Modelling the dispersal of invasive species using landscape variables

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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE (Ph.D.) IN FORESTRY ENGINEERING AND NATURAL RESOURCES

2023

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Financial Institutions:

FCT Doctoral Programme (Sustainable Forests and Products, SUSFOR) PD/BD/142960/2018

FCT - Programa Pessoa - TC-05/10

European Union - HOMED project - grant 771271

2023

Acknowledgements

This section is dedicated to all the people who helped or contributed to the development of my thesis. Without them I would not have been able to finish this chapter of my life and so I'd like to give my thanks.

First and foremost, I would like to thank my three advisors, who made it all possible and express my utmost gratitude.

Professor José Carlos Franco, for the steady support during the whole time, with his calm and collected demeanor and help in structuring the thesis work and invaluable help in the writing.

Doctor Hervé Jactel, for providing me great support and counseling with his great insight and organizational skills, along with helping me with my first conference oral presentation, and of course welcoming me to work with him at Bordeaux.

Finally, the greatest support even before the start of the thesis, Professor Manuela Branco, who envisioned and supervised all my work from the start. She was the one responsible for introducing me to the research opportunities and research contacts that made it all possible. She accompanied every aspect of the thesis work, allowing me the freedom and help to develop the research skills I have now and making sure I was able to finish the thesis.

Doctor Christelle Robinet for giving me the opportunity to work with her and for modelling teachings, which were invaluable for the thesis and for my future.

Doctor Mário Santos for the opportunity, support, teaching and patience given to me for developing the scientific article and in finishing the thesis.

The tremendous help from my work colleagues from ISA. Sofia Branco offered great help throughout the thesis in many research activities. André Garcia helped in pretty much all the fieldwork, along with the invaluable ability for finding a way to make things work in the field. Catarina Afonso for the great company in the scientific courses we attended, being a great company.

I'd like to thank the Bordeaux INRAE research department for the warm welcome I received on the three times I came, and all the members of it. I'd like to especially thank Dr. Inge van Halder for all the help in the work, even helping me in my first failed attempt at reaching the research center.

Also, I would like to thank all my closest friends for their continuous support and great company throughout the last years.

Finally, I would like to thank my family for having always supported my academic career. Special thanks are due to my father who helped a lot in the statistical analysis across all my research works, but also in the writing, formatting the thesis and by being a great father. Also, I would like to thank my sister for having drawn for me the chapter pictures, my mother for always supporting me and lending me her car for the fieldwork and my beautiful dog Simba for being the best.

Contents

Abstract

Biological invasions are a great threat for ecosystems functioning and human welfare. In many cases, the more realistic strategy in the combat of invasive species is controlling their spatial expansion.

Our work focused on studying the role of the landscape structure towards invasive species dispersal. For this we developed three different dispersal models using landscape factors and ecology concepts. Models were applied to two highly relevant invasive species for Europe, the pine wood nematode (PWN) and the african citrus psyllid. To understand the role of landscape heterogeneity towards the spread of the PWN, we studied the dispersal trajectories of the vector, *M. galloprovincialis*. We used least-cost path analysis to estimate the beetle's behaviour response to the different land uses from a mark-release-recapture essay conducted in a heterogenous forest. The importance of isolated host trees for invasive species spread was tested in two spatio-temporal models for the dispersal of *T. erytreae* at large and local scales. At large scale, the role of human activities for the invasion patterns of *T. erytreae* in Portugal was also addressed. The inclusion of human-mediated spread and isolated host trees improved the F1-Scores of the model validation with the official reports as observations. For the local scale model, in an heterogenous agriculture region, we developed a spatio-temporal model, using an epidemiological approach for the chain of tree infections, to explain the dispersal of the species across the landscape. This model further allowed incorporating different management strategies.

Altogether, our findings highlighted the importance that landscape structure, and its elements, can have for invasive species dispersal, making their inclusion invaluable for invasive species modelling. Additionally, we were able to contribute with promising novel methodologies and modelling approaches for invasive biology, including a tool for pinpointing the origin of captured individuals in trapping grids.

Keywords: Invasive species; dispersal; landscape heterogeneity; spatio-temporal model; pest management.

Resumo

As invasões biológicas representam uma grande ameaça ecologicamente e para o bem-estar humano. Frequentemente, a estratégia mais realista no combate a espécies invasoras é controlar a sua expansão.

O nosso trabalho centrou-se no estudo do papel da estrutura da paisagem na dispersão de espécies invasoras. Desenvolvemos três modelos de dispersão utilizando fatores da paisagem e conceitos ecológicos. Estes modelos foram aplicados a duas espécies invasoras de grande importância para a Europa, o nemátodo da madeira do pinheiro (NMP) e a psila dos citrinos africana. Para estudar o efeito da heterogeneidade da paisagem na dispersão do NMP, estudámos as trajetórias de voo do vetor *Monochamus galloprovincialis*. Utilizámos análises do caminho de custo mínimo para estimar o comportamento de voo do inseto com os diferentes tipos de uso do solo, com um ensaio de marcação, largada e recaptura dentro de uma floresta heterogénea. A importância de árvores hospedeiras isoladas para a dispersão de espécies invasoras foi estudada com dois modelos espaço-temporais para a dispersão de *T. erytreae* em grande escala e escala local. A importância da dispersão mediada por atividades humanas para a invasão de *T. erytreae* em Portugal também foi estudada. A inclusão de dispersão mediada pelo homem e das árvores hospedeiras urbanas melhoraram os F1-*Scores* da validação do modelo. Finalmente foi desenvolvido um modelo espaço temporal em escala local, numa região agrícola em Portugal. Este modelo teve uma abordagem epidemiológica, para explicar a dispersão da espécie na região. Este modelo permitiu ainda a incorporação de diferentes estratégias de gestão.

Os nossos resultados destacam a importância da estrutura da paisagem para a dispersão de espécies invasoras. Além disso, contribuímos com metodologias promissoras para a modelação de espécies invasoras e uma ferramenta para a gestão de espécies invasoras.

Palavras-chave: Espécies invasoras; dispersão; heterogeneidade da paisagem; modelo espaço temporal; gestão de pragas.

Resumo Alargado

As invasões biológicas representam atualmente uma das maiores ameaças para a biodiversidade e para o bem-estar humano. A atenção sobre as invasões biológicas aumentou, em especial, nas últimas décadas devido à tendência crescente do número de novas introduções e acumulação de espécies exóticas a nível mundial. Tanto durante a sua expansão, como após o seu estabelecimento em vastas áreas, as espécies invasoras podem causar impactos diretos e indiretos negativos sobre os ecossistemas e/ou sobre a atividade humana, potencialmente devastadores.

Após o seu estabelecimento, a erradicação de espécies invasoras é uma estratégia com pouco sucesso devido às áreas afetadas e ações tardias sobre a população invasora. Só a deteção precoce, permitindo controlar as populações numa fase inicial do seu estabelecimento, poderá possibilitar o sucesso da sua erradicação sem esforços avultados. Após esta fase, e na maioria dos casos, a solução de gestão mais realista e mais eficaz centra-se no controlo da expansão da população da espécie invasora. Para o desenvolvimento de medidas de gestão na fase de expansão, torna-se muito útil e importante o desenvolvimento de ferramentas de modelação da dispersão das espécies invasoras. Os modelos de dispersão permitem melhorar a compreensão e prever os padrões de dispersão das espécies. Estes modelos podem ainda ser muito úteis para o desenvolvimento de estratégias mais eficazes para a sua gestão.

O nosso trabalho centrou-se em estudar a importância da estrutura da paisagem na dispersão de espécies invasoras. Para isso, desenvolvemos três modelos de dispersão utilizando fatores da paisagem e conceitos ecológicos. Estes modelos foram aplicados a duas espécies invasoras de grande importância para a Europa, o nemátodo da madeira do pinheiro (NMP), *Bursaphelenchus xylophilus*, e a psila africana dos citrinos, *Trioza erytreae*.

O NMP foi introduzido na Europa em 1999, em Portugal. Este patogéneo causa a doença da murchidão dos pinheiros que rapidamente conduz à morte de árvores infetadas vulneráveis à doença. O NMP representa uma grande ameaça económica e ecológica para as florestas de pinhal da Europa. Em Portugal. a

principal espécie florestal hospedeira é o Pinheiro-bravo, *Pinus pinaster*. O NMP é propagado entre árvores por insetos vetores do género *Monochamus* (Col: Cerambycidae). A espécie autóctone, *Monochamus galloprovincialis*, é o único vetor do NMP conhecido da Europa.

Para estudar o potencial papel da heterogeneidade da paisagem na dispersão do NMP, estudámos o efeito de diferentes usos do solo sobre as trajetórias de voo do vetor, *M. galloprovincialis*. Foi conduzido um ensaio de marcação, com largada e recaptura do inseto vetor, numa paisagem heterogénea de folhosas e resinosas, no sudoeste de França. Esta paisagem combinou plantações de pinheiro-bravo, áreas de corte-raso e áreas isoladas com floresta mista e floresta de folhosas. As largadas foram feitas num ponto central e as capturas foram efetuadas numa rede de 36 armadilhas iscadas com feromonas, dispostas em quadricula. Os dados permitiram modelar as trajetórias de dispersão dos insetos dentro da paisagem usando o conceito dos caminhos de custo mínimo. Com a modelação das trajetórias, fizemos a estimativa dos níveis de resistência oferecidos por cada uso do solo sobre a dispersão do vetor. A modelação e análise de caminhos de custos mínimos mostrou que os insetos evitaram áreas com espécies florestais não hospedeiras. Pelo contrário, as áreas de corte raso não tiveram influência sobre o comportamento de dispersão da espécie. Adicionalmente, foi desenvolvido um método para localizar o ponto da origem dos insetos, utilizando as coordenadas das armadilhas e valor de recapturas para estimar o baricentro ponderado. Este método de localização do baricentro foi significativamente melhorado com a adição de informação dos níveis médios da resistência da paisagem próxima das armadilhas. Em conclusão, este estudo realçou a importância da aplicação de conceitos de ecologia da paisagem para a modelação das espécies invasoras, contribuindo com mais evidências sobre o potencial da heterogeneidade da paisagem e dos processos ecológicos em reduzir a dispersão de pragas invasoras.

A psila africana dos citrinos *T. erytreae* foi introduzida na Europa em 2015, nomeadamente em Espanha e em Portugal. Esta espécie representa uma grande ameaça económica para a produção de citrinos na Europa, em particular, pelo facto de *T. erytreae* ser um dos vetores da doença *Huanglongbing* ou *citrus* *greening* nos citrinos. Esta é considerada a pior doença de citrinos do mundo, sendo capaz de causar perdas catastróficas de produção.

Desde a sua deteção em Portugal continental, na cidade do Porto, *T. erytreae* expandiu-se de forma rápida e direcionada para o Sul do país, ao longo da costa de Portugal. Esta região litoral é também onde há maior concentração de população humana.

Desenvolvemos um modelo espaço temporal para simular a dispersão da espécie ao longo do território português. Um dos objetivos consiste em estudar a importância de atividades humanas e de árvores isoladas de citrinos encontradas em áreas urbanas em Portugal, para explicar os padrões de invasão observados. Esta modelação combina um modelo de reação-difusão combinado com um modelo de dinâmica da população para simular a dispersão natural da espécie, assim como um modelo estocástico para simular a dispersão de longa distância facilitada por atividades humanas. O modelo teve em conta a disponibilidade de árvores hospedeiras, a partir de dados da distribuição de pomares de citrinos, e a possibilidade de inclusão de árvores hospedeiras em meio urbano, estimadas por meio de imagens do *Google Street View*. A dispersão da psila foi simulada desde 2015 até 2021 com diferentes combinações de parâmetros do modelo: dois níveis de fecundidade; inclusão ou não da dispersão de longa distância mediada por atividades humanas; inclusão ou não de árvores hospedeiras urbanas. O modelo mostrou ser uma boa ferramenta para simular a dinâmica de invasão de *T. erytreae.* A incorporação de dispersão de longa distância mediada por atividades humanas melhorou significativamente a validação do modelo, utilizando os relatórios oficiais do DGAV como dados de observação. A inclusão das árvores hospedeiras urbanas também aumentou significativamente o F1-Score na validação do modelo, demonstrando a importância que plantas hospedeiras isoladas podem ter na dispersão de espécies invasoras, funcionando como *stepping stones*. Finalmente, foi demonstrado o potencial da utilização de imagens do Google Street View, como uma ferramenta eficiente para estimar a densidade de árvores urbanas e periurbanas.

Para a dispersão de *T. erytreae* a nível local, desenvolvemos um modelo autómato celular com abordagem epidemiológica espaço temporal para simular a invasão de espécie dentro de uma região de agricultura heterogénea. A paisagem virtual do modelo foi dividida em células de 5 metros x 5 metros, com cada célula a representar a presença ou ausência de uma árvore de limoeiro. A dispersão de *T. erytreae* pela paisagem no modelo foi simulada através da cadeia de infeções de limoeiros. O modelo permitiu estudar o papel da estrutura da paisagem e de ações de gestão sobre o sucesso da invasão e dispersão da espécie.

O desempenho do modelo foi avaliado comparando os resultados da simulação com as observações de campo. Através de análise estatística, estudámos o papel de cada variável da paisagem (densidade de limoeiros, proporção de áreas urbanas, fragmentação dos pomares de limoeiros) nos diferentes indicadores de dispersão. A eficácia da gestão agrícola foi testada comparando a dispersão da espécie entre simulações com e sem aplicações de inseticidas. Foi verificado que os limoeiros dispersos em áreas urbanas residenciais desempenharam um papel significativo em promover a capacidade de dispersão entre pomares de limoeiros da *T. erytreae*, tendo um efeito positivo para o aumento da conetividade de habitat na região. A densidade de limoeiros promoveu a taxa de dispersão da espécie e do crescimento da população. O efeito negativo da fragmentação dos pomares de limoeiro foi verificado para a taxa de infeção de limoeiros, mas não para a dispersão máxima da espécie, provavelmente devido ao impacto contrário de conectividade feito pelos limoeiros urbanos. Os tratamentos de inseticidas aplicados nos pomares na região não se mostraram eficazes em controlar a dispersão da psila dentro da região, mas mostraram potencial na redução do nível de infeção da espécie dentro dos pomares e, portanto, os seus impactos. Este estudo demonstra, assim, o potencial desta nova abordagem simples e intuitiva para modelação de espécies invasoras, utilizando uma perspetiva epidemiológica. Devido à simplicidade e plasticidade da metodologia, esta pode ser adaptada para outros casos de estudo com diferentes espécies de pragas, diferentes locais de estudo, ou escala temporal. Adicionalmente, a componente visual deste modelo permite que seja utilizado como ferramenta de gestão e de educação para agricultores ou *stakeholders*, para testar cenários hipotéticos.

Os estudos destacaram a importância dos processos ecológicos que ocorrem em diferentes escalas espaciais na dispersão de espécies invasoras. Estes

processos ecológicos foram influenciados pela estrutura da paisagem, representada pelas variáveis de composição e configuração da paisagem. Conseguimos, assim, demonstrar a importância que variáveis de paisagem e a aplicação de conceitos da ecologia da paisagem podem ter para o estudo de invasões biológicas. Finalmente, contribuímos com o desenvolvimento de metodologias e conceitos inovadores para a modelação de espécies invasoras e aplicações práticas para a gestão de espécies invasoras.

Palavras-chave: Espécies invasoras; dispersão; heterogeneidade da paisagem; modelo espaço temporal; gestão de pragas.

Chapter 1. Introduction

1.1. Biological Invasions: Trends, Causes and Impacts

Biological invasions are nowadays considered as one of the greatest threats to biodiversity and human welfare across the globe (Kenis et al., 2009; Bellard et al., 2016; Pyšek et al., 2020). There has been a growing concern over invasive species in the last decades due to the alarming rising trend of exotic species introductions and accumulation (Fig. 1.1) (Seebens et al., 2015, 2017). This trend is mainly attributed to globalization of international trade, alongside environmental changes, and climate change (Chapman et al., 2017; Sardain et al., 2019; Skendžić et al., 2021). The start of the increasing global trend of exotic species introductions goes back to the 15th century, stemming from the advancement on means of sail, allowing humans to travel across large distances, between continents. This allowed the travel of large transport ships across the world with large entourages of people along with high volumes of materials, crop plants, animals, contributing for intentional or accidental introductions of exotic species into new areas across the globe, with some of these species becoming invasive (di Castri 1989; Nentwig & Josefsson, 2010; Seebens et al., 2017).

Exotic species are non-native organisms that have been introduced outside of their native distribution. Most invasive species are introduced exotic species that become established, being able to reproduce and disperse by themselves (Simberloff, 2010). These are deemed invasive when they pose a significant menace to the local native ecosystems, biodiversity, or human interests. It should be noted that invasive species can be native species whose incidence, impact or geographical distribution is notably increasing, with climate change being one of the leading causes behind its more recent increase (Kenis et al., 2009; Simberloff, 2010).

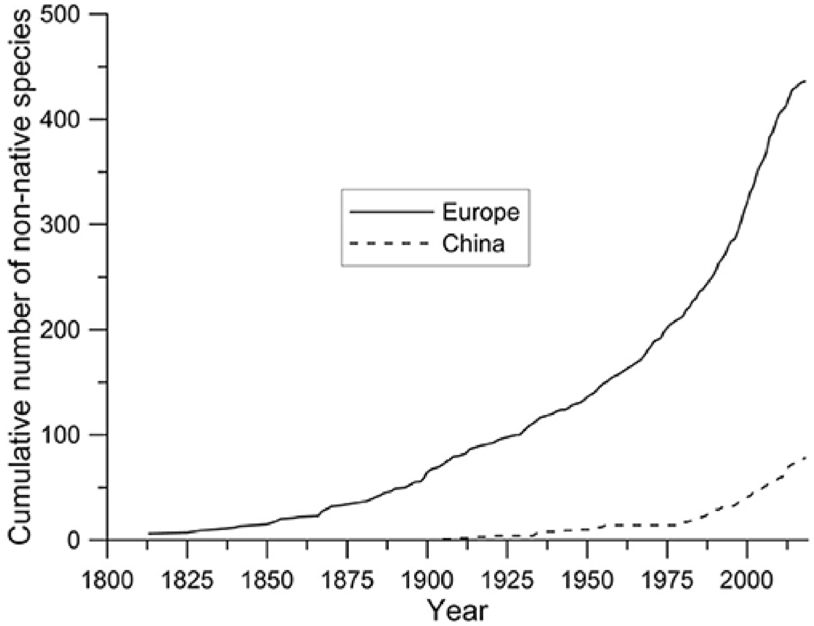

Fig. 1.1 - Cumulative number of detections (i.e., new establishments) of exotic insect species associated to woody plants in Europe and China over time (Source: Roques et al., 2020).

Invasive species can directly affect native ecosystems and biodiversity such as through herbivory towards native plants or as a predator or a parasitoid towards native prey or host species, or by hybridization with native species. Invasive species can also indirectly affect the native biodiversity, by resource competition, interference on trophic chains, carrying harmful pathogens or parasites for the native species or inducing significant ecosystem modification (Kenis et al., 2009; Simberloff, 2010).

Biological invasions can also be a source of enormous negative impacts towards human welfare across the globe (Pimentel et al., 2005; Mazza et al., 2014). These include direct economic costs due to production losses in the agricultural or in the forestry sector, pest management costs, or economic and social costs due to the introduced species posing a threat to ecosystem services, such as habitat loss caused by the *Melaleuca quinquenervia* tree species in South Florida, or even negative impacts on human health, such as exotic ticks that can transmit serious diseases (Keirans & Durden, 2001; Pimentel, 2005; Charles & Dukes, 2007; Mazza et al., 2014; Fantle-Lepczyk et al., 2022).

To take into consideration the impact of biological invasions at a global scale, the average cost of biological invasions in the United States of America from 2010-20 was estimated to be at least 21 billion US dollars per year (Fantle-Lepczyk et al., 2022), while in Europe, it was estimated to be at least 12.5 billion Euros per year (Kettunen et al., 2009). Additionally, the global realized and potential economic costs of biological invasions was recently estimated to be around 2.3 trillion US dollars (Zenni et al., 2021).

Insects represent a large part of the exotic fauna worldwide, being one of the most diverse group of organisms in the world. Yet have, so far, received disproportionately less attention in research regarding their invasive biology (Kenis et al., 2009; Bradshaw et al., 2016). A well-known example of an invasive insect species with disastrous ecological and economic impact is the emerald ash borer, *Agrilus planipennis* (Fairmare), in North America, first discovered in Michigan and nearby Ontario in 2002, presumably introduced from China (Cappaert et al., 2005; Bray et al., 2011; Keever et al., 2013; Siegert et al., 2014). The species is currently found in 29 states of the USA (USDA, 2023), having already killed tens of millions of ash trees and is a threat for most of the 8.7 billion ash trees throughout North America. The economic cost of this invasive species was estimated to be $10.7 billion, between 2009 and 2019 (Kovacs et al., 2010) alongside the vast ecological impacts.

Invasive species often possess certain traits that contribute to their success in establishing spreading and outcompeting the native species in the new environments, including: i) high reproductive capacity - rapid population growth, multiple generations per year, large number of offspring within a short period; ii) adaptability - phenotypic plasticity, tolerance for a wide range of environmental and climatic conditions, being generalist with a wide range of food type; iii) high dispersal ability, enabling to spread over large distances, either by their own means, or passive dispersal by wind, water, animal vectors, or human activities such as trade and transport (Simberloff, 1989; Pheloung et al., 1999; Copp et al., 2005; Pyšek & Richardson, 2007; Nentwig et al., 2009; Vall-llosera & Sol, 2009; Jarošík et al., 2015). Additionally, exotic species may benefit from escaping from their natural enemies or diseases in the new environment, granting them an additional competitive advantage over the local native species (Keane & Crawley, 2002).

Some authors consider that specific taxa-independent characteristics that facilitate successful establishment and spread of invasive species have not yet been unambiguously identified (Arim et al., 2006). Independently of the factors that facilitate the establishment and spread, the invasion process will roughly follow the same stages, independently of the invader's taxa.

1.2. Invasion phases and management strategies

After the species introduction, the second phase of invasion is the species establishment in the new environment, with the creation of a self-sufficient population with low spread. This will be followed by an expansion phase with increasing spread rates, and finally a saturation phase where spread rates reach a plateau. This halt in the expansion rate is usually attributed to the saturation of space availability, limited by the natural biogeographical limitations. During the establishment and its expansion, the exotic species may cause significant negative impacts towards the region's ecosystems and the human population, becoming an invasive species (Arim et al., 2006; Hui & Richardson, 2017).

Managing and controlling invasive species is crucial to mitigate their negative impacts and preserve the stability and health of ecosystems. Management strategies can vary depending on the specific invasive species and the ecological context (Yokomizo et al., 2009; Büyüktahtakın & Haight, 2017). However, there are general management strategies corresponding to the invasive process. The first stage is the prevention, carried out by understanding the pathways leading to the species entry in the area, and followed by applying regulations, policies, and security inspections to prevent exotic species introduction (Haack et al., 2014; Genovesi et al., 2015). Once the species escapes this first scrutiny, to avoid the second stage, i.e., the establishment, it is crucial to set up systems for detecting and monitoring the invasive species before its establishment. Raising awareness among the public, stakeholders, and relevant industries about the risks associated with invasive species is also important for the prevention of entry and establishment of the invasive species (Silvertown, 2009; Aceves-Bueno et al., 2017; Shackleton et al., 2019). In ideal circumstances prevention or prompt eradication would be the most effective strategy for dealing with invasive species, preventing the introduction and establishment of the invasive species (Mehta et al., 2007; Branco et al., 2023). This is achieved through international quarantine measures, such as banning imports of materials from contaminated countries or requiring these goods only to be imported with adequate phytosanitary treatments (Haack et al., 2014; Sequeira and Griffin 2014; Sikes et al., 2018). Yet, even with the adoption of biosecurity systems for the detection and interception of potentially dangerous organisms from trade and travel routes, a large proportion of these organisms continue to arrive undetected in many countries (Brockerhoff et al., 2006; Meurisse et al., 2019). For these failed interceptions, eradication would be the best alternative for pest species with high expected impact.

During the establishment phase, eradication attempts may have a high chance of success if the distribution of the invaded organism is still very restricted, such as when the invader organism is restricted to the primary plant material of its introduction (Fig. 1.2) (Branco et al., 2023). However, if the species population is already widespread, the cost of eradication, in most cases, exceeds its benefits, due to its much lower success rate (Tobin et al., 2014). Several factors have been found to be associated with the success of an eradication programme, among which, the early detection of the pest species combined with quick and decisive actions, with continuous efforts from start to finish (Pluess et al., 2012; Liebhold et al., 2016; Branco et al., 2023). Just like for interception, eradication success is dependent on the early detection of the pest species since its introduction. The need for early detection leads to one of the biggest challenges in the fight against biological invasions (Mehta et al., 2007; Reaser et al., 2020). This has proven to be particularly challenging, since the presence of a newly introduced invasive species is often only noticeable when its impact is already substantial, which happens when the species is already well established and significantly spread, making its eradication near impossible (Fig. 1.2). Furthermore, the introduced species are usually unexpected, making their detection even more unlikely (Lodge et al., 2006). For this, sentinel plant programmes have started to gain attention in the last years, either used for pest species risk assessment (Britton et al., 2010; Eschen et al., 2018) or pest species surveillance (Stevens 2008; Manfield et al., 2019).

During the expansion phase, measures mostly target the containment of the species, to reduce the population, and slow down the geographical spread of the species. The success in both can ultimately reduce the invasive species potential costs towards the ecosystem and human welfare (Simberloff 1997; Sharov & Liebhold, 1998; Sharov et al., 2002) and buy time to find more effective and specific measures for combating the species.

Dispersal modelling aims at improving the understanding of the dispersal mechanisms of invasive dynamics, which is crucial for the three starting stages of invasion (Srivastava et al., 2019). For the introduction phase, risk analysis of more likely or dangerous introduction locations improves monitoring efforts. To combat invasive species establishment, locating the starting point of the invasion could prove very valuable for neutralizing the invasion, before the species can establish and spread. Finally for the dispersal phase, models aiming at predicting the dispersal of the invasive species, help improve the success of eradication and control measures (Liang et al., 2014; Elith, 2017; Srivastava et al., 2019).

After the spreading phase, the species spatial distribution will be extensive, with the need of integrated control methods applied for the pest species control. These control methods may include chemical control or biological control, namely through classical biological control by introducing natural enemies from the invasive species native range, to reduce invasive species populations. Additionally, in some cases, restoration measures may be applied, to restore and rehabilitate affected ecosystems, such as by reducing habitat degradation caused by the invasive species (Wittenberg & Cock, 2005; Miller & Hobbs, 2007). The management strategies employed in each phase should be designed according to the specific characteristics of the invasive species and the available resources. Understanding how these processes occur for each specific species at each stage is thus crucial. In this work we dedicate our attention to the dispersal, i.e., spread phase, and the role of landscape features towards the invasive species dispersal behaviour, using two model invasive species.

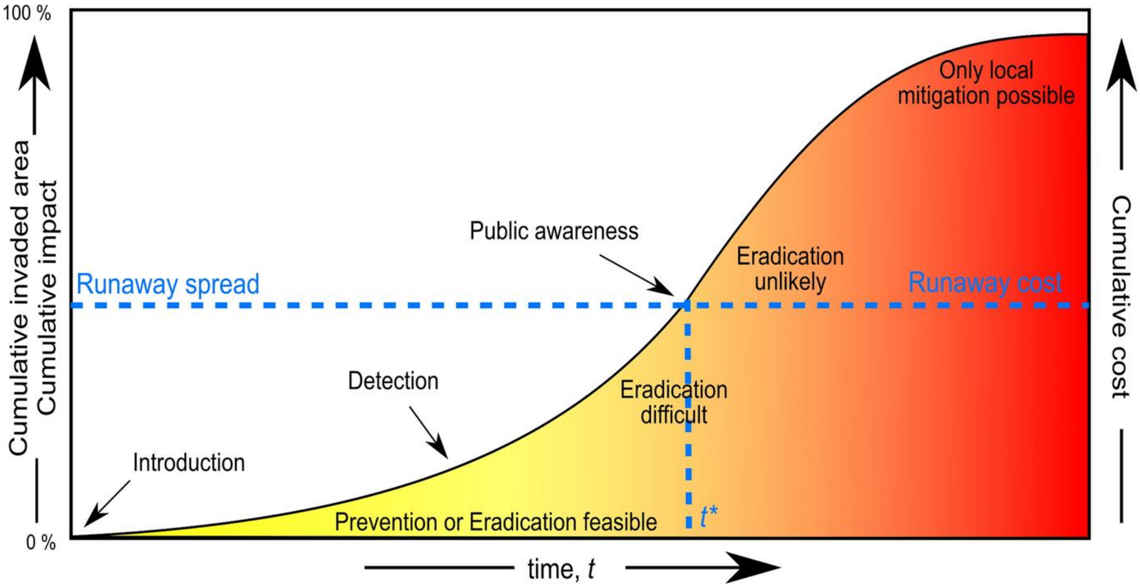

Fig. 1.2 - The classical invasion curve. This relationship displays a generalized invasive exotic population response over time *t*, after its introduction and establishment into a new environment. As the population expands and spreads, the cumulative area invaded (reported as a percentage of the total invaded area), cumulative impacts, and costs (damage and management) are assumed to follow a logistic curve, assuming cumulative cost saturation in the long term. There is a key point in time for management introduction at $t=t^*$, where the cost of inaction is exactly half of the cost in the scenario where management is never introduced. We refer to this as a *runaway* point, where management transitions from delayed to severely delayed and thus from difficult to unlikely (Source: Ahmed et al., 2022).

1.3. Dispersal of insect species: context and definitions

The dispersal of organisms across the landscape plays a fundamental role in species population ecology (Baguette et al., 2013). Naturally, individuals actively disperse to improve their fitness, in search of food, mating or emigrating to other habitat areas with more favourable conditions, such as more food resources, lower competition, natural enemies and reduced inbreeding (Baguette et al., 2013). In the case of insect species, these are known for using sensory cues, both visual and olfactory, for finding favourable host plants during food foraging or in migration (Huber, et al., 2000; Jones et al., 2019). Additionally, some insects have been shown to use visual and olfactory cues to identify and avoid non-host trees (Zhang & Schlyter, 2004; Jactel et al., 2011, Kerr et al., 2017), improving the species effectiveness at finding host trees, with non-host trees acting as barriers for the species dispersal. Due to this, a mixed forest has been suggested to have the potential of disrupting host finding, creating an olfactory barrier from the presence of non-host trees (Zhang & Schlyter, 2004), which could prove invaluable for management strategies to prevent or slow down the spread of pest species (Raffa et al., 1993). Additionally, in a mixed forest, the effect of top-down controls from natural enemies has been shown to be stronger (Castagneyrol & Jactel, 2012; Ampoorter et al., 2020; Staab & Schuldt; 2021). The higher abundance and richness of natural enemies' species found in mixed forest is explained by the "enemies" hypothesis. The hypothesis states that the alternative food resources and shelter offered by more complex habitats promote the natural enemies' communities, which in turn will control prey population (Root, 1973; Castagneyrol & Jactel, 2012; Ampoorter et al., 2020; Staab & Schuldt, 2021).

Yet, individuals can also disperse through passive mechanisms, such as being transported by other vector organisms, phoresia, or by abiotic mechanisms such as the wind or flowing water. In some cases, these passive mechanisms can be very important for a species dispersal ability, as found for the dispersal of small insects aided by the wind (Compton et al., 2002; Leitch et al., 2021) or human activity as in the case of the spread of the pine wood nematode in China or the yellow legged wasp in Portugal (Robinet et al., 2009; Verdasca et al., 2021).

1.4. Studying and modelling the dispersal of invasive insect species

Currently, our understanding of the landscape's effect towards emerging insect dispersal behaviour is relatively poor. Hence improving the understanding of invasive insect species dispersal is paramount to improve the monitoring and control efforts towards these species.

During the expansion phase of invasive species, the spread can be divided in short and long-distance dispersal mechanisms (Tobin & Robinet, 2022). Short-distance dispersal is related with the species natural dispersal abilities, such as its flight ability, while long-distance dispersal is associated with passive dispersal by external vectors, such as human activity, other animals or aerial dispersal aided by the wind (Gippet et al., 2019).

The study of long-distance dispersal is challenging due to the stochasticity behind it, which may result in uncertain and potentially biased estimates of the true underlying processes (Koralewski et al., 2021; Tobin & Robinet, 2022). Regarding the study of short-distance dispersal of animals in the landscape, radio tracking is the most accurate method, but it is not possible for small-sized species, like most insects, due to the equipment's weight (Robinet et al., 2019). There are several other methods for studying insect dispersal behaviour and ability in the field such as flight observation, telemetry, mark-release-recapture (MRR), flight mills, colonization patterns and genetic studies across the landscape (Ranius 2006; David et al., 2014).

Insects during their dispersal will react to the different elements of the surrounding landscape. Some types of land-use may encourage dispersal, while others may slow down or even hinder its dispersal. These notions are based on the concept of functional landscape connectivity (Tischendorf & Fahrig 2000), which states that different land-use types offer different friction values for the species dispersal. This will result in different levels of dispersal inhibition or facilitation depending on landscape features (Fig. 1.3) (Zeller et al., 2012). Currently, this concept has mainly been applied for species conservation studies (Fig. 1.3) (Bunn et al., 2000), such as for studying the effect of landscape structure on the inter-connectivity between local Iberian lynx populations (Ferreras 2001) or for the study of dispersal routes in the landscape used by the California tiger salamander (Wang et al., 2009). The concept of functional landscape connectivity has been also applied in other unrelated study areas, such as road planning optimization or studying historical human traveling routes (Choi et al., 2009; Gustas et al., 2019).

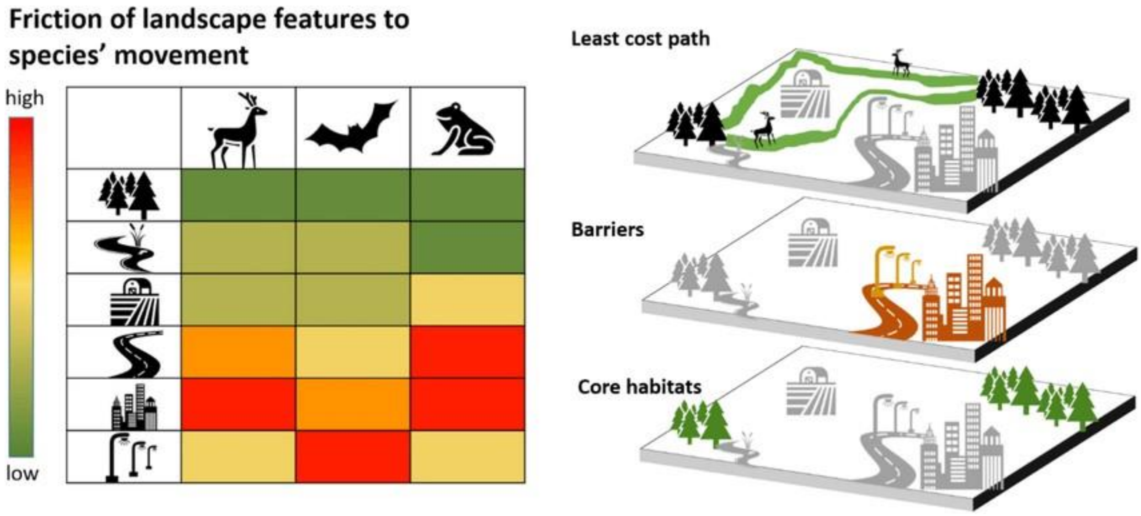

Fig. 1.3 - Representation of a species' least cost path between core habitat patches estimated with the friction of landscape features to the species' movement (Source: Honeck et al., 2020)

While the general idea would indicate that land-use types corresponding to the species habitat would facilitate dispersal, several studies verified the opposite in some cases (Lutscher & Musgrave, 2017; Crone et al., 2019). This is justified by the fact that individuals may be inclined to remain in the favourable habitat patches, which would then slow down its dispersal. Oppositely, unfavourable habitat patches may promote the species dispersal. In case the organisms need to search for more favourable areas, the presence of less favourable areas may promote a faster and longer dispersal of the species, potentially accelerating the species overall dispersal in the landscape.

Still, landscape heterogeneity likely has a very significant role in insect species dispersal behaviour, in some cases slowing it down (Rigot et al., 2014) and in others accelerating the dispersal of insect species (Lustcher & Musgrave, 2017; Saura et al., 2014).

The first spatial models developed for the spread of invasive species was published by Skellam in 1951, using a reaction-diffusion model (RD) to address the spread of muskrats (*Ondrata zibethicus*) in central Europe (Skellam, 1951). Reaction-diffusion models remain to be the most common approach up to date for modelling invasive species spatial spread (Higgins & Richardson, 1996; Volpert & Petrovskii, 2009). Spread models using stage-based integro-difference equation (IDE) were a more recent development (Kot et al., 1996). These models separate the species growth and dispersal into separate stages. These models were pioneer in demonstrating the importance of long-distance dispersal in the invasion process, even being a rare event (Kot et al., 1996; Lewis 1997; Neubert & Caswell, 2000).

Yet, dispersal models generally do not consider management strategies and, more importantly, the fine landscape features are ignored, by generalizing the entire landscape, assuming homogenous landscapes for the species spread (With, 2002). This is a major flaw when simulating invasion spread dynamics. Effectively, it is known that both individual dispersal and demography are largely affected by the landscape patchwork present in the real world (With & King, 1999; Andrew & Ustin, 2010). with Local landscape heterogeneity and diversity having been shown to affect individual dispersal and demography (Oliver et al., 2010, Rigot et al., 2014).

Still, there is no clear consensus on which landscape characteristics are the major players for the species-specific population dynamics and spread. Moreover, species population spread, settlement and extinction in the landscape are the product of multiple processes at various landscape scales, making the study of species responses to landscape dynamics much more complicated to understand and simulate (With, 2002).

1.5. The studied invasive species

This thesis concentrates on two insect species, a forest and an agricultural invasive species, highly relevant in Europe for their socio-economic and ecological impacts, i.e., the pine wood nematode (PWN), *Bursaphelenchus xylophilus,* causal agent of the pine wilt disease, whose insect vector in Europe is the *Monochamus galloprovincialis* (Olivier) (Fig. 1.4a), and the African citrus psyllid, *Trioza erytrae* (Del Guercio) (Fig. 1.4b), vector of the citrus huanglongbing disease (HLB). Below, is briefly described the invasion ecology and expected impact of the two insect species.

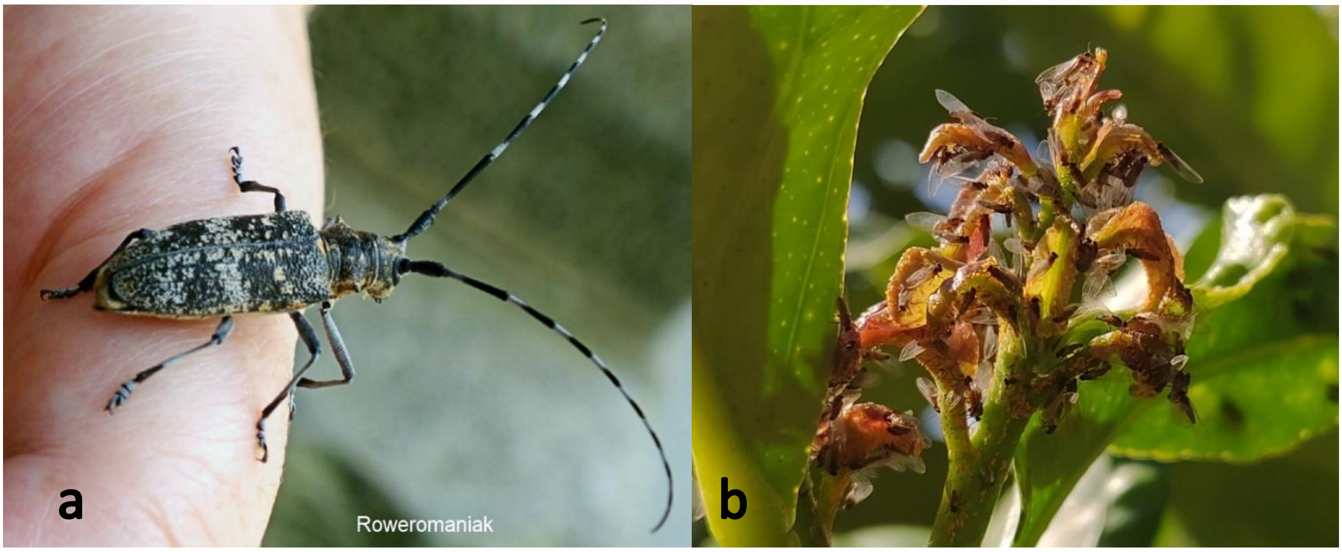

Fig. 1.4 – a) Photograph "*Monochamus galloprovincialis"* depicting an adult pine sawyer beetle, (photo by Roweromaniak), distributed under a CC BY_SA 3.0 license. b) A lemon tree leaf shoot crowded by several adult African citrus psyllid and eggs, (photo by Pedro Nunes).

1.5.1. The Pine Wood Nematode

The PWN, *Bursaphelenchus xylophilus* (Steiner & Buhrer), is a well-known example of an invasive species with large ecological and economic impacts (Evans et al., 1996). This nematode is indigenous to North America, where it causes no noticeable damage to American pines, as they are adapted to it. On the contrary, the nematode greatly affects non-American pine species (Futai, 1979; Zhao et al., 2008), causing the pine wilt disease, which leads to tree death within a few weeks or months. PWN preferred hosts are pine trees (*Pinus* spp.) but also can attack other conifer genera, such as *Abies*, *Cedrus*, *Chamaecyparis*, *Larix*, *Picea* and *Pseudotsuga* (Evans et al., 1996).

The PWN was first identified in Louisiana, USA, in 1929 (Steiner & Buhrer, 1934). It was first found outside its natural range in 1969, detected in Japan (Kiyohara & Tokushige, 1971). Since then, it has spread, within the American continent, to Canada and Mexico (Knowles et al., 1983; Dwinell, 1993), and to Eastern Asia, in China, Taiwan and Korea (Yi et al., 1989; Zhao et al., 2008). In Europe, the PWN was detected in 1999, in Portugal mainland (Mota et al., 1999). A few years later, it was detected in Madeira islands and in Spain (EPPO 2009; Abelleira et al., 2011; Fonseca et al., 2012).

The pine wood nematode does not colonize host trees on its own. It uses an insect vector as means of transport, from one tree to another, to allow its inoculation. In all regions where the PWN has been introduced, the insect vectors are longhorn beetles of the genus *Monochamus* (Coleoptera, Cerambycidae) (Linit, 1988; Evans et al., 1996). Whilst this genus includes more than 100 species, for each region only a few major vector species have been identified. The most important vector species for the American and Asian continents are *M. carolinesis* (Olivier) and *M. alternatus* (Hope), respectively, though there have been identified other less effective vectors (Evans et al., 1996). Only five *Monochamus* species are known to be native to Europe and, so far, only *M. galloprovincialis* (Olivier) was identified as being a vector of the PWN (Naves, 2008; Sousa et al., 2001).

The transmission of the PWN is done by adult beetles. The immature stages larvae and pupae develop inside the tree trunk. If the tree is infested by the PWN, the nematodes will migrate into the adult beetle's tracheal system infecting the insect. Thus, the beetles emerging from an infested tree will likely carry the PWN. Young adult beetles, after emerging from a tree, will then feed on pine shoots of healthy trees for sexual maturation and during the rest of their adult life. If the beetle is carrying the PWN, it may transmit it through the bark wounds inflicted during its feeding on the tree shoots. These wounds will be used by the nematodes to penetrate the vascular tissues of the tree and colonize a new tree (Linit 1990; Naves et al., 2007a). Gravid beetle females lay eggs in the bark of decaying trees, where they can also transmit nematodes, through the wounds inflicted to the tree shoots in the egg laying process (Naves et al., 2007b). The transmission of PWN will induce the pine wilt disease on the tree, which causes a quick death for the tree (Fig. 1.5).

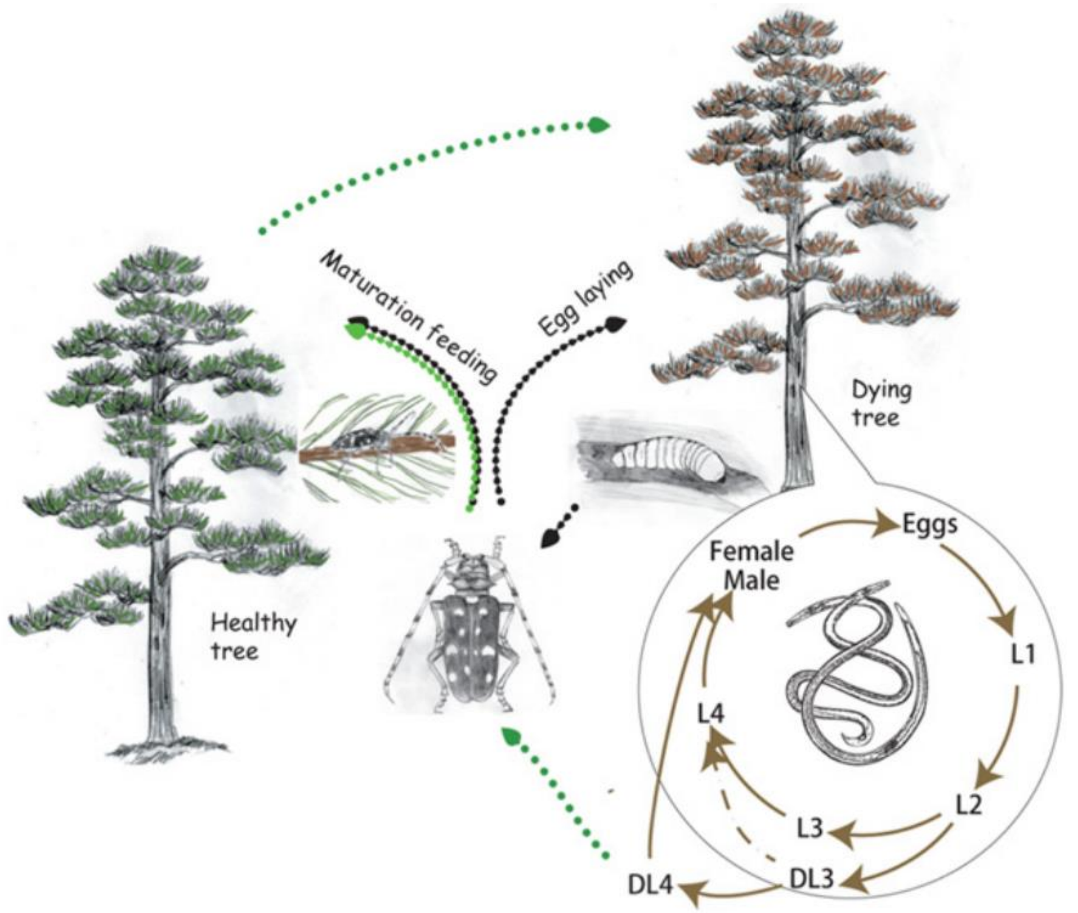

Fig. 1.5 - Diagram summarizing the life cycle of the pine wilt nematode (Source: Jones et al., 2013).

In Europe, the PWN spread in the landscape is mirrored by the dispersal of *M. galloprovincialis* through the forest landscape. *M. galloprovincialis* is a widely distributed insect species, ranging from Western Europe to the Caucasus (Petersen-Silva et al., 2014). Its immature stages grow on decaying pine trees or fallen pine logs. Therefore, supply of dead and decaying pine material is beneficial for *M. galloprovincialis*, required for the species oviposition and supplied in abundance by the deadly disease. On a local scale, the flight dispersal ability of *M. galloprovincialis* can be extended on average to around 16 km, during the insect's lifetime (David et al., 2014; Robinet et al., 2019). However, the spread can be greatly increased by human activities, especially through the transport of wood containing both the vectors and the nematodes (Robinet et al., 2009). So far, the eradication of the PWN in Europe is imposed by the removal of all host trees in 500 m around an infested tree, as requested by EU regulation

(Implementing Decision 2012/535/EU). However, this eradication strategy has not been effective, mainly due to the high dispersal capacity of the insect vector (Robinet et al., 2020). It is therefore of paramount importance to better understand the behaviour and dispersal capabilities of the insect vector to predict the location of new foci of PWN infestations, or to slow down its spread if eradication of new foci fails.

The invasion of PWN in Portugal has been quite impactful. The maritime pine, *Pinus pinaster* Aiton is the second most important forest trees species in Portugal and is highly susceptible to the pine wilt disease, being the main tree species host in Europe (Naves et al., 2016). Highly restrictive measures were implemented to reduce the risk of introduction of the PWN in other countries, with huge economic costs for Portugal (European-Union, 2008; Tóth, 2011). If PWN spread to the rest of Europe would not have been controlled, the direct costs, from cumulated wood loss, from 2008 to 2030, were estimated to reach 22 billion euros, with a reduction of social welfare of 218 million euros (Soliman et al., 2012). Due to this imminent threat, all countries in the EU and Switzerland were mandated to maintain monitoring trapping grids to detect as soon as possible an eventual spread of the PWN in Europe (Commission Implementing Decision 2012/535/EU of 26 September 2012). Thus, to improve the effectiveness of the management actions against this pathogen, there is an urgent need to study the vector species dispersal abilities and behaviour, and to improve future surveillance and containment efforts against this threat.

A major step forward in controlling the spread of PWN would be the early detection of insect vectors carrying the nematode, which can be achieved using pheromone traps (Álvarez et al., 2016). If beetles carrying the nematode are detected on separate traps, then the location of the infestation site from which they originated might be estimated. This could involve the implementation of trapping networks, allowing the triangulation of an area of probable origin of insects trapped in the surrounding landscape (e.g., fixed grid triangulation) (Pierce 1994; Arbogast et al., 1998). Even though the use of monitoring traps for the early detection of the PWN is currently mandatory to all EU members (Commission Implementing Decision 2012/535/EU of 26 September 2012), so far there are few scientific contributions towards the optimization of monitoring trapping networks, especially regarding trap density (Torres-Vila et al., 2015).

1.5.2. The African Citrus psyllid

The African citrus psyllid, *Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae) is a small exotic sap-sucking insect, native of tropical Africa (Moran & Blowers, 1967). Its main host plants are Rutaceae plants, such as citrus, displaying preference for lemon trees (*Citrus limon* L.) over sweet orange trees (*Citrus sinensis* L.) (Aubert 1987). The psyllid was recently introduced in Continental Europe, in 2014 and 2015, respectively in Spain and Portugal, in the north-western region of the Iberian Peninsula (Monzó et al., 2015; Pérez-Otero et al., 2015; DGAV 2021). The appearance of this invasive species has drawn major attention, as it is one of the two known vectors of HLB, also known as the greening disease, considered the most damaging citrus disease in the world (McClean & Oberholzer, 1965; Cocuzza et al., 2016). The disease is caused by the bacteria *Candidatus liberibacter* spp. (Bové 2006; Gottwald 2010), not yet present in Europe. The typical symptoms caused by the disease include yellowing of the shoots, asymmetric blotchy mottle on leaves, and inward leaf curl with vein corking (Fig. 1.6, Das et al., 2021). Additionally, tree's growth may be reduced, along with sour fruit and early leaf-drop and die back, often causing tree death in 3 to 8 years after the tree becomes symptomatic (Das et al., 2021). Illustratively, since the introduction of HLB in Florida, orange production in the region dropped by 74%, between 2005 and 2019, causing the downsizing of the entire industry. (Singerman & Rogers 2020; Das et al., 2021).

Since its detection in Portugal, the *T. erytreae* distribution has rapidly expanded southwards along the coastal area. The spread was fast, despite the phytosanitary measures implemented by the Ministry of Agriculture to contain its spread, including the ban of the movement of citrus material across the country and mandatory application of insecticide treatments by owners of citrus plants (DGAV 2015).

There is a lack of recent and relevant studies for Europe on the biology and dispersal ability of this species. Most studies were conducted more than 40 years ago in South Africa. Still, we know that this psyllid species has a very high invasive potential due to its high fecundity (between 327 and 827 average eggs laid per female), no diapause, rapid growth, multivoltine cycle (up to 8 generations per year) and long longevity of adults, even during unfavourable conditions, up to 82 days during cold weather, which increases their resilience to unfavourable events and management actions (Moran & Blowers, 1967; Catling 1969a, 1972; Tamesse & Messi, 2004). The species reproduction is mainly limited by the host's leaf phenology, since viable egg laying only occurs on young leaf shoots, with the nymph's leaf feeding causing typical pit galls in the leaves (Fig. 1.7). Hence, leaf phenology is considered one of the most important factors behind the species dynamics (Catling, 1969b; Catling, 1972). Like most insects, temperature conditions can influence *T. erytreae* survival, growth, and development (Sinclair et al., 2012). For *T. erytreae*, low temperatures (10-12ºC) and extreme high temperatures, over 32ºC, limit the species growth. These extreme temperatures, along with low relative air humidity, significantly increase the mortality rate of immature stages, with younger stages being more vulnerable (Catling 1969a; Green & Catling, 1971; Catling 1973; Aidoo, 2022). This is easily verified on the field, with populations dropping during summer periods exhibiting high temperature and low relative air humidity, designated as fatal days, which justifies the species preference for a cool moist weather (Catling, 1969a; Green & Catling, 1971).

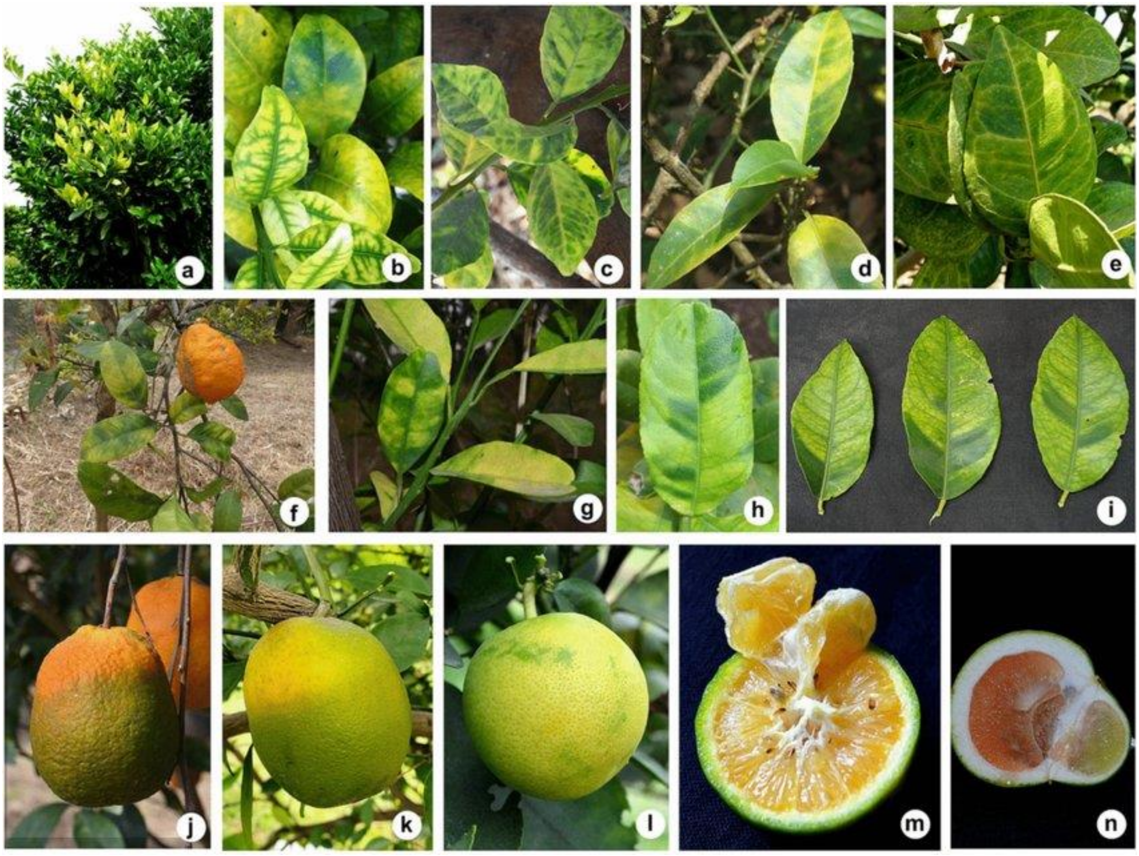

Fig. 1.6 – Different symptoms associated with Huanglongbing (citrus greening disease) in different citrus cultivars grown in India. Symptoms observed on the leaves: ‘Yellow shoot’: (a); ‘Blotchy mottle’ leaf symptoms: (b, c, d, e, f, g, h, i). Symptoms observed on the fruit: Stylar end greening (j & k), Lopsided fruit showing blotchy mottle on fruit surface (l), fruit showing aborted seeds (m), and lopsided, pink-fleshed fruit (n) (Source: Das et al., 2021).

Fig. 1.7 – *Trioza. erytreae* on lemon tree's leaf shoots. a) Typical pit galls caused by nymphal feeding of *T. erytreae*, protruding to the upper face of the leaf; b) Nymphs of *T. erytreae* on the lower face of a leaf, inside each pit gall (Photos by Pedro Nunes).

Regarding the species dispersal ability, it was estimated that *T. erytreae* could fly over 1.5 km, but this was under the lack of host plants (Samways & Manicom, 1983; Van den Berg & Deacon, 1988). Additionally, since this species is a small winged insect, its aerial dispersal is prone to being aided by wind currents, as was shown in the case of the Asian citrus psyllid, *Diaphorina citri* (Kuwaiama) and other similar insect species (Close et al., 1978; Aubert & Hua, 1990; Antolínez et al., 2022).

The citrus industry in Portugal represents around 19,000 ha of land use, mostly of sweet orange, followed by lemon orchards, concentrated in the south of the country (INE, 2021). The introduction of the greening disease could potentially have a devastating economic effect in Portugal and even more in Spain, being the largest citrus producer in Europe (USDA, 2021). Furthermore, it is very common to find citrus plants in urban and peri-urban residential landscapes, both in Portugal and Spain, including in the streets and in home gardens and backyards (Duarte 2012). These trees represent another potential source of host tree distribution for the pest species along with citrus orchards. It has been found in some cases that scattered host plants can play an important role in the spread of invasive species (Rossi et al., 2016). It should be noted that we could not find any study regarding the distribution, characterization, or valuation of residential citrus trees in Portugal.

1.6. Thesis hypothesis, objectives, and outline

The main objectives of this thesis are the following:

1) Provide evidence regarding the importance of landscape composition and configuration and its underlying ecological processes, for the dispersal of invasive species.
2) Development of different concepts and methodologies for the study of invasive species dispersal using landscape variables.
3) Development of practical implementations for invasion management using landscape variables.

We will use landscape variables for the modelling of invasive species in three case studies, with different model species (*Bursaphelenchus xylophilus* and *T. erytreae)*, contexts, spatial scales, and different modelling approaches.

We will study the following three hypotheses:

- **Landscape heterogeneity can slow down the spread of invasive species.**
  To test this hypothesis, we will evaluate key ecological processes related with landscape heterogeneity, which could impact the dispersal of the PWN vector species, *M. galloprovincialis* and *T. erytreae*.
- **The invasion spread of *T. erytreae* in Portugal was mediated by the distribution of isolated host trees.**
  For this hypothesis, we will test the effect of isolated host trees from residential urban areas for the invasive spread of *T. erytreae* at both local and large scales.

- **Human-mediated spread played a large role in the observed rapid invasion of *T. erytreae* in Portugal**.
  To evaluate this process, we will compare the effect of human-mediated spread towards explaining the invasion spread of *T. erytreae* in Portugal.

The thesis is structured in five different chapters, illustrated in Fig. 1.8, starting with the current introductory chapter, followed by three chapters in the form of scientific articles based on each of the three case studies, and ending with a chapter with the main conclusions.

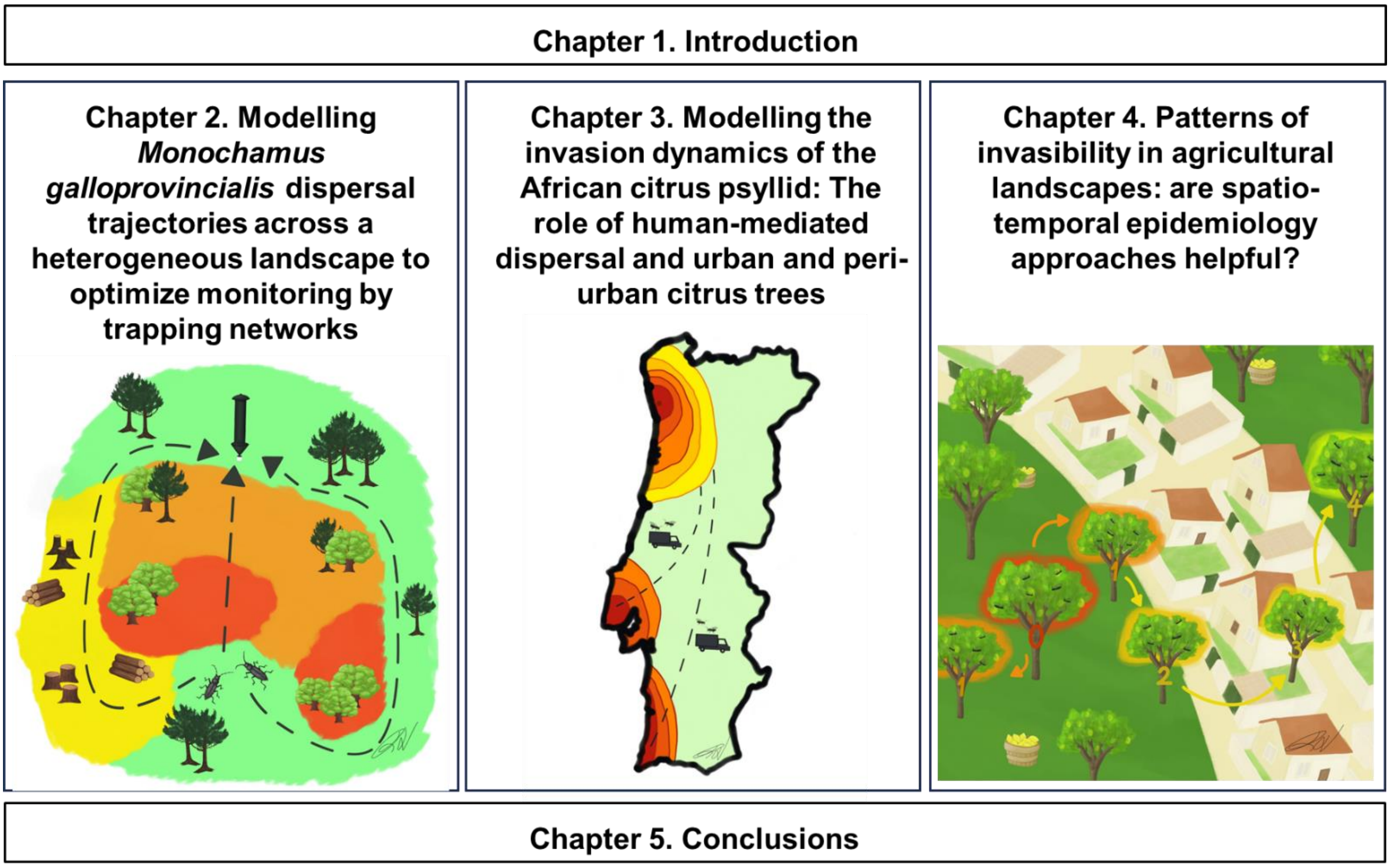

Fig. 1.8 – Overall structure of the thesis (pictures by Rita Catela Nunes).

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Chapter 2. Modelling *Monochamus galloprovincialis* dispersal trajectories across a heterogeneous landscape to optimize monitoring by trapping networks

Published in Landscape Ecology

Nunes, P., Branco, M., Van Halder, I., & Jactel, H. (2021). Modelling *Monochamus galloprovincialis* dispersal trajectories across a heterogeneous landscape to optimize monitoring by trapping networks. *Landscape Ecology*, *36*, 931-941.
https://doi.org/10.1007/s10980-020-01188-1

Abstract

Context

The pine wood nematode (PWN) is an invasive species which was introduced into Europe in 1999. It represents a major economic and ecological threat to European forests. In Europe, the maritime pine is the main host and *Monochamus galloprovincialis is* its only vector.

Objectives

Our goal was to analyze the effect of landscape heterogeneity on the vector's dispersal. We further aimed at developing a new method to locate the origin of insects captured in a systematic network of pheromone traps.

Methods

A mark-release-recapture experiment was carried out in a heterogeneous landscape combining maritime pine plantations, clear-cuts and isolated patches of broadleaved and mixed forests in the southwest of France.

Least-cost path analysis was used to model dispersal trajectories and assign friction values to each land-use type in the landscape. We used the trap's geographical coordinates, capture levels and mean friction values of neighbouring patches to calculate a weighed barycentre and the position of the release of marked beetles.

Results

Least Cost Path modelling revealed the vector's tendency to avoid habitat patches such as mixed or deciduous forests and not avoid clear-cuts. The weighted barycentre method was greatly improved when the friction values of the trap's surrounding land-uses were used.

Conclusions

Our study demonstrates the value of applying landscape ecology concepts and methods to improve our understanding and prediction of pest invasion processes. A practical application is the design of systematic grids of pheromone traps to locate the infection focus from which PWN vectors originate in a newly colonized area.

Keywords: Pine wood nematode, landscape heterogeneity, least cost pathway, barycentre, flight

2.1. Introduction

In the last decades, the number of non-native pest species that have become established outside their native range has dramatically increased worldwide (Seebens et al., 2017; Walther et al., 2009). A small portion of these species can become invasive, some with high ecological and economic costs in the agricultural and forestry sectors (Pimentel et al., 2005; Kenis et al., 2009). The pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer) is a well-known example of an invasive species with great ecological and economic impacts (Evans et al., 1996). This nematode is indigenous to North America, where it causes no noticeable damage to American pines. On the contrary, the nematode greatly affects non-American pine species (Futai, 1979; Zhao, 2008), causing pine wilt disease, which leads to tree death within a few weeks or months. The PWN was first detected in Japan in 1969 (Tokushige & Kiyohara, 1971), then in China, Taiwan, and South Korea in the eighties (Yi et al., 1989; Liou et al., 1999). In Europe, the PWN was detected in 1999 in Portugal mainland (Mota et al., 1999). A few years later, it was detected in Madeira islands (Portugal) and Spain (EPPO, 2009; Abelleira, 2011; Fonseca et al., 2012).

The pine wood nematode cannot colonize host trees on its own. It needs an insect vector to transport it from one tree to another and then allow it to be inoculated. In all regions where the PWN has been introduced, the insect vectors are longhorn beetles of the genus *Monochamus* (Linit, 1988; Evans et al., 1996). In Europe, only *Monochamus galloprovincialis* (Olivier) has been reported to be a vector of the PWN (Sousa et al., 2001). Young adult beetles feed on pine shoots of healthy trees for sexual maturation. During this phase, the beetles produce bark wounds, which are used by the nematodes to penetrate the vascular tissues of the tree (Linit, 1990; Naves et al., 2007(a)). Gravid beetle females lay eggs in the bark of decaying trees, where they can also transmit nematodes (Naves et al., 2007(b)). The PWN thus spreads through the dispersal of its insect vector. On a local scale, dispersal by flight of *M. galloprovincialis* can extend on average around 16 kilometers during the insect’s lifetime (David et al., 2014, Robinet et al., 2019). However, the spread can be greatly increased by human activities, especially through the transport of wood containing both the vectors and the nematodes (Robinet et al., 2009). So far, the eradication of PWN has been mainly done by the removal of contaminated trees. However, it was recently demonstrated that clear-cutting susceptible trees 500 m around an infested tree, as requested by EU regulation to eradicate the PWN, is not effective, mainly due to the high dispersal capacity of the insect vector (Robinet et al., 2020).

It is therefore of paramount importance to better understand the behavior and dispersal capabilities of the insect vector in order to predict the location of new foci of PWN infestation, or to slow down its spread if eradication of new foci fails. During their dispersal phase, insects generally react to landscape elements. Some types of land use can enhance dispersal, while others slow it down or even hinder it. According to the concept of functional landscape connectivity (Tischendorf & Fahrig, 2000), land use types have different friction values which result in different levels of dispersal inhibition (Zeller et al., 2012). This concept has been mainly applied for organisms of conservation interest and much less for pest insects (Bunn et al., 2000; Ferreras, 2001; Wang et al., 2009). In general, it is assumed that land-use types corresponding to the species habitat facilitate dispersal, but several studies indicate the opposite (Crone et al., 2019, Lutscher et al., 2017), because individuals might prefer to stay in favorable habitat patches while moving faster through unfavorable ones. It is therefore likely that landscape heterogeneity can have a significant role in slowing down (Rigot et al., 2004) or accelerating the dispersal of insect species.

Different methods exist to study insect dispersal in the field such as observation of flying insects, telemetry, mark-release-recapture (MRR) experiments, colonization patterns or genetic studies (Ranius, 2006). Telemetry has been tested recently for *Monochamus alternatus* (Hope), but it was found to be unable to track over long distances (Zhang et al., 2020). MRR with baited traps was found to be relevant for *M. galloprovincialis* although within a short spatial range (Álvarez et al., 2015; Sanchez-Husillos et al, 2015, Jactel et al., 2019). In addition, MRR data alone do not permit a functional interpretation of flight behavior through different landscape elements since it only provides information on the release and recapture points. To determine the effect of landscape composition and configuration on dispersal behavior, MRR data should be combined with modelling tools, such as Least Cost Path analyses (LCP). These tools allow testing the effect of different land-use friction values on recapture rates (Adriaensen et al., 2003).

A major step forward in controlling the spread of PWN would be the early detection of insect vectors carrying the nematode, which can be achieved using pheromone traps (Álvarez et al., 2016), and then the location of the infestation site from which they originated. This could involve the implementation of trapping networks, allowing the triangulation of an area of probable origin of insects trapped in the surrounding landscape (e.g., fixed grid triangulation, Pierce 1994, Arbogast et al., 1998). Even though the use of monitoring traps for the early detection of the PWN is currently mandatory to all EU members (Commission Implementing Decision 2012/535/EU of 26 September 2012), so far there were very few scientific contributions towards the optimization of monitoring trapping networks, especially regarding trap density (Torres-Vila et al., 2015).

In this study, we organized a mark-release-recapture experiment of *M. galloprovincialis* beetles, using a systematic grid of pheromone traps deployed in a heterogeneous forest landscape. Recapture data were used to fit an LCP model to assess the friction value of different types of land use, with respect to flight dispersal of the insect vector. We estimated correlations between insect recapture rates and i) the distance of a direct flight trajectory from the release point to the trap position or ii) the distance of a longer flight trajectory but minimizing dispersal costs. Our hypothesis was that the insects would avoid flying through non-habitat patches represented by e.g., broadleaved or mixed-species woodlands.

We then used the evaluated friction value for each land use type to calculate an average friction value in a buffer around each trap. We calculated the coordinates of the barycenter of the trap's positions in the grid, weighted by the recapture levels and the average value of friction around the traps. Our hypothesis was that by proceeding in this way we could approach the coordinates of the point of insect release and thus propose a method of predicting the location of the original focus of the captured insects.

This study therefore represents an original application of landscape ecology concepts to better study and predict the risk of spread of an invasive alien species in realistic forest landscapes.

2.2. Material and Methods

2.2.1. Study area

The study was carried out in the south-west of France in the 'Landes de Gascogne' forest. This region of one million ha is dominated by even-aged plantations of the native maritime pine *Pinus pinaster* Ait, which is the main host tree of the pine wood nematode in Europe (Naves et al., 2016). Broadleaved woodlands are rare and found along rivers or as scattered patches of a few hectares. They are generally dominated by oak species (*Quercus robur* or *Quercus pyrenaica*). Open areas in the landscape are mainly represented by pine clear-cuts, maize fields, firebreaks and powerlines. The local climate is temperate oceanic Sub-Mediterranean with mean annual temperature of 14°C and a mean total annual precipitation of 944 mm.

2.2.2. Site selection and landscape mapping

Within the Landes de Gascogne Forest we selected a study site of 183 ha in the municipality of Saint Jean d'Illac, with a heterogeneous landscape composed of different land-uses (different ages of pine stands, clear-cuts, mixed forests, and broadleaved forests (coordinates of the centre 44°48'16.721''N, 0°51'2.329''W).

Land-use types of the study site were mapped in ArcGIS using aerial photos of 2018 (i.e., the year of the study) with a pixel size of 50 cm as background layer. We distinguished 13 land-use types that could be recognized on these photos and that could be of ecological relevance for the dispersal behaviour of *Monochamus* beetles (see Appendix S2.1 for land-use description and Fig.2.1). Landscape mapping was checked in the field for the patches visible from forest roads.

2.2.3. Beetle's origin and releases

We released marked *M. galloprovincialis* adults reared from infested logs (i.e., adult immatures) or collected in traps in pine stands (i.e., adult matures).

Maritime pine logs or branches infested with *M. galloprovincialis* larvae were collected in spring 2018 and stored outside in tents. From Mid-May on, tents were inspected daily to collect newly emerged adult beetles. They were kept in the laboratory in plastic boxes separated by sex and fed until release with fresh maritime pine shoots. The released beetles had an age of one to seven days and are hereafter called "immatures". They were marked with POSCA® paint on the elytra using a different code for each release date and a mark on the thorax coding for 'immature beetle'. Previous release studies (Robinet et al., 2019) showed that marks did not affect beetle flight performances.

We also collected beetles with baited traps in maritime pine stands outside the study area. The age of these beetles was unknown, but they were at least 15 days old since they reacted to the pheromone and were thus sexually mature (Jactel et al., 2019). They were also marked with a painted code for each release date and a code for 'mature beetle'.

All marked beetles were released at a fixed point in a mature maritime pine stand in the centre of the study landscape. In total 3162 beetles were released (2747 immature and 415 mature).

2.2.4. Recaptures

We placed 36 traps (Cross Vane® type) in a regular grid pattern within the study landscape, with a mean distance of 170 m between traps in a total area of about 1 Km^2. Because of field conditions the distance between traps varied somewhat (between 130 and 220 m). The 36 cross traps were baited with Galloprotect 2D ®, a commercial product that includes the aggregation pheromone (2-undecyloxy-1-ethanol) and kairomonal substances (2-undecyloxy-1-ethanol, ipsenol and 2-methyl-3-buten-1-ol) (Jactel et al., 2019), the lures were replaced once in the summer. The collecting vial contained an insecticide. Traps were checked twice a week, between 13 June and 8 August 2018.

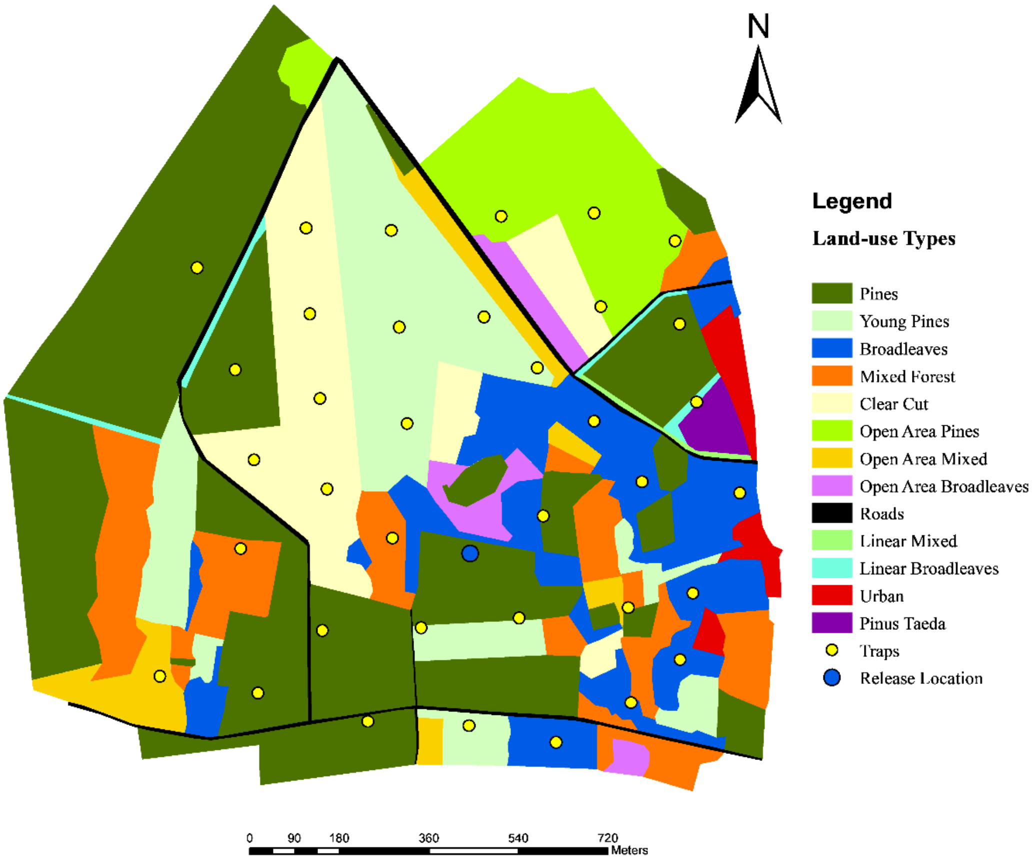

Fig. 2.1 – Map of the study landscape and its classification in 13 different land-use classes (see SM1 for detailed definition), the 36 trap positions for recapturing the beetles, *Monochamus galloprovincialis*, and the release point of the beetles.

2.2.5. Creating the Least Cost Distance Model

We calculated Least Cost Pathways from the release point to all 36 traps in ArcMap using the "cost path polyline function". This procedure provides for each trap (i) a value representing the minimum total cost to reach it (i.e., Path Cost i, PathCi). The cost depends on the distance and the friction value of each land-use type between the release point and the trap (Adriaensen et al., 2003). The algorithm calculates the path through the landscape with the lowest total cost. To find the friction value (Fi) of each land use type, we went through a model optimization process. We assumed that the number of catches in each trap (beetles' recapture value of trap i, *BRi*) would depend on the least cost pathway between the release point and this trap. Multiple scenarios with different friction values for the different land use types were used to calculate the corresponding PathCi. For each scenario, we calculated the correlation between PathCi and log transformed values of *BRi*. We iteratively modified the friction value of the different land-uses until reaching the maximum value of the coefficient of determination R^2.

First, we tested scenarios for which each land use type was tested separately for low, medium and high friction values (“1”,”4”,”8”) respectively (within a scale of 1 to 9), while keeping the other land-uses at value 1. We also tested friction values higher than 9, but they did not change the resulting paths. Second, according to the results (i.e., R^2 value), land-use types were grouped into three categories of friction values (1 - Low, 4 - Medium, 8 - High). Third, within each of the three categories, we incrementally changed the friction values (e.g., ±1), keeping the values constant in the other two categories, until we reached the maximum value of R^2. Last, we repeated the procedure for the other two categories. The complete optimization process is described in detail in the supplementary material (Tables S2.2).

2.2.6. Re-finding the position of the release point using the recapture levels in the grid of traps

We investigated whether we could re-find the position of the release point of marked beetles using the location of the traps and their level of recapture. The objective of this computation was to simulate a situation where a grid of traps was set up to detect the position of a focus of infested trees (from which beetles originate) in the landscape. To estimate the coordinates of the release point (simulating beetles’ emergence from the infestation focus), we used the method of weighted barycentre that is commonly applied to find the centroid of a system of several points in a given two-dimensions space, taking into account the weight (or size) of the points. Here we used the trap recaptures as weight. The general formula is the following:

$$X_B = \frac{\sum_{i=1}^{n} x_i . w_i}{\sum_{i=1}^{n} w_i}$$

$$Y_B = \frac{\sum_{i=1}^{n} y_i . w_i}{\sum_{i=1}^{n} w_i}$$

With X_B and Y_B being the coordinates of the weighted barycentre, x_i and y_i the coordinates of the points (here of the traps) and w_i the weight of the points, here the recapture of marked beetles in the traps (*BRi*). This method based on Euclidian distances between traps is further called the EUC-method.

In a second step, we tested whether we could improve the estimation of the coordinates of the release point (barycentre of the traps) by taking into account the difficulty of the beetles to reach the traps, according to the friction value of the surrounding land-uses. For that, we used our best estimates of friction values per land-use type to calculate the mean friction value in a buffer radius around each trap (*Fi*). For this, we tested the results between different buffer radius values of 50, 100, 150 and 200 meters and compared the accuracy of resulting estimations. The results were compared with an ANOVA type 1 analysis.

We thus adapted the calculation of the coordinates of the barycentre using the following formula:

$$X_B = \frac{\sum_{i=1}^{n} x_i . BR_i . F_i}{\sum_{i=1}^{n} BR_i \, . F_i}$$

$$Y_B = \frac{\sum_{i=1}^{n} y_i . BR_i . F_i}{\sum_{i=1}^{n} BR_i \, . F_i}$$

With F_i being the mean value of friction values in a buffer around each trap (*i)*. This method based on Euclidian distances between traps and the results of Least Cost Pathway analysis for surrounding trap friction was further called the LCP method.

The accuracy of the barycentre estimation methods was calculated as the distance (Dist), in meters, from the estimated barycentre to the real release point. The precision of each method was calculated as the 95% confidence interval of the Dist values, also calculated for all possible *n*=36 subsets of *n*-1=35 traps (Jackknife resampling technique).

2.2.7. Effect of reducing the number of traps on the accuracy of estimating the location of the release point

We studied the effect of reducing the number of traps on the accuracy of barycentre estimation. Following the same approach of a systematic trapping grid, we calculated the barycentre estimations for subsets of 5x5, 4x4, and 3x3 traps. In addition, we took the precaution of evenly distributing the traps across the landscape. For that, we divided the study landscape in 25, 16 or 9 quadrants, and we re-sampled one trap per quadrant. All possible combinations of one trap per quadrant were considered, resulting in 2049, 93312 and 230400 combinations respectively for the 25, 16 and 9 trap grids. For each trap combination, we calculated the barycentre coordinates, using the EUC and the LCP methods. The mean distance between the estimated barycentre coordinates and the release point (Dist) was then calculated for each density of traps in the systematic grid as a measure of accuracy and the precision of each method was calculated as the 95% confidence interval of the Dist values. These and the previous calculations were all done with Microsoft Excel, using the “list all combinations” function from the Kutools add-on.

2.3. Results

2.3.1. Recaptures and Least Cost Path model

In the 36 traps, 68 marked beetles were recaptured (i.e., 2.2 % of total number of released beetles), 53 immature and 15 mature beetles. The recaptures were unevenly distributed across the study area with 13 traps with no beetles recaptured and two traps with 17 and 15 recaptured beetles (47%).

The optimization process for determining land-use friction values based on the highest R^2 between the least cost path costs (PathCi) of the traps and their recapture values (*BRi*) resulted in the selection of the best scenario (P19), with a R^2 of 0.63, p-value < 0.0001 (SM2). The R^2 using the Euclidean distance method was much lower (0.27). The corresponding friction values for each land use type are shown in Fig. 2.2. Open areas and mature pine stands had the lowest friction values (1-2), clear-cuts of pine stands, young pine plantations, broadleaves, urban and *Pinus taeda* had intermediate frictions values (3-5) while linear woodlands with broadleaves and mixed pine and broadleaved forest had the highest values (9). These friction values were used for the rest of LCP analyses.

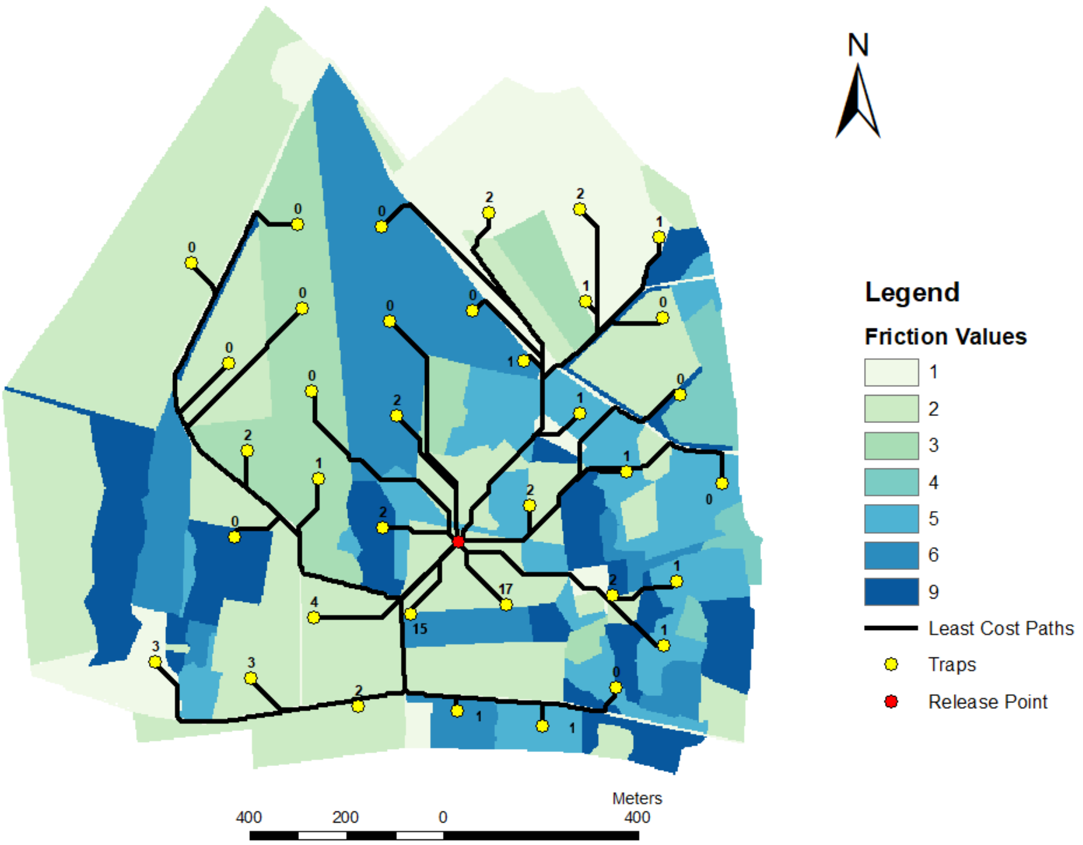

Fig. 2.2 - The least cost paths between the release point and each of the 36 traps calculated with the least cost pathway model using the set of land-use type friction values from scenario P19. Friction values of each land-use type: 1 – Open Area Mixed, Open Area Pines and Roads; 2 – Pines and Open Area Broadleaves; 3 – Clear Cut; 4 – Urban and *Pinus taeda* 5 – Broadleaves; 6 – Young Pines; 9 – Linear Broadleaves, Linear Mixed and Mixed Forest. The number of captured beetles is indicated per trap.

2.3.2. Re-finding the position of the release point using the recapture levels in the grid of traps

We verified that the buffer radius values had no significant effect on the accuracy of barycenter estimates (Dist) with the LCP method (p-value > 0.995, Appendix

S2.3). Thereafter, the LCP results used will be those based on a buffer radius of 100 m. This allowed including a larger number of surrounding land-uses, without overlapping between surrounding buffers of two adjacent traps.

Using only the coordinates and recapture data of the 36 traps to calculate weighted barycenter's, the accuracy was Dist = 31.0 m (Euclidean distance estimation method). The accuracy was improved with a reduction of Dist to 15.1 m when the friction values of the surrounding landscape (within a buffer of 100 m) were considered, (the Least Cost Path estimation method) (Fig 2.3). Using the jackknife resampling method for the 36 traps trial, both estimation methods offered similar precision, with 95% confidence intervals of 2.7 and 2.4 meters respectively for the LCP and EUC method (Fig 2.3).

For both estimation methods, the accuracy (Dist) sharply decreased as the number of traps decreased (Fig. 2.3). LCP method always provided higher accuracy over EUC method. Yet, the gain in accuracy of the LCP method over the EUC method decreased with the reduction of trap number, from 51%, 30%, 25% to 13% for grids of 36, 25, 16 and 9 traps respectively (Fig. 2.3). The two estimation methods had similar precision values (Fig. 2.3).

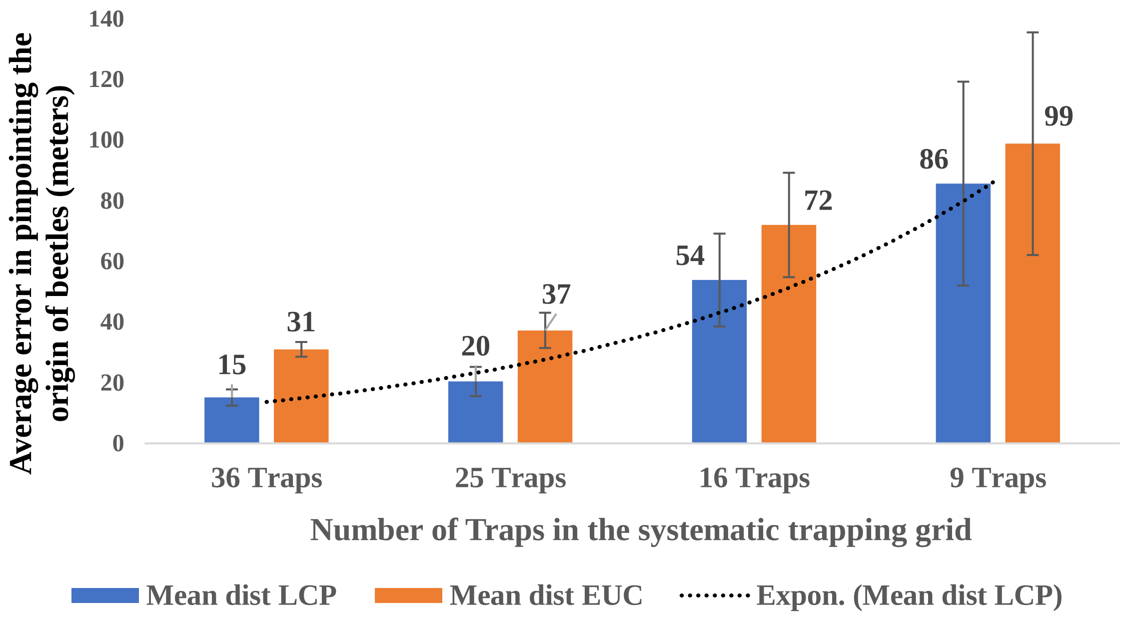

Fig. 2.3 – Average error in pinpointing the origin of beetles, i.e. distance between the real location of the release point of marked beetles and the estimated location (mean and standard error of Dist) using a weighted barycentre calculation with trap recapture data only (EUC method, orange bars) or trap recapture data and friction values of surrounding landscape (using the 100m radius buffer) around traps (LCP method, blue bars), for different trap densities.

2.4. Discussion

Combining a mark-release-recapture experiment in a heterogeneous landscape and least-cost pathways simulations, we were able to show that *Monochamus galloprovincialis*, the insect vector of the pine wood nematode, modifies its flight behavior in response to certain elements of the landscape and that this information can be used to improve the design of a trapping network to find the localization of infestation sites. The uneven distribution of the captured beetles in the trapping grid (Fig. 2.2.) was in agreement with the heterogeneity of the landscape surrounding the site of release and the distance to the release point.

2.4.1. Dispersal of the insect vector of the pine wood nematode across realistic landscapes

More specifically, it emerged from our modelling analysis that some habitat types would offer a greater resistance to *M. galloprovincialis* movement than others. Open areas with scattered pine trees received the lowest friction value (=1), suggesting that beetles move fast through these types of land uses, perhaps due to reduced obstacles to flight.

Mature pines had a slightly higher friction value (=2). They represent the main habitat of the insect vector and are therefore not avoided by dispersing beetles. However, favorable habitats with abundant feeding resources may also slow down insect dispersal due to feeding stops (Crone et al., 2019, Lutscher et al., 2017).

Interestingly clear-cuts of former pine plantations had a friction value (=3) only slightly higher than the one of mature pine stands, which indicates that they are not avoided by flying beetles. This result is consistent with the findings of Bakke (1985) and Schroeder (2013) for the conifer bark beetle *Ips typographus*. Schroeder (2019) obtained similar trap captures of *M. galloprovincialis* in clear-cut areas and pine stands. Etxebeste et al., (2016) also reported longer flight distances of *M. galloprovincialis* in fragmented than in continuous pine landscapes. The *M. galloprovincialis* non-avoidance of clear-cuts by flight, has important implications for the eradication strategy of the pine wood nematodes as it confirms that EU recommendation for clearcutting 500m around infected trees would not prevent the insect vectors from dispersing (Robinet et al., 2020). Young pine stands had a relatively high resistance value (=6) in our study. Their dense structure may impede insect dispersal and they do not provide reproduction resources.

All the land-use types containing high density of non-host tree species, principally broadleaved trees like oaks, had high friction values for the dispersal of *M. galloprovincialis*. The avoidance of patches of non-habitat may be explained by the lack of attractive chemical cues for the beetles, as they are attracted to pine terpenes, particularly during the maturation phase of young adults (Giffard et al., 2017). In addition, broadleaved trees might emit non-host volatiles that are commonly used by conifer-specialist insects to identify and avoid non-habitats (Jactel et al., 2011), according to the semiochemical diversity hypothesis (Zhang and Schlyter 2004).

However, our study had some limitations. Only one landscape was used to calculate LCP, which might reduce the generality of our results. Replicating the mark-release-recapture experiments in different landscapes of different configuration would obviously be of interest to better ascertain our estimates of friction values, although these experiments are very work intensive, especially because the recapture rates are always low for *M. galloprovincialis* (see Robinet et al., 2019 and 2020, Etxebeste et al., 2016). Another approach would be sensitivity tests based on merging certain types of land use (similar in terms of vegetation cover) to verify their effects on the estimation of friction values. Additionally, behavioral experiments, with radio telemetry, would be necessary to fully disentangle the effect of friction caused by a non-suitable habitat and the retention effect caused by an attractive habitat. Finally, the activation of pheromone traps in different land-use types, coupled with an analysis of trap captures taking into account the amount of habitat and non-habitat patches in their surroundings (Martin-Garcia et al., 2011) could provide an indirect verification of friction values.

Nevertheless, our study clearly showed that landscape composition has an effect on *Monochamus* dispersal. The effect of landscape heterogeneity on dispersal will however depend on the presence of landscape elements promoting or impeding movement and the configuration of these elements in the landscape. In theory, landscape heterogeneity can stimulate or slow down the dispersal of invasive species, depending not only on the proportion and distribution of different habitat types in the landscape but also on the variability of dispersal parameters, including the existence of long-distance dispersal events (O'Reilly-Nugent et al., 2016). However, very few empirical studies exist to validate these hypotheses. For example, Rigot et al. (2014) showed that the rate of spread of the invasive scale *Matsucoccus feytaudi* was slowed by the heterogeneity of the forest landscape using long-term monitoring of the invasion front. Another possible approach is the use of process-based dispersion models. For instance, integrating behavioral aspects such as the avoidance of non-habitat patches, would improve the realism of the individual-based model of the flight dispersal of *M. galloprovincialis* (Robinet et al., 2019), which could be then used to simulate flight trajectories in more or less heterogeneous landscapes.

2.4.2. Systematic trapping networks for the monitoring of the insect vector of the pine wood nematode

In the management of invasive species, early detection is a key element for successful eradication and containment. Soon after the arrival of a species into a new area, starts the establishment phase (Simberloff, 1997, Liebhold & Tobin, 2008) and it is well recognized that control actions need to be taken during this phase, while the invasive population has a limited distribution, in order to increase their cost-effectiveness (Simberloff, 1997). Therefore, there is a pressing need to improve the early detection of invasive species and the ability to predict the most likely locations where alien species are established in surveyed landscapes. For forest pests, this means to detect the individual trees or cluster of trees that are being colonized. This is particularly important for invasive forest pests because eradication methods often rely on the removal of host trees in demarcated areas. In the particular case of PWN, EU regulations require the felling of all susceptible trees within a buffer zone of 500m around any infected tree but it was recently suggested to rather focus on the cutting of individual trees (Robinet et al., 2020). To improve the capacity for early detection of the arrival of PWN in new forest areas we made two assumptions: 1) it will be useful to detect the nematode as carried by its insect vector, which can be trapped, in complement with the detection of the first infected trees, which are likely to be isolated in the landscape and difficult to spot; 2) the setting up of a systematic grid of traps will make it possible not only to capture the first *Monochamus* carrying the invasive nematode but also to locate the source of infestation from which they originate, thanks to a triangulation method.

Using a mark-release recapture trial with a systematic grid of traps and a calculation of the Euclidean weighted barycenter using trap coordinates and catches, it was possible to pinpoint the origin of the beetles with good accuracy (31 meters). However, the method was significantly improved when the landscape composition around each trap was taken into consideration by assigning different levels of resistance (friction) to beetle dispersal to different land-use types. The location of the release point was then predicted with a remarkable accuracy of 13m.

Establishing high-density trap grids is not realistic given the cost of installation and assessments. By simulating a reduction in trap density in systematic grids, we showed that with only 9 traps spread over 180 ha, i.e., one trap per 20 ha, we could still predict the location of the original insect outbreak with an accuracy of 86 m, i.e., in an area of about 2.5 ha. We believe that restricting the search area for infected trees with PWN dieback symptoms to an area of 2.5 ha instead of 180 ha is a real progress in optimizing the early detection of infestation spots. However, further simulations in a larger array of landscape configurations, and considering installation and maintenance costs, still need to be carried out to optimize an operational detection method based on systematic trap networks (Augustin et al., 2004; Mercader et al., 2013; Elkinton et al., 2014; Wilson et al., 2017; Sylla et al., 2017). This approach is likely to be particularly relevant for the surveillance of risk areas, including buffer zones established in the periphery of contaminated regions, such as those currently located on the border of Portugal and Spain.

2.5. Conclusions

Our study demonstrates the value of applying landscape ecology concepts and methods to improve our understanding and prediction of pest invasion processes. By using the least cost pathway method to analyze the results of a mark release-recapture experiment, we were able to demonstrate the importance of landscape composition and configuration for the dispersal of the PWN insect vector. The two main findings are that clear cuts of pine plantations did not disturb its flight path and that patches of non-habitat, composed mainly of broadleaved species, were avoided, imposing longer flight trajectories, and probably reducing the spread of the disease. A practical application of these results is that we can now better design systematic trap networks and interpret their results, taking into account the composition of the surrounding landscape. We thus propose an innovative method to locate the most likely area of origin in the landscape of trapped insects that carry the nematode. This approach should now be applied in a wider range of landscape composition, with other types of land-uses and landscapes, with different degrees of compositional and configurational heterogeneity, in order to be able to generalize its application, especially in the main areas at risk of nematode establishment.

Acknowledgements

We would like to thank Victor Rebillard and Fabrice Vétillard very much for their help in collecting, rearing, marking, releasing, and recapturing the beetles.
We gratefully acknowledge support for this work from the project PINASTER (French Ministry of Agriculture), the EU project REPHRAME KBBE.2010.1.4- 09 (FP7 Project), the EU project HOMED (H2020, grant agreement no. 771271) and from the PESSOA project (TC-05/10).

P. Nunes was supported by SUSFOR (PD/00157/2012) doctoral grant funded by the Foundation for Science and Tecnhonology (FCT) (PD/BD/142960/2018). Both P. Nunes and M. Branco are supported by CEF, a research unit funded by Foundation for Science and Technology (FCT), Portugal (UID/AGR/00239/2019 and UIDB/00239/2020).

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Supplementary material

Appendix S2.1 - Land Uses in The Study Area

Table S2.1.1 – Description of the study area's heterogenous landscape divided by land use types, and their overall spatial representation.

Land use types	Description	Area
Pines	"Mature" *Pinus pinaster* monoculture plantations (more than 8 years old).	36%
Young Pines	Juvenile *Pinus pinaster* monoculture plantations (below 8 years old trees).	14%
Broadleaves	Dense broadleaf forest areas composed mainly of *Quercus robur* and *Quercus pyrenaica*.	11%
Mixed Forest	Dense forest areas with mixed composition of both broadleaf and conifer tree species, mainly *Quercus* spp. and *Pinus pinaster*.	10%
Clear Cut	*Pinus pinaster* stand recently chopped down.	10%
Open Area Pines	Low tree density areas composed of conifer tree species, mainly *Pinus pinaster* (tree density below 20%).	8%
Open Area Mixed	Low tree density areas composed of both broadleaf and conifer tree species, mainly *Quercus robur* and *Pinus pinaster* (tree density below 20%).	3%
Open Area Broadleaves	Low tree density areas composed of broadleaf tree species, mainly *Quercus robur* (tree density below 20%).	2%
Roads	Forest roads or low traffic rural roads.	2%
Linear Mixed	Dense tree lines composed of both broadleaf and conifer tree species, mainly *Quercus robur* and *Pinus pinaster*.	1%
Linear Broadleaves	Dense tree lines composed of broadleaf tree species, mainly *Quercus robur* and *Quercus pyrenaica*.	1%
Urban	Urban structures (Buildings)	1%
Pinus taeda	Dense forest plantation solely composed of *Pinus taeda*.	1%

Appendix S2.2 – Land-use Types Friction Value Optimization Process

During this model optimization process, scenarios were created with different combinations of friction values for the land-use types in the landscape. The comparison between scenarios was made using the R^2 value obtained for each scenario based on the correlation between the least cost path value obtained for each trap and the recapture values of each trap (Log (Recap + 1)).
Models with higher R^2 values were interpreted to be better models for the flight pattern of the insects on the landscape.

Step 1 – Grouping of Land-use Types Based on Path Friction Value

Part 1

First, scenarios were created of all combinations in which each land-use type friction value was individually tested with a friction value of 4 (Table S2.2.1) or 8 (Table S2.2.2), while the other land-use type friction values were set at 1. Increase or decrease of the R^2 of each scenario was used as the comparison tool between the scenarios.

Table S2.2.1 – The resulting R^2 values of each scenario (M1-M13) developed for estimating the type of resistance each land-use type offers towards the beetle's dispersal, using a medium friction value (4). The model's performance was compared using the R^2 values.

Land-use Types	Control	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13
Pines	1	4	1	1	1	1	1	1	1	1	1	1	1	1
Open Area Pines	1	1	4	1	1	1	1	1	1	1	1	1	1	1
Open Area Mixed	1	1	1	4	1	1	1	1	1	1	1	1	1	1
Open Area Broadleaves	1	1	1	1	4	1	1	1	1	1	1	1	1	1
Roads	1	1	1	1	1	4	1	1	1	1	1	1	1	1
Young Pines	1	1	1	1	1	1	4	1	1	1	1	1	1	1
Clear Cut	1	1	1	1	1	1	1	4	1	1	1	1	1	1
Broadleaves	1	1	1	1	1	1	1	1	4	1	1	1	1	1
Mixed Forest	1	1	1	1	1	1	1	1	1	4	1	1	1	1
Pinus Taeda	1	1	1	1	1	1	1	1	1	1	4	1	1	1
Urban	1	1	1	1	1	1	1	1	1	1	1	4	1	1
Linear Broadleaves	1	1	1	1	1	1	1	1	1	1	1	1	4	1
Linear Mixed	1	1	1	1	1	1	1	1	1	1	1	1	1	4
R^2	0.263	0.073	0.184	0.232	0.252	0.252	0.311	0.289	0.359	0.300	0.263	0.263	0.268	0.267

Table S2.2.2 – The resulting R^2 values of each modelling scenario (H1-H13) developed for estimating the type of resistance each land-use type offers towards the beetle's dispersal, using a high friction value (8). The model's performance was compared using the R^2 values.

Land-use Types	Control	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13
Pines	1	8	1	1	1	1	1	1	1	1	1	1	1	1
Open Area Pines	1	1	8	1	1	1	1	1	1	1	1	1	1	1
Open Area Mixed	1	1	1	8	1	1	1	1	1	1	1	1	1	1
Open Area Broadleaves	1	1	1	1	8	1	1	1	1	1	1	1	1	1
Roads	1	1	1	1	1	8	1	1	1	1	1	1	1	1
Young Pines	1	1	1	1	1	1	8	1	1	1	1	1	1	1
Clear Cut	1	1	1	1	1	1	1	8	1	1	1	1	1	1
Broadleaves	1	1	1	1	1	1	1	1	8	1	1	1	1	1
Mixed Forest	1	1	1	1	1	1	1	1	1	8	1	1	1	1
Pinus Taeda	1	1	1	1	1	1	1	1	1	1	8	1	1	1
Urban	1	1	1	1	1	1	1	1	1	1	1	8	1	1
Linear Broadleaves	1	1	1	1	1	1	1	1	1	1	1	1	8	1
Linear Mixed	1	1	1	1	1	1	1	1	1	1	1	1	1	8
R^2	0.263	0.052	0.109	0.202	0.252	0.236	0.270	0.263	0.359	0.305	0.263	0.263	0.269	0.270

Based on the increase of R^2 from each individually tested land-type (Table S2.2.1 & Table S2.2.2), we attributed each land-use type to one of the following groups: Group 1 = Highest R^2 at friction = 1: “Pine Forest”, “Open area mixed”, “Open area pines”, “Open area broadleaves”, “Roads”

Group 2 = Highest R^2 at friction = 4: “Young pines”, “Clear-cut”

Group 3 = Highest R^2 at friction = 8: “Linear mixed”, “Linear broadleaves”

Group 4 = Highest R^2 at friction = 4 and 8: “Broadleaves”, “Mixed Forest”

Note: *“The land-use types “*Pinus taeda*” and the “Urban” had limited distribution in the study landscape, their contribution towards the paths between any trap and the release point is practically non-existent. This resulted in their friction values having no significant impact to the R^2. Since our study does not permit to accurately test their effect, these two land-use type friction values were locked at the value of 4, as an intermediate value and were not tested any further.”

Part 2

For group 4, the two land-use type’s friction values in the group were tested again between the value of 4 and 8, but now using all the other land-use type values established by the groups made in part 1. For this, 4 different scenarios were tested, representing each possible combination for the 2 land-use types using the cost value of 4 or 8 (Table S2.2.3).

Table S2.2.3 – The resulting R^2 values of each modelling scenario (C1-C4) developed to estimate the model's performance improvement with the change of friction values, between medium (4) or high (8), for the Broadleaves Forest areas and Mixed Forest areas. The model's performance was compared using the R2 values.

Land-use Types	C1	C2	C3	C4
Pines	1	1	1	1
Open Area Broadleaves	1	1	1	1
Open Area Mixed	1	1	1	1
Open Area Pines	1	1	1	1
Roads	1	1	1	1
Young Pines	4	4	4	4
Clear Cut	4	4	4	4
Broadleaves	4	8	8	4
Mixed Forest	4	8	4	8
Pinus Taeda	4	4	4	4
Urban	4	4	4	4
Linear Broadleaves	8	8	8	8
Linear Mixed	8	8	8	8
R^2	0.404	0.483	0.470	0.564

These results allowed us to merge the land-use types in Group 4 into existing groups. Since the scenario that produced the best result had broadleaves cost value at 4 and mixed forest cost value at 8 (C4); "Broadleaves" were moved to Group 2 and "Mixed Forests" were moved to Group 3.

Step 2 – Friction value Group Optimization

Using the 3 groups of land-use types established in step 1. Each land-use type in a group was separately tested in for values close to their previously assigned cost value.

First, we started with group 1, with the assigned cost value of 1. Two different scenarios were created in which the friction values of the 5 land-use types varied between 2 and 3. The developed scenarios offered lower R^2 results than the original, therefore the friction values of the land-use types inside this group were kept at 1 (Table S2.2.4).

For group 2, assigned friction values of 4, three different scenarios were created with different friction values of the 3 land-use types between 3, 5 and 6. All tested scenarios offered lower R^2 results compared to the original scenario, hence the friction value of the land-use types of groups 2 were kept at 4 (Table S2.2.4).

Finally, for group 3, with assigned friction values of 8, three different scenarios were created in which different friction values for the 3 land-use types were tested between 6, 7 and 9. There was an improvement of R^2 in the scenario with friction value of 9 (scenario C8) and therefore the friction value of the land-use types in group 3 was changed to 9 (Table S2.2.4).

Table S2.2.4 – Least cost path model scenarios where the change of friction value for each group of land-use types was tested towards explaining the trap's recapture values, using the R2 value to compare each model's performance.

Land-use Types	C5	C6	C7	C8	C9	C10	C11	C12
Pines	1	1	1	1	1	1	2	3
Open Area Broadleaves	1	1	1	1	1	1	2	3
Open Area Mixed	1	1	1	1	1	1	2	3
Open Area Pines	1	1	1	1	1	1	2	3
Roads	1	1	1	1	1	1	2	3
Young Pines	5	6	3	4	4	4	4	4
Broadleaves	5	6	3	4	4	4	4	4
Clear Cut	5	6	3	4	4	4	4	4
Pinus Taeda	4	4	4	4	4	4	4	4
Urban	4	4	4	4	4	4	9	9
Mixed Forest	8	8	8	9	7	6	4	4
Linear Broadleaves	8	8	8	9	7	6	9	9
Linear Mixed	8	8	8	9	7	6	9	9
R^2	0.535	0.506	0.562	0.571	0.558	0.184	0.478	0.379

Step 3 – Individual Friction value Optimization

Using the land-use friction values obtained from the group testing in Step 3, we created scenarios aiming to enhance the model (R^2) through individual optimization of the friction value of each land-use type. This optimization was accumulative, meaning that changes during the optimization process will be ongoing throughout the whole process.

We started with the individual optimization of the friction values of land-use types in group 1, Which were all currently assigned with a friction value of 1.

The five different land use types were individually tested with scenarios in which their friction value was changed between the values of 2 and 3 (Table S2.2.5).

1. The Pines land-use type friction value of 2 offered the best results. The friction value of this land-use type was then changed to 2 for the remainder of the process.
2. The Roads land-use type friction cost remained best at the original cost of 1, no changes were made.
3. The Open Area Broadleaves land-use type friction value offered the best results at 2, The friction value of this land-use type was changed to 2 for the remainder of the process.
4. The Open Area Mixed land-use type friction cost remained best at the original cost of 1, no changes were made.
5. The Open Area Pines land-use type friction cost remained best at the original cost of 1, no changes were made.

Table S2.2.5 – Least-Cost path modelling simulations for the variation of the friction values of the land-use types in group 1 ("Pines", "Open Area Broadleaves", "Open Area Mixed", "Open Area Pines" and "Roads"), towards the model's performance (R^2).

Land-use Types	P1	P2	P5	P6	P7	P8	P9	P10	P3	P4	P11	P12	P13	P14
Pines	2	3	2	2	2	2	2	2	2	2	2	2	2	2
Open Area Broadleaves	1	1	2	3	2	2	2	2	1	1	2	2	2	2
Open Area Mixed	1	1	1	1	2	3	1	1	1	1	1	1	1	1
Open Area Pines	1	1	1	1	1	1	2	3	1	1	1	1	1	1
Roads	1	1	1	1	1	1	1	1	2	3	1	1	1	1
Young Pines	4	4	4	4	4	4	4	4	4	4	3	5	6	7
Broadleaves	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Clear Cut	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Pinus Taeda	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Urban	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Linear Broadleaves	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Linear Mixed	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Mixed Forest	9	9	9	9	9	9	9	9	9	9	9	9	9	9
R^2	0.584	0.471	0.609	0.589	0.589	0.555	0.565	0.519	0.499	0.492	0.587	0.616	0.618	0.616

The same was done for the three different land-use types in group 2, but with the friction values of the scenarios being between the values of 3, 5, 6 and 7 (Table S2.2.6).

1. The Young Pines land-use type friction value of 6 offered the best results. The friction value of this land-use type was then changed to 6 for the remainder of the process.
2. The Broadleaves land-use type friction value of 5 offered the best results. The friction value of this land-use type was then changed to 5 for the remainder of the process.
3. The Clear-cut land-use type friction value of 3 offered the best results. The friction value of this land-use type was then changed to 3 for the remainder of the process.

Table S2.2.6 – Least-Cost path modelling simulations for the variation of the friction values of the land-use types in group 2 ("Young Pines", "Broadleaves" and "Clear Cut"), towards the model's performance (R^2).

Land-use Types	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22
Pines	2	2	2	2	2	2	2	2	2	2	2	2
Open Area Broadleaves	2	2	2	2	2	2	2	2	2	2	2	2
Open Area Mixed	1	1	1	1	1	1	1	1	1	1	1	1
Open Area Pines	1	1	1	1	1	1	1	1	1	1	1	1
Roads	1	1	1	1	1	1	1	1	1	1	1	1
Young Pines	3	5	6	7	6	6	6	6	6	6	6	6
Broadleaves	4	4	4	4	3	5	6	7	5	5	5	5
Clear Cut	4	4	4	4	4	4	4	4	3	5	6	7
Pinus Taeda	4	4	4	4	4	4	4	4	4	4	4	4
Urban	4	4	4	4	4	4	4	4	4	4	4	4
Linear Broadleaves	9	9	9	9	9	9	9	9	9	9	9	9
Linear Mixed	9	9	9	9	9	9	9	9	9	9	9	9
Mixed Forest	9	9	9	9	9	9	9	9	9	9	9	9
R^2	0.587	0.616	0.618	0.616	0.590	0.622	0.611	0.589	0.627	0.610	0.595	0.584

Finally, the same was done for the three different land use types in group 3, but with the friction values used for the scenario creation varying between the values of 7 and 8 (Table S2.2.7).

1. The Linear Broadleaves land-use type friction cost remained best at the original cost of 9, no changes were made.
2. The Linear Mixed land-use type friction cost remained best at the original cost of 9, no changes were made.
3. The Mixed Forest land-use type friction cost remained best at the original cost of 9, no changes were made.

Table S2.2.7 – Least-Cost path modelling simulations for the variation of the friction values of the land-use types in group 3 ("Linear Broadleaves", "Linear Mixed" and "Mixed Forests"), towards the model's performance (R^2).

Land-use Types	P23	P24	P25	P26	P27	P28
Pines	2	2	2	2	2	2
Open Area Broadleaves	2	2	2	2	2	2
Open Area Mixed	1	1	1	1	1	1
Open Area Pines	1	1	1	1	1	1
Roads	1	1	1	1	1	1
Young Pines	6	6	6	6	6	6
Broadleaves	5	5	5	5	5	5
Clear Cut	3	3	3	3	3	3
Pinus Taeda	4	4	4	4	4	4
Urban	4	4	4	4	4	4
Linear Broadleaves	8	7	9	9	9	9
Linear Mixed	9	9	9	7	9	9
Mixed Forest	9	9	9	9	8	7
R^2	0.627	0.626	0.627	0.627	0.622	0.617

With the model optimization process completed, the scenario that enveloped all our changes and offered the highest model performance was scenario P19, with a R^2 value of 0.627 (Table S2.2.8)

Table S2.2.8 – Model simulation’s Friction values of each land-use type that offered the highest explanatory ability towards the trap recapture values (R^2).

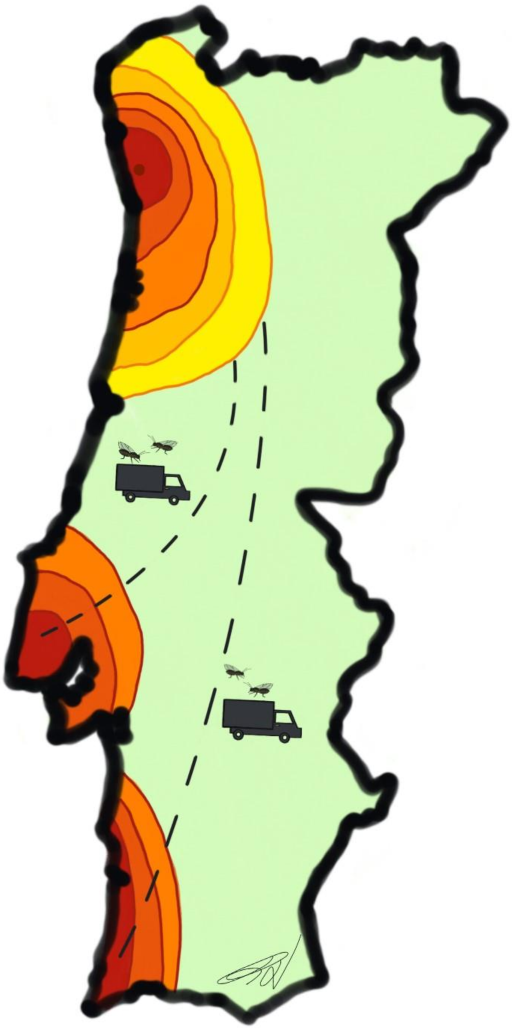
Land-use Types	Scenario P19
Pines	2
Open Area Broadleaves	2
Open Area Mixed	1
Open Area Pines	1
Roads	1
Young Pines	6
Broadleaves	5
Clear Cut	3
Pinus Taeda	4
Urban	4
Linear Broadleaves	9
Linear Mixed	9
Mixed Forest	9
R^2	0.627

Appendix S2.3 – Estimation of the infestation barycenter based on trapping grids with the trap reduction.

Table S2.3.1 - The calculated mean Distance (meters) between the estimated barycenter coordinate and the original release location coordinate. The estimated barycenter was calculated using the LCP method for four different buffer distances (50, 100, 150 and 200 meters). Additionally, the gain in accuracy (%) of the use of the LCP method over the EUC method is displayed alongside the 95% margin of error (ME) of both methods.

Traps	Buffer	Mean dist LCP	Mean dist EUC	Gain in Accuracy (%)	ME (95%) LCP	ME (95%) EUC
36	50m	12.99	30.99	58.08	2.46	2.43
	100m	15.11		51.25	2.69	
	150m	12.53		59.57	2.55	
	200m	12.27		60.41	2.52	
25	50m	20.38	37.25	45.28	3.81	5.81
	100m	26.05		30.08	4.83	
	150m	26.40		29.14	4.76	
	200m	25.17		32.44	4.74	
16	50m	50.87	72.01	29.36	13.41	17.22
	100m	53.85		25.21	15.30	
	150m	54.53		24.27	15.75	
	200m	57.61		20.00	17.08	
9	50m	79.59	98.82	19.46	28.97	36.72
	100m	85.65		13.33	33.62	
	150m	88.93		10.01	36.07	
	200m	90.87		8.04	37.31	

Chapter 3. Modelling the invasion dynamics of the African citrus psyllid: The role of human-mediated dispersal and urban and peri-urban citrus trees



Published in Neobiota

Nunes, P., Robinet, C., Branco, M., & Franco, J.C. (2023). Modelling the invasion dynamics of the African citrus psyllid: The role of human-mediated dispersal and urban and peri-urban citrus trees. *NeoBiota*, *84*, 369-396. https://doi.org/10.3897/neobiota.84.91540

Abstract

The African citrus psyllid*, Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae), is native to tropical Africa and invasive species in North America and Europe. The main host plants are citrus, displaying a preference for lemon trees. This psyllid was recently detected in the Northwest region of the Iberian Peninsula, both in Spain and Portugal. Here, we used a model combining a reaction-diffusion model to a stochastic long-distance dispersal model to simulate the invasion dynamics of *T. erytreae* in Portugal. The psyllid spread in Portugal was simulated between 2015 and 2021 for different combinations of model parameters: two fecundity levels; spread with and without stochastic long-distance dispersal; single or two introductions of *T. erytreae*; and considering or not the urban and peri-urban citrus trees, besides citrus orchards, estimated using Google Street view imagery. The incorporation of long-distance human mediated dispersal significantly improved the F1-Score in the model validation using the official reports as the observed data. Concomitantly, the dispersal rate of *T. erytreae* in Portugal was on average about 66 km/year, whereas removing long-distance dispersal events, the observed mean was 7.8 ± 0.3 km/year. The dispersal was mainly towards the South along the coastline, where the human population is concentrated. The inclusion of the estimated citrus trees outside orchards areas significantly increased the F1-Score in the model validation, revealing the importance these isolated host plants hold as stepping stones for the specie's current invasion and possibly for other species alike.

Keywords: invasive, insect vectors, isolated trees, models, psyllids, non-native species, spread, *Trioza erytreae*

3.1. Introduction

Pest species introductions outside the native range have significantly increased in the last decades worldwide (Walther et al., 2009; Seebens et al., 2017; Turner et al., 2021). This is mainly attributed to the intensification of global trade and human travel (Brockerhoff & Liebhold, 2017; Roques 2010). Some of these introduced species are invasive, causing high ecological and/or economic impact in agricultural or forest ecosystems (Pimentel et al., 2005; Kenis et al., 2009; Zenni et al., 2021).

The African citrus psyllid, *Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae), is a small sap-sucking insect, native to tropical Africa (Moran & Blowers 1967). The main host plants of *T. erytreae* are Rutaceae, such as citrus, displaying a preference for lemon trees (*Citrus limon*) over sweet orange trees (*Citrus sinensis*) (Aubert 1987). The psyllid was recently introduced in Continental Europe. It was detected in the Northwest region of the Iberian Peninsula, both in Spain in 2014, and in Portugal in 2015 (Monzó et al., 2015; Pérez-Otero et al., 2015; DGAV 2015). The introduction of this invasive species has drawn major attention, as it is a vector of the huanglongbing (HLB), also known as the greening disease (McClean & Oberholzer, 1965), considered the most damaging citrus disease in the world, caused by gram negative bacteria, *Candidatus liberibacter* spp. (Bové, 2006; Gottwald, 2010), which is not yet present in Europe. Illustratively, since HLB was introduced in Florida, orange production dropped by 74%, between 2005 and 2019 (Singerman & Rogers, 2020). In Europe, *T. erytreae* and HLB are classified as A2 (Annex II B) and A1 quarantine pests (Annex II A), respectively (EU, 2019; EPPO, 2022a; 2022b).

Since its detection, *T. erytreae* has been expanding southwards along the Portuguese coastal area, despite the phytosanitary measures that were implemented by the Ministry of Agriculture to contain its spread (DGAV 2015). It has a high invasive potential due to its high fecundity (between 327 and 827 eggs per female), no diapause, and multivoltine biological cycle (up to 8 generations per year) (Catling 1969, 1972; Moran & Blowers, 1967; Tamesse & Messi, 2004). However, the number of yearly generations can be reduced to 3 in hot and dry summer conditions or other unfavorable conditions for the host leaf flushing, as

T. erytreae reproduction requires the availability of young leaf shoots (Catling 1972; Tamesse & Messi, 2004; Cocuzza et al., 2017). Adults of *T. erytreae* were estimated to fly up to 1.5 km, especially if forced by external factors, such as lack of leaf flushes (Samways & Manicom, 1983; Van den Berg & Deacon, 1988). Its dispersal may be further aided by wind currents, as was shown in the case of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Aubert & Hua, 1990; Antolinez et al., 2021). Human activities, such as the transportation of fruit or plant material, may also be involved in the long-range dispersal of the species (Aubert & Hua, 1990; Antolinez et al., 2021). This has been shown for other agricultural and forest pests (Shigesada & Kawasaki, 1997; Tobin & Blackburn, 2008; Robinet et al., 2009), including the Asian citrus psyllid (Halbert et al., 2010).

Citrus orchards in Portugal represent around 19,000 ha, mostly concentrated in the South, the major production region (EU 2021). However, besides citrus orchards, it is common to find citrus plants in urban and peri-urban landscapes, all over the country, including urban trees in villages and cities, mostly in central and southern Portugal, but also trees in home gardens and backyards (Duarte, 2012). These citrus trees are not included in the official statistics and may represent a significant area, within the global spatial distribution range of citrus plants in Portugal, which has not been estimated. They may have an important role in the dispersal of *T. erytreae*. Indeed, scattered host plants could play an important role in the spread of invasive species in general, as it was clearly demonstrated for the pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Rossi et al., 2016). For *T. erytreae*, isolated citrus trees may be a reservoir of the psyllid (Van den Berg et al., 1991), and they are expected to influence the connectivity between the fragmented citrus producing lands.

A few studies used the bioclimatic suitability of the different geographic regions of Portugal and Spain to predict the potential spread of *T. erytreae* (Benhadi-Marín et al., 2020, 2022; Paiva et al., 2020). Paiva et al., (2020) used the water vapour pressure deficit (based on the results of Green & Catling (1971)) to predict climate suitability for the psyllid, in Portugal, based on data collected from 18 weather stations, distributed along the country. Benhadi-Marín et al., (2020) carried out a pest risk analysis modelling approach to predict the expected spread of *T. erytreae* in the Iberian Peninsula. They compared three models, (1) a radial range expansion model, (2) a hybrid model of logistic growth and radial rate, and (3) the deterministic version of the dispersal kernel model. The kernel model with two hypothetical entry points (Vila Nova de Arousa, in Spain and Porto, in Portugal) showed to accurately predict the distribution of the psyllid with respect to latitude, five years after its detection. More recently, the same research team refined the approach used previously (Benhadi-Marín et al., 2022), by: improving the spatial data resolution (1 km); including a physical barrier (altitude of 400 m) and long-distance dispersal events (cells up to 500 km apart were allowed to be colonized) for modeling purposes; extending the prediction to 30 years after the introduction of *T. erytreae* in the Iberian Peninsula; simulating different scenarios (very low, low, medium, and high spread). Using this approach, Benhadi-Marín et al., (2022) identified three key risk areas, one in Portugal, the citrus growing areas of Setúbal, and two in Spain, Huelva and the potential citrus corridor that connects Guipúzcoa, claiming that these areas should have special attention for the monitoring of *T. erytreae.*

To explore the role of human activities in the spread of *T. erytreae* in continental Portugal, we used a model that combined a reaction-diffusion model to simulate the natural spread of the species and a stochastic long-distance dispersal model to simulate human mediated dispersal of the species. Reaction-diffusion models have been commonly used for simulating the spatial spread of invasive species as they describe both population growth and population dispersal in spatially explicit way to provide an estimate of population density over time and space (e.g., Shigesada & Kawasaki, 1997). These models describe diffusive dispersal (e.g., dispersal into adjacent habitats), but cannot describe jumps at long distances. To model explicitly a stratified dispersal (allowing both diffusive dispersal and long-distance jumps), we thus combined a reaction-diffusion model to a stochastic long distance dispersal model. This approach has been previously used to explore the role of human mediated dispersal in expanding insect populations (e.g., Robinet et al., 2019). To our knowledge, this is the first time this modeling approach is used for *T. erytreae.* Our specific objectives were: (i) to understand the role that human-mediated spread has played in the current invasion of the psyllid in Portugal; (ii) to highlight the importance of trees outside citrus orchards in the psyllid's spread and the general importance that isolated trees data can have for large-scale pest species modelling. With this aim, we provided an important innovation in the utilization of Google Street view imagery to estimate citrus trees density in urban and peri-urban areas. Finally, we tested the hypothesis of multiple introductions of *T. erytreae*, as suggested by Ruíz-Rivero et al., (2021).

3.2. Material and Methods

3.2.1. Model development

A reaction-diffusion model was developed to simulate the local spread of *T. erytreae* since its arrival in Portugal for the whole continental area of the country. This type of modelling is commonly used for describing the spatial spread of invasive species (e.g., Shigesada & Kawasaki, 1997). The model which incorporates the dispersal of the species and the population's growth can be expressed using the following Fisher equation:

$$\frac{\partial N}{\partial t} = D\left(\frac{\partial^2 N}{\partial x^2} + \frac{\partial^2 N}{\partial y^2}\right) + rN\left(1 - \frac{N}{K}\right)\mathrm{e} \qquad \text{eq.1}$$

where N is the population density of *T. erytreae* (km^{-2}), dependent on time t and spatial location (x,y); D is the coefficient of diffusion (km^2 /year); r is the population growth rate ($year^{-1}$); and K is the carrying capacity (km^{-2}). The model exhibits a travelling wave with a constant spread rate (C; km/ year) defined by:

$$C = 2\sqrt{rD} \qquad \text{eq.2}$$

where C, K, and r were all estimated beforehand (see text below), while D was assumed to be homogeneous across Portugal, calculated based on eq. 2 (similarly to Robinet et al., 2017), considering C and r of the area infested by *T. erytreae* in the end of 2015.

The model described was applied over a grid of 5km×5km resolution, from 2015 to 2021 with a yearly time step.

The population dynamics and spread parameters were obtained based on previous research studies on the biology and ecology of the psyllid, so that model simulation could be then validated using the independent presence/absence of observed data.

3.2.2. Spread rate and carrying capacity

The estimate of the local spread rate *C* (6 km/year) was based on the maximum flight capacity of *T. erytreae* determined by Van den Berg and Deacon (1988), i.e., 1.5 km, multiplied by 4, the estimated number of yearly generations that T. erytreae can have in Porto, where *T. erytreae* was first detected.

The carrying capacity *K* was estimated for each cell in the model, as the product of the average maximum capacity of *T. erytreae* per host tree, k_{tree}, and the estimated number of citrus trees in the cell. k_{tree} was determined using data from Catling (1972), corresponding to the mean number of individuals per citrus tree (adults, nymphs and eggs), observed in a 3-year study, under favourable conditions, using the following formula:

$$k_{tree} = \text{Adults} + (0.289 \times \text{Nymphs} + (0.289 \times 0.95 \times \text{Eggs})) \qquad \text{eq. 3}$$

where 0.289 is the nymphal survival rate at natural conditions (Caitling 1970) and 0.95 is the egg viability in optimum environmental conditions (Catling 1972; Van der Berg et al., 1991) to obtain k_{tree} = 2719 adults of *T. erytreae* per tree.

To estimate the spatial density of citrus trees in orchards, throughout the country, we used the data from the 2019 agricultural census (INE 2021). This dataset does not include citrus plants in urban and peri-urban areas, as well as isolated citrus trees in rural landscapes. The dataset from INE (2021) provides the area of citrus orchards per county. The density of citrus trees in orchards was assumed, for simplicity, to be the same all over the territory, i.e., 400 citrus trees/ha, considering 5 m x 5 m per tree. Although citrus tree spacing may vary between 5m x 4 m or lower and 7m x 5 m (Cavaco & Calouro, 2005, Vacante & Gerson, 2012), we considered for simplicity a median value of 5 m x 5 m. The citrus tree density for each 25 km^2 grid cell of the model was estimated based on spatial data from the Land Use and Occupancy Mapping - COS2018, (available at https://www.dgterritorio.gov.pt/) and the data from the 2019 agriculture census, (INE 2021), about the area of citrus orchards.

As data on the density of citrus trees in urban and peri-urban areas was not available from the 2019 agricultural census (INE 2021) and considering its possible influence on the dispersal of *T. erytreae,* we developed an innovative approach to estimate it. We used the spatial data of the COS2018 dataset, to classify the urban and peri-urban areas. Then, we divided these areas into three different classes: Vertical urban areas; Horizontal urban areas; and Discontinuous urban areas (see S1). For each class, the mean density of citrus trees was estimated using Google Street view imagery to survey the number of visible trees in randomly selected polygon areas extracted from COS2018 spatial dataset along the country (Rousselet et al., 2013; Berland & Lange, 2017). The estimations were made using the survey counts of citrus trees in each area, weighted against the sample area sizes. Only areas with good image quality were used. We surveyed at least 250 ha for each of the three urban classes considered to provide a confident estimation of citrus tree density. All the surveys were conducted by the first author.

3.2.3. Growth rate

To calculate the growth rate, *r*, for *T. erytreae* in Portugal, we used climatic modelled data collected for 30 years (Palma 2017). This data was collected for each centroid of a grid of 25km x 25km covering Portugal. The climatic variables considered were the daily mean temperature, daily maximum temperature, and the daily minimum relative air humidity. The average daily climatic data was grouped into three periods per month. Each period had 10 days, except the last third of each month, which varied from 8 to 11 days, depending on the month. These periods are henceforth called as “10-day periods”, needed to calculate *T. erytreae* survival rate, using the method developed by Catling (1969).

For each 10-day periods, the number of “viable days” (i.e., the number of days above the lower temperature threshold for development) was calculated. Temperatures were estimated based on the average of the 30 years of the climatic data. A lower temperature threshold of 12.0ºC was considered, based on citrus tree growth not occurring below these temperature values (Webber 1943; Kumar 1977), as well as the inability of *T. erytreae* female oviposition and larva growth (Catling, 1973). An upper temperature threshold was not used since we took into consideration the effect of high temperature and low humidity in weather survival.

Weather Survival (*WS*%) was further calculated for each 10-day period, using the mean Vapour Pressure Deficit (*VPD*) of the three days with the highest daily values of maximum temperature, using Saturated Vapour Pressure (*SVP*) (Green & Catling, 1971; Murray 1967):

$$VPD = ((100 - RH)/100) \times SVP \qquad \text{eq. 4}$$

$$SVP = 610.7 \times 10^{7.5Tmax/(237.3+Tmax)} \qquad \text{eq. 5}$$

$$\text{Weather Survival } (WS\%) = 0.0308\ X_3^2 - 4.1825\ X_3 + 137.7709 \qquad \text{eq. 6}$$

where X_3 is the mean value of the *VPD* in millibars, of the three days with the highest maximum temperatures during the 10-day period.

For the model calculations, we used Weather Mortality (*WM*%):

$$WM = 1 - (WS/100) \qquad \text{eq. 7}$$

For each area, the number of possible yearly generations was then estimated, as the sum of life cycle progress rounded down from each yearly 10-day period from February until the end of September, the most important period of leaf flushing for citrus trees in Portugal (Paiva et al., 2020). Life Cycle Progress was calculated by dividing the average viable days and the estimated total life cycle duration in days for each 10-day period, calculated using the average temperature of the viable days (*VD*) and the life cycle duration (*G*), that is the expected total number of days to successfully complete the insect life cycle from egg to adult.

$$\text{Life Cycle Progress} = VD / G \qquad \text{eq. 8}$$

The life cycle duration *G*, in days, was calculated based on the number of days needed to complete egg incubation (*Idays*) plus the number of days needed to complete nymphal growth (*Ndays*) based on the equations proposed by Catling (1969). To this period, we added 5 days of the pre-oviposition period, being the mean of the pre-oviposition time of the species (Van der Merwe 1923; Catling 1973), and another 10 days to reach the peak of oviposition (Catling 1973).

$Idays = 4.9763 + 3.3443 \times 0.8452^{Tmed-20}$ eq. 9

$Ndays = 16.7974 + 5.2726 \times 0.7843^{Tmed-20}$ eq. 10

$G = Idays + Ndays + 15$ eq. 11

The growth rate at a given time period *t* in the year, R_t was calculated as:

$R_t = f/2 \times (1-WM) \times (1- 0.8\ WM) \times (1- 0.3\ WM) \times (1- 0.15\ WM) \times (1- 0.075\ WM) \times 0.289$ eq. 12

where *f* is the female fecundity, estimated by the average number of eggs per female. Due to the different fecundity estimates reported in the literature, we tested two different values, i.e., 827 and 327 eggs per female (Moran & Blowers, 1967; Catling, 1969), in the model. The mean fecundity value was multiplied by egg viability rate, estimated as 0.95 in optimal environmental conditions (Catling 1972). We assumed a sex ratio of 1:1 (Begemann, 1984). *WM*, 0.8 *WM*, 0.3 *WM*, 0.15 *WM*, 0.075 *WM* correspond to the weather mortality for the 1st, 2nd, 3rd, 4th and 5th instars, based on the fact that larval stages increasingly become more resilient to the adverse environmental conditions (Catling, 1972). Finally, 0.289 is the natural survival rate from egg to adult in "perfect conditions", with the presence of natural enemies, according to Catling (1970).

For the remaining generations, we considered a geometric growth, $N_t = N_{t-1} R_t$

The resulting $R^G = \prod R_t$, where *G* is the maximum potential generations in each cell per year, will reflect the total growth potential of the species in the area for one year (Pointeau et al., 2021). The intrinsic yearly growth rate of the cell is then calculated as follows:

$r = \ln(R^G)$ eq.13

We repeated this procedure for each cell of the model.

A cold limitation factor was further generated considering the reproduction of this species is limited by extremely cold temperatures. Cold and long winter periods can hinder population reproduction and limit the species' capacity to stay in the region throughout the entire year. Thus, we consider that regions having on average less than 2 days of viable days (i.e., with a mean daily temperature above 12.0ºC), for the three consecutive winter months (December, January, and February), were not suitable for *T. erytreae* to establish. The model forced that in these areas, the number of *T. erytreae* was always 0. The period of three months is based on the maximum longevity of 82 days recorded for the species during winter (Caitling, 1969).

3.2.4. Long distance dispersal

Modelling long-distance dispersal is always very challenging since it is based on stochastic and relatively rare dispersal events. To model human-mediated long-distance dispersal, we calculated the number of long-distance dispersal events (*NB*) that occur in each year, which was randomly chosen using the following equation:

$$NB = 1 + e \qquad \text{eq. 14}$$

where *e* denotes an independent and identically distributed random variable with a Poisson distribution with mean $\boldsymbol{\lambda} = 5\ \ln((P/2642) + 1/\ln(2))$, *P* is the number of cells estimated to be infested at a given time *t*, and 2642 is the total number of cells that can be infested in the model.

We used the Poisson distribution as it is a simple discrete distribution that is often used to model jump processes (Hooten & Wikle, 2008). The baseline mean $\boldsymbol{\lambda}$ is defined as a concave increasing function of *P*, with a minimum value of 1.8 when $P = 0$, and a maximum value of 4.5 when $P = 2642$. In this way, the number of simulated long-distance jumps increases with the area infested, taking relatively realistic values compared to the spread pattern observed.

For parameter testing, besides the simulations using the baseline $\boldsymbol{\lambda}$ for medium-frequency jumps, we also considered low- and high-frequency jumps, as well as the case of no jumps, as described in Table 3.1,

For each long-distance dispersal event, the model randomly chooses a cell that is not yet infested (N<1), with a growth rate r>1 and a suitable human population density. The minimum human density threshold (*H*) allowing the arrival of a long-distance jump was set to 125 habitants/km^2, using the same threshold considered for the spread of the yellow-legged wasp in France (Robinet et al., 2017), which also delimit urban areas relatively well in Portugal as well, being mostly in the coastal areas of the country, represented as dark red areas in Fig. 3.1. A recent study showed that both the distance to roads and urbanization intensity play an important role in spatial and temporal dynamics of the dispersal of the Asian citrus psyllid, *D. citri* in California (Bayles et al., 2022). We did not use road network data nor urbanization intensity due to lack of reliable complete data available for Portugal. Instead, we used human population density (data from 2017 available at https://www.ine.pt/), as it was demonstrated to being a suitable proxy for road traffic in Portugal (Barata 2012).

Cells infested by such long-distance jumps received an arbitrary set of 50 individuals.

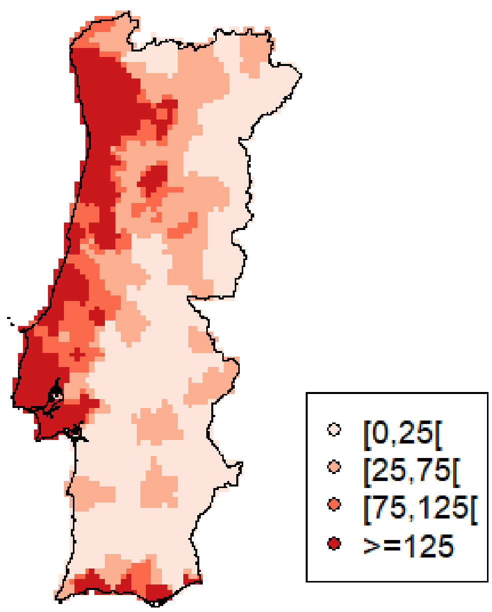


Fig. 3.1 – Distribution of the human population density in Portugal (number of inhabitants/km^2) in 2017. The human population threshold of 125 habitants/km^2 was used to characterize locations where long-range distance dispersal could occur in the model.

3.2.5. Multiple introductions

A molecular study on the genetic diversity of *T. erytreae* populations in the Iberian Peninsula suggested the possibility of multiple introductions of the psyllid (Ruíz-Rivero et al., 2021). Two main genetic clusters were observed along the Portuguese coast. Based on these results, we defined two possible scenarios in the model: 1) one single introduction of *T. erytreae*, in the North, Porto city in 2014; 2) two introductions, the first in the North, Porto city in 2014, and a second in the region of Lisbon in 2017, which has an international port and airport. For the second introduction scenario, we added 1000 individuals to a specific cell in the Lisbon area, where *T. erytreae* was first detected in the region (data provided by DGAV reports, DGAV (2021)).

3.2.6. Model running and validation

We combined estimates of local spread and growth with estimates of long-distance dispersal. We applied these models on a grid that covers Portugal with a spatial resolution of 5 km x 5 km. The simulation began in 2014 in one cell in the Porto region with 1000 individuals for the initial condition of the invasion, which was later discovered already spreading, in 2015.

Since the first detection of *T. erytreae* in Porto, in 2015, the Portuguese National Plant Protection Authority, has been monitoring the species spread at the parish level, with the deployment of a trapping protocol surrounding the infested areas within a 3 km buffer range, with yellow-sticky traps and additional, monitoring within 10 km range. In addition, they also included reports from citizens and stakeholders, after confirming their authenticity. Reports are publicly available whenever there are newly infested areas (DGAV, 2021). This data set was provided to us by DGAV. We compiled it yearly and used it to validate our model, as well as determining the dispersal capacity, from 2015 until the end of 2021. All the parameters used for the model were estimated independently from this presence/absence observed data, allowing for independent validation.

To validate the model and identify the dispersal scenarios that best fit the observed data, the species' spread was simulated between 2014 and 2021 for different combinations of model parameters (Table 3.1).

For the simulations considering long-distance dispersal (stochastic sub-model), we ran 300 replicate simulations using randomly generated long-distance events.

Table 3.1 – Parameters and scenarios tested in the modelling.

Model parameters	Scenarios tested	Details
Long-distance dispersal (LDD)	No, Low, Medium, High	No: No LDD Low: $\boldsymbol{\lambda} = 5 \ln((P/2642) + 1/\ln(2)) / 2$ Medium: $\boldsymbol{\lambda} = 5 \ln((P/2642) +1/\ln(2))$ High: $\boldsymbol{\lambda} = 5 \ln((P/2642) + 1/\ln(2)) \times 2$
Fecundity (Fecund)	Low, High	Low: 327 eggs/female High: 827 eggs/female
Number of introductions of *T. erytreae* (LIS)	True False	True: Two introductions; in Porto in 2014 and in Lisbon 2017 False: One introduction in Porto
Host trees available (Urb)	True False	True: Trees from orchards, plus trees from urban and peri-urban areas False: Trees from orchards only

We consider that a cell is infested when $N \geq 1$ individual.

For the replication of simulations considering long-distance dispersal, we calculated the percentage of simulations that classified each cell as infested. For each cell, if 50% or more simulations predicted the infestation, then the model classifies those cells as infested. Thereafter, for each model, using the simulation data from 2021, we calculated the F1-Score performance criteria (Chinchor & Sundheim, 1993), using the following equation:

$$\text{F1-Score} = \frac{2\ x\ (precision\ x\ recall)}{precision + recall} = \left(\frac{TP}{TP + \frac{1}{2}(FP + FN)}\right) \quad \text{eq.15}$$

where *TP* is the sum of true positives, *FP* is the sum of false positives, and *FN* is the sum of false negatives. F1-Score is the harmonic average of precision and recall.

We compared models with different parameters using the model's performance criteria, F1-Score. We also calculated standard errors for each model's F1-Score, using 2000 bootstrap simulations taken from the 300 original simulations of each stochastic model. These were used to obtain the p-values for testing the equality of the F1-Score for pairwise comparisons between two models. The p-value was calculated by the inversion of the bootstrap normal confidence interval for the difference in means (Thulin 2021) using the following equation:

p-value = $2(1 - \Phi^{-1}(|F1a - F1b|/\sqrt{SEa^2 + SEb^2}))$ eq.16

where Φ^{-1} denotes the inverse cumulative distribution function of the standardized normal distribution, F1*a* and F1*b* are the F1-Scores of model a and model b, respectively, and *SEa* and *SEb* are the standard error values of model a and model b, respectively.

We also calculated the Area Under the Curve (AUC) of the receiver operating characteristic plots (Fielding & Bell, 1997) and the Youden index of each model (Youden 1950), but they led to the same conclusions as the F1-Score.

The tested parameters were the two different female fecundity rate values (Low or High), the inclusion or not of long-distance dispersal events (Yes or No), the frequency of the long-distance dispersal events (Low, Medium, High), the inclusion of the estimated residential urban citrus trees (Yes or No), and the occurrence of a second introduction of *T. erytreae* in Lisbon (Yes or No) (Table 3.1).

We compared the estimated local spread rate value against the observed short-range dispersal based on presence-absence data reports by the Portuguese Plant Protection Authority (DGAV, 2021). For each year, we calculated the mean least distance between all newly infested parishes centroids and past infested parish centroids (*DP*). Infested parishes attributed to long-distance dispersal were removed from the short-range dispersal rate calculation. We identified such parishes, when their *DP* was higher than 30 km or was higher than the distance between its centroid and a long-distance dispersal parish centroid. 30 km is an arbitrary distance value that is significantly higher than the yearly estimated flight capacity of *T. erytreae* and three times the 10 km radius used by the Portuguese Plant Protection Authority for the species monitoring (DGAV, 2021). Finally, with the average value of *DP* from each year, we calculated the average short-range dispersal rate of *T. erytreae* in Portugal for each year and in total using all *DP* values independently of the year of infestation. Furthermore, to calculate the total dispersal capacity to the east and to the south of the country, the main directions the species could spread in Portugal, we used the distance between the infestation origin and the furthest parish towards the east and the south, as was done for the spread pattern of *V. velutina* (Verdasca et al., 2021). Additionally, we calculated the total infestation area of the infested parishes along the invasion years.

Model simulation running, validation and all statistical analysis were done with the statistical language R version 4.2.0 (R Core Team, 2022). Data and code are available upon request.

3.3. Results

3.3.1. Spread rate

The reports of the Portuguese Plant Protection Authority (DGAV, 2022) denote a fast dispersal of *T. erytreae* in Portugal (Fig. 3.2). Between 2015 and 2021, the African citrus psyllid was able to spread mostly southward, along the coastal area of Portugal, covering a maximum distance of about 461 km, between Porto and western Algarve, and a cumulative area of about 14,239 km^2 (Fig. 3.2). This corresponds to an average of about 65.9 km/year and 2034 km^2/year. The dispersal towards the East was only 100 km (14 km/year). However, removing long-distance dispersal events, the observed mean dispersal rate of *T. erytreae* in Portugal was 7.8±0.3 km/year (Table 3.2). The estimated short-range dispersal capacity used in our model simulation was 6 km/year, which turned out very close to the observed data (ranging from 5.6 to 10.4 km/year) (Table 3.2)

Table 3.2 – Short-range yearly mean dispersal distance (± SE) and area of *Trioza erytreae* in Portugal, between 2015 and 2021, based on the reports published by the Portuguese Plant Protection Authority (DGAV, 2022).

Short-range dispersal rate	2015	2016	2017	2018	2019	2020	2021	Mean
Mean dispersal distance (km)	10.4±1.3	7.5±2.8	8.5±0.8	5.6±0.6	7.9±0.7	8.1±0.6	5.6±0.6	7.8±0.3
Dispersal area (km^2)	384.7	698.4	1604.5	1612.0	1075.2	4401.7	4462.0	2034.1

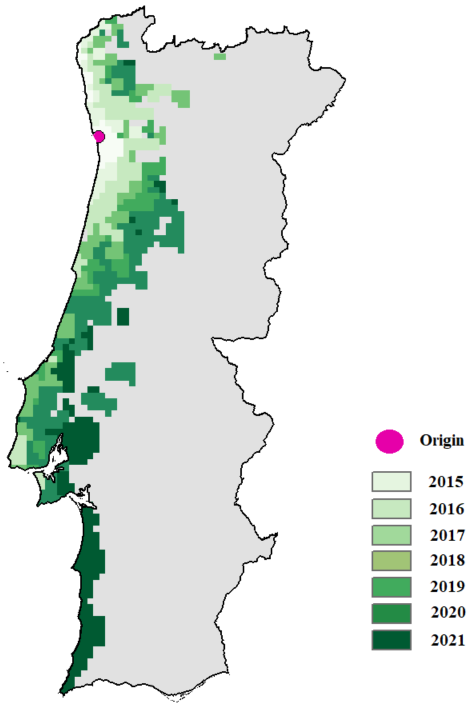


Fig. 3.2 – Spatio-temporal representation of Portugal's invasion by *Trioza erytreae*, between 2015 and 2021. Elaborated based on data from DGAV reports.

3.3.2. Growth rate

Our estimates of the number of yearly *T. erytreae* generations in Portugal varied from 3 to 4 generations per year. The estimated grow rate (*r*) of *T. erytreae* in the Portuguese territory was found to be higher along the coast area (Fig. 3.3). A different female fecundity rate had a major impact on the growth rates estimated, especially in the interior central and southern regions (Fig. 3.3). The model included a cold limiting factor, portraying areas whose winter was deemed as too extreme for *T. erytreae* survival, where the growth rate was 0. The cold limited areas are all located in the northern interior part of the country (Fig. 3.3), where most areas are mountainous, and the climate is colder, especially in the winter.

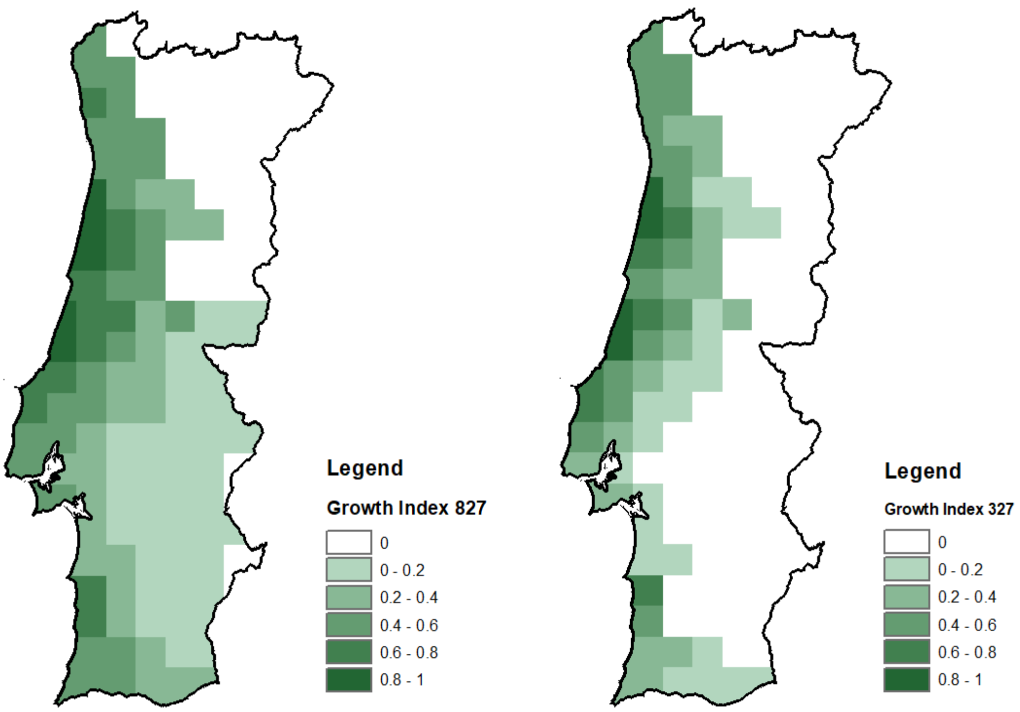


Fig. 3.3 - The estimated growth index of *Trioza erytreae* in Portugal, considering two fecundity levels: 827 eggs per female (left); 327 eggs per female (right)

3.3.3. Host availability

According to the most recent agriculture census of Portugal, there is a total surface of 21,681 ha of citrus orchards in continental Portugal, 74% of which located in the South, in the Algarve region (INE 2021). We estimated a total of 11,993,645 citrus trees in orchards and 7,427 trees in urban and peri-urban areas (Fig. 3.4). The estimated citrus-trees density in Vertical, Horizontal, and Discontinuous urban areas were 0.37, 3.2 and 5.14 trees per hectare, respectively (see Table S3.1 in supplementary material section).

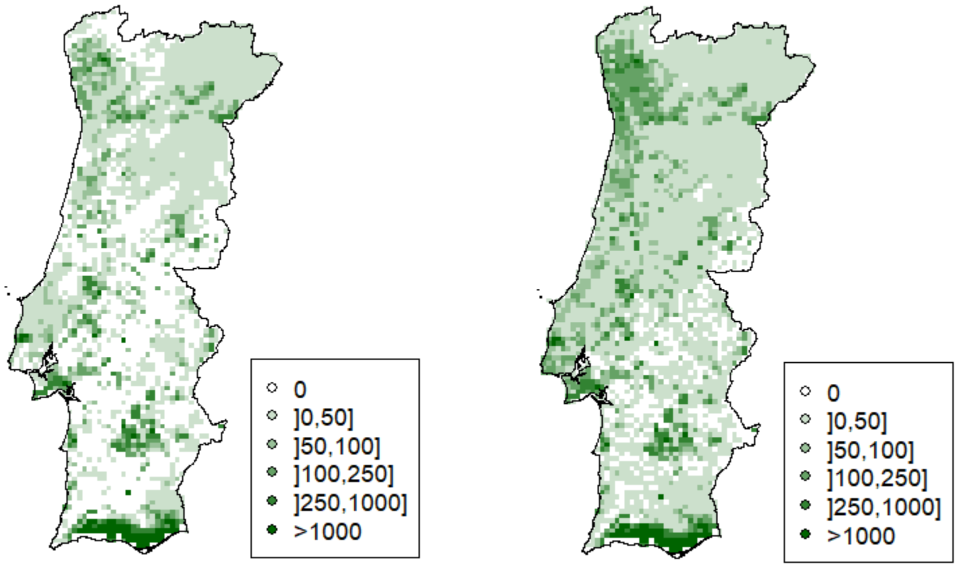


Fig. 3.4 – The spatial representations of the estimated citrus trees density (number of trees/km^2) in Portugal. The left map represents the estimation of citrus trees density in Portugal based on the area of citrus orchards reported in the last agricultural census (INE 2021), while the right map was obtained using both the data from the agricultural census (INE 2021) and our estimates of the number of citrus trees in urban and peri-urban areas, based on Google Street imagery.

3.3.4. Model Validation

The model simulations that included long-distance dispersal fit the observed data better than those that did not (Table 3.3; see Tables S3.2 for p-values). This is shown by the significantly higher F1-Score between every model with the same combination of parameters besides the long-distance dispersal, independently of the frequency considered, i.e., low, medium, or high (e.g., model 6 F1-Score = 0.733 vs model 30 F1-Score = 0.803, p-value < 0.001; Table 3.3, Table S3.2.1). The difference is even greater if the second introduction scenario is not considered (e.g., model 5 F1-Score = 0.583 vs model 29 F1-Score = 0.801, p-value < 0.001; Table 3.3, S3.2.1).

Table 3.3 – The 32 different model simulations covering all parameter combinations and the corresponding F1-Scores for the model validation against the 2021 observed data.

	Parameters				Statistics	
Simulations	LDD	Fecundity	Urban trees	Second introduction	F1-Score	SE
1	No	low	Yes	No	0.530	0.0
2	No	low	Yes	Yes	0.669	0.0
3	No	low	No	No	0.421	0.0
4	No	low	No	Yes	0.546	0.0
5	No	High	Yes	No	0.583	0.0
6	No	High	Yes	Yes	0.733	0.0
7	No	High	No	No	0.463	0.0
8	No	High	No	Yes	0.596	0.0
9	low	low	Yes	No	0.791	0.0035
10	low	low	Yes	Yes	0.790	0.0029
11	low	low	No	No	0.640	0.0055
12	low	low	No	Yes	0.640	0.0060
13	low	High	Yes	No	0.804	0.0023
14	low	High	Yes	Yes	0.795	0.0023
15	low	High	No	No	0.689	0.0026
16	low	High	No	Yes	0.688	0.0024
17	medium	low	Yes	No	0.786	0.0043
18	medium	low	Yes	Yes	0.794	0.0036
19	medium	low	No	No	0.622	0.0065
20	medium	low	No	Yes	0.643	0.0053
21	medium	High	Yes	No	0.800	0.0024
22	medium	High	Yes	Yes	0.800	0.0024
23	medium	High	No	No	0.687	0.0021
24	medium	High	No	Yes	0.684	0.0021
25	High	low	Yes	No	0.789	0.0037
26	High	low	Yes	Yes	0.794	0.0027
27	High	low	No	No	0.615	0.0090
28	High	low	No	Yes	0.637	0.0058
29	High	High	Yes	No	0.801	0.0024
30	High	High	Yes	Yes	0.803	0.0023
31	High	High	No	No	0.683	0.0026
32	High	High	No	Yes	0.686	0.0018

Different frequencies of long-distance dispersal events (low, medium and high) were not consistent in model improvement in all parameter combinations (Table 3.3, Table S3.2.2).

The inclusion of the estimated urban and peri-urban citrus trees significantly increased the model performance, with significantly higher F1-Score values in every model combination (e.g., model 30 F1-Score = 0.803 vs model 32 F1-Score = 0.686, p-value < 0.001; Table 3.3, Table S3.2.3).

The scenario of considering a second introduction was beneficial only for the simulations not using long-distance dispersal, when compared with similar models (e.g., model 6 F1-Score = 0.733 vs model 5 F1-Score 0.583, p-value < 0.001; Table 3.3, Table S3.2.4). The same was not true for model simulations considering long-distance dispersal. The parameter was sometimes not significant (e.g., model 9 F1-Score = 0.791 vs model 10 F1-Score = 0.790, p-value = 0.97), sometimes beneficial (e.g., model 19 F1-Score = 0.622 vs model 20 F1-Score = 0.643, p-value = 0.011) and sometimes negative towards model performance (e.g., model 13 F1-Score = 0.804 vs model 14 F1-Score = 0.795, p-value = 0.006; Table 3.3, Table S3.2.4).

Finally, model simulations that considered high fecundity (827 eggs per female) performed better those with low fecundity (327 eggs per female) in 6 out of 8 parameter combinations. In two cases, changing fecundity did not significantly affect model performance (e.g., model 22 F1-Score = 0.800 vs model 18 F1-Score = 0.794, p-value = 0.126; Table 3.3, Table S3.2.5).

Overall, the model simulations with the highest performance were 13, 21, 22 and 30, showing no significant differences (Table S3.2.6). All these models used long-distance dispersal, high female fecundity, urban citrus trees, but differed in the long-distance dispersal frequency and in considering the second introduction of *T. erytreae*.

Altogether, and considering the temporal evolution between 2015 and 2021, our best model simulations showed a high concordance between the observed and predicted distribution of *T. erytreae* over the seven years after its detection in Portugal (Fig. 3.5).

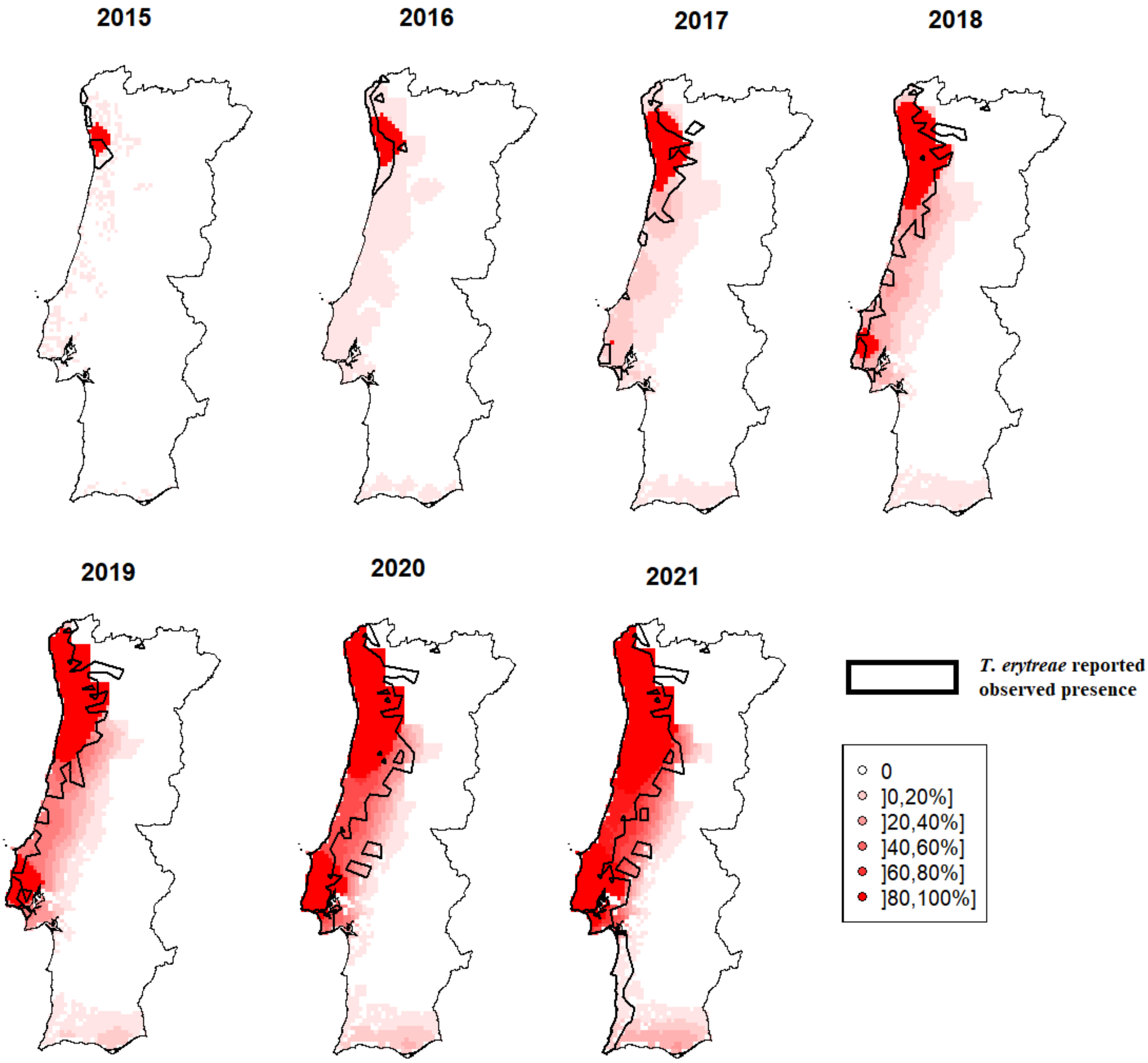


Fig. 3.5 – Spatio-temporal dynamics of *Trioza erytreae* spread in Portugal from 2015 up to 2021, predicted using 300 replicate simulations of model simulation #30. The color gradient represents the probability of each grid cell to be infested according to the model, calculated as the relative number of simulations that predicted the area's infestation. The border of the observed distribution area of *T. erytreae* is represented by a black line (elaborated based on data obtained from DGAV reports) to allow visual assessment of model performance.

3.4. Discussion

The major result of this study is that human-mediated dispersal and citrus trees outside orchards play an important role in the spread of *T. erytreae* in Portugal. Hereafter, we discuss in more detail these results as well as other findings.

3.4.1. Role of human-mediated dispersal

Human-mediated dispersal is a well-known documented phenomenon, recognized as a key issue in invasion science (Ricciardi et al., 2017; Bullock et al., 2018; Gippet et al., 2019). Human activities leading to insect dispersal can be divided into three pathways: Contamination, hitchhiking and harvesting (Pergl et al., 2017; Gippet et al., 2019). For the spread of *T. erytreae* we believe the major pathways behind human-mediated dispersal of the species would be hitchhiking, as suggested for the invasion of *D. citri* in Southern California (Bayles et al., 2017) and the invasion of the yellow legged wasp, *Vespa velutina* in Portugal (Verdasca et al., 2021). In this pathway, adults' psyllids would be accidentally attached to a vehicle vector, from where they may allow being transported farther away from their flight capacity, increasing the potential dispersal capacity of the species. This dispersal pattern coincides with higher dispersal along the coastline, where the human population is denser. It also reflects the distribution of North-South highways along the coast. Additionally, the movement of infested citrus plants is another possible pathway for the dispersal of *T. erytreae* in Portugal.

Since its detection, in 2015, the African citrus psyllid was able to spread mostly southward, along the coastal area of Portugal, covering a maximum distance of about 461 km, between Porto and western Algarve, in seven years, corresponding to an average of about 66 km/year. This dispersal rate is about 4 to 8 times higher the values reported for other Hemiptera, such as the hemlock woolly adelgid, *Adelges tsugae* Annand (Adelgidae) (8-13 km/year), and the beech scale, *Cryptococcus fagisuga* Lindinger (Eriococcidae) (14-15 km/year) (Liebhold & Tobin, 2008). However, without considering long-distance dispersion events, the observed mean dispersal rate of *T. erytreae* in Portugal was 7.8

km/year, similar to the spread rate of the other insect cases (Liebhold & Tobin, 2008).

These results highlight that the spread of *T. erytreae* corresponds to a combination of short-range and occasional long-range dispersal events. This dispersal pattern, called “stratified dispersal” is commonly observed in the spread of invasive insect species (Liebhold & Tobin, 2008). For *T. erytreae,* the inclusion of long-distance dispersal events greatly improved model performance in predicting the observed data (Table 3.3). In the current study, the large difference between the mean global dispersal rate (66 km/year) and the mean diffusion dispersal rate, estimated excluding the long-distance dispersal events (7.8 km/year), greatly highlights the importance of long-distance dispersal events for the species spread, which often are anthropogenic (Liebhold & Tobin, 2008).

Likewise, the predicted and observed spread of *T. erytreae* along the coastal area of Portugal is also related with the high population density in the area, mostly between Porto and Setubal regions (Fig. 3.1), where long-distance dispersal events were concentrated. The role of human mediated dispersal was also reported in the yellow-legged hornet’s rapid expansion along the coast of Portugal, attributed to the density of motorways (Verdasca et al., 2021). Nevertheless, motorways density and vehicle traffic are correlated with population density (Barata 2012). Although human-mediated movement of insect life stages is usually the dominant modality of long-distance dispersal, other mechanisms may also be involved, such as the wind, which has not been considered here. For example, Antolínez et al., (2022) showed that the dispersal of the Asian citrus psyllid, *D. citri* may be influenced by wind speed. Oppositely, wind direction was not found to be a significant factor in an experimental trial on *D. citri* dispersal conducted by Lewis-Rosenblumet al., (2015). Nevertheless, Bayles et al., (2017) suggested that the observed spread pattern of the psyllid in California could be related with the prevailing wind direction, but without supporting a definitive conclusion. Future studies should investigate the possibility of assisted dispersal of psyllids in the upper wind, as an additional long-distance dispersal mechanism.

Our model provided contrasting results regarding the hypothesis of additional introductions of *T. erytreae* in Portugal during its invasion, as suggested by Ruíz-

Rivero et al., (2021) based on the genetic diversity of *T. erytreae* populations. When not using long-distance dispersal in the model, including a second introduction improved model performance towards predicting *T. erytreae* spread in Portugal (Table 3.3). Yet, when coupled with the long-distance dispersal parameter, the effect of a second introduction on model performance was inconsistent. This was shown by the best model simulations (13, 20, 21 and 30), that either used or did not use the second introduction parameter. This is likely due to long-distance dispersal diluting the importance of the introduction in the model since both have a similar impact on the model. In fact, the approach used to include in the model an additional introduction of *T. erytreae* was basically based on an input of individuals (1,000) in a defined cell of Lisbon area, which is not much different from a non-random long-distance dispersal event. This outcome further reveals that secondary introductions of invasive species might be frequently misled with long-distance dispersals. Nevertheless, multiple introductions are more than just an addition of individuals to the founding invasive population. They may have an important role in incrementing genetic diversity of the invasive population, compensating the low genetic variability associated with founder effects (Handley et al., 2011). If this increment of genetic diversity is related with new adaptive traits, such as higher fecundity and/or survival rates, then additional introductions are expected to influence the dispersal dynamics of the invasive population. This scenario could be considered in the model, by changing the parameters fecundity and survival. In this respect, it is interesting to note that the higher fecundity value tested was associated with a higher performance of the model.

3.4.2. Role of urban and peri-urban citrus trees

Urban areas often facilitate the introduction, establishment and spread of non-native species, in biological invasions (Cadotte et al., 2017; Gaertner et al., 2017; Hui et al., 2017; Padayachee et al, 2017). Their green areas, including ornamental trees, public gardens, parks and backyard gardens, may function as stepping stones for non-natives species to disperse and invade agroecosystems (Hui et al., 2017). In Portugal, citrus trees are one of the most common plant species present in urban and peri-urban landscapes, used as ornamental plants in street trees, public gardens, parks, as well as food plants in backyard gardens. Using an innovative method based on Google Street View imagery, we estimated the spatial distribution of those citrus trees outside orchards and its density according to Urban Areas typology (Table S3.1). Our results showed that these citrus trees played a very important role in the dispersal of *T. erytreae* along the country (Table 3.3, Table S3.2.3). For large areas of the observed distribution of the psyllid in 2021, where citrus orchards are almost non-existent (Fig. 3.2), the major source of host plants are the citrus trees in the urban and peri-urban areas (Fig. 3.4). This corroborates the previous claim that ornamental host species can contribute to connecting fragmented citrus producing lands, as well as act as reservoir areas for the psyllid, especially in the frame of management actions in citrus producing lands (van der Berg et al., 1991).

Similarly, a recent study in California (Bayles et al., 2022), where citrus trees are also common ornamental and food plants in urban and peri-urban areas, also pointed out the importance that these trees played in the invasion dynamics of the Asian citrus psyllid, including its spill over between urban and agricultural habitats (commercial citrus orchards).

3.4.3. Biological factors

We found no recent information in the literature regarding female fecundity and no data available from Portugal on this parameter. For the modelling scenarios, we used two values provided in old literature, one considering a high fecundity of 827 eggs/female (Catling 1973) and an alternative one estimating a lower fecundity of 327 eggs/female (Moran & Blowers, 1967) (Table 3.3, Table S3.2.5). Our modelling results showed significant differences according to this parameter, with the simulations using the higher fecundity performing significantly better than those with the lower fecundity. This outcome evidences the relevance to retrieve this type of basic biological data for the understanding of population invasion dynamics. Regrettably, this biological data is sometimes scarce and with low sampling power. Also, biological information may change with populations and differ in the invaded range. As these data is essential for modelling potential spread over several generations, we recommend that efforts should be spent on collecting such biological information on the invaded range of the species.

3.4.4. Model performance

Globally, our model was able to predict most of the spatio-temporal dynamics of *T. erytreae* spread quite well, except the recent invasion of southwestern area in 2021, in the coast of Alentejo and west coast of Algarve, for which the model predicted a low colonization probability of 17% by the psyllid (Fig. 3.5). This low probability associated to a long-distance dispersal event, into the referred region, is explained by the low human population density in the region. However, a high seasonal touristic flow from the North occurring in this coastal area during Spring and Summer periods was not considered in our model. This large movement of people, including many residents from *T. erytreae* infested areas may favour hitchhiking mechanisms of dispersal (Gippert et al., 2019). Nevertheless, the observed presence of the psyllid in the area consisted mainly of small colonies or damage in isolated trees or small groups of trees in backyards and gardens (Amílcar Duarte, University of Algarve and Celestino Soares, DRAPALG pers. com., 2021). Furthermore, even if the model predicts low probability of invasion there, the probability is above 0, so it does not predict absence (Fig. 3.5). This infestation results from relatively rare and stochastic events, which are difficult to predict with a high probability.

The fast spread of *T. erytreae* in Portugal occurred despite the efforts carried out by the Portuguese Plant Protection Authority to contain its dispersal and eradicate it. The measures implemented included the delimitation of demarcated areas, being the infested areas plus a buffer zone surrounding the infested areas with trap placement and active monitoring, along with various other control measures within the infested areas (DGAV, 2021). Such control measures have not been accounted in our spread model simulations, and this may explain the reason why some areas in Southern and inner Portugal, with relatively moderate predicted infestation probabilities, were not invaded by *T. erytreae* (Fig. 5). The apparent failure of the model in some parts of the country may result from a certain level of success of the implemented control measures.

3.5. Conclusions

Our model showed to be a good tool for simulating the invasion dynamics of *T. erytreae.* It was able to predict the observed spread of *T. erytreae* in Portugal from 2015 to 2021, when considering long-distance human-mediated dispersal and urban and peri-urban citrus trees. Our results support the hypothesis of human-mediated spread being a key-factor in the fast invasion of *T. erytreae* in Portuguese territory. This was highlighted by the fast spread pattern favouring the southern axis, mostly along the coastal area, where there is higher human population density, which was considered for the long-distance dispersal events in the model. Other factors possibly involved, such as the wind should be considered in future studies. Our results did not support the hypothesis of a second invasion event of *T. erytreae* in Portugal. However, this hypothesis cannot be excluded based on our results, since our model was not primarily designed to test the hypothesis.

Additionally, our work showed that citrus trees from urban and peri-urban environments had a very important role in the spread of *T. erytreae* in Portugal. This is highlighted by the major impact that they had on model performance, considering their very low relative number in comparison with the estimated orchard trees. Our results contribute to highlighting the importance that isolated host trees can have for species invasive dynamics. These trees are generally disregarded due to lack of statistical data. Finally, we showed that Google Street view imagery can be an efficient tool to estimate the density of urban and peri-urban trees.

Acknowledgements

Thanks are due to Direção Geral de Agricultura e Veterinária (DGAV) for sending us the reports about the invasion of T. erytreae. We also thank Amilcar Duarte (University of Algarve) and Celestino Soares (Direção Regional de Agricultura e Pescas do Algarve) for providing information on the invasion of the coastal area of Alentejo and Algarve by T. erytreae and Luis Catela Nunes for advice in the statistical analysis. Thanks, are also due to the editor and reviewers for their comments and suggestions, which allowed us to improve an earlier version of the manuscript. Forest Research Centre (CEF) (UIDB/00239/2020) and the Laboratory for Sustainable Land Use and Ecosystem Services—TERRA (LA/P/0092/2020) are research units funded by FCT, Portugal.

Pedro Nunes received support from the PESSOA project (TC-05/10) and a SUSFOR (PD/00157/2012) doctoral grant funded by the Foundation for Science and Technology (FCT), Portugal (PD/BD/142960/2018). This study was supported by HOMED project, which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 771271.

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Supplementary material

The dataset used in this study is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

The following data sources were used:

Cos2018, Land Use and Occupancy Map 2018 – Available at https://www.dgterritorio.gov.pt/

Agricultural census of 2019 (INE 2021) - Available at https://www.ine.pt/xurl/pub/437178558

Human population density in Portugal from 2017 – Available at https://www.ine.pt/

The R script and the data needed to run the model are available at https://zenodo.org/record/7096566

Table S3.1.1 – Estimated citrus tree density of each residential urban area class, the sampled area used for their estimation in hectares and the description of the urban and peri-urban area classes.

Urban areas	Estimated density (trees/ha)	Sampled area (ha)	Description
Vertical	0.37	360.9	Plot areas with continuous buildings that occupy a surface area over 50% of the plot and with a height greater than or equal to 3 floors.
Horizontal	3.20	293.4	Plot areas with continuous buildings that occupy a surface area over 50% of the plot and with a height lower than 3 floors.
Discontinuous	5.14	329.1	Plot areas with discontinuous residential buildings, representing between 30 to 80% of the total plot area, along with areas with vegetation, bare ground, backyard gardens or agriculture.

Tables S3.2.1-6 – Tables with the p-values of the F1-Score pair-comparisons between the different simulated *T. erytreae* invasion models (1-32). With the model simulation parameters represented as: LDD – Long distance dispersal (Yes or No), Urb – Inclusion of the citrus trees outside of orchard areas (Yes or No), LIS – Inclusion of a second *T. eytreae* introduction in Lisbon, Fecund – Use a high or low female fecundity rate (827 vs 327 eggs/female).

Table S3.2.1 – p-values of the F1-Score pair-comparisons between simulated models not using Long-distance dispersal (LDD) and model simulations using LDD with frequency ranging from low, medium and high.

Comparison between models not using and using LDD				LDD frequency		
LDD	Urb	LIS	Fecund	Low	Medium	High
No	Yes	No	low	<0.001	<0.001	<0.001
No	Yes	Yes	low	<0.001	<0.001	<0.001
No	No	No	low	<0.001	<0.001	<0.001
No	No	Yes	low	<0.001	<0.001	<0.001
No	Yes	No	High	<0.001	<0.001	<0.001
No	Yes	Yes	High	<0.001	<0.001	<0.001
No	No	No	High	<0.001	<0.001	<0.001
No	No	Yes	High	<0.001	<0.001	<0.001

Table S3.2.2 – p-values of the F1-Score pair-comparisons between simulated models using different Long-distance dispersal frequencies, low, medium and high.

			p-values		
Urb	LIS	Fecund	Low vs Med	Low vs High	Med vs High
Yes	No	low	0.419	0.725	0.636
Yes	Yes	low	0.446	0.369	0.987
No	No	low	0.031	0.016	0.525
No	Yes	low	0.678	0.695	0.403
Yes	No	High	0.192	0.271	0.848
Yes	Yes	High	0.131	0.028	0.516
No	No	High	0.573	0.120	0.249
No	Yes	High	0.213	0.393	0.629

Table S3.2.3 – p-values of the F1-Score pair-comparisons between simulated models using and not using the estimated urban citrus trees in the model.

LDD	LIS	Fecund	p-values
No	No	low	<0.001
No	Yes	low	<0.001
No	No	High	<0.001
No	Yes	High	<0.001
low	No	low	<0.001
low	Yes	low	<0.001
low	No	High	<0.001
low	Yes	High	<0.001
medium	No	low	<0.001
medium	Yes	low	<0.001
medium	No	High	<0.001
medium	Yes	High	<0.001
High	No	low	<0.001
High	Yes	low	<0.001
High	No	High	<0.001
High	Yes	High	<0.001

Table S3.2.4 – p-values of the F1-Score pair-comparisons between simulated models with or without a second introduction of *Trioza erytreae* in 2017 in the model.

LDD	Urb	Fecund	p-values
No	Yes	low	<0.001
No	No	low	<0.001
No	Yes	High	<0.001
No	No	High	<0.001
low	Yes	low	0.976
low	No	low	0.967
low	Yes	High	0.006
low	No	High	0.870
medium	Yes	low	0.160
medium	No	low	0.011
medium	Yes	High	0.898
medium	No	High	0.374
High	Yes	low	0.258
High	No	low	0.041
High	Yes	High	0.564
High	No	High	0.422

Table S3.2.5 – p-values of the F1-Score pair-comparisons between simulated models using *Trioza erytreae* higher or low female fecundity estimates (827 vs 327 eggs/female) for the model.

LDD	**Urb**	**LIS**	p-values
No	Yes	No	<0.001
No	Yes	Yes	<0.001
No	No	No	<0.001
No	No	Yes	<0.001
low	Yes	No	0.001
low	Yes	Yes	0.162
low	No	No	<0.001
low	No	Yes	<0.001
medium	Yes	No	0.004
medium	Yes	Yes	0.126
medium	No	No	<0.001
medium	No	Yes	<0.001
High	Yes	No	0.007
High	Yes	Yes	0.016
High	No	No	<0.001
High	No	Yes	<0.001

Table S3.2.6 – p-values of the F1-Score pair-comparisons between the best performing models, using F1-Score as defining criteria.

Models	13	14	21	22	29	30
13	1.000	0.006	0.192	0.239	0.1	0.589
14	0.006	1.000	0.170	0.131	0.119	0.028
21	0.192	0.170	1.000	0.898	0.848	0.437
22	0.239	0.131	0.898	1.000	0.949	0.516
29	0.271	0.119	0.848	0.949	1.000	0.564
30	0.589	0.028	0.437	0.516	0.564	1.000

Chapter 4. Patterns of invasibility in agricultural landscapes: are spatio-temporal epidemiology approaches helpful?

This chapter is based on a joint work by Pedro Nunes, Manuela Branco, José Carlos Franco, and Mário Santos.

Abstract

The African citrus psyllid, *Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae), is an invasive pest which was recently introduced in Europe. This species attacks several *Citrus* species, with preference for lemon, and is vector of huanglongbing, the most damaging citrus disease, caused by the bacteria *Candidatus liberibacter*. We developed a spatio-temporal epidemiological approach to simulate the spread of *T. erytreae* in a heterogenous agriculture region. The model's virtual landscape was divided by patches, each representing the presence or absence of a lemon tree. The spread of *T. erytreae* across the model's landscape was simulated using the chain of tree infections. The model allowed to study the role of landscape structure and the effectiveness of management actions towards the species invasion success and dispersal.

Model performance was evaluated by comparing the simulation results with field observations. We used statistical analysis to study the role of each landscape variable (lemon tree density, proportion of urban areas, orchards fragmentation) towards the different spread indicators. Human management effectiveness was tested by comparing the spread with and without insecticide applications. The scattered lemon trees from residential areas were found to play a significant role towards the dispersal of *T. erytreae*. The chemical management actions applied in the region were not found to be effective towards the species dispersal, but with potential in reducing the impacts of the pest species. Here, we present a simple and intuitive new take for modelling invasive species, using an epidemiological approach, which may further consider management options. The procedure can be further applied to other species, environments and for simulation what if scenarios.

Keywords: Epidemiological model; invasive species dispersal; African citrus psyllid; landscape variables; Pest management.

4.1. Introduction

Biological invasions are currently one of the major drivers of global biodiversity loss, and of economic losses in agricultural and forest ecosystems (Pimentel et al., 2005; Pyšek et al., 2020; Diagne et al., 2021). The introduction of invasive exotic species in ecosystems can threaten the native biota and the ecosystem's functioning, with the potential to greatly impact both the environment and human welfare (Simberloff et al., 2013; Diagne et al., 2021). Many invasive species are agricultural insect pests that can affect agriculture by causing major yields losses and control costs (Bradshaw et al., 2016). A well-known example is the widely dispersed Mediterranean fruit fly, *Ceratitis capitata* (Wiedmann), one of the world's most destructive pests (Liquido et al., 1990; Szyniszewska & Tatem, 2014).

The invasion process can be divided in three phases, the entrance of a species in a new area, the establishment of a population and the dispersal to neighbouring regions (Liebhold & Tobin, 2008). In detail, the spread of an invasive species occurs through short and long-distance dispersal mechanisms (Tobin & Robinet, 2022). Short distance dispersal is related with the species dispersal abilities, while long-distance dispersal is often associated with passive dispersal by vectors, such as human activity, animals, water flow or wind (Gippet et al., 2019). Besides species traits, other factors may play a role in species dispersal patterns, including ecological and behavioural processes, related with the landscape (Conradt & Roper, 2006; Christie & Knowles, 2015). In general, insects react to the surrounding landscape, with different land uses, having a role in the organism dispersal behaviour. For example, habitat preference will promote organisms to disperse in search of suitable habitat areas. In reverse, insects may remain in those areas that are most suitable, refraining its dispersal. Such behaviour was verified for the flight trajectory of *Monochamus galloprovincialis* (Olivier) in a heterogenous landscape (Nunes et al., 2021). Furthermore, landscape features and spatial patterns, such as fragmentation, may affect the connectivity among suitable habitat patches, potentially affecting the patterns of a species dispersal within the landscape (Opdam, 1991; Schtickzelle et al., 2006). Additionally, dispersal is, for most species, density-dependent, i.e., population densities may influence dispersal dynamics. Most frequently, individuals tend to disperse from areas with high population densities to areas with low population densities (Harman et al., 2020). Thus, reducing population levels, by adequate control measures, in the frontier of expansion may allow slowing the spread of invasive species rendering environmental and economic benefits (e.g., Sharov et al., 2002). Additionally, environmental factors linked with activity and life cycles, such as temperature and the leaf phenology of host plants, may also affect the dispersal of herbivore insects (Aidoo et al., 2022).

Our work focused on understanding the factors that could interfere in the short-distance dispersal of the African citrus psyllid*, Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae) in a recently invaded European agricultural landscape. This is a small sap-sucking insect species, native to tropical Africa (Moran & Blowers, 1967). The main host plants are from the Rutaceae family, including lemon, *Citrus limon* and sweet orange, *Citrus sinensis* (Aubert, 1987). The psyllid was recently introduced in the northwest of the Iberian Peninsula (Monzó et al., 2015; Pérez-Otero et al., 2015; DGAV, 2015). This introduction has drawn major attention, as *T. erytreae* is a vector of huanglongbing (HLB), also known as the greening disease, considered the most damaging citrus disease (McClean & Oberholzer, 1965; Cocuzza et al., 2017). The disease is caused by the bacteria *Candidatus liberibacter* spp. (Bové, 2006; Gottwald, 2010), which has not yet been reported in Europe. *Trioza erytreae* and HLB are classified as A2 and A1 quarantine pest species, respectively (EU, 2019; EPPO, 2022a; 2022b). *T. erytreae* has a high invasive potential, due to high fecundity (between 327 and 827 eggs per female), no diapause and multivoltine cycle (up to 8 generations per year) (Kumar, 1977; Moran & Blowers, 1967; Catling, 1969a, 1972; Tamesse & Messi, 2004). Multivoltinism is supported on a fast growth cycle, with short nymphal maturation development, varying between 17 to 47 days, depending on the temperature (Moran & Blowers, 1967; Catling, 1972; Van den Berg, 1991).

Yet, since oviposition and larval growth can only occur in leaf shoots of host trees, the species reproduction is limited by the availability of leaf shoots (Catling, 1972; Cifuentes-Arena et al., 2018). Consequently, the number of yearly generations can be reduced to three, in locations with long periods with less favourable conditions for continuous host leaf flushing, such as hot and dry summer or extended cold winter (Catling, 1969b; Tamesse & Messi, 2004). These conditions are both negative for the species oviposition and nymphal growth (Catling, 1973), altogether affecting the number of yearly generations (Nunes et al., 2023).

An eradication program was implemented in Portugal, with several restrictions to citrus plants trade and mandatory application of insecticides on infested citrus trees in regions where the species was detected (DGAV, 2015). Despite these measures, *T. erytreae* spread rapidly from North to South, mostly along the coastal area of Portugal, with an average rate of 7.8 km per year, between 2015 and 2021 (Nunes et al., 2023).

Modelling the dispersal of invasive species is a relevant tool which can be used not only to understand and predict the spread but also help developing management strategies to contain invasive species expansion and their impacts. Spatial models for describing the dynamics of invasive species were first proposed by Skellam (1951), with the development of a reaction-diffusion model (RD) for the spread of muskrats, *Ondrata zibethicus* in central Europe. Until now, reaction-diffusion models remain the most common approach to modelling invasive species spread (Higgins & Richardson, 1996; Shigesada & Kawasaki, 1997; Robinet et al., 2017). However, empirical dispersal data for a wide range of insect species has shown leptokurtic or “fat-tailed” distributions, with rare long-distance dispersal events (Liebhold & Tobin, 2008).

Reaction-diffusion models can be improved by incorporating population growth and long-distance stochastic events, as developed by Robinet et al (2017) for the dispersal of the yellow-legged hornet, *Vespa velutina* (Lepeletier) in France. Still, these models typically do not consider management strategies and assume that the landscape is homogenous (Kot et al., 1996). This can be a major defect when simulating spatio-temporal dynamics, since individual dispersal and demography can be largely affected by the landscape patchwork (Andrew & Ustin, 2010). In fact, the effect of local landscape heterogeneity and diversity on individual dispersal and demography has been confirmed by various studies (Oliver et al., 2010; Rigot et al., 2014).

One potential alternative for simplifying the modelling also allowing understanding the landscape dynamics of invasive species is using a Cellular

Automata (CA) model, a concept introduced by Ulam and Von Neumann in the 1940s (Neumann, 1966; Hogeweg, 1988). These models provide a spatial framework to simulate local behaviour according to a set of rules of neighborhood, cell states and time constraints. Altogether these variables allow studying the behaviour of complex systems (Hogeweg, 1988). These models allow the implementation and replication of spatial forms, which is enhanced by the link with geographic information systems (GIS) in CA models. This way, CA have been applied to diverse ecological questions, including epidemic propagation (Sirakoulis et al., 2000), urban growth (Van Vliet et al., 2009) and forest fires (Encinas et al., 1997). However, applications focused on invasive species are scarce, especially in the scope of landscape dispersal dynamics.

Here we use an innovative method, by developing a spatio-temporal epidemiological cellular based model to simulate the spread of *T. erytreae* in a recently invaded region in Portugal, namely to: (1) test the suitability of this epidemiological approach to model the dispersal of a pest species in a heterogenous landscape; (2) study the importance of the landscape structure, using habitat composition and configuration variables, towards the invasive species dispersal success; (3) support relevant management actions to halt or reduce the species spread.

4.2. Material and Methods

4.2.1. Study area

To implement our model, we select the region of Mafra, in the District of Lisbon (Fig. 4.1A), where *T. erytreae* was first detected in 2018, sounding the alarms in an economically important region for lemon production (INE 2021; Fig. 4.1B). This is a heterogeneous agricultural landscape with a total area of 47.4 Km^2, consisting of commercial lemon orchards, and other land use / land cover (LULC), such as other agricultural crops (dominated by vineyards and pear orchards), forest (*Eucalyptus globulus, Pinus pinaster* and *Pinus pinea)*, and urban areas (Fig. 4.1B). The lemon orchards encompass about 2.5 Km^2 (5.3% of regional area), with individual orchards ranging from 0.25 ha to 8.5 ha (Fig. 4.1B).

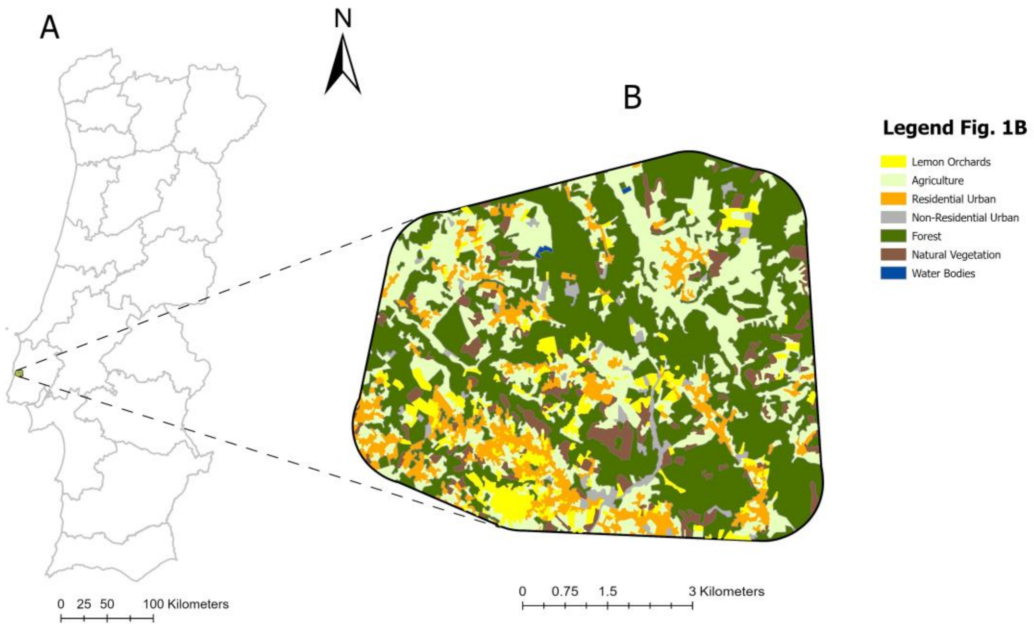

Fig. 4.1 – (A) Location of the study area in Portugal; (B) the study area landscape, divided within colours to depict land use/cover classes (LULC): yellow, lemon orchards; light green, other agriculture crops (vineyards and pear orchards); orange, residential urban areas; grey, non-residential urban areas; dark green, forests (*Eucalyptus globulus, Pinus pinaster* and *Pinus pinea*); brown, grasslands, shrublands and scrublands; blue, rivers and reservoirs.

4.2.2. Field Data Surveys

For monitoring the spread of *T. erytreae* in the study area, three field surveys were implemented in 2019: in spring, 10th of May (shortly after the species presence was first reported in the area); in summer, 15th of July; and in autumn, 10th of November. In detail, 20 trees were monitored in each of 30 sampled lemon orchards, distributed within the study area. The sampled lemon orchards were selected from farmers belonging to Frutoeste (https://frutoeste.pt/en/), the major farmers association in the region. Monitoring consisted of visual inspection of tree's canopy, searching for the presence of any sign of *T. erytreae*, either alive individuals (eggs, nymphs, or adults), dead or the distinctive signs of leaf damage caused by its nymphs (see appendix S4.1 in supplementary materials section) (Moran & Blowers, 1967; Cocuzza et al., 2017). Sampled trees were selected in the central area of the orchard and spaced between each other by 2 or 3 trees, depending on the orchard's size, to have a minimum buffer of two lines of trees from the hedge of the orchard.

4.2.3. Model

The model was designed to simulate spatio-temporal dynamics of infection, possible outbreaks, and the subsequent spread of *T. erytreae* within the agricultural landscape during the first year of the invasion. Each simulation lasted 310 days (time units), starting on the first of January and ending on the 10th of November, corresponding to the last monitoring field survey.

The model was developed using NetLogo software (version, 6.2.2., Wilensky 1999) and the simulations were run with the nlrx package (Salecker et al., 2019) of R software (version 4.2.0, R Core Team 2022).

4.2.4. Virtual landscape

The virtual landscape was divided within 25m^2 square patches (1,896,268 patches) and the discrete simulations were updated daily. To simplify the structure and the computational complexity, each patch represents a LULC, in the case of lemon orchards, a lemon tree. For this, we considered 5mx5m as the average lemon-tree spacing in lemon orchards (Nunes et al., 2023). The study area's Landscape information was retrieved from the COS 2018 (available at https://www.dgterritorio.gov.pt/). This information was complemented with google earth satellite imagery for details concerning the distribution of lemon orchards and residential urban areas (Fig. 4.1B). For each patch of lemon orchards, a lemon tree was assigned. On the other hand, lemon trees in the residential urban areas were randomly distributed, with a 1.3% chance for each urban patch to have a lemon tree assigned to it, based on the 5.14 citrus trees/ha density estimated previously (Nunes et al., 2023). No lemon trees were assigned to the other LULC.

4.2.5. Conceptualization of the dispersal of *Trioza erytreae* in the landscape

The chain of tree infections in the virtual landscape was used as the indicator of the invasive species spread across the landscape like an epidemiological susceptible-infectious-susceptible SIS-model (Fu & Milne, 2003; Pastor-Satorra et al., 2015). A SIS-model describes the dissemination of a pathogenic organism from an infectious host to healthy susceptible individuals in a population. In the present work, the lemon trees are the hosts, which become susceptible when producing leaf flushing, to the affecting organism, *T. erytreae* (Fig. 4.2). They are infected by nearby infectious trees, becoming latent lemon trees (carrying immature forms), and will over time become infectious lemon trees (carrying adult forms) (Fig. 4.2).

Hence, Lemon trees during the model simulations can either be susceptible (i.e., have active leaf growth), or non-susceptible (no active leaf growth, making it resistant to being infected) (Fig. 4.2). Additionally, lemon trees can be latent (infected with immature forms) and/or infectious (infected with adult forms), as a single lemon tree may carry both immature and adult forms.

This epidemiological approach allowed us to tackle the spread of the psyllid without using *T. erytreae* population dynamics, enabling a simpler and more intuitive model, while maintaining the focus on the landscape. More details of the model can be found on the supplementary material.

4.2.6. Leaf flush phenology

Trioza erytreae development and oviposition can only occur on young leaf shoots, as older leaves are not suitable (Catling, 1972). Thus, citrus phenology is one of the most important factors regulating population dynamics of the psyllid (Catling, 1972; Tamesse & Messi, 2004; Cocuzza et al., 2017).

Leaf phenology was incorporated in the model indicating the lemon tree's susceptibility to being infected. In the model, each lemon tree has a daily chance to begin active leaf growth (flush) or rejuvenate their active leaf growth (in case they already have active leaf growth). Daily chance varied for each month of the year. The monthly chance was calculated, using referenced data of monthly citrus leaf flushing patterns (appendix S4.5, Milosavljević et al., 2018).

Additionally, in the model, the active leaf growth only lasts 25 days. After 25 days, the lemon tree loses its active leaf growth and becomes non-susceptible. This was adapted from the findings of leaf suitability for the oviposition of *Diaphorina citri* (Kuwayama), which we adopted for *T. erytreae* (Cifuentes-Arenas et al., 2018, appendix S4.1). More details regarding the model simulations can be found in appendix S4.7.

4.2.7. Temperature simulation

The development rate and the life expectancy of *T. erytreae* are both related to the air temperature. Higher air temperatures speed up the growth of the immature stages of *T. erytreae*, potentially increasing the number of yearly generations (Green & Catling, 1971), and possibly decreasing the longevity of adult forms as found for *Diaphorina Citri* (Kuwayama) (Catling & Annecke, 1968; Catling, 1973; Liu & Tsai, 2000; Urbaneja-Bernat et al., 2020). The mean air temperature was incorporated in the model to simulate the maturation rate of the immature forms of *T. erytreae* and the life expectancy of *T. erytreae* adult forms.

The daily mean air temperature (*tmed*) was assumed to be the same for the entire study area. It was simulated using an equation to capture its seasonal pattern over the year. The equation was formulated as a polynomial of degree 5 in time (days) and estimated by ordinary least squares using a dataset of the daily weather in Lisbon (Humberto Delgado Airport weather station) in 2018 (Wunderground, 2021). To capture the randomness of daily mean temperatures over time, we added to the previous polynomial equation a random term modelled as an auto-regressive model of order 2. For both terms we used the Gretl statistical software (https://gretl.sourceforge.net/). We provide additional details in appendix S4.6.

4.2.8. *Trioza erytreae* growth

In the model, the species growth is reflected in two mechanisms: 1) by the transition of latent lemon trees to infectious lemon trees, (the maturation process of immature forms carried by the lemon trees), and 2) the time for an infectious tree no longer being infectious (the death of the adult forms due to age) (Fig. 4.2).

4.2.8.1. Immature forms development

For the maturation of the immature forms, *T. erytreae* egg and nymphal development equations were combined into a daily immature stage development equation, as a function of the mean daily air temperature (*tmed*) (Catling, 1973):

Daily maturation development rate = 1 / (4.9763 + 3.3443 x $0.8452^{(tmed\ -\ 20)}$ + 16.7974 + 5.2726 x $0.7843^{(tmed\ -\ 20)}$)

where *tmed* is the daily mean air temperature (ºC).

Latent trees over time accumulate maturation development (TriozaDevelop), which dictates when *T. erytreae* maturation is completed (TriozaDevelop > 1.0), with the latent lemon tree becoming an infectious tree (Fig. 4.2). A pre-oviposition period is randomly calculated, for every lemon tree when it becomes an infectious lemon tree, varying between 3 to 7 days, which will determine the period needed to pass for the infectious lemon trees to truly start infecting susceptible lemon trees (Moran & Blowers, 1967; Van den Berg, 1990). More details regarding the model simulations can be found in appendix S4.7.

4.2.8.2. Aging of adult forms

To simulate the aging of *T. erytreae* adult forms in the model, we used an equation of the daily adult aging, as a function of the mean daily air temperature (*tmed*):

Daily adult aging rate = 1 / (97.534 - (3.077 x *tmed*))

where *tmed* is the daily mean air temperature (ºC).

This equation was estimated using a linear regression model, based on data for the adult longevity of *D. citri* (appendix S4.3; Liu & Tsai, 2000). Infectious trees over time accumulate adult aging (DonatorDevelop), which dictates when adults have reached their life expectancy and die (DonatorDevelop > 1.0), with the infectious lemon trees no longer being an infectious lemon tree.

4.2.9. *Trioza erytreae* spread

T. erytreae dispersal in the landscape was represented as the infection of lemon trees across the virtual landscape. Each susceptible lemon tree has a daily chance to be infected, becoming a latent lemon tree, dependent on existing an active infectious lemon tree within *T. erytreae* flight capacity.

The daily tree infection probability was defined as 4%, based on the findings of daily tree infection rates in an orchard found for *T. erytreae* (Samways & Manicom, 1983). Flight capacity of *T. erytreae* was deemed to be 300 meters based on 89% of trap capture findings having been in that distance range (van den Berg & Deacon, 1988). Thus, transmission from an infectious tree to a susceptible one was defined to occur for distances up to 300m (appendix S4.4).

4.2.10. Insecticide treatments

In the study area, lemon orchard's farmers sprayed insecticides with varying frequency and insecticide products. We incorporated human management in the model in the form of insecticide treatments in the lemon orchards.

The spray time period, frequency, safety period between applications and insecticide effectiveness were all defined using regional information of insecticide management of the 30 monitored lemon orchards (appendix S4.2).

Each lemon orchard had a daily 3% chance of being sprayed with insecticide, between the 10^{th} of May and the 15^{th} of October, with a minimum 14-day interval between insecticide application (based on the maximum safety period of used insecticide products) (appendix S4.2). This 3% daily chance was calculated based on, the mean yearly treatments, period of spraying and safety period:

$$DI = (Ym / ((DE - DS) - (Ym \times S))) \times 100$$

DI - Daily insecticide application chance in an orchard (%); Ym- Yearly mean treatments; DE- Date of end of applications; DS– Date of start of applications; S- Safety period.

In addition, insecticide effectiveness in controlling *T. erytreae* was defined from the range of active ingredients used, and their known effectiveness on immature and adult forms, based on Molina et al., (2022). This way, insecticide effectiveness in removing *T. erytreae* from trees was parametrised in the model depending on the active ingredient (type-spray) used in the orchard. Using the list of products (appendix S4.2) we determined that there was 50% chance for the orchard insecticide applications to use non-effective substances (success rate of 0% for all forms) and a 50% chance for the use of effective substances (success rate of 69% and 79% against immature and adult forms, respectively) (appendix S4.2). Successfully treated latent lemon trees and infectious lemon trees will lose the corresponding insect form(s), (Fig.4.2; Fig. 4.3; appendix S4.7 for more details regarding the model simulations).

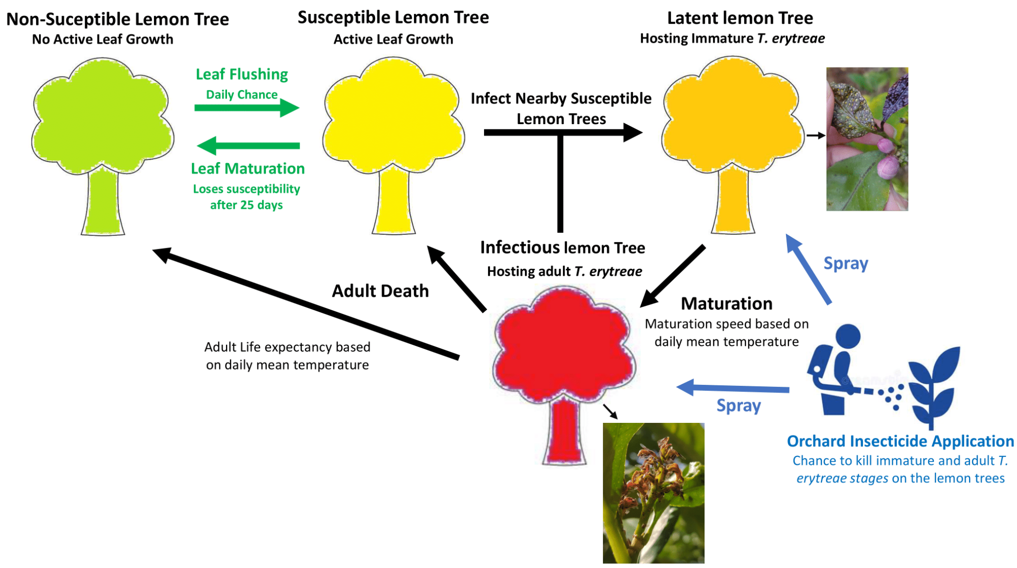

Fig. 4.2 – Conceptualization of the spatio-temporal cellular based model for simulating the landscape dynamics of *Tryoza erytreae*. Green arrows represent the mechanisms related with tree leaf phenology; black arrows represent mechanisms related with growth of *T. erytreae* in the host trees; and blue arrows correspond to human management of the insect pest. Non-susceptible lemon trees become susceptible with the start of leaf flushes, enabling the oviposition of *T. erytreae*. Susceptible lemon trees, depending on the distance from an infectious tree, will have a probability of becoming infected (Latent lemon tree).

Any susceptible tree (or infected) becomes non-susceptible at the end of the leaf flushing period. A latent lemon tree (no *T. erytreae* adults present) will become an infectious lemon tree (*T. erytreae* adults present). Infectious tree, eventually, will lose its infectious state, due to the death of the psyllid adults (dependent on daily mean temperature), reverting to a susceptible or non-susceptible state, depending on the presence (or not) of leaf flushes. Finally, insecticide sprays have the potential to alter the infectious state of a tree, turning it back to a susceptible lemon tree or non-susceptible lemon tree, depending on the presence (or not) of leaf flushes, respectively.

4.2.11. Model Simulations

Before the simulation starts, we select if insecticide application in citrus orchards will be used in the model (scenario 1 or not (scenario 0). After the initialization, the model's setup is processed as illustrated in Fig. 4.3, starting with Clearing all cell's variables and the global variables and ending with the random selection of the patient 0 lemon tree.

Afterwards, the simulation runs, with all lemon trees starting as non-susceptible and non-infected lemon trees. At each day, the model will run a sequence of steps illustrated in detail in Fig. 4.3, starting with the global variables of time and mean daily air temperature and ending with Insecticide applications. At the 45th day, the invasion is started with the infection of the patient 0 lemon tree with immature forms, turning into a latent lemon tree. The Simulations end at the end of the 310th day. More details regarding the model simulations can be found in appendix S4.7.

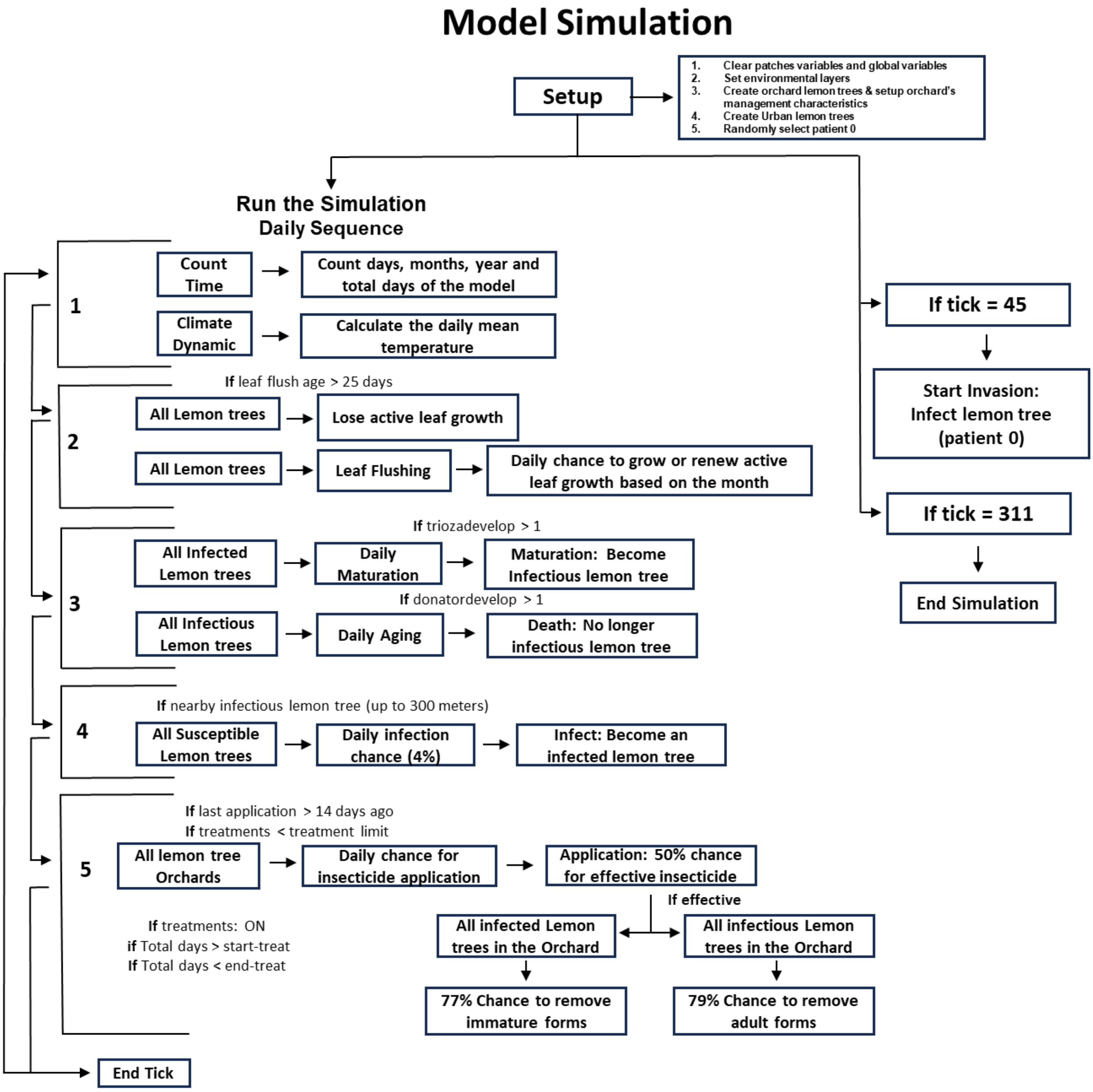

Fig. 4.3 – Overview of the simulation steps. After selecting whether treatments are included in the model or not (scenario 0 or 1), the simulation begins with the setup sub-model: 1 – Reset all cell's variables; 2 - set environmental layers for the virtual landscape; 3 – Create lemon trees in orchard LULC cells; 4 – Set up the orchard's management characteristics; 4 – Create lemon trees in urban LULC cells; Randomly select the patient 0 lemon tree. After the setup sub-model, the Run the Simulation sub-model is started, with the following daily sequence processes: step 1, the global variables of time and of the mean daily air temperature are updated; step 2, Susceptible lemon trees with active leaf growth over 25 days old, lose their active leaf growth. Additionally, all lemon trees have a daily chance to grow or rejuvenate active leaf growth (based on monthly leaf

phenology); step 3, Latent trees accumulate daily development, based on the mean daily air temperature. If the maturation threshold is reached, the tree becomes an infectious tree, and its oviposition period is calculated. For infectious trees, daily aging is accumulated. If the aging threshold is reached, then the lemon tree is no longer an infectious lemon tree; step 4, all susceptible lemon trees have a daily 4% chance to being infected, becoming a latent lemon tree, provided there is a nearby infectious lemon tree (=<300m); step 5, each lemon orchard has the chance to be sprayed by insecticide (3%), provided the right time period and safety period has been completed (14 days). The insecticide spray has a 50% chance to use effective products. If effective, for all lemon trees in the orchard, the insecticide will have a 67% success rate against immature forms and 79% success rate against adult forms, removing them from the lemon trees in case of success. More details regarding the model simulations can be found in appendix S4.7.

4.2.12. Model data statistical analysis

4.2.12.1. Model performance evaluation

Model performance evaluation was tested using independent data collected in the Barreiralva area, i.e., the lemon orchards´ area within Mafra region where *T. erytreae* was first detected (Fig.4.1B). The field data, collected in 11 (Barreiralva orchards) out of the 30 monitored orchards in Mafra region, were compared against model simulation results. Specifically, we compared two indicators of the invasion success of *T. erytreae*: (1) the relative number of infected orchards in the area (i.e., the proportion of orchards with at least one currently or previously infected tree from the total number of orchards in the area) (RelOrchard); (2) the relative number of infected lemon trees (i.e., the proportion of lemon trees infected at least once) divided by the number of lemon trees the area (RelInfect).

To endeavour mimicking reality, simulations were performed incorporating insecticide applications and considering the initial occurrence of *T. erytreae* on the 15th of February (defined considering the first spring leaf flushing period): one lemon tree, in the centre of each of the two suspected starting locations of the invasion, hosted newly laid eggs of immature *T. erytreae* (*Trioza* develop = 0).

The simulation lasted until the 10th of November, corresponding to the date of the last field survey and the last leaf-flushing period of the year (Syvertsen et al., 1981; Milosavljević et al., 2018).

4.2.12.2. Insecticide application effectiveness

For testing the effectiveness of insecticides' application in hindering the spread of the species in the landscape, two scenarios were considered (60 independent simulations each): Scenario 0, no insecticide application; Scenario 1, with insecticide applications. Three indicators were monitored at the end of the simulation: (1) Maximum Distance (MaxDist), the maximum distance between the invasion starting point and the faraway lemon tree infected at least once; (2) Relative Infected lemon tree area (RInfArea), the area of lemon trees infected at least once, divided by the surrounding total area in a radius of 1800 m, from the starting point; (3 RelinfTree, the total number of infected or infectious lemon trees at the end of the simulation (310th day), divided by the surrounding total area in a radius of 1800 m, from the starting point. This radius limit was defined as no lemon tree became infected beyond 1800 m, from the starting point in any simulation. The initial occurrence of *T. erytreae* in the model was defined for the 15th of February (starting on a randomly chosen latent lemon tree in the study area) and finished on the 10th of November. We used the independent-samples t test (Gerald, 2018) for each of the three indicators. We tested if the distribution of the indicators was significantly different between the two scenarios (*n*=60), which were used as the grouping variables. These analyses were run using the SPSS 28.0 (SPSS, 2021).

4.2.12.3. Land use / Land cover correlations with the invasion patterns

To understand the importance of the surrounding landscape towards the species spread, scenario 1 was implemented (118 independent simulations) and indicators MaxDist, RInfArea, RInfTree were related, within the 1800 m radius, with three different landscape metrics, namely: (1) lemon tree density (Td), (2) relative urban area (Ur) and (3) the lemon orchard fragmentation index (Fg) being

(1 – Effective lemon orchard mesh size (m_{eff})) (Jaeger, 2000). Model parameters were defined as the previous "Insecticide Application Effectiveness".

Multivariate linear regression models were used to infer the explanatory power and influence of the landscape metrics on the indicators of spread (MaxDist, RInfArea and RInfTree). Only predictors with Generalized Variance Inflation Factor lower than 5 were retained (Neter et al., 1996). All statistical analyses were carried out using the SPSS 28.0 software (SPSS, 2021) and the R statistical software (v4.2.1; R-Core-Team, 2022), using the packages MuMln (Bartón, 2020) and R commander (Fox & Bouchet-Valat, 2020).

4.3. Results

4.3.1. Model performance evaluation

The relative number of infected orchards in Barreiralva region increased linearly in our simulation from about 47% in March up to 100% in November. The simulated data mimicked very well the observed field data (Fig. 4.4). Concerning the simulations of the percentage of infected trees, the values well depicted the observations carried out in July and November (with 10% or less deviance; Fig 4.5). In May, the deviance was 18%. Generally, the simulations of the percentage of infected trees increased faster than the observed values. The simulations started with lower values in May and attained higher values in November.

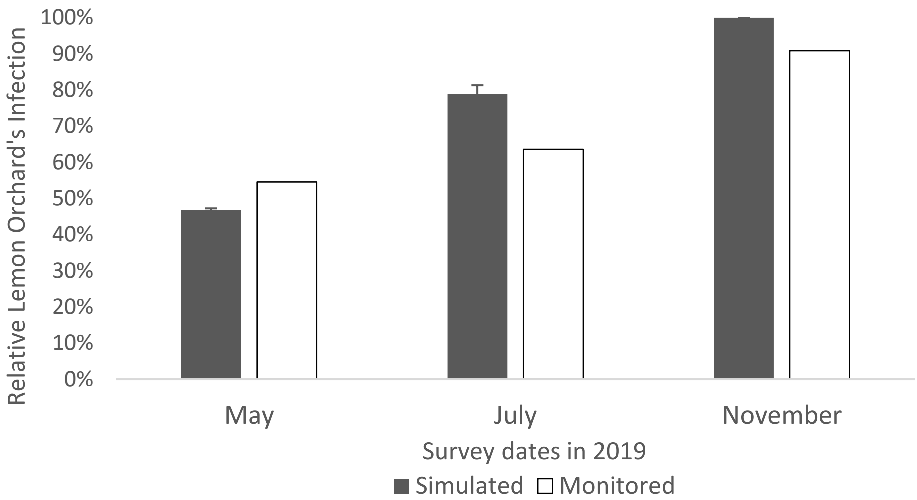

Fig 4.4 – Simulated and observed lemon orchard's infestation (95% confidence intervals) in Barreiralva, in the three survey dates, i.e., 15th of May, 15th of July and 10th of November of 2019 (*n*=11).

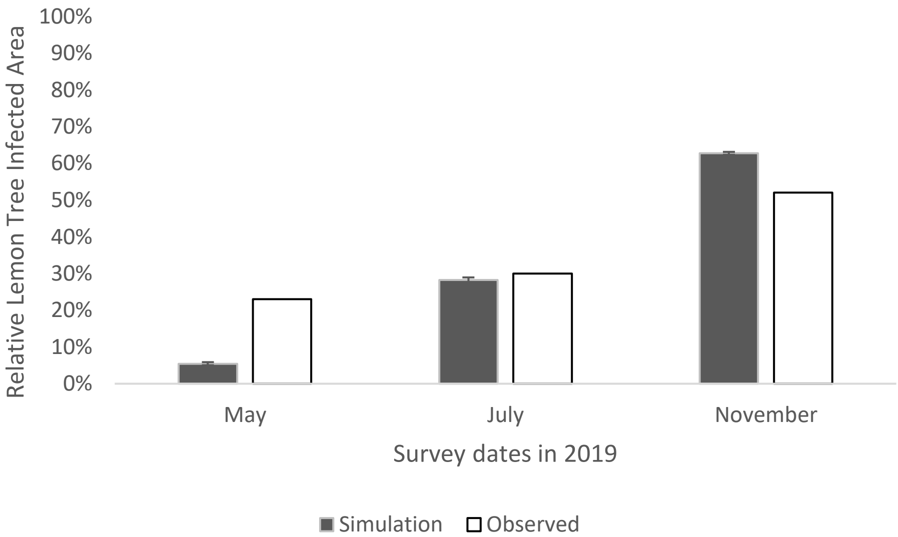

Fig 4.5 – Simulated and observed relative lemon tree infection (95% confidence intervals) in Barreiralva, in the three survey dates, i.e., 15th of May, 15th of July and 10th of November of 2019 (*n*=11).

4.3.2. Insecticide application effectiveness

Insecticide applications did not have a significant effect in the maximum distance of dispersion (MaxDist) (scenario 1, M = 1378.2, SD =307.6; scenario 0, M =1419.4, SD =42.8; t (118) = 0.706, p = 0.441; Fig. 4.6A). On the other hand, significant differences between scenarios were observed for the proportion area of lemons trees infected at least once (RInfArea) (scenario 1, M = 0.0268, SD = 0.0177; scenario 0, M = 0.0348, SD = 0.02283; t(118) = 2.192, p < .001) (Fig. 4.6B) and for the relative final population (RInfTree) (scenario 1, M = 0.01184, SD = 0.00153; scenario 0, M = 0.0234, SD = 0.01469; t(118) = 2.192, p = 0.003) (Fig. 4.6C).

The mean values of each of the three invasion indicators for the two different tested scenarios along the running of the simulations are presented in Fig. 4.6.

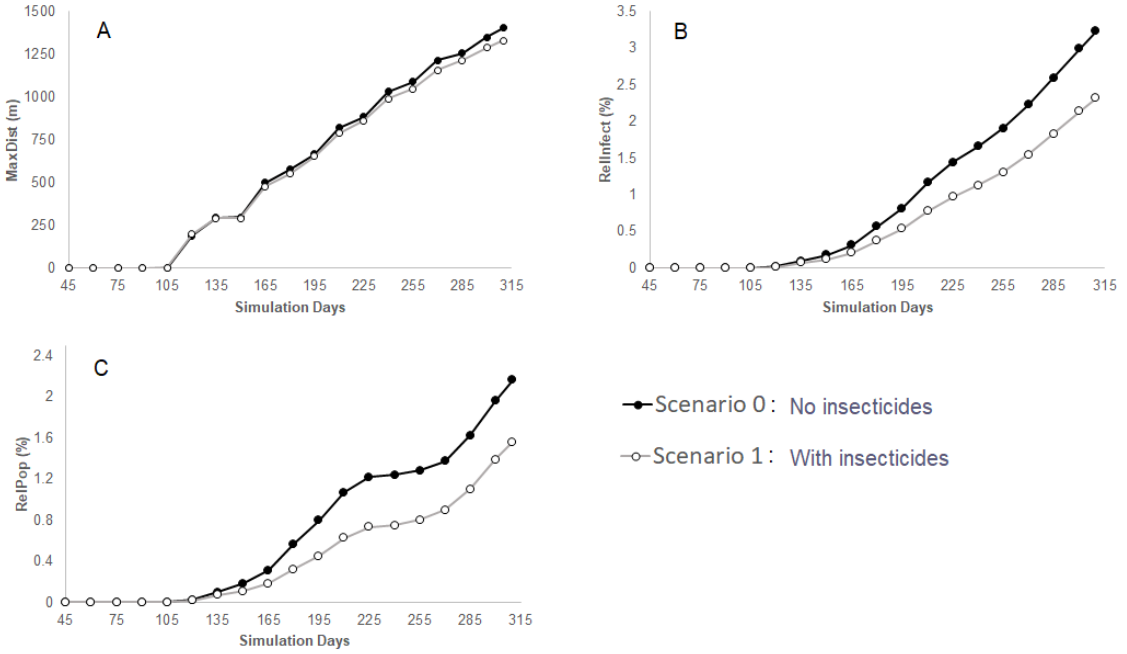

Fig 4.6 – Effect of the inclusion of insecticide treatments on *Trioza erytreae* invasion success, comparing the mean values of the three invasion indicators, for the simulations ran for each of the two tested scenarios (Scenario 0 - No treatments, *n*=60; and Scenario 1 - with Insecticide treatments, *n*= 60), along the running period of the model simulations: A) MaxDist, the maximum distance between the invasion starting point and the furthest away lemon tree infected at least once; B) RInfArea, the proportion area of lemons trees infected at least once, in a radius of 1800m, from the starting point, and C) RInfTree, the number of infected or infectious lemon trees by the end of the simulation, divided by the surrounding total area in a radius of 1800 m, from the starting point. Invasion start was set up at 45th day of the model and the simulations were set to end on the 310th day.

4.3.3. Landscape's role in the invasion patterns

All three landscape variables, i.e., lemon tree density (Td), relative urban area (Ur) and lemon orchard fragmentation index (Fg) affected the proportion area of lemons trees infected (RInfArea) and the specie's relative population (RInfTree) (Table 4.1).

The maximum distance of dispersion (MaxDist) significantly increased with the relative urban area (Ur) (Table 4.1). Yet it was not found to be significantly correlated with either the lemon tree density (Td), nor did the lemon orchard fragmentation index (Fg).

The relative area of lemon trees infested (RInfArea) was positively related with lemon tree density (Td), relative urban area (Ur) and with lemon orchard fragmentation index (Fg). Likewise, the specie's relative population (RInfTree) was positively related with Td, Ur and Fg.

Examples of model simulations, represented by the spatial infection range in the landscape, are represented in Fig. 4.7.

Table 4.1 – Summary of the unstandardized coefficients of the models developed for the invasion indicators (MaxDist - the maximum distance between the invasion starting point and the faraway lemon tree infested at least once; RInfArea - the area of lemon trees infected at least once, divided by the surrounding total area in a radius of 1800 m, from the starting point; and RInfTree - the number of infected or infectious lemon trees by the end of the simulation, divided by the surrounding total area in a radius of 1800 m, from the starting point), using three landscape metrics as the covariates of the models (Td – Lemon tree density in a radius of 1800 m, from the starting point, Ur – Relative urban area in a radius of 1800m, from the starting point, Fg – Lemon orchard fragmentation index (1 – the effective mesh size of the lemon tree orchard patches in a radius of 1800m, from the starting point (Jaeger., 2000)). Significance values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; standard errors in parentheses; t = t-score (*n*=118).

Model: Dependent variable:	(1) MaxDist	(2) RInfArea	(3) RInfTree
Td	-	0.186***(0.035)	0.138***(0.020)
	-	t = 5.363	t = 6.973
Ur	1007.397***(91.387)	0.118***(0.031)	0.082***(0.018)
	t = 11.039	t = 3.801	t = 4.633
Fg	-	-0.035***(0.006)	-0.021***(0.003)
	-	t = 5.699	t = 6.024
Constant	136.061***(12.399)	-0.012***(0.003)	-0.009***(0.001)
	t = 10.973	t = -4.765	t = -6.240
R^2	0.512	0.712	0.797
AIC	928.744	-1113.767	-1244.355

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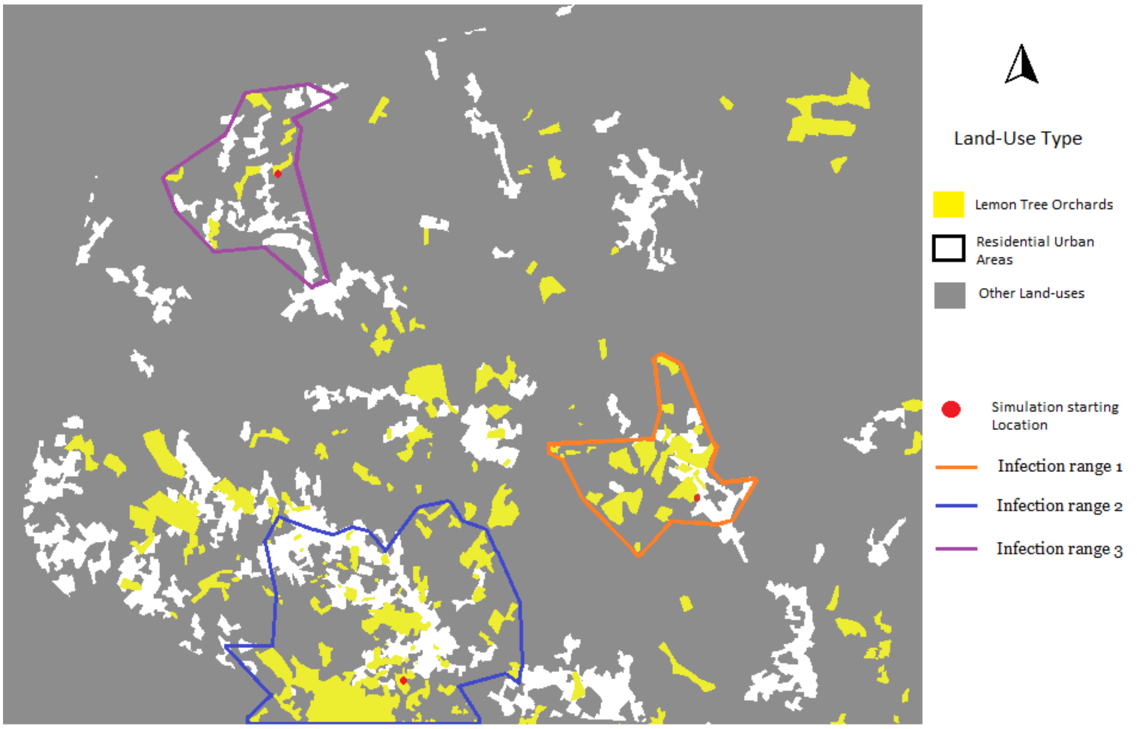

Fig. 4.7. Examples of the infection range of three different model simulations (1 - orange, 2 -blue and 3-purple), on three different starting locations, represented by the red circles. The model simulations were run for scenario 1 (with insecticide treatments) with the infestation starting on the 15th of February (45th day) and the simulation ending on the 10th of November (310th day). Yellow - Lemon orchard areas, White - Residential urban areas, and Grey - Other land-use areas.

4.4. Discussion

The recent introduction and rapid spread of *T. erytreae* is a great threat for the citrus industry in Europe (Bové 2006; Gottwald 2010; Cocuzza et al., 2017). We studied the importance of the surrounding landscape composition and structure towards the invasion success of *T. erytreae,* in a realist heterogeneous agricultural landscape. With this aim, we developed a novel spatio-temporal cellular based epidemiological modelling approach. This model allows to study the species spread in the landscape, as well as to simulate management options with relevant practical application.

4.4.1. Model performance

The epidemiological approach developed for modelling *T. erytreae* dispersal was able to portray the invasion pattern of the species in Mafra region, in the first year after its detection. It was quite accurate at simulating the observed spread of the species between habitats areas across the studied landscape, represented by the different lemon orchards in the region (Fig. 4.4). The model was less accurate at simulating the tree infection rates, with a seemingly higher infection rate then the observed in the field (Fig. 4.5). This was not surprising due to the model's simplification of population dynamics and the species dispersal, along with the limited specie's dispersal information we had access (Fig. 4.5). The simplification of population dynamics likely reduced the model's accuracy in tree infection, since the infection rate of susceptible trees was independent from the density of the specie's population density, represented by the number of surrounding infectious trees. Additionally, the number of observed infected lemon trees may have been underestimated from the reality, since orchard farmers regularly prune the trees young shoots, potentially removing the evidence of the species past presence in the form of leaf damage (appendix S4.1; Tucker et al., 1994; Intrigliolo & Roccuzzo, 2011).

4.4.2. Impact of pest management in the invasion success

Regarding the model's potential to test management scenarios, this was easily accomplished, incorporating human management from orchard farmers, in the form of insecticide spraying.

The pest management simulations indicated that *T. erytreae* spread in the region (MaxDist) was not significantly affected by pest management practices. Yet, the relative area of infected lemon trees (RInfArea) and the relative insecticide applications. Meaning that pest management likely did not hinder the species ability to spread across the orchards in the region but did affect the overall species population and the trees infection rate in the orchards. The inability to control the species spread is possibly due to the species high fecundity and rapid growth (adults have relatively long lifespan with continuous oviposition), in the absence of effective natural enemies (Catling, 1970; Pérez-Rodríguez et al., 2019). Also, the high variation in the number of insecticide sprays observed among farmers may also have reduced pest management general effectiveness (Aida, 2018; Noy & Jabbour, 2020). In fact, the number of insecticide sprays carried per orchard and year ranged from zero to seven and many of the used sprays are not effective against *T. erytreae*, being directed to other citrus pests, such as mites, scale insects and the citrus flower moth, *Prays citri* (Millière), the key-pest in the region (da Silva et al., 2006). Therefore, the impact on *T. erytreae* populations from chemical control in the region is expected to be very heterogeneous in space and time. In addition, insecticide effectiveness may also be affected by the common overlapping of different developmental stages (Coccuza et al., 2017; Molina et al., 2022). Finally, the scattered lemon trees from residential urban areas in the region represent not only a pathway for the species dispersal, but also a refuge from which *T. erytreae* may recolonise neighbouring lemon orchards, since these trees are usually not sprayed (Van den Berg et al., 1991). These results are in accordance with a recent review on the factors influencing the success or failure of eradication of non-native pests of woody plants in Europe (Branco et al., 2023), in which chemical control was reported to have a low success rate (25%).

It must be noted, since our model was focused on the dispersal on the landscape, the simplification of population dynamics, may have led to an underestimation of the effect of insecticides, since model the infection rate of susceptible trees in the model is independent from the number and density of surrounding infectious trees.

4.4.3. Role of Landscape characteristics in invasion success

Our results showed that all three indicators of invasion, MaxDist, RInfArea and RInfTree were positively related with the proportion of residential urban areas (Ur), supporting the hypothesis that isolated citrus trees in urban areas favour the spread of *T. erytreae* in invaded areas. These isolated citrus trees may act as stepping stones across separated habitat patches, improving habitat connectivity in heterogenous environments, and the species dispersal across the landscape (Saura et al., 2014; Rossi et al., 2015; Cadavidad-Florez et al., 2020; Rocha et al., 2021). Additionally, isolated trees may further stimulate organism dispersal due to the limited resources single trees offer. The importance of isolated trees in invasion dynamics has been observed with other insect pests, such as *Hylastes ater* (Payk) and the pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Rossi et al., 2016; Be et al., 2017).

In a previous study (Nunes et al., 2023), we showed that the involvement of isolated citrus trees, from urban and peri-urban areas, in the spread of *T. erytreae* across the Portuguese coast was highly significant. It was evident that in areas where citrus orchards were notably scarce, the primary supply of host plants originates from citrus trees within urban and peri-urban regions. However, in Mafra region, the landscape shows a different pattern. Lemon orchards cover about 5.3% of the area and residential urban areas constitute a host habitat network for *T. erytreae* across the region (see Fig. 4.1B). Therefore, in such a landscape, we suggest that isolated lemon host trees in urban and peri-urban areas may function as ecological corridors, incrementing the connectivity among fragmented lemon orchards, thus favouring the spread of the psyllid.

Regarding lemon tree density, while it did not significantly affect the species maximum spread (MaxDist), likely related with the holistic approach that does not consider *T. erytreae* population dynamics. It was shown to impact the species dispersal and growth ability across the lemon trees of the region, increasing both tree infection (RinfArea), and the species population (RInfTree). This result was anticipated, as resource limitation was not considered by our model neither for the host trees nor the pest species: a higher presence of host trees directly corresponds to an increase in the number of trees infected during the simulation (RInfArea) and higher final population level provided by the higher number of infected trees (RInfTree). The Habitat Amount Hypothesis (Fahrig, 2013) corroborates the obtained results: the habitat amount within the surrounding landscape is the main driver behind species abundance (and richness). The increase of the species abundance in turn results in higher density of adults, likely increasing the species dispersal capacity for the future, as species dispersal can be related with population density (Fye, 1983; Harman et al., 2020), with the over-crowding or absence of resources potentially forcing long-distance dispersal, as suggested by van den Berg & Deacon (1988). In addition, the increase of adult populations will increase the number of individuals with exceptionally higher dispersal abilities, as found in the release trials of *T. erytreae* in no-host conditions (Van den Berg & Deacon, 1988).

Similar results were obtained considering the lemon orchard fragmentation index (Fg). Orchard fragmentation was positively correlated with RelInfestArea and RInfTree. This was expected, since orchard fragmentation will reduce habitat connectivity, negatively impacting *T. erytreae* dispersal in the landscape, along with forcing the dispersal to small habitat areas, both can be related with metapopulational island effects (Hanski, 1998; Tobin et al., 2023).

Finally, the small-scale study area used with the prevalence of habitat area likely diminished the role of landscape configuration for dispersion, comparatively with a study area where habitat areas were to be rarer and more fragmented, which has been verified in previous habitat connectivity studies (McIntyre & Wiens, 1999). Additionally, the high dispersal range and high reproductive rates of *T. erytreae* and the prevalent distribution of urban areas in the study area, with host trees offering stepping stone dispersal pathways, likely counteracted the negative effect of habitat fragmentation and the importance of tree density towards the species dispersal.

4.4.4. Final remarks

One of the main computational challenges we faced for modelling *T. erytreae* invasive dynamics was its population dynamics, including high reproductive rates, different instars with varying development speeds and survival rate dependent on environmental factors. Additionally, its multivoltine nature and relatively high dispersal ability and the limited understanding of its dispersal behaviour made it tough to use a biological derived spatio-temporal model (Cocuzza et al., 2017). Our epidemiieological approach supported on the infection of its host, the lemon trees, simplifies the population dynamics and focuses on the species dispersal and impact across the landscape. As far as we know, only one other work presented a similar approach, even if focused on management actions inside orchards (Atallah et al., 2012). The developed modelling approach was able to portray the invasive pattern of the species observed in the Mafra region, in the first year after its detection (Fig. 4.4 and 4.5).

In the end, we were able to show the potential of the simple and intuitive nature of our modelling approach, allowing it to be easily adapted for other studies, such as simulating different management scenarios, different spatial or temporal models, other unrelated pest species or even for work outside invasion ecology. Additionally, the visual component of our model, allows to be used as a supporting management tool for farmers and stakeholders, incorporating what-if scenarios.

Finally, to improving the model's ability to simulate *T. erytreae* infection dynamics, there would be a need to further calibrate the model. We would suggest focusing on the infection component of the model. Specifically making a susceptible lemon tree's likelihood of being infected also take into consideration: (1) the number of surrounding infectious trees (sources of adults); (2) the availability of other susceptible trees in the region (competition); (3) The distance of the source trees, declining the infection rate with the distance from the infectious trees.

5.1. Conclusions

Our results supported the hypothesis that scattered host trees from residential areas play a significant role towards the *T. erytreae* invasion success, counteracting the negative effect of landscape heterogeneity, by acting as a stepping-stone between patches in the landscape. The proportion of lemon orchards within the landscape was the main driver for the species spatio-temporal dynamics, championing the idea that the area of suitable habitat seems more important than its composition and arrangement. On the other hand, even if management actions were not able to halt the invasive spread across the landscape, tree infection and species population decreased, possibly reducing future costs from *T. erytreae.*

A new take for modelling invasive species dispersal was presented and tested with success, supported on an epidemiological approach, that might be characterised by its simplicity, intuitive methodology and flexibility. As the method is adaptable to other geographical locations, spatial scales and even for other ecological studies, we are confident that it will pave the way for more research in this scope. In fact, testing the approach in other systems but also as a risk analysis tool concerning the greening disease might prove (or not) the methodology's potential and limitations outside its original idea.

Acknowledgements

Thanks are due to the Frutoeste agriculture cooperative, and in particular Eng. Nuno Farracho, Eng. Rosário Antunes, and the lemon farmers for allowing us to monitor their lemon orchards and providing treatment information. Also, we would like to thank Prof. Luis Catela Nunes for advice in the statistical analysis and Dr André Garcia for the assistance in the monitoring field work. Forest Research Centre (CEF) (UIDB/00239/2020) and the Laboratory for Sustainable Land Use and Ecosystem Services—TERRA (LA/P/0092/2020) are research units funded by FCT, Portugal.

P. Nunes was supported by the SUSFOR (PD/00157/2012) doctoral grant funded by the Foundation for Science and Technology (FCT) (PD/BD/142960/2018). This study was supported by the HOMED project, which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 771271.

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Supplementary Material

Appendix S4.1

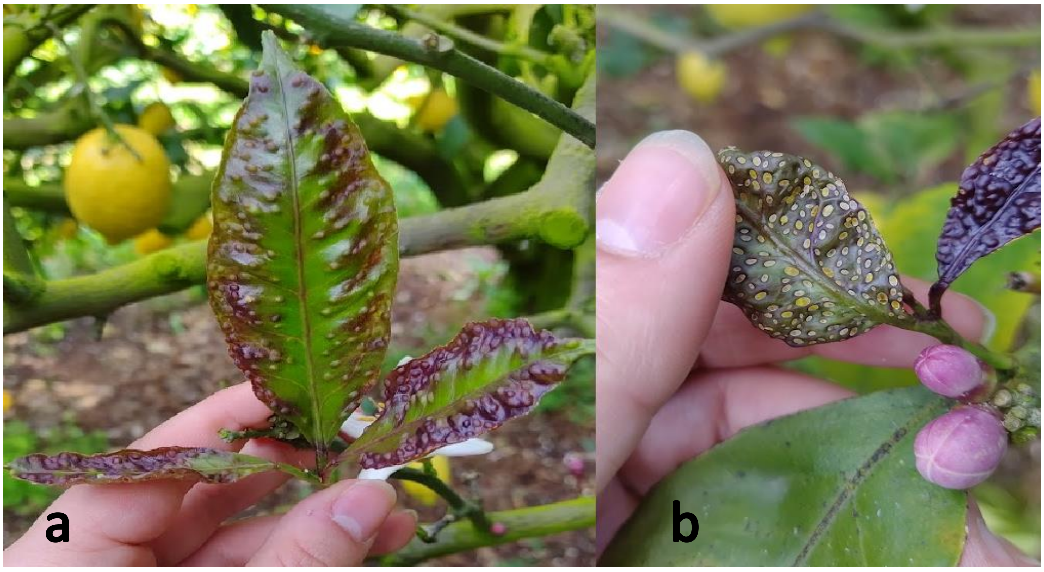

Fig. S4.1.1 - *Trioza erytreae* on lemon tree's leaf shoots. a) Typical pit galls caused by nymphal feeding, protruding to the upper face of the leaf; b) Nymphs on the lower face of a leaf, inside each pit gall, (Photos by Pedro Nunes).

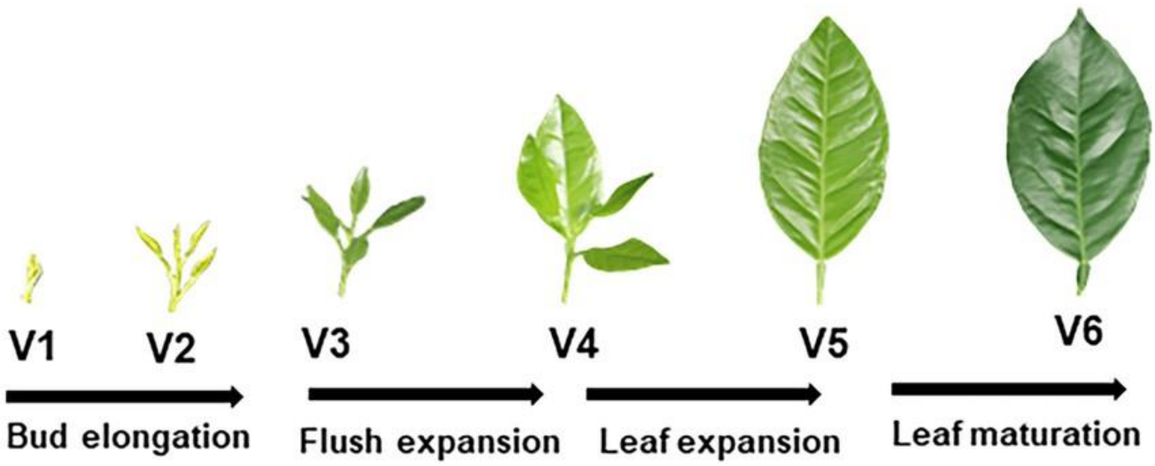

Fig. S4.1.2 – The developmental stages of citrus leaves. *Diaphorina citri* nymphal growth was found to be successful on all leaf flushing stages, except V5 and V6. A citrus leaf reaches the V5 stage at around 25 days old, where leaf expansion rate is significantly reduced (Source: Cifuentes-Arenas et al., 2018).

Appendix S4.2

Table S4.2.1 - Regional Information of the insecticide treatment frequency in the 30 monitored orchards in Mafra, Portugal, provided by the Frutoeste agriculture cooperative.

Orchard Name	Yearly Insectide Applications
Casa dos Palheiros	3
Loureira	7
Poço da Serra	4
Pomar Novo	0
Parque Paintball	0
Tuço	5
Casal da Ervideira	1
Serra	3
Charruada Terço	5
Charruada G	3
Carrasqueira B	5
Casal Mato Cima	4
Charruada A	3
Charruada B	4
Charruada F	5
Pucariça	4
Talhões	3
Vale das Pereira	0
Poço da Serra Novos	6
Casal Zambujeiro	1
Tagarros	1
Centieira	4
Fonte Santa	2
Panasqueira	5
Quinta da Gamanha	4
Quinta do Pelim	3
Palame A	5
Palame - Cova	5
Palame Novos	4
Veneno	5
Average	3.47
Standard Deviation	1.80

Table S4.2.2 - Regional Information of the total number of each insecticide product used in the 30 monitored orchards in Mafra, Portugal, provided by the Frutoeste agriculture cooperative and their reported efficacy at controlling *T. erytreae* according to Molina et al., (2022).

List of the most used insecticide products in the monitored orchards in 2019				Efficiency against *T. erytreae* forms		Safety period (days)
Active Ingredient	Product	Uses	Efficient	Nymphs	Adults	
abamectin	Boreal	24	No	0%	0%	7 - 10
acetamiprid	Epik	21	Yes	**69.40%**	79.30%	14
Emamectin benzoate	Affirm	24	No	0%	0%	7
Lambda-cyhalothrin	Judo; Karate Zeon	51	Yes	99.20%	**78.90%**	7
tebufenozide	Mimic	24	No	0%	0%	7

Insecticide products reference sources:

Boreal – https://ascenza.pt/pt-pt/products/borealr

EPIK – https://sipcam.pt/produto/epik-sl/

AFFIRM – https://www.syngenta.pt/product/crop-protection/insecticida/affirm

JUDO - https://ascenza.pt/pt-pt/products/judor

KARATE ZEON – https://www.syngenta.pt/product/crop-protection/insecticida/karate-zeon

MIMIC - https://www.lusosem.pt/produtos-fitofarmaceuticos/inseticidas/mimic

Appendix S4.3

The adult longevity of *T. erytreae* as a function of the mean air temperature was estimated using a linear regression model based on longevity data for female *Diaphorina citri* adults recorded at six different temperatures (Liu & Tsai, 2000):

Estimated adult longevity = 97.534 - (3.077 * *tmed*)

where *tmed* is the average air temperature (ºC).

Table S4.3.1 – Recorded oviposition (eggs per female, mean ± SE) and longevity (days, mean ± SE) of female *D. citri* at six temperatures (Source: Table 4 in Liu & Tsai, 2000).

Table 4. Oviposition (eggs per female, mean ± SE) and longevity (days, mean ± SE) of female D. citri *at six temperatures*

Temp. (°C)		*N*	Mean longevity of female	Mean no. eggs per female
15		18	88.3 ± 4.31	171 ± 25.1
20		22	50.6 ± 2.61	494 ± 50.5
25		25	39.7 ± 1.39	626 ± 22.3
28		21	34.7 ± 1.13	748 ± 34.7
30		25	33.5 ± 1.08	316 ± 30.9
33		23	28.7 ± 1.38	67 ± 10.3
	F		98.4	70.2
	df		5, 128	5, 128
	P		<0.001	<0.001

Appendix S4.4

Trioza erytreae dispersal capacity in the model derived from the fact that only 11.6% of the *T. erytreae* organisms reached distances over 300m in a host absence release trial with the help of prevailing winds (Van den Berg & Deacon, 1988).

Table S4.4.1 –Captured female and male adults in yellow sticky traps in a release trial, with a grid of traps surrounding, with the release point of about 10,000 individuals, in host absence conditions and prevailing winds (Van den berg & Deacon, 1988).

Distance (m)	Females	Males	Total	Ratio
1 – 300m	155	141	296	88.4%
300 – 1600m	25	14	39	11.6%

Appendix S4.5

For the leaf flushing phenology of the model, we calculated a daily chance for leaf flushing dependent on the month. This was calculated using the monthly citrus leaf flushing patterns (Milosavljević et al., 2018), and dividing the percentage of canopy bearing leaf flush growth by 25 days. 25 days is the longevity of suitability of leaf growth flushes used in the model (Cifuentes-Arenas et al., 2018)

Table S4.5.1 - Monthly flushing patterns for citrus trees (Sweet orange trees and Lemon trees) combined from 10 trees in different 10 study sites in California. For each tree, four canopy samples were retrieved for the presence or non-presence of leaf flush growth (North, South, East, West). The percentage of canopy bearing leaf flush growth was calculated as the number of canopy samples with flush growth / (number of flush growth canopy samples + non-flush growth canopy samples) × 100 (Milosavljević; 2018).

Month	Citrus tree canopy bearing leaf flush (%)
January	22.5%
February	90%
March	58.75%
April	15%
May	22.75%
June	30%
July	31.25%
August	14%
September	22.25%
October	27.5%
November	12.5%
December	7.5%

Appendix S4.6

The daily mean air temperature (*tmed*) was assumed to be the same for the entire study area. It was simulated using an equation to capture its seasonal pattern over the year. The equation was formulated as a polynomial of degree 5 in time t (days) and estimated by ordinary least squares using a dataset of the daily weather in Lisbon (Humberto Delgado Airport weather station) in 2018 (Wunderground, 2021). To capture the randomness of daily mean temperatures over time, we added to the previous polynomial equation a random term modelled as an auto-regressive model of order 2, also estimated by ordinary least squares. The simulation model to generate *tmed* in each day t is described in the following equations:

$tmed_t = Sazo_t + r_t$

where the seasonal component Sazo is given by

$Sazo_t = (10.2078 + 27.9757 * (t / 360) - 274.813 * (t / 360) ^ 2 + 1141.28 * (t / 360) ^ 3 - 1591.07 * (t / 360) ^ 4 + 697.627 * (t / 360) ^ 5)$

and the random autoregressive component is given by

$r_t = -0.00190633 + 0.912862 * r_{t-1} - 0.233528 * r_{t-2} + e_t$

with r_{t-1} denotes the value of r_t from the previous day, and r_{t-2} the value of r_t from two days ago, and e_t is an independent random error with a Normal distribution with mean zero and standard deviation 1.551146.

The daily mean air temperature for 2018 and the estimated seasonal pattern are presented in the following figure.

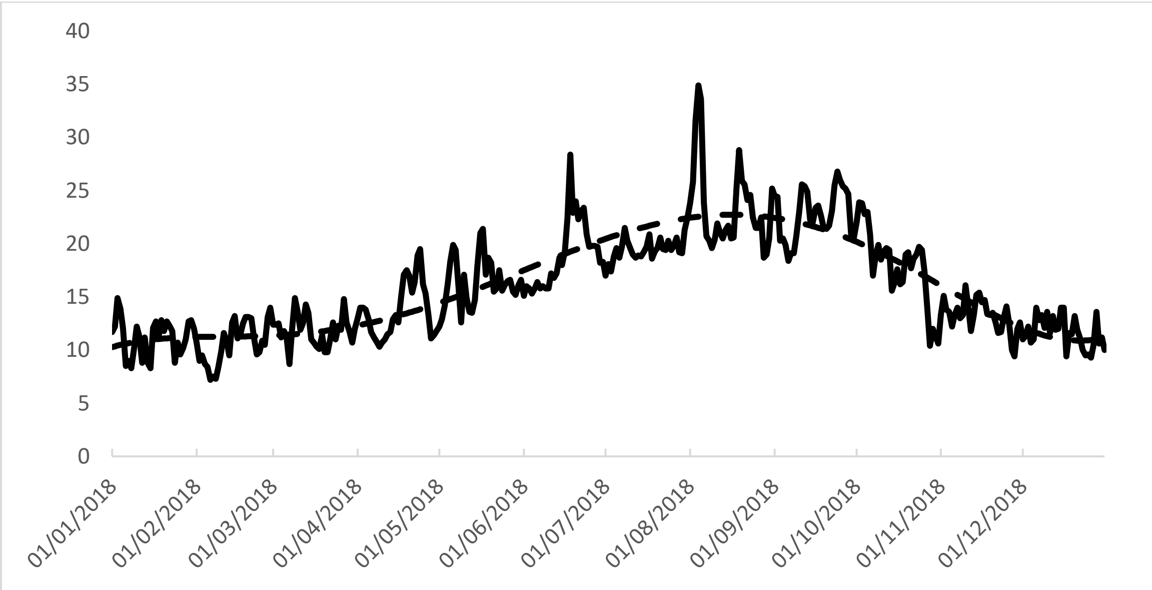

Fig. S4.6.1 Daily mean air temperature of 2018 in Lisbon (Wunderground, 2021) and the estimated seasonal pattern.

One simulation of *tmed* from the model is presented in the figure below.

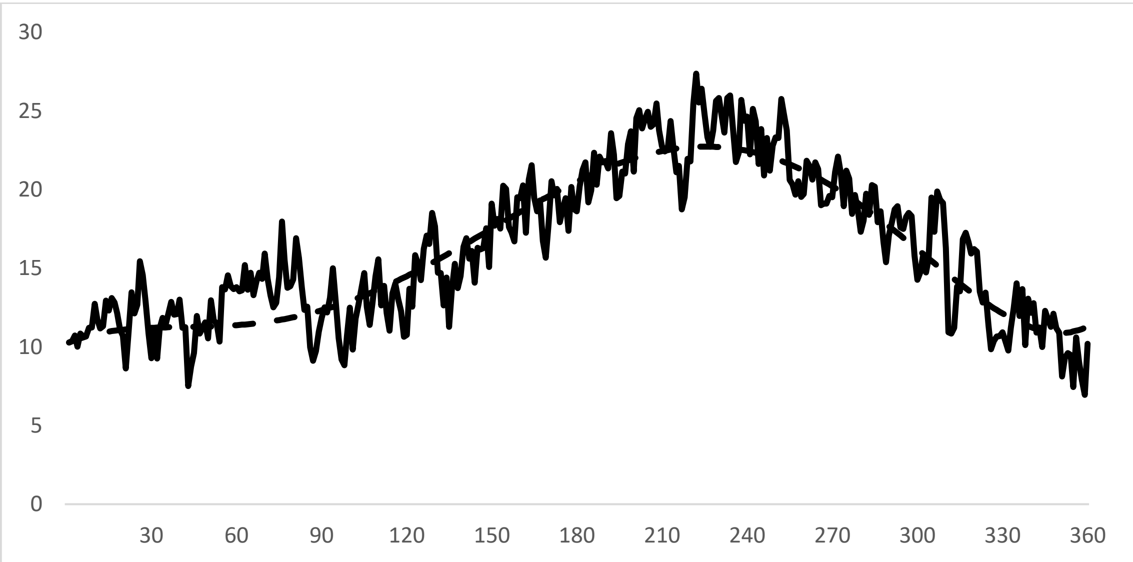

Fig. S4.6.2 *tmed* simulated from the model for one year (360 days) and the estimated seasonal pattern.

Appendix S4.7

Entities, state variables and scales & Process overview and scheduling

Entities, state variables and scales

The model includes only one type of conceptual entities, the patches that compose the modelled landscape. Patches are cells of a grid defining habitat suitability represented as presence or non-presence of a host plant tree. In the case of patches that represent host plant, they are also characterized by state variables related to their current suitability as hosts for the pest species and when infested, the pest species current state in the tree.

Table S.4.7.1 - Description of the model's conceptual entities and state variables of the patches.

Patches		
State variables	**Description**	**References and data sources**
Identity	Each patch has a unique identification defined by the respective coordinates (pxcor, pycor).	
Size	Each patch has an area of 25 km^2 (5 km × 5 km).	
Orchard	Lemon orchard identification number: Each lemon orchard is represented by the union of spatially connected lemon orchard patches.	Landscape characterization based on COS 2018 and Google earth satellite imagery
Limon	Presence of a Citrus Tree or not	Ceia et al., 2015
Urban	Urban area identification number	COS 2018
Flush	[0, 1] – Active leaf growth or not – Determinant for the lemon tree to be susceptible (1) or not (0).	Milosavljević, I et al., (2018).
FlushTime	Time in days since the active leaf growth started. 25 days until the leaves are no longer viable for oviposition.	Cifuentes-Arenas, Juan Camilo, et al., (2018)
Trioza	[0, 1] – Presence or Absence of immature forms of *T. erytreae* in the lemon tree. Determinant for the lemon tree to be latent (1) or not (0).	
TriozaDevelop	Accumulation of the daily maturation development towards the maturation of immature forms, dictating when they fully develop into adults.	Catling, H. D. (1973)
Donator	[0, 1] - Presence or Absence of adult forms of *T. erytreae* in the lemon tree. Determinant for the lemon tree to be infectious (1) or not (0).	
DonatorDevelop	Accumulation of the daily adult aging, determining the duration of the infectious state of the tree.	Liu & Tsai (2000)
DonatorTime	Age of the adult to consider the pre-oviposition period of the species.	
Pre-Oviposition	[3 – 7] Randomly assigned between 3 to 7 days, Infectious trees only become active infectious trees after the DonatorTime > Pre-oviposition.	Annecke and Cilliers 1963; Moran Blowers 1967; Catling 1973
ProbInfection	[0 – 9999] - Daily randomly generated number for citrus trees, dictating the chances for the tree to be infested by a nearby infectious lemon tree.	
Treatments	Accumulated yearly number insecticide treatment applications done in the patch.	
Limit	Number of maximum yearly insecticide sprays for the orchard tree, assigned at the start of the model based on regional data.	Based on real yearly Insecticide application data of orchard owners of the region
TimeTreat	Time in days since the last insecticide spray used in the patch, used to take into consideration the safety period between insecticide treatments.	
Spray-Chance	[0 – 100] Daily randomly generated number for orchard trees to dictate when will each orchard receive an insecticide treatment.	
Type-Spray	[0, 1] Dictates whether the insecticide spray will use effective products against *T. erytreae* (1), or non-effective (0)	Based on Insecticide application products of orchard owners of the region and their efficacy (Molina et al., 2022).
Infestations	Accumulated number of separate times that the lemon tree was infected during the simulation.	

The size of each patch corresponds to 25m^2 (5x5m) and the study area represents an area of 47.4 Km^2. The region landscape can be divided into three land-use classes (LULC): Lemon orchards, urban area, and other land types. All Lemon orchards patches each represent one lemon tree, while urban patches have a 1.3% chance of representing an urban citrus tree which are randomly assigned at the beginning of the model, based on the 5.14 urban trees/ha urban citrus trees density in peri urban environments (Nunes et al., 2023). In this model there are no moving components, the agents being the citrus trees which are immovable patches.

Each tick represents 1 day with the months being comprised by 30 days and one year as 360 days. Each simulation lasts 310 days (310 time units), representing the surveyed time period, along with, representing the yearly time period of citrus tree flushing in Mediterranean climates (Milosavljević, et al., 2018; Syvertsen et al., 1981).

Process overview and scheduling

Before the simulation starts, insecticide treatments are selected, or not, to be included in the model (Scenario 0 or Scenario 1). After that, the “Setup” sub-model is run. Next, the “Run the Simulation” sub-model is processed, as represented in Fig S4.7.1.

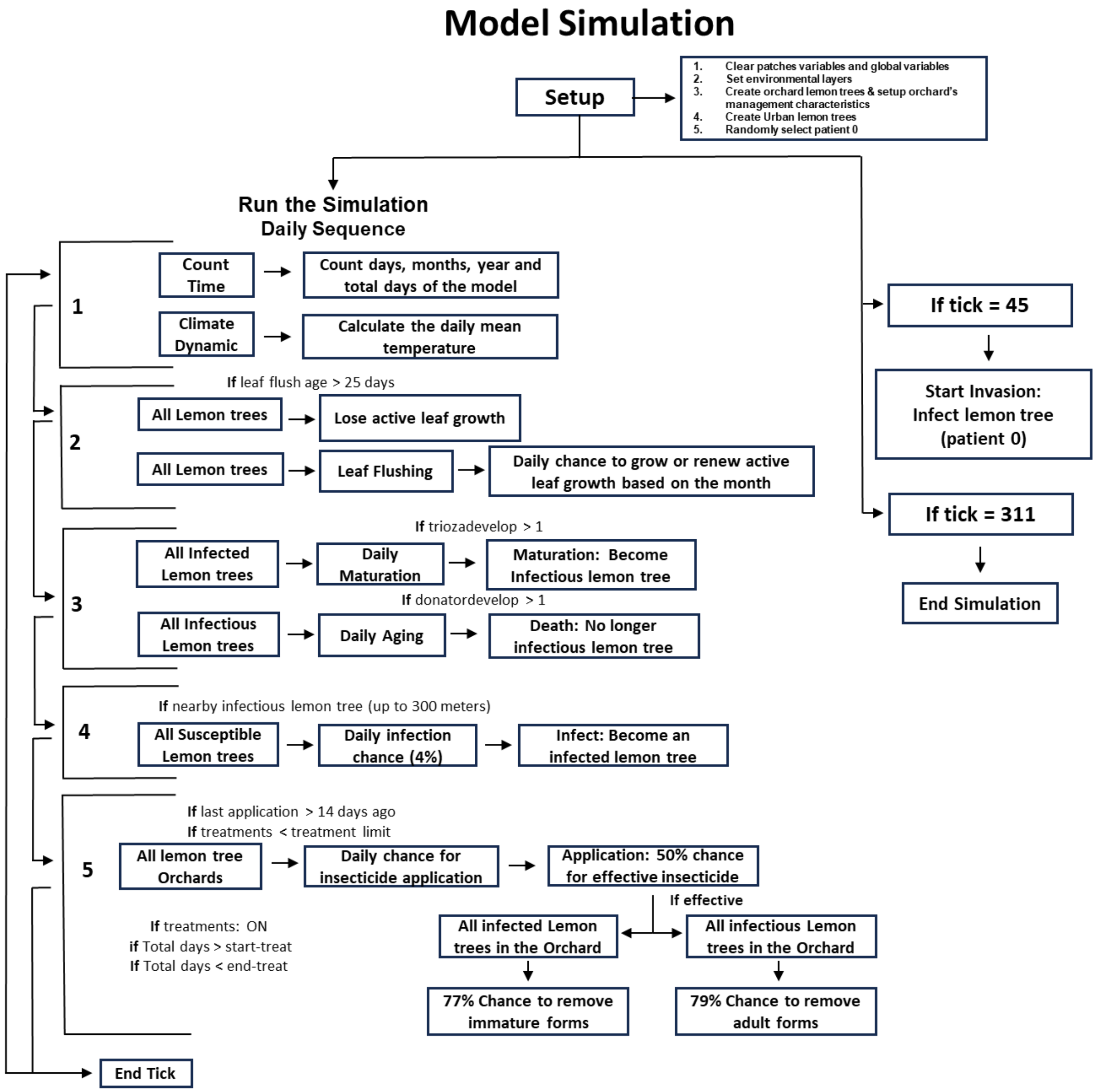

Fig S.4.7.1: Illustration of the processes behind the model running divided by the SETUP sub-model and the Run the Simulation sub-model. (Same as Fig. 4.3 above).

The "Setup" sub-model performs the following steps:

Setup

1. Reset the following patch variables by setting them to 0 (limon, Trioza, infestations, Donator, Donatordevelop, TriozaDevelop, DonatorTime, Flush, FlushTime, treatments, TimeTreat). Also reset all global variables: day = 0, month = 1, year = 1, total-days (t) = 0; r1 = 0, r2 = 0, sazo = 10.2078; *tmed* = 10.2078.
2. Load up the environmental layer of residential urban areas and the environmental layer of lemon orchards to create the virtual landscape.
3. Create lemon trees in orchard areas, by turning all lemon orchard LULC patches into lemon trees (limon = 1). Additionally, for each lemon orchard, set a yearly limit of insecticide applications, using a random number from a normal distribution, with mean 3.47 and standard deviation of 1.80. Based on treatment frequency in the region (appendix S.4.2).
4. Create lemon trees in urban areas, assigning for every urban LULC patch, a 1.3% chance to become a lemon tree (limon = 1).
5. Randomly select a random lemon tree in the study area to be the first initially infected lemon tree of the simulation (patient 0).

After the "**Setup"** sub-model, the "**Run the Simulation"** sub-model is started, with the following daily sequence processes, being repeated until the start of tick 311:

Run the Simulation

1 – Count time and Temperature Simulation

1. The time is modelled in discrete time steps (1 day), over which discrete events occur. Each month has 30 days.
2. The daily mean air temperature (*tmed*) is simulated using the equation presented in appendix S4.6.

2 – Leaf Flushing

1. For every lemon tree with active leaf growth, the age is checked. In case the age has exceeded 25 days old (FlushTime > 25), the tree loses its active leaf growth (flush = 0), becoming a non-susceptible lemon tree (Cifuentes-Arena et al., 2020).
2. Every lemon tree with no active leaf growth has the chance to start active leaf growth (Flush= 1). The chance to start active leaf growth is based on the equation presented in appendix 4.5.
3. Every lemon tree with active leaf growth (Flush = 1) has a daily chance to rejuvenate its active leaf growth, resetting its age (FlushTime = 0). The daily chance is the same as the previous process.
4. Leaf active growth age (FlushTime) of all lemon trees is updated by adding 1 day.
5. Invasion Start - if of tick being 45, the invasion of *T. erytreae* in the landscape begins. The previously selected patient 0 lemon tree is infected by immature psyllid forms (Trioza = 1; TriozaDevelop = 0).

3 – *T. erytreae* growth

1. The daily maturation development rate is updated daily based on the mean air temperature using the following equation: Daily maturation development rate = 1 / ((4.9763 + (3.3443 x $0.8452^{(tmed - 20)}$ + (16.7974 + 5.2726 x $0.7843^{(tmed - 20)}$))))
2. All latent lemon trees (Trioza = 1) add the value of the daily maturation development rate to the accumulation of maturation development patch variable (TriozaDevelop).
3. The daily adult aging rate is also updated daidly based on the mean air temperature using the following equation: Daily adult aging rate = 1 / (97.534 - (3.077 x *tmed*)).

4. All infectious lemon trees (Donator = 1) add the value of the daily adult aging rate to the accumulation of adult aging patch variable (DonatorDevelop).
5. Any latent lemon tree with TriozaDevelop exceeding 1.0, will become an infectious tree, losing the immature forms (Trioza = 0, Triozadevelop = 0), and gaining adult forms (Donator = 1). In addition, they are assigned a randomly calculated the pre-oviposition value varying from 2 to 7 (Pre-Oviposition).
6. Any infectious lemon tree with DonatorDevelop exceeding 1.0, will no longer be an infectious tree, losing the adult forms (Donator = 0, DonatorTime = 0; DonatorDevelop = 0).

4 – Infection

1. All susceptible non infected lemon trees (flush = 1 and Trioza = 0) have a 4% chance to be infected (Samways & Manicom, 1987), in case there is an active infectious lemon tree nearby (Donator = 1, DonatorTime > Pre-Oviposition and distance =< 300m) (Van den Berg & Deacon, 1988). In case of infection, the infected lemon tree becomes a latent lemon tree (Trioza = 1, TriozaDevelop = 0, increase infestations by 1).
2. Each active infectious lemon tree (Donator = 1, DonatorTime > Pre-Oviposition) will infect itself if susceptible to being infected (Flush = 1 and Trioza = 0). This way becoming both an infectious and latent lemon tree (Trioza = 1; TriozaDevelop = 1; Increase Infestations by 1; Donator = 1)

5 – Insecticide Treatment

1. For each lemon orchard, it is attributed whether in this day, the insecticide product used would be effective (spray-type = 1) or not against (spray-type = 0) *T. erytreae*, with a 50% chance for each outcome.
2. Each lemon orchard has a 3% daily chance to be sprayed by insecticide if its yearly treatment limit has not yet been reached and if the last treatment was more than 14 days ago.

3. If a lemon orchard is chosen to be sprayed, and the insecticide product used is effective (spray-type = 1), then each lemon tree will have a 66% chance to remove immature forms (Trioza) and a 79% chance to remove adult forms (Donator).
4. At the end, all trees increase the time of last treatment by 1 (TimeTreat), signifying the passage of time.

In the end of the daily sequence of processes, the tick is finished, and step 1 is restarted, until tick 411th is reached, ending the **Run the simulation** sub-model.

Chapter 5. Conclusions

In this thesis, we aimed at understanding the role that landscape composition and configuration may play in the spread of invasive species. For this, we developed different modelling approaches to study the dispersal of invasive species, using landscape factors and landscape ecology concepts, at both local and large scale, based on two model species, i.e., the pine wood nematode*,* causal agent of the pine wilt disease vectored by *M. galloprovincialis*, and a pest of orchard trees, *T. erytreae*, the vector of the citrus greening disease.

In this final chapter, we provide a summary of the main achievements and discuss the studied research hypothesises, the methodological contributions and innovations from our work along with providing some practical implications and directions for future research.

5.1. The effect of landscape heterogeneity in the spread of invasive pests

The main hypothesizes of our work was that landscape heterogeneity can slow the spread of invasive species. This hypothesis is based on various ecological processes associated with the increase of landscape heterogeneity, that may potentially be detrimental for insect spread. We focused on the following three ecological processes related to landscape heterogeneity, that may affect dispersal, in the case of *M. galloprovincialis*: (1) Increase of non-host trees at a stand level contributes to visual and semiochemical disruption of beetle's ability to finding host trees in the forest; (2) Higher plant species diversity can promote natural enemies' abundance and richness, strengthening the top-down control by natural enemies, thus reducing pest survival; (3) Increase of habitat fragmentation, leading to the increase of non-favourable areas in the landscape, reducing the connectivity between habitat areas.

We showed that at stand level non-host tree habitats are avoided by *M. galloprovincialis* in its dispersal. Forest areas with higher non-host tree proportion (broadleaf forests and mixed forests) had higher resistance levels against the beetle's dispersal, while areas with a low proportion of non-host trees (pine areas, open pine areas and clear-cut areas) had the lowest friction. This reveals the potential repellent effect that non-host trees can have towards the beetle's dispersal. This is likely explained by the role of non-host tree species sensory cues inducing visual and/or semiochemical disruption of the beetle's ability to find suitable hosts. This effect is highlighted by the high resistance level found on mixed forest areas, demonstrating the potential effect of adding non-host trees to reduce the attractiveness of a habitat area. Additionally, the higher resistance level found on mixed forest over broadleaf forest areas might be explained by the natural enemy's top-down control. A mixed forest has a higher tree species diversity, which can promote natural enemy's abundance and diversity in the area, strengthening the top-down control of the beetle's population by natural enemies. Furthermore, higher tree species diversity could increase the diversity of sensory cues in the area, increasing the effect of sensory confusion for the beetle's host finding.

Finally, we also provided evidence that habitat fragmentation impacts the pest species dispersal at local scale, for both *M. galloprovincialis* and *T. erytreae*. In the case of *M. galloprovincialis*, the habitat fragmentation found in the heterogenous forest area reduced the connectivity of favourable habitat areas. The pattern of discontinuous habitat areas with non-habitat areas across the landscape was shown to alter the beetles flight trajectories, utilizing least-cost path analysis. The beetles were forced to utilize longer alternative trajectories for host searching to avoid the non-habitat areas, likely slowing down its spread at the landscape level. For *T. erytreae*, citrus orchard fragmentation also influenced the rate of the infestation progress in the study area.

Taking into consideration all our findings, we can conclude that landscape heterogeneity can influence the dispersal of an insect pest. Yet, its effect is not straightforward, as it will depend on the positive or negative nature of the landscape elements towards the insect dispersal, which depends on the insect species and landscape configuration. Past studies have shown contrasting effects of landscape heterogeneity towards different species dispersal, even for the same species in different landscape types (Roland, 1993; Wood et al., 2010).

Hence, increasing land-use diversity may not necessarily reduce invasive species spread, evidenced by the inconsistent effect of non-habitat areas. In case of *M. galloprovincialis*, broadleaf and mixed forests were found to be deterrent for the species dispersal, while open areas such as clear-cuts did not affect the species dispersal ability or even facilitating the spread, shown by areas with low host tree density facilitating the spread for both *M. galloprovincialis* (open pine areas) and *T. erytreae* (urban and peri-urban areas). The promotion of insect species dispersal by the absence of host trees has also been reported for *T. erytreae*, where psyllids released in non-host conditions were found to disperse in longer distances in search of habitat (Samways & Manicom, 1983; Van den Berg & Deacon, 1988). This further highlights the need to improve our overall understanding of the ecological processes behind insect species dispersal and their interaction with the landscape characteristics.

5.2. The role of isolated host trees in the spread of invasive pests

Isolated host tree patches or scattered trees can function as stepping stones, improving the habitat connectivity in fragmented environments (Saura et al., 2014). Additionally, these may promote long-range distance dispersal, expanding the species dispersal range, allowing the species to reach other favourable habitat areas. This topic has been studied extensively in conservation biology, as the creation or conservation of these patches has been found to play a large role in species conservation (Saura et al., 2014; Cadavidad-Florez et al., 2020; Rocha et al., 2021). We hypothesized that isolated host trees can have an important role in the spread of invasive pests. This hypothesis was tested for the invasion of *T. erytreae* in Portugal, at both local and large scales.

We found that both for local and large scale, the distribution of isolated host citrus trees, from urban and peri-urban areas, played a major role in explaining the observed invasion patterns of *T. erytreae* in Portugal. In both cases, the distribution of the isolated host trees was shown to promote the expansion rate of the insect pest, improving habitat connectivity. At the large-scale level, not incorporating isolated host trees in the model, significantly decreased the species spread rate and the model's performance at predicting the invasion dynamics, showcasing the negative effect that habitat fragmentation could have played for the species spread. The distribution of these isolated citrus trees in residential urban areas represented a continuum of hosts along the coastal area of Portugal, completely changing the invasive spread dynamics, allowing the spread of *T. erytreae* even at low host density conditions.

At the landscape level, we showed that the proportion of surrounding urban areas with isolated citrus trees also played a key role in increasing the expansion range of *T. erytreae*. Again, isolated host trees served as stepping stones countering the constraints imposed by local orchard fragmentation, improving the chances for the insect pest to reach other citrus orchards. Furthermore, these isolated trees have the potential to serve as refuges for population growth in managed agriculture environments (Van den berg & Deacon, 1988). In fact, our results supported this hypothesis. Additionally, our model showed that the insecticide application of the orchards in the region did not significantly affect the species spread, which may be explained by the refuge and dispersal role from the considerably distributed urban areas in the region, alongside the high dispersal and reproductive ability of the psyllid and the high variation in management effort observed in the region, which can affect the effectiveness of pest management (Aida, 2018; Noy and Jabbour, 2020).

5.3. The role of human-assisted dispersal in the spread of invasive pests

The role of anthropogenic pathways in the spread of invasive insect pests have increasingly been recognized as a key factor in invasion science (Ricciardi et al., 2017; Bullock et al., 2018; Gippet et al., 2019). Human activities that lead to the dispersal of insect pests can be divided into three main pathways: contamination, hitchhiking and harvesting (Pergl et al., 2017; Gippet et al., 2019). To explain the invasive rapid spread of *T. erytreae* across the coastal area of Portugal, we focused on hitchhiking activities associated with rare stochastic long-range dispersals in regions with high human density.

The findings of our study lend support to the idea that human-mediated spread played a crucial role in the rapid invasion of *T. erytreae* in the Portuguese territory. The evidence for this is underscored by the swift spread pattern, which predominantly favoured the southern axis and closely followed the coastal regions where human population density is higher. In our model, we considered that long-distance dispersal events were facilitated by human activities. While there may be other factors, such as wind, that could have been involved, we recommend their inclusion in future research efforts.

5.4. Methodologies and Innovation

Our case studies highlighted the significance of ecological processes occurring at different spatial scales in driving the dispersal of invasive insect pests. These ecological processes were found to be influenced by landscape composition and configuration variables. Therefore, the use of these variables in studying invasive species dispersal, along with applying concepts from landscape ecology, was shown to be extremely valuable.

With each case study, we aimed at developing innovative methodologies and modelling approaches for invasion biology. In chapter 2, we displayed the potential in the use of least-cost path analysis methods for invasive species studies and contributed towards optimization of trapping grid systems for pest detection, with an innovative method for locating the most likely area of origin in the landscape of the trapped insects, along with trap-density reduction analysis.

In chapter 3, we contributed with the first spatio-temporal dispersal model of *T. erytreae*, along with the first study and evidence of the role of human-assisted dispersal in the invasion spread of *T. erytreae*. Additionally, we provided the first known study showcasing the importance of urban and peri-urban citrus host trees in the invasive spread of *T. erytreae* in Portugal and demonstrated the potential of Google Street view imagery as a tool for estimating the density of urban and peri-urban trees. Finally, the developed model highlighted the potential of modelling stratified dispersal, combining a diffusive dispersal model with a stochastic long distance dispersal model.

Finally, in chapter 4, we showcased the potential of the developed approach for modelling insect pest dynamics using an innovative new take with an epidemiological cellular automaton model approach.

5.5. Practical implications

We believe our results further cement the potential benefit from increasing tree species diversity in forest areas, increasing the likelihood of existing non-host trees for future invaders, potentially hindering their invasion process. One way to improve the odds of planting non-host trees is in selecting a pool of different tree species with as much phylogenetic variation as possible (Bertheau et al., 2010). However, planting these trees must be done with great precaution, as they also pose as a risk of accidentally introducing new invasive specialist insect's species, as found with eucalypt pest species in California (Paine et al., 2010).

At landscape level, it seems advisable to increase compositional heterogeneity in order to prevent the dispersal of invasive pests while providing complementary habitats for their natural enemies. Improving landscape heterogeneity would pave the way for the implementation of the "land sparing" approach, i.e., the spatial separation of ecological and economic functions in parcels of different land use and management types that are particularly favourable to these functions. This land sparing principle does, however, pose problems of territorial governance, particularly regarding the decision to change land use to maximize a particular function. This can lead to loss of income if, for example, a patch of agricultural land must be converted into a nature reserve. For this, compensation measures should be envisaged, or payments for ecosystem services should be granted, which is not yet the case for forests, contrary to EU programmes for promoting organic farming in Europe (Dayoub & Korpela, 2019; Lefebvre & Langrell, 2015).

The methodology developed for locating the most likely origin of captured organisms from a trapping grid has the potential to be adapted for improving both invasive and pest species monitoring, and the management of these species. Additionally, we suggest using spread models to identify the more likely invasion areas, i.e., hot spots of invasion risk. We suggest increasing the number of trapping grids, targeting all these high-risk areas, but with reduced number of traps, as large grids were not found to be cost effective.

The findings of both human-mediated dispersal and spread facilitated by the isolated host trees in residential urban areas, showed the importance of considering these processes for the monitoring, surveillance, and control of pest species.

Finally, the simple and intuitive epidemiologic modelling approach developed for *T. erytreae* could be easily adapted for other studies, such as simulating different monitoring or management scenarios, at different spatial or temporal scales. Additionally, the visual component of this model allows it to be used as a management tool for practitioners and stakeholders, with what-if scenarios.

5.6. Future directions

For the future, it would be interesting to apply the developed methodology of trap monitoring, which pinpoints the most likely origin of captured insects, to other types of pests and landscapes in order to better validate the approach. It could then be applied for the monitoring and surveillance trapping grids of forest and agriculture pest species with the aim of improving the effectiveness and reducing the economic and environmental impact of management actions, such as large clear-cutting and insecticide spraying (Cayuela, 2011; Robinet et al., 2020).

It would be also important to test the ecological processes affecting dispersal behaviour of invasive pest species in more direct manners, such as laboratory trials to study the response of target organisms to a large variety of different tree species odours and their combinations, or direct studies describing natural enemies' spatial distribution and impact towards pest species, along with testing the correlation with landscape metrics.

Specifically for the control of Pine wood nematode spread, it would be important to find which non-host tree species release chemical substances deterrent for *M. galloprovincialis* dispersal, to find the most cost-effective manner of hindering the dispersal of the PWN using these trees species in pine plantations (Van Halder et al., 2022).

The successful application of these findings is dependent on the acceptability of landscape changes by stakeholders and the society. Therefore, it would be imperative to understand the willingness of stakeholders for different potential management actions and the required incentives, developing studies focused on evaluating the economic and social costs and benefits of landscape planning actions, along with studying the effectiveness of incentive programmes or government regulations. A transdisciplinary approach, combining ecological and social sciences, is thus necessary.

Regarding *T. erytreae* potential invasion in Europe, the development of risk analyses for other countries in Europe would help to improve invasion scenarios, which would allow to identify likely introduction hotspots, areas of higher risk of spread and then better allocate funding for monitoring. This would speed up the application of control actions, increasing their success (Branco et al., 2023).

Finally, it would be interesting to apply the developed epidemiological model to other pest species and environments, such as *Diaphorina citri* in North America, the other psyllid vector species of the greening disease, or even for simulating the introduction of the greening disease in Portugal and other parts of Europe. This would enable us to assess the potential and limitations of this methodology under a wider range of conditions.

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