance development. Microsatellite data also suggest that different greenhouse crop producers within a village (PJ1-5) have come to share a relatively homogeneous whitefly population over time. When combining this evidence for homogeneity, challenges associated

with inter-greenhouse movement by whitefly in Finland with variation in mortality from tested compounds described in this paper it appears that insecticide use patterns continue to be a leading factor contributing to the creation of variability in response to insecticides.

Table 1. Physical and chemical qualities of the compounds used in the study.

Compound	Product	Manufacturer	Chemical group	Mode of action
Imidacloprid [a]	Confidor WG-70	Bayer CropScience	neonicotinoid	inhibits acetylcholine receptors in the central nervous system, causing paralysis followed by death
Pymetrozine [b]	Plenum WG-50	Syngenta	pyridine-azomethine	neuroactive insecticide that inhibits the feeding system by preventing insects from inserting their stylus into the plant tissue leading to death from starvation
Spiromesifen [c]	Oberon SC-240	Bayer CropScience	tetronic acid derivative	inhibits lipid synthesis, interferes with development of the egg and immature stages and reduces adult female fecundity

[a] Available for use in Finland since 2000.
b Plenum WP-25, formulation was available for use in Finland from 2000 until 2007. Thereafter, Plenum WG-50 was available from 2007 until 2013.
c Available for use in Finland since 2009.

Table 2. Abbreviations of *T. vaporariorum* population names, their original host plants and cultivars, date of sampling and testing, analyses conducted and number of whiteflies tested (N). Each population was sampled from different crop production company.

Population	Original host	Cultivar	Sampling / Testing dates	Compounds tested	N
REF	Bean	Canadian Wonder	5.2012 / 9.2012 5.2012 / 8.2012 5.2012 / 9.2012	Imidacloprid Pymetrozine Spiromesifen	1387 964 891
BL	Tomato	Encore	5.2012 / 9.2012 5.2012 / 8.2012	Imidacloprid Pymetrozine	1545 964
PJ 1	Tomato	Encore	6.2011 / 11.2011 5.2012 / 9.2012 5.2012 / 8.2012	Spiromesifen Imidacloprid Pymetrozine	2056 1526 945
PJ 4	Tomato	Dometica	6.2011 / 11.2011 5.2012 / 9.2012 5.2012 / 8.2012	Spiromesifen Imidacloprid Pymetrozine	1223 2041 914
PJ 5	Tomato	Dometica	6.2011 / 11.2011 5.2012 / 9.2012 5.2012 / 8.2012	Spiromesifen Imidacloprid Pymetrozine	1676 2036 926
TJ 1	Cucumber	Logica	6.2011 / 11.2011	Spiromesifen	1625
PR	Tomato	Careza	6.2011 / 11.2011 5.2012 / 9.2012 5.2012 / 8.2012	Spiromesifen Imidacloprid Pymetrozine	1126 924 907
ML	Tomato	DRW	5.2012 / 9.2012 5.2012/ 8.2012	Imidacloprid Pymetrozine	1411 901

Table 3. Variation among *T. vaporariorum* populations' susceptibility to insecticides (used in present study) reported in the literature. Susceptible population from bean, UK corresponds to our reference strain obtained from N. Karatolos in 2012.

Insecticide [REF]	Population	Host	Application method	LC_{50} (mg L^{-1} a.i.)	RR	Country
Imidacloprid [1]	Sampled	strawberry	foliar	298 [a]	-	California
	Sampled	strawberry	systemic	87.4 [b]	-	California
Imidacloprid [2]	Susceptible	bean	foliar	8.12	1	UK
	Sampled	rose, tomato, cucumber	foliar	60.2-193	7.41-23.8	UK, Spain, Italy
Imidacloprid [3]	Susceptible	cucumber	foliar	94.4		Greece
	Sampled	cucumber	foliar	138-417.5	1.5-4.4	Greece
Imidacloprid [4]	Sampled	cucumber	foliar	211	-	China
	Sampled	cotton, pumpkin, vegetable marrow	foliar	52.5-65.3	-	China
Imidacloprid [5]	Susceptible	bean	systemic	5.3	1	UK
	Sampled [c]	tobacco	systemic	3	0.57	UK
	Sampled	fuchsia	systemic	4.9-8.6	0.93-1.62	UK
Imidacloprid [6]	Susceptible	bean	systemic	8.12	1.00	UK
	Sampled	bean	systemic	3.96-29.7	0.49-3.66	UK, Netherlands
Imidacloprid [7]	Susceptible	bean	foliar	17.2	1	UK
	Sampled	tomato, vegetables, aubergine, ornamentals	foliar	44.9-374	2.61-21.8	UK, Spain, Turkey, China, Germany
Pymetrozine [7]	Susceptible	bean	foliar	38.8	1	UK
	Sampled	tomato, vegetables, aubergine, ornamentals	foliar	160-792	4.13-20.4	UK, Spain, Turkey, China, Germany
Spiromesifen [8]	Susceptible	bean	foliar	0.61	1	UK
	Sampled	ornamentals and vegetables	foliar	2.75 - 15.7	4.49-25.7	UK, Turkey, Germany

[a] µg a.i. mL−1
[b] mg a.i. plant−1
[c] No previous imidacloprid exposure in the field

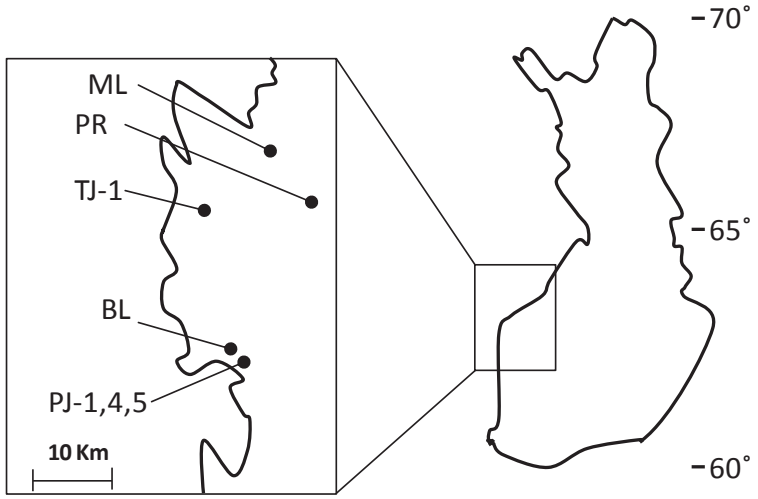


Fig. 1 Map of sampling locations in Finland. Letters refer to populations described in Table 2.

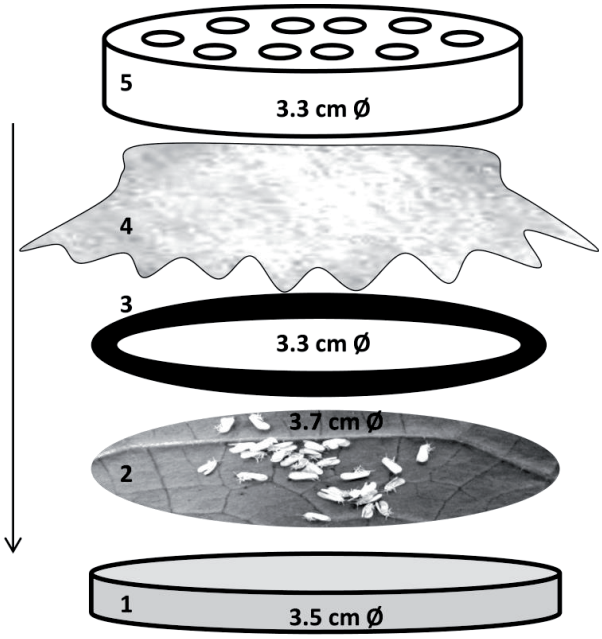
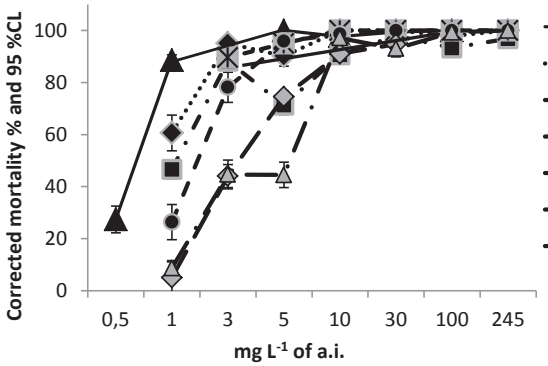
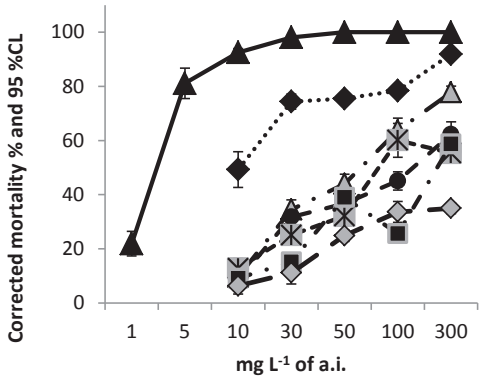


Fig. 2 Schematic of petri dishes used for the adulticide bioassays: 1 - petri dish (0.5 cm deep, 3.5 cm in diameter) containing 1% agar, 2 - leaf disc treated with insecticides covered with 30 immobilized adult whiteflies, 3 - rubber seal (3.3 cm in diameter), 4 - thin breathable nonwoven milk filter paper (Hygia™), 5 - perforated (~10 holes, 2mm in diameter) and petri dish lid (1 cm deep, 3.3 cm in diameter). After construction, the petri dishes were inverted to imitate the natural leaf position (see text).



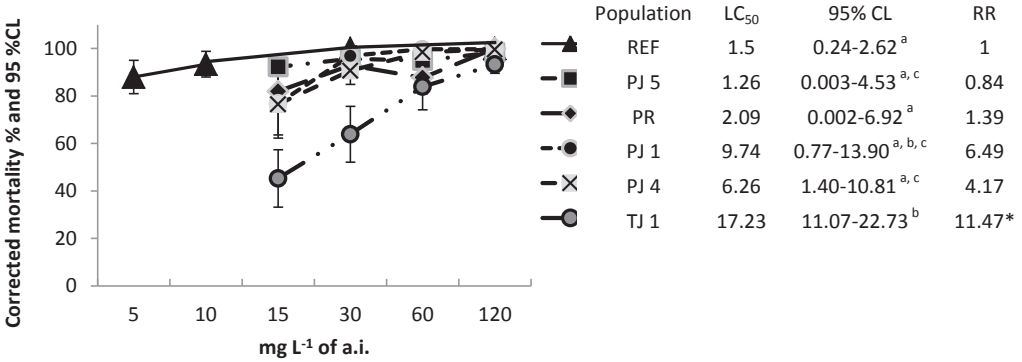
Population	LC_{50}	95% CL	RR
REF	0.42	0.10-1.20 [a]	1
BL	0.61	0.10-1.99 [a, c]	1.48
PJ 5	0.44	0.02-1.26 [a]	1.05
ML	0.91	0.08-1.66 [a, c]	2.18
PJ 1	1.61	1.30-1.92 [c]	3.88
PR	3.34	2.32-4.51 [b]	8.04
PJ 4	4.34	3.35-5.54 [b]	10.46

Fig. 3 Variation in Shneider-Orelli's corrected mortality of adult whitefly populations after imidacloprid treatment. Bars show 95 % confidence limits. Response of each population is described by LC_{50} (mg L^{-1} of a.i.) and their 95% confidence limits (CL), as well as resistance ratios (RR) relative to REF population. Shared letters indicate overlapping 95% CL and no significant difference between LC_{50}.



Population	LC_{50}	95% CL	RR
REF	17.14	13.68-20.59 [a]	1
BL	7.88	2.45-14.26 [a]	0.46
PJ 4	66.99	53.42-85.00 [c]	3.91
PJ 1	128.17	88.97-219.58 [b]	7.48
ML	133.88	83.86-294.83 [b, c, d]	7.81
PJ 5	218.97	139.45-473.60 [b, d]	12.78
PR	680.65	290.36-5723.83 [d]	39.72

Fig. 4 Variation in Shneider-Orelli's corrected mortality of adult whitefly populations after pymetrozine treatment. Bars show 95 % confidence limits. Response of each population is described by LC_{50} (mg L^{-1} of a.i.) and their 95% confidence limits (CL), as well as resistance ratios (RR) relative to REF population. Shared letters indicate overlapping 95% CL and no significant difference between LC_{50}.



Population	LC_{50}	95% CL	RR
REF	1.5	0.24-2.62 [a]	1
PJ 5	1.26	0.003-4.53 [a, c]	0.84
PR	2.09	0.002-6.92 [a]	1.39
PJ 1	9.74	0.77-13.90 [a, b, c]	6.49
PJ 4	6.26	1.40-10.81 [a, c]	4.17
TJ 1	17.23	11.07-22.73 [b]	11.47*

Fig. 5 Variation in Shneider-Orelli's corrected mortality of 2nd instar whitefly nymphs on spiromesifen treated leaves. Bars indicate 95 % confidence limits. Response of each population is described by LC_{50} (mg L^{-1} of a.i.) and their 95% confidence limits (CL), as well as resistance ratios (RR) relative to REF population. Shared letters indicate overlapping 95% CL and no significant difference between LC_{50}. * RR of TJ 1 was obtained from comparison with LC_{50} of REF population from tomato and could be lower when compared to LC_{50} of REF population from cucumber, which was not available for this study.

ACKNOWLEDGEMENTS

The authors thank the BIOINT Doctoral Programme at the University of Jyväskylä, the Oiva Kuusisto Foundation and the Ministry of Agriculture for financial support; MTT personnel, Lina Dragunaitė, Heli Honkanen and Susanna Rantakari for technical assistance; the greenhouse crop producers of Ostrabothnia and crop advisor Jenny Granfors (ProAgria) for collaboration; Nikolaos Karatolos (Rothamsted Research) for sharing the susceptible reference population; two anonymous reviewers that gave valuable comments to improve the manuscript; Emily Knott for English revision and comments on the manuscript; and the organizers of the productive 1st International Whitefly Symposium.

REFERENCES

1 Denholm I, Cahill M, Dennehy TJ and Horowitz AR, Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*; **353**: 1757-1767 (1998).

2 Byrne FJ, Denholm I, Birnie LC, Devonshire AL and Rowland MW. Analysis of Insecticide Resistance in the Whitefly, *Bemisia tabaci*. In *Resistance '91: Achievements and Developments in Combating Pesticide Resistance*, ed. by Denholm I, Devonshire AL and Hollomon DW. Springer Netherlands: Dordrecht, pp. 165–178 (1992).

3 van Lenteren JC, van Roermund HJW and Sütterlin S, Biological Control of Greenhouse Whitefly (*Trialeurodes vaporariorum*) with the Parasitoid *Encarsia formosa*: How Does It Work? *Biological Control*; **6**: 1-10 (1996).

4 Whalon ME, Mota-Sanchez D and M. HR. Arthropod Pesticide Resistance Database (2013).

5 Bi JL and Toscano NC, Current status of the greenhouse whitefly, *Trialeurodes vaporariorum*, susceptibility to neonicotinoid and conventional insecticides on strawberries in southern California. *Pest Management Science*; **63**: 747-752 (2007).

6 Gorman K, Devine G, Bennison J, Coussons P, Punchard N and Denholm I, Report of resistance to the neonicotinoid insecticide imidacloprid in *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *Pest Management Science*; **63**: 555-558 (2007).

7 Gorman K, Slater R, Blande JD, Clarke A, Wren J, McCaffery A and Denholm I, Cross-resistance relationships between neonicotinoids and pymetrozine in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science*; **66**: 1186-1190 (2010).

8 Karatolos N, Denholm I, Williamson M, Nauen R and Gorman K, Incidence and characterisation of resistance to neonicotinoid insecticides and pymetrozine in the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae). *Pest Management Science*; **66**: 1304-1307 (2010).

9 Gorman K, Hewitt F, Denholm I and Devine GJ, New developments in insecticide resistance in the glasshouse whitefly (*Trialeurodes vaporariorum*) and the two-spotted spider mite (*Tetranychus urticae*) in the UK. *Pest Management Science*; **58**: 123-130 (2002).

10 Karatolos N, Williamson MS, Denholm I, Gorman K, ffrench-Constant R and Nauen R, Resistance to spiromesifen in *Trialeurodes vaporariorum* is associated with a single amino acid replacement in its target enzyme acetyl-coenzyme A carboxylase. *Insect Molecular Biology*; **21**: 327-334 (2012).

11 Fuog D, Fergusson SJ and Flückiger C. Pymetrozine: A Novel Insecticide Affecting Aphids and Whiteflies. In *Insecticides with Novel Modes of Action Mechanisms and Application* ed. by Ishaaya I and D D. Springer: Berlin, pp. 40-49 (1998).

12 Greenberg SM, Jones WA and Liu T-X, Tritrophic Interactions Among Host Plants, Whiteflies, and Parasitoids. *Southwestern Entomologist*; **34**: 431-445 (2009).

13 van Lenteren JC and Noldus LPJJ. Whitefly-plant relationships: behavioural and ecological aspects. In *In: Whiteflies: their bionomics, pest status and management Intercept Ltd, Andover (1990) 47-49* (1990).

14 Liang P, Cui J-Z, Yang X-Q and Gao X-W, Effects of host plants on insecticide susceptibility and carboxylesterase activity in *Bemisia tabaci* biotype B and greenhouse whitefly, *Trialeurodes vaporariorum*. *Pest Management Science*; **63**: 365-371 (2007).

15 IRAC. IRAC Susceptibility Test Methods Series. Method No: 015 (12a), version 3 (2009).

16 Cahill M, Byrne FJ, Gorman K, Denholm I and Devonshire AL, Pyrethroid and organophosphate resistance in the tobacco whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bulletin of Entomological Research*; **85**: 181-187 (1995).

17 Nauen R, Koob B and Elbert A, Antifeedant effects of sublethal dosages of imidacloprid on *Bemisia tabaci*. *Entomologia Experimentalis et Applicata*; **88**: 287-293 (1998).

18 IRAC. IRAC Susceptibility Test Methods Series. Method No: 016 (12c), version 3 (2009).

19 Liu T-X, Toxicity and efficacy of spiromesifen, a tetronic acid insecticide, against sweetpotato whitefly (Homoptera: Aleyrodidae) on melons and collards. *Crop Protection*; **23**: 505-513 (2004).

20 Gu Y and L S, Life Table of Experimental Population of White House Fly *Trialeurodes vaporariorum* (Westwood). *Journal of Qingdao Agricultural University*; **2** 2006).

21 Schneider-Orelli O. *Entomologisches Praktikum: Einführung in die land- und forstwirtschaftliche Insektenkunde*. Sauerländer, Aarau, (1947).

22 SPSS. PASW Statistics for Windows: Chicago: : SPSS Inc (2009).

23 Wang KY, Kong XB, Jiang XY, Yi MQ and Liu TX, Susceptibility of immature and adult stages of *Trialeurodes vaporariorum* (Hom., Aleyrodidae) to selected insecticides. *Journal of Applied Entomology*; **127**: 527-533 (2003).

24 Rauch N and Nauen R, Identification of biochemical markers linked to neonicotinoid cross resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Archives of Insect Biochemistry and Physiology*; **54**: 165-176 (2003).

25 Rao Q, Xu Y-h, Luo C, Zhang H-y, Jones CM, Devine GJ, Gorman K and Denholm I, Characterisation of Neonicotinoid and Pymetrozine Resistance in Strains of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from China. *Journal of Integrative Agriculture*; **11**: 321-326 (2012).

26 Byrne DN and Bellows TS, Whitefly Biology. *Annual Review of Entomology*; **36**: 431-457 (1991).

27 Berlinger MJ, Lehmann-Sigura N and Taylor RAJ, Survival of *Bemisia tabaci* adults under different climatic conditions. *Entomologia Experimentalis et Applicata*; **80**: 511-519 (1996).

28 Nauen R, Vontas J, Kaussmann M and Wölfel K, Pymetrozine is hydroxylated by CYP6CM1, a cytochrome P450 conferring neonicotinoid resistance in *Bemisia tabaci*. *Pest Management Science*; **69**: 457-461 (2013).

29 Xie W, Wang S, Wu Q, Feng Y, Pan H, Jiao X, Zhou L, Yang X, Fu W, Teng H, Xu B and Zhang Y, Induction effects of host plants on insecticide susceptibility and detoxification enzymes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science*; **67**: 87-93 (2011).

30 Mullin CA and Croft BA, Host-Related Alterations of Detoxification Enzymes in *Tetranychus urticae* (Acari: Tetranychidae). *Environmental Entomology*; **12**: 1278-1282 (1983).

31 Longhurst C, Babcock JM, Denholm I, Gorman K, Thomas JD and Sparks TC, Cross-resistance relationships of the sulfoximine insecticide sulfoxaflor with neonicotinoids and other insecticides in the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Pest Management Science*; **69**: 809-813 (2013).

32 Servìn-Villegas R, García-Hernández JL, Murillo-Amador B, Tejas A and Martínez-Carrillo JL, Stability of insecticide resistance of silverleaf whitefly (Homoptera: Aleyrodidae) in the absence of selection pressure. *Folia Entomol Mex*; **45**: 27-34 (2006).

33 Nauen R, Stumpf N and Elbert A, Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Science*; **58**: 868-875 (2002).

V

LOW LEVELS OF MITOCHONDRIAL DNA AND SYMBIONT DIVERSITY IN THE WORLDWIDE AGRICULTURAL PEST, THE GREENHOUSE WHITEFLY *TRIALEURODES VAPORARIORUM* (HEMIPTERA: ALEYRODIDAE)

by

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