

# Distribution of alien tetrapods in the Iberian Peninsula

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Academic editor: S. Bertolino | Received 18 June 2020 | Accepted 27 November 2020 | Published 11 January 2021

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**Citation:** Ascensão F, D'Amico M, Martins RC, Rebelo R, Barbosa AM, Bencatel J, Barrientos R, Abellán P, Tella JL, Cardador L, Anadón JD, Carrete M, Murgui E, Fernandes P, Santos SM, Mira A, da Luz Mathias M, Tiago P, Casabella E, Reino L, Paulo OS, Pereira HM, Capinha C (2021) Distribution of alien tetrapods in the Iberian Peninsula. NeoBiota 64: 1–21. <https://doi.org/10.3897/neobiota.64.55597>

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## Abstract

We present a dataset that assembles occurrence records of alien tetrapods (amphibians, reptiles, birds and mammals) in the Iberian Peninsula, a coherent biogeographically unit where introductions of alien species have occurred for millennia. These data have important potential applications for ecological research and management, including the assessment of invasion risks, formulation of preventive and management plans, and research at the biological community level on alien species. This dataset summarizes inventories and data sources on the taxonomy and distribution of alien tetrapods in the Iberia Peninsula, comprising known locations from published literature, expert knowledge and citizen science platforms. An expert-based assessment process allowed the identification of unreliable records (misclassification or natural dispersion from native range), and the classification of species according to their status of reproduction in the wild. Distributional data was harmonized into a common area unit, the 10 × 10 km Universal Transverse Mercator (UTM) system (n = 6,152 cells). The year of observation and/or year of publication were also assigned to the records. In total, we assembled 35,940 unique distribution records (UTM × species × Year) for 253 species (6 amphibians, 16 reptiles, 218 birds and 13 mammals), spanning between 1912 and 2020. The species with highest number of distribution records were the Mediterranean painted frog *Discoglossus pictus* (n = 59 UTM), the pond slider *Trachemys scripta* (n = 471), the common waxbill *Estrilda astrild* (n = 1,275) and the house mouse *Mus musculus* (n = 4,043), for amphibians, reptiles, birds and mammals, respectively. Most alien species recorded are native to Africa (33%), followed by South America (21%), Asia (19%), North America (12%) and Oceania (10%). Thirty-six species are classified by IUCN as threatened in their native range, namely 2 Critically Endangered (CR), 6 Endangered (EN), 8 Vulnerable (VU), and 20 species Near Threatened (NT). Species maps are provided in DataSet1, as well R code and GIS layers to update them as new records are obtained.

## Keywords

Alien terrestrial vertebrates, biological invasions, Iberian Peninsula, invasive species, Portugal, Spain

## Introduction

The human-mediated introduction of species into regions outside their native range is an important component of global change. Alien species (*sensu* Essl et al. 2018) are responsible for the decline and extinction of native species, economic losses and human health problems (Clavero and García-Berthou 2005; Tatem et al. 2006; Hulme 2009; Simberloff et al. 2013) and are leading to irreversible changes to the diversity and distribution of life on Earth (Simberloff et al. 2013; Capinha et al. 2015). Alien species can impact receiving ecosystems, even if simply through competition with native species for space, food, water or other resources. Although impacts perceived as ‘significant’ are generally recorded for only a subset of alien species, i.e. the ‘alien invasive species’ (*sensu* IUCN 2000), many ongoing invasions may have simply not yet spread to the point when impacts become noticeable. Furthermore, several invasions may have already been set in motion, leading to impacts in the near future (Essl et al. 2011). Some alien species may be currently in a lag-phase, during which little or no increase in distributional ranges is observed; that may be followed by an increase-phase in which their occurrence and invasiveness rises rapidly (Aikio et al. 2010; Essl et al. 2011; Russell and Blackburn

2017). Hence, data on alien species occurrence is crucial and a first step to understand the main drivers shaping their distribution (Abellán et al. 2017; Ascensão et al. 2020) and delineate effective management actions and policies accordingly (Abellán et al. 2017; Hattab et al. 2017; Carboneras et al. 2018; Ascensão et al. 2020).

In natural environments, alien tetrapods (amphibians, reptiles, birds and mammals) can compete with, and predate, native species. The American mink (*Neovison vison*), for example, led to significant population declines of ground nesting birds (e.g. the black-headed gull *Chroicocephalus ridibundus* or the common tern *Sterna hirundo*), and small mammals (e.g. the European water vole *Arvicola amphibius* and the Pyrenean desman *Galemys pyrenaicus*) in its introduced range (Craik 1997; Aars et al. 2001). Also, the rose-ringed Parakeet (*Psittacula krameri*), invasive in Iberia, is highly aggressive toward the tree-dwelling greater noctule bat (*Nyctalus lasiopterus*) when trying to occupy their tree cavities. Rose-ringed parakeet aggressions often result in noctule death, causing population declines and disruption of the complex social behavior of this bat species (Hernández-Brito et al. 2018). Likewise, the pond slider (*Trachemys scripta*) feeds on several native species of plants and animals, and it potentially competes with native turtles, such as the endangered European pond turtle (*Emys orbicularis*), for food, basking and nesting sites (Cadi and Joly 2003, 2004; Balzani et al. 2016). On the other hand, the economic impacts of tetrapods can be striking. For example, in Italy the coypu (*Myocastor coypus*) caused over 11 Mio € in damages during 1995–2000 and similar developments have been suggested for Spain (Panzacchi et al. 2007). Likewise, the monk parakeet (*Myiopsitta monachus*) or the rose-ringed Parakeet are considered important avian pests (Kumschick and Nentwig 2010; Senar et al. 2016; Reys et al. 2018).

Here, we provide a first compilation of the distribution of the alien tetrapods in Iberian Peninsula. This region integrates the Mediterranean biodiversity hotspot, harboring about half of the European plant and terrestrial vertebrate species (Myers et al. 2000). It is also a region where many alien species are becoming common and spreading, but no cross-taxonomic assessment on distribution patterns has been performed to date, except for birds (Abellán et al. 2016, 2017; Ascensão et al. 2020). The dataset here described contains information on the known occurrences of alien amphibians, reptiles, birds and mammals in this region. We considered all known species with individuals occurring freely in cities and in the countryside (not in captivity), but we discarded domestic species. For each species, we have also included information on current status of establishment in the Iberian Peninsula, classifying each species as ‘established’, ‘not established’, or ‘uncertain’. The applications of these data range from supporting the development of measures for the prevention and management of biological invasions to undertaking species- and community-level ecological research. Specifically, the assembled data allows more detailed research on the distribution of single alien species and of the spatial patterns of richness and composition of alien species assemblages at the regional scale (Ascensão et al. 2020). These potential applications are of specific relevance for a number of species currently being targeted by national and EU-level legislation.

## Metadata

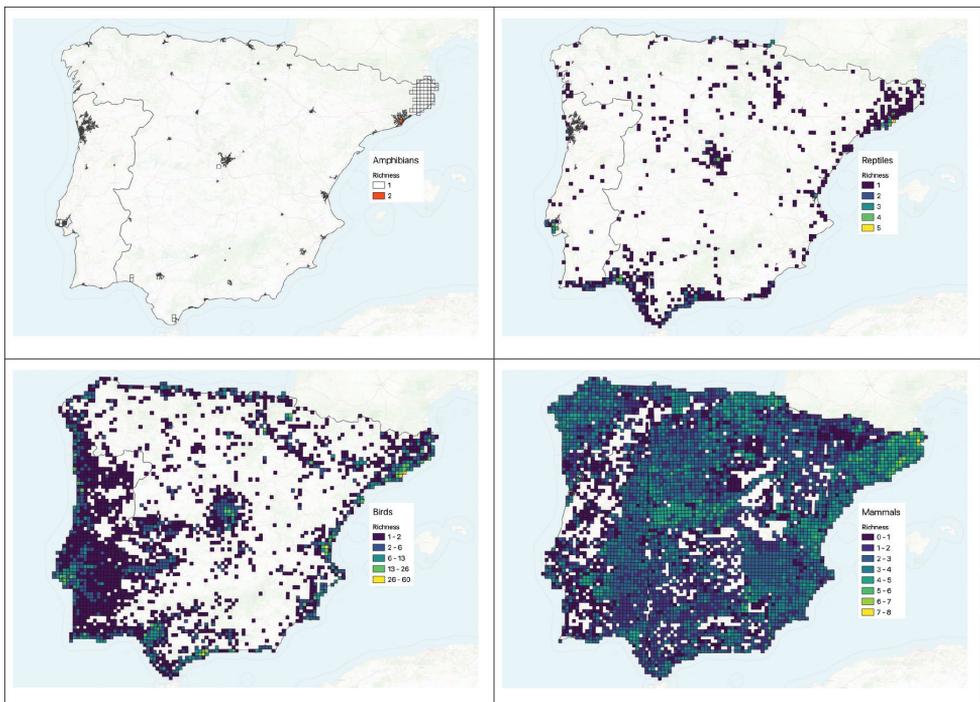
### Data set descriptors

#### A. Data set identity

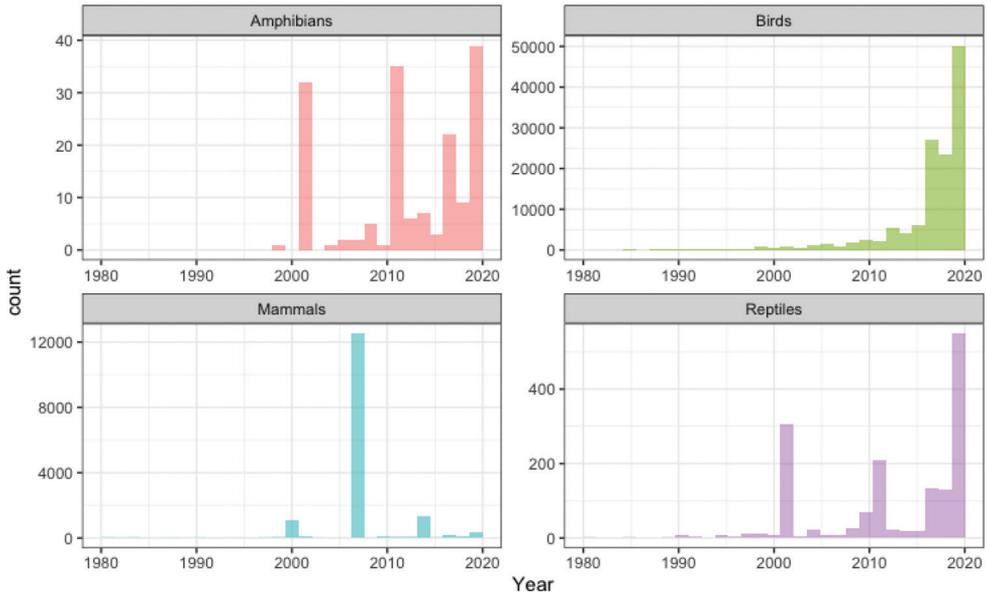
Registry of alien tetrapods (terrestrial and freshwater vertebrates) in the Iberian Peninsula.

#### B. Data set description

This dataset summarizes inventories and data sources on the distribution of alien tetrapods in the Iberian Peninsula, from 1912 onwards, comprising known locations from published literature, expert knowledge and citizen science platforms. An expert-based filtering process allowed the identification of unreliable records (e.g., misclassification or natural dispersion from native range). Distributional data was harmonized into a common area unit, the Universal Transverse Mercator (UTM) system ( $n = 6,152 \times 10 \times 10$  km cells). The dataset consists of one file, containing a  $15 \times 159,677$  matrix of values, including information on species, location, time, current establishment status and source. In total, we assembled 35,940 unique distribution records (UTM  $\times$  species  $\times$  Year) for 253 species (6 amphibians, 16 reptiles, 218 birds and 13 mammals) (Fig. 1), spanning between 1912 and 2020 (Fig. 2). Fifty-six species (22%) are known to re-



**Figure 1.** Richness of alien tetrapods in Iberian Peninsula, by taxonomic Class.



**Figure 2.** Yearly distribution of records on non-native tetrapods in the Iberian Peninsula. Year in x-axis refers to the most accurate timeframe information available and may indicate the time of publication (e.g., Spanish Atlas of Mammals in 2007), or the actual time of observation for the data (e.g., from citizen science platforms). Between 1912 and 1980 there are 138 records (<0.1% of total), not shown.

produce in the wild (established in Iberia), whereas for 98 species (39%) there is no evidence for being established, and for 99 species (39%) the establishment status is uncertain. The species with the highest number of distribution records were the Mediterranean painted frog *Discoglossus pictus* ( $n = 59$  UTM), the pond slider *Trachemys scripta* ( $n = 471$ ), the common waxbill *Estrilda astrild* ( $n = 1,275$ ) and the house mouse *Mus musculus* ( $n = 4,043$ ). Most alien species recorded are native to Africa (33%), followed by South America (21%), Asia (19%), North America (12%) and Oceania (10%). Thirty-six species are classified by IUCN as threatened in their native range, namely 2 Critically Endangered (CR), 6 Endangered (EN), 8 Vulnerable (VU), and 20 species Near Threatened (NT). The file is labelled as “Data\_AscensãoEtAl\_Neobiota.csv”.

## 1. Principal investigators

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## Research origin descriptors

### A. Overall project description

#### 1. Identity

This dataset assembles and harmonizes all known locations of alien terrestrial and freshwater vertebrates (tetrapods) in the Iberian Peninsula by UTM cell (ca. 10 × 10 km). The taxonomy and origin are also provided for each species.

#### 2. Originators

The data was collected under the project “The role of transportation in Biological Invasions” funded by Infraestruturas de Portugal Biodiversity Chair (ref02035004). Methodology was developed by Fernando Ascensão and César Capinha. All authors participated in data collection and validation process.

#### 3. Period of study

Data was collected from 01/01/2017 to 10/08/2020. Collected data included records from 1912 to 2020.

#### 4. Objectives

The primary objective of the present work was to compile a registry of non-native vertebrates (tetrapods) present in the Iberian Peninsula. The applications of these data range from supporting the development of measures for the prevention and management of biological invasions to undertaking species- and community-level ecological research. Specifically, the assembled data allows more detailed research on the distribution of single alien species and of the spatial patterns of richness and composition of alien species assemblages at the regional scale. These potential applications are of specific relevance for a number of species currently being targeted by national and EU-level legislation.

#### 5. Source of funding

Infraestruturas de Portugal Biodiversity Chair (ref02035004) funded the project “The role of transportation in Biological Invasions”. F. Ascensão was also funded by Fundação para a Ciência e Tecnologia – grant SFRH/BPD/115968/2016.

### Summary of “The role of transportation in Biological Invasions” project

The naturalization of non-native species is now one of the main mechanisms responsible for altering the biosphere, causing profound changes in the structure and functioning of ecosystems. Given the increasing mobility of people and goods, concomitant with the increase in road and rail networks, and consequent increase in the number of introductions

(intentional or not) of non-native species in new locations, it is expected that these changes will continue worsening sharply in the future. The main objective of this project was to deepen the knowledge about the biogeographic patterns that are emerging as a result of this growing mix of species. Part of the data has been published in Ascensão et al. (2020).

## **B. “Specific” subproject description**

### **1. Site description:**

#### a. Site type

Data was collected for all of the Iberian Peninsula, namely continental areas of Portugal and Spain, together with Gibraltar (a British Overseas Territory located at the southern tip of the Iberian Peninsula).

#### b. Geography

Continental areas of Portugal and Spain, together with Gibraltar (a British Overseas Territory located at the southern tip of the Iberian Peninsula).

#### c. Habitat

The region includes terrestrial and freshwater habitats.

#### d. Geology, landform

The region includes various geological types, ranging from Ediacaran to the Quaternary.

#### e. Watersheds, hydrology

All river systems in the Iberian Peninsula.

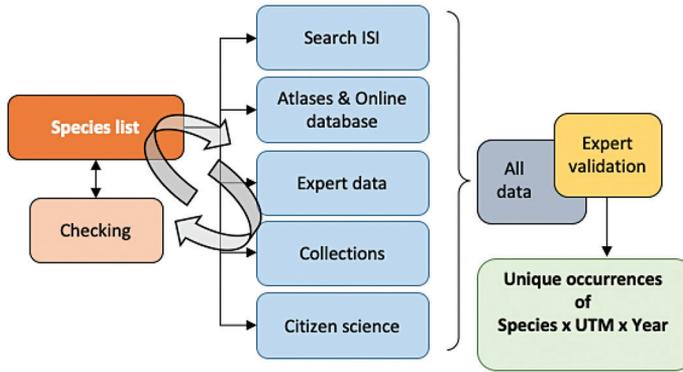
#### f. Climate

Climatic conditions in the study area range from Mediterranean (most part) to Atlantic (northern region).

## **2. Experimental or sampling design**

### a. Design characteristics

Data was obtained following the framework depicted in Fig. 3, through extensive data source search, from scientific literature to online databases, museum collections and by requesting unpublished data to experts on alien species and from citizen science



**Figure 3.** Framework of collection of occurrence records of alien tetrapod species in the Iberian Peninsula.

platforms. Data was then validated through an expert-based procedure to ensure the accuracy and validity of the occurrence records.

b. Data collection period, frequency, etc.

Basic data collection period was 01/01/2017 to 10/08/2020.

### 3. Research methods

The first step was to identify all tetrapod species occurring in the Iberian Peninsula. We first listed these species by searching in key publications, including national atlases from Portugal (Matias 2002; Equipa Atlas 2008; Loureiro et al. 2008; Catry et al. 2010; Bencatel et al. 2017) and Spain (Barbadillo et al. 1999; Pleguezuelos et al. 2002; Martí and del Moral 2003), and from the recent assessments dedicated to alien birds in the Iberian Peninsula by Abellán et al. (2016). The listing was updated whenever the indication of additional alien species was found during the occurrence data search. All names were standardized according to the IUCN ([www.iucnredlist.org](http://www.iucnredlist.org)), but we retained the alternative names to perform subsequent data searches.

A collection of experts was gathered (all authors of this study) to discuss the cryptogenic status of some species. This resulted in a list of 406 species potentially occurring in the Iberian Peninsula. After debate among the authors, a few species listed as alien in some data sources were not considered because there was substantial uncertainty about their nativity in the region (Table 1).

Occurrence data was searched in multiple types of sources, including published literature (atlases, research articles, databases), from citizen science data portals and through requests of unpublished data to institutions and experts.

**Published literature:** The ISI Web of Science (<https://apps.webofknowledge.com>) was searched using the search term: “TS = ((*list of species names including alternative ones*

**Table 1.** Species for which there was debate among the authors about whether or not they should be considered native, and for which it was finally decided not to consider as such.

Species	Support
Edible frog <i>Pelophylax</i> kl. <i>Esculentus</i>	Highly difficult to distinguish from <i>Rana perezi</i> , requires genetic data.
Spur-thighed tortoise <i>Testudo graeca</i>	Unclear origin (Graciá et al. 2013)
False smooth Snake <i>Macroprotodon cucullatus</i>	Unclear origin (Loureiro et al. 2008)
Egyptian mongoose <i>Herpestes ichneumon</i>	Unclear origin (Gaubert et al. 2011)
European mink <i>Mustela lutreola</i>	Unclear origin (Clavero 2014; Maran et al. 2016)

separated by “OR”) AND CU = (Portugal OR Spain)) AND DOCUMENT TYPES: (Article OR Book OR Book Chapter OR Data Paper OR Proceedings Paper OR Review)”. The search was then refined by using the filters RESEARCH AREAS: (ZOOLOGY OR ENVIRONMENTAL SCIENCES ECOLOGY) AND WEB OF SCIENCE CATEGORIES: (ZOOLOGY OR ECOLOGY OR BIODIVERSITY CONSERVATION). The timespan was 2002 (inclusive) onward. We restricted the search to 2002 because previous records are expected to be compiled in the Atlases (see below). The searches were last updated on August 10<sup>th</sup>, 2020. This resulted in a collection of 767 references, of which 199 articles were identified from their abstracts as potentially containing useable location data. Finally, occurrences of alien species were retrieved from 65 publications (Table 2).

For Portugal, occurrence data further included information from atlases (Bencatel et al. 2017; Catty et al. 2010; Equipa Atlas 2008; Loureiro et al. 2008; Matias 2002); and for Spain information was also obtained from the online database ‘Inventario Español de Especies Terrestres’ (IEET 2014). This database compiles information from several sources, including atlases and other databases (Table 3). Additional information for Spain was obtained from Barbadillo et al. (1999), Martí and del Moral (2003) and Pleguezuelos et al. (2002).

**Citizen science:** We further collected information from four different citizen science platforms, including ‘Biodiversity4all’ (Biodiversity4All 2020), ‘Proyecto Avis’ (Varela et al. 2014; URL: [proyectoavis.com](http://proyectoavis.com)), ‘Colectivo Ornitológico Cigüeña Negra’ (COCN; URL: <http://bd.cocn.eu>), eBird (eBird 2020; Sullivan et al. 2009), and iNaturalist (iNaturalist 2020). The records from Proyecto Avis, COCN and Biodiversity4all were provided directly by their administrators, P. Tiago and E. Casabella, in August 2020. The records from eBIRD were downloaded from its site (full dataset) in August 2020. iNaturalist, records were downloaded using the R packages ‘rgbif’ (Chamberlain et al. 2016) in R environment (R Core Team 2020) in August 2020.

**Table 2.** Studies from which locations of alien terrestrial vertebrates were retrieved.

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**Table 3.** Sources of ‘Inventario Español de Especies Terrestres’ (IEET 2014), and the number of records retrieved from each source. Base de Datos AHE – amphibian and reptile database of the Spanish Herpetological Association; PASER – Spanish Bird banding monitoring program; SACRE – Spanish Common Bird Sensus Program; SECEM – Spanish Society for the Conservation and Study of Mammals.

Source	Records
Base de Datos AHE (2011)	446
Pleguezuelos et al. (2002). Atlas y Libro Rojo de los Anfibios y Reptiles de España.	805
Madroño et al. (2004). Libro Rojo de las Aves de España.	660
PASER (2008)	4
PASER (2009)	3
PASER (2010)	1
PASER (2011)	4
SACRE (2008)	18
SACRE (2009)	13
SACRE (2010)	15
SACRE (2011)	40
Palomo (2007). Atlas y Libro Rojo de los Mamíferos Terrestres de España.	12,513
SECEM 2009–2013	3

**Unpublished data:** In addition to the data directly sourced from published literature and online platforms, unpublished occurrence data were obtained through contacts with multiple experts (co-authors).

**Data treatment:** We considered all records with geographic coordinates and/or Universal Transverse Mercator (UTM) 10 × 10 km identified. All records were standardized into

individual  $10 \times 10$  km cells of the Universal Transverse Mercator (UTM) grid system. All data records compiled had coordinates with low ( $<1$  km) coordinate uncertainty.

**Accuracy and validity of the occurrence records:** The following procedures were carried out for the final version of the database to ensure the accuracy and validity of the occurrence records. For each species, we built a distribution map in the Iberian Peninsula, identifying the UTM cells. These maps were sent to the panel of experts who confronted the mapped data with their own knowledge about the distribution of the species. All species maps were evaluated and validated by the consulted experts. We note that for some species, the data obtained are unlikely to provide a comprehensive representation of their distribution. This is particularly clear for *Rattus norvegicus* and *Mus musculus*, which likely have much wider distributions. The scarcity of records for these species should be explained in part by their lower appeal by citizen scientists, thus having fewer records on citizen science platforms than other more iconic species.

**Status of establishment:** Based on our collective knowledge, we distinguished those species known to reproduce in the wild (established), from those for which there is still no evidence of reproduction (not established), and those for which reproduction is uncertain.

#### 4. Project personnel

Principal investigators:

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#### Data set status and accessibility

##### A. Status

###### 1. Latest update

August 2020.

###### 2. Metadata status

Metadata are complete.

## **B. Accessibility**

### **1. Storage location and medium**

The data set is available online through the current publication and through Zenodo (DOI: 10.5281/zenodo.4018706). We also provide as Suppl. material 4 the R code and GIS layers to update the maps presented in Suppl. material 2. Original data files exist on the authors' personal computers in MS Excel format.

### **2. Contact person(s)**

Fernando Ascensão: (*current address*) Centre for Ecology, Evolution and Environmental Changes Faculdade de Ciências da Universidade de Lisboa Edifício C2, 5º Piso, Sala 2.5.46 Campo Grande 1749-016 Lisboa Portugal. Email: [fjascensao@fc.ul.pt](mailto:fjascensao@fc.ul.pt).

### **3. Copyright restrictions**

None.

### **4. Proprietary restrictions:**

None.

## **Data structural descriptors**

### **Data Set Files**

#### **1. Identity**

The dataset consists of 1 file (MS Excel document), named “55597\_0R-3-A\_Dataset SM-1.csv” (Suppl. material 1), containing one worksheet. Each species record (line) also has the origin and IUCN conservation status. In Suppl. material 2, we also provide the distribution maps for each species. The R file, also in Suppl. material 2, contains the necessary code to replicate the maps using updated information.

#### **2. Size**

The size of “55597\_0R-3-A\_Dataset SM-1.csv” has 20.8 MB.

#### **3. Format and storage mode**

The file type is MS Excel. No compression scheme was employed.

#### **4. Header information**

In the worksheet, a single header row includes the species' taxonomic classification (i.e., four headers: Class, Order, Family and Species name), common name, the species origin (Africa, Asia, S. America, C. America, N. America, Europe, Oceania), reproduction in the wild (known, not established and uncertain), the IUCN conservation status, and the UTM. Time information is shown in three columns: 'Year\_publication' refers to the year of publication of Atlases, books, reports and scientific papers. 'Year' refers to most accurate time of observation, frequently presented as an interval e.g., '2010–2019' and '2002(before)'. The column 'Year\_numeric' is the most conservative numeric number of Year, in the previous examples would be 2019 and 2002, respectively. The column 'Source' indicates the reference from which the information was obtained. Finally, the 'key' column indicates the unique ID of citizen science platforms, allowing the online visualization of the records. For example, the key = 58020496 in iNaturalist can be retrieved by the URL: [www.inaturalist.org/observations/58020496](http://www.inaturalist.org/observations/58020496), showing a common slider.

#### **5. Alphanumeric attributes**

Alphabetic character fields.

#### **Supplemental descriptors**

##### **A. Data acquisition**

###### **1. Data forms or acquisition methods**

Available online through the current publication.

###### **2. Computer programs and data-processing algorithms:**

We provide an R script in Suppl. material 4 which allows updating the maps of species occurrences. GIS information is also provided in Suppl. material 3.

#### **Acknowledgements**

We thank Dr. Cristiane Bastos-Silveira for her help in collecting mammal data from the National Museum of Natural History in Lisbon. FA was funded through a post-doctoral grant from Fundação para a Ciência e Tecnologia (FCT, SFRH/BPD/115968/2016). RCM work was carried out in the framework of REN Biodiversity Chair, funded by REN (Redes Energéticas Nacionais, S.A.) and FCT. MLM thanks

to FCT/MCTES for the financial support to CESAM (UID/AMB/50017/2019), through national funds. Work supported by National Funds through FCT-Fundação para a Ciência e a Tecnologia in the scope of the project UIDB/50027/2020.

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## Supplementary material I

### Dataset

Authors: Fernando Ascensão, Marcello D'Amico, Ricardo C. Martins, Rui Rebelo, A. Márcia Barbosa, Joana Bencatel, Rafael Barrientos, Pedro Abellán, José L. Tella, Laura Cardador, José D Anadón, Martina Carrete, Enrique Murgui, Pedro Fernandes, Sara M. Santos, António Mira, Maria da Luz Mathias, Patrícia Tiago, Eduardo Casabella, Luís Reino, Octávio S. Paulo, Henrique M. Pereira, César Capinha

Data type: table

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Link: <https://doi.org/10.3897/neobiota.64.55597.suppl1>

## Supplementary material 2

### Maps

Authors: Fernando Ascensão, Marcello D'Amico, Ricardo C. Martins, Rui Rebelo, A. Márcia Barbosa, Joana Bencatel, Rafael Barrientos, Pedro Abellán, José L. Tella, Laura Cardador, José D Anadón, Martina Carrete, Enrique Murgui, Pedro Fernandes, Sara M. Santos, António Mira, Maria da Luz Mathias, Patrícia Tiago, Eduardo Casabella, Luís Reino, Octávio S. Paulo, Henrique M. Pereira, César Capinha

Data type: Maps

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Link: <https://doi.org/10.3897/neobiota.64.55597.suppl2>

## Supplementary material 3

### GIS

Authors: Fernando Ascensão, Marcello D'Amico, Ricardo C. Martins, Rui Rebelo, A. Márcia Barbosa, Joana Bencatel, Rafael Barrientos, Pedro Abellán, José L. Tella, Laura Cardador, José D Anadón, Martina Carrete, Enrique Murgui, Pedro Fernandes, Sara M. Santos, António Mira, Maria da Luz Mathias, Patrícia Tiago, Eduardo Casabella, Luís Reino, Octávio S. Paulo, Henrique M. Pereira, César Capinha

Data type: GIS data

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Link: <https://doi.org/10.3897/neobiota.64.55597.suppl3>

## Supplementary material 4

### R code

Authors: Fernando Ascensão, Marcello D'Amico, Ricardo C. Martins, Rui Rebelo, A. Márcia Barbosa, Joana Bencatel, Rafael Barrientos, Pedro Abellán, José L. Tella, Laura Cardador, José D Anadón, Martina Carrete, Enrique Murgui, Pedro Fernandes, Sara M. Santos, António Mira, Maria da Luz Mathias, Patrícia Tiago, Eduardo Casabella, Luís Reino, Octávio S. Paulo, Henrique M. Pereira, César Capinha

Data type: R code

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Link: <https://doi.org/10.3897/neobiota.64.55597.suppl4>



# Arboreta reveal the invasive potential of several conifer species in the temperate forests of western Europe

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Academic editor: R. Hufbauer | Received 30 June 2020 | Accepted 6 January 2021 | Published 20 January 2021

**Citation:** Fanal A, Mahy G, Fayolle A, Monty A (2021) Arboreta reveal the invasive potential of several conifer species in the temperate forests of western Europe. *NeoBiota* 64: 23–42. <https://doi.org/10.3897/neobiota.64.56027>

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## Abstract

Identifying emerging invasive species is a priority to implement early preventive and control actions. In terms of the number of invasive tree species, forestry represents the second largest pathway of introduction, with an invasive debt likely existing for alien conifers in Europe. In the early 1900s, a network of arboreta was established in southern Belgium to assess the wood production potential of prospective conifer and broadleaved species. Here, we use eight arboreta as natural experiments to identify alien conifers presenting invasive behavior. Through systematic sampling, we quantified the natural regeneration of alien conifers and recorded local environmental variables. For each species, regeneration density, dispersal distances, and age structure were analyzed. Generalized mixed effects models were fitted to test the effect of planted area and tree-stand type on regeneration. The environmental space occupied by regenerating alien conifers was evaluated using principal component analysis. Out of 31 planted alien species, 15 (48%) were identified in natural regeneration, of which eight (26%) exhibited important regeneration density and dispersal distances. The most invasive species were *Tsuga heterophylla* and *Abies grandis*, confirming earlier field observations. Both large planted areas and areas planted with alien conifer species increased the density of regeneration. Species that had the highest regeneration density tolerated a wide range of environmental conditions, including shaded understory, which could lead to the invasion of mature, undisturbed forests. This study showed that 17% of the studied alien conifers are potentially invasive because they show important regeneration, long-distance dispersal, and, of importance, have already produced offspring that have matured and are capable of creating new satellite populations. In conclusion, our results provide a guideline for future planting operations, recommending extreme caution when planting these species in the temperate forests of Western Europe.

## Keywords

Arboretum, dispersal, gymnosperm, invasiveness, non native trees, propagule pressure, regeneration

## Introduction

Early identification of emerging invasive species remains one of the most challenging issues in invasion science. Following numerous introductions worldwide for ornamental or production purposes, many tree species have since been recognized as invasive (Dodet and Collet 2012; Richardson et al. 2014). Rejmánek (2014) identified 76 tree species exhibiting invasive behavior in Europe. Alien woody species have the capacity to modify the structure of invaded ecosystems substantially and cause extensive ecological and economical damage (Lamarque et al. 2011; Pyšek 2016). In addition, management actions are often taken too late, when the species are already widespread and when the management costs of mitigation are prohibitive (Rejmánek and Pitcairn 2002; van Wilgen and Richardson 2014).

Most problematic tree species in Europe were introduced decades or centuries ago (Nyssen et al. 2016) and actively spread by human. In parallel, some dispersed outside their cultivation areas and spread *via* small satellite populations over kilometers (Mack 2005; Pyšek and Richardson 2012). Delays between the installation of these satellite populations and their capacity to reproduce create an important lag phase between the introduction of a species in a new area and its invasion of natural habitats (Wangen and Webster 2006). In Germany, this lag phase has been estimated to last 170 years on average for trees (Kowarik 1995). Future invasive species might, therefore, already have been introduced but might not have completed the naturalization–invasion continuum, yet. This time-delayed invasion is referred to as the invasion debt (Rouget et al. 2016). Because introduction events increased during the second half of the 20<sup>th</sup> century, an invasion debt, without doubt, exists in Europe for trees (Essl et al. 2011) and must be evaluated to anticipate new invasions (Richardson and Rejmánek 2011).

When the number of introduction events increases, so does the probability of naturalization (Heger 2016). Along with the propagule pressure, several functional traits can help predict the invasiveness of plant species such as an important SLA, growth rate, height, germination rate and fitness (van Kleunen et al. 2010; Lamarque et al. 2011; Kutlvašr et al. 2019). For conifers specifically, Richardson and Rejmánek (2004) identified a small seed mass, short juvenile period and short intervals between large crops as traits associated with invasiveness.

The forestry sector has been introducing alien tree species for centuries in Europe for timber production, including many conifers from Asia and north America (Krumm and Vítková 2016; Gil-Moreno 2018). Species selected for forest plantations often originate from regions with a similar climate and present high growth rates, two factors contributing to enhanced invasiveness (Richardson and Rejmánek 2004). Introduced species are also grown in large-scale plantations using cultivation techniques that enhance survival rates, which lowers the probability of local extinctions and creates a massive propagule pressure, increasing the probability of them escaping from cultivation (Mack 2005; Křivánek et al. 2006; Pyšek et al. 2014). For example, in the Czech Republic, 25% of tree species introduced for forestry have become invasive (Pyšek 2016). Most invasive trees in Europe are light-demanding and have better

invasion success in disturbed habitats (Richardson and Rejmánek 2004; Meloni et al. 2016). However, shade-tolerant species also exhibit invasive behavior once introduced to mature forests with low disturbance. For instance, *Prunus serotina* invaded the undergrowth of forests in western Europe (Hernandez et al. 2016).

Conifers in particular have been introduced to many areas and were widely planted for timber production, providing substantial opportunity for invasion (Richardson and Rejmánek 2004; Broncano et al. 2005). Globally, 36 species of conifers are already considered invasive (Richardson and Rejmánek 2004) with alien conifers used in commercial forestry having a significantly higher probability of escaping cultivation compared to species not used for timber production (Essl et al. 2010). Conifers are still not often perceived as problematic in Europe because their invasions have been primarily documented in the southern hemisphere so far. Yet, studies indicate that alien conifers are showing invasive behaviors in Europe, too (Carrillo-Gavilán and Vilà 2010; Essl et al. 2010). If the planting of alien species in European forests continues, which is likely to happen with the dieback of several native species, it is necessary to distinguish high risk species from those that are unlikely to become invasive (Dodet and Collet 2012; Heger 2016).

Forest trials and arboreta offer the opportunity to monitor the regeneration dynamic of exotic species, acting as sentinel sites of which careful observations could facilitate the detection of new invasions (Carrillo-Gavilán and Vilà 2010). These areas are also likely to act as sources of propagules and sites of entry for new invasions (Richardson and Rejmánek 2004; Brundu and Richardson 2016). During the 19<sup>th</sup> century, the Belgian Forest Department started to diversify forests plantations with exotic species. A network of 23 forest arboreta was set up between 1890 and 1914 throughout the country to monitor the growth and wood production potential of both native and alien species, especially ones from Japan and the west coast of North America (Nysen et al. 2016). The arboreta were implemented in various ecological regions to cover the diversity of climates and soil types of the country. Every arboretum differed in its setup and list of species. Unfortunately, the geographical origin of the seeds remains unknown. Recently, a new interest in these arboreta emerged and new inventories were performed in 2016 to study the production potential of exotic species in the light of climate change (Lhoir and Scholzen 2017). In several of these arboreta, no management actions of the understory were implemented over the last 15 years except for clearing the pathways. The natural regeneration is therefore mostly untouched.

In this study, we aimed to identify alien conifer species presenting invasive potential. To do so, we systematically quantified the natural regeneration of alien species in and around eight selected arboreta. Richardson et al. (2000) defined invasive plant species as species producing reproductive offspring in very large numbers and at considerable distances from parent plants. By combining information on tree density, realized dispersal, and the size structure of the natural regeneration of alien conifers, we assessed their invasive potential. Specifically, we evaluated i) the density of natural regeneration and the realized dispersal distances from nearest parent trees; ii) whether a diverse size structure exists in the natural regeneration of species that regenerated;

and iii) the influence of tree-stand type and environmental conditions on the regeneration density of species of highest concern. The correlation between the regeneration density and traits linked to invasiveness in previous studies was also assessed.

## Material and methods

### Study area

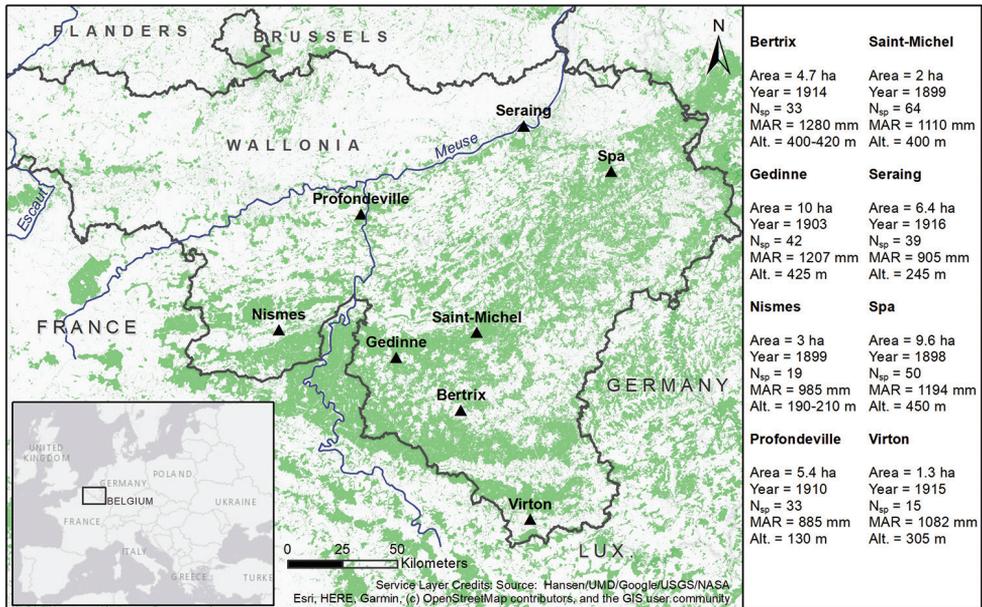
The study area covered the Walloon Region in Southern Belgium (49.5966°N to 50.5705°N latitude, 4.5469°E to 5.8852°E longitude). Eight arboreta, further referred to as “sites”, were selected (Fig. 1) based on three criteria: i) at least 15 planted alien conifer species, ii) no management actions in the understory that would have influenced the natural regeneration for the last 15 years, and iii) information being available on plantation dates.

In this study species were considered alien when they did not naturally occur in continental Europe. Sixty-nine percent of the total planted area within the arboreta was occupied by alien conifers. Only 8% percent was planted with European conifers (mainly *Picea abies* and *Abies alba*). The remaining area was planted with native and alien broadleaves. All the arboreta consist of forest ecosystems, even though a few small clearings with solitary individuals could be found. Thus, the planted area varied greatly across species, from 6 m<sup>2</sup> to 9.1 ha.

### Sampling procedures

Field sampling was conducted from April to July 2018. Sampling was systematic and covered the entire arboreta and a 100-m buffer, representing a total of 129.5 ha. For each arboretum, a 30×30 m grid was applied and a plot was installed at each intersection, generating 1565 plots. Sampling plots consisted of circles of 2-m radius. Plots situated on roads, ponds, private land, and recent forest plantings were excluded along with sites with insecure access, such as rocky scree. In total, 1109 plots were sampled in forested areas (from 71 to 244 plots per arboretum). In each plot, all individuals of alien conifer species (from young seedlings to adult trees) were recorded and their height measured from the ground to the tip of the main stem. They were then assigned to the following size classes: class 0 for seedlings between 0 and 0.3 m high, class 1 for saplings between 0.3 m and 1.3 m high (height of measurable diameter at breast height, DBH), class 3 for trees higher than 1.3 m but with DBH smaller than 5 cm, class 4 for trees with DBH between 5 and 9.9 cm, and so on for every 5 cm increment in DBH.

Identifying seedlings was sometimes challenging and 1878 fir seedlings (including 850 in only one plot) were excluded from further analyses, as it was not possible to determine species with certainty due to their stage of development (probably *A. grandis* or *A. alba*). The regeneration data for *Abies* species was therefore underestimated.



**Figure 1.** Location and description of the arboreta used in this study (triangle symbols) on a background map of tree cover in 2000 (Hansen et al. 2013). For each arboretum, the following characteristics were obtained: Year = year of first plantings,  $N_{sp}$  = number of alien conifer species planted in the arboretum, MAR = Mean Annual Rainfall between 1981 and 2010 obtained from the Royal Meteorological Institute of Belgium (RMI, n.d.), and Alt. = Altitude (m).

We measured environmental variables that influence the settlement of species (Dyderski and Jagodziński 2018). The thickness of litter (mm) was measured with a ruler at four different places in the plot and the mean was calculated (ranging from 0 to 100 mm, median = 20 mm, mean = 24.14 mm). The pH was measured with a pH-kit on the field in the center of the plot with a precision of 0.5 units (range: 4 to 6.5, median = 4.0, mean = 4.3). Canopy openness was assessed with a spherical convex densiometer in four cardinal directions (Forestry Supplier spherical crown densiometer, Convex – Model A), and ranged from 0.2 to 90.2 % (median = 7.2 %, mean = 11.1 %). Soil drainage was attributed from the plot geographical coordinates based on the Digital Soil Map of Wallonia (Bah et al. 2007; Service Public de Wallonie 2019). Soils ranged from being excessively well-drained (1) to poorly drained (5). Soil drainage classes are defined according to soil morphological attributes, more precisely the depth of appearance of gleyic color pattern reflecting the presence of stagnant water (Bah et al. 2007). Most soils were excessively well-drained (median = 1, mean = 1.89). Out of the 1109 prospected plots, the tree-stand type was defined: 545 plots were under coniferous stands, 557 plots were under broadleaved species (mainly *Fagus sylvatica* in the buffer zones), and seven plots were in open areas (clear-cuts).

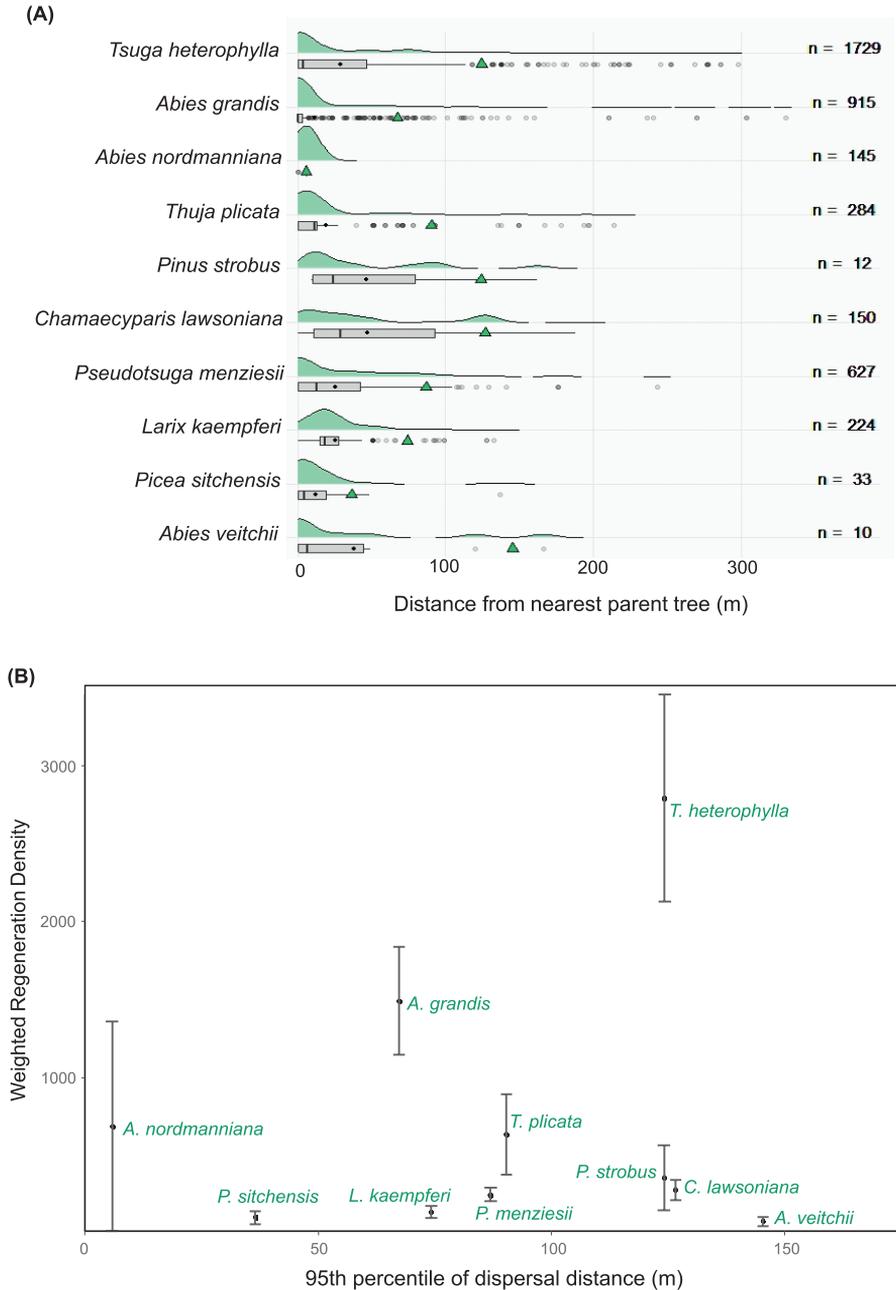
## Statistical procedures

A generalized linear mixed effect model (GLMM) with Poisson family was used to determine whether there was a significant influence of several variables on the regeneration ability of alien species regenerating in at least two sites. The lme4 package was used (Bates et al. 2015). The fixed variables were the species, the area of plantation, the time since plantation, the distance to the nearest parent trees, and the tree-stand type (broadleaves, open areas, European conifers or exotic conifers). The exact number of planted trees per species was unknown. We therefore used the area of plantation as a proxy for the propagule pressure, as the density of plantation was similar for the coniferous species. The site and the plot nested within the site were included as random effects. Zero-inflation was tested and not detected. A significant  $p$  value threshold was set at 0.05. An ANOVA with the “car” package was performed on the regression result (Fox and Weisberg 2019).

The two first key determinants of invasiveness that we analyzed were the density of regeneration and dispersal distances from the closest parent trees. Regeneration Density (RD) was calculated for every species as the mean number of individuals per ha. For the capacity of regeneration of different species to be comparable, we calculated the Weighted Regeneration Density (WRD) which represented the density of regenerating individuals per ha for 1 ha planted of the same species. The WRD was calculated by dividing the regeneration density (RD) in each plot by the planted area of species in the corresponding arboretum. Because WRD is the density of individuals ( $\text{indiv.ha}^{-1}$ ) divided by an area (ha), the unit is  $\text{indiv.ha}^{-1}.\text{ha}_{\text{planted}}^{-1}$ . For each species in each plot, the realized dispersal distance (DD) was measured as the distance to the nearest planted parent trees with ArcMap v. 10.5.1 (ESRI 2019). For species with at least 10 individuals found in the regeneration, boxplots and density plots of the distribution of dispersal distances were constructed. As long-distance dispersal events are of major importance in the invasion process, the 95<sup>th</sup> percentile of distribution of distances was represented to characterize the tail (Higgins and Richardson 1999; Monty et al. 2013). A “summary plot” (Fig. 2B) combining the WRD and 95<sup>th</sup> percentile of dispersal distances was built to characterize the behaviors of species visually regarding these two aspects of invasiveness. The plots and analyses were performed using R software (R Core Team 2020).

Richardson et al. (2000) delineated a threshold of 100 m in 50 years as a rule of thumb for the dispersal of an alien plant defined as invasive (Richardson and Rejmánek 2004; Nygaard and Øyen 2017). To compare dispersal observed in the arboreta with the threshold provided in this definition, dispersal distances over 50 years (DD50) were also calculated. Dispersal distances (DD) for every individual were divided by the time since planting minus the age of maturity of the species, and were then multiplied by 50 (Eq. 1). Data on the age of maturity were compiled from Kattge et al. (2011), Petit et al. (2017), and Forestry Commission Scotland (2015).

$$DD50 = \frac{DD \times 50}{\text{time since planting} - \text{age of maturity}} \quad (\text{Eq. 1})$$



**Figure 2.** Regeneration density and dispersal of alien conifers **A** boxplots and density plots of dispersal distances for species of which at least 10 individuals were recorded. Species are ordered in descending order using WRD. The total number of individuals per species (n) is indicated on the right. The mean (point) and median (vertical bar) are indicated. The 95<sup>th</sup> percentile was also represented with a green triangle **B** comparison of species based on mean WRD  $\pm$  standard error ( $\text{indiv. ha}^{-1} \cdot \text{ha}_{\text{planted}}^{-1}$ ) and 95<sup>th</sup> percentile of dispersal distances (m).

Because an invasive species must be capable of producing mature offspring, the size structure of natural regeneration was also observed. A table on size structure was constructed for the 10 species with at least 10 measured individuals to examine the viability of the natural regeneration.

We investigated whether the most invasive species occupy a wide range of environmental conditions. We selected species presenting a combination of important regeneration density ( $WRD > 100 \text{ indiv. ha}^{-1} \cdot \text{ha}_{\text{planted}}^{-1}$ ), high dispersal distance ( $\text{Perc. } 95 > 50 \text{ m}$ ), and a developed size structure with older individuals ( $DBH > 10 \text{ cm}$ ). To detect environmental gradients through the measured plots, we performed a principal component analysis (PCA) on the environmental matrix containing all plots and the four quantitative environmental variables using the *ade4* package (Dray and Dufour 2007). The plots in which the selected species were regenerating were projected in the environmental space made by the first two Principal Components. Density lines for each species on the two axes of the PCA were drawn, allowing us to delineate the environmental space occupied by each species. This method is widely used to estimate niche overlap of species (Broennimann et al. 2012).

Finally, data was gathered for two traits associated with invasiveness, namely the seed mass and the maximal height of the species, both linked to the capacity to disperse at long distances (Richardson and Rejmánek 2004; Kutlvař et al. 2019). Data was compiled from Greene and Johnson (1993), Kattge et al. (2011) and Johnson and More (2014). As a Shapiro-Wilk test rejected the normality of our variables, we performed a non-parametric Kendall correlation test on these two variables related to the Weighted Regeneration Density of all species planted in at least half of the arboreta.

## Results

In total, 1109 plots were surveyed and 4148 individuals recorded, from small seedlings to mature trees over 60 cm of DBH. Due to the size of the sampling plots, we never found more than one non-planted tree with a  $DBH > 20 \text{ cm}$  in one plot. These individuals belonged to 31 alien conifer species planted between 1898 and 1916 in eight arboreta across the Walloon Region (Table 1). For 15 of the planted species, no regeneration was detected, while six species had less than 10 individuals recorded across all sites. In contrast, some species presented abundant regeneration. The most frequent seedlings encountered were *Tsuga heterophylla* and *Abies grandis*. The planted area, time since planting and distance to the nearest parent trees significantly affected the density of regeneration (Table 2). We further used the Weighted Regeneration Density (WRD) for between-species comparison.

*Tsuga heterophylla* was the most represented alien conifer in natural regeneration with a WRD of  $2794.0 \text{ indiv. ha}^{-1} \cdot \text{ha}_{\text{planted}}^{-1}$ . This species was followed by *Abies grandis* ( $WRD = 1493.8 \text{ indiv. ha}^{-1} \cdot \text{ha}_{\text{planted}}^{-1}$ ), *Abies nordmanniana* ( $688.3 \text{ indiv. ha}^{-1} \cdot \text{ha}_{\text{planted}}^{-1}$ ) and *Thuja plicata* ( $637.8 \text{ indiv. ha}^{-1} \cdot \text{ha}_{\text{planted}}^{-1}$ ).

**Table 1.** List of species planted in at least four of the eight selected arboreta. **N sites planted** = number of arboreta where species were planted. **Native distribution** of species is also given. **Planted area** = total planted area of species in all sites. **N sites found** and **N plots** are the number of sites (arboreta) and plots (1109 plots in total) where the natural regeneration of species occurred. **N indiv.** = number of trees recorded in natural regeneration. For each plot, the regeneration density (**RD**) in  $\text{indiv. ha}^{-1}$  and weighted density of regeneration (**WRD**) in  $\text{indiv. ha}^{-1} \cdot \text{ha}^{-1}$  were calculated, and the mean is given in the table. The median, maximum, and 95<sup>th</sup> percentiles of dispersal distance distributions are given (**Median DD**, **Max DD** and **Perc. 95 DD**). The maximum dispersal distance over 50 years (**Max DR50**) and the 95<sup>th</sup> percentile (**Perc. 95 DR50**) were calculated.

Species	Native distribution	Planted area	N sites planted	N sites found	N plots	N indiv.	Mean RD	Mean WRD	Median DD	Perc. 95 DD	Max DD	Perc. 95 DR50	Max DR50
<i>Tsuga heterophylla</i>	North America	2,1	8	6	136	1729	1240,7	2794,0	3,8	124,3	298,0	84,3	201,3
<i>Abies grandis</i>	North America	1,803	8	6	103	915	656,6	1493,8	0,0	67,4	330,1	39,5	177,5
<i>Abies nordmanniana</i>	Caucasus	0,581	6	2	4	145	126,2	688,3	5,8	5,8	5,8	4,03	4,0
<i>Thuja plicata</i>	North America	1,567	8	4	39	284	203,8	637,8	11,2	90,3	213,8	54,9	127,2
<i>Pinus strobus</i>	North America	0,325	6	2	9	12	10,7	357,8	23,9	124,2	162,2	73	95,4
<i>Chamaecyparis lausoniana</i>	North America	2,088	8	5	46	150	107,6	279,4	28,7	126,7	187,7	77,3	120,3
<i>Pendulstuga menziesii</i>	North America	9,011	8	6	177	627	449,9	248,8	12,8	87,0	243,3	40,4	95,0
<i>Chamaecyparis obtusa</i>	Japan	0,08	5	1	2	4	4,7	243,8	7,0	7,0	7,0	4,5	4,5
<i>Larix kaempferi</i>	Japan	3,247	8	3	39	224	160,7	136,6	18,1	74,3	132,3	49,5	88,2
<i>Abies cilicica</i>	Middle-East	0,09	4	1	1	3	4,2	117,0	2,9	2,9	2,9	2,3	2,3
<i>Chamaecyparis pisifera</i>	Japan	0,236	6	1	1	6	4,6	116,3	0,8	0,8	0,8	0,5	0,5
<i>Picea sitchensis</i>	North America	0,789	4	2	9	33	37,1	104,2	4,2	36,5	136,7	33,8	126,5
<i>Abies homolepis</i>	Japan	0,336	6	2	2	4	3,3	84,8	0,0	15,9	18,7	15,9	25,2
<i>Abies veitchii</i>	Japan	0,578	5	3	7	10	9,3	76,6	6,6	145,4	166,0	125	133,9
<i>Abies procera</i>	North America	0,352	5	1	1	0,1	0,9	20,7	0,0	0,0	0,0	0	0,0
<i>Picea orientalis</i>	Caucasus	0,294	7	1	1	1	0,8	8,5	5,7	5,7	5,7	4,4	4,4
<i>Abies concolor</i>	North America	0,294	5	0	0	0	0	0	—	—	—	—	—
<i>Abies numidica</i>	North Africa	0,122	4	0	0	0	0	0	—	—	—	—	—
<i>Cedrus libani</i>	Middle-East	0,049	4	0	0	0	0	0	—	—	—	—	—
<i>Cryptomeria japonica</i>	Japan	0,265	8	0	0	0	0	0	—	—	—	—	—
<i>Metasequoia gypsoseoides</i>	Asia	0,281	6	0	0	0	0	0	—	—	—	—	—
<i>Picea engelmannii</i>	North America	0,236	4	0	0	0	0	0	—	—	—	—	—
<i>Picea glauca</i>	North America	0,14	4	0	0	0	0	0	—	—	—	—	—
<i>Picea jezoensis</i>	Asia	0,107	5	0	0	0	0	0	—	—	—	—	—
<i>Picea boyanuae</i>	Japan	0,234	6	0	0	0	0	0	—	—	—	—	—
<i>Picea rubens</i>	North America	0,143	4	0	0	0	0	0	—	—	—	—	—
<i>Picea torano</i>	Japan	0,115	4	0	0	0	0	0	—	—	—	—	—
<i>Pinus ponderosa</i>	North America	0,141	4	0	0	0	0	0	—	—	—	—	—
<i>Sequoiadendron giganteum</i>	North America	0,244	7	0	0	0	0	0	—	—	—	—	—
<i>Tsuga canadensis</i>	North America	0,238	5	0	0	0	0	0	—	—	—	—	—
<i>Xanthocyparis nootkatensis</i>	North America	0,045	4	0	0	0	0	0	—	—	—	—	—

**Table 2.** Results of the generalized linear mixed effect model on the count of regeneration. Estimates, standard errors, Z values and p values are given for fixed effects.

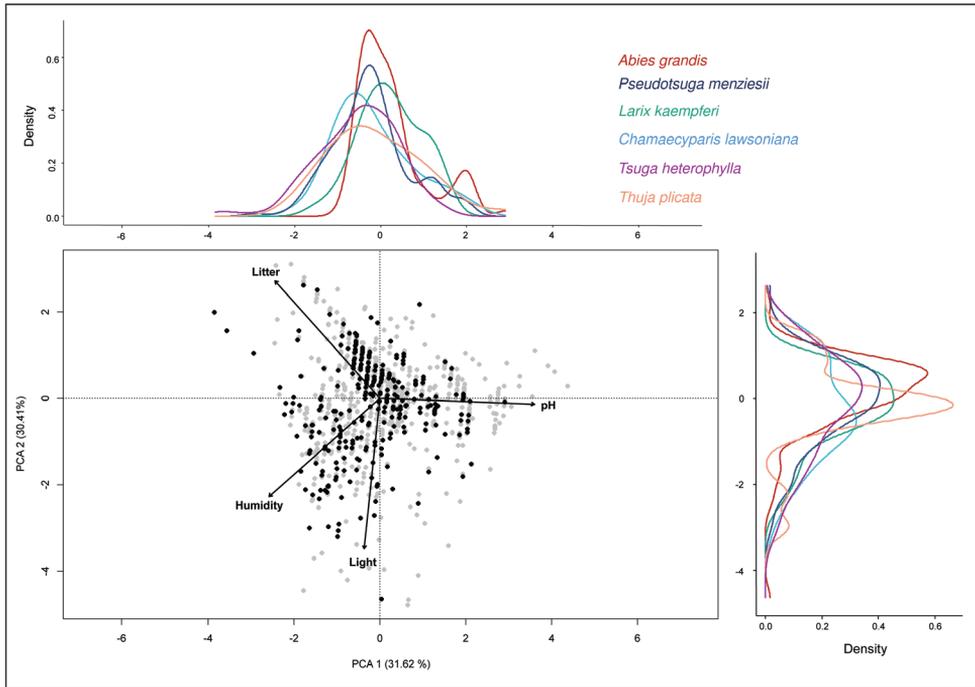
Variable	Estimate	Std. Error	Z value	p value
Species	(base)			
<i>Abies grandis</i>	-3.17E+00	4.63E-01	-6.85	< 0.001
<i>Abies balsamea</i>	-5.55E-01	1.17E-01	-4.744	< 0.001
<i>Abies nordmanniana</i>	-2.28E+00	3.20E-01	-7.121	< 0.001
<i>Abies veitchii</i>	-9.86E-01	1.09E-01	-9.078	< 0.001
<i>Chamaecyparis lawsoniana</i>	-6.29E-01	1.12E-01	-5.61	< 0.001
<i>Larix kaempferi</i>	-9.07E-01	1.85E-01	-4.9	< 0.001
<i>Picea sitchensis</i>	-2.79E+00	2.82E-01	-9.902	< 0.001
<i>Pseudotsuga menziesii</i>	-2.40E+00	1.10E-01	-21.872	< 0.001
<i>Thuja plicata</i>	-2.74E-01	9.48E-02	-2.894	0.00381
<i>Tsuga heterophylla</i>	1.84E+00	8.61E-02	21.336	< 0.001
Canopy type	(base)			
Broadleaves	1.28E+00	8.64E-02	14.862	< 0.001
European conifers	1.46E+00	7.58E-02	19.259	< 0.001
Exotic conifers	3.61E+00	1.73E-01	20.869	< 0.001
Open areas	5.24E-02	3.15E-03	16.638	< 0.001
Time since plantation	5.56E-05	3.24E-06	17.179	< 0.001
Surface planted	-2.16E-02	5.69E-04	-37.956	< 0.001
Distance from plantation				

**Table 3.** Size class distribution of percentages for species with more than 10 recorded individuals. The two first classes are composed of individuals smaller than 1.3 m, for which DBH could not be calculated. The other classes were based on DBH intervals (cm). Classes were aggregated to improve readability.

Species	N	Height (m)			DBH (cm)   H > 1.3 m					
		0–0.3	0–1.3	< 5	5–10	10–20	20–30	30–50	> 60	
<i>A. grandis</i>	939	53.2	34.6	11.4	0.5	0.2	0	0	0	
<i>A. nordmanniana</i>	145	98.6	1.4	0	0	0	0	0	0	
<i>A. veitchii</i>	10	30	40	30	0	0	0	0	0	
<i>C. lawsoniana</i>	163	39.3	30.7	20.2	8.0	1.2	0	0	0.6	
<i>L. kaempferi</i>	227	52.4	31.7	14.5	0	0.4	0.9	0	0	
<i>P. menziesii</i>	623	64.5	23.6	7.7	1.9	1.3	0.6	0	0.3	
<i>P. sitchensis</i>	15	100	0	0	0	0	0	0	0	
<i>P. strobus</i>	9	100	0	0	0	0	0	0	0	
<i>T. heterophylla</i>	1732	80.1	9.2	9.5	0.8	0.2	0.1	0.1	0	
<i>T. plicata</i>	287	49.1	38.3	10.8	0.7	0.3	0	0.7	0	

Ten species had at least 10 seedlings recorded in the natural regeneration. They tended to be found close to parent trees (Fig. 2A). However, the seedlings of nine species were sometimes detected at >100 m distance from possible parent trees. Four species had a 95<sup>th</sup> percentile for dispersal distance distribution exceeding 100 m. Only *Abies nordmanniana* displayed very low dispersal distances, with all recorded individuals occurring within 6 m of planted parent trees. The maximal DD exceeded 200 m for the seedlings of *Tsuga heterophylla*, *Pseudotsuga menziesii*, and *Thuja plicata*, and even 300 m for *Abies grandis*.

For the same 10 species with 10 recorded individuals, size structure was used to investigate the survival of the regeneration. Ninety-three percent of recorded trees in natural regeneration were <1.3 m high. All individuals of *P. sitchensis* and *P. strobus* were seedlings <0.3 m high (Table 3). However, older trees with a DBH >20 cm were detected for *C. lawsoniana*, *P. menziesii*, *T. plicata*, *L. kaempferi*, and *T. heterophylla*.



**Figure 3.** Distribution of alien conifers in the environmental space. Regeneration of six conifers in the environmental space made by the two first axes of the PCA. The circle of correlation of four environmental variables was projected on the graph: pH, litter thickness, canopy openness (referred to as “Light”), and soil drainage class (referred to as “Humidity”). The percentage of explained variance for each Principal Component is indicated. Dots represent all plots of the eight arboreta. Black dots are those in which at least one of the six species is regenerating. Density lines are drawn for each species along the two axes of the PCA.

Though conifers and broadleaved stands were almost equally represented in the plot data, alien conifers mainly regenerated under coniferous stands. Specifically, 69% of individuals were found under exotic conifers, 18 % under European conifers, 7% in clear-cut areas, and only 6% under broadleaved species. Open areas and exotic coniferous stands significantly increased the regeneration count of alien conifers (Table 2).

From the principal component analysis (Fig. 3), two environmental gradients were identified and were regulated by soil pH (first Principal Component, PC1) and canopy openness (PC2). Wet soil tended to be more acidic. Litter was thicker on acidic plots with low light availability. Along these gradients, we projected the presence of six species showing a combination of important WRD ( $>100 \text{ indiv. ha}^{-1} \cdot \text{ha}_{\text{planted}}^{-1}$ ), high dispersal distance (perc. 95  $> 50 \text{ m}$ ), and developed size structure (individuals  $>10 \text{ cm DBH}$ ): *Tsuga heterophylla*, *Abies grandis*, *Thuja plicata*, *Chamaecyparis lawsoniana*, *Larix kaempferi*, and *Pseudotsuga menziesii*. We found that these species occupied a wide range of environmental conditions of the arboreta, including areas with low canopy openness. Ecological niches and optimums of presence were rather similar for the six species. *Abies grandis* also occurred on dry and basic soils.

Kendall's correlation highlighted a significant positive relationship between the height of species and their WRD ( $\tau=0.459$ ,  $z=3.096$ ,  $p$  value = 0.002). On the other hand, the correlation was not significant for the seed mass ( $\tau = -0.064$ ,  $z=-0.411$ ,  $p$  value = 0.681).

## Discussion

This study demonstrated that alien conifers naturally regenerated in each arboretum that was visited, sometimes in dense patches of seedlings. Of the 31 alien species considered, 16 were detected regenerating. Eleven species (35%) had a Weighted Regeneration Density of more than 100  $\text{indiv}\cdot\text{ha}^{-1}\cdot\text{ha}_{\text{planted}}^{-1}$ . The planted area and the time since plantation both had a positive significant effect on the count of regeneration, confirming the important influence of the propagule pressure on the regeneration of alien species (Lockwood et al. 2009; Pyšek et al. 2009).

Most species primarily regenerated close to parent trees. Long dispersal events of over 100 m were detected for nine species. For the prolific species *Tsuga heterophylla*, five percent of regeneration occurred past 124 m, and some even reached 300 m one century after planting. Thus, long-distance dispersal events are frequent for this species. The 95<sup>th</sup> percentile of dispersal distance also exceeded 100 m for *P. strobus*, *C. lawsoniana*, and *Abies veitchii*. However, the prospected area was limited, with even longer distances from the closest parent trees being possible. Our estimates of long-dispersal distances can therefore be considered conservative. Given the importance of long-distance dispersal events in the invasion process, more exhaustive inventories of the dispersal potential of these species along transects are required until no individual is found for a given distance lapse (Higgins and Richardson 1999).

The weighted regeneration density and the dispersal distance are useful tools for monitoring the invasive behavior of alien conifers. However, as invasive species must maintain viable populations, the age structure of natural regeneration must be incorporated (Wilson et al. 2014). For *A. nordmanniana*, the high number of individuals was attributed to a single large germination event resulting in hundreds of seedlings of less than one-year-old being detected in one plot; 142 out of the 145 individuals recorded were young seedlings beneath a parent tree, indicating that most regeneration is not viable. *Pinus strobus* and *P. sitchensis* seedlings were recorded at further distances, but only seedlings smaller than 0.3 m were found. In comparison, *T. heterophylla*, *P. menziesii*, *A. grandis*, *C. lawsoniana*, *L. kaempferi*, and *T. plicata* also tended to exhibit large germination events beneath parent trees but older trees were also recorded (see Table 3), including mature ones. Thus, these species likely have the capacity to create new satellite populations.

The question of whether some species cross the benchmark of 100 m dispersal distance over 50 years was evaluated in this study. Richardson et al.(2000) stated that, for a species to be invasive, there must be “clear evidence that it regenerated naturally and recruited seedlings more than 100 m from parent plants”. This distance is associated

with a time-lapse of 50 years since introduction, and reproductive offspring must be found beyond 100 m. These events involve the tail of the dispersal curve, as only a small number of long-distance dispersal events producing reproductive offspring is necessary to create a new population at a far distance. Individuals of *A. grandis*, *T. heterophylla*, *T. plicata*, *C. lawsoniana*, *P. sitchensis*, and *A. veitchii* occurred over a DD50 of 100 m. If no mature individuals were measured, individuals taller than 1.3 m were recorded for *A. grandis* and *T. heterophylla* over the specified distance. It means they survived the most vulnerable seedling stage, and could potentially grow to sexual maturity.

Six species exhibited high invasive potential based on the three studied factors: *T. heterophylla*, *A. grandis*, *T. plicata*, *C. lawsoniana*, *L. kaempferi*, and *P. menziesii*. They were selected for the environmental analysis. Once projected on the PCA, these six species occupied a large proportion of the environmental space encountered at the surveyed sites, and displayed generalist behavior across common environmental conditions. Of note, *T. heterophylla* preferentially regenerated on acidic soils, supporting existing knowledge on the ecological preferences of this species (Rooney et al. 2000). In comparison, *A. grandis* tolerated drier soils, which might be beneficial under climate change as water stress is likely to become more frequent in the near future (Campioli et al. 2009). An important regeneration was recorded in open areas resulting from clear cuts. Interestingly, these six species also exhibited shade tolerance during regeneration with many individuals occurring in plots with low canopy openness, allowing them to spread in closed forest ecosystems. Martin and Marks (2006) demonstrated that invasions of undisturbed forests by shade-tolerant alien species frequently occur but require a longer time span than invasions in disturbed habitats, resulting in their invasiveness often being underestimated. The combination of a generalist behavior across soil types and shade-tolerance could allow these alien conifers to invade mature, undisturbed forests. This phenomenon is likely to be facilitated by areas planted with conifers. Consequently, because of the capacity of conifers to transform habitat, increasing the proportion of coniferous stands in wood production forests might accelerate the invasion rate of alien conifers (Jagodźiński et al. 2015). In 2011, 48 % of the southern Belgium forest was planted with conifers (Lecomte 2017). *Pseudotsuga menziesii* represented 6 % of the surface inventoried by the Belgian permanent forest inventory in 2011, far more than the other species highlighted in our study, and its proportion has increased by 52% since 2001. *Larix* sp., *A. grandis*, *T. plicata*, *T. heterophylla* and *C. lawsoniana* together represent a marginal section inventoried surface in 2011, and it is difficult to know the real extent of these species in public and private forests (Bauwens 2020). However, trials with alien species are becoming more frequent (Richardson et al. 2014), and exotic conifers are more and more considered as replacement species to compensate for the die-back of native productive species. For example, *C. lawsoniana* and *T. plicata* are selected for the REINFFORCE arboreta network, aiming to collect data on the growth of alien species in view of the diversification of European Atlantic temperate forests in light of climate change (Orazio et al. 2013).

Our sampling covered a large diversity of environmental conditions met in southern Belgium forests, from calcareous to acidic soils, from forests dominated by native

broadleaves to spruce plantations. These species can potentially invade a large proportion of forest lands, especially productive lands planted with conifers and managed with clear-cut regimes. However, this study did not cover the full diversity of temperate forests in Western Europe, with wider gradients potentially generating greater differences in the environmental space occupied by each species.

These six highlighted species also exhibit invasive behavior in other European countries (Rejmánek and Richardson 2003; Richardson and Rejmánek 2004; Broncano et al. 2005; Orellana and Raffaele 2010; Forestry Commission Scotland 2015). In western Norway, *T. heterophylla* is considered to be a very invasive conifer due to its high potential for spreading into neighboring stands and clear cuts (Oyen 2001). Plantations of *T. heterophylla* generate intense shade with few plants being able to live beneath them (Harmer et al. 2011). Galoux (1951) demonstrated the high regeneration capacity of *T. heterophylla* in Belgian arboreta, mentioning dense regeneration patches that occur beneath seed-bearers and in the neighboring plantations. The same author also stressed the abundant seed production and regeneration potential of *C. lawsoniana*, *P. menziesii*, and *A. grandis*. As the report was written in the middle of the 20<sup>th</sup> century, we know that the natural regeneration of these species has been ongoing for at least 70 years in the arboreta.

A small seed mass and an important maximal height have been linked to a better invasion success of plants in previous studies (Richardson and Rejmánek 2004, van Kleunen et al. 2010; Kutlvař et al. 2019). Both traits are linked with the capacity of species to spread at long distances. We did find a positive correlation between the maximal height and the Weighted Regeneration Density, but not with the seed mass. Dawson et al. (2011) surveyed exotic plants escaping from a tropical botanical garden. They concluded that propagule pressure was of greater significance than the functional traits in the establishment of alien plants in natural habitats. In a study conducted in North America, Pyšek et al. (2015) concluded that the importance of biological traits is highly dependent on the invasion stage, and often over-estimated. Further investigation on the role of functional traits on the invasiveness should be led in the local conditions of the Belgian arboreta, including the relative growth rate and specific leaf area, to test whether similar conclusions can be drawn.

The species exhibiting an important invasive potential in our study could be part of the invasion debt *sensu* Rouget et al. (2016) in Belgian forests. The important lag phase might be misleading concerning the potential impact of alien conifers, especially *T. heterophylla*. Twenty percent of the studied species exhibited invasive tendencies and they will certainly continue to expand in the future, especially if planting effort increases. In comparison, we did not detect any regeneration for half of the studied species. If foresters want to diversify forest plantations, they should avoid introducing species with high invasiveness and prefer native species or low-risk alien species (Brundu et al. 2020).

We identified species that were likely to become invasive based on small forest trials. The effect of mass plantings was not addressed. However, we demonstrated that the size of planted areas positively impacts regeneration density. Previous studies showed that propagule pressure has the potential to overwhelm ecological resistance

of ecosystems to invasions (Von Holle and Simberloff 2005). Even species considered to be dispersal-limited but with strong potential for wood production might cross a propagule pressure threshold and become invasive in the future due to high planting intensity (Richardson et al. 2004; Jagodziński et al. 2018).

Ennos et al. (2018) demonstrated that using non-native species for wood production and the diversification of forests presents great ecological and economic risks, potentially to the detriment of native tree species and associated biodiversity. Based on experience in countries with longer histories of using alien conifers, along with objectives to prevent further ecological damage, risk analyses of introduced alien conifers must be performed by monitoring old forest trials and arboreta (Richardson and Rejmánek 2004).

## Conclusion

Given the observed natural regeneration and dispersal of alien conifers in the old forest arboreta of southern Belgium, we recommend exercising caution when planting them in western temperate Europe. Half of the studied species regenerated, with almost 20% of these exhibiting an invasive behavior. Species showing the highest risk of being invasive were *T. heterophylla* and *A. grandis*, and to a lesser extent *C. lawsoniana*, *T. plicata*, *L. kaempferi*, and *P. menziesii*. Species with more limited dispersal capacities or a lesser proportion of mature trees, such as *A. nordmanniana*, *P. strobus*, *P. sitchensis*, and *A. veitchii*, could become of concern if planted at large scales. The results show that forest arboreta act as entry points for invasive species, especially now that more forestry trials are being set up to compensate for the die-off of native productive species. Thorough monitoring of alien conifers introduced for wood production is therefore needed to take early action for control and avoidance of larger introductions.

## Acknowledgements

Maps of the arboreta were provided by the “Arboreta” project run by the Earth and Life Institute of UCLouvain (Scholzen and Lhoir 2018). We thank Moran Durtschi-Guillemot for providing field assistance. We would also like to thank the “Division de la Nature et des Forêts”, more precisely the Forest Sections of Profondeville, Viroinval, Wellin, Saint-Michel, Spa, Seraing, Virton and Neufchâteau.

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# A multiregional assessment of transnational pathways of introduction

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Academic editor: J. Hierro | Received 11 November 2020 | Accepted 10 December 2020 | Published 20 January 2021

**Citation:** McGrannachan CM, Pagad S, McGeoch MA (2021) A multiregional assessment of transnational pathways of introduction. *NeoBiota* 64: 43–67. <https://doi.org/10.3897/neobiota.64.60642>

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## Abstract

Information on the pathways by which alien taxa are introduced to new regions is vital for prioritising policy and management responses to invasions. However, available datasets are often compiled using disparate methods, making comparison and collation of pathway data difficult. Using a standardised framework for recording and categorising pathway data can help to rectify this problem and provide the information necessary to develop indicators for reporting on alien introductions. We combine the Convention on Biological Diversity's Pathways Categorisation Scheme (CPC) with data compiled by the Invasive Species Specialist Group (ISSG) to report on multiregional trends on alien introduction pathways over the past 200+ years. We found a significant increase in the documented number of multiregional alien introduction events across all pathways of the CPC's three hierarchical levels. The 'escape' pathway is the most common documented pathway used by alien taxa. Transport stowaways via shipping-related pathways are a rapidly increasing contribution to alien introductions. Most alien introduction events were of unknown pathway origin, highlighting the challenge of information gaps in pathway data and reiterating the need for standardised information-gathering practices. Combining the CPC framework with alien introduction pathways data will standardise pathway information and facilitate the development of global indicators of trends in alien introductions and the pathways they use. These indicators have the potential to inform policy and management strategies for preventing future biological invasions and can be down-scaled to national and regional levels that are applicable across taxa and ecosystems.

## Keywords

Aichi Target 9, alien taxa, Convention on Biological Diversity, introduction event, introduction pathway, propagule pressure

## Introduction

Expansion and increased intensity of global trade and human movement has exacerbated global species invasions (Essl et al. 2015; Early et al. 2016). Worldwide increases in the number of alien species are likely to continue (Seebens et al. 2017), meaning it is crucial that the pathways by which alien species are transported and introduced to new locations, and how these change in relative importance over time, are identified, understood and better managed (Essl et al. 2015; Chapman et al. 2017). Pathways of introduction are the means by which alien species are transported intentionally or unintentionally outside of their natural geographic range (Richardson et al. 2010; Turbelin et al. 2017). A pathway approach to risk assessment for invasive alien species focuses primarily on identifying introduction pathways to (i) develop early detection and preventative strategies, with the aim to reduce or eliminate the propagule pressure of alien species (Faulkner et al. 2016; Padayachee et al. 2017; Pergl et al. 2017), and (ii) to prioritise investment in managing pathways responsible for the highest propagule loads or particular high risk species (McGeoch et al. 2016). Additionally, a pathway approach can be important in the absence of species-specific data, or when suitable control efforts for individual species are unachievable (Hulme et al. 2008; Padayachee et al. 2017). Accounting for introduction pathways is therefore fundamental for developing relevant management and policy strategies that minimise the introduction, spread and impact of alien species (Hulme et al. 2008).

Efforts to categorise alien species via their pathways of introduction have culminated in the development of a standardised pathway categorisation framework (Harrower et al. 2017). Using this framework, pathways of introduction and spread are classified as intentional or unintentional and encompass three introduction mechanisms: the importation of a commodity, the arrival via a transport vector (through a dispersal corridor resulting from human activity), and the natural spread from a neighbouring region where the species is alien (UNEP 2014; Essl et al. 2015). The foundation of this framework is the six pathway introduction categories (release, escape, transport-contaminant, transport-stowaway, corridor and unaided) originally proposed by Hulme et al. (2008), which encompass 32 specific pathway subcategories of introduction (for example, agriculture, horticulture and ship ballast water). This ‘Convention on Biological Diversity (CBD) Pathways Categorisation’ (CPC) (*sensu* Harrower et al. 2017) incorporates standardised terminology and guidelines for pathway categorisation and is applicable at a global scale and across different taxonomic groups (Harrower et al. 2017; Tsiamis et al. 2017). The CPC has now been validated by application to alien introductions at national (South Africa; Faulkner et al. 2016), continental (Europe; Pergl et al. 2017; Tsiamis et al. 2017) and global scales (167 cities worldwide; Padayachee et al. 2017). Importantly, the intention of this scheme is, *inter alia*, to assist global reporting as well as country Parties to the CBD to respond to the Strategic Plan for Biodiversity 2011–2020 (UNEP 2014). In particular, this is relevant to achieve and report on Aichi Target 9 by 2020, such that *invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated and measures are in place to manage pathways to prevent their introduc-*

*tion and establishment* (Convention on Biological Diversity 2010). Whereas monitoring pathways of invasion was not included in the previous global indicator framework for invasive alien species (McGeoch et al. 2010), doing so has now become central to reporting on policy targets for biological invasion (McGeoch and Jetz 2019).

Developing information on pathways introductions using a standardised framework is currently a priority for several reasons. First, preventing the introduction and spread of alien and potentially invasive species is the first line of defence in the management of biological invasions. Managing the early stages of the invasion process (i.e. transport and introduction) that focus on prevention is more cost-effective than reactive, post-introduction management of species (Leung et al. 2002; Rout et al. 2011; Kumschick and Richardson 2013). Nonetheless, management, policy and research that targets the transport and introduction stages of invasion remain relatively under-represented compared to the invasion stages of establishment and spread (Puth and Post 2005; Early et al. 2016; Chapman et al. 2017).

Second, information on the pathways of species introductions has not, to date, been consolidated into a readily available or accessible form (Saul et al. 2017). Harmonising and identifying discrepancies between data sources is crucial for informing alien species policy and management (Seebens et al. 2020). For example, a recent comparison of European pathway data between the European Alien Species Information Network (EASIN) and the CPC revealed that the pathway subcategories of ~ 5,500 alien species registered with EASIN did not directly align with CPC subcategories (Tsiamis et al. 2017). These types of discrepancies can compound the already high level of uncertainty when identifying and assigning pathways to individual species introductions, particularly for unintentional pathways (e.g. transport-contaminant; transport-stowaway) that may be inadequately documented (Essl et al. 2015).

Third, information on introduction pathways contributes directly to biosecurity policy and regulations, including regulating the criteria for the import and trade of alien species (Burgiel et al. 2006; Leung et al. 2014; Hulme 2015). For example, a blacklist (banned from importation) or whitelist (permitted importation) approach has been adopted by many countries as a response to the global trade in ornamental nursery stock, which is the primary means of introduction of alien plants (Dehnen-Schmutz 2011; Essl et al. 2011; Hulme et al. 2017). Pathway information informs prioritisation of biosecurity interventions by identifying pathways that pose relatively high invasion risk in terms of both propagule load (Brockerhoff et al. 2014) and high risk species (Pergl et al. 2017; Roy et al. 2014) and further informing the development of preventative management strategies and policy at multiple scales (Pyšek et al. 2011; Faulkner et al. 2016). However, few comprehensive pathway-focused policies have been implemented at any administration level, and those that are in place tend to target the release and escape pathways (Essl et al. 2015).

Finally, information on pathway changes over time can, with appropriate modelling and interpretation (McGeoch and Jetz 2019), be used to develop indicators for reporting on alien introduction trends (Rabitsch et al. 2016; Wilson et al. 2018). While the importance of some pathways can remain constant over several decades

(e.g. shipping), other pathways (e.g. horticulture) may increase in importance (Ojaveer et al. 2017; Zieritz et al. 2017). These changes may reflect updated legislation for the importation of species, or the increasing global trade of certain commodities (Zieritz et al. 2017; Seebens et al. 2018), and are important for monitoring the effectiveness of biosecurity policy and implementation, such as Aichi Biodiversity Target 9 as well as Sustainable Development Goal 15.8 (Rabitsch et al. 2016).

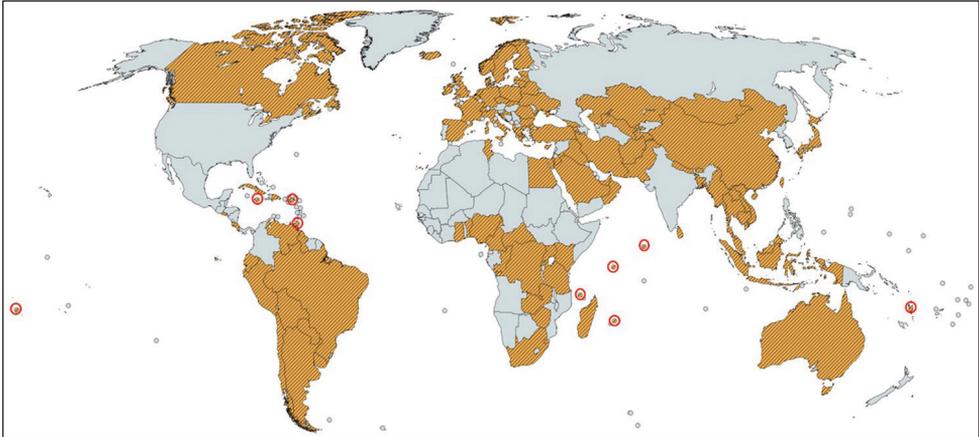
To date, pathway analysis has been conducted for specific regions (e.g. South Africa, Europe; Faulkner et al. 2016; Pergl et al. 2017), environments (e.g. urban; Padayachee et al. 2017), taxonomic groups (e.g. invertebrates, plant pests; Chapman et al. 2016; Houghton et al. 2016) or specific pathway(s) (Kumschick et al. 2016; Tingley et al. 2018). Although several assessments have shown changes in pathways of invasion over time (Rabitsch et al. 2013; Ojaveer et al. 2017; Zieritz et al. 2017), these are restricted to specific taxonomic groups or geographic locations (but see Rabitsch et al. 2016). Building on these regional and taxon-specific efforts, here we conduct a cross-taxonomic, multiregional analysis of information available on transnational introduction pathways that incorporates all major groups, environments and pathways, to quantify decadal trends in invasion reported via these pathways since 1800. We use a hierarchical, standard categorisation of pathways (Harrower et al. 2017) so that the results may in future be appropriately modelled, compared, downscaled to regions and countries, and form a baseline for future reporting of trends in invasion pathways. We specifically ask (1) are recorded invasive alien species introductions largely intentional or unintentional? (2) What pathways of introduction and spread are responsible for alien species introductions? (3) What pathway subcategories are alien species using to move about?

## Methods

### Data used

Introduction records compiled from the Global Register of Introduced and Invasive Species (GRIIS) by the ISSG were used as the underlying data for the analysis of pathway trends. The GRIIS dataset provides verified and annotated country checklists of alien and invasive species (Pagad et al. 2018). In addition to species names, each record includes taxonomy, the environment/system in which the species occurs, the provenance/origin of the species, evidence of impact (yes/no), date of introduction or first record, type of introduction, pathways of introduction, mechanism of impact, and references for source information. GRIIS Version 2016.2 includes draft checklists for all 196 countries that are party to the CBD.

Data for 18746 introduction events, involving 4832 alien species in 101 countries, and occurring between the years 1300 and 2017, were available and adequate to conduct a pathways assessment (Fig. 1). Here we define an introduction event as a recorded introduction of an alien species in a country outside of its native range. Each introduction event included the date of first introduction or first record of a



**Figure 1.** The 101 countries (orange) used to conduct the global pathways assessment. Red open circles indicate small island nations ( $n = 9$ ) (<https://mapchart.net/>; accessed 30 July 2019).

species and contained data on either all or some of the following information types: (1) introduction being intentional or unintentional (i.e. ‘pathway type’); (2) ‘pathway category’ (escape, release, transport as contaminant or stowaway, corridors, unaided or unknown); (3) ‘pathway subcategory’ (further details of specific vectors within each pathway category). The data include Animalia, Bacteria, Chromista, Fungi, Plantae, Protozoa and Virus taxa. The 101 countries cover six regions: Africa, Asia, Europe, North America, Oceania and South America (Figure 1; Suppl. material 3: Table S1). These countries encompass a range of different sizes, development status and climatic regions and thus are geographically representative of global data.

A further 5113 species are known to be introduced to the selected 101 countries via known pathways but were not included in analysis as they do not have authoritative information on dates of introduction or first record. These species were therefore excluded and we concentrated on the 4832 species for which the date of introduction in these 101 countries is known. The total number of introduction events currently estimated is  $\sim 98422$ , involving  $\sim 10800$  species (including the 5113 species mentioned above). These events, besides known invasive species, include weeds, agricultural pests and diseases, and other non-invasive aliens for which no pathway information or dates of introduction are known.

Information and data on pathways of introduction were extracted during 2016/2017 from sources used to compile national checklists (see Pagad et al. 2018 for information on the general data collation and entry process). Information sources ranged from scientific peer-reviewed literature, databases, reports both published and unpublished and research data. Textual information describing pathways of introduction were documented and then reviewed for categorisation. These categories were inserted into the data collection templates. Because the CPC is relatively new, some of the information from the data sources used pathway terminology that did not fully align with the CBD framework. In these cases, it was necessary to interpret

the pathways within the CBD framework, using literature-based pathway information as a guide. This enabled all data to be compiled using the standard categorisation of pathways endorsed by the Parties to the CBD (UNEP 2014). These categories were inserted into the data collection templates.

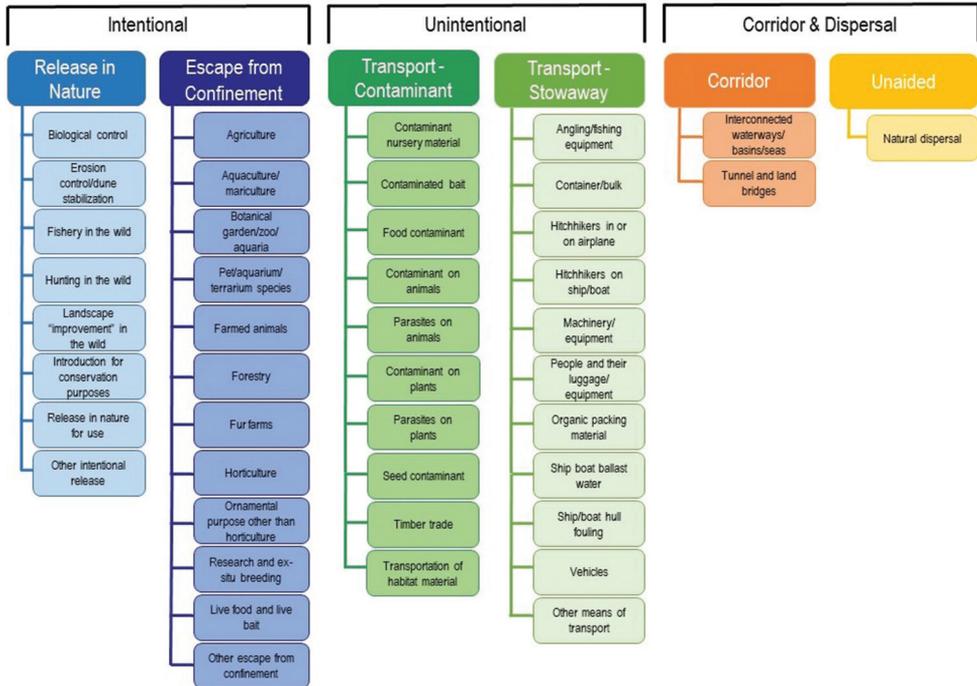
Information on dates of introduction or first record and information related to the three levels of the pathway hierarchy for the actual introduction event were recorded - pathway type, pathway category and pathway subcategory. Each introduction event was temporally classified using centuries and decades as classifiers (Suppl. material 2: Appendix S1). First introduction records were aggregated by decade beyond 1800. Decadal scales are appropriate because there is often a lag between detection and reporting events. All records prior to 1800 were aggregated as 'Pre-1800'. Records from the most recent decade were classified as '2011 plus'.

## Pathway categorisation

We used the definitions and descriptions of introduction pathways contained in Harrower et al. (2017). This document is the most up-to-date guideline for interpreting the definitions of the CPC and provides examples of the CBD Pathways Categorisation's application to species information (Harrower et al. 2017). The definitions and descriptions were revised and modified by a panel of experts, using comparisons of the CPC pathway descriptions to descriptions used in (1) the Global Invasive Species Database (GISD), (2) the Delivering Alien Invasive Species Inventories for Europe (DAISIE) database, (3) the Great Britain's Non-Native Species Information Portal (GBNNSIP) database, and (4) the EASIN information platform (Harrower et al. 2017). Of particular benefit is the distinction between pathway subcategories that appear to overlap. For example, the 'Contaminant on Plants' subcategory seemingly overlaps with the 'Contaminant nursery material' and 'Transportation of habitat material' subcategories. The Harrower et al. (2017) guideline defines and describes the difference between these pathways and treats them in a prescribed order of precedence for category allocation. For example, the 'Contaminant on plants' subcategory is defined to contain all contaminants on plants that are not related to the nursery trade, where 'Contaminated nursery material' is given precedence over 'Contaminant on plants' (Harrower et al. 2017). Despite some shortcomings of the CPC framework, particularly the uncertainty involved in interpreting some subcategories (Faulkner et al. 2020; Pergl et al. 2020), it is a reliable framework with which to report on introduction trends at a transnational level. The CPC framework is still relatively new (2014) and its further development and adoption will facilitate its use as a standardised tool for reporting on alien introductions (Pergl et al. 2020).

## Analysis of trends

For pathway types (i.e. intentional or unintentional introductions), we report trends in terms of both total recorded introduction events for each decade, as well as cumulative introduction events documented between 1800 and 2017. Pathway categories are reported



**Figure 2.** Overview of the hierarchical, standard categorisation of pathways. Six pathway categories and 44 pathway subcategories are broadly categorised into a) intentional transport and introduction of taxa, b) pathways of unintentional introduction and c) pathways by which taxa move to new regions, without direct transportation by humans (i.e. Pathway types). Adapted from Harrower et al. (2017).

as total number of introduction events per decade for each category. We also report cumulative introduction events for pathway categories, using 1970 as a baseline year. This date was chosen for its comparability with the 1970 baseline used for CBD global biodiversity indicators in Butchart et al. (2010). The dominant pathway subcategories are reported as cumulative introduction events from 1800 to 2017.

We used generalized linear models (negative binomial distribution with log link function) to quantify changes in the recorded number of introduction events over time (introduction events ~ decade). This was conducted at all introduction pathway levels (pathway type, pathway category, pathway subcategory). For subcategories, only the pathways with more than 100 introduction events ( $n = 18$  subcategories) were considered.

## Results

### Pathway types

There was a total of 8172 (43.59%) intentional and 10574 (56.41%) unintentional documented introduction events of alien species across the 101 countries (Table 1).

**Table 1.** Summary of introduction pathways and their documented introduction events. Bracketed numbers represent the number of subcategories categorised as “other”.

Total number of documented introduction events: 18746			
Introduction Pathway	# of Documented introduction events	% of Total documented introduction events	Rank
<b>Pathway type</b>			
Intentional	8172	43.59	2
Unintentional	10574	56.41	1
<b>Pathway category</b>			
Release	1078	5.75	5
Escape	7094	37.84	1
Transport-contaminant	1982	10.57	3
Transport-stowaway	1581	8.43	4
Corridors	828	4.42	5
Unaided	148	0.79	6
Unknown	6035	32.19	2
<b>Pathway subcategory</b>			
Release: Biological control	109	0.58	18
Release: Fishery in the wild	248	1.32	14
Release: Landscape/flora/fauna improvement	342	1.82	11
Release: Other subcategories (4)	110	0.59	n/a
Release: Release in nature for use	229	1.22	15
Escape: Agriculture	1193	6.36	3
Escape: Aquaculture/mariculture	1010	5.39	4
Escape: Forestry	273	1.46	13
Escape: Horticulture	3581	19.10	2
Escape: Ornamental purpose	475	2.53	7
Escape: Pet/aquarium species	288	1.54	12
Escape: Other subcategories (6)	204	1.09	n/a
Transport-contaminant: Seed contaminant	654	3.54	6
Transport-contaminant: Timber trade	190	1.01	16
Transport-contaminant: Other subcategories (8)	310	1.65	n/a
Transport-stowaway: Hitchhikers on ship/boat	412	2.20	10
Transport-stowaway: Ship/boat ballast water	447	2.38	8
Transport-stowaway: Ship/boat hull fouling	422	2.25	9
Transport-stowaway: Other subcategories (8)	82	0.44	n/a
Corridors: Interconnected waterways/basins/seas	827	4.41	5
Corridors: Other subcategories (1)	0	0.00	n/a
Unaided: Natural dispersal across borders	137	0.73	17
Unknown: Unknown	7203	38.42	1

Since 1800, steady and significant increases in both documented intentional and unintentional introduction events have occurred (Table 2; Figure 3a, b). From 1800 to 1900, both pathway types showed similar cumulative increases in introduction events, but with more documented intentional introduction events than unintentional events (Figure 3b, Table 3). Post 1900, the overall number of documented unintentional introductions per decade was higher than intentional introductions (Figure 3a, b; Table 3). Decadal increases in documented introduction events ranged between 5.79% (1800–1810) and 23.15% (1860–1870) for intentional introductions and between 7.19% (1800–1810) and 24.68% (1890–1900) for unintentional introductions (Table 3). The average decadal increase in intentional and unintentional introductions was 13.12% and 15.29%, respectively (Table 3). The decade of

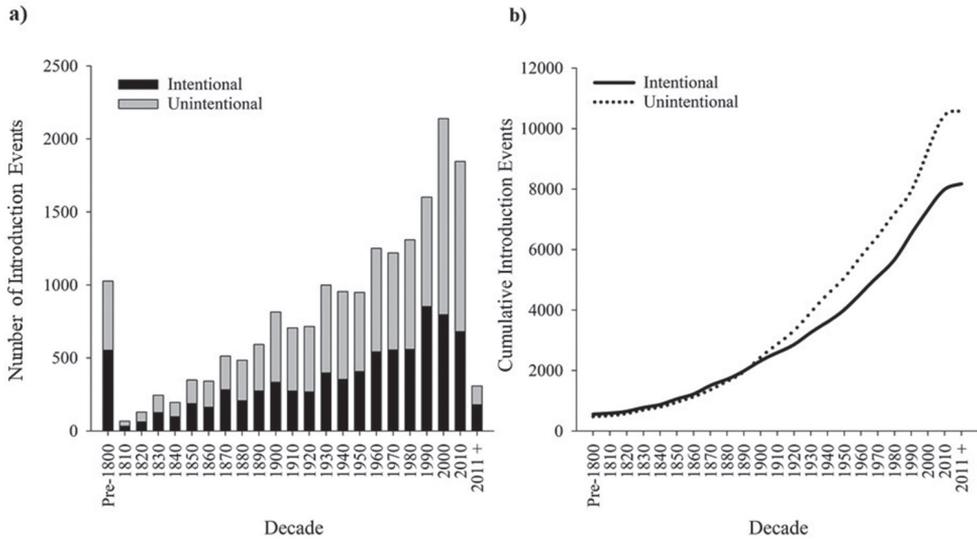
**Table 2.** Decadal increase in documented intentional and unintentional introduction events for the period 1810 to 2011. + or – signs in the right-most column indicate a higher (+) or lower (-) decadal growth rate of intentional introductions when compared to unintentional introductions.

Decade	Documented intentional introduction events	Documented unintentional introduction events	Intentional decadal growth (%)	Unintentional decadal growth (%)	Difference between intentional / unintentional decadal growth rates
Pre-1800	553	473	n/a	n/a	n/a
1810	585	507	5.79	7.19	-1.40
1820	646	575	10.43	13.41	-2.98
1830	772	693	19.50	20.52	-1.02
1840	869	790	12.56	14.00	-1.43
1850	1056	951	21.52	20.38	+1.14
1860	1218	1129	15.34	18.72	-3.38
1870	1500	1359	23.15	20.37	+2.78
1880	1707	1636	13.80	20.38	-6.58
1890	1981	1953	16.05	19.38	-3.32
1900	2314	2435	16.81	24.68	-7.87
1910	2587	2868	11.80	17.78	-5.98
1920	2854	3316	10.32	15.62	-5.30
1930	3251	3918	13.91	18.15	-4.24
1940	3603	4521	10.83	15.39	-4.56
1950	4009	5064	11.27	12.01	-0.74
1960	4550	5773	13.49	14.00	-0.51
1970	5105	6437	12.20	11.50	+0.70
1980	5663	7188	10.93	11.67	-0.74
1990	6516	7937	15.06	10.42	+4.64
2000	7313	9279	12.23	16.91	-4.68
2010	7993	10445	9.30	12.57	-3.27
2011 >	8172	10574	2.24	1.24	+1.00
<b>Average</b>	n/a	n/a	<b>13.12</b>	<b>15.29</b>	<b>3.10</b>
<b>Std Dev.</b>	n/a	n/a	<b>4.70</b>	<b>5.25</b>	<b>2.17</b>

1991–2000 had more documented introduction events than any other decade in the time series (Figure 3a).

### Pathway categories

The documented number of introduction events for each pathway category has increased significantly per decade since 1800 (Table 2; Figure 4a). The ‘escape’ pathway is the most prevalent pathway by which species introductions are known to occur (37.84%), followed by ‘unknown’ pathway introductions (32.19%; Table 1; Figure 4a). Post 1970 trends show both escape and unknown pathways increased dramatically in cumulative number of introduction events, with 3177 and 2350 additional events, respectively, occurring between 1970 and 2017 (Figure 4b). This is equivalent to 81.38% (escape) and 58.14% (unknown) of the total number of pre-1970 documented introduction events. The remaining five pathway categories had fewer cumulative introduction events compared to escape and unknown pathways, the highest being ‘transport-contaminant’ (1982 events by 2017) and the lowest ‘unaided’ (148 events by 2017; Figure 4c). Of these five pathways, ‘transport-stowaway’ showed the steepest cumulative increase in introduction events post 1970 (Figure 4c).



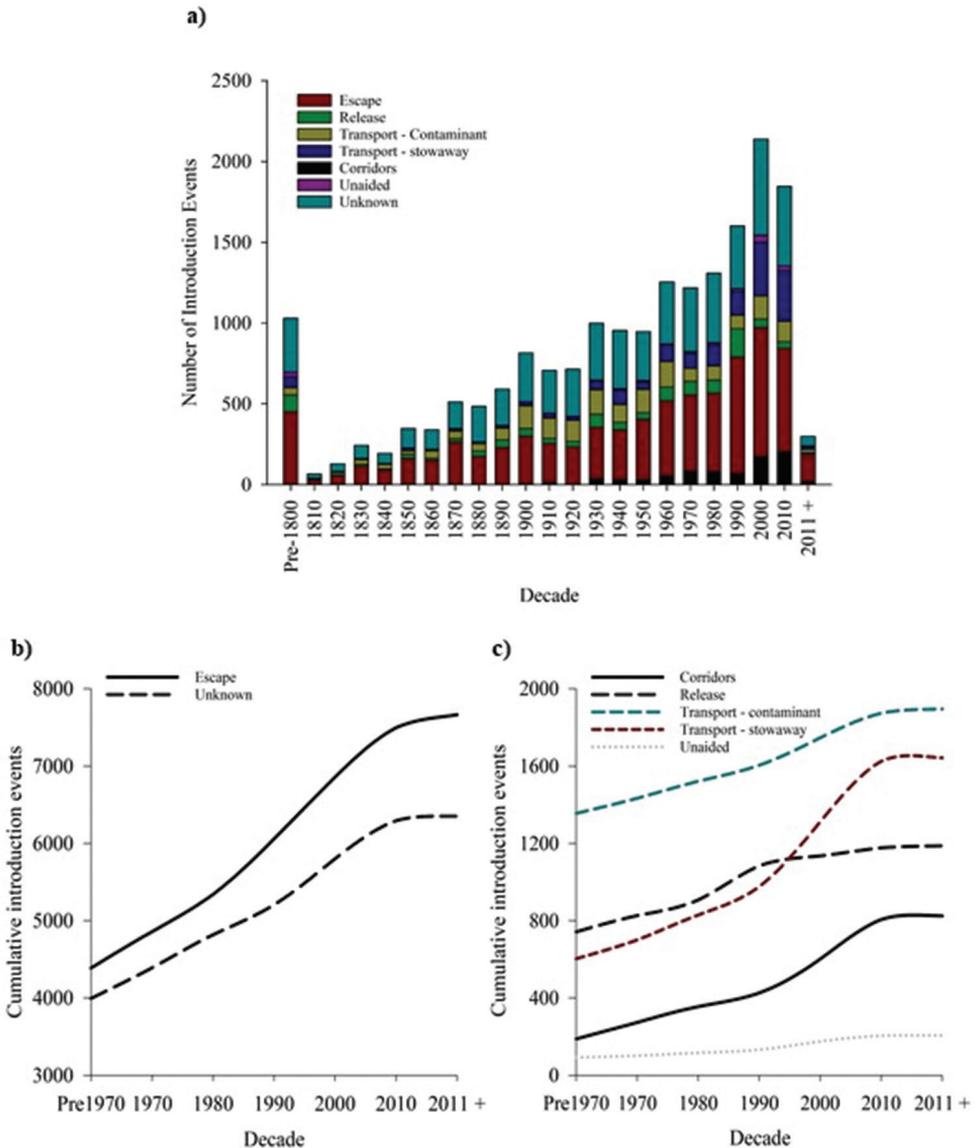
**Figure 3.** Decadal changes in the documented number of intentional and unintentional introductions of alien species for 101 countries. Trends in introduction events ( $n = 18746$ ) involving over 4800 alien species are shown as: **a** number of documented introduction events, and **b** the cumulative number of documented introduction events. An introduction event in this figure represents one species introduced outside of its known native range for the first time and into one of the 101 countries in the pool.

### Pathway subcategories

The 18 pathway subcategories with more than 100 introduction events since 1800 ranged from 109 to 7203 records (Table 1). The top 18 subcategories were representative of all pathway categories. ‘Unknown’ was the pathway subcategory associated with the most documented introduction events (7203; 38.42%), followed by three subcategories from the escape pathway: ‘horticulture’ (3581; 19.10%), ‘agriculture’ (1193; 6.36%) and ‘aquaculture/mariculture’ (1010; 5.39%; Table 1; Figure 5a). Many of the subcategories showed sharp rates of increase, particularly from the beginning of the twentieth century, including ‘hitchhikers on ships’, ‘ship ballast water’, ‘ship hull fouling’ and ‘interconnected waterways’ (Figure 5b, c; Suppl. material 1: Figure S1a). In comparison, most subcategories from the escape pathway (except horticulture) had slower cumulative introduction rates, including ‘agriculture’, ‘aquaculture/mariculture’, ‘forestry’, ‘ornamental purpose other than horticulture’ and ‘pet/aquarium species’. All but one subcategory (‘release in nature for use’) significantly increased in introduction events per decade since 1800 (Table 3).

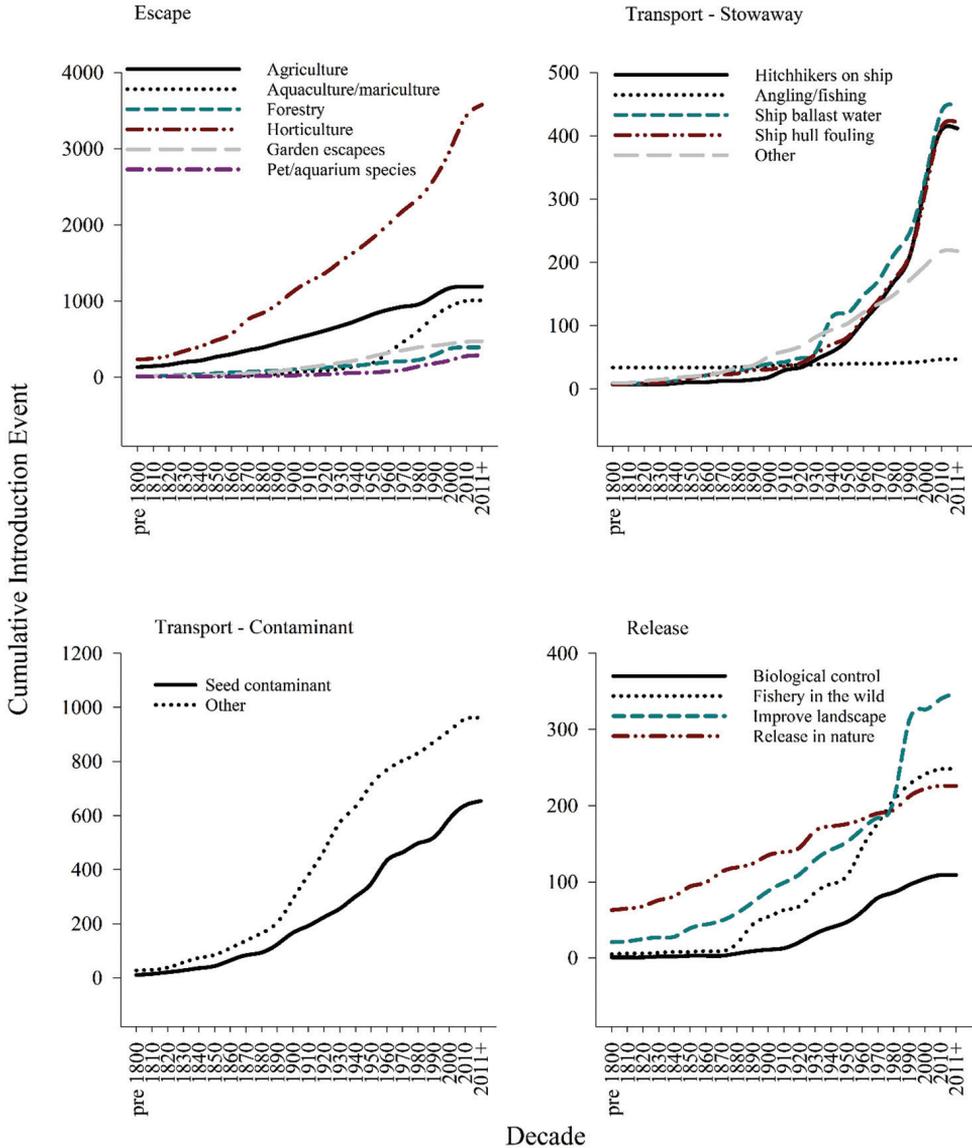
### Discussion

We used the CBD pathways categorisation framework and a multiregional dataset encompassing a range of taxonomic groups to report on decadal changes in introduction pathways reported for alien species since 1800. We highlighted the significant increase



**Figure 4.** Changes in the six main pathway categories (as well as the number of introductions via unknown pathways) **a** the documented number of introduction events ( $n = 18746$ ) of alien species per decade since 1800 for 101 countries **b–c** cumulative number of documented introduction events by pathway since 1970 (note different scaling on y-axes).

of documented events for almost every pathway at each of the three hierarchical levels of the CPC. Unintentional introductions have increased over intentional introductions since the beginning of the twentieth century. However, ‘Escape’ – an intentional pathway - is the most common pathway category documented, particularly for pathway sub-



**Figure 5.** Changes in the dominant pathway subcategories across decades. Cumulative number of documented introduction events (note different scales on y-axes). The 18 pathway subcategories shown are those with most (> 100) introduction events (see Suppl. material 1: Figure S1 for ‘corridors: interconnected waterways/basins/seas’, ‘unaided: natural dispersal across borders’ and ‘unknown’ pathway subcategories not shown).

categories related to plant and aquatic cultivation. This shows that intentional pathways are still an important source of alien introductions. The vast majority of documented introduction events, however, are unknown (38.42%), which emphasises the high level of uncertainty involved in categorising and managing alien species introduction pathways.

**Table 3.** Trends in recorded introduction events by pathway across the period 1800 to 2017. Generalized linear model results (family = negative binomial, link = log). Significant  $p$  values ( $p < 0.05$ ) shown in bold.

Pathway type	Slope coefficient	Std. Error	Df	$z$	$p$
Intentional	0.011	0.001	20	8.427	< <b>0.001</b>
Unintentional	0.013	0.002	20	8.568	< <b>0.001</b>
<b>Pathway category</b>					
Release	0.012	0.002	20	5.764	< <b>0.001</b>
Escape	0.011	0.001	20	8.608	< <b>0.001</b>
Transport - contaminant	0.01	0.002	20	5.493	< <b>0.001</b>
Transport - stowaway	0.021	0.002	20	11.25	< <b>0.001</b>
Corridor	0.035	0.003	20	10.156	< <b>0.001</b>
Unaided	0.026	0.003	20	7.787	< <b>0.001</b>
Unknown	0.01	0.002	20	6.408	< <b>0.001</b>
<b>Pathway subcategory</b>					
Release: Biological control	0.017	0.004	20	4.858	< <b>0.001</b>
Release: Fishery in the wild	0.016	0.004	20	4.253	< <b>0.001</b>
Release: Landscape "improvement"	0.012	0.002	20	5.075	< <b>0.001</b>
Release: Release in nature for use	0.001	0.002	20	0.421	0.674
Escape: Agriculture	0.004	0.002	20	2.017	<b>0.045</b>
Escape: Aquaculture/mariculture	0.028	0.003	20	8.975	< <b>0.001</b>
Escape: Forestry	0.006	0.002	20	2.858	<b>0.004</b>
Escape: Horticulture	0.009	0.001	20	7.632	< <b>0.001</b>
Escape: Ornamental purpose	0.012	0.002	20	5.429	< <b>0.001</b>
Escape: Pet/aquarium species	0.024	0.003	20	8.628	< <b>0.001</b>
Contaminant: Seed contaminant	0.01	0.002	20	5.644	< <b>0.001</b>
Contaminant: Timber trade	0.032	0.005	20	6.581	< <b>0.001</b>
Stowaway: Hitchhikers on ship/boat	0.028	0.003	20	8.316	< <b>0.001</b>
Stowaway: Ship/boat ballast water	0.021	0.003	20	7.633	< <b>0.001</b>
Stowaway: Ship/boat hull fouling	0.023	0.003	20	8.457	< <b>0.001</b>
Corridors: Interconnected waterways	0.035	0.003	20	10.155	< <b>0.001</b>
Unaided: Natural dispersal	0.025	0.003	20	7.514	< <b>0.001</b>
Subcategory unknown	0.01	0.002	20	6.408	< <b>0.001</b>

## Accidental and deliberate introduction events

Prior to the twentieth century, the cumulative rate of increase for both intentional and unintentional introduction events documented were virtually identical (Figure 3b). The beginning of the twentieth century saw unintentional surpass intentional introductions, a trend that has continued up to the present. The increase in unintentional introductions is likely due to the rise in international trade, which is widely acknowledged as an important factor in allowing alien species to successfully establish in novel geographic regions (Levine and D'Antonio 2003; Perrings et al. 2005; Yemshanov et al. 2012; Chapman et al. 2017). In particular, the accidental transport of inconspicuous taxa, such as fungi, microorganisms, pathogens and invertebrates are often associated with global trade, including live plant imports and importation via shipping (Brockerhoff and Liebhold 2017; Chapman et al. 2017; Okabe et al. 2017). Studies from multiple regions including Europe (Chapman et al. 2017; Pergl et al. 2017; Saul et al. 2017), Asia (Hong et al. 2012; Okabe et al. 2017), the US (Liebhold et al. 2012) and the Antarctic (Osyczka et al. 2012; Houghton et al. 2016) have found that these taxonomic groups are more often associated with unintentional pathways. Increases in

trade volume and the subsequent rise in accidental introductions of alien species may counteract existing national biosecurity and phytosanitary measures (Brockerhoff and Liebhold 2017). It is therefore important to improve measures for monitoring unintentional introduction pathways to effectively address the ongoing occurrence of accidental alien introductions. Interestingly, although unintentional introductions surpassed intentional introductions, escape (an intentional introduction pathway category) had most associated introduction events (excluding unknown events). This highlights that the prevention and management of intentional introductions are of equal importance to those of unintentional introductions, especially given that the impact realised by alien taxa has been associated more frequently with intentional than unintentional introductions (Pergl et al. 2017).

### Introduction pathway categories and subcategories

Our findings corroborate previous studies of alien introduction pathways in several ways. First, ‘escape’ is overall the most common documented pathway category by which alien species are introduced (Turbelin et al. 2017). Second, ‘transport-stowaway’ is becoming an increasingly important introduction pathway, particularly for marine stowaways (Zieritz et al. 2017). Finally, records of introduction events via unknown pathways are prevalent in existing databases and presents an ongoing problem for assessing alien introductions (Katsanevakis and Moustakas 2018). Our global perspective takes into consideration alien species from multiple taxonomic groups but supports similar findings from studies focussing on specific taxonomic groups or regions.

Escape was the most prevalent pathway, with records almost doubling between 1970 and the present (Figure 4b). Escape has been identified as the most frequent introduction pathway across all taxa at global (Turbelin et al. 2017) and national (South Africa; Faulkner et al. 2016) scales, for plants at country- (Czech republic; Pyšek et al. 2011; USA; Lehan et al. 2013) and city-scales (Padayachee et al. 2017) and for both plants (Pergl et al. 2017) and vertebrates in Europe (Saul et al. 2017; Roy et al. 2019) and globally (Saul et al. 2017, van Kleunen et al. 2018). However, the escape introduction pathway has been shown as less important for some taxonomic groups, such as marine species (Molnar et al. 2008; Katsanevakis et al. 2013) or terrestrial arthropods (Essl et al. 2015). Our results highlight the importance of the escape pathway at a multiregional level and emphasise the ongoing need for better containment procedures and greater public awareness of the risks involving escaped organisms, particularly ornamental plants (Ricciardi et al. 2017; Saul et al. 2017, van Kleunen et al. 2018).

Horticulture is the most important pathway subcategory of alien plant introductions (Turbelin et al. 2017, van Kleunen et al. 2018) and was the pathway subcategory with the largest and fastest increase in introduction events (Figure 5a). Agriculture was the second most important pathway subcategory and is also recognised as an important contributor to alien plant introductions (Mack and Erneberg 2002; Richardson et al. 2003). Both horticulture and agriculture are pathway subcategories specific to plants (Harrower et al. 2017) and their combined, high proportion of recorded intro-

ductions in the dataset (see Table 1) supports previous studies that show escape (from horticulture or agriculture) is an important pathway for plants.

The importance of escape as an introduction pathway for faunal species is reflected by the high number of introduction events attributed to escape from aquaculture/mariculture (e.g. fish farms) compared with the pet/aquarium trade. Aquaculture/mariculture had the third most introduction events, while records attributed to the pet/aquarium trade remained relatively stable across the assessed time-period (Figure 5a). Aquaculture was found to be the highest contributing pathway to freshwater alien species introductions in Europe (Nunes et al. 2015) and an important pathway for alien invasions of European seas (Nunes et al. 2014). The ecological impacts of invasion via aquaculture can be severe (Naylor et al. 2001; Keller et al. 2011) and given the aquaculture sector is one of the fastest growing global primary industries (Teletchea and Fontaine 2014), it is also likely that alien introductions via this pathway will continue to rise.

Subcategories of the transport-stowaway category were among those with largest growth in alien introductions since 1970 (Figure 5b). In particular, there was a sharp rise in the post-1970 introduction of marine stowaways as hitchhikers on ships, in ship ballast water or as ship hull fouling, which saw 67%, 62% and 67%, respectively. The importance of marine/aquatic pathways is also reflected in the sharp rise in introduction events by interconnected waterways since 1970 (Suppl. material 1: Figure S1a). Interconnected waterways were found to be an important pathway in the Mediterranean Sea, largely via the Suez Canal (Zenetos et al. 2012). These increases in alien introductions are likely due to the continued expansion of tourism and international shipping (Early et al. 2016; Turbelin et al. 2017). The introduction of marine and freshwater alien taxa via shipping-related transport has been confirmed as an important source of ongoing propagule pressure in many parts of the world, including the Mediterranean region, Northwest Europe, the Northeast Pacific and Australia (Tingley et al. 2017; Zieritz et al. 2017; Anil and Krishnamurthy 2018).

A key challenge in attempting to decipher trends in alien introductions is uncertainty in the specific pathways used by species (Katsanevakis et al. 2013; Essl et al. 2015). This is particularly problematic for unintentional introductions via transport contaminants or stowaways, and for smaller organisms such as marine invertebrates that are at a higher risk of going unnoticed or undocumented (Essl et al. 2015; Ojaveer et al. 2017; Zieritz et al. 2017). The results shown here demonstrate the problem clearly: the total number of introduction events where a pathway category was unknown far exceeded all other known pathway categories (Figure 4b–c). The exception to this was the ‘escape’ pathway, an intentional pathway category that surpassed the number of unknown introduction events (Figure 4b). Furthermore, ‘unknown’ was the highest-ranked pathway subcategory in terms of the number of introduction events and was almost double that of the second-ranked subcategory (Horticulture; Table 1). These results corroborate previous studies that have demonstrated and highlighted the risk that uncertainty poses to introduction pathway datasets and trends (Zenetos et al. 2017; Katsanevakis and Moustakas 2018).

There are several reasons why uncertainty in pathway identification and trends occurs. Often, the lack of historical introduction records (i.e. pre-mid twentieth century; Ojaveer et al. 2017) can result in gaps in datasets that can particularly impact the interpretation of introduction temporal trends (McGeoch et al. 2010; Katsanevakis et al. 2013; Galil et al. 2018). Usually this occurs due to decreased scientific effort or reduced awareness of the need to record alien species introductions (Ojaveer et al. 2017).

In many cases, multiple pathways are equally tenable as the cause of alien species introductions to a new region (Minchin 2007). This makes assigning the correct pathway difficult and decisions may be entirely based on the interpretations or assumptions of assessors (Zenetos et al. 2012). In other instances, the species' ecology may be used to infer an introduction pathway (Zenetos et al. 2012). A representative example of this is the introduction of marine species into the Mediterranean Sea via the Suez Canal. Several pathway subcategories could feasibly be responsible for new introductions into the Mediterranean, including species as hitchhikers on ships, through ship ballast water or hull fouling, or through natural dispersal through the canal (Katsanevakis et al. 2013). These types of uncertainty can potentially over- or under-emphasise certain pathways, causing trends to be misrepresented at both global and regional scales. Using a confidence score in allocating pathways may provide a cautionary approach to the compilation of pathway data that helps identify which species, pathways or regions are particularly susceptible to uncertainty (Essl et al. 2015). A focus on improving monitoring of these identified species, pathways or regions may aid efforts to alleviate uncertainty in pathway data. Confidence scores have been successfully integrated into other alien-focused, standardised frameworks, such as the Environmental Impact Classification of Alien Taxa (EICAT; Blackburn et al. 2014; Hawkins et al. 2015) and have recently been used in assessing alien introduction pathways in Europe (Pergl et al. 2020).

The compilation of pathway data from multiple countries or regions can also be a source of uncertainty. Data is often unavailable in many countries, due to a lack of adequate monitoring, data collection efforts or funding (Latombe et al. 2017). Compiling data at national or regional levels usually requires a well-established network of contacts and managing these networks can expend a great deal of time and effort (Zenetos et al. 2017). Furthermore, having multiple pathway data sources will result in multiple ways in which the data is formatted, leading to discrepancies between data. Enacting a standardised framework such as the CPC to filter and arrange pathway data will ensure that trends in introductions of alien species are reported accurately. This is crucial if pathways of introduction are to be considered as an accurate indicator for alien species invasions (Wilson et al. 2018). Given that trends in pathway introductions change over time and across regions, the accuracy and standardisation (or lack thereof) of data can greatly benefit or hinder monitoring and biosecurity efforts (McGeoch et al. 2016; Latombe et al. 2019).

Developing indicators from standardised pathway data is necessary for accurate reporting of alien introduction trends. These indicators can then be used to identify the shortcomings in invasive alien species management and policy targets and help improve legislation for dealing with biological invasions (McGeoch et al. 2010; Hulme

2015). Predictive tools such as risk assessments and horizon scanning can incorporate pathway indicators to better estimate the susceptibility of regions to invasion and identify those species that will pose the greatest introduction threat (Hulme 2015; Rabitsch et al. 2016). The continual input of new pathway data will be needed to ensure that indicators remain up to date and to prevent policy decisions relying on historical pathway patterns (Latombe et al. 2019). Given that the Strategic Plan for Biodiversity 2011–2020 is coming to an end, and the 2021–2030 phase is about to begin, the development and testing of pathway indicators for tracking invasive alien species trends becomes increasingly urgent (Rabitsch et al. 2016).

## Conclusion

We propose that the CBD Pathway Categorisation framework is a suitable tool for providing standardised information on alien introduction pathways. This information can then be used to report on pathway trends and their changes across time, taxa, habitats and geographic scales. However, the high number of cases where introduction pathways are unknown will remain a significant challenge to the reporting and documentation of alien introductions (Latombe et al. 2019). Despite this, the CPC framework can enable countries to improve recording and reporting of alien introductions and assist in developing strategies to reduce the impacts of alien introductions beyond the Strategic Plan for Biodiversity 2011–2020.

## Acknowledgements

MAM acknowledges support from the Australian Research Council (DP200101680).

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## Supplementary material 1

**Figure S1. Cumulative introduction events between 1800 and 2017 for a) interconnected waterways and natural dispersal pathway subcategories and b) unknown pathway subcategories**

Authors: Chris M. McGrannachan, Shyama Pagad, Melodie A. McGeoch

Data type: multimedia

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Link: <https://doi.org/10.3897/neobiota.64.60642.suppl1>

## Supplementary material 2

**Appendix S1. Guidelines for the recording of dates of introduction/ first record and conversion of dates to whole numbers**

Authors: Chris M. McGrannachan, Shyama Pagad, Melodie A. McGeoch

Data type: methodology

Explanation note: Gives a more in-depth explanation as to the process of recording introduction and first records for the pathways data.

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Link: <https://doi.org/10.3897/neobiota.64.60642.suppl2>

### Supplementary material 3

**Table S1. List of 101 countries used in the analysis of introduction pathway trends**

Authors: Chris M. McGrannachan, Shyama Pagad, Melodie A. McGeoch

Data type: country list

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Link: <https://doi.org/10.3897/neobiota.64.60642.suppl3>



# Border interceptions of forest insects established in Australia: intercepted invaders travel early and often

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Academic editor: M. Hill | Received 5 November 2020 | Accepted 6 January 2021 | Published 21 January 2021

**Citation:** Nahrung HF, Carnegie AJ (2021) Border interceptions of forest insects established in Australia: intercepted invaders travel early and often. *NeoBiota* 64: 69–86. <https://doi.org/10.3897/neobiota.64.60424>

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## Abstract

Invasive forest insects continue to accumulate in Australia (and worldwide) and cause significant impacts through costs of prevention, eradication and management, and through productivity losses and environmental and biodiversity decline. We used our recent non-native Australian forest insect species inventory to analyse border interception rates (2003–2016) of established species, and link interception frequencies with biological traits, historical establishment patterns, commodities and countries of origin. The strongest predictor of interception frequency was year of establishment. Polyphagous species were more likely to be intercepted, as were more concealed species, although this latter likely reflects the higher interceptions of bostrichid borers and other wood-boring Coleoptera relative to other taxa. Interceptions occurred more often for species native to Asia; in contrast, interceptions from other regions were more likely to be of species invasive there. While interception frequencies did not provide a good overall indicator of contemporaneous species establishments, wood and bark borers were more closely linked for establishments and interceptions. The first fifty forest insect species to establish comprised 85% of all border interceptions of established species between 2003 and 2016, while the most-recent fifty species represented just 6% of interceptions. We suggest that early-establishing species are among the “super-invaders” that continue to move globally, while more recent invasive species may be exploiting new trade pathways, new commodity associations, or changes in dynamics in their countries of origin.

## Keywords

Biosecurity, exotic, nonindigenous species, non-native, quarantine

## Introduction

International trade and travel pose an increasing risk of the movement of non-native species. Forest insect invasions are among the most wide-ranging and high-impact unintended outcomes of this globalised economy (Brockerhoff et al. 2006), causing significant impacts to planted and native forests via costs associated with their prevention, detection (Mayo et al. 2003), eradication (Brockerhoff et al. 2010) and management (Cameron et al. 2018), and severe impacts on forest productivity (Moser et al. 2009), ecosystem functions (Clark et al. 2010), ecosystem services (Boyd et al. 2013) and biodiversity (Liebhold et al. 2017), as well as negatively influencing property prices and trade (Holmes et al. 2009; Aukema et al. 2011; Lovett et al. 2016).

Australia has recorded an average of one new non-native forest insect (those associated with plantation, amenity and native trees, and timber) establishment per year over the last 135 years (Nahrung and Carnegie 2020), with one species (*Sirex noctilio*) costing AUD\$35M in losses and control (Cameron et al. 2018), while another two (*Hylotrupes bajulus* and *Marchalina hellenica*) cost AUD\$45M in eradication/containment since 2003 (Carnegie and Nahrung 2019). There are increased costs associated with post-border detections compared with the prevention of arrival (Epanchin-Niell et al. 2015; Reaser et al. 2020), and hence, it is important to identify high-risk invasion pathways with a view to reducing risk (Byers et al. 2005; McGeoch et al. 2016). Given conflicting reports on the utility of border interceptions to predict invasion risk (e.g. Brockerhoff et al. 2006; Haack 2006; Caley et al. 2014; Lee et al. 2016), the recent initiation of a National Forest Biosecurity Surveillance Strategy in Australia (Department of Agriculture and Water Resources 2018), and the ongoing risk of invasive insects to Australia's forests, we sought to examine potential relationships between border interceptions and established forest and timber insects in Australia. To this end, we used historical and contemporaneous data to identify patterns that may help to understand invasions and potentially reduce future incursions. For example, a better understanding of pathway-commodity-taxa relationships can assist with designing surveillance tools for early detection within areas of high risk (Poland and Rassati 2019).

Biological invasions are generally considered in three distinct phases: arrival, establishment and spread (Liebhold and Tobin 2008). We have previously explored non-native forest insect establishment and spread (Nahrung and Carnegie 2020) and post-border detections and responses to recent incursions of forest insects in Australia (Carnegie and Nahrung 2019); here we add contemporaneous arrival of these established non-native insects to our examination of Australian non-native insect invasion processes. We used our recently compiled database (Nahrung and Carnegie 2020) to examine border interception patterns for recent and historical established insect species in relation to biological traits, invasion history and phylogeny. Interceptions are defined as by ISPM 5 (FAO 2019): the detection of a pest during inspection – in this case at the border. We use our results to identify – at least among those already established – taxa that are more likely to be intercepted, pathways that are likely to be used, and origins that represent higher likelihood of interceptions occurring to inform emerging forest biosecurity arrangements in Australia.

## Materials and methods

Insects of forest-relevance (amenity, plantation and native trees, and timber-in-service pests) that established in Australia over the last 135 years were taken from Nahrung and Carnegie (2020), a database that includes the year of first recorded occurrence, host range, distribution and impact collated from records and literature. The number of interceptions of each insect species was extracted from the Australia-wide Department of Agriculture, Water and Environment (DAWE) border interception database (2003–2016), accessed under a formal data-sharing deed with HFN. These interception data comprise air, sea and mail border detections made during inspection by phytosanitary personnel at ports of entry associated with international cargo, travellers and mail. Available details included country of origin, and commodity-association, which were categorised to geographic region and broad commodity (dried (including woven plant material, dried fruit, seeds, nuts and grains) and fresh plant material (including nursery stock, fresh flowers, fruit and vegetables), wood packaging (pallets, dunnage, and crates) and wood products (logs, timber, furniture and artefacts), non-host commodity (hitch-hiking)). Within these commodity classes, the data were further partitioned as to whether they comprised commercial (cargo) or non-commercial (baggage, mail and personal effects) pathways. The Australian state/territory in which the interception occurred was recorded and included in some analyses.

Descriptive summaries of interception frequencies at Order and Family levels were prepared, as well as by native range and shipment origin. Frequencies were compared using goodness of fit two-way Chi-square tests where required and where sample sizes were high enough to allow comparison. Family-level analyses only considered families for which at least three species were established, or more than ten interceptions were recorded.

Traits previously noted to be important in forest invasions (body size, concealment, host-associated lifestages (Nahrung and Swain 2014) and parthenogenetic reproduction (Niemelä and Mattson 1996)) were determined for each established species from literature. Polyphagy, impact, year of establishment and number of Australian states and global regions where each insect is also invasive were taken from Nahrung and Carnegie (2020) and further used in trait analyses. Non-multidimensional scaling (nMDS) and analysis of similarity (ANOSIM) based on an index of association matrix (Clarke and Gorley 2015) of these traits was used to compare intercepted and non-intercepted species groups, with similarity percentage (SIMPER) analysis subsequently used to identify the traits that contributed the most to group separation (Clarke 1993). The software used for these multivariate analyses was Primer 7 (V 7.0.13, PRIMER-e). Spearman rank correlation was used to examine relationships between the number of interceptions and numerical trait scores. These were further examined using Mann-Whitney U-tests testing comparing trait ranks between binary groups “intercepted” and “not-intercepted”. For intercepted species, geographic origin and commodity associations were also examined. These analyses were performed using IBM SPSS V26.

Finally, to test the hypothesis that interception frequency can be used as a predictor of establishment as a surrogate of propagule pressure (*sensu* Caley et al. 2014;

Eschen et al. 2014), we compared interceptions and establishments over the same period for which our interception data were available (2003–2016).

We acknowledge the limitations of the border interception data including a lack of information on relative inspection rates and import volumes, difficulties in accurately identifying different insect lifestyles and potential differences in inspection rates and methods between jurisdictions. The insects were destroyed as part of usual biosecurity processes.

## Results

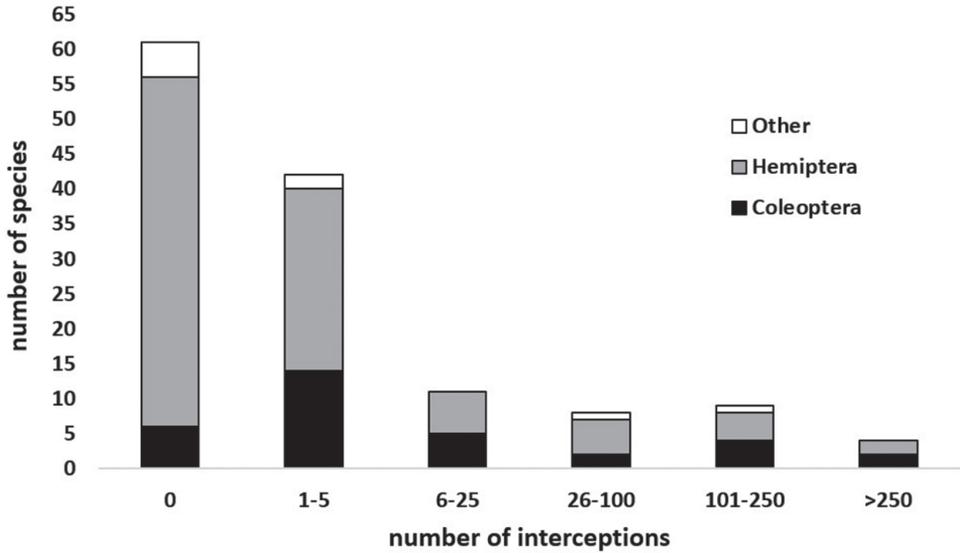
A total of 4,013 interceptions were made of 74 of the 135 forest insect species established in Australia (Suppl. material 1). There were 1,954 interceptions of the established Coleoptera, 1,815 interceptions of established Hemiptera, and 244 of established species in other Orders (Hymenoptera (4), Lepidoptera (179), Thysanoptera (61)). Significantly fewer of the established species that primarily impact forestry (28/70) were intercepted than species that affect other industries as well as forestry (46/65) ( $\chi^2_1 = 12.9$ ,  $P = 0.0003$ ). Most established species were never (41%) or rarely (1–5 times) (35%) intercepted (Figure 1), with significantly more species of established Coleoptera intercepted (27/33) than species of Hemiptera (43/93) ( $\chi^2_1 = 12.5$ ,  $P < 0.001$ ).

For families represented by three or more species, there were no interceptions of any of the three established species in each of the Adelgidae, Cicadellidae and Tenthredinidae (Figure 2, Table 1). The Bostrichidae was the most-intercepted family, with all six established species intercepted – five in at least six Australian states/territories – and an average of 262 interceptions per species (Table 1). In contrast, the Aphididae had high numbers of interceptions representing the lowest proportion of established species, with 77% of established species never intercepted (Table 1). Within the Hemiptera, a significantly higher proportion of scale insects (Diaspididae, Coccidae, Pseudococcidae) were intercepted than aphids (72%) ( $\chi^2_1 = 17.1$ ,  $P < 0.001$ ) (Table 1).

Interception frequencies varied by native range, with higher intercepted: unintercepted ratios for species that originated from Asia-Pacific and South America than for species whose native range was Europe or North America (Figure 3).

Based on the similarity (index of association) of trait scores (body size, concealment, host-associated lifestyles, sexual/asexual or partial asexual reproduction, polyphagy, impact, year established, distribution within Australia and global distribution), ANOSIM showed a significant difference between established species that were intercepted and those that were not intercepted ( $R = 0.17$ ,  $P = 0.001$ ) with nMDS showing a slight separation between groups (Figure 4a) and SIMPER analysis revealing that ‘year established’ contributed 79% of the dissimilarity between groups. Group separation was maintained ( $R = 0.19$ ,  $P = 0.001$ ) when ‘other’ taxa were removed (Figure 4b), with ‘year established’ contributing 80% to dissimilarity between intercepted and non-intercepted taxa.

The number of border interceptions per established species was negatively correlated with their year of establishment ( $\rho = -0.4$ ,  $P < 0.001$ ), with intercepted species hav-

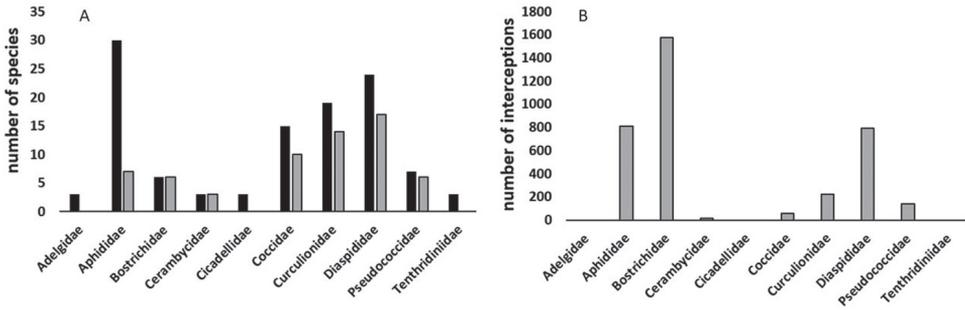


**Figure 1.** Frequency histogram showing the number of times established forest insects were intercepted at the Australian border between 2003 and 2016. Total number of interceptions = 4,013. “Other” orders include species of Lepidoptera (2), Thysanoptera (2) and Hymenoptera (5).

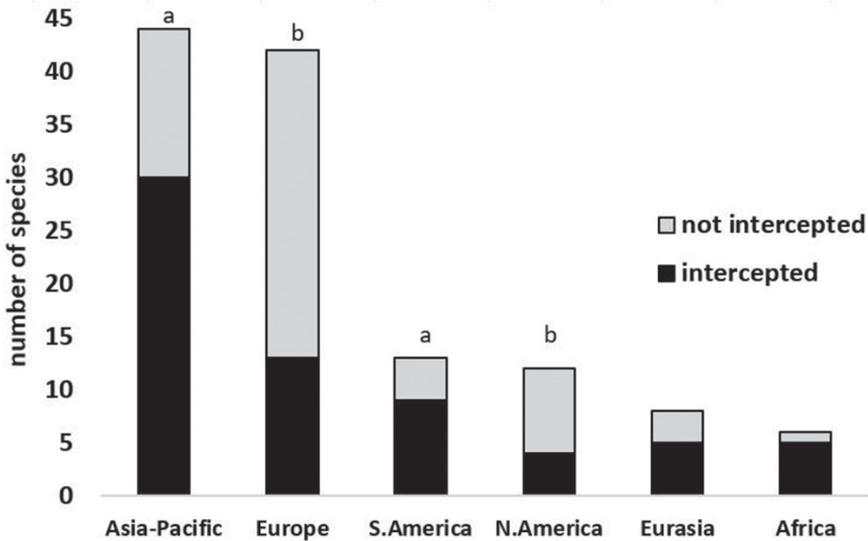
**Table 1.** Number of established species, intercepted species and total number of interceptions (2003–2016) per family for forest insect species established in Australia. Only families with >3 established species or >10 interceptions were tabled. COL=Coleoptera; HEM=Hemiptera; OTH=other orders (Lepidoptera, Thysanoptera).

Order	Family	N species established	N species intercepted (%)	N interceptions	Interceptions/established sp
COL	Anobiidae	1	1	15	15.0
	Bostrichidae	6	6 (100)	1573	262.2
	Cerambycidae	3	3 (100)	16	5.3
	Curculionidae	19	14 (73.6)	224	11.8
	Dynastidae	1	1	55	55.0
	Ptinidae	1	1	68	68.0
HEM	Aphididae	30	7 (23)	813	27.1
	Coccidae	15	10 (66.7)	60	4.0
	Diaspididae	24	17 (70.8)	796	33.2
	Pseudococcidae	7	6 (85.7)	139	19.9
OTH	Noctuidae	1	1	179	179.0
	Thripidae	1	1	61	61.0

ing established in Australia significantly earlier (median establishment year 1926) than those that were not intercepted (median 1952) (Mann-Whitney U-test,  $U = 1387.5$ ,  $P < 0.001$ ) (Figure 5); similarly, a significantly higher proportion of the species that established prior to 1900 was intercepted than for the species that established since the 1940s ( $\chi^2_1 = 0.02-8.5$ ,  $P = 0.004-0.9$ ). The first fifty forest insect species to establish comprised 85% of all border interceptions between 2003 and 2016, while the most-recent fifty species represented just 6%.



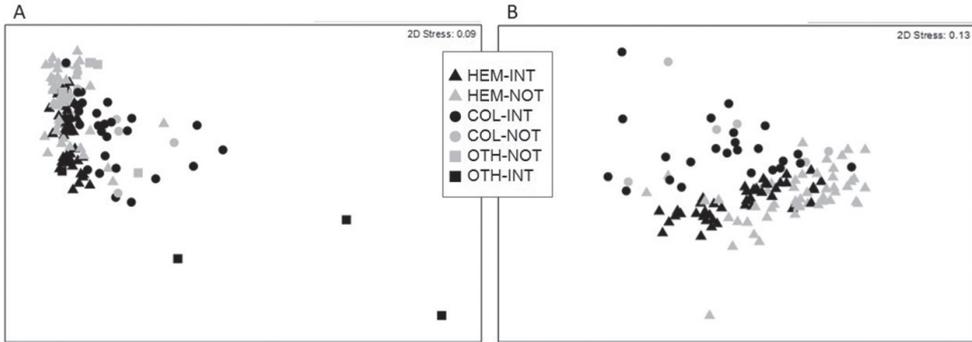
**Figure 2.** Number of established (black) and intercepted (grey) species (A), and number of interceptions (B) between 2003 and 2016 of invasive forest species in families with >3 species established in Australia.



**Figure 3.** Relative number of species intercepted and not intercepted between 2003 and 2016 of forest-related insect species established in Australia according to their native range. Letters above bars designate significant differences between frequencies of intercepted/not intercepted taxa for regions with sufficient data to enable comparison.

As well as interception probability being associated with time since establishment, it was also significantly related to polyphagy (Spearman rank correlation,  $\rho = 0.49$ ,  $P < 0.001$ ), with those species that were intercepted having significantly broader host ranges than those that were not intercepted (Mann-Whitney U-test,  $U = 3394.5$ ,  $P < 0.001$ ). Similarly, insects with a broader geographic distribution within Australia (Spearman rank correlation,  $\rho = 0.49$ ,  $P < 0.001$ ) and globally ( $\rho = 0.37$ ,  $P < 0.001$ ) were more likely to be intercepted than those with smaller distributions.

This relationship with prior distribution may be reflected in the number of interceptions where shipment origin was recorded ( $n = 3,821$ ), where insects detected from North



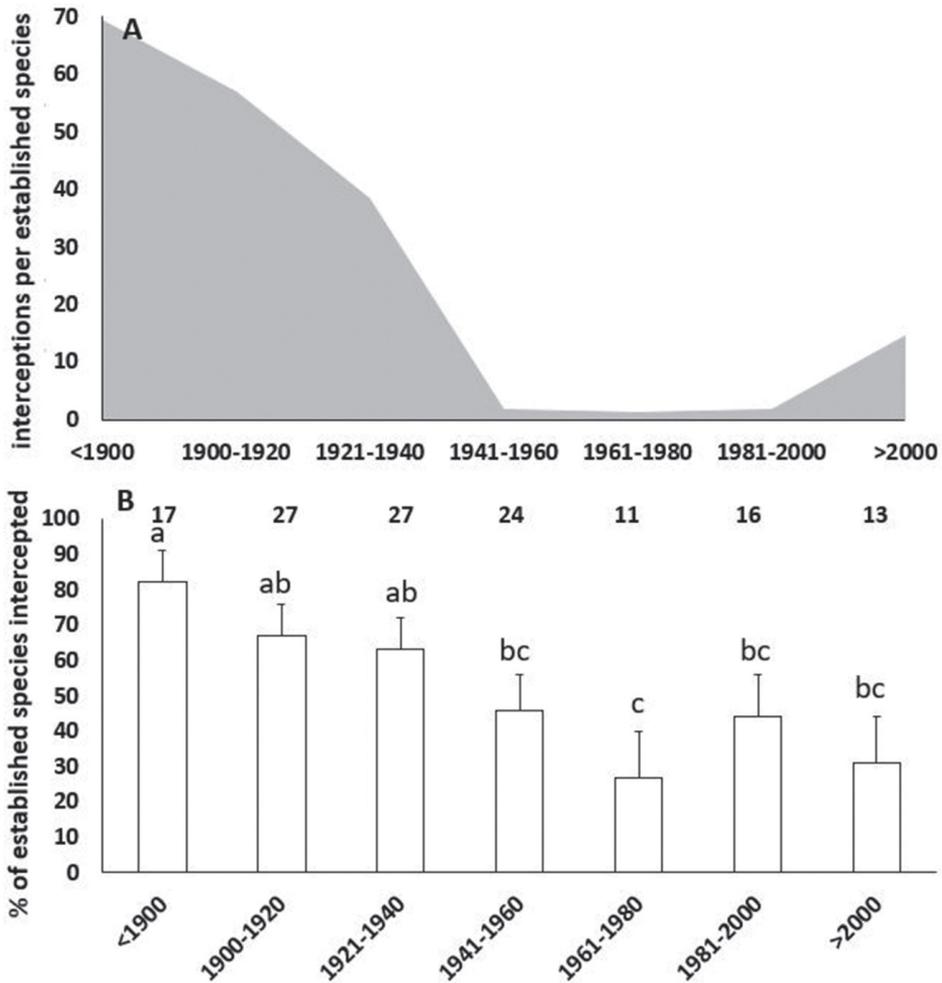
**Figure 4.** nMDS plots based on the index of association of traits of non-native Hemiptera (triangles), Coleoptera (circles) and insects from other orders (squares) **(A)** and Hemiptera and Coleoptera only **(B)** established in Australia and whether they were intercepted (INT) (black) or not intercepted (NOT) (grey) at the border between 2003 and 2016.

**Table 2.** Most frequently intercepted (>100 times between 2003 and 2016) established non-native forest-related insects in Australia. Forest-specific species are marked with an asterisk, with those causing moderate impact marked with two asterisks. N is the number of times each species was intercepted, and year is the first recorded establishment in Australia.

Species	Order	Family	N	Year
<i>Dinoderus minutus</i> **	Coleoptera	Bostrichidae	564	1915
<i>Minthea rugicollis</i> **	Coleoptera	Bostrichidae	529	1924
<i>Macrosiphum euphorbiae</i>	Hemiptera	Aphididae	373	1920
<i>Aonidiella aurantiae</i>	Hemiptera	Diaspididae	365	1896
<i>Aphis gossypii</i>	Hemiptera	Aphididae	222	1902
<i>Pseudaulacaspis pentagona</i>	Hemiptera	Diaspididae	195	1898
<i>Helicoverpa armigera</i>	Lepidoptera	Noctuidae	179	1885
<i>Heterobostrychus aequalis</i> *	Coleoptera	Bostrichidae	179	2013
<i>Lyctus brunneus</i> *	Coleoptera	Bostrichidae	169	1899
<i>Myzus persicae</i>	Hemiptera	Aphididae	161	1903
<i>Naupactus cervinus</i>	Coleoptera	Curculionidae	160	1934
<i>Hemiberlesia lataniae</i>	Hemiptera	Diaspididae	157	1897
<i>Sinoxylon anale</i> **	Coleoptera	Bostrichidae	131	1924

America, Europe and New Zealand were mostly of species that were invasive in those regions (i.e. representing possible bridgehead movement) (Figure 6). However, the highest numbers of intercepted species were in shipments from Asia-Pacific, and most were of species native to that region. The highest proportion of interceptions from Africa and South America were of species that were not recorded as being established in those regions.

In parallel, the more regions from which a species was intercepted, the more interceptions of that species occurred (Spearman rank correlation,  $\rho = 0.71$ ,  $P < 0.001$ ). The most commonly-intercepted species are listed in Table 2, of which five species are primarily forestry pests, with three considered of moderate impact. Primarily forest pests, including high priority pests not yet established in Australia, will be examined further in another study (Nahrung and Carnegie in prep). The median establishment

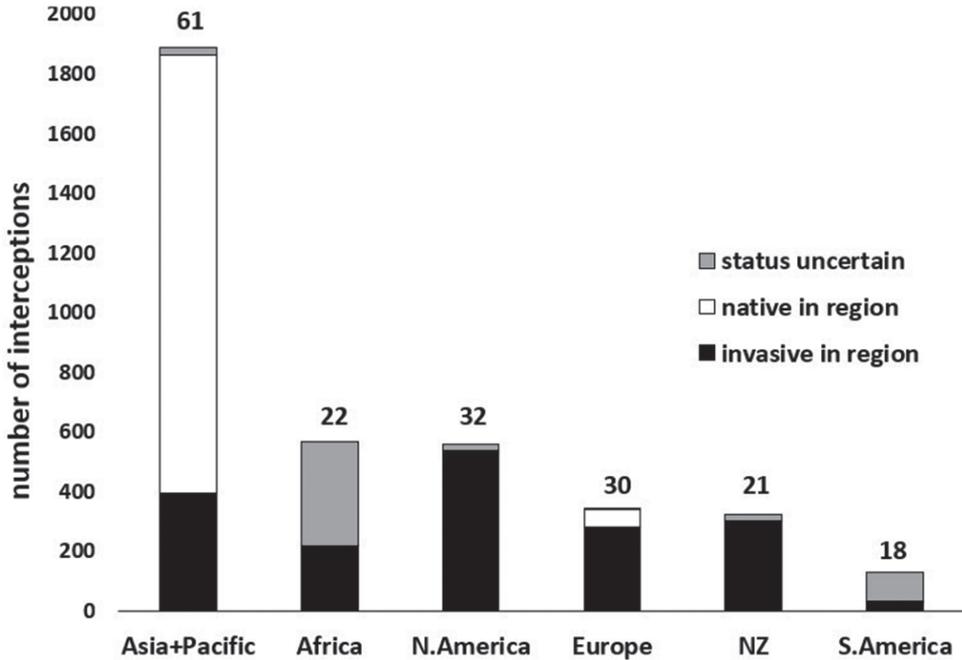


**Figure 5.** Number of border interceptions per non-native forest insect species that established in Australia in 20-year intervals (**A**) and the percentage (+SE) of established species that were intercepted according to when they established (**B**). Number of species that established in each time period above the bars in 5B.

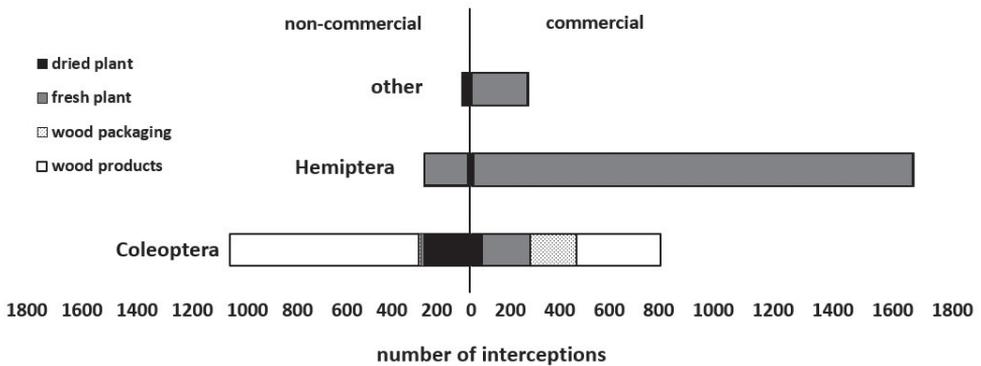
year for the most highly-intercepted species was 1903, compared to 1929 for species intercepted <100 times, and 1952 for non-intercepted species (Table 2).

Of the other biological traits considered, concealed species were more likely to be intercepted (Spearman rank correlation,  $\rho=0.29$ ,  $P=0.001$ ) and species that were more parthenogenetic were less likely to be intercepted ( $\rho = -0.27$ ,  $P = 0.002$ ); these patterns likely reflect the very high interceptions of wood-borers (concealed, sexual) and the under-representation of intercepted aphids (free-living, parthenogenetic) among established taxa.

There were very strong commodity associations between taxa, with Hemiptera almost completely (98%) associated with fresh plant material (e.g. nursery stock, fruit,



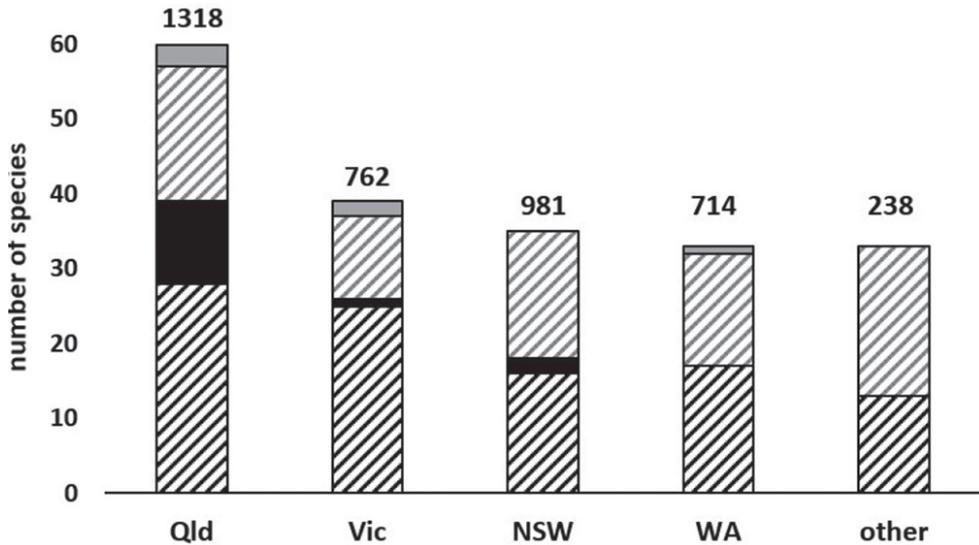
**Figure 6.** Number of interceptions of established forest insects in Australia from different regions, and the status of the species intercepted in that region (see Nahrung and Carnegie 2020). Numbers above bars indicate the total number of species intercepted from that source region.



**Figure 7.** Number of interceptions of established forest species of Hemiptera, Coleoptera and other orders (Hymenoptera, Lepidoptera, Thysanoptera) on different commodities on non-commercial (baggage, mail, personal effects) and commercial (cargo) pathways between 2003 and 2016.

foliage) and Coleoptera largely (64%) associated with wood (e.g. packaging, timber, furniture, and artefacts) (Figure 7).

About 90% of interceptions of Hemiptera were made in commercial cargo, in contrast to Coleoptera where 60% of interceptions were associated with non-



**Figure 8.** Number of established species of Hemiptera (black) and Coleoptera (grey) intercepted in Queensland (Qld), Victoria (Vic), New South Wales (NSW), Western Australia (WA), South Australia (SA), Northern Territory (NT), and Tasmania (Tas), with unique species in solid colour. Total number of interceptions per state is above the bars.

commercial pathways (baggage, mail, personal effects) ( $\chi^2_1 = 988$ ,  $P < 0.001$ ); this is again likely a reflection of the high interception rate of bostrichid borers. Only about 5% of interceptions were made on non-host commodities (ie hitch-hikers).

Within Australia, one-third of all border interceptions of established species was made in Queensland. Overall, 59% of established species were intercepted at the border of the first state that they were recorded as established in, with ten species intercepted in at least six states/territories, and twenty species intercepted in only one state. Queensland had the highest number of interceptions, the highest number of species intercepted, and the highest number of unique interceptions (Figure 8).

Four of the eleven species that established during the interception data collection period (2003 to 2016) were intercepted in that timeframe, three of which were Coleoptera. Only one species was intercepted more than three times – and its establishment date is dubious (see discussion). Of the other three species, only two interceptions were made in the period prior to their discovery in Australia, such that only one interception of one of the four moderate-high impact pest species was made prior to their establishment (Table 3). Two-thirds (126) of these interceptions were made in commercial cargo.

## Discussion

Just over half (55%) of the non-native forest and timber insects established in Australia since 1885 were intercepted at the border between 2003 and 2016, with one-third of

**Table 3.** Non-native forest insects established in Australia 2003–2016 and number of border interceptions (N) of each in this timeframe and prior to establishment in parentheses. Those causing moderate impact are marked with one asterisk, those with high impact with two.

Species	Order	Family	N	Year
<i>Nematus oligospilus</i> *	Hymenoptera	Tenthredinidae	0	2003
<i>Psyllopsis fraxinicola</i>	Hemiptera	Psyllidae	0	2003
<i>Hylotrupes bajulus</i> **	Coleoptera	Cerambycidae	2 (1)	2004
<i>Corythucha ciliata</i> *	Hemiptera	Tingidae	3 (0)	2006
<i>Cinara pilicornis</i>	Hemiptera	Aphididae	0	2008
<i>Tuberolachmus salignus</i>	Hemiptera	Aphididae	0	2010
<i>Chaitophorus leucomelas</i>	Hemiptera	Aphididae	0	2011
<i>Xylosandrus crassiusculus</i>	Coleoptera	Curculionidae	2 (1)	2011
<i>Heterobostrychus aequalis</i>	Coleoptera	Bostrichidae	179 (157)	2013
<i>Shivaphis celti</i>	Hemiptera	Aphididae	0	2013
<i>Marchalina hellenica</i> **	Hemiptera	Margarodidae	0	2014

contemporaneous establishments being intercepted in the same period. In contrast to the USA (McCullough et al. 2006), significantly more Coleoptera were intercepted than Hemiptera, although more Hemiptera were established. Bostrichid borers were the most highly intercepted family both here and in Wylie and Yule (1977)'s Australian study, and are likewise over-represented in interceptions globally (Turner et al. in review). This is reflected in our trait analyses, which indicated that concealed species were more likely to be intercepted than free-living species. Sessile concealed taxa such as wood borers are protected from desiccation and extreme temperatures and may be more likely to survive transportation (Sopow et al 2015). Frass and holes left by wood borers may provide visual cues that increase detectability that mobile insect lifestages do not, although not all borers do this (e.g. siricid wasps (Burnip et al. 2010)). Alternatively, the over-representation of concealed species in interceptions could reflect the importance of wood borers as quarantine pests (Lawson et al. 2018) and that wood products and packaging are high-risk commodities that may attract added scrutiny (Kenis et al. 2007).

Brockerhoff et al. (2006, 2014) and Haack (2006) described positive relationships between interceptions (propagule pressure) and establishments among bark and wood borers, and indeed, 88% of wood and bark borers historically established in Australia, and all three that established in our data timeframe were intercepted. Cerambycid borers comprised one-third of species in common between establishments and interceptions in Europe (Eschen et al. 2015), while Turner et al. (2020) described the Cerambycidae as having a small per arrival establishment probability relative to interception probability (and, similar to our results, that aphids had lower ratio of interception probability to establishment probability). Caley et al. (2014) also found higher interception rates of established Coleoptera in Australia, so it appears that interception rates may be more reflective of establishments for beetles (or that they are simply more detectable) – particularly wood and bark borers – compared with other taxa.

Overall, however, like Caley et al. (2014) we found that border interceptions did not provide a good predictor of incursion risk in Australia, at least during the time frames studied. Both studies also identified a similar pattern of interceptions with

historically established species, which Caley et al. (2014) attributed as a proxy of propagule pressure. We further consider this pattern as evidence for a suite of ‘super-invaders’ *sensu* Turner et al. (in review): species that are almost ubiquitous in global pathways with an invasive status among several world regions. Thus, although commonly used as a predictor for invasions and a proxy for propagule pressure, it may be that higher interception rates are more reflective of invasion success, than a predictor of it, at least among these species. For example, the top 5 of the 74 species intercepted here accounted for over half of all interceptions, are all invasive elsewhere (in an average of 4.4 other world regions), and established in Australia prior to 1924. Although biosecurity practices were less stringent in that timeframe with unregulated movement of live plants (the Australian federal government introduced its first Quarantine Act in 1908 (Maxwell et al. 2014)), trade and travel were also markedly lower, less diverse, and restricted to movement by sea. Over 80% of the species that could only have arrived by sea were still travelling that way between 2003 and 2016. Nahrung and Carnegie (2020) found that earlier-establishing species had broader global non-native distributions, further corroborating the notion that intercepted species have travelled ‘early and often’, leading to a self-accelerating process in which invasion begets invasion (Bertelsmeier and Keller 2018).

Polyphagy was also a correlate of interception frequency in our study, with insect species with a broader host range intercepted more often than those with a narrow host range – presumably a direct relationship with the more commodities on which a species feeds, the more pathways available and the more likely to be intercepted. While earlier-establishing species were more polyphagous than later-establishing species (Nahrung and Carnegie 2020), we found very strong relationships with establishment time and interception likelihood – year of establishment was the strongest contributor to group separation.

A notable exception to the patterns we found for interception frequency and establishment date and invasive distribution within Australia was *Heterobostrychus aequalis*, the lesser auger beetle, whose establishment status in Australia has been controversial, with several sources citing it as present in Australia prior to our listed establishment date of 2013 (see Wylie and Peters 2016); we therefore submit that it was in fact, elusive, rather than absent and likely established earlier. *Lyctus discedens* was unusual in its low interception rate, early establishment, and non-invasiveness in other global regions. It was also the only species established prior to 1900 that was not intercepted by sea between 2006 and 2013: we recommend its taxonomy be reviewed as its status is unclear (see Borowski 2020; R. Wylie pers. comm.).

As expected, live plants and wood products were responsible for the vast majority of interceptions, hosting mostly Hemiptera and Coleoptera, respectively, with both recognised major pathways for forest insect invasions (Liebhold et al. 2012; Lovett et al. 2016; Lawson et al. 2018; Meurisse et al. 2019) and subject to strict regulations regarding importation to Australia (Department of Agriculture and Water Resources 2015). Coleoptera were more likely to be associated with non-commercial pathways (baggage, mail and personal effects) than Hemiptera. This may reflect Australia’s strict biosecurity

messaging to travellers regarding carrying fresh plant products, and a relatively lower public awareness of potential risks posed by unprocessed wooden materials.

Interceptions from Asia-Pacific accounted for over half of all interceptions of our established forest taxa and represented the highest proportion of regional native species. Wylie and Yule (1977) and Lawson et al. (2018) also reported higher numbers of border interceptions in goods originating from Asia. The number and taxonomic composition of established forest insects is similar between those originating from Europe and Asia (Nahrung and Carnegie 2020) but this similarity was not reflected in interceptions, with significantly more native Asian species intercepted than native European species. This is likely a reflection of higher trade volumes and smaller geographic distance with Asia, as found for ant invasions in Australia (Suhr et al. 2019). Further, most interceptions from all countries but Asia were apparently invasive to those regions – representing so-called bridgehead movement, increasingly recognised as a conduit to invasions globally (Bertelsmeier and Keller 2018). The patterns observed in Nahrung and Carnegie (2020) for higher establishments of Asian-origin species in northern Australia is perhaps also reflected in their interceptions, with 90% of intercepted Asian-Pacific species recorded at the Queensland border, compared with 57% or less in the other states. As trade diversifies in commodities among world regions, and as exotic plant species are planted in new regions, opportunities for new pathway associations and new arrivals arise (Brockerhoff and Liebhold 2017; Lantschner et al. 2020) – this may in part explain the 14-fold difference in numbers of interceptions between the first fifty species established and the most recent fifty species established. The lower frequency of recently-established species in interception pathways compared to long-established species could reflect a number, or a combination, of situations. It may reflect the reality that some pests arrive through non-commercial pathways (e.g. Paine et al. 2010; Essl et al. 2015), or that pathways considered ‘lower risk’ may attract less attention due to a risk-return principle (e.g. Kenis et al. 2007), or represent inspection ‘gaps’ (Bacon et al. 2012).

## Conclusion

This study concentrated on species that are already established in Australia. A separate study will consider interceptions across an expanded range of species, and include the high priority pests of forest significance not yet established in Australia (Nahrung and Carnegie in prep.). However, here we have demonstrated clear relationships with interception frequency and time since establishment, polyphagy and invasiveness in other regions that provide further evidence for the notion of ‘super-invaders’ that established early and continue to be moved in international trade and travel, as well as the over-representation of Bostrichidae in interceptions and establishments (Turner et al. in review). Our results may be used to revise stakeholder engagement strategies, consider the role of emerging pathways in risk assessments, and to support ‘over-the-horizon’ surveillance and biosecurity networks in neighbouring regions.

## Acknowledgements

We thank staff at the Department of Agriculture, Water and the Environment, particularly Brendon Reading, for access to border interception data and Chris Howard and Matthew Gordon for critical comments on the manuscript. We also thank Rebecca Turner (Scion), Francisco Tovar (PHA) and Andy Howe (USC) for additional comments on the manuscript. HFN was in receipt of an Advance Queensland Industry Fellowship through the Queensland Department of Innovation and Tourism Industry Development, supported by the University of the Sunshine Coast, Department of Agriculture and Fisheries, National Sirex Coordination Committee, Forest and Wood Products Australia, Plant Health Australia and HQPlantations Pty Ltd. AJC acknowledges support from Forestry Corporation of NSW.

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## Supplementary material I

### **Non-native insect species established in Australia, traits and interceptions**

Authors: Helen F. Nahrung, Angus J. Carnegie

Data type: interceptions and traits of established forest species.

Explanation note: The supplementary data file contains the list of established non-native forest insects in Australia, their taxonomic placement, traits used in analyses and number of border interceptions.

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Link: <https://doi.org/10.3897/neobiota.64.60424.suppl1>

# Urban host plant utilisation by the invasive *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) in northern Utah

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Academic editor: D. Pureswaran | Received 27 October 2020 | Accepted 13 January 2021 | Published 28 January 2021

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**Citation:** Holthouse MC, Spears LR, Alston DG (2021) Urban host plant utilisation by the invasive *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) in northern Utah. NeoBiota 64: 87–101. <https://doi.org/10.3897/neobiota.64.60050>

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## Abstract

The invasive and highly polyphagous brown marmorated stink bug, *Halyomorpha halys* (Stål), is a severe agricultural and urban nuisance pest in North America. Since its initial invasion into Utah in 2012, *H. halys* has become well established in urban and suburban locations along the western foothills of the Wasatch Front in northern Utah. Bordering the Great Basin Desert, this area is unique from other North American locations with *H. halys* due to its high elevation (> 1200 m), aridity (30-year mean RH = 53.1%; dew point = -1.9 °C) and extreme temperatures (the 30-year mean minimum and maximum in January and July in Salt Lake City range from -3.1 to 3.6 °C and 20.3 to 32.4 °C, respectively). To document which plant species harbour *H. halys*, surveys were conducted in 17 urban/suburban sites in four counties during 2017 and 2018. *Halyomorpha halys* was more abundant in Salt Lake and Utah counties than in the more northern counties of Davis and Weber and was found on 53 plant species, nine of which hosted two or more developmental stages in both years. The majority of hosts were in the families Fabaceae, Rosaceae and Sapindaceae. Northern catalpa, *Catalpa speciosa* (Warder), was the most consistent host, supporting a majority of *H. halys* detections in all life stages; thus we identify it as a sentinel host. Twenty-nine species were novel hosts for *H. halys* in North America; of these, *Acer ginnala* Maxim, *Populus tremuloides* Michx., *Prunus armeniaca* X *domestica* ‘Flavor King’ and *Prunus virginiana* ‘Schubert’ were detected with two or more life stages of *H. halys* in both years. Peak populations of *H. halys* occurred from mid-June to mid-September. We describe *H. halys* plant utilisation by life stage and seasonal period to aid future detection and management of this invasive insect in the greater Intermountain West region.

## Keywords

Brown marmorated stink bug, *Catalpa speciosa*, host plant, Intermountain West, sentinel host, survey

## Introduction

Native to Asia, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) has become an urban nuisance and severe agricultural pest in many parts of the world (Garipey et al. 2014; Haye et al. 2015; Hoebeke and Carter 2003; Leskey et al. 2012; Macavei et al. 2015; Maistrello et al. 2016). In North America, *H. halys* has been detected in 46 U.S. States and four Canadian Provinces and observed on over 170 plant species, including a wide variety of ornamental trees, woody shrubs, vegetables, row crops and speciality fruit crops (StopBMSB.org, Haye et al. 2015). Research shows that optimal development is achieved when *H. halys* has access to multiple host species, especially those with both foliage and reproductive structures present (Acebes-Doria et al. 2016). Documentation of preferred *H. halys* hosts and plant communities is critical for studying its dispersal into novel geographic regions, such as Utah. Plant surveys for *H. halys* and other polyphagous invasive species have documented ornamental hosts and unmanaged wooded areas near suburban regions as critical for initial population establishment (Bakken et al. 2015; Branco et al. 2019). Urban and suburban areas also offer overwintering sites for *H. halys* in human-made structures, especially in areas with low winter temperatures, heavy snow accumulation and few natural overwintering sites (e.g. dead tree stands) (Lee et al. 2014).

In northern Utah, surveys in 2017 and 2018 were initiated to document plant species harbouring *H. halys* egg mass, nymph and adult life stages and their seasonal occurrence. Surveys were conducted along the urbanised western foothills of the Wasatch Front, which is considered part of the greater Rocky Mountain Range and stretches 258 km south from the Idaho border to central Utah. Approximately 80% of Utah's human population lives within 25 km of the Wasatch Range, creating a band of urban and suburban sprawl between the western mountain foothills and the eastern edge of the Great Basin Desert where much of Utah's vegetable and fruit crop production occurs (data.census.gov, nass.usda.gov). Established urban populations of *H. halys* in northern Utah present risks to speciality and field crops. For example, early season feeding by *H. halys* on tart cherry (*Prunus cerasus* 'Montmorency') fruit can invoke substantial abscission and yield loss (Schumm et al. 2020), while injury to a wide variety of vegetable and small fruit crops in urban farms reduced product quality and yields (Z. Schumm, personal communication).

The high elevation (> 1200 m), aridity (30-year mean RH = 53.1%; dew point = -1.9 °C) and extreme seasonal temperature fluctuations of northern Utah (the 30-year mean minimum and maximum in January and July in Salt Lake City range from -3.1 to 3.6 °C and 20.3 to 32.4 °C, respectively) (ncdc.noaa.gov, climate.usu.edu, worldclimate.com) present a novel environmental setting for *H. halys*. Many other regions of the world with established *H. halys* populations, especially those in North America, include more humid and lower elevation habitats (Bariselli et al. 2016; Faúndez and Rider 2017; Garipey et al. 2014; Rice et al. 2014). Plant surveys in northern Utah will provide insights into *H. halys* invasion of other inter-mountainous regions, including identification of novel plant hosts. Documentation of primary, or sentinel, host plant

species (those that support two or more life stages of *H. halys*) can aid in supporting further research and development of targeted management practices for *H. halys* (Mansfield et al. 2019).

## Materials and methods

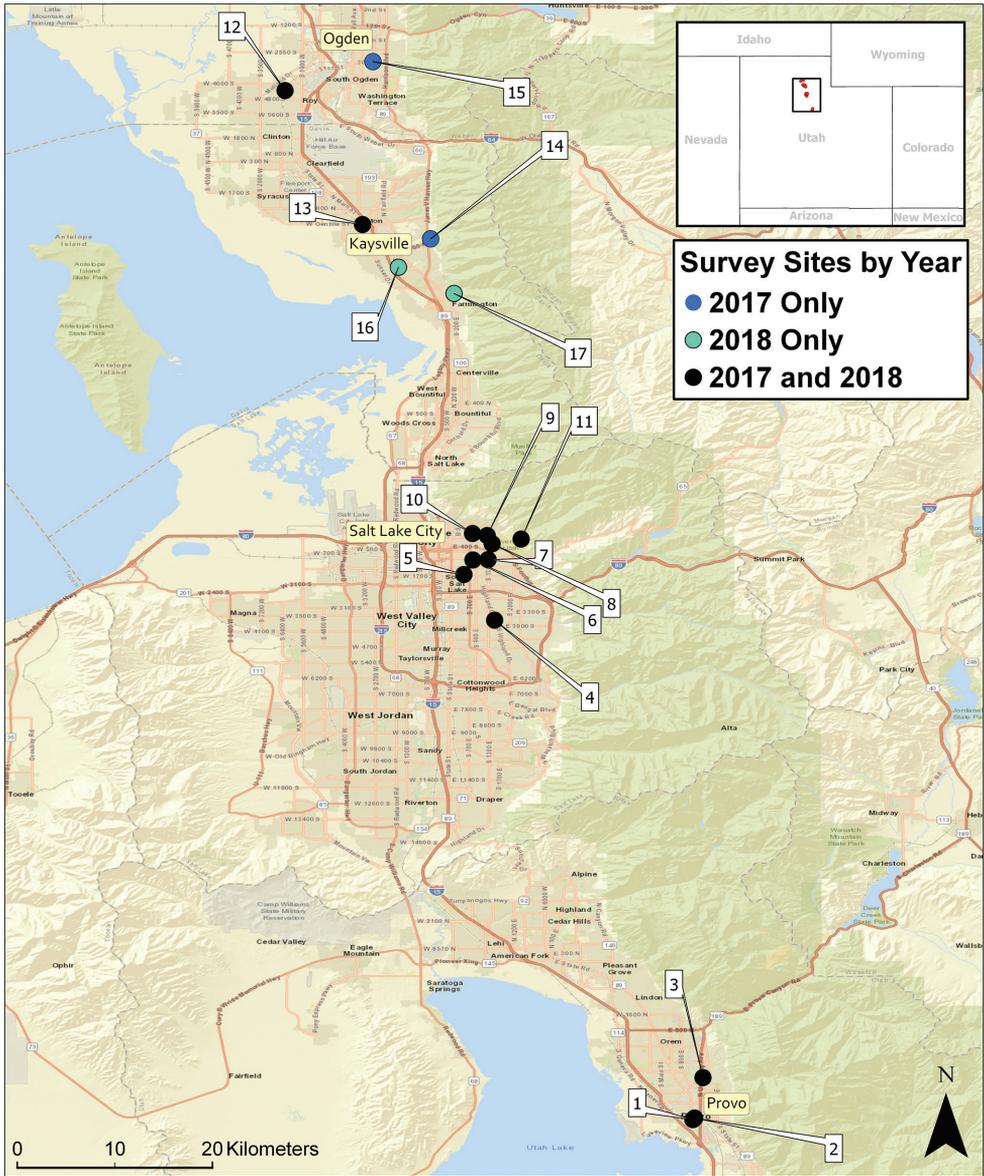
A total of 17 urban and suburban host plant survey sites were selected in 2017 and 2018, based on previous positive *H. halys* collections in Davis, Salt Lake, Utah and Weber Counties (Fig. 1). Two sites were replaced in 2018 due to lack of stink bug detections in 2017 for a total of 15 sites per year. Sites were sampled bi-weekly from 16 May to 24 August 2017 and from 8 May to 22 August 2018. In both years, a subset of six sites was surveyed until the last week of September to provide later seasonal data for sites with higher *H. halys* populations. In 2017, these six sites were numbers 3, 5, 7, 8, 9 and 10; in 2018, they were 3, 4, 7, 9, 10 and 13 (Fig. 1).

At each site, a line sampling transect, 200 m long by 40 m wide, was established. Twenty of the total available plants within each transect were randomly selected regardless of species and surveyed by one or two observers using visual inspection (e.g. underside of leaves, limbs and tree trunks) and beating sheets (BioQuip Products Inc., Rancho Dominguez, CA) for 3 min (Bakken et al. 2015). Pole pruners and a stepladder (3 m standing height) were used to examine 3–5 m height of tree canopies. For small-sized plants where all foliage could be fully inspected in less than 3 min, observers moved to the next plant upon completion. When *H. halys* was detected, plants were inspected for an additional 7 min to estimate densities of each life stage observed (egg, nymph and adult) for a total observation time of 10 min. Mean *H. halys* counts per plant species and year were calculated to provide a relevant comparison of host plant preference; however, as plant species were not equally represented in transects, the mean number of *H. halys* per visual sample is provided (Table 1). Means were not compared statistically.

Each plant surveyed within a site was assigned a unique serial number and re-sampled on bi-weekly visits, providing insights into seasonal phenology of *H. halys* on the representative plant species. Each surveyed plant was tracked with the mapping application Collector by Esri and data were transferred into ArcGIS Online and ArcGIS Pro for management and visualisation (Esri, Redlands, CA). Plant identifications were confirmed by the Utah State University Intermountain Herbarium (UTC), where voucher specimens of each species are archived.

## Results

A total of 53 plant species from 17 families were observed with one or more *H. halys* life stages present between May and September of 2017 and 2018 (Table 1). Of these, 29 are novel hosts according to the national StopBMSB.org plant species repository.

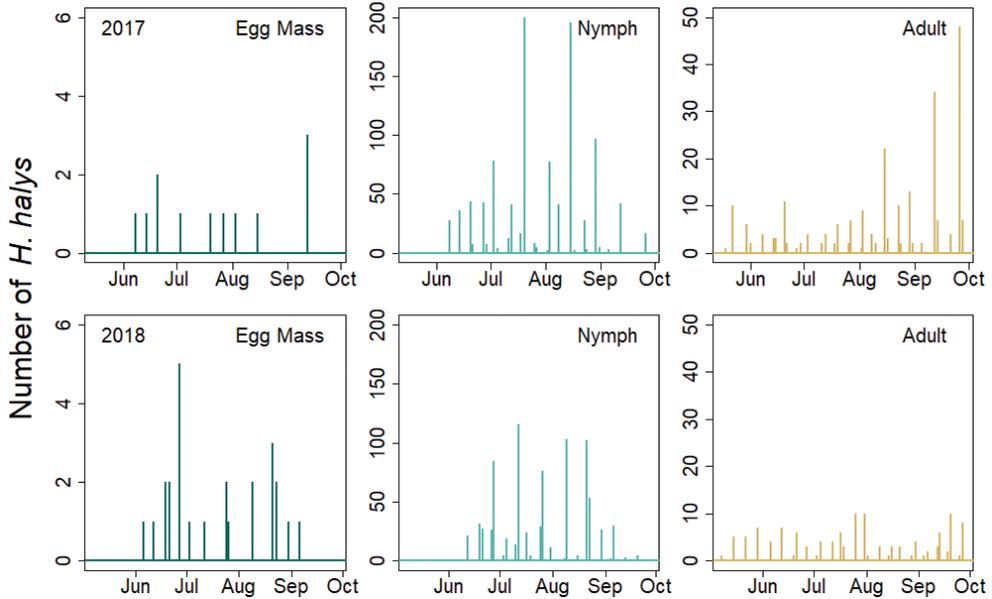


**Figure 1.** Map of 17 host plant survey sites in northern Utah, 2017 and 2018. Black dots represent sites that were visited in both years, blue dots represent sites visited only in 2017 and turquoise dots represent sites visited only in 2018. Geographical coordinates are as follows: Site 1: 40°13'44.7"N, 111°39'56.2"W; Site 2: 40°13'49.9"N, 111°39'50.6"W; Site 3: 40°16'05.2"N, 111°39'22.6"W; Site 4: 40°41'33.6"N, 111°50'53.8"W; Site 5: 40°44'06.4"N, 111°52'35.9"W; Site 6: 40°44'55.0"N, 111°52'05.8"W; Site 7: 40°44'56.1"N, 111°51'15.8"W; Site 8: 40°45'49.1"N, 111°51'02.1"W; Site 9: 40°46'16.5"N, 111°51'18.6"W; Site 10: 40°46'23.4"N, 111°52'07.1"W; Site 11: 40°46'04.8"N, 111°49'25.8"W; Site 12: 41°11'03.7"N, 112°02'29.2"W; Site 13: 41°03'35.9"N, 111°58'12.3"W; Site 14: 41°02'48.1"N, 111°54'26.2"W; Site 15: 41°12'40.3"N, 111°57'37.8"W; Site 16: 41°01'13.0"N, 111°56'13.1"W; and Site 17: 40°59'45.5"N, 111°53'08.5"W.

**Table 1.** Mean number of *H. halys* egg masses (E), nymphs (N) and adults (A) present per sample\* of plant species during surveys in northern Utah, 2017 and 2018. Plant species in bold were documented with two or more *H. halys* life stages in 2017 and 2018. The number of times each plant species was surveyed (no. of unique specimens × no. of visits) is found in the column labelled (n). The NS term indicates no surveys were conducted on the indicated plant and year.

Family name	Scientific name	Year							
		2017				2018			
		(n)	E	N	A	(n)	E	N	A
Apocynaceae	<i>Vinca major</i> <sup>‡</sup>	40	–	0.05	–	37	–	–	–
Araliaceae	<i>Hedera helix</i> <sup>‡</sup>	35	–	–	0.08	32	–	0.16	–
Berberidaceae	<i>Mahonia repens</i> <sup>‡</sup>	18	–	–	0.06	29	–	–	–
Bignoniaceae	<i>Campsis radicans</i> <sup>‡</sup>	10	–	–	0.10	8	–	0.13	0.13
	<b><i>Catalpa speciosa</i></b>	284	0.05	8.81	2.70	288	0.11	7.22	0.78
Caprifoliaceae	<i>Lonicera maackii</i> <sup>‡</sup>	10	–	0.20	–	10	–	0.10	–
Cornaceae	<i>Cornus alba</i> ‘Elegantissima’ <sup>†</sup>	10	–	0.10	0.10	10	–	–	–
Cupressaceae	<i>Thuja plicata</i> <sup>‡</sup>	11	–	–	–	11	–	0.18	0.09
Fabaceae	<i>Caragana arborescens</i>	9	–	0.11	0.11	8	–	–	0.38
	<b><i>Cercis canadensis</i></b>	26	0.04	0.81	–	27	–	0.07	0.15
	<i>Gleditsia triacanthos</i>	10	–	0.10	–	10	–	0.10	–
	<i>Gleditsia triacanthos</i> var. <i>inermis</i>	24	–	–	–	30	–	–	0.03
	<b><i>Robinia pseudoacacia</i></b>	36	–	0.03	0.08	47	–	0.02	0.02
	<i>Robinia pseudoacacia</i> ‘Purple Robe’	10	–	–	0.10	11	–	–	–
	<i>Fagus sylvatica</i> ‘Purpurea Tricolor’ <sup>†</sup>	30	0.03	0.03	0.20	30	–	–	–
Fagaceae	<i>Quercus macrocarpa</i> ‘Urban Pinnacle’ <sup>†</sup>	17	–	–	–	11	–	–	0.05
	<i>Tilia cordata</i> <sup>‡</sup>	8	–	0.13	–	8	–	–	–
Malvaceae									
Oleaceae	<i>Forsythia X intermedia</i> ‘Lynwood Gold’ <sup>†</sup>	NS	NS	NS	NS	8	–	0.06	–
	<i>Fracinus pennsylvanica</i>	39	–	0.03	0.05	32	–	–	0.03
	<i>Ligustrum vulgare</i> <sup>‡</sup>	29	–	0.10	–	22	–	–	–
	<i>Syringa vulgaris</i> <sup>‡</sup>	62	–	0.10	0.10	62	–	–	0.05
Rosaceae	<i>Amelanchier X grandiflora</i> <sup>†</sup>	18	–	–	–	16	–	0.06	–
	<i>Crataegus mollis</i> <sup>†</sup>	11	–	–	0.09	11	–	–	–
	<i>Crataegus monogyna</i>	8	–	0.13	0.13	8	–	–	–
	<b><i>Malus domestica</i></b>	55	–	0.35	0.47	53	–	0.25	0.89
	<i>Malus floribunda</i> <sup>‡</sup>	8	–	0.13	0.75	8	–	–	0.13
	<i>Malus</i> ‘Prairiefire’ <sup>†</sup>	8	–	–	0.63	8	–	–	0.13
	<i>Malus sylvestris</i> <sup>‡</sup>	18	–	0.06	–	16	–	–	–
	<b><i>Prunus armeniaca X domestica</i> ‘Flavor King’<sup>†</sup></b>	28	–	0.21	0.14	22	–	0.05	0.18
	<i>Prunus avium</i>	35	–	0.03	0.03	29	–	0.10	–
	<i>Prunus cerasifera</i>	9	–	–	–	8	–	0.13	–
	<i>Prunus cerasifera</i> ‘Nigra’ <sup>‡</sup>	73	–	0.04	0.08	76	–	–	0.03
	<i>Prunus domestica</i>	11	–	–	0.55	21	–	0.71	0.05
	<i>Prunus persica</i>	51	–	–	–	35	–	0.11	0.17
	<b><i>Prunus virginiana</i> ‘Schubert’<sup>†</sup></b>	61	0.02	0.30	0.30	54	0.06	0.31	0.28
	<i>Pyrus calleryana</i>	24	–	–	–	30	–	0.03	–
	<i>Rosa acicularis</i> <sup>‡</sup>	89	–	0.03	0.03	109	–	–	–
	<i>Spiraea japonica</i> ‘Goldflame’ <sup>†</sup>	10	–	0.30	–	10	–	0.20	–
Salicaceae	<i>Populus angustifolia</i> <sup>‡</sup>	NS	NS	NS	NS	8	–	0.25	–
	<b><i>Populus tremuloides</i><sup>‡</sup></b>	49	–	0.12	0.53	39	–	0.11	0.23
	<i>Salix purpurea</i> <sup>‡</sup>	10	–	0.20	–	10	–	–	–
Sapindaceae	<i>Acer freemanii</i>	10	–	0.10	–	10	–	–	–
	<b><i>Acer ginnala</i><sup>‡</sup></b>	9	–	2.78	1.78	8	–	0.25	0.13
	<i>Acer grandidentatum</i> <sup>‡</sup>	14	0.06	–	–	16	–	–	–
	<i>Acer negundo</i>	78	–	–	0.03	112	–	0.02	0.06
	<i>Acer nigrum</i> <sup>‡</sup>	8	–	0.25	0.13	NS	NS	NS	NS
	<i>Acer palmatum</i> ‘Fireglow’	18	–	–	0.06	18	–	0.06	–
	<b><i>Acer platanoides</i></b>	127	–	0.26	0.06	118	–	0.03	0.04
	<i>Acer platanoides</i> ‘Crimson King’ <sup>‡</sup>	17	–	0.04	0.35	26	–	–	–
	<i>Acer rubrum</i>	8	–	–	–	8	–	0.13	–
	Scrophulariaceae	<i>Buddleia davidii</i>	25	–	0.04	0.12	24	–	–
Ulmaceae	<i>Ulmus pumila</i> <sup>‡</sup>	70	–	–	0.06	80	–	–	0.05
Vitaceae	<i>Vitis Vinifera</i>	28	–	–	–	28	–	0.04	–

\*All plants were sampled for a minimum of 3 min; plants with one or more *H. halys* life stages were sampled for an additional 7 min, a total of 10 min. †Novel host plant species for *H. halys* documented in Utah (as compared to current North American literature).



**Figure 2.** Total number of *H. halys* per life stage observed during plant surveys in northern Utah from May through to September, 2017 (top row) and 2018 (bottom row). Tick marks on the x-axis represent the beginning of a month. Note the unique y-axis scales for each life stage.

Seven plant species were documented with two *H. halys* life stages present across both years: *Acer ginnala* Maxim., *Acer platanoides* L., *Cercis canadensis* L., *Malus domestica* Borkh., *Populus tremuloides* Michx., *Prunus armeniaca* X *domestica* ‘Flavor King’ and *Robinia pseudoacacia* L. Two species, *Catalpa speciosa* (Warder) and *Prunus virginiana* ‘Schubert’, had all three *H. halys* life stages present in both years. The majority of *H. halys* observed were found on *C. speciosa*, comprising 91% of all *H. halys* detected in this study. Plant species without observations of *H. halys* are listed in Table 2. Additional plant species with *H. halys* detections in northern Utah, observed external to these surveys, are listed in Table 3.

*Halyomorpha halys* egg masses were detected in low numbers (< 40 masses) in both survey years (Table 1), with detections beginning the first week of June and continuing into early September (Fig. 2). Most egg masses were found on *C. speciosa*, followed by *P. virginiana* ‘Schubert’ and only single sightings on *C. canadensis*, *Fagus sylvatica* ‘Purpurea Tricolor’ and *Acer grandidentatum* Nutt (Table 1). Egg masses were difficult to detect due to their cryptic colouration and small size, which likely contributed to under-representation of this life stage in surveys. Nymphs were the most prevalent life stage detected and were observed between June and late September (Fig. 2). Nymphs were found on 44 of the total 53 plant species, with the highest numbers found on *C. speciosa* (Table 1). Fewer adults were detected compared to nymphs, but adults were observed throughout the entire duration of survey periods in both years, with peak detections in September 2017 (five times higher than in September 2018) and Au-

**Table 2.** Plant species without *H. halys* detections during surveys in northern Utah, 2017 and 2018. Surveys (*n*) indicates the number of times a species was sampled.

Family name	Scientific name	Surveys ( <i>n</i> )
Adoxaceae	<i>Sambucus cerulea</i>	8
	<i>Viburnum opulus</i>	16
Amaryllidaceae	<i>Allium aflatumense</i>	35
Anacardiaceae	<i>Cotinus coggygria</i>	20
	<i>Rhus typhina</i>	7
	<i>Rhus typhina</i> 'Laciniata'	56
Apocynaceae	<i>Asclepias syriaca</i>	7
Asteraceae	<i>Artemisia tridentata</i>	15
Berberidaceae	<i>Berberis thunbergii</i> var. <i>atopurpurea</i> 'Rose Glow'	15
	<i>Berberis vulgaris</i>	21
Betulaceae	<i>Betula nigra</i>	7
	<i>Betula papyrifera</i>	17
Cannabaceae	<i>Celtis occidentalis</i>	15
Caprifoliaceae	<i>Lonicera</i> X <i>heckrottii</i> 'Goldflame'	17
	<i>Symphoricarpos albus</i>	21
Celastraceae	<i>Euonymus alatus</i>	30
	<i>Euonymus fortunei</i>	48
Cornaceae	<i>Cornus alba</i> 'Siberica'	24
	<i>Cornus kousa</i>	15
	<i>Cornus sericea</i>	19
Cucurbitaceae	<i>Cucumis sativus</i>	9
Cupressaceae	<i>Juniperus chinensis</i>	15
	<i>Metasequoia glyptostroboides</i>	16
Elaeagnaceae	<i>Elaeagnus angustifolia</i>	23
Fabaceae	<i>Cladrastis kentukea</i>	8
	<i>Cladrastis lutea</i>	15
	<i>Maackia amurensis</i>	8
Fagaceae	<i>Quercus gambelii</i>	74
	<i>Quercus rubra</i>	8
Ginkgoaceae	<i>Ginkgo biloba</i>	21
Grossulariaceae	<i>Ribes alpinum</i>	21
Hydrangeaceae	<i>Philadelphica</i> X <i>virginialis</i>	8
Juglandaceae	<i>Juglans regia</i>	53
Lamiaceae	<i>Nepeta cataria</i>	16
Lauraceae	<i>Lindera benzoin</i>	15
Magnoliaceae	<i>Liriodendron tulipifera</i>	21
	<i>Magnolia denudata</i>	15
Malvaceae	<i>Alcea rugosa</i>	56
	<i>Hibiscus syriacus</i>	7
	<i>Tilia platyphyllos</i>	16
	<i>Tilia tomentosa</i>	8
Oleaceae	<i>Forsythia</i> X 'Northern Sun'	8
	<i>Syringa</i> 'Bailbelle'	8
	<i>Syringa reticulata</i> 'Ivory Silk'	8
	<i>Syringa vulgaris</i> 'Ludwig Spaeth'	8
	<i>Syringa</i> X <i>hyacinthiflora</i>	15
Plantaginaceae	<i>Penstemon strictus</i>	16
Rosaceae	<i>Crataegus crus-galli</i> var. <i>inermis</i>	16
	<i>Crataegus laevigata</i> 'Paul's Scarlet'	14
	<i>Crataegus</i> X <i>lavellei</i>	16
	<i>Fragaria vesca</i>	15
	<i>Malus ioensis</i>	33
	<i>Physocarpus opulifolius</i> 'Dart's Gold'	23
	<i>Physocarpus opulifolius</i> 'Diablo'	8
<i>Prunus americana</i>	8	
	<i>Prunus dulcis</i>	14

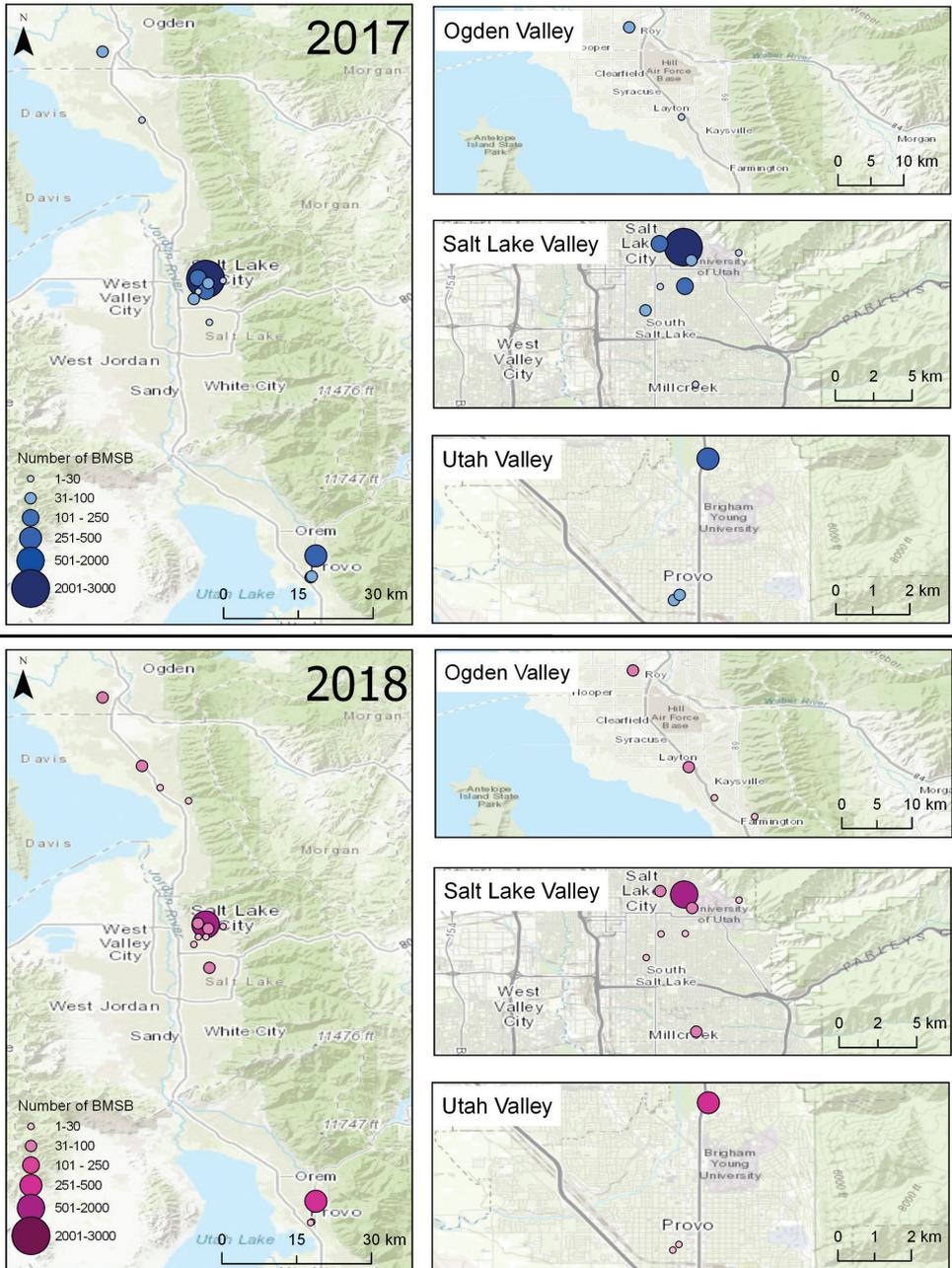
Family name	Scientific name	Surveys (n)
Rosaceae	<i>Prunus mahaleb</i>	16
	<i>Prunus X cistena</i>	19
	<i>Pyrus pyrifolia</i>	7
	<i>Rosa</i> spp.	168
Rosaceae	<i>Prunus virginiana</i>	59
	<i>Sorbus alnifolia</i>	8
Rutaceae	<i>Tetradium daniellii</i>	8
Salicaceae	<i>Populus deltoides</i>	7
	<i>Salix lasiolepis</i>	8
Sapindaceae	<i>Acer campestre</i>	18
	<i>Acer griseum</i>	21
	<i>Acer saccharinum</i>	7
	<i>Aesculus hippocastanum</i>	16
	<i>Aesculus X carnea</i> 'Briotii'	8
	<i>Koelreuteria paniculata</i>	8
Saxifragaceae	<i>Astilbe X arendsi</i> 'White Gloria'	17
Simaroubaceae	<i>Ailanthus altissima</i>	40
Solanaceae	<i>Capsicum annuum</i> 'Big Bertha'	9
	<i>Lycium barbarum</i>	19
	<i>Solanum melongena</i>	25
Ulmaceae	<i>Ulmus americana</i> 'Lewis & Clark'	8
	<i>Ulmus parvifolia</i> 'Emer II'	8
	<i>Ulmus propinqua</i> 'Emerald Sunshine'	8
Vitaceae	<i>Parthenocissus quinquefolia</i>	66

**Table 3.** Additional *H. halys* host plant species documented in northern Utah, but extramural to the surveys in this study, 2017–2020.

Family name	Scientific name
Asteraceae	<i>Helianthus annuus</i>
Boraginaceae	<i>Borago officinalis</i>
Cucurbitaceae	<i>Cucurbita pepo</i>
Fabaceae	<i>Phaseolus vulgaris</i>
Lamiaceae	<i>Ocimum basilicum</i>
Moraceae	<i>Morus alba</i>
Poaceae	<i>Zea mays</i>
	<i>Zea mays</i> 'Everta'
	<i>Prunus armeniaca</i>
Rosaceae	<i>Prunus cerasus</i>
	<i>Prunus persica</i>
	<i>Pyrus communis</i> 'Williams'
	<i>Rubus idaeus</i>
Salicaceae	<i>Populus fremontii</i>
Solanaceae	<i>Solanum lycopersicum</i>

gust and September 2018 (Fig. 2). Adults were found on 36 plant species, most commonly on *C. speciosa*, with sporadic high density sightings on several species within the families Rosaceae and Sapindaceae, specifically those within the genera *Acer* and *Malus* (Table 1). Total numbers of nymphs and adults detected were nearly 1.5 times greater in 2017 than in 2018 (3,611 in 2017 and 2,515 in 2018) (Figs 2, 3).

In general, sites surveyed in Salt Lake and Utah Counties had higher densities of *H. halys* in both years than sites in Weber and Davis Counties to the north (Fig. 3). The site containing the highest densities of *H. halys* was in the Avenues neighbourhood (Site 9) of Salt Lake City. This area contains street blocks lined with large, mature ornamental trees and is within 3 km of the University of Utah campus where *H. halys* was



**Figure 3.** The total number of *H. halys* detected at survey sites in 2017 (top left) and 2018 (bottom left). The maps on the right show magnified views of Ogden Valley (Weber and Davis counties), Salt Lake Valley (Salt Lake County) and Utah Valley (Utah County).

originally detected in Utah in 2012. Site 3 in northern Provo, Utah County, had the second highest density of *H. halys* sightings. Both locations were next to large apartment buildings with several *C. speciosa* trees in close proximity.

## Discussion

Surveys in northern Utah for *H. halys* have documented several prominent host plant species belonging to the families Bignoniaceae, Fabaceae, Rosaceae and Sapindaceae. These families, along with their most commonly encountered genera (*Catalapa*, *Cercis*, *Malus*, *Prunus* and *Acer*), have been documented as beneficial hosts for *H. halys* in other regions of North America (Hoebeke and Carter 2003; Bakken et al. 2015; Bergmann et al. 2016) and Asia (Lee et al. 2013). Twenty-nine of the plant species observed in this northern Utah study were novel *H. halys* host detections for North America (StopBMSB.org). Some novel hosts, such as the native *P. tremuloides*, supported both nymph and adult *H. halys* on the bark and foliage. No direct observation of feeding or plant damage was recorded; however, *P. tremuloides* may be an important host for *H. halys* establishment in Utah as it is a commonly planted ornamental tree and known to sustain biodiversity, native habitat and other ecosystem services in the intermountain region (Rogers et al. 2020). Our surveys were only conducted in urban areas; therefore, further study is needed to confirm the potential for wild *P. tremuloides* to support *H. halys*, including other intermountain areas where this tree is an important native plant.

*Acer ginnala* was a novel host with consistent nymph and adult detections in both survey years, especially from May to June. This early season preference could be due to nutrient availability and plant health, as many *A. ginnala* experience foliar chlorosis in mid to late summer due to a lack of iron from alkaline soils in Utah (Mengel 1994), possibly making this host less desirable in the mid and later season. The only host species, besides *C. speciosa* to exhibit all three life stages of *H. halys*, was *P. virginiana* ‘Schubert’, which is also a novel host species. This association is likely due to its large plantings in northern Utah residential areas, attractive fruiting structures for feeding and dense protective canopy. This species is also exploited by several native stink bug species, specifically *Chinavia hilaris* (Say). However, the Utah native *P. virginiana*, did not host *H. halys* during these surveys, suggesting the ornamental *P. virginiana* ‘Schubert’ is a more suitable host, possibly because it offers a larger canopy and is more common near *H. halys* overwintering sites, such as human-made structures. More extensive sampling of the native *P. virginiana* is suggested to support a more comprehensive comparison.

The most common and consistent host plant for *H. halys* in northern Utah is the northern catalpa, *C. speciosa*; the highest number of egg masses, nymphs and adults were found on this host in both survey years. Our observations support other surveys in North America and Eurasia where *H. halys* was common on *C. speciosa* (Bakken et al. 2015; Musolin et al. 2018). Resources of this plant that seem to attract *H. halys* are its large leaves, flowers and reproductive pod structures. The undersides of the large leaves are especially advantageous for *H. halys* oviposition. As a primary and sentinel host, *C. speciosa* is a target for prevention, detection and management practices against the spread and further establishment of *H. halys* into agricultural lands in Utah. Contrary to other reports in North America that document *Ailanthus altissima* (Mill.) Swingle as a prominent host plant (Rice et al. 2014; Bakken et al. 2015; Bergmann et

al. 2016), our surveys of this species (sampled 40 times in 2018 only, Table 2) did not detect *H. halys*. We project that the host status of *A. altissima* could change in Utah given its apparent preference by *H. halys* in other regions of North America.

The occurrence and abundance of certain plant species impacted the survey results, as stated for *P. virginiana* ‘Schubert’ above. This is largely due to *H. halys* quickly dispersing by flight (Wiman et al. 2015) and using plants for a variety of functions (Bergmann et al. 2016), including resting between flights. Therefore, it is reasonable to assume that more abundant plant species in an urban landscape are more likely to harbour dispersing adults. *Acer plantanoides* was a major component of urban vegetation cover in all of the surveyed counties in northern Utah, likely contributing to some of its observed *H. halys* abundance. A similar association can likely be applied to common ornamental plant species in families Fabaceae, Rosaceae and Sapindaceae. We did observe some exceptions; a notable one being the genus *Rosa*, with few *H. halys* detections.

Although *H. halys* has been detected on plants, in pheromone traps and by the public in multiple locations in Utah, established populations are primarily concentrated along the Wasatch Front (west side of the Rocky Mountain range). To date, the highest densities of *H. halys* reside within Salt Lake and Utah Counties. The concentration of *H. halys* in the larger metropolitan areas of Salt Lake and Utah Counties is most likely due to its original detection and establishment in Salt Lake City with expansion into nearby urban centres. These urban areas offer overwintering shelter in human structures (Lee et al. 2014), wooded areas with mature ornamental hosts (Bakken et al. 2015) and human-mediated vectors of transport (e.g. Interstate 15, Union Pacific Railroad) (Wallner et al. 2014). Urban centres with high populations of *H. halys* are in close proximity to northern Utah’s agricultural production areas, especially speciality fruit and vegetable crops which are at risk of feeding damage by *H. halys* (Schumm 2020; Schumm et al. 2020). Identification of ornamental plants that harbour *H. halys* in the urban-agricultural interface is critical for providing information for preventative management decisions and for better management of future crop invasions.

Using *C. speciosa* and other prominent host plants identified in this study as sentinel hosts, property owners and land managers in Utah, as well as other surrounding States in the greater Intermountain West, can more accurately track the invasion and establishment by *H. halys* (Mansfield et al. 2019). Beyond host plant species data, these surveys provide a temporal context for *H. halys* development across its multiple life stages in northern Utah. Nymphs were observed in significantly higher numbers than egg masses and adults from mid-June through to early September. This suggests that Utah growers and land managers should initiate monitoring using beat sheets or traps in May or early June, with treatment needs assessed from June through to September with consideration of crop and harvest timelines.

Interestingly, overall populations of *H. halys* nymphs and adults decreased from 2017 to 2018. The reason for this population decline is unknown. No major differences in relative humidity, temperature and cumulative degree-days occurred between the two survey years when utilizing the predictive phenology model of Nielsen et al. (2008, 2016). For example, in Salt Lake City where the majority of sites were located,

the mean minimum/maximum temperatures and minimum relative humidity in May of 2017 and 2018 were 7.7/21.9 °C and 24.4% and 9.3/23.6 °C and 24.2%, respectively. In September 2017 and 2018, the mean minimum/maximum temperatures and minimum relative humidity was 10.2/24 °C and 29.7% and 11.2/28.6 °C and 13.6%, respectively (climate.usu.edu). Temperatures and relative humidity were more similar between years in the June through to August survey periods and fell between environmental conditions observed in May and September. Regardless, *H. halys* populations are still relatively low in Utah compared to other regions of North America where climate conditions are more favourable for *H. halys* (Rice et al. 2014; Nielsen et al. 2016). Extreme high temperature and low humidity are known to negatively affect *H. halys* survival and reproduction (Nielsen et al. 2008; Haye et al. 2014; Fisher et al. 2020).

Another limiting factor could be egg mortality by parasitoid wasps. *Trissolcus japonicus* Ashmead, a parasitoid of *H. halys* native to its home range, was first detected in Utah in Salt Lake City in June 2019 and expanded its abundance and range in 2020 (Holthouse et al. 2020; K. Richardson, personal communication). However, egg mass parasitism rates by native wasps in northern Utah surveys were similar between 2017 and 2018 and *T. japonicus* was found only after these host plant surveys were conducted. Another organism that may have caused this population decrease is *Nosema maddoxi* Becnel, Solter, Hajek, Huang, Sanscrainte and Estep (Hajek et al. 2017). This microsporidian is known to cause mortality in *H. halys* adults and nymphs and was detected in wild-caught *H. halys* specimens in Salt Lake City and Provo, Utah in 2017 (Preston et al. 2020; C. Preston, personal communication). However, in 2018, dissections of 141 adult *H. halys*, collected from several locations in Salt Lake City and Provo, Utah, revealed no *N. maddoxi* spores, implying the microsporidian was absent or collection/dissection methods were ineffective in detecting its presence (M. Holthouse, unpublished data). Despite our inability to explicitly define a cause for declines in *H. halys* populations along the Wasatch Front since 2017, this trend has continued into 2020 (M. Holthouse, unpublished data).

## Conclusion

Plant surveys for the invasive brown marmorated stink bug, *H. halys*, within the urban landscape of northern Utah, have revealed 53 host plant species from 17 families capable of harbouring one or more developmental life stages of the insect. Of these plant species, *C. speciosa*, northern catalpa, harboured the predominance of *H. halys* eggs, nymphs and adults across survey sites and years. Peak numbers of *H. halys* nymphs, the most abundant life stage, occurred between June and early September in both years with highest densities in Salt Lake and Utah Counties. A notable novel host is *P. tremuloides*, an important native tree in the Intermountain West and other interior western regions. We documented that *H. halys* can be found season-long on a wide variety of managed ornamental plants and identified 29 novel host species in northern Utah.

## Acknowledgements

We thank Kate Richardson, Hanna Kirkland, Chelise Dever, Zachary Schumm, Ben Steadman, Lily Bourett, James Withers, Loren Linford, Stephanie Hall, Erin Berdahl and Ryan West for their assistance with field research. Special thanks to Michael Piep, Kristian Valles and the Utah State University Intermountain Herbarium for help with plant identification and curation. Funding was provided by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Specialty Crop Research Initiative under award number 2016-51181-25409; USDA Specialty Crop Block Grant; Utah Department of Agriculture and Food; Western Sustainable Agriculture Research and Education program under award number 2017-38640-26913 and subaward number [GW18-106]; and Utah State University Extension. This research was supported by the Utah Agricultural Experiment Station, Utah State University and approved as journal paper number 9395.

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# Predicting terrestrial dispersal corridors of the invasive African clawed frog *Xenopus laevis* in Portugal

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Academic editor: W.Rabitsch | Received 25 October 2020 | Accepted 17 December 2020 | Published 28 January 2021

**Citation:** Ginal P, Moreira FD, Marques R, Rebelo R, Rödder D (2021) Predicting terrestrial dispersal corridors of the invasive African clawed frog *Xenopus laevis* in Portugal. NeoBiota 64: 103–118. <https://doi.org/10.3897/neobiota.64.60004>

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## Abstract

Invasive species, such as the mainly aquatic African clawed frog *Xenopus laevis*, are a main threat to global biodiversity. The identification of dispersal corridors is necessary to restrict further expansion of these species and help to elaborate management plans for their control and eradication. Here we use remote sensing derived resistance surfaces, based on the normalised difference vegetation index (NDVI) and the normalised difference water index (NDWI) accounting for behavioural and physiological dispersal limitations of the species, in combination with elevation layers, to determine fine scale dispersal patterns of invasive populations of *X. laevis* in Portugal, where the frog had established populations in two rivers. We reconstruct past dispersal routes between these two invaded rivers and highlight high risk areas for future expansion. Our models suggest terrestrial dispersal corridors that connect both invaded rivers and identify artificial water bodies as stepping stones for overland movement of *X. laevis*. Additionally, we found several potential stepping stones into novel areas and provide concrete information for invasive species management.

## Keywords

Amphibian, distribution, invasive species management, NDVI, resistance kernel

## Introduction

Worldwide, invasive species are a main threat to biodiversity (e.g. Fritts and Rodda 1998; Rosenzweig 2001; Chornesky and Randall 2003; Davis 2003; Doherty et al. 2016), having also played a role in global amphibian decline (e.g. Gibbons et al. 2000; Chornesky and Randall 2003; Falaschi et al. 2020). Some invasive amphibians are known to have disastrous impacts on native ecological communities and to alter sensitive ecological relationships through competition for resources, predation or spread of infectious diseases (Kraus 2009; Kraus 2015 and references therein; Measey et al. 2016 and references therein; Kumschick et al. 2017).

The African clawed frog (*Xenopus laevis*) is native to southern Africa and has been moved worldwide as a model organism for laboratory research (Measey et al. 2012; van Sittert and Measey 2016). This species has unique physiological and demographical traits, including a tolerance to saltwater and eutrophic conditions and behavioural adaptations, such as terrestrial migration or the ability to burrow into substrate to persist in drought and extreme temperature events. This trait combination confers it with an enormous invasive potential (for a thorough review, see Measey et al. 2012; Sousa et al. 2018; Scalera et al. 2019). Recently, it was ranked second amongst all invasive amphibian species considering its environmental and socio-economic impacts (Measey et al. 2016).

To date, *X. laevis* has established invasive populations in numerous countries across four continents due to both deliberate and accidental release of laboratory animals and the pet trade (Measey et al. 2012). In Europe, it successfully invaded lotic and lentic freshwater habitats (Moreira et al. 2017) and established populations are known in the U.K., France, Sicily (Italy) and Portugal (see Measey et al. 2012 and references therein). Comparisons of mitochondrial DNA suggest that all the Portuguese frogs resulted from presumably a single introduction event and descend from animals exported from the South-western Cape clade, in Mediterranean from South Africa (De Busschere et al. 2016). Correlative SDM approaches revealed the Mediterranean region of Portugal as climatically highly suitable for *X. laevis* (Measey et al. 2012; Ihlow et al. 2016). However, while the populations in Sicily and France are spreading fast (Faraone et al. 2008; Louppe et al. 2017), the expansion of the Portuguese populations was comparatively slow – approximately 30 years after the introduction event, the species was still confined to a 30 km<sup>2</sup> region (Sousa et al. 2018).

Dispersal is essential for successful spread of an invasive species (cf. Blackburn et al. 2011). Animal dispersal occurs at different life stages and is triggered to evade competition, to acquire resources, to reduce mortality or for reproduction (Bowler and Benton 2005 and references therein; Van Dyck and Baguette 2005). Amphibians are often referred to as poor dispersers, but some species disperse over considerable distances of more than 10 km, for example, from the terrestrial habitats to spatially disjunct breeding sites for migration (e.g. Avise 2000; Smith and Green 2005 and references therein). Traditionally, *X. laevis* was thought to be strictly aquatic with all life stages inhabiting the same aquatic environment, constraining the invasive potential of the species to connected aquatic habitats. However, there is now sufficient evidence that

the species migrates overland amongst various types of lentic and lotic water bodies, which significantly expanded our view of the species' dispersal and, therefore, invasive potential (see Measey et al. 2016 for recent review). Terrestrial movement seems to be constrained to a fraction of the population (21–36% [Measey and Tinsley 1998]), involves adults of both sexes (De Villiers and Measey 2017), occurs mostly nocturnally, involves Euclidean distances up to 2.36 km (De Villiers 2015) and a maximum velocity of 0.2 km/h (Measey and Tinsley 1998). It is known that a drying habitat or the reduction of resources due to high numbers of conspecifics can lead to mass migration events, although other potential factors that trigger terrestrial dispersal remain unknown (Measey et al. 2016).

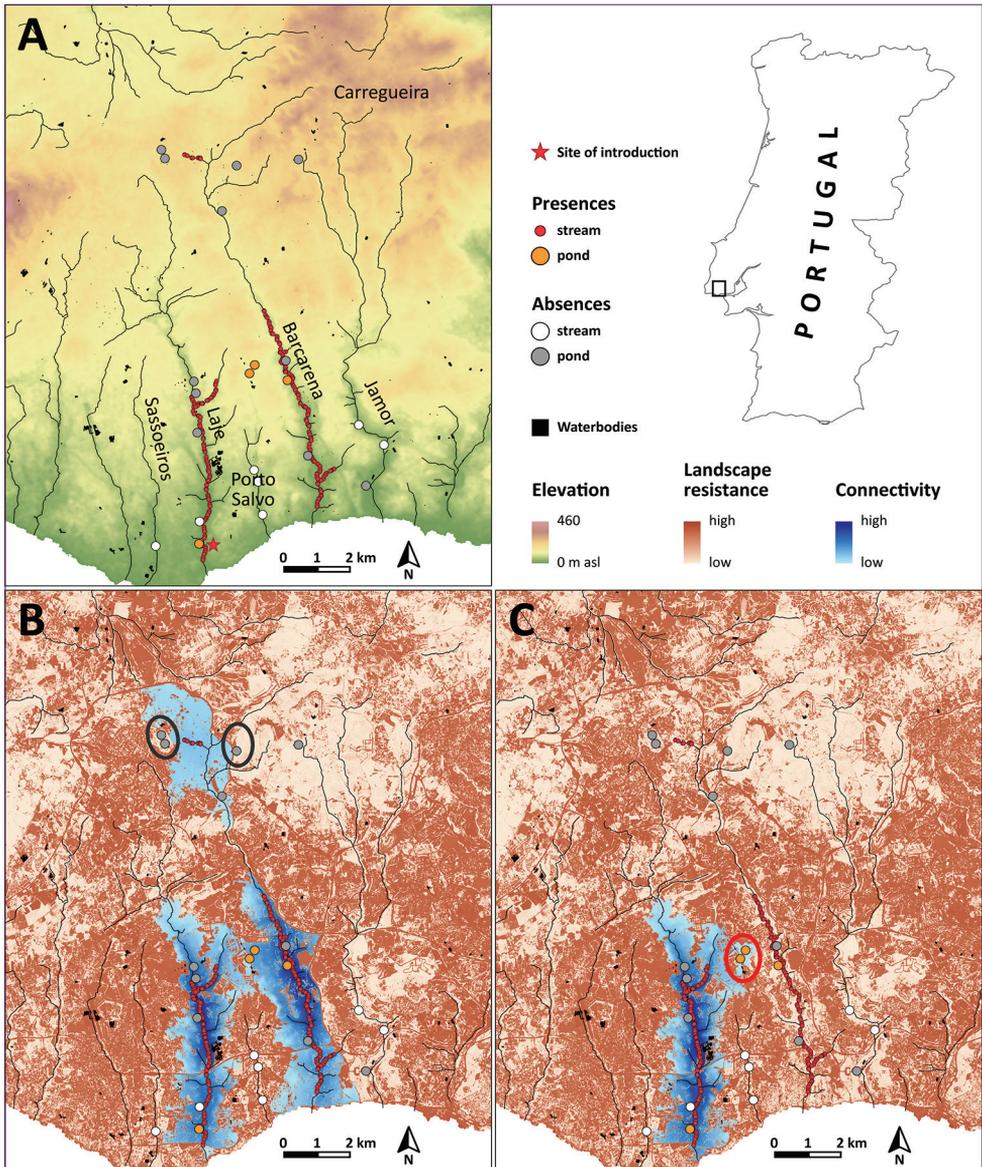
To predict dispersal pathways and, therefore, be able to block or hamper further expansion of an invasive species, it is possible to build resistance surfaces that reflect different costs for a species to move through the landscape using vegetation cover, elevation, slope or other landscape features (Landguth et al. 2012). As a multitude of paths may exist between two points, either multiple low-cost paths or smoothed output paths using a probability-density function can be considered (Cushman et al. 2009; Pinto and Keitt 2009). The latter approach allows a variety of smoothing functions (Gaussian, Epanechnikov, uniform, triangle, biweight, triweight and cosine function) referred to as kernel density estimations (Li and Racine 2007).

In the present study, we used occurrence records of *X. laevis* in Portugal and fine scale remote sensing data to build landscape resistance kernels that predict the influence of landscape structure on the dispersal dynamics of this invasive frog. Landscape resistance is subsequently used to identify past dispersal routes and to highlight areas at risk of future invasion by *X. laevis*. This study provides insights into the role of landscape configuration on dispersal patterns and provides a tool for future management of this species, as well as of others with similar dispersal patterns.

## Materials and methods

### Study area

West Portugal is characterised by a Mediterranean-type climate (Rubel and Kottek 2010). The first record of *X. laevis* in western Portugal occurred in Laje River, which runs through a densely-urbanised part of Oeiras County ca. 20 km west of Lisbon, in 2006 (Sousa et al. 2018) (Fig. 1A). However, this introduction likely occurred much earlier and is probably the result of accidental escape from nearby research laboratories after the strong winter floods of 1979/80 (Rebelo et al. 2010; Sousa et al. 2018). Due to its cryptic lifestyle combined with a lack of interest in the wildlife of urban rivers, the species established and spread along the river, undetected for more than 25 years (Sousa et al. 2018). The frog then spread from Laje River into a close parallel-flowing second river (Barcarena), where it was found in 2008 (Rebelo et al. 2010). The maximum invaded area by the frog on the river sections occurred along 5.86 km in the main



**Figure 1.** Study area **A** overview of study area: Elevation and important locations (i.e. the two invaded rivers and other localities that could be threatened by invasion). Features as presence and absence points (streams and ponds), water bodies and the site of introduction are highlighted **B** areas of low and high risk of invasion: Landscape resistance and the connectivity (including all presences as starting points) of the study area. Features as presence and absence points (streams and ponds) and water bodies are highlighted. Two areas of low but possible risk of invasion are surrounded by black circles **C** reconstruction of past dispersal from Laje into Barcarena River: Landscape resistance and the connectivity (including only presences from Laje River as starting points) of the study area. Features as presence and absence points (streams and ponds), and water bodies are highlighted. The golf-course ponds, which were used as stepping stones into Barcarena River, are surrounded by a red circle.

stream of Laje plus one of its tributaries and 6.39 km in the main stream of Barcarena plus three tributaries (Moreira et al. 2017). The invaded river basins are roughly 3 km apart, but the headwaters of two small tributaries of Laje River are nearby (ca.1 km) to the headwaters of a tributary of Barcarena River (Fig. 1A). Both rivers are permanent, approximately 10 m wide in most stretches and 2 m deep in summer in the deepest stream pools (Moreira et al. 2017). The tributaries inhabited by *X. laevis* are also permanent, ca. 1 m wide and up to 1.5 m deep (Moreira et al. 2017). Both rivers flow into the Tagus estuary and, although *X. laevis* can tolerate a moderate salinity, it seems unlikely that it has used this path to cross between both river basins (Sousa et al. 2018). The area has just a few still and artificial water bodies, most of them 20–110 m apart from the streams (Moreira et al. 2017). Three large golf-course ponds are located between the headwaters of two tributaries flowing in opposite directions, one to each river. The invasive populations seem constrained to the two rivers, some of their tributaries, the three golf-course ponds and some man-made ponds, covering a total area of approximately 30 km<sup>2</sup>.

## Landscape resistance

We calculated fine scale resistance kernels to determine connectivity and predict potential overland dispersal for the invasive Portuguese population. We used literature-based GPS data of confirmed presences (Rebelo et al. 2010), updated them to a total of 201 locations and added 19 confirmed absences along the streams, as well as in isolated ponds, according to our own field research. To define the study area, we chose a circular buffer of 5 km around the GPS-points, which is about twice the maximum documented terrestrial dispersal distance during a dispersal event of the frog (Measey 2016).

## Remote sensing derived resistance surfaces

We obtained high resolution multispectral satellite imagery containing the invaded Portuguese distribution range (625 km<sup>2</sup> × 4 title IDs = 2500 km<sup>2</sup>) as A3 products of the RapidEye satellite (Blackbridge 2014). We used the satellite images with title-IDs 2956913, 2956914, 2957013 and 2957014 and card IDs 26196070, 26196539, 26196860, 26195477, 26196831, 26196078, 26196867, 26196894 and 26196746. The dataset contains four orthorectified raster images, each with five remote sensing channels (blue: 440–510 nm, green: 520–590 nm, red: 630–685 nm, red edge: 690–730 nm, NIR: 760–850 nm wavelengths). Each raster image covers an area of 625 km<sup>2</sup>. The spatial resolution is 5 m grid cell size and spatial accuracy is 10 m (Blackbridge 2014). The subsequent corrections were applied to the five raw remote sensing channels: ‘top of atmosphere correction’ (TOA), ‘cloud cover correction’ and ‘histogram correction’ using the packages LANDSAT (Goslee 2011), RASTER (Hijmans 2015) and LMODEL2 (Legendre 2014) for R according to the product specifications. Using RS TOOLBOX (Leutner and Horning 2016) and the above-mentioned packages for R, the raster images for each study area were mosaicked before the ‘Normalised

Difference Vegetation Index' (NDVI) as a measure for vegetation cover and the 'Normalised Difference Water Index' (NDWI), showing differences in the water content of vegetation, were computed.

Based on remote sensing variables using a threshold-based water detection method, the larger still and flowing freshwater bodies within the study area were detected (Klemenjak et al. 2012; Tetteh and Schönert 2015). To improve the algorithms' capacity to detect water, the precise locations of all verified water bodies were identified manually in Google Earth and used to train a bioclim model using the DISMO package for R (Hijmans, Phillips et al. 2013; R Core Team 2017). The output of the bioclim model was again verified by hand. Small water bodies that had not been detected by this measure were georeferenced by hand using Google Earth. We used only one randomly selected occurrence record per  $50 \times 50$  m grid cell because computation time increases exponentially with the number of species occurrences and the size of the study area. We collected seven presences comprising two invaded river sections (Laje 5.6 km and Barcarena 6.3 km) and identified 612,536 locations potentially adequate for invasion.

We calculated resistance surfaces by combining NDVI and NDWI scores giving higher priority to vegetation cover, but acknowledging that humid areas may be preferred by the frogs (i.e.  $NDVI + NDWI / 10$ ). We applied an inverse monomolecular transformation using relevant functions of the RESISTANCEGA package for R (Peterman 2014, Peterman et al. 2014) to account for the higher expected permeability of areas covered by humid vegetation. The equation of the inverse monomolecular transformation is  $y = -r(1 - \exp^{-bx})$ , with  $r$  = resistance surface, which is controlled by shape ( $x$ ) and magnitude ( $b$ ) parameters that are varied during optimisation (see Peterman 2018 for more details). This transformation appreciates that the permeability of vegetated areas may not shift too much with declining vegetation cover. However, resistance increases exponentially in more open landscapes, especially in the Mediterranean climate, characterised by hot and dry summers. As our resistance surface is based on vegetation and humidity indices, we can differentiate various states of vegetation, but less so for bare soil. Bare soil usually falls within an NDVI range between 0.1–0.2 and plants will always have positive values between 0.2 and 1. Therefore, an exponential function counterbalances the differences in the discrimination ability of our indices. The resistance surfaces were scaled to range from 0–1. Subsequently, a threshold was determined to detect man-made surfaces, such as roads and buildings by comparing all scores between 0.70–0.85 in steps of 0.01 to areal pictures of the same area and reclassifying all scores above the best-matching threshold to a resistance of 50. This threshold does not differentiate between roads, which are semi-permeable and buildings which represent absolute barriers. A score of 50 allows the frogs to cross a maximum of up to 6 grid cells of 5 m (ca. 30 m) man-made surfaces, making roads semi-permeable, but areas with a high density of man-made structures become impermeable.

## Elevation data

Laboratory trials, using *X. laevis* individuals from Portugal, were used to quantify the effect of slope on dispersal. These trials showed an increasing difficulty to overcome

slopes, with 60 degrees as the upper limit. An elevation layer with a spatial resolution of 30 m derived from the 'Advanced Spaceborne Thermal Emission and Reflection Radiometer' 'Global Digital Elevation Model' (ASTER GDEM) was obtained from the online database of the NASA Land Processes Distributed Active Archive Centre (LP DAAC) of the USGS/Earth resources observation and science (EROS) centre (<https://lpdaac.usgs.gov>). We re-sampled it to a resolution of 5 m using bidirectional interpolation, available in the RASTER package (Hijmans 2015) for R.

## Resistance kernels

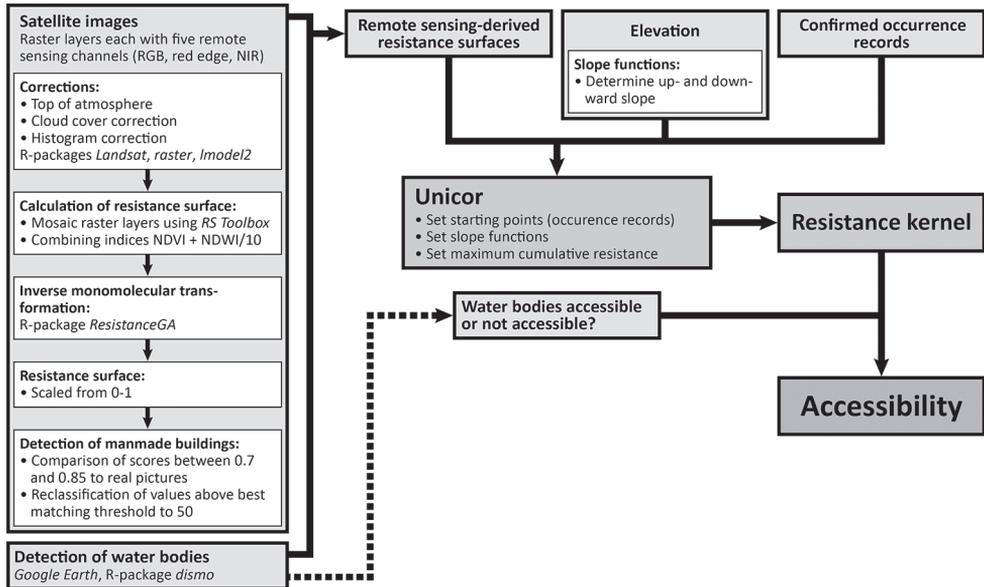
The remote sensing derived resistance surfaces, in combination with the elevation data, were used to calculate resistance kernels that quantify permeability of the landscape after Compton et al. (2007), using the UNICOR package for python (Landguth et al. 2012; Fig. 2). The resistance kernel approach combines a kernel density estimator with a directional least-cost matrix to produce a multidirectional probability distribution representing variability in habitat quality (Compton et al. 2007). This measure considers land use and elevation derived from remote sensing data and equals higher permeability with higher connectivity as it suggests a higher probability of a dispersing frog to arrive at the water bodies (Compton et al. 2007).

Based on the laboratory trials, the slope function was defined so that an upward slope of 60 degrees is the maximum, while downward slopes were considered as generally permeable (upward slope function as determined, based on trials:  $y = 3.1051 e^{0.038x}$ , scaled to 0–1; downward resistance = 0; settings UNICOR: Type\_Direction = Hiking; 6;-3).

Based on capture-mark-recapture data from South Africa (De Villiers 2015), we determined the maximum cumulative resistance, which is observed in the field using least cost paths, calculated with the same set of remote sensing derived resistance plus elevation data. For this, we used the satellite images with the title-IDs 3423406, 3423407, 3423307 and 3423207 and Card-IDs 26195795, 26195801, 26195803 and 26196899. The highest cumulative resistance detected was used to parameterise the dispersal models for the study area (maximum cumulative resistance = 308). Model accuracy was evaluated by extracting the values of the resistance kernel for the used presence records. Further, true absence records were used in the same way to check if they were in- or outside of the predicted kernel area.

## Results

UNICOR outputs show the cumulative density of optimal paths buffered by the kernel density estimation (Fig. 1C). As for model accuracy, the 201 presence records had a mean cumulative density of  $51.21 \pm 20.02$  (range: 2.19–80.32), while, for the 19 absence records, this was  $19.21 \pm 28.13$  (range: 0–70.52). Eleven of the 19 absences (57.9%) were outside of the kernels' range. The resistance kernels also located functionally well-connected water bodies and complexes in close vicinity to the existing populations (Fig. 1B – invaded ponds).



**Figure 2.** Schematic figure of methodological approach for the calculation of resistance kernels

The known *X. laevis* populations, occupying the two rivers, are evidently constrained by landscape resistance and high permeability was attributed only to the valley bottoms around the river beds of Laje and Barcarena. Importantly, our results explain the current distribution of the species, including its absence from nearby streams and locate the probable contact route between the two invaded basins, supporting the hypothesis of a natural colonisation of Barcarena by overland dispersal. In fact, areas of low (but still possible) permeability connect the two valleys at two locations, but the isolated animals found upstream of Barcarena seem to have no connectivity with the main downstream population (Fig. 1B).

## Discussion

With this work, it was possible to reconstruct the most probable past dispersal routes, terrestrial corridors for overland dispersal and water bodies that function as stepping stones, fostering the *X. laevis* invasion. Additionally, we found potential stepping stones into novel areas, now considered of high invasion risk.

## Pace of invasion

Despite its dispersal abilities, which include terrestrial movements up to 2.36 km (De Villiers 2015), an ascertained maximum velocity of 0.2 km/h (Measey and Tinsley 1998) and the apparently ideal climatic conditions (Measey et al. 2012; Ihlow et al.

2016), the *X. laevis* invasion in Portugal was quite slow. This is probably the result of a mostly aquatic (lotic) dispersal route, rather than the terrestrial overland dispersal documented on the species' original range (De Villiers and Measey 2017). Dispersal along flowing waters – and, in this case, opposed to the water flow in the first river to be colonised (Fig. 1A) – is affected by a combination of the species' dispersal ability, the location of the introduction site, the hydrological regime and landscape resistance.

The landscape of the Laje and Barcarena basins is hostile to a semi-terrestrial frog (see below). In fact, only a few isolated ponds were colonised (Fig. 1C); several ponds at 50 to 80 m from the streams were not reached by *X. laevis*. A few triggers of *X. laevis* terrestrial movement have been identified (De Villiers and Measey 2017). One of the most important triggers seems to be population density, which is here relatively low (Moreira et al. 2017), probably due to the low habitat quality – heavily polluted urban rivers. This low abundance also explains why the species went undetected for more than 25 years (Sousa et al. 2018). Although *X. laevis* can live, disperse through and successfully reproduce in flowing waters (Lobos and Jaksic 2005; Courant et al. 2017; Moreira et al. 2017), these seem not to be ideal for the species. Lotic habitats have been mostly identified as pathways for dispersal, while breeding is commonly referred to take place in lentic water bodies, like pools or ponds (Fouquet and Measey 2006; Faraone et al. 2008; Measey 2016). In Portugal and probably due to the poor habitat quality of the two streams and/or presence of predatory fish, the number of metamorphs is much lower in lotic than in lentic environments; metamorph size is also smaller, whereby reaching the reproductive size takes longer (Moreira et al. 2017).

Some features of the Mediterranean climate may have also contributed to the slow dispersal. The annual period where terrestrial dispersal could take place is not certain, as the mostly dry and hot summers seem too risky for terrestrial movement. The mild winters could be very suitable for dispersal overland, because these Mediterranean streams are typically subject to high water level variability; the rainy winters regularly cause river floods (Boix et al. 2010), spreading the species along the river valley. However, the site of initial introduction was located downstream, close to the mouth of the River Laje (Fig. 1A), meaning that, until the colonisation of Barcarena, only upstream dispersal was possible. Events like the 1979/80 flood, when the species escaped in Laje River, could boost its dispersal, but hardly upstream.

## Distribution

We found that the modelled landscape connectivity correlates well with the distribution of this frog. In the areas of high connectivity along the river-beds of Laje and Barcarena rivers, the species' dispersal is hampered by  $> 22^\circ$  slopes and  $> 60^\circ$  slopes seem to be nearly unconquerable. Landscape connectivity along large parts of the river sections is further constrained by cement walls instead of natural riverbank. Further, the rivers have several physical barriers like waterfalls, hampering the connectivity amongst populations and reducing landscape permeability (Sousa et al. 2018). In fact, the lack of continuity upstream of the Barcarena basin (Fig. 1C) results from a ~250 m long

tunnel through which the stream flows beneath two highways (detected as roads by remote sensing). Frogs were recently (June 2020) found just upstream and downstream of the tunnel and are very probably able to pass through the tunnel.

Away from the riverbeds, connectivity decreases very quickly along the steep, non-urbanised slopes to the very low connectivity of the highly-urbanised plateaus. If the frogs manage to leave the stream, they become hampered or blocked by traffic and buildings. This complex topology constrains connectivity amongst the invaded locations and the few other water bodies within the study area. According to our model, topography and urban areas are therefore sufficient to explain the non-colonisation of the three nearest streams – Sassoeiros to the west, Jamor to the east and Porto Salvo in between the two colonised rivers (Fig. 1C).

### Past dispersal routes

Due to road and building constructions after the year 2000, the maps that we used for this work may not depict all the dispersal corridors that were available in the 1980s and 1990s. However, our models show that Barcarena was very probably invaded from Laje by frogs that dispersed overland and used the golf-course ponds as stepping stones. According to the model, dispersing frogs may have used two small tributaries to reach the golf-course ponds. The northernmost tributary is the strongest candidate as a past dispersal route, given the large population that was found there. The golf ponds were dug and filled in 2002 and are located exactly in the single suitable corridor identified. As noted by other authors on less hilly landscapes (cf. Faraone et al. 2008), small water bodies can be used as stepping stones during the rainy season to reach suitable habitat. However, we cannot fully exclude other non-accounted factors, such as the intentional release by amphibian keepers.

### Areas of high risk

All factors, low habitat quality, restricted availability of water bodies and hampered dispersal ability, probably explain the comparatively slow invasion of *X. laevis* in Portugal. Still, the species has managed to colonise two rivers and this work suggests that it used artificial water bodies as stepping stones on a terrestrial pathway in a densely-urbanised area, highlighting the risks of further invasions.

Possible stepping stones for dispersal into other streams should be identified, monitored and, if possible, altered (e.g. by encircling them with walls, since there are no natural ponds in the area) to hamper further overland spread. The Jamor River basin and Carregueira Mountain are two semi-natural regions not yet invaded, located east and northeast of the currently-invaded area (Figure 1A). Jamor River is apparently protected by a high landscape resistance (Fig. 1C) and is, therefore, not naturally reachable by terrestrially dispersing *X. laevis*. However, landscape resistance is low at Carregueira, a small forested mountain range with farms and golf-courses (including lakes), which

also contains the headwaters of Jamor River. Currently, this area is not in the range of the connectivity kernel, but a *X. laevis* invasion is possible starting at its western edge. In the northern limit of the Barcarena population, there are also several small water bodies that could be used as possible stepping stones into novel areas.

The current eradication plan for this species in Portugal (Sousa et al. 2018) can be informed by this study. Regular monitoring of the water bodies that are within reach of the species, according to our model, is strongly advised. If possible, toad barriers could be built on the main pathways for overland dispersal, particularly at the edges of Carregueira and around the northern isolated population, which should effectively block further spread. As the number of colonised sites is reduced by the eradication programme, we recommend to verify (and if possible, to block) all paths within a radius of 5 km around the colonised water bodies (double the species currently known maximum terrestrial dispersal ability during a dispersal event) to minimise expansion risk. Our results are also relevant for other countries where *X. laevis* occurs, highlighting the importance of blocking strategic overland routes of dispersal, either by using toad fences or by draining ponds that may be used as stepping stones.

### Advantages and limitations

The fine-scale remote sensing derived resistance surfaces, based on NDVI and NDWI, in combination with elevation layers, allowed us to reconstruct potential past dispersal routes between the two invaded rivers and highlighted areas at high risk of invasion. This provides a detailed map highlighting areas which are threatened by invasion and knowledge of potential corridors for the invasive species. However, the computational power and time needed for this method increases with the number of starting points and with the resolution of raster layers. Furthermore, this approach is based on species-specific knowledge about biology and physiology and model accuracy strongly depends on evaluation by experts. Some very fine scale dispersal barriers may remain undetected by remote sensing, such as waterfalls with seasonally varying intensities or smooth walls. These landscape features may further restrict the dispersal potential on a local scale.

### Acknowledgements

This study was funded by ERANET BiodivERsA (project title: “Invasive biology of *Xenopus laevis* in Europe: ecology, impact and predictive models”) and the Deutsche Forschungsgemeinschaft (DFG RO 4520/3-1) for which we are very grateful. All distribution data were collected under the scope of the “Plano de erradicação de *Xenopus laevis* nas ribeiras do Concelho de Oeiras”, coordinated by Instituto da Conservação da Natureza e das Florestas (Portugal). In addition, we thank BlackBridge for providing the high resolution RapidEye satellite images used for this study free of charge. Further, we want to thank Erin Landguth, Morris Flecks and Ursula Bott for technical assistance.

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# Invasive plant benefits a native plant through plant-soil feedback but remains the superior competitor

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Academic editor: E. Wandrag | Received 18 August 2020 | Accepted 12 January 2021 | Published 28 January 2021

**Citation:** Buerdell SL, Milligan BG, Lehnhoff EA (2021) Invasive plant benefits a native plant through plant-soil feedback but remains the superior competitor. *NeoBiota* 64: 119–136. <https://doi.org/10.3897/neobiota.64.57746>

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## Abstract

Plant soil feedback (PSF) occurs when a plant modifies soil biotic properties and those changes in turn influence plant growth, survival or reproduction. These feedback effects are not well understood as mechanisms for invasive plant species. *Eragrostis lehmanniana* is an invasive species that has extensively colonized the southwest US. To address how PSFs may affect *E. lehmanniana* invasion and native *Bouteloua gracilis* growth, soil inoculant from four sites of known invasion age at the Appleton-Whittell Audubon Research Ranch in Sonoita, AZ were used in a PSF greenhouse study, incorporating a replacement series design. The purpose of this research was to evaluate PSF conspecific and heterospecific effects and competition outcomes between the invasive *E. lehmanniana* and a native forage grass, *Bouteloua gracilis*. *Eragrostis lehmanniana* PSFs were beneficial to *B. gracilis* if developed in previously invaded soil. Plant-soil feedback contributed to competitive suppression of *B. gracilis* only in the highest ratio of *E. lehmanniana* to *B. gracilis*. Plant-soil feedback did not provide an advantage to *E. lehmanniana* in competitive interactions with *B. gracilis* at low competition levels but were advantageous to *E. lehmanniana* at the highest competition ratio, indicating a possible density-dependent effect. Despite being beneficial to *B. gracilis* under many conditions, *E. lehmanniana* was the superior competitor.

## Keywords

Below-ground interactions, black grama, invasion ecology, Lehmann lovegrass, plant competition, plant invasion, soil microbiota

## Introduction

Plant-soil feedbacks (PSFs; Bever et al. 1997) are interactions between a plant and the biotic and abiotic components of soil that affect plant growth, reproduction, and survival. One plant can create a PSF that affects itself, other conspecifics, or other co-occurring species. Positive PSFs result in increased growth, reproduction, or survival from increases in nutrient availability by arbuscular mycorrhizal fungi or mineralizing microbes (Klironomos 2002), while negative feedbacks result in decreased growth, reproduction, or survival from increases in herbivores, parasites, or pathogens (Bever et al. 1997; Petermann et al. 2008). Plant-soil feedback interactions may affect biodiversity and ecosystem functioning (Mack and Bever 2014), species coexistence (van der Putten et al. 2013), community dynamics (Teste et al. 2017), and competition (Kulmatiski et al. 2008).

Plant-soil feedback and competition are not always independent processes and should not be considered separately (Casper and Castelli 2007). Understanding such interactions is essential for the management and restoration of invaded grassland ecosystems (Wolfe and Klironomos 2005; Eviner and Hawkes 2008). Furthermore, PSFs may change over time (Bartelt-Ryser et al. 2005). For example, Reynolds et al. (2003) determined that positive PSFs influenced early successional communities, resulting in homogenization while negative PSFs resulted in increased diversity. Plant-soil feedback effects may remain after removal of the invasive species, limiting the growth of native species by creating a biotic legacy effect that influences successional changes (Kardol et al. 2007) or the restoration of native species (Kardol and Wardle 2010).

*Eragrostis lehmanniana* (Lehmann lovegrass), is an invasive, perennial C4 bunchgrass that reproduces both sexually and asexually. It was introduced into the United States from South Africa (Cox 1992) as part of efforts to restore degraded rangelands. A seed production program initiated by the USDA-Soil Conservation Service in 1937 led to extensive seeding in northern Mexico, west-Texas, New Mexico, and Arizona (Cox et al. 1982). The only area with extensive documentation of *E. lehmanniana* population expansion is Arizona where *E. lehmanniana* had established on approximately 145,000 hectares in Arizona by 1986 (Cox and Ruyle 1986). It is now known to have formed large monospecific stands in many areas of Arizona and New Mexico. Many of these areas were formerly dominated by the native grass species, *Bouteloua gracilis* (blue grama), a drought tolerant perennial C4 grass of high forage value (Gould 1951).

The expansion of *E. lehmanniana* in the southwestern United States is likely a result of several factors. In addition to the ability to increase tiller production in response to drought (Fernández and Reynolds 2000), *E. lehmanniana* produces great quantities of wind and water-dispersed seeds (Sumrall 1990). Fire is ineffective for control because canopy burning increases *E. lehmanniana* seedling establishment (Biedenbender and Roundy 1996). Even though only one genetic line was introduced into the U.S., and therefore *E. lehmanniana* has limited genetic diversity, it shows a high degree of phenotypic plasticity (Schussman et al. 2006). While phenotypic plasticity, prolific reproduction, and positive response to fire contribute to the success of *E. lehmanniana*

in the U.S., interactions with below ground biota may also facilitate invasion via PSF. In addition, many invasive plant species competitively suppress natives (Levine et al. 2003), and while *E. lehmanniana* likely can competitively suppress native rangeland grasses, few studies have experimentally explored the importance of *E. lehmanniana* competition on community structure that results from *E. lehmanniana* invasion.

As yet, we lack an understanding of the role of plant-mediated soil biotic changes, such as PSF, and their effects on interspecific competition in *E. lehmanniana* invasions in native grasslands. *Eragrostis lehmanniana* invasion may be increasing in part because of PSF, and PSF mediated competition. However, little is known about *E. lehmanniana* PSF, so it is equally possible that these feedbacks could be negative and ultimately limit *E. lehmanniana* invasion. Interspecific competition may also play a role in *E. lehmanniana* invasion, but how PSF influences interspecific competition is not yet understood. Evaluating the influence of PSF on competitive interactions between *E. lehmanniana* and native grasses will enhance understanding of PSF effects on plant competition and supply information that may be invaluable for rangeland restoration in the U.S. southwest.

The goal of this study was to evaluate the roles of competition and PSF in *E. lehmanniana* invasion over an invasion chronosequence. To address this goal, we determined how *E. lehmanniana* PSFs vary over time since invasion and affect competitive interactions between *E. lehmanniana* and the native grass *B. gracilis*. Three questions framing this study were: 1) How does the age of established populations of *E. lehmanniana* affect the strength and direction of *E. lehmanniana* PSF on itself and on *B. gracilis*? 2) How do PSFs created by *E. lehmanniana* affect *B. gracilis* growth? 3) How do PSFs created by *E. lehmanniana* affect competition between *E. lehmanniana* and *B. gracilis*? We predicted: 1) PSF benefits of *E. lehmanniana* to itself and conspecifics would dissipate as time since invasion increased, 2) PSFs created by *E. lehmanniana* would reduce *B. gracilis* biomass production, and 3) PSFs created by *E. lehmanniana* would provide an advantage to *E. lehmanniana* in competitive interactions with *B. gracilis*.

## Methods

### Study area

The Appleton-Whittell Research Ranch (Fig. 1) is managed by the National Audubon Society as a cooperative effort among the National Audubon Society, The Research Ranch Foundation, Bureau of Land Management, The Nature Conservancy, Resolution Copper Company, and the U.S. Forest Service (Kennedy and Robinett 2013). Located near Sonoita, Arizona in Madrean mixed grass prairie, grazing has been excluded from the 8000 acres since 1968 (Kennedy and Robinett 2013).

According to Breckenfeld and Robinett (2001), on the Appleton-Whittell Research Ranch, loamy upland ecological sites occur as mesa tops and fan terraces with neutral to slightly acid pH. A six-inch clay horizon is covered by one to three inches of gravelly



**Figure 1.** Loamy upland sampling sites at Appleton-Whittell Audubon Research Ranch, Sonoita, AZ, USA. Inset shows location of Appleton-Whittell Audubon Research Ranch in Arizona, USA. Dates are the estimated dates of Lehmann lovegrass (*Eragrostis lehmanniana*) invasion. Map data 2021 Google.

sandy loams. *Bouteloua* species dominate areas not invaded by nonnative *Eragrostis* species. Native grasses include *Bouteloua gracilis*, *B. curtipendula* (sideoats grama), and *B. chondrosioides* (sprucetop grama) and are mixed with *Aristida* (threeawn), *Lycurus setosus* (wolftail), *E. intermedia* (plains lovegrass), and *Bothriochloa barbinodis* (cane beardgrass) (Breckenfeld and Robinett 2001).

### Inocula collection

Soil inocula were collected from four loamy upland sites (Fig. 1, Table 1) on the Appleton-Whittell Research Ranch (AWRR) in Sonoita, AZ in 2017 (Fig. 1) on October 23, 2017. Three of these sites had been invaded by *E. lehmanniana* and one site was uninvaded. *E. lehmanniana* invaded these sites in 1949, 1985–2001, and 2003–2006. Ranges of invasion ages are based on best estimates from staff at the AWRR. At each invaded site, we collected 50 ml soil samples from 0–10 cm immediately below the root crown for each of 60 *E. lehmanniana* plants. Crown circumference of each plant was recorded. A hand trowel, sterilized with 70% ethanol solution between samples, was used to collect soil and transfer soil to 50 ml Falcon centrifuge tubes. In the uninvaded site, we collected 50 ml soil samples from the rhizosphere of 60 *B. gracilis* plants using a sterilized hand trowel. A total of 240 soil samples were collected and stored in

**Table 1.** Locations of loamy upland soil sample collection sites at Appleton-Whittell Audubon Research Ranch, Sonoita, AZ, USA.

Site	North American Datum 1983	Universal Transverse Mercator (UTM)	
	Latitude / Longitude	Easting	Northing
Uninvaded	31.571065, -110.492825	547152	3495008
2003–2006	31.580104, -110.489865	545463	3494981
1985–2001	31.591461, -110.498605	547515	3495289
1949	31.575366, -110492261	547712	3496438

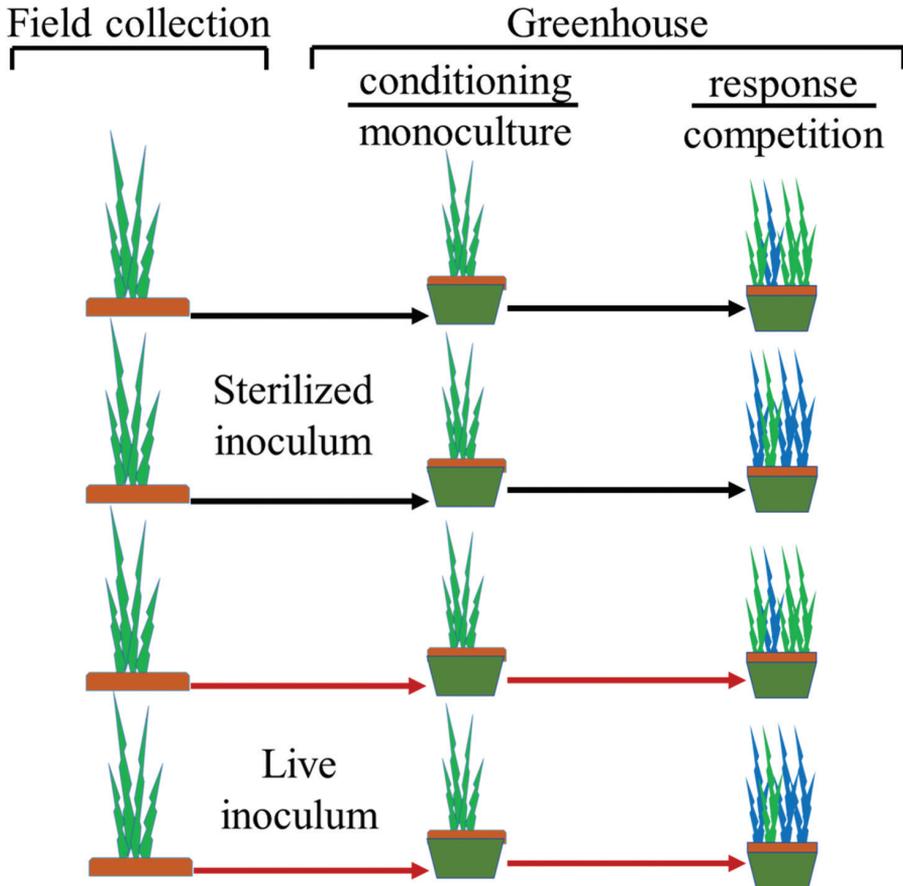
an insulated cooler for transportation and kept at 26 °C for 30 days before use. Because soils were dry when collected, storage at room temperature was unlikely to lead to any rapid biological transformations. This storage temperature approximated the historical mean monthly temperature at the sample collection site when samples were collected. Any potential increase in soil respiration due to the difference in temperature was prevented by lack of available moisture (Conant et al. 2004).

### Inoculum and bulk soil preparation

One half of the soil samples from each of the four locations was randomly selected to be used as “live” inoculum. The remainder was sterilized by autoclave for use as “sterile” control inoculum for the PSF experiment. A local sandy loam soil collected from the Chihuahuan Desert Rangeland Research Center near Las Cruces, NM was used as the growing media. We collected 0–20 cm of the soil surface and then double steam pasteurized the soil in a Heavy Duty Pro-Grow Soil Sterilizer at 88–93 °C over 48 consecutive hours with mixing at 24 h. Soil was added to the soil sterilizer in layers, with each layer being wetted initially and at 24 h after the start of pasteurization, when soil was mixed for the second round of pasteurization.

### Experimental design

We conducted a two-phase plant-soil feedback experiment (Fig. 2, Brinkman et al. 2010). *Eragrostis lehmanniana* conditioned soil microbial communities in Phase 1. Growth of *B. gracilis* and *E. lehmanniana* indicated response to microbial communities in Phase 2. We used a randomized complete block design with five levels of competition, four invasion times, two soil treatments (living and sterile), four plants per pot, and six replicates for a total of 240 pot-level experimental units. On November 25, 2017, three percent inoculum was added to 97 % pasteurized sandy loam field soil to create a total of 1L of medium per pot. The use of soil inoculum isolates the effect of soil biota from other soil properties (Brinkman et al. 2010). Each pot was inoculated with one soil sample collected from one plant. Treatment with live, non-autoclaved *B. gracilis* inoculum yielded a single uninvaded treatment, and treatments with live, non-autoclaved *E. lehmanniana* inoculum from three invaded sites yielded three invaded treatments.



**Figure 2.** Experimental design for plant-soil feedback experiment based on Brinkman et al. (2010) combined with de Wit and van der Bergh (1965) replacement series for plant competition experiment.

### Greenhouse experiment – conditioning phase

In Phase 1, the conditioning phase, all pots were seeded with *E. lehmanniana*. Because a high amount of plant biomass was desired to facilitate the proliferation of soil microbes from minimal inoculum, *E. lehmanniana* was seeded at a high density (100–150 seeds per pot). This resulted in approximately 50 to 75 plants per pot. While there were different numbers of *E. lehmanniana* plants per pot, this likely did not affect biomass produced, as the density was high enough to ensure the effect of the law of constant final yield (Weiner and Freckleton 2010). To provide time for the microbial community from the inoculant to proliferate throughout the soil, plants were grown for 12 weeks during the conditioning phase. After 12 weeks, all aboveground biomass was harvested by clipping at soil level, oven-dried at 60 °C for 48 h, and weighed. The pots were air-dried at ambient greenhouse temperature for two weeks to ensure that *E. lehmanniana* plants were dead prior to Phase 2.

## Greenhouse experiment – response phase

In Phase 2, we used a replacement series design (de Wit and Van den Bergh 1965) to determine competition effects between species, and the effects of site, defined by *E. lehmanniana* invasion age, on competition. This design, common in plant competition studies, holds plant density constant and varies the relative proportion of two species, A and B. If species A is the superior competitor, the relative yield of species B in competition with species A will be less than when species B is grown in monoculture. Likewise, the relative yield of species A will be higher in competition with species B than it would be when grown in monoculture. In our experiment, plant density was held constant at four plants per pot while one of five ratios, 0:4, 1:3, 2:2, 3:1, 4:0, of *E. lehmanniana* and *B. gracilis* was randomly assigned to each pot from Phase 1, stratified by site. Treatments were divided equally among five blocks. Each pot was divided into four sections using wooden popsicle sticks cut to fit in the pot. This ensured seeds from the two species remained separate for germination. Approximately 25 *E. lehmanniana* and *B. gracilis* seeds were sown in the randomly assigned section of the soil surface. Preliminary germination trials indicated *Bouteloua gracilis* seeds needed three more days for germination than *E. lehmanniana* seeds (data not shown). Therefore, *B. gracilis* seeds were sown three days prior to *E. lehmanniana* so all plants would emerge simultaneously. Two weeks after emergence, when it was possible to identify seedling species, seedlings were thinned so that each pot contained only four equidistant plants. Phase 2 plants were grown for 12 weeks at which time the aboveground biomass was harvested and dried as described for Phase 1.

In both phases, pots were watered daily to maintain a moist growth environment. Pots were fertilized once between Phase 1 and Phase 2 with 20 ml Miracle-Gro Water Soluble All Purpose Plant Food (24-8-16).

## Plant-soil feedback

Plant-soil feedback values were calculated using above-ground biomass per pot in each phase. We had six replicates for each level of competition, inoculum, and invasion age. Within each set of matching combinations of competition and invasion age, we randomly paired each of six sterile pots with one of the six live pots to calculate one PSF value for each pair of pots. This resulted in a total of six PSF values for each factor and treatment following Petermann et al. (2008). Plant-soil feedback values were calculated using untransformed biomass values as:

$$\text{PSF} = \ln \left( \frac{\text{biomass live inoculum}}{\text{biomass sterile inoculum}} \right) \quad (1)$$

whereas biomass was the above-ground plant material in a single pot. This formula was chosen based on recommendations in Brinkman et al. (2010) so all feedback scores were symmetrical around zero. Feedbacks are described from the plant perspective and

aligned with common usage in PSF research (Brinkman et al. 2010; van der Putten et al. 2016). Positive and negative PSF values indicate higher and lower biomass production with live inoculum, respectively.

### Replacement series and relative yield

Relative yield (RY) and relative yield total (RYT) were calculated according to de Wit and Van den Bergh (1965):

$$RY = \left( \frac{Y_x}{Y_m} \right) \quad (2)$$

$$RYT = \left( \frac{Y_{ix}}{Y_{im}} \right) + \left( \frac{Y_{jx}}{Y_{jm}} \right) \quad (3)$$

where  $Y_x$  is yield in mixture and  $Y_m$  is yield in monoculture for relative yield and where  $i$  and  $j$  are *E. lehmanniana* and *B. gracilis*, respectively, for relative yield total. Relative yields instead of absolute yields were used because the biomass produced by the two species were qualitatively very different (Jolliffe 2000).

### Statistical analysis – invasion chronosequence and plant-soil feedback

In the response phase, PSFs were analyzed as a function of species, site, and competition as fixed effects, block as random effect, and all two- and three-way interactions of fixed effects using a linear mixed-effects model with PSF as a normally distributed response variable. Conditioning phase biomass and crown circumference were evaluated as covariates but were not significant and were removed from the model. Data were subset for specific comparisons when an interaction term was significant. In addition, data from monocultures were analyzed as a function of species and site as fixed effects, block as random effect, and factorial interactions of all fixed effects using a linear mixed-effects model with PSF as the response variable. Data were then subset by species and analyzed as a function of site as a fixed effect and block as a random effect using a linear mixed-effects model with PSF as the response variable. When significant differences in mean PSF were detected among site and treatment, we used post hoc testing using Tukey's Honest Significant Difference (H.S.D.,  $p < 0.05$ ) to identify treatments with different effects.

### Statistical analysis – competition with and without plant-soil feedback

Lovegrass-grama competition without PSF was analyzed using paired t-tests that tested the null hypothesis that the actual relative yield was equal to the expected relative yield at each competition ratio and for each species. To test if *E. lehmanniana* PSF

provided an advantage to *E. lehmanniana* in competitive interactions with *B. gracilis*, we analyzed the significance of the difference between mean relative yield in sterile vs living soil for a given *E. lehmanniana* : *B. gracilis* ratio using the same linear mixed-effects model and Tukey H.S.D *post hoc* tests with relative yield as the gamma-distributed response variable.

All data were analyzed using IBM SPSS Statistics 25 (IBM, 2018). Validity of models was assessed with plots of fitted vs. residuals to check for constant variance and to ensure there were no negative fitted values. A Levene test and visual assessment of residuals were used to ensure homoscedasticity. Normal probability (Q-Q) plots were used to ensure the random effects were normally distributed. Wald chi-square statistics were calculated for linear mixed models using SPSS MIXED (IBM, 2018).

## Results

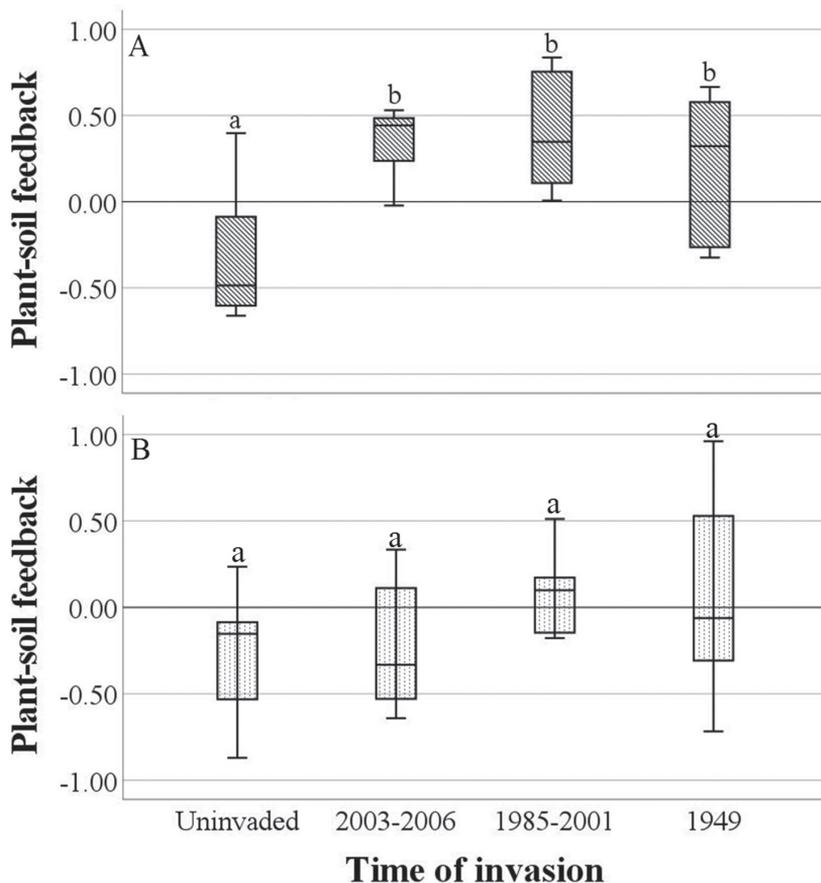
### Invasion chronosequence and plant-soil feedback

While conditioning biomass showed a weak, positive correlation to inoculum source-plant crown circumference ( $r = 0.129$ ,  $p = 0.016$ ), there was no evidence of relationship between source-plant crown circumference and above-ground biomass produced in the response phase. Therefore, crown circumference was excluded as a covariate for subsequent analyses. Neither *E. lehmanniana* nor *B. gracilis* response phase biomass was affected by *E. lehmanniana* conditioning phase biomass. Conditioning phase biomass was not correlated with response phase PSF for either species. Therefore, conditioning phase biomass was not included as a covariate for response phase analysis.

Plant soil feedbacks on *B. gracilis* in soil from the uninvaded area were significantly different from PSFs on *B. gracilis* in invaded soils ( $F_{3,20} = 9.488$ ,  $p < 0.001$ , Fig. 3a). Plant-soil feedbacks developed in uninvaded soils were negative and resulted in decreased *B. gracilis* above-ground biomass. Plant-soil feedbacks on *B. gracilis* were positive in all invaded sites and resulted in increased above-ground biomass (Fig. 3a). Plant-soil feedbacks on *E. lehmanniana* were not significantly different from zero (Fig. 3b) over all invasion times.

### Competition without plant-soil feedback

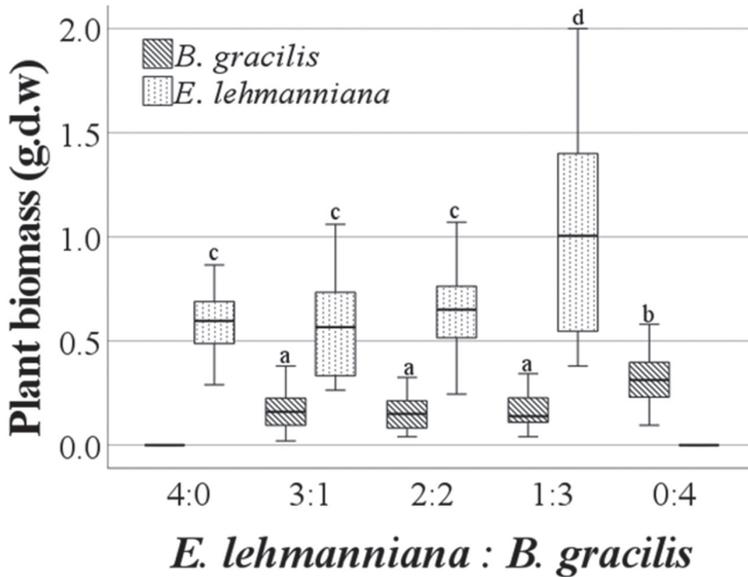
To evaluate competition independently of PSF, mean above-ground biomass per plant was evaluated across competition ratios for sterile treatments (Fig. 4), using pooled data from all sites. When grown in sterile soil inoculum, *E. lehmanniana* mean per plant biomass was equal across *E. lehmanniana* : *B. gracilis* ratios of 4:0, 3:1, and 2:2, yet increased at 1:3 ( $F_{3,89} = 10.932$ ,  $p < 0.001$ , Fig. 4). In contrast, when grown in sterile soil inoculum, *B. gracilis* mean per plant biomass was equal across *E. lehmanniana* : *B. gracilis* ratios of 3:1, and 2:2, 1:3, and increased at 0:4 (Fig. 4,  $F_{3,90} = 12.475$ ,  $p < 0.001$ ).



**Figure 3.** Plant-soil feedbacks for **A** *Bouteloua gracilis* and **B** *Eragrostis lehmanniana* monocultures grown in soils conditioned for 12 weeks by *E. lehmanniana*. Plants were grown for 12 weeks prior to harvesting in the response phase. Soil inoculum collected from sites of known lovegrass invasion times on Appleton-Whittell Audubon Research Ranch, Sonoita, AZ. ( $n = 6$ ). Similar letters over the bars indicate no difference in plant-soil feedbacks between invasion times. The boxes represent 25–75% interquartiles. The bold black lines inside the box represent the medians. Top and bottom whiskers indicate the maximum and minimum values, respectively. Values greater than zero indicate that a species performed better on live soil than on sterile soil, and vice versa.

### Competition with plant-soil feedbacks

Data were pooled for each site because relative yield competition outcomes for each ratio did not vary across sites ( $p > 0.05$ ). Live inoculum had little effect on relative yield of either species (Fig. 5), except at the highest (3:1) *E. lehmanniana*: *B. gracilis* ratio. *B. gracilis* yielded less than its hypothetical yield, as demonstrated by the yield line shifting to below the hypothetical expected yield line (Fig. 5). *Eragrostis lehmanniana* displayed the opposite trend with its relative yield shifted to above its hypothetical expected yield line (Fig. 5). Across competition levels, relative yield total was only slightly less than expected for all sites combined.

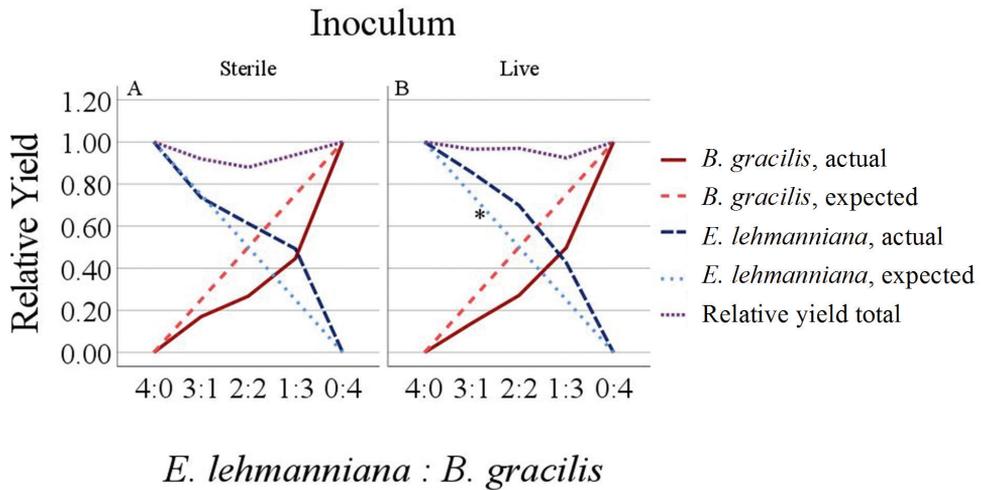


**Figure 4.** Median per plant above-ground *Bouteloua gracilis* and *Eragrostis lehmanniana* biomass (grams dry weight) produced in a replacement series competition experiment in soils conditioned for 12 weeks by *E. lehmanniana* with sterile inoculum. Plants were grown for 12 weeks prior to harvesting. Within species, significant differences among *E. lehmanniana* ( $F_{3,89} = 10.932$ ;  $p < 0.001$ ) and *B. gracilis* ( $F_{3,90} = 12.475$ ;  $p < 0.001$ ) biomass are represented by letters, with similar letters over the bars indicating no difference in mean biomass between competition ratios. The boxes represent 25–75% interquartiles. The bold black lines inside the box represent the medians. Top and bottom whiskers indicate the maximum and minimum values, respectively.

## Discussion

The goal of this study was to evaluate the roles of PSF and competition in *E. lehmanniana* invasion into *B. gracilis* communities over time. We estimated net plant-soil feedbacks to determine the influence of *E. lehmanniana* invasion age on *B. gracilis* growth and competitive outcomes between the two species. Our results showed that *E. lehmanniana* invasion created interspecific PSFs that benefited *B. gracilis*. However, this effect was only present when *B. gracilis* was grown in soils conditioned with inocula from *E. lehmanniana* invaded communities. *Bouteloua gracilis* growth was inhibited when grown in soil conditioned by *E. lehmanniana* with inoculum from the native *B. gracilis* community, indicating that during the initial phases of an invasion, *B. gracilis* would suffer a negative PSF. Plant-soil feedbacks on *E. lehmanniana* were not significantly different from zero. Despite being beneficial through PSF to *B. gracilis* under many conditions, *E. lehmanniana* outcompeted *B. gracilis* over all competition levels. We found no significant differences in competition outcomes between live and sterile inoculum from *E. lehmanniana* populations of four invasion ages that would indicate PSF influences competition, apart from the highest ratio of *E. lehmanniana* to *B. gracilis*.

The addition of fertilizer in our experiment may have ameliorated negative PSF (Brinkman et al. 2010). Thus, it is possible that the positive PSF documented on



**Figure 5.** Replacement series competition study of relative yields of *B. gracilis* and *E. lehmanniana* across competition ratios grown in soil cultured with **A** sterile and **B** live inoculum. Soils were conditioned for 12 weeks by *E. lehmanniana*. Plants were grown for 12 weeks prior to harvesting in the response phase. Soil inoculum collected from sites of known lovegrass invasion times on Appleton-Whittell Audubon Research Ranch, Sonoita, AZ. The expected (hypothetical) lines represent the relative yield that would be expected if species did not compete with one another (e.g., the relative yield for a species A, planted with a competitor B, at A:B planting ratios of 0:4, 1:3, 2:2, 3:1 and 4:0 are 0, 0.25, 0.5, 0.75 and 1, respectively). If one species is outcompeted, it will yield less than expected and its curve will shift to below the expected line. The line for relative yield total will be convex for facilitation or concave for competition. The superior competitor will yield more than expected and its curve will shift to above its expected line. The asterisk indicates the proportion of *E. lehmanniana* at which plant soil feedbacks increased *E. lehmanniana* yield relative to yield in the sterile soil pots.

*B. gracilis* are the result of fertilization overcoming the negative affect of nutrient depletion as a feedback mechanism. Desert soils are often nitrogen poor (Peterjohn and Schlesinger 1991), and fertilizer addition can have great effect on plant growth especially in a greenhouse setting (Manning et al. 2008). Nutrient addition may also have decreased any positive feedback effects by reducing the benefit of microbes that assist in nutrient acquisition or increasing plant pathogen populations (Revillini et al. 2016). Nonetheless, we felt that fertilization was necessary after the conditioning phase where plants showed signs of nutrient deficiency. Further, fertilization increases plant-plant competition (Rajaniemi 2002) and facilitated the testing of *B. gracilis* – *E. lehmanniana* competition under the necessarily short time frame and limited growth space of a greenhouse PSF experiment (Forero et al. 2019).

### Invasion chronosequence and plant-soil feedback

Though the mean *E. lehmanniana* PSF values indicated the potential for PSF to become more positive over time, the ages of established populations of *E. lehmanniana* did not significantly affect the strength and direction of *E. lehmanniana* PSF. Plant-soil feedbacks

effects on grasses are predominantly negative and 70 % of 329 experiments have resulted in negative PSF effects (Kulmatiski et al. 2008). Our mean *E. lehmanniana* intraspecific PSF effects ranged between -0.259 and 0.961, values higher than the -0.53 average for nonnative perennials reported by Kulmatiski et al. (2008). Diez et al. (2010) found that as invasive plant residence time and spread increased, PSFs became more negative; however, relatively few studies have evaluated PSF over decades of invasion residence time, as we have done. Many invasive plants develop greater negative PSF over time (Bever 2003; Reinhart and Callaway 2006). *Eragrostis lehmanniana* intraspecific PSFs were neutral across 68 years of invasion, suggesting this species may be unresponsive to soil microbiota.

Te Beest et al. (2009) suggested that the ability to increase plant performance in soils conditioned by heterospecifics may be a mechanism favoring invasion, especially for plants that easily disperse into new habitats via seed or propagule dispersal. *Eragrostis lehmanniana* PSFs were detrimental to *B. gracilis* in uninvaded soils. We hypothesize that once *E. lehmanniana* individuals become established they may condition the soil to the detriment of *B. gracilis*, facilitating further establishment and spread of *E. lehmanniana*. *Bouteloua gracilis* may subsequently respond positively to *E. lehmanniana* PSF, but the superior competitive ability of *E. lehmanniana* will negate any beneficial PSF effect on *B. gracilis*.

Many previous studies have shown that plants tend to perform better in soils conditioned by heterospecifics (Kulmatiski et al. 2008), and our results only partially support this idea. *E. lehmanniana* PSF conferred a benefit to *B. gracilis* in soils that were previously invaded by *E. lehmanniana*. However, in uninvaded soils, heterospecific feedbacks negatively affected *B. gracilis* performance. In a meta-analysis to determine the relative importance of competition and PSF, Lekburg et al. (2018) suggested that in resource-limited environments facilitative interactions are likely to be enhanced by PSFs. The combination of inter- and intraspecific PSF effects may potentially help maintain diversity and contribute to invasion resistance (Klironomos 2002; Reinhart et al. 2003; Te Beest et al. 2009). The ability to create monocultures despite beneficial interspecific PSF effects indicates that *E. lehmanniana* possesses other, more effective, traits for invasion, such as competitive ability.

## Plant-soil feedback and competition

Though PSF can modify competitive interactions and vice versa (Casper and Castelli 2007), *Eragrostis lehmanniana* was a stronger competitor than *B. gracilis* regardless of PSF effects. Most exotic plant species exert a strong competitive effect against native plant species (Levine et al. 2003) and *E. lehmanniana* is no exception. Though *E. lehmanniana* competitively suppressed *B. gracilis*, competition between these two species reduced total relative yield, likely due to intraspecific competitive suppression by *E. lehmanniana*. Additional research is needed to fully understand the importance of this interaction in *E. lehmanniana* invasions.

*Eragrostis lehmanniana* PSFs affected competition only when at 75% *E. lehmanniana* density. At lower densities, the effects of competition were much greater than

PSF effects. Apart from the highest ratio of *E. lehmanniiana* to *B. gracilis*, we found no differences in outcomes of competition between live and sterile inoculum from *E. lehmanniiana* populations of four invasion ages that would indicate PSF influences competition. Similarly, when investigating how community context altered plant–soil feedback between the non-native invasive forb *Lespedeza cuneata* and co-occurring native prairie species, Crawford and Knight (2017) found that a beneficial intraspecific PSF effect had no effect on competitive outcomes. However, Lekberg et al. (2018) determined that at low densities, PSF was overwhelmed by the strength of competition. We found that PSF was overwhelmed at low *E. lehmanniiana* density, yet influenced competition at higher density, indicating a density-dependent effect that likely contributes to the invasiveness of *E. lehmanniiana*. Even without PSF, *E. lehmanniiana* is the superior competitor in *E. lehmanniiana*-*B. gracilis* interactions.

Wubs and Bezemer (2017) found that competitive hierarchies are altered by PSF if conditioned by a single species. However, if multiple species have conditioned the soil, plant evenness increases due to the PSF-induced similarity of competitive ability across species (Wubs and Bezemer 2017). Future research in this system should include individual and combined conditioning by *B. gracilis* as well as *E. lehmanniiana* and should investigate the resultant competitive outcomes between the two species. Our results differ from Xue et al. (2018) who determined that PSF effects are enhanced by interspecific competition. In our study, PSF influenced competition only in soils developed from the oldest site and only at high *E. lehmanniiana* proportions.

Based on previous understanding (Casper and Castelli 2007), our results explain some variation within the PSF interactions at the seedling stage of *B. gracilis* and *E. lehmanniiana*. Future research should attempt to quantify PSF interactions of these species over a longer growth period to determine if *E. lehmanniiana* continues to provide a benefit via PSF to *B. gracilis* as plants mature and if competition outweighs this benefit. By utilizing soil inoculum from mature plants in established populations, our study helps develop the understanding of the changes in PSF potential over years of population habitation.

To further elucidate the function of PSFs in plant invasions, future research should include growth of *E. lehmanniiana* in soil conditioned by heterospecific and conspecific individuals at varying plant densities. The mechanisms by which *E. lehmanniiana* interacts with specific soil microorganisms also needs investigation. In addition, differences in biomass allocation resulting from soil conditioning by conspecifics and heterospecifics may influence reproduction and competitive ability, influencing range expansion (Te Beest et al. 2009) and have yet to be investigated in *E. lehmanniiana*.

## Conclusions

We rejected our prediction that PSF benefits of *E. lehmanniiana* to itself and conspecifics would dissipate as time since invasion increased. Plant-soil feedbacks provided no benefit to *E. lehmanniiana*, nor did this change over time. With respect to our

prediction that *E. lehmanniana* PSFs would inhibit *B. gracilis* biomass production, we determined that contrary to our prediction, *B. gracilis* benefited from PSFs under all conditions except uninvaded. Our third prediction that *E. lehmanniana* competition would be enhanced by PSF was only partially confirmed. Plant-soil feedback did not provide an advantage to *E. lehmanniana* in competitive interactions with *B. gracilis* at low competition levels but were advantageous to *E. lehmanniana* at the highest competition ratio, indicating a possible density-dependent effect.

Plant and soil-microbial communities are responsive to biotic and abiotic conditions that affect associated plants (Ehrenfeld et al. 2005). Conditions such as climate shifts, plant species assemblages, and herbicide use may change the diversity of associated mycorrhizae and microbial functional groups. These changes in turn affect both plant and community function. Determining the occurrence of PSFs in an exotic species such as *E. lehmanniana* is the first step in defining the functional significance of changes in the microbial community structure on invaded communities and ecosystems.

## Acknowledgements

We thank Kirsten Romig of the Jornada Experimental Range for seedling identification, the Jornada Basin LTER for graduate student support, and Darren James of the Jornada Experimental Range for statistical advice. We are grateful to Appleton-Whittell Audubon Research Ranch for allowing us access for sampling, to Linda Kennedy for helping us to locate appropriate sampling sites on the ranch, and Cristina Francois for additional information regarding the ranch. This work was partially supported by the USDA National Institute of Food and Agriculture, Hatch project NMLEhnhoff-17H.

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# Invasive ants reduce nesting success of an endangered Hawaiian yellow-faced bee, *Hylaeus anthracinus*

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Academic editor: J. Sun | Received 23 September 2020 | Accepted 21 December 2020 | Published 28 January 2021

**Citation:** Plentovich S, Graham JR, Haines WP, King CBA (2021) Invasive ants reduce nesting success of an endangered Hawaiian yellow-faced bee, *Hylaeus anthracinus*. NeoBiota 64: 137–154. <https://doi.org/10.3897/neobiota.64.58670>

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## Abstract

Hawaii has a single group of native bees belonging to the genus *Hylaeus* (Hymenoptera: Colletidae) and known collectively as Hawaiian yellow-faced bees. The majority of the 63 species have experienced significant declines in range and population. In 2016, seven species received federal protection under the Endangered Species Act of 1973. Competitors and predators, such as invasive bees, wasps and ants, are thought to be important drivers of range reductions and population declines, especially at lower elevations where more non-native species occur. We evaluated the effects of invasive ants on nesting *Hylaeus anthracinus* using artificial nest blocks that allowed us to track nest construction and development. The blocks were placed in pairs at 22 points encompassing three sites on the north and east sides of Oahu. One block in each pair was treated with a sticky barrier to prevent access by ants, while the other block remained untreated. From December 2015 to December 2016, we monitored 961 individual nests in the blocks. Seventy percent of nests in control blocks were invaded by ants. Nests in treated blocks were more likely to produce at least one adult than nests in untreated blocks (38% vs. 14%, respectively). In untreated blocks, ants were the most common cause of nest mortality followed by lack of development, displacement (primarily by the competitor *Pachodynerus nasidensis*) and presumed pathogens. The invasive ant, *Ochetellus glaber* was the only observed nest predator, although the big-headed ant, *Pheidole megacephala* was also present. *Hylaeus anthracinus* inhabits coastal strand habitat which occurs in a narrow band just above the high tide line. Nests at one site were destroyed due to a high wave event, highlighting this species' vulnerability to sea level rise. Additionally, no adult bees or nests were observed at the points where yellow crazy ants, *Anoplolepis gracilipes* were established. An increased understanding of the factors limiting Hawaii's yellow-faced bees will provide information for future conservation efforts that may include landscape-scale ant control, habitat restoration and translocations.

**Keywords**

*Anoplolepis gracilipes*, *Hylaeus anthracinus*, insect conservation, invasive ants, *Ochetellus glaber*, solitary bees, yellow crazy ants

**Introduction**

The Hawaiian archipelago has a single group of native bees (Hymenoptera, Colletidae, *Hylaeus*), known collectively as Hawaiian yellow-faced bees. A monophyletic radiation produced at least 63 species, all of which are endemic to one or more islands in the archipelago (Magnacca 2011). Hawaiian yellow-faced bees were once one of the most abundant and widespread insect groups in the Hawaiian Islands (Perkins 1899). The group evolved with elements of the flora to form mutualistic plant/pollinator relationships (Howarth 1985; Hopper 2002; Daly and Magnacca 2003). There are few ecological studies of Hawaiian *Hylaeus* and the limited information available indicates significant declines in population and range (Magnacca 2007; Daly and Magnacca 2003; Magnacca and King 2013). In 2016, seven species received federal protection under the United States Endangered Species Act of 1973 (USFWS 2016).

Loss of both foraging and nesting habitat, competition with introduced Hymenoptera and predation by introduced arthropods may contribute to population and range reductions in Hawaiian yellow-faced bees (Hopper 2002; Lach 2008; Wilson and Holway 2010; Sahli et al. 2016; Ing and Mogren 2020). Invasive ants are thought to be a significant threat because Hawaiian yellow-faced bees, like the rest of Hawaii's endemic arthropods, are thought to have evolved in the absence of ants (Perkins 1899; Krushelnycky et al. 2005). *Hylaeus* populations may be suppressed in areas where invasive ants are present (Cole et al. 1992; Sahli et al. 2016). Invasive ants compete with *Hylaeus* bees for floral nectar and *Hylaeus* bees avoid flowers when certain ants are present (Lach 2008). The relative importance of ant predation versus resources competition on *Hylaeus* is unknown (Magnacca 2007).

Hawaiian yellow-faced bees are cavity nesters that use dead, hollow stems in vegetation or holes on the ground in soil, sand, coral rubble and under rocks (Cole et al. 1992; Magnacca 2007). They lack specialised mouthparts for excavating and are thought to be reliant on other species for initiation of holes in wooden substrates (Magnacca 2007). Females line nests with a cellophane-like, membranous material composed of lipid polymer and protein (Espelie et al. 1992). They provision cells with pollen carried in their crops and there are often multiple cells in a single nest (Daly and Coville 1982). Given the difficulty of finding and monitoring *Hylaeus* nests, very little is known about nesting ecology and factors limiting nest success. Some Hawaiian *Hylaeus* are known to use artificial wooden nest blocks (Daly and Coville 1982).

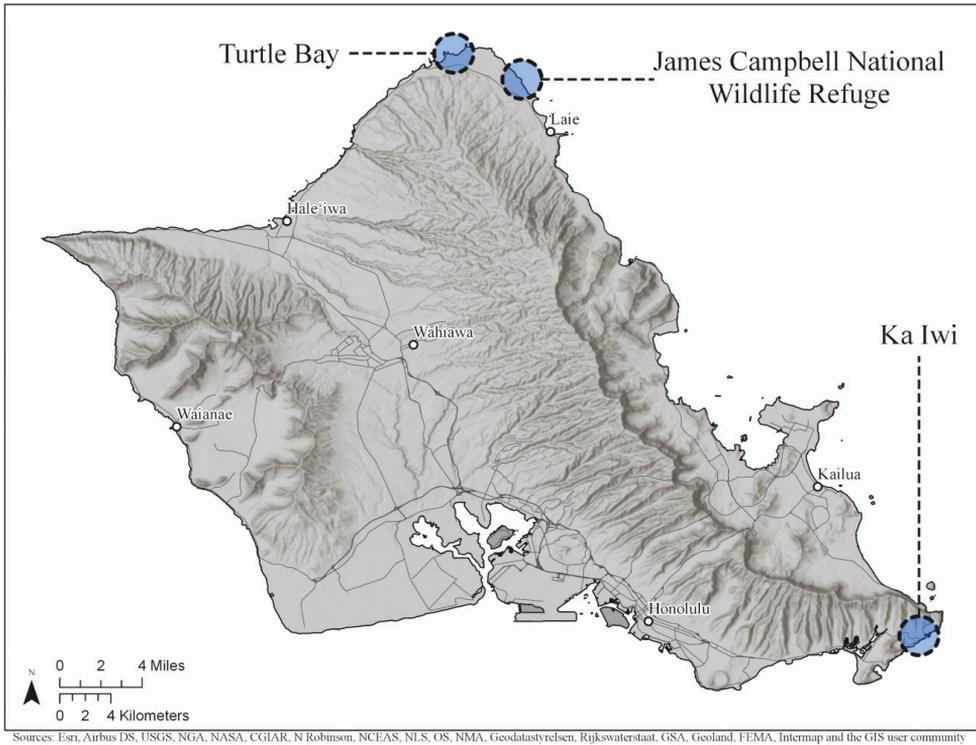
We focused on one endangered species, *Hylaeus anthracinus* (F. Smith, 1853), which primarily occurs in narrow bands of coastal habitat just above the high tide line on Oahu, Lanai, Maui, Molokai and Hawaii Island (Magnacca 2007). The species has been observed

nesting in both hollow stems and coral rubble (Graham and King 2017). No studies have attempted to estimate nesting success or describe brood number, developmental time or causes of mortality. We used artificial blocks with removable clear tubing that allowed us to unobtrusively track nest construction and development. We used a paired experimental design to evaluate the effects of invasive ants on nesting success at three sites on Oahu. We hypothesised that nests in blocks that excluded ants (i.e. treatment blocks) would have increased nesting success and produce more adults compared to nests in blocks that could be accessed by ants (i.e. control blocks). The artificial nest block design allowed us to collect additional information on nest architecture, developmental time and causes of mortality.

## Study sites and methods

### Study sites

Three study sites were selected, based on the presence of known *H. anthracinus* populations. Vegetation at the three sites consisted of coastal strand dominated by the native shrub *Scaevola taccada* (Gaertn.) Robx. and the introduced tree *Heliotropium foertherianum* (Hilger & Diane). Two sites [Turtle Bay (21.706075, -157.996561) and James Campbell Wildlife Refuge (JCNWR, 21.689633, -157.948752)] were on the northern coast of Oahu and one site (Ka Iwi, 21.292859, -157.660334) was on the southern shore (Figure 1). The number of monitoring points ( $n = 22$ ) differed at the sites depending on the extent of available habitat with 15 points at JCNWR (labelled 1–15), five at Ka Iwi (labelled 16–20) and two points at Turtle Bay (labelled 21 and 22). All points were separated by a minimum of 15 metres. The two points at Turtle Bay were on a narrow peninsula, while the 15 points at JCNWR were along a long stretch of coastline (Figure 2). Based on surveys conducted in advance of nest-block deployment, the invasive black household ant [*Ochetellus glaber* (Mayr, 1862)] and the big-headed ant [*Pheidole megacephala* (Fabricius, 1793)] were present at all sites. The two species appear to partition the habitat; the twig-nesting *O. glaber* was primarily found in the vegetation, while the ground-nesting *P. megacephala* was found on the ground and was only occasionally observed foraging in the vegetation. The yellow crazy ant [*Anoplolepis gracilipes* (F. Smith, 1857)] was invading James Campbell National Wildlife Refuge from the east and the species was present at three of the 15 points at JCNWR (i.e. 13, 14 and 15). This species is ground-nesting, but was seen in high numbers foraging in the vegetation. Each of the three study sites also supported populations of invasive bees and wasps including, but not limited to, *Apis mellifera* Linnaeus, 1758 (Apidae), *Ceratina smaragdula* (Fabricius, 1787) (Apidae), *Hylaeus strenuus* (Cameron, 1897) (Colletidae), *Lasiglossum* sp. (Halictidae), *Megachile* sp. (Megachilidae) and *Pachodynerus nasidens* (Latreille 1812) (Halictidae). All Hymenoptera were initially captured and examined with a hand lens and/or microscope to observe primary characters. The Pacific Invasive Ant Key (<http://idtools.org/id/ants/pial/>) was used to identify ants and bees were identified using various taxonomic resources (Michener 2000; Daly and Magnacca 2003;



Sources: Esri, Airbus DS, USGS, NGA, NASA, CGIAR, N Robinson, NCEAS, NLS, OS, NMA, Geodatastyrelsen, Rijkswaterstaat, GSA, Geoland, FEMA, Intermap and the GIS user community

**Figure 1.** Three field sites, Turtle Bay, James Campbell National Wildlife Refuge and Ka Iwi (clockwise from top left) used to study the effects of invasive ants on nesting Hawaiian yellow-faced bees (*Hylaenus anthracinus*) on Oahu, Hawaii. The number of monitoring points (2, 15 and 5, respectively) varied due to extent of habitat.

Snelling 2003) and verified through comparison with existing specimens at the University of Hawaii and/or Bishop Museum Entomology collections and consultation with taxonomic experts (K. Magnacca). Specimens were deposited at the University of Hawaii Insect Museum (UHIM).

## Experimental design

We used artificial nest blocks to evaluate the effects of invasive ants on *H. anthracinus* nests. Each wooden block (a section of untreated lumber 30 cm × 3.8 cm × 8.9 cm) had 20 potential nest sites, 10 on each side (Figure 3). Holes were drilled and lined with removable transparent plastic tubes creating artificial nest cavities that had an inner diameter of 4.3 mm and depth of 60 mm. This depth and diameter were based on data collected from dissections of natural nests (Graham and King 2017). The blocks were hung from vegetation using 8 mm diameter rope (Figure 4). Blocks were hung in pairs at the 22 monitoring points within two metres of each other. The rope suspending one randomly-selected block in each pair was treated with a sticky barrier



**Figure 2.** Coastal strand vegetation dominated by the native *Scaevola taccada* and the introduced *Heliotropium foertherianum* at James Campbell National Wildlife Refuge (JCNWR).

(Tree Tanglefoot) to prevent access by ants, while the other block remained untreated. Effort was made to ensure the blocks hung freely and were not in contact with any vegetation that would enable access by ants. We monitored blocks twice each week from December 2015 to December 2016.

During each monitoring session, the status of each potential nest site was assessed by carefully pulling out each transparent plastic tube and observing any nests. When nests were observed, we recorded the number of cells in the nest, whether a larva or pupa was present in each cell and any other relevant observations, such as discolouration or runny consistency of pollen, evidence of predation or provision raiding or the presence of moisture. Each nest was tracked over its entire development to determine its fate (i.e. the final stage reached). Nests were classified as successful if at least one adult *H. anthracinus* appeared to have emerged from the nest, failed if they did not produce at least one adult bee or unknown if the fate could not be determined.

When possible, we identified the likely cause of failure for each failed nest. Potential causes of failure included depredation by ants, lack of development (no larvae observed), presumed pathogen infection, displacement by invasive bee or wasp nests and flooding by rain or seawater. Presumed pathogen infection was based on abnormal colouration or consistency of pollen provisions and may have included fungal, bacterial



**Figure 3.** Treatment (left) and control (right) blocks hanging in *Scaevola taccada*. The rope suspending treatment blocks was treated with a sticky barrier (Tree Tanglefoot) to prevent invasive ants from accessing the blocks.

or viral pathogens, amongst which we did not distinguish. Although we checked nests twice a week, we undoubtedly missed some nesting attempts (i.e. bees started nests that were depredated before the next monitoring event) and we were not able to attribute outcomes to all nests. Nests in which only a back seal was observed with no pollen provisions were not counted as nests and not included in analyses.



**Figure 4.** Hawaiian yellow-faced bee (*Hylaeus anthracinus*) adult female and nest with three cells in plastic tubing from an artificial nest block on Oahu, Hawaii.

## Data analysis

A paired t-test and a Wilcoxon signed-rank test for matched pairs were used to evaluate differences in the number of successful nests and the number of adults produced, respectively, in control vs. treatment blocks at each point ( $n = 20$ ). All statistical tests were done using JMP Version 14 (SAS Institute Inc., Cary, NC, 1989–2020). The two Turtle Bay points were flooded during a high wave event three months into the project and were excluded from analyses because no nests produced adults in treatment or control blocks and *H. anthracinus* was no longer observed at the site through the end of the study in December 2016. The differences between the number of successful nests in control vs. treatment blocks were normally distributed (Shapiro-Wilk test:  $W = 0.954$ ,  $P = 0.43$ ), enabling the use of a paired t-test. We hypothesised that the mean difference between pairs of observations would be greater than zero.

We used a Wilcoxon signed-rank test for matched pairs as a non-parametric equivalent to a paired t-test to evaluate differences in number of adults produced from control vs. treatment blocks because data were not normally distributed (Shapiro-Wilk test:  $W = 0.89$ ,  $P = 0.029$ ). We hypothesised that the mean difference between the number of successful nests in control blocks and the number of successful nests in treatment blocks would be greater than zero.

## Results

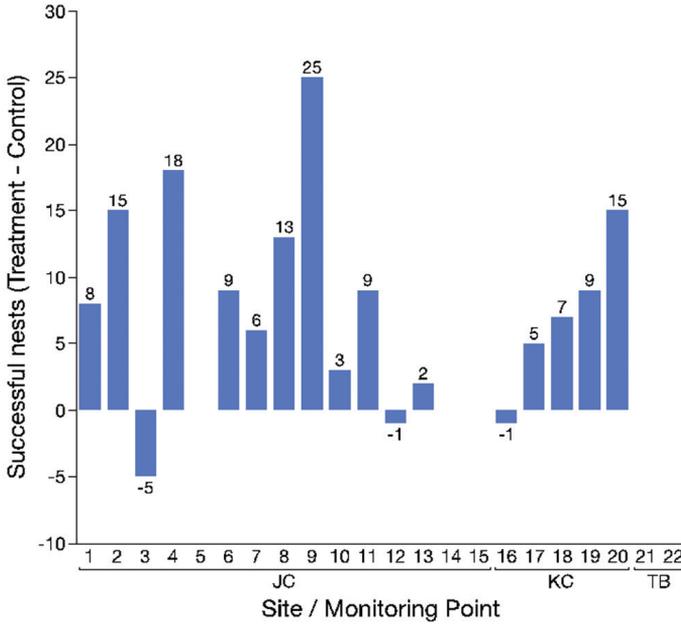
We observed a total of 961 *H. anthracinus* nests from 22 points at three study sites from December 2015 to December 2016. This included 686 nests at JCNWR, 253 nests at

Ka Iwi and 22 nests at Turtle Bay. The first sign of nest initiation was the appearance of a cellophane-like lining that sealed the back of the nest tube; this is typical in the family Colletidae. After this back lining was made, the females would deposit pollen, presumably lay an egg and then seal the chamber off and sometimes begin another cell immediately (Figure 4). The number of cells observed in a nest ranged from zero (back seal with some pollen, but no further progression) to eight with a mean of 2.59 and median of two cells. This distribution was right skewed and most nests had either one (31%) or two (23%) cells. Most nests were found after one or more cells were complete, but in 17 nests with between one and three cells, we were able to record accurate data, within 48 hours from time of nest initiation to within 48 hours of adult emergence. For those 17 nests, at least one adult emerged after an average of 37.6 days (range 29–52 days).

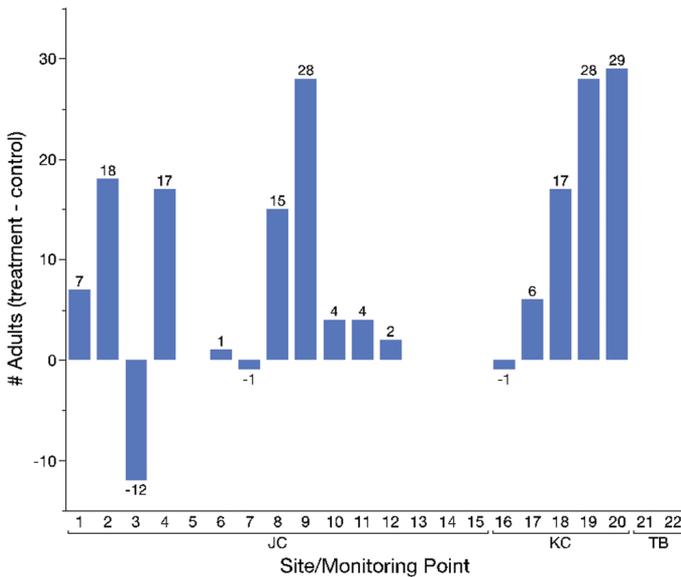
We were able to determine if the nest was successful or failed in 927 of the 961 nests and were able to attribute causes (i.e. successful, depredated, lack of development, pathogen, nesting by invasive wasp, flooded or adult unable to emerge) to 889 nests. In some cases when nests failed, the cause was unclear. For example, a nest may have failed due to the growth of a pathogen and was then invaded by ants. In these cases, we knew the nest failed, but were not able to attribute an outcome. In control and treatment blocks combined, 235 of the 889 (26.4%) nests produced at least one adult and the remaining 682 failed (76.7%). Of the failed nests, 654 (95.9%) could be attributed to a cause including depredated by ants ( $n = 324$ , 47.5%), lack of development ( $n = 201$ , 29.5%), pathogen ( $n = 52$ , 7.6%), nesting by an invasive wasp ( $n = 62$ , 9%), flooded ( $n = 13$ , 1.9%) or adult unable to emerge ( $n = 2$ , 0.3%) and 38 (5.6%) could not be attributed to a cause.

We found support for both of our hypotheses. Treatment blocks from which ants were excluded produced a higher number of successful nests than control blocks that could be accessed by ants (paired t-test:  $t$ -ratio = 4.05,  $DF = 19$ ,  $P > t = 0.0003$ ). The pattern was similar at individual sites, with treatment blocks having significantly higher nest success at both JCNWR (paired t-test:  $t$ -ratio = 3.20,  $DF = 14$ ,  $P > t = 0.0032$ ) and Ka Iwi (paired t-test:  $t$ -ratio = 2.68,  $DF = 4$ ,  $P > t = 0.0275$ ). In treatment blocks, 38.2% (174 of 456) of nests produced at least one adult, compared to only 14.1% (61 of 433) of nests in control blocks (Table 1, Figure 5). Not only were more nests successful in treatment blocks, but they also produced more adults compared to control blocks ( $11.7 \pm 2.69$  vs.  $4.4 \pm 1.15$ , respectively, Wilcoxon Signed Rank:  $S = 75.0$ ,  $P = 0.0012$ , Table 1, Figure 6). This pattern was also similar at both JCNWR: Wilcoxon Signed Rank:  $S = 38.5$ ,  $P = 0.01$ ) and Ka Iwi (Wilcoxon Signed Rank:  $S = 6.5$ ,  $P = 0.06$ ).

The main reason nests in control blocks failed was predation by invasive ants. Throughout the project, we observed ant predation while it was occurring in 274 instances and each time, *O. glaber* was the culprit. We found that ants invaded 70.2% (304 of the 433 nests) of the nests in control blocks (Table 1). Nests are often multi-celled and rarely ( $n = 12$ ) ants invaded a nest, but at least one adult still emerged. Invasive ants breached treatment blocks on eight occasions: twice when treatment blocks fell to the ground due to strong winds, once when a branch was touching a treat-



**Figure 5.** Difference in number of successful nests in control vs. treatment blocks at James Campbell National Wildlife Refuge (JC), Ka Iwi (KC) and Turtle Bay (TB). Numbers above zero indicate points where there were more successful nests in the treatment block (Paired t-test: t-ratio = 4.05, DF = 19,  $P > t = 0.0003$ ). The two Turtle Bay points did not have successful nests due to flooding.



**Figure 6.** Difference in number of adults produced by control vs. treatment blocks at James Campbell National Wildlife Refuge (JC), Ka Iwi (KC) and Turtle Bay (TB). Numbers above zero indicate points where there were more adults produced in treatment blocks (Wilcoxon Signed Rank:  $S = 75.0$ ,  $P = 0.0012$ ). The two Turtle Bay points did not have successful nests due to flooding.

**Table 1.** Summary of control and treatment blocks invaded by invasive ants at Turtle Bay, James Campbell National Wildlife Refuge (JCNWR) and Ka Iwi on Oahu, Hawaii. The two Turtle Bay points were flooded during a high wave event three months into the project and were excluded from analyses because no nests produced adults in treatment or control blocks.

Site	Block	# points	# Successful nests	# adults produced	Mean # adults/ nest	Mean #adults/ block	# Nests invaded by ants	Total nests
Turtle Bay	Control	2	0	0	0	0	9 (64.2%)	14
Turtle Bay	Treatment	2	0	0	0	0	0	8
JCNWR	Control	15	47 (15.1%)	71	0.22	4.7	234 (75.2%)	311
JCNWR	Treatment	15	122 (37.3%)	152	0.46	10.1	10 (3.1%)	310
Ka Iwi	Control	5	14 (12.7%)	25	0.23	5	61 (56.5%)	108
Ka Iwi	Treatment	5	52 (37.7%)	104	0.75	20.8	10 (7.2%)	138
All sites	Control	22	61 (14.1%)	96	0.21	4.4	304 (70.2%)	433
All sites	Treatment	22	174 (38.2%)	256	0.54	11.6	20 (4.4%)	456

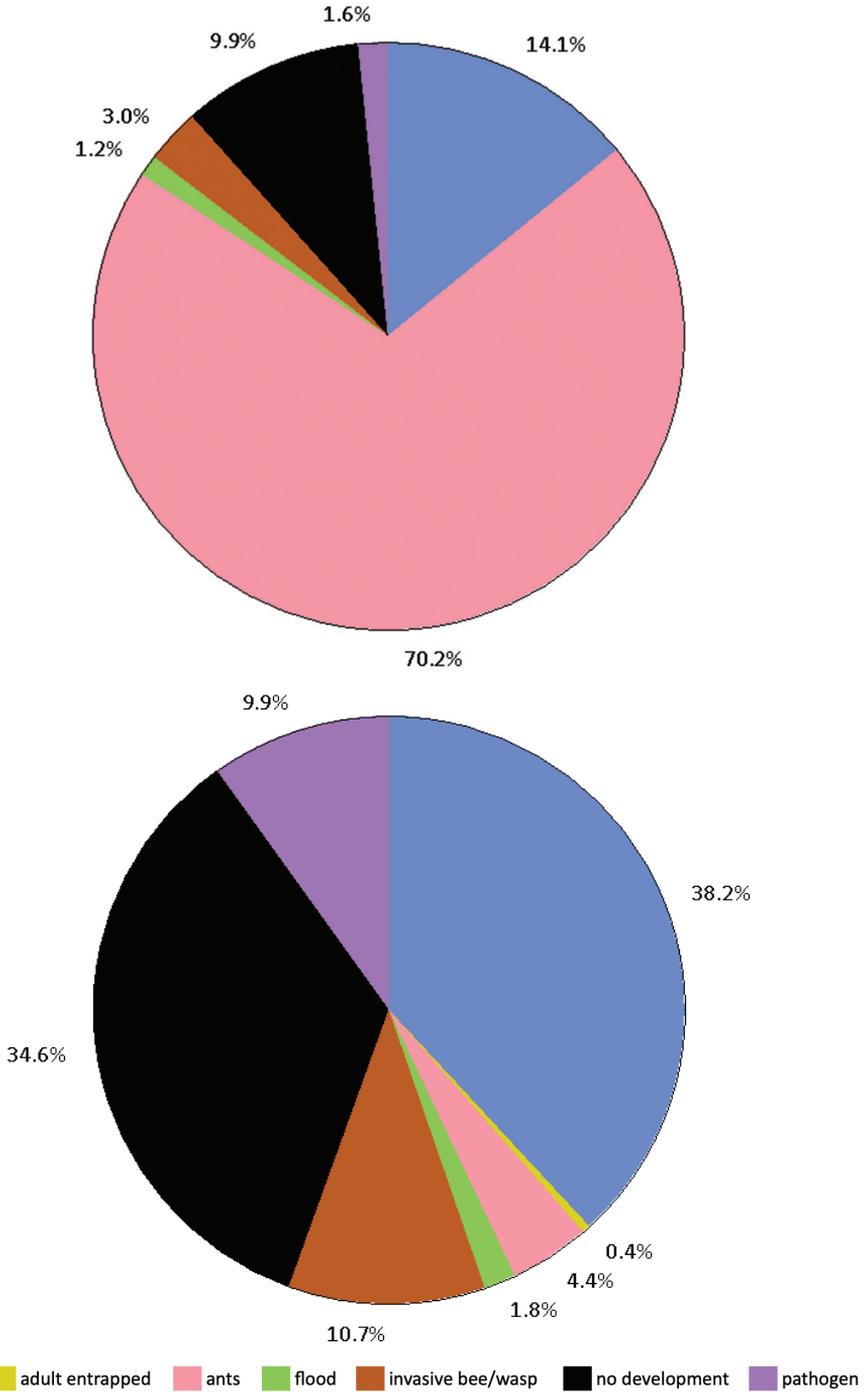
ment block, once when a block was colonised by a winged *O. glaber* queen and four times when the sticky barrier was compromised due to debris covering it. This affected 20 nests in six treatment blocks and was discovered and addressed within one to two days. The treatment block at monitoring point 3 at JCNWR was breached by ants on three different occasions causing the failure of at least three nests (Figures 5, 6).

Following predation by ants, lack of development was the second leading cause of failure in control blocks and the primary cause of failure in the treatment blocks. In control blocks, where ant predation accounted for 70.2% of failed nests, lack of development accounted for 9.9%, nesting by invasive bee or wasp accounted for 3% and the remaining two outcomes (pathogen or flooding) accounted for less than 2% each (Figure 7). In treatment blocks, nests primarily failed due to lack of development (34.6%), nesting by invasive wasps (10.7%) and then pathogens (9.9%) (Figure 7). We observed *H. anthracinus* nests being taken over by two invasive wasp or bee species: the keyhole wasp *P. nasidens* (n = 61) and leafcutter bee *Megachile* sp. (n = 1).

Between 3 December 2015 and 24 February 2016, we found 22 nests at the two points on the Turtle Bay property. All nests failed either due to predation by invasive ants (n = 9) or due to inundation (n = 13) during a large swell on 24 February 2016. As with JCNWR and Ka Iwi, only *O. glaber* was observed depredate nests. We monitored the Turtle Bay site for adults and nests through the end of the study in December 2016 and we did not observe *H. anthracinus* return; however, we did observe the invasive *H. strenuus* recolonising the site.

## Discussion

Yellow-faced bee (*Hylaeus* sp.) populations in areas where invasive ants are present are known to be smaller than populations in uninvaded areas (Krushelnicky 2014; Sahli et al. 2016). In these cases, ant depredation of native *Hylaeus* nests was suspected (Cole et al. 1992), but was not documented or quantified. We found that invasive ants reduced both nest success of *H. anthracinus* and the number of adults produced per successful



**Figure 7.** Nest outcomes including causes of failure in control (n = 433, top) and treatment (n = 456, bottom) blocks across the three sites (Turtle Bay, James Campbell National Wildlife Refuge and Ka Iwi) on Oahu, Hawaii.



**Figure 8.** *Ochetellus glaber* depredating a nest in an artificial nest block on Oahu, Hawaii.

nest. Invasive ants depredated 70.2% (304 of the 433 nests) of the nests in unprotected control blocks and were, by far, the greatest cause of nest failure (Figure 7). Nest predation by ants was substantial at all three sites, ranging from 56.5% at the Ka Iwi site to 64.2% at Turtle Bay and 75.2% at JCNWR (Table 1).

In the areas where *P. megacephala* and *O. glaber* were the dominant ant species, *O. glaber* was regularly seen forming recruiting lines into nests and removing the contents including pollen, eggs, larvae and pupae (Figure 8). In all instances where depredation by ants was observed ( $n = 274$ ), *O. glaber* was the culprit, even though *P. megacephala* was common at all sites. The two species seemed to segregate into different niches, with *P. megacephala* more commonly observed on the ground while *O. glaber*



**Figure 9.** Yellow crazy ant (*Anoplolepis gracilipes*) investigating a nest tube in one of the nesting blocks on Oahu, Hawaii.

was primarily observed in the vegetation. The only observation of *P. megacephala* depredating a nest tube occurred on 11 September 2016 at JCNWR and it did not involve a nest of *H. anthracinus*. In that instance, we observed more than 100 *P. megacephala* depredating a nest of an unidentified wasp filled with paralysed spiders. However, we did observe a *P. megacephala* worker attempting to enter a *H. anthracinus* nest in a tube we had temporarily removed from a block and placed on a fence post. In this case, the adult female bee was blocking entrance to the nest and prevented the ant from accessing the nest. We commonly observed females in nests during nest construction, but rarely after construction was completed, thus nests are unattended throughout most of their development.

Hawaiian yellow-faced bees are known to nest in dead, hollow stems or holes on the ground in soil, sand, coral rubble and under rocks (Cole et al. 1992; Magnacca 2007; Magnacca 2020). We saw large numbers of *H. anthracinus* nesting in the coral rubble just above the high tide line at the Ka Iwi site. We did not observe this at our other sites. One possible explanation for persistence of ground nests at Ka Iwi is the lower populations of *P. megacephala* along the coastline at Ka Iwi. We did not measure ant densities at each site, but we often noted seeing fewer ants on the ground in the coral rubble area at Ka Iwi compared to our other sites. Although *H. anthracinus* can co-exist with *P. megacephala* at some density (Magnacca and King

2013), *P. megacephala* may play an important role in excluding them from ground nesting. Since our study used nest blocks hanging in shrubs, it did not evaluate the impact of ants on ground nesting.

While *H. anthracinus* populations may be able to persist with *O. glaber* and *P. megacephala*, we found no evidence that it can co-exist with the yellow crazy ant (*A. gracilipes*). *Anoplolepis gracilipes* was invading JCNWR from the east and was present in high densities at two points (14 and 15) and at low densities at point 13 at the beginning of our study (Figure 9). Although the vegetation structure and plant species composition was similar at these three points, *H. anthracinus* adults were not observed in the area and no nests were found at points 14 and 15 and only two nests were found in the treatment block at point 13 over the course of the year-long study (Figure 5). By the end of our study, the range of *A. gracilipes* had expanded and the species was also present in low densities at points 11 and 12. This amounts to an expansion of approximately 60 m in one year and represents a significant threat to the long-term persistence of *H. anthracinus* at this site.

*Hylaeus anthracinus* appeared to have few defences against invasive ants. One type of behaviour we observed that may be beneficial in deterring ants involved the female adult bee remaining stationary at the entrance of the nest facing outwards. It is unclear if adult bees seen exhibiting this behaviour are simply resting or actively guarding the nest from kleptoparasites or other parasites or predators. Regardless of its origins, we observed this behaviour preventing ants from accessing nests on several occasions. However, female bees were typically observed in their nests only during active construction. Once all nest cells were completed and sealed, the bees were no longer present, leaving nests vulnerable to ant predation during brood development.

Our study design and monitoring methods had weaknesses. Although we monitored the nest boxes twice a week for a year, some bee nests were initiated and depredated in between visits, so it is possible the number of failed nests and those depredated by ants is an underestimate. Alternatively, it is possible that our estimate of nest predation by ants is higher than that which occurs in a natural setting. As outlined in the Methods section, our wooden blocks had 20 pre-drilled holes, 10 on each side. In control blocks, it is possible that, once invasive ants found the block, they were more likely to depredate all nests present in the block. This contrasts to natural conditions where nests are distributed in hollow stems across a host plant and are likely to be further apart and require additional foraging effort on the part of the ants. Similarly, the structure of the nest blocks may have increased the likelihood of invasive competitors easily utilising the available nest holes and/or of pathogens spreading quickly between nests. Additionally, nests in treatment blocks appeared to fail at a higher rate due to lack of development (34.5%) than nests in control blocks (9.9%). We surmise that high levels of predation by ants in control blocks across all available nests may mask what would otherwise be higher numbers of nest failures due to lack of development. The seemingly high number of nests that failed to develop may have been associated with elevated levels of moisture in the plastic tubing used in our artificial nests, although we lack data from natural nests for comparison. A comparison of nest success

rates in a variety of artificial nest materials, including more porous or breathable tubes, would be valuable.

While this study was not designed to evaluate other threats to *H. anthracinus*, the loss of all surviving nests ( $n = 13$ ) and apparently the entire population, on the peninsula at Turtle Bay due to inundation during the large swell on 24 February 2016 illustrates and documents their vulnerability to storms, large swell events and sea-level rise compared to most species. Although this species was widespread historically (Perkins 1899), currently, *H. anthracinus* is only observed in the narrow strip of coastal strand vegetation above the high tide line on Oahu. Populations are unlikely to move inland as sea-level rises because of development and the presence of degraded habitat dominated by invasive species. A study using high-resolution dynamic model experiments found an increased frequency of tropical cyclones from 1980 to 2018 over the Central Pacific (i.e. the area around Hawaii and extending east and south) that could only be explained by factoring in human-accelerated climate change (Murakami et al. 2020). Based on this, we expect continued increased storm frequency in the Central Pacific. The coastal flooding and erosion that accompany these storms could be detrimental to *H. anthracinus* populations.

The lowland arthropod fauna of Hawaii has been largely eclipsed by non-native species and less than 5% of arthropod species in coastal areas on Oahu are native (Plentovich 2010). Invasive ants, bees and wasps (e.g. *A. mellifera*, *C. smaragdula*, *C. dentipes*, *Lassioglossum* sp., *H. strenuus* and *P. nasidans*) now dominate pollinator webs in coastal areas of the Hawaiian Islands (Hopper 2002; Shay et al 2016; Shell et al. 2017; Shay and Drake 2018). Not only do these invasive pollinators compete with *H. anthracinus* for floral resources (Lach 2008; Ing and Mogren 2020), but they may also compete for nest sites. Introduced pollinators have been found to utilise the same plants and similar dimensions for nest cavities in wild nests (Graham and King 2017). We observed three invasive hymenopteran species nesting in our artificial nest boxes: *P. nasidans*, *Megachile* sp. and *H. strenuus*. Cumulative impacts of such nest site competition are unknown; however, it is reasonable to assume that the presence of these invasive competitors places additional stresses on coastal-dwelling endangered yellow-faced bees, like *H. anthracinus*.

## Conclusion

Once widespread, most species of Hawaiian yellow-faced bees are now extremely rare and, given their small size and cryptic nature, very little is known about their nesting ecology, including factors limiting nest success. *Hylaeus anthracinus* is one of a limited number of native terrestrial invertebrates persisting in coastal areas of the main Hawaiian Islands, but its populations are sparse and patchily distributed. We found that invasive ants had a severe to catastrophic effect on nesting *H. anthracinus*, depending on the ant species present. In our study, the invasive ant *O. glaber* depredated the majority (70%) of bee nests built in unprotected nest blocks. In areas invaded and with high densities of yellow crazy ant (*A. gracilipes*), no nests were initiated and no adult

bees were observed, suggesting that invasion by these ants may completely exclude *H. anthracinus*. The compounding effects of predation by invasive ants, habitat loss and resource competition with invasive Hymenoptera are unknown, but all are likely to have contributed to the decline of *H. anthracinus*. The few remaining *H. anthracinus* populations on Oahu are constrained to a narrow strand of coastal habitat just above the high tide line, making the species vulnerable to sea level rise and increased storm frequency and intensity, both of which are predicted as the climate changes.

Hawaiian yellow-faced bees evolved with elements of the flora to form mutualistic plant/pollinator relationships (Howarth 1985; Hopper 2002; Daly and Magnacca 2003). Without the onset of active management for this species, we expect populations to further decline as invasive ants, especially yellow crazy ants, continue to expand their distribution (Chen 2008). Ecological restoration of coastal ecosystems in Hawaii is necessary to improve and expand habitat for Hawaiian yellow-faced bees and other native invertebrates in support of existing plant/pollinator relationships. Safe and effective ant control and/or eradication methods as part of habitat restoration are needed to allow the recovery of Hawaiian yellow-faced bees. Additional testing of novel control technologies like RNA interference, *Wolbachia*-based approaches and improved delivery methods for toxicant baits are needed to control invasive ants at a landscape scale. Once suitable habitat is identified or areas are restored, translocation could be used to expand the range of this and other Hawaiian yellow-faced bees.

## Acknowledgements

Thanks to Jackie Smith and Ryan Colle for assistance with data collection. We also want to thank the staff at James Campbell National Wildlife Refuge, Hawaii Department of Land and Natural Resources – State Parks Division and Turtle Bay Resort for allowing access and supporting this project. Thanks also to Paul Krushelnycky of the University of Hawaii, Karl Magnacca of Oahu Army Natural Resources Program and two anonymous reviewers for comments which improved this manuscript. Financial support was provided by the U.S. Fish and Wildlife Service's Pacific Island's Coastal Program, the State of Hawaii – Department of Land and Natural Resources and the Department of Defense Legacy Resource Management Program.

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# Interaction of traffic intensity and habitat features shape invasion dynamics of an invasive alien species (*Ambrosia artemisiifolia*) in a regional road network

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Academic editor: G. Karrer | Received 18 September 2020 | Accepted 2 February 2021 | Published 24 March 2021

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**Citation:** Lemke A, Buchholz S, Kowarik I, Starfinger U, von der Lippe M (2021) Interaction of traffic intensity and habitat features shape invasion dynamics of an invasive alien species (*Ambrosia artemisiifolia*) in a regional road network. NeoBiota 64: 155–175. <https://doi.org/10.3897/neobiota.64.58775>

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## Abstract

Road corridors are important conduits for plant invasions, and an understanding of the underlying mechanisms is necessary for efficient management of invasive alien species in road networks. Previous studies identified road type with different traffic volumes as a key driver of seed dispersal and abundance of alien plants along roads. However, how the intensity of traffic interacts with the habitat features of roadsides in shaping invasion processes is not sufficiently understood. To elucidate these interactions, we analyzed the population dynamics of common ragweed (*Ambrosia artemisiifolia* L.), a common non-indigenous annual species in Europe and other continents, in a regional road network in Germany. Over a period of five years, we recorded plant densities at roadsides along four types of road corridors, subject to different intensities of traffic, and with a total length of about 300 km. We also classified roadsides in regard to habitat features (disturbance, shade). This allowed us to determine corridor- and habitat-specific mean population growth rates and spatial-temporal shifts in roadside plant abundances at the regional scale. Our results show that both traffic intensity and roadside habitat features significantly affect the population dynamics of ragweed. The combination of high traffic intensity and high disturbance intensity led to the highest mean population growth whereas population growth in less suitable habitats (e.g. shaded roadsides) declined with decreasing traffic intensity. We conclude that high traffic facilitates ragweed invasion along roads, likely due to continued seed dispersal, and can compensate partly for less suitable habitat features (i.e. shade) that decrease population growth along less trafficked roads. As a practical implication,

management efforts to decline ragweed invasions within road networks (e.g. by repeated mowing) should be prioritized along high trafficked roads, and roadside with disturbed, open habitats should be reduced as far as possible, e.g. by establishing grassland from the regional species pool.

### **Keywords**

Disturbance, habitat type, human-mediated dispersal, interaction, population dynamics, road ecology, seed dispersal, shading

## **Introduction**

Plant invasions are a global phenomenon closely linked to human activities and related transportation network infrastructures (Bradley et al. 2012; Seebens et al. 2015; Chapman et al. 2017). A large number of studies revealed the importance of road corridors for the spread of invasive plant species (e.g. von der Lippe and Kowarik 2007; Brisson et al. 2010; Joly et al. 2011; Dar et al. 2015; Manee et al. 2015; Okimura et al. 2016; Follak et al. 2018a) and identified several important anthropogenic dispersal vectors as reviewed by Ansong and Pickering (2013). These vectors are commonly related to construction work, road maintenance and roadside management, agriculture, transportation industry and private vehicles. Depending on factors like vector velocity (Taylor et al. 2012), seed accrual rate (Rew et al. 2018) and seed adhesion time (Bajwa et al. 2018) the achieved dispersal distances can range from some tens of meters to hundreds of kilometers for road vehicles (von der Lippe and Kowarik 2007; Taylor et al. 2012; Lemke et al. 2019). An estimation from experimental data on dispersal by adhesion to vehicles revealed that approximately every hundredth seed transported by road vehicle is likely to be dispersed more than 5 km in road networks (Taylor et al. 2012). As regional dispersal corridors, roads therefore probably play an important role for plant invasions (Vicente et al. 2014). Observed distribution patterns of alien plant species in road networks suggest traffic volume as one key variable related to the dispersal process (Vakhlamova et al. 2016; Barlow et al. 2017; Geng et al. 2017; Horvitz et al. 2017). At a local scale, traffic intensity is known to affect the diversity and composition of roadside vegetation (Truscott et al. 2005; Jaźwa et al. 2016), and distribution patterns of invasive alien species as well (Lemke et al. 2019).

Traffic volume has also been shown to trigger abiotic parameters like pollutant load (e.g. Jantunen et al. 2006; Neher et al. 2013; Mikołajczak et al. 2017) or the level of physical disturbance on road verges (Truscott et al. 2005; Johnson et al. 2006). Compared to the surrounding habitats, roadsides are physically, chemically and biologically altered environments (Forman and Alexander 1998) receiving more light, additional moisture from road drainage and sometimes additional mineral nutrition from adjacent agricultural fields. As a negative effect in this habitat, plants are stressed by pollutants from traffic exhausts and by de-icing salt that also affects soil pH (Forman and Alexander 1998; Seiler 2000; Hofman et al. 2012; Gentili et al. 2017). Habitat quality of roadsides is locally modulated by adjacent land- use patterns that may result in differences in shading or disturbance intensity (Christen and Matlack 2009; Speziale et al. 2018). We expect that both shading and the availability of open habitats at road

verges are critical for the establishment of roadside populations because most plant species growing on roadsides are commonly categorized as herbaceous generalists (Coffin 2007) or light, drought and disturbance tolerating ruderal specialists (Sera 2010; Gade 2013; Bochet and Garcia-Fayos 2015; Kalwij et al. 2015).

While several vectors of human-mediated dispersal at roadsides are well understood, their effects on regional population dynamics of alien plants along roads and their interplay with habitat quality and adjacent land use have hardly been studied. A better understanding of interactions between traffic-related and habitat-related drivers of plant invasions along roads would also support the early detection of alien species, their control and related management measures – if necessary – in management at local or regional scales (Cabra-Rivas et al. 2015; Lembrechts et al. 2017).

We use *Ambrosia artemisiifolia* L. (henceforth common ragweed) as a model species to elucidate relationships between traffic- and habitat-related features of road corridors. Common ragweed is an annual ruderal plant species that is well adapted to roadside habitats (Essl et al. 2015). Seedling recruitment is highly increased by frequent disturbance of these sites and specimens can reach large biomass (Fumanal et al. 2008; MacDonald and Kotanen 2010). Common ragweed develops best under full light conditions but tolerates lower light conditions, yet with reduced fitness (Montagnani et al. 2017). Understanding invasions by common ragweed is important, since this species severely affects human health by allergenic pollen; it is also an important agricultural weed (Bullock et al. 2012; Essl et al. 2015; Sölter et al. 2016).

The spread of common ragweed is limited by low natural dispersal rates (barochory; Essl et al. 2015; Lemke et al. 2019) and a reduced plant growth at deeply shaded habitats (e.g. forest understoreys; Joly et al. 2011; Qin et al. 2012). While natural dispersal rates cannot explain the rapid range expansion of common ragweed throughout Europe (Essl et al. 2009; Ozaslan et al. 2016; Skalova et al. 2017), there is growing evidence about the different human-mediated dispersal processes that support its invasion success. First introduction and repeated new invasion foci resulted mainly from contaminated seed lots of grain, oil seed or bird food (Montagnani et al. 2017). Several modes of dispersal related to traffic corridors have been shown to transport relevant numbers of seeds, sometimes over long distances, e.g. soil movement for construction purposes (Brandes and Nitzsche 2006), adhesion and loss of diaspores from mowing machinery (Vitalos and Karrer 2009), seed losses from trucks that carry contaminated grain or soil (Nawrath and Alberternst 2010) and finally direct dispersal by the slipstream behind vehicles and adhesion to tires (von der Lippe et al. 2013; Lemke et al. 2019) as a common and predictable process at the local scale. While there is increasing information and experimental evidence about these dispersal vectors, the interacting effects of traffic and site conditions on the spread of common ragweed on a regional scale are still poorly understood. In this study, we address the population dynamics of common ragweed on a regional scale by performing a road-network analysis to reveal the spatial-temporal dynamics in roadside populations of common ragweed. We systematically compare plant abundances between different road corridor types over a period of years and evaluate the contribution of traffic density and habitat type, and their interactions, on the dynamics of these roadside populations.

Based on our multi-annual approach we test the following hypotheses: (a) traffic intensity affects the expansion and densification of common ragweed populations along roads, resulting in growth rates linked to the corridor type (from high to low traffic intensity); (b) habitat type affects the population growth of common ragweed resulting in higher plant densities on disturbed roadsides compared to undisturbed roadsides and lower plant densities for shaded compared to un-shaded habitats; and (c) depending on the corridor type, the interaction between traffic- and habitat-related factors leads to changes in population growth in similar habitat types.

## Material and Methods

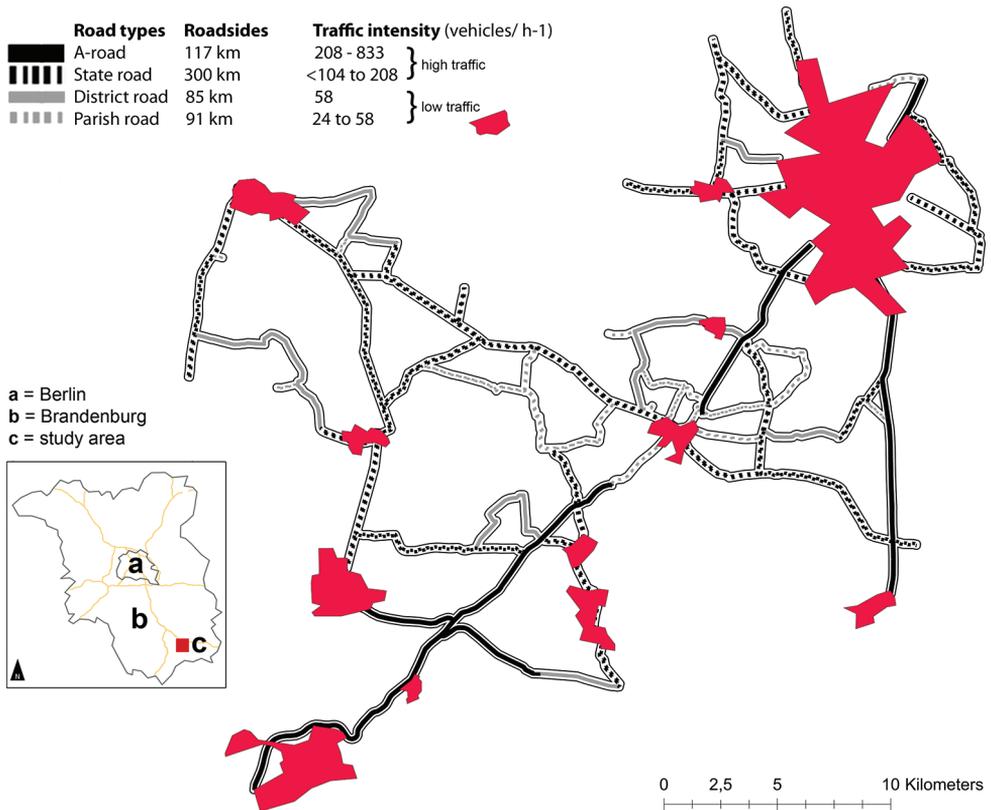
### Study species

Common ragweed (*Ambrosia artemisiifolia* L.) was chosen as a model species because its spatial distribution patterns are closely related to human activities – especially to transportation corridors, with rail or road traffic likely functioning as major dispersal vectors (Bullock et al. 2012). The species is native to parts of the United States and southern Canada and has spread to several parts of the world (Makra et al. 2015; CABI 2016). It was introduced to Europe in the 19<sup>th</sup> century and is now naturalized in several countries (Montagnani et al. 2017). Its allergic pollen affects public health in several European countries where it is most abundant in southeast Central Europe, north Italy and southeast France (Smith et al. 2013; Lake et al. 2017; Rasmussen et al. 2017). Preventive management is also needed for less invaded countries like Germany to reduce future health costs (Born et al. 2012). In consequence, management efforts target common ragweed, both nationally and internationally (Bullock et al. 2012; Sölter et al. 2016).

Common ragweed grows in a range of open and disturbed habitats like wastelands, old fields or agricultural areas and along transportations corridors (Essl et al. 2015). While its natural dispersal is limited to distances around 1 meter (Lemke et al. 2019), the rapid spread of common ragweed in its introduced range can be explained by several human-mediated dispersal vectors both in Europe (Essl et al. 2015) and in North America (Joly et al. 2011). Seeds of common ragweed are mainly dispersed unintentionally with the transport of contaminated commodities like sunflower seed, soil movements and traffic or roadside management (Vitalos and Karrer 2009; von der Lippe et al. 2013; Lemke et al. 2019). Before it was regulated in the EU, contaminated birdfeed was a major pathway for repeated introductions (Bullock et al. 2012).

### Study region

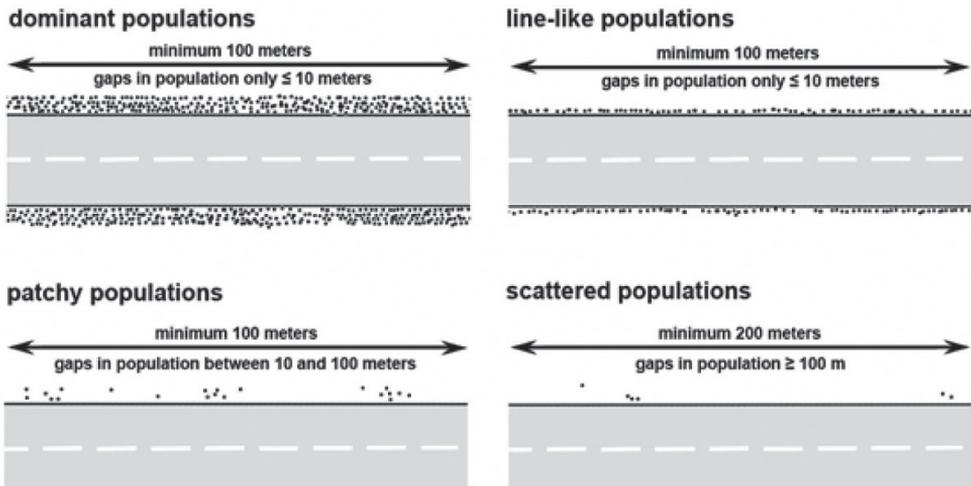
The study region (35 km × 35 km; road network of 300 km) is embedded in the historical region of *Niederlausitz* (Federal State of Brandenburg, Germany) and one of the hotspots of common ragweed in Germany (Buters et al. 2015). This area includes



**Figure 1.** The study area (c) is situated in the south east of the Federal State of Brandenburg, Germany (b). The mapped road network consists of four road types (black line: a-road, black dotted line: state road; grey line: district road; grey dotted line: parish road). Based on official and additional vehicle counts we pooled a-road and state road to *high traffic intensity roads* and district road and parish road to *low traffic intensity roads*. Settlements are displayed in red.

many populations along the local road network and on adjacent agricultural and rural sites (Brandes and Nitzsche 2007) where the species has been observed as an agricultural weed since the 1970s (pers. com. staff members of Agrargenossenschaft Drebkau, 2010). A first systematic absence-presence mapping of common ragweed in the region of Cottbus-Drebkau-Calau revealed that about half of the mapped roadsides (60 km of 125 km) were colonized, yet with different occupancy patterns in the two years of the census (Nitzsche 2010). Starfinger (2008) also reported a high number of linearly spaced roadside populations in this region, mostly consisting of small plants of about 20 cm in height. Lemke et al. (2019) found that most of the plants grow in the first 0.5 m to the road border.

Within the road network analyzed in this study (Fig. 1), local road maintenance staff mowed all roadsides twice a year, independently of the traffic intensity of the road corridor, with a first service in June and a second service in autumn (September to



**Figure 2.** Visualization of the four population density classes of common ragweed mapped in the road network survey. Note that the minimum length for a change in recorded plant density during the mapping was different for ‘scattered populations’ (200 m) compared to the other three density classes (100 m). Dominant populations differed from line-like populations in their lateral expansion perpendicular to the roadway.

October). Staff members were aware of the presence of this plant in the road network (pers. com. road maintenance staff, 2009 and 2012). During the survey, we did not notice any adjustment of management (e.g. frequency or timing of mowing) for the different corridor types or population densities of common ragweed.

## Roadside survey

Our field survey included five semi-quantitative mappings of common ragweed roadside populations in the years 2008 to 2012. In each of these five years, we conducted a census in summer (July to August) when common ragweed was best visible along the roads and easy to distinguish from the other roadside vegetation by its characteristic greenish color. The total length of mapped roadsides was about 300 km. We classified four plant density categories of common ragweed (Fig. 2) to evaluate population dynamics between the respective years. For each census we mapped the roadsides visually from a slowly moving car (Starfinger 2008) and captured the density categories of common ragweed plants as well as related adjacent landmarks like field entries or forest borders to precisely locate the mapped common ragweed patches.

We classified four road types according to traffic volume (Fig. 1), based on existing data for a-roads and state roads (Landesbetrieb Straßenwesen Brandenburg 2010) and on personal vehicle counts along district roads and parish roads 2008 (Lemke, unpublished). For an overall measure of traffic intensity, we merged a-roads (208 to 833 vehicles/h) and state roads (<104 to 208 vehicles/h) to roads with high traffic volume because for both road categories the official vehicle count was beyond our ‘medium traffic’ threshold (58 vehicles/h; see Lemke et al. 2019). Likewise, we combined district

roads and parish roads to roads with low traffic volume (24 to 58 vehicles and <24 vehicles, respectively).

In 2012, we conducted an additional roadside habitat mapping from a slowly moving car to capture habitat features that we expected to potentially limit ragweed populations in road corridors. The underlying classification of roadside habitats was chosen after a thorough inspection of the different characteristics of roadsides in the field. First, we differentiated shaded from un-shaded (i.e. sunny) habitats. We assigned all roadsides to the category “shaded” that were shaded most of the day by close tree lines, tree stretches or single trees, or by an adjacent forest canopy, given that all of these elements clearly reduce light availability on the ground – which we hypothesized to limit common ragweed establishment. Since we expected a dense vegetation cover to limit the population establishment of common ragweed as well, we further differentiated the sunny roadside sections into disturbed and undisturbed sections according to a vegetation cover of <50% and of >50%, respectively, assuming that most kinds of disturbance lead to open soil patches along roads. We were not able to differentiate the shaded roadsides into disturbed and undisturbed sections as these sections were generally characterized by a sparse vegetation layer that made it impossible to visually distinguish disturbances from the slowly mowing car. However, we argue that in this habitat type recruitment is limited by shade rather than by the limitation of safe sites as the share of open soil was high throughout the road network. This classification resulted in three well distinguishable habitat types with recurring vegetation elements. The **undisturbed roadsides** frequently contained species of ruderal grasslands like *Poa pratensis*, *Lolium perenne*, *Bromus hordeaceus*, *Achillea millefolium* or *Rumex crispus*. In **disturbed roadsides**, these species gradually drop out and typical disturbance indicators appear such as *Poa annua*, *Stellaria media*, *Conyza canadensis* and *Matricaria discoidea*. Along the **shaded roadsides**, some of the competitive grassland species of the undisturbed sections still occur, supplemented by some species of shade-tolerant ruderal vegetation, such as *Geum urbanum*, *Chaerophyllum temulum* or *Chelidonium majus*.

## Data processing and data analysis

We used a geographic information system (ArcMap of ArcGIS 10.3.1, ESRI, Redlands, California) to integrate the density distribution mappings of common ragweed as roadside line features to a digital form of the regional road network (OpenStreetMap and contributors, CC-BY-SA). In a second step, we split this network into 3-m-long spatial units resulting in 198,327 single road cells of 3 m length longitudinal to road direction. Each cell contained information about road type, traffic direction, roadside habitat and plant density for the seasons 2008, 2009, 2010, 2011 and 2012.

*Road sections:* To better understand how common ragweed interacts with variations in roadside habitats and traffic intensity within the road network at a regional scale, we split the entire network into 49 *road sections* based on existing junctions and t-junctions (a-road:  $n = 14$ , state road:  $n = 16$ , district road:  $n = 8$ , parish road:  $n = 11$ ). We defined these sections as our main investigation units for analyses on temporal and spatial changes in the number of road cells colonized by common ragweed. Within

their boundaries, each of the units is homogeneous in relation to the type of corridor or traffic intensity.

*Road cells:* To get a deeper insight into the roadside population dynamics we used *road cells* as a second level unit within our main unit *road section* in the sense of a high-precision investigation of the interactions between traffic intensity and habitat feature at the local scale. As we wanted to uncover plant density variations individually for each *road cell*, we encoded the mapped plant densities (none, scattered, patchy, line-like, dominant) into numbers (0, 1, 2, 3, 4) and calculated the differences in population density between the years ( $n = 4$  sub sets of *periods*, i.e. 2008/2009, 2009/2010, 2010/2011, 2011/2012). Each *road cell* now included information about plant density in the prior season, the difference between the two seasons (*change in population density*) and the plant density in the post season. To cover only those road cells where change in ragweed density could potentially be observed, we filtered the road cells so that either the prior season or the post season had to be non-zero.

*Binomial generalized linear mixed model:* To assess the effects of road type and year on the invaded portion of the road sections, we performed a binomial generalized linear mixed model ('glmer' from the r-package *lme4*) with the counts of invaded and uninvaded road cells in each section as a dichotomous response vector. We used *road type* (a-road, state road, district road, parish road) and *year* (2008, 2009, 2010, 2011, 2012) and their interaction as fixed effects and the nesting of *road sections* in *year* as a random factor.

*Linear mixed-effects model:* To unravel the effects of traffic and habitat on the dynamics of ragweed density, we used a linear mixed-effects model ('lmer' from the r-package *lme4*) with the categorical variables *roadside habitat* (disturbed, undisturbed, shaded) and *traffic intensity* (high traffic, low traffic) as fixed effects. As random effects we used again *road sections* nested in *year* to account for temporal and spatial nesting of the *road cells*. Here, *year* is used as a covariate and no longer as a fixed effect, as we were interested in the interactions between habitat feature and traffic intensity independently of annual dynamics. As response we used the *change in population density* based on the encoded population density categories (see paragraph on roadside cells). This vector ranged from “-4” (population in the *road cell* decreases from level ‘4’ to ‘0’ [‘dominant’ to ‘uninvaded’]) to “+4” (population in the *road cell* increases from level ‘0’ to ‘4’). A vector value of “+1” for example would display an increase of plant density in the *road cell* by a whole factor level (e.g. 0 to 1, 1 to 2, 2 to 3, 3 to 4). This variable showed a normal distribution and hence a linear model was chosen.

## Results

### Traffic intensity and population expansion

As hypothesized, traffic intensity was related to the expansion of common ragweed populations within the road network. At the landscape scale, common ragweed colonized a portion of between 0.24 to 0.51 (a-road), 0.41 to 0.53 (state road), 0.29 to

0.35 (district road) and 0.46 to 0.36 (parish road) of all road cells in the respective corridor type between 2008 and 2012 (Fig. 3).

In the binomial generalized linear mixed model the *year* of investigation has the strongest effect on the number of colonized road cells while *road type* alone is not a significant predictor (Table 1). However, a significant interaction between year and road type indicates that the annual change in colonized roadsides differs significantly between road types. After the first year, we detected a system-wide strong increase of the mean colonization share (a-road = +0.19; state road = +0.09; district road = +0.12; parish road = +0.16). Beside this initial increase we found, for the remaining period, the lowest interannual variations along the a-road (+0.07 for 2009/2010, -0.02 for 2010/2011, +0.02 for 2011/2012) and the highest variations along the parish road (-0.08, -0.15, -0.03). Furthermore, the colonization share for the high traffic corridors a-road and state road remained stable after 2008, with only minor fluctuations. The colonization of road cells in the low traffic corridor types showed a different pattern, with a weak decreasing trend in the district roads, and a sharp drop in the parish roads (Fig. 3).

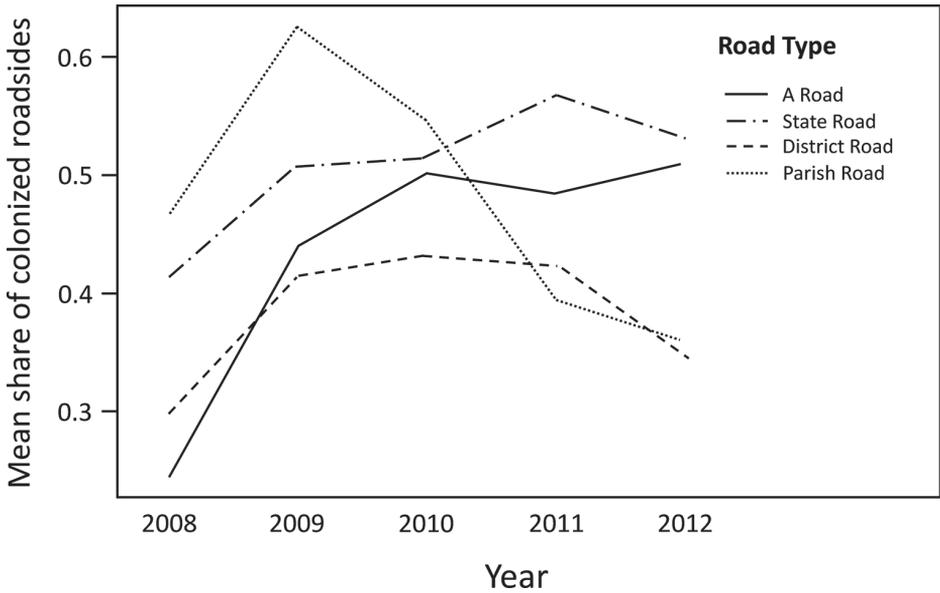
### Habitat features, traffic intensity and population growth

Population density in already invaded road sections increased over the entire road network during the study but this process was strongly modulated by both traffic intensity and habitat type. Based on the linear-mixed model, both factors, *roadside habitat* and *traffic intensity*, significantly affect the mean change in population density of common ragweed in the road cells (Table 1). However, the most significant effect on change in population density is the interaction between *traffic intensity* and *roadside habitat* (Table 1), suggesting that some features related to the number of passing vehicles modulate the habitat effect within road corridors.

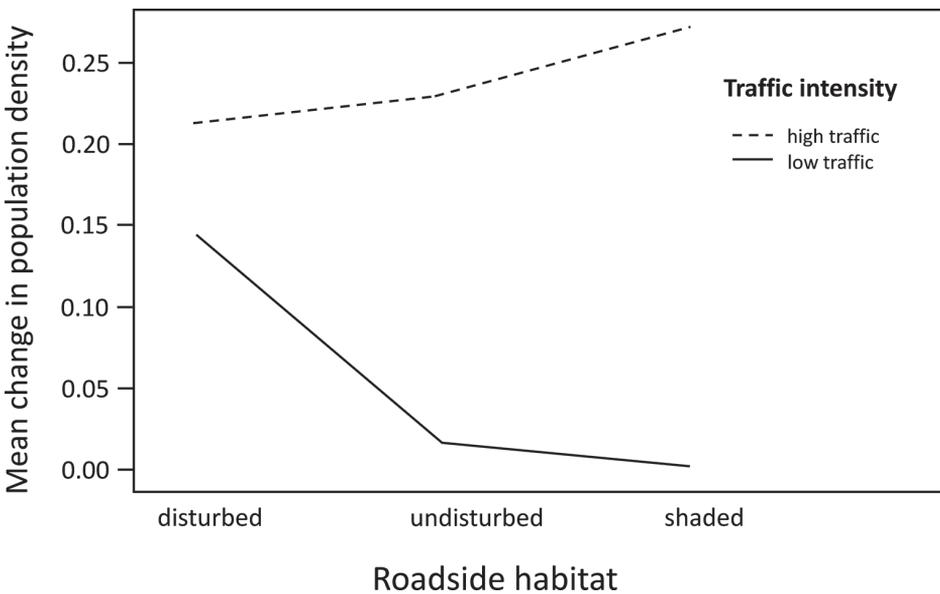
The interaction plot in Fig. 4 illustrates how the mean change in population density differed between the three habitat types *disturbed*, *undisturbed* and *shaded* in relation to the two traffic intensities. In general, there is a higher change in population density within high traffic corridors. While we found a lower change in density for undisturbed and shaded habitats compared to disturbed roadsides within low traffic corridors, the change was even higher in these habitats within high traffic corridors.

**Table 1.** Effects of a) Road type and year on the proportion of roadside cells colonized by *Ambrosia artemisiifolia* and b) Traffic intensity and roadside habitat on annual change in population density of *Ambrosia artemisiifolia* in 49 roadside sections.

Model	$\chi^2$	df	p
<b>a) Colonized roadside cells (binomial glmm)</b>			
Road Type	7.19	3	0.066
Year	14.68	4	0.0054
Road Type:Year	21.32	12	0.046
<b>b) Annual Change in population density (linear mixed model)</b>			
Traffic intensity	7.62	1	0.006
Roadside habitat	88.53	2	< 0.001
Traffic intensity:Roadside habitat	120.33	2	< 0.001



**Figure 3.** Proportion of roadsides in different types of road corridors, colonized by *Ambrosia artemisiifolia* in five years. Mean values are based on road section specific proportion of colonized road cells (n = 49 road sections in the road network).



**Figure 4.** Mean change in population density of *Ambrosia artemisiifolia* between subsequent years in invaded road corridors with different traffic intensities. High traffic is merged from the corridor types a-road and state road, low traffic is merged from district road and parish road. Mean values are based on road cell specific population density categories (n = 303994 road cells in the road network).

## Discussion

Plant invasions at higher spatial scales are shaped by highly complex dispersal pathways which interact with landscape characteristics (Pauchard and Shea 2006; Muthukrishnan et al. 2018). How such interactions modulate population growth in invasive species is a clearly understudied question. Here we show that the long-term dynamics in roadside populations in a common invasive species are shaped by an interplay of habitat features and traffic intensity. We found that a combination of high traffic intensity and high disturbance intensity of roadside habitats leads to high population growth in common ragweed. As a second insight, population growth in less suitable habitats with reduced availability of light and open soil, declines with reduced traffic intensity.

In general, a multitude of dispersal vectors shape plant dispersal in road networks (Kowarik and von der Lippe 2007; Auffret et al. 2014). The spread of common ragweed along roads, for example, is mainly fostered by agricultural machineries (Karrer 2014), soil movements, roadside maintenance and road traffic (Montagnani et al. 2017). Especially the two latter vectors promote plant invasions in road corridors by i) increasing the plant density in already existing plant patches and ii) supporting the colonization of empty patches – visible as a rapid range infilling at a regional scale (Warren et al. 2013; Rauschert et al. 2017). Rapid range expansions, on the other hand, rather depend on a high proportion of rare long distance dispersal events (LDD; Nathan et al. 2008). In the case of common ragweed, mainly agricultural machineries and contaminated soil transports are expected to support LDD events (Bullock et al. 2012; Essl et al. 2015) whereas mowing machines are supposed to act mainly at a regional scale (“medium dispersal”; Essl et al. 2015). A previous study demonstrated that road traffic modulates the population dynamics of ragweed at roadsides far beyond the reach of natural dispersal of the species, although the dispersal distances of the seeds are usually limited to the local scale (Lemke et al. 2019).

As a main insight from this study, traffic intensity seems to modulate the inter-annual variation of the spatial occupancy patterns of common ragweed within the regional road network. In the observation period 2009–2012, the percentage of colonized road cells was increasing or rather stable along high traffic roads (a-road, state road; Fig. 3). In contrast, the number of occupied road cells continuously decreased in the low-traffic parts of the road network during the same period (Fig. 3). This may be due to an interaction at the local scale. As illustrated in Fig. 4, disturbance and light availability are related to the colonization of roadside cells by common ragweed. Changes in these (or other) habitat features can lead to modified distribution patterns in roadside plants as shown by Dostálek et al. (2014). Increase in vegetation cover and shade limits the colonization success of common ragweed along roads (Fig. 4; Joly et al. 2011), while such change may favor other species. The perennial *Asclepias syriaca*, for example, preferably colonizes unpaved roadsides and those bordered by forests and grassland in Austria (Follak et al. 2018b)

The sudden increase in the overall occupancy of common ragweed in the road network after the first observation period (2008–2009, Fig. 3) seems to be a system-wide

event which might be related to interannual changes of the recruitment dynamics as well as to an unusually high seed production in the preceding year. Recruitment could have been fostered by the dry and warm summer of 2008 as these climatic conditions had visible negative effects on the competing roadside vegetation even in the following year while common ragweed is usually unaffected by warm and dry summers. Reduced competitive ability of the roadside vegetation is well known as a cause for increased recruitment success of common ragweed (Karrer and Milakovic 2016; Gentili et al. 2017). In the same vein, the warm summer could have fostered seed productivity in common ragweed, resulting in higher seed transport by all possible human mediated dispersal vectors in the system (e.g. by agricultural machinery, mowing machinery, vehicles and losses from soil transports).

Preferences for disturbed, open habitats correspond to the pioneer character of common ragweed (Montagnani et al. 2017). In contrast to many other pioneer plants, the spread of common ragweed is not facilitated by wind-dispersed seeds, pointing to human-mediated dispersal as key for the colonization of road networks (Essl et al. 2015). There are several possible vectors of human-mediated dispersal that could be related to traffic intensity. First, the number and speed of passing vehicles is positively related to direct dispersal by tires and the slipstream of cars and trucks (Lemke et al. 2019). Second, losses from trucks that carry soil or grain contaminated with seeds of common ragweed are a source for the establishment of new populations that is likely to increase with traffic intensity (Nawrath and Alberternst 2010). Third, the very relevant seed transport by mowing machinery (Vitalos and Karrer 2009) could indirectly be related to traffic intensity as different road types usually receive a slightly different maintenance by mowing. As our investigated road network receives the same frequency of mowing, seed dispersal by mowing machinery could still differ due to different types of mowing machines in the different road types. While all these traffic related dispersal vectors have been acknowledged as important agents in moving seeds of common ragweed, the interplay between traffic intensity and habitat features of roadsides – and its importance for population dynamics – has not yet been quantified at a regional scale.

Our linear mixed model revealed significant interactions between habitat features and traffic intensity and the population dynamics of common ragweed in the regional road network. However, the effect of habitat was considerably larger than that of traffic (Table 1). This may explain the declining plant density in low traffic road cells with less suitable habitat features (Fig. 4). Here, newly emerging populations are obviously more prone to local extinction due to interspecific competition (Patracchini et al. 2011; Gentili et al. 2017; Cardarelli et al. 2018; Lommen et al. 2018a) and a decreased plant performance (MacDonald 2009; Qin et al. 2012). However, disturbed low traffic road cells show a constantly high population growth in the observed road network. These results add evidence to previously demonstrated positive effects of disturbance on seedling recruitment and establishment on ruderal sites (Fumanal et al. 2008) and highlight the importance of disturbance events in road corridors as a driver for common ragweed invasions. Surprisingly, high traffic road cells displayed a consistently high population growth rate even in shaded and less disturbed road

sections. This trend can be attributed to the additional influx of diaspores due to traffic-mediated dispersal (Lemke et al. 2019) that obviously leads to the densification of populations even under less suitable habitat conditions. In the same vein, an increased propagule pressure from abundant roadside plant populations has been shown to partly compensate for seed traits that usually do not promote long-distance dispersal (von der Lippe and Kowarik 2012).

Bullock and colleagues (2018) propose to differentiate dispersal processes in spatial networks into “human-vectored dispersal” (HVD) and “human-altered dispersal” (HAD). Local impacts on population dynamics (e.g. by traffic-mediated dispersal) can thereby be identified as aspects of HVD. Still, the network-wide distribution of ragweed can be related to HAD as it is affected by the predominant land-use structures. A next step would be to concurrently analyze short-term and long-term changes in landscape patterns (e.g. temporary change in land use, construction works) in regard to spatiotemporal invasion patterns. While Christen and Matlack (2009) did not find any hints for a conduit function of roadside habitats, our study suggests that roadsides can function, depending on traffic intensity, either as habitat or as conduit for plant invasions. Roadsides with low traffic therefore offer only a habitat within the scope of natural population dynamics and thus limited dispersal functions whereas high traffic roadsides enable an increased population density for common ragweed with greater spread potential. The interaction between traffic-mediated seed dispersal and local habitat features is thus an important mechanism in understanding plant invasions in road networks at the landscape scale.

## Conclusions

The drivers of roadside invasions by common ragweed are not yet fully understood, although there is increasing evidence of the separated effects of dispersal by traffic and road maintenance and habitat features (Vitalos and Karrer 2009; Bullock et al. 2012; Essl et al. 2015; Montagnani et al. 2017; Lommen et al. 2018b; Lemke et al. 2019). Our study adds further insights into these processes based on a detailed sampling of population data at a regional scale. As an innovation, we linked the assessment of dispersal vectors (traffic intensity) and habitat features (disturbance, shade) with population dynamics of common ragweed in a roadside network over a period of five years.

In our study, population growth of common ragweed proceeded even on roadsides with less suitable habitat conditions – but only along high-traffic roads. This indicates seed dispersal by vehicles and by road maintenance can compensate, at least partly, for less favorable habitat conditions. As a future direction, a threshold in traffic intensity and maintenance for a continued population growth along roadsides should be identified, based on more detailed data.

Our results on the interaction between traffic, roadside habitats and population dynamics have practical implications for habitat and population management to halt *Ambrosia* invasions along the road network. Depending on traffic intensity, colonized

roadsides can serve both as a stepping stone habitat and as conduit for common ragweed invasions in road networks as already indicated by Nawrath and Alberternst (2010) and Karrer et al. (2011). Management measures that aim at local eradication of *Ambrosia* populations should give priority to reducing the established roadside populations and their seed bank in critical parts of the road network. In the light of our results, most critical parts of the network with a high potential for traffic-mediated dispersal are high traffic roads with established populations and a high share of disturbed roadsides. This is in accordance with recommendations for prioritization of control measures along high-ranked roads in Austria (Karrer et al. 2011). For parts of the road network where the focus is on weakening and suppressing ragweed populations, an adjusted mowing regime is recommended, to prevent flowering and fruit set (Milakovic and Karrer 2016; Lommen et al. 2018b). Another way of containing the invasion is to establish a dense vegetation layer that impedes common ragweed germination and seedling establishment (Karrer and Milakovic 2016). Meanwhile our results indicate that shading, e.g. by tree plantations at roadsides, may not be sufficient in this respect, as dense traffic could partly compensate for the effect of shading on population growth. Measures should therefore aim at establishing a competitive herbaceous layer from appropriate seed mixtures that have been shown to effectively control *Ambrosia* establishment (Karrer and Milakovic 2016).

## Acknowledgements

The present study was conducted at the Technische Universität Berlin. We thank Konstantin Etling for mapping the roadside habitats and for digitalizing the data set and Gerhard Karrer and two anonymous reviewers for helpful comments on a previous version. We also thank Kelaine Vargas for improving the English. AL acknowledges funding from the Julius Kühn Institute, Braunschweig for part of the work presented here. SB received funding from the German Federal Ministry of Education and Research BMBF within the Collaborative Project “Bridging in Biodiversity Science-BIBS” (funding number 01LC1501A-H). We acknowledge support by the Open Access Publication Fund of TU Berlin.

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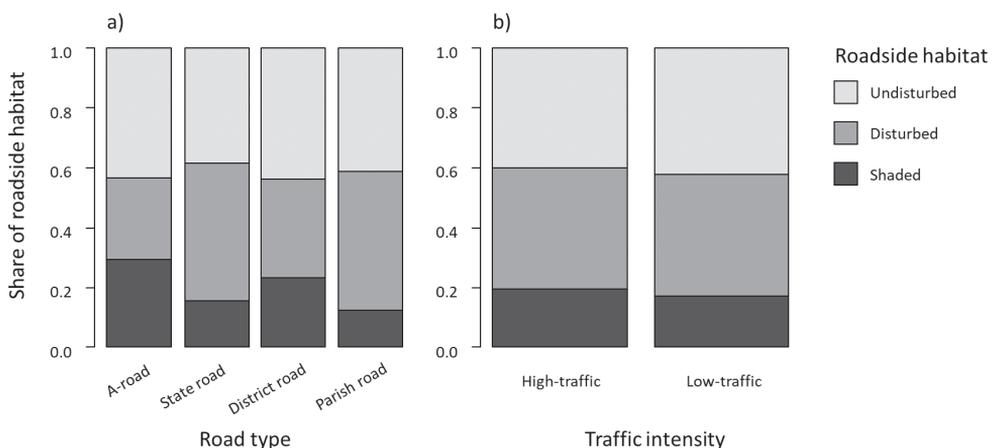
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## Appendix I



**Figure A11.1.** Distribution of roadside habitat types within **a** road types **b** categories of traffic intensity over the entire road network.



# Ornamentals lead the way: global influences on plant invasions in the Caribbean

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Academic editor: M. von der Lippe | Received 9 January 2021 | Accepted 16 March 2021 | Published 2 April 2021

**Citation:** Rojas-Sandoval J, Ackerman JD (2021) Ornamentals lead the way: global influences on plant invasions in the Caribbean. *NeoBiota* 64: 177–197. <https://doi.org/10.3897/neobiota.64.62939>

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## Abstract

Understanding the historical factors associated with the invasion success of alien species in a region may help us to identify sources, vectors, and pathways that are more likely to originate new invaders. Here, we gather data for traits related to the history of introduction (e.g., continent of origin, reason for introduction, and date of introduction) of 616 alien plant species listed as invasive on 18 island groups across the Caribbean region. We used these data to evaluate how human activity has influenced plant invasions on Caribbean islands over time and whether invasion success could be driven by traits of the introduction process. We found that significantly more invasive plants (54%) were intentionally introduced for ornamental reasons than for any other purpose. Most invaders in the Caribbean are native to Asia, South America, and Africa and the cumulative number of invasive species in this region has been steadily increasing during the last 200 years, but since 1850, this trend has been led by species introduced as ornamentals. We also found a significant association between continent of origin and reason of introduction, with more invaders than expected being ornamentals from Asia and America, and forage species from Africa. Our results show that introduced ornamentals are successfully invading all major habitats across the Caribbean, exacerbating conservation issues and threatening native biodiversity. Armed with knowledge of origins and reasons for introductions, effective biosecurity actions as well as control and management strategies can be better targeted to address the problem of invasive species in the region.

## Keywords

Alien flora, horticulture, introduction pathways, invasion history, invasion success, residence time, year of introduction

## Introduction

Globalization and the intensification of international trade, travel, and transport are leading to an unprecedented increase in the number of alien species translocated into new regions (Westphal et al. 2008; Hulme 2009; Early et al. 2016; Chapman et al. 2017), breaking down biogeographical barriers and homogenizing global biotas (Meyerson and Mooney 2007; Sax and Gaines 2008; Winter et al. 2010; Capinha et al. 2015; Turbelin et al. 2017). For the last few centuries, the numbers of established alien species from different taxonomic groups have increased around the world and are projected to continue in the future (Seebens et al. 2017, 2020). At the same time, changes in climate and land-use (e.g., expansion of agriculture and urban areas) are transforming and degrading natural habitats making them more susceptible to biological invasion (Mooney and Hobbs 2000; Bradley et al. 2010). Besides being a consequence of globalization, biological invasions are also major drivers of global biodiversity change, threatening the conservation of native biodiversity and human livelihoods (Pyšek et al. 2020).

Over 13,000 alien species of vascular plants have established persistent wild populations in areas outside their native range (van Kleunen et al. 2015; Pyšek et al. 2017). Most of these alien plants were originally introduced intentionally (van Kleunen et al. 2020) and a subset of them have overcome specific barriers to their survival, establishment, and dispersal and are actively spreading into new areas where they are identified as invasive species (Blackburn et al. 2011). Among the factors contributing to successful naturalization and invasion of alien species are propagule pressure, residence time, disturbance, biogeographic, climatic and socioeconomic traits of the recipient region, and intrinsic attributes of introduced species (Daehler 2003; Wilson et al. 2007; Lockwood et al. 2009; Moravcová et al. 2015; Essl et al. 2019). Consequently, the pool of alien species in a given region often depends on historical factors related to the type, intensity, and frequency of human activities in the area (Lambdon et al. 2008; Zenni 2014; van Kleunen et al. 2020). Historically, activities such as agriculture, forestry, and horticulture are well-known for the common practice of moving plant species well beyond their native distribution ranges (Inderjit 2005; Hulme 2009; Richardson and Rejmánek 2011). There is also robust evidence in the literature showing that planting practices affect invasion success and disproportionately more invaders are recruited from species introduced through horticulture than other means (Reichard and White 2001; Dehnen-Schmutz et al. 2007; Hulme et al. 2018; van Kleunen et al. 2018). Within this context, studies attempting to identify means of introduction and the historical and geographical determinants driving invasion success are crucial to help us identify regions, vectors, and pathways more likely to originate potential new invaders as well as to design science-based strategies for the prevention and control of current and future invasions.

Globally, islands are hotspots of naturalized alien species richness across multiple taxonomic groups (Dawson et al. 2017) and insular regions with high per capita GDP, high human population densities, and high levels of anthropogenic disturbance

are supporting the most invasive alien species (Kueffer et al. 2010; Essl et al. 2019). While these drivers are strong, different archipelagos have unique properties associated with biological invasions that are not necessarily shared globally (Kueffer et al. 2010). The Caribbean region, comprising the Bahamas, Greater and Lesser Antilles, and islands off the coast of northern South America, is considered a global biodiversity hotspot with high priority for conservation due to its biological richness and high levels of endemism (Myers et al. 2000; Maunder et al. 2008). These islands share a complex geological and biogeographical history that has resulted in a unique distribution of their biodiversity (Santiago-Valentin and Olmstead 2004; Roncal et al. 2020). They also share an intricate human, political, and socio-economic history that has led to high levels of anthropogenic disturbance, multiple introductions of alien species over centuries, and extensive deforestation resulting in habitat-loss and degradation of biodiversity (Maunder et al. 2008; Rojas-Sandoval et al. 2017, 2020). Currently, the expansion of urban areas, massive tourism developments, and high human population densities are accelerating social-ecological changes and generating unprecedented pressure on Caribbean natural resources (Dixon et al. 2001; Grandoit 2005). Owing to the combination of these unique circumstances, Caribbean islands are an excellent model to assess the link between introductions of alien species and human activities and the potential role of historical and geographical factors driving invasion success. On the other hand, assessments of the history of plant invasions are still limited for the Caribbean region, highlighting gaps in knowledge that need to be addressed.

In this study, we used data for traits related to the history of introduction (e.g., continent of origin, reason for introduction, and date of introduction) of alien plant species classified as invasive on 18 island groups in the Caribbean region to: (1) assess how human activity has influenced plant invasions in the Caribbean during the last 200 years, (2) identify how many invasive species were introduced from different continents and for certain purposes and whether these patterns of introductions have changed over time, and (3) evaluate whether invasive species introduced for certain purposes are invading a wider range of habitat types. We expected plant taxa native to tropical regions to be the dominant invaders in the Caribbean following the premise that climate matching between native and introduced range is one of the few factors that consistently predicts invasion success (Thuiller et al. 2005; Hayes and Barry 2008; Bellard et al. 2016; Cabra-Rivas et al. 2016). We also expected species introduced for ornamental purposes to dominate the pool of invaders on these islands based on robust evidence in the literature indicating that from all the introduction pathways, ornamental trade is the largest source of invasive plants worldwide (Hulme et al. 2018; van Kleunen et al. 2018). By evaluating the introduction history of invasive alien species on these islands and the potential associations among continent of origin, reason of introduction, and preferred habitats invaded, we attempt to identify whether there are specific regions (sources) or reasons for introduction (specific economic and/or environmental uses) related to invasion success. These analyses would enable one to target management strategies linked to those sources and uses that represent higher invasion risk.

## Methods

### Data collection

To compile our dataset, we searched for relevant literature on Scopus, Google Scholar, and CAB Direct. Searches were performed in English, French, and Spanish with no restriction on publication year, using the following keywords: invasive, exotic, alien, non-native, naturalized AND plant, vegetation, and flora AND West Indies, Caribbean, Lesser and Greater Antilles. Additional references were identified using specialized invasive databases and by manually scanning the reference lists from the retrieved publications. The final dataset included only alien spermatophyte species that were listed as invasive in the original source consulted and are spreading beyond the point of introduction (Suppl. material 1: Appendix S1 contains the list of all sources consulted). Species occurring exclusively in captivity or under cultivation, hybrids, and unreliable records were excluded. The resulting dataset comprised invasive plant species from 18 Caribbean island groups including Anguilla, Aruba, the Bahamas, Bonaire, Cuba, Curacao, Dominican Republic, Guadeloupe, Jamaica, Martinique, Puerto Rico, St. Barthélemy, St. Eustatius, St. Lucia, St. Martin (including the Dutch and French parts of the island), Saba, Trinidad and Tobago and the Virgin Islands (including the British and U.S. Virgin Islands).

Our dataset includes the taxonomic family of each invasive plant species and the following descriptive parameters:

1. Continent (or region) of origin: Each species was classified with respect to where it is native (Africa, Asia, South America, North America, Australia-Pacific region, Europe, and West Indies). For each continent, species were also classified as “tropical origin” (species whose region of origin occurs entirely within the tropics or includes either the tropics of Cancer or Capricorn) and “temperate origin” (species whose region of origin occurs exclusively in temperate regions at latitudes  $>35^\circ$ ).

2. Life form: We classified species as either aquatic herbs, grasses, herbs, shrubs, trees (including palms), succulents or vines. In addition, herbaceous and woody habits were distinguished.

3. Reason for introduction: For each species we categorized the main reason for introduction as follows: (i) agroforestry, (ii) agriculture/food (species introduced for human consumption), (iii) forage (including forage and fodder for domestic animal food), (iv) ornamental, (v) soil conservation (including species introduced for erosion control and dune stabilization), and (iv) timber production.

4. Habitat type: Each species was classified according to the natural habitat that they have invaded on Caribbean islands. The habitat types evaluated are: (i) drylands (including dry forest, cactus thickets and Caribbean semiarid shrublands), (ii) moist forest, (iii) rainforest (including wet forest, high montane forest, and rainforest), and (iv) wetlands (including swamps, mangroves and seasonal flooded coastal forest).

Some of these categories are not mutually exclusive and one species could be assigned to multiple categories. For example, species introduced for multiple purposes

were assigned to each of them. Similarly, if the native distribution range of one species covers more than one continent or if the species is invading more than one habitat, it was assigned to each of them. Species complementary data were obtained from the National Plant Germplasm System (GRIN-Global) and other local and international sources and websites (Suppl. material 1: Appendix S2 for a complete list of the sources consulted).

We also determined the date of introduction for each plant species in our dataset. However, for Caribbean islands obtaining data on the exact dates of introduction of alien species is very difficult due to the lack of detailed historical records. Thus, we decided to use the “minimum residence time” as a conservative approach to have an estimate of the latest possible date when each species could have entered the Caribbean region. For this, we searched online herbarium records of the U.S. National Herbarium (US) and the New York Botanical Garden (NY), two herbaria with extensive collections from the Caribbean region. From these herbarium collections we extracted the date of “the earliest available record” for each species on a Caribbean island and used it as a surrogate for its “date of introduction”.

## Data analyses

Descriptive statistics and contingency table analyses were used to describe the profile of invasive plant species. We used  $\chi^2$  tests to evaluate differences in the observed and expected numbers of invasive species introduced from different regions (continents of origin) and for different purposes. For these comparisons, the expected number of invasive species in each category was evaluated as the mean number of species from all the categories. To evaluate whether the invasion success of alien plant species could be related to factors associated with their history of introduction, we used our dataset to construct the following interaction matrices: (1) continent of origin  $\times$  reason of introduction, (2) continent of origin  $\times$  habitats invaded, (3) life-form  $\times$  habitat invaded, (4) reason for introduction  $\times$  habitats invaded and (5) reason for introduction  $\times$  habit. Then, matrices were analyzed as contingency tables using generalized linear models with log-link function and Poisson distribution of errors (Crawley 2007). To evaluate temporal variation in the number of invasive species the cumulative number of species was regressed against the date of introduction. We also evaluated temporal variation in the cumulative number of invasive plant species introduced from each continent and by the different reasons of introduction. We performed all statistical analyses in *R* version 3.6.2 (R Development Core Team 2020) using the *MASS* package (Venables and Ripley 2002) and the *Circlize* package (Gu et al. 2014) to generate visualizations of the flows of invasive species.

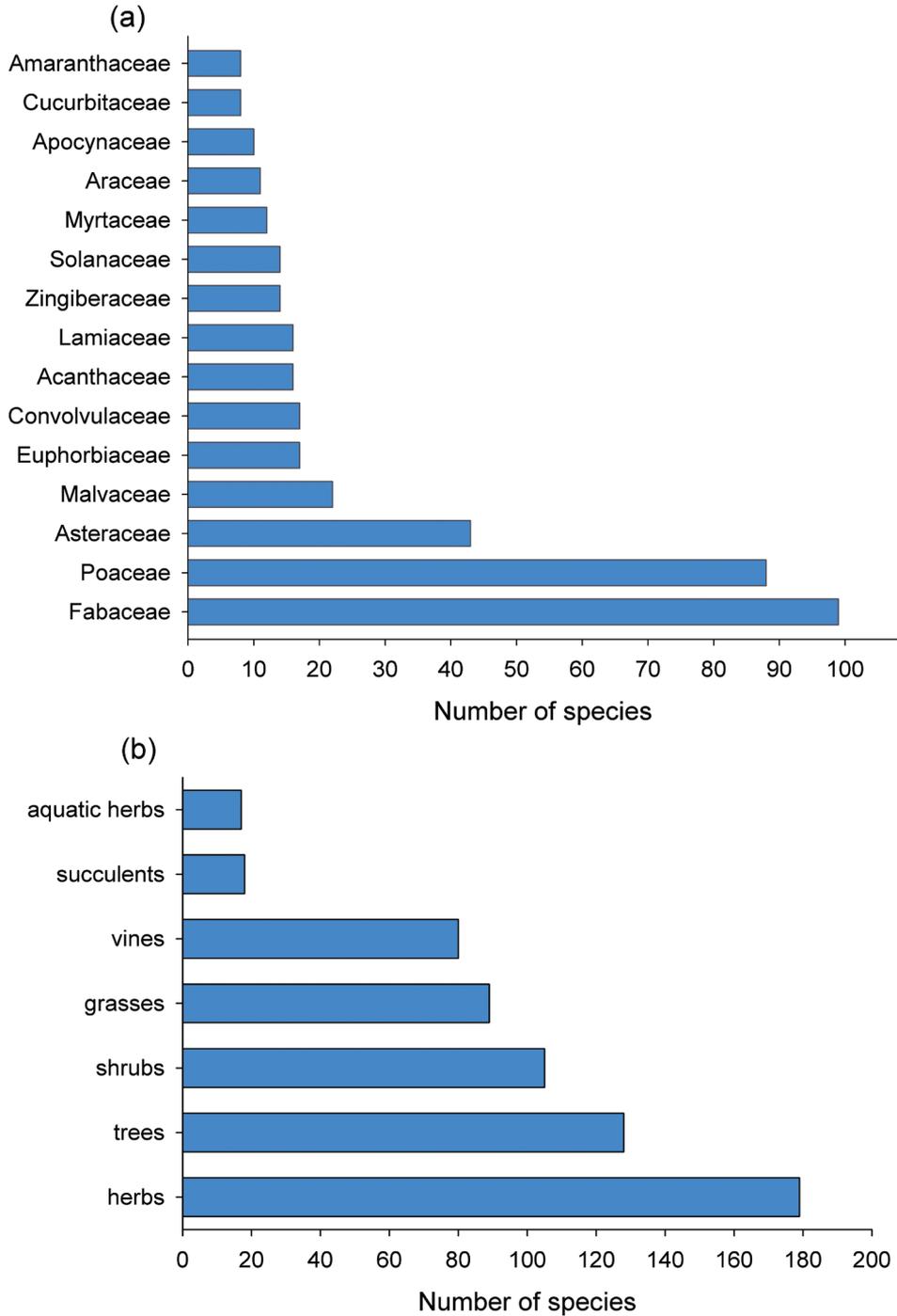
## Results

We found 616 alien plant species from 402 genera and 102 families that are classified as invaders on at least one of the islands included in this study (Suppl. material 1: Fig. S1). Plant families with the largest number of invasive species are Fabaceae,

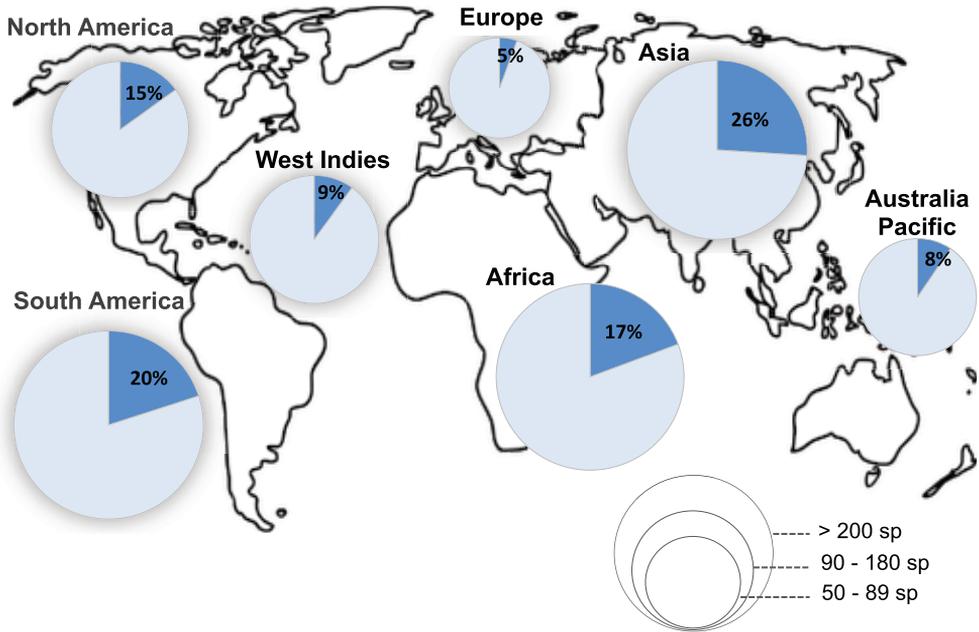
Poaceae, and Asteraceae and these accounted for 37% of all invaders (Fig. 1a). The number of invasive species within each life-form category differed significantly ( $\chi^2 = 229.3$ ,  $df = 6$ ,  $p < 0.0001$ ) with a prevalence of herbs (29%) and trees (21%) followed by shrubs (17%), grasses (14%), vines (13%), succulents (3%) and aquatic herbs (3%). Fewer invasive species than expected were aquatic herbs and more species than expected were herbs (Fig. 1b). Regarding habit, 55% of all invasive species are herbaceous and 45% are woody species. For the continent of origin, we found that invasive plants in the West Indies originated from all continents, but the number of species introduced from each region varied significantly ( $\chi^2 = 253.5$ ,  $df = 6$ ,  $p < 0.0001$ , Fig. 2). Asia, South America, and Africa contributed significantly more invasive species than expected while fewer than expected invasive species came from Europe. As expected, most invaders on Caribbean islands originated from tropical regions across the different continents but the group is dominated by species coming from tropical regions in Asia, South America, and Africa (Suppl. material 1: Fig. S2). For example, 85% of the total number of invasive species introduced from Asia (281 species) has its origin (native distribution range) in tropical regions of Asia. Similar results were identified for most continents including the other two major donors of invasive species, South America (82% species with tropical origin) and Africa (78% species with tropical origin) (Suppl. material 1: Fig. S2).

We were able to determine the pathway of introduction for 605 out of 616 invasive species in our dataset. We found that 111 species were unintentional introductions (18%) and 494 species were intentionally introduced (82%). Of the latter, we detected clear significant differences for the reasons of introduction ( $\chi^2 = 528.7$ ,  $df = 5$ ,  $p < 0.0001$ , Fig. 3a), with considerably more species being introduced as ornamentals than by any of the other purposes. Species introduced as ornamentals accounted for 54% of all invaders followed by species introduced for forage (17%), agriculture/food (13%) and agroforestry (9%). Species introduced for soil conservation and timber production only contributed 5% and 2%, respectively. Our results also showed that across the Caribbean islands, invaders occurred in all the habitat types (Suppl. material 1: Fig. S3), but when we normalized each habitat type by habitat area (considering all the islands included in the study), we found that the number of invasive species per unit area is slightly higher in drylands compared to moist forests and rainforests (Fig. 3b). Wetlands are the habitats with the lowest number of invasive species per unit area.

Of the 616 species included in our dataset, we found herbarium records for 523 species that had been collected from Caribbean islands. We used those records to evaluate the temporal variation in species introductions. These data showed that there has been a steady increase in the cumulative number of invasive species introduced into the Caribbean in the last 200 years (Fig. 4a) with the most rapid increase occurring between 1850 and 1925. We also found that more than 79% of all invasive species are long-term residents in the Caribbean and have minimum residence times of more than 100 years (Fig. 4b). There is also a steady increase in the cumulative number of species being introduced for different purposes (Fig. 4c) but this increase has been led



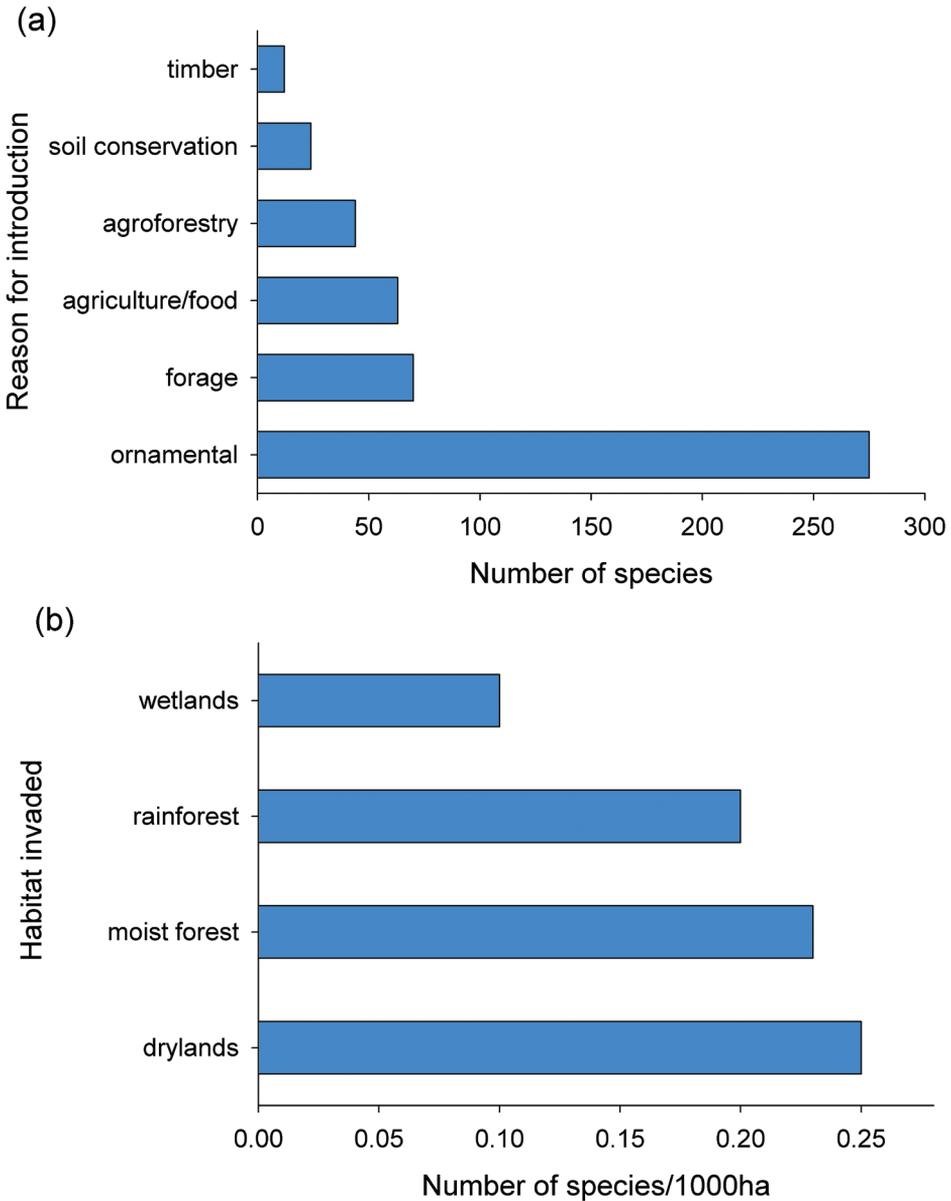
**Figure 1.** Number of invasive plant species on Caribbean Islands grouped by **a** plant families with the largest numbers of invasive species and **b** primary life-forms.



**Figure 2.** Continent of origin of invasive plant species on Caribbean islands. The size of the circle indicates the number of invasive species originating from each continent and the percentage in the circle represents the contribution of each continent to the total pool of invasive plant species on Caribbean islands. In this map, North America comprises Canada, USA and Mexico, and South America includes the Central and South America countries.

by species introduced for ornamental reasons. Our data showed that ornamental introductions have been increasing steeply since 1850 and remain at high levels (Fig. 4c). For the continent of origin, we did not detect a real sequence of introductions from one continent or another. Species introduced from Asia, America, and Africa have been gradually increasing since 1880 while species introduced from the remaining regions have nearly ceased since the mid-20<sup>th</sup> century (Fig. 4d). We assume any effect of local collecting bias would be minimal and not affect general outcomes as our data have broad spatial (18 islands) and temporal (>200 yrs) coverage.

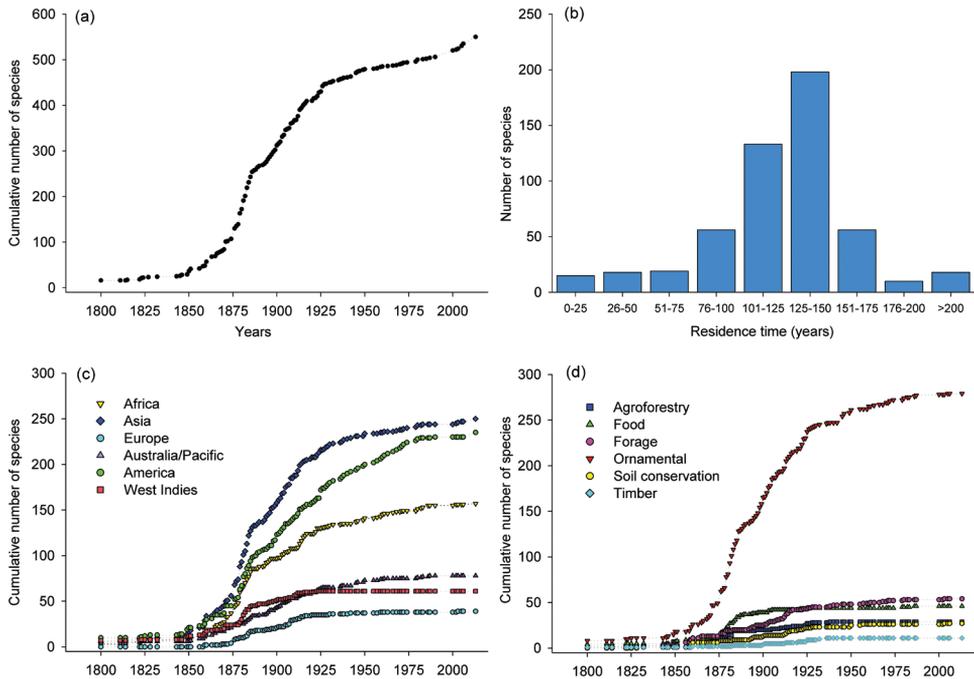
For the different interaction matrices evaluated, we found significant differences for the association between continent of origin and reason for introduction ( $\chi^2=134.7$ ,  $df=25$ ,  $p<0.0001$ , Fig. 5). Our combined results showed that more species than expected were ornamentals introduced from Asia and America, and more species than expected were introduced from Africa to be cultivated as forage. We also detected significant differences for the interaction between reason for introduction and habitat invaded ( $\chi^2=28.2$ ,  $df=15$ ,  $p=0.02$ , Fig. 6), with more habitats invaded by species introduced as ornamentals than for any other purpose. For the other interaction matrices analyzed we found no significant associations ( $p>0.05$  in all cases, Suppl. material 1: Figs S4, S5).



**Figure 3.** Number of invasive plant species on Caribbean islands considering **a** the reason of introduction and **b** the habitat types that they are invaded normalized by the total area cover by each habitat type.

## Discussion

By analyzing the reason of introduction and the geographical origin of invasive species in the Caribbean, we show that while many species were unintentional introductions or deliberately introduced for practical reasons related to land management,

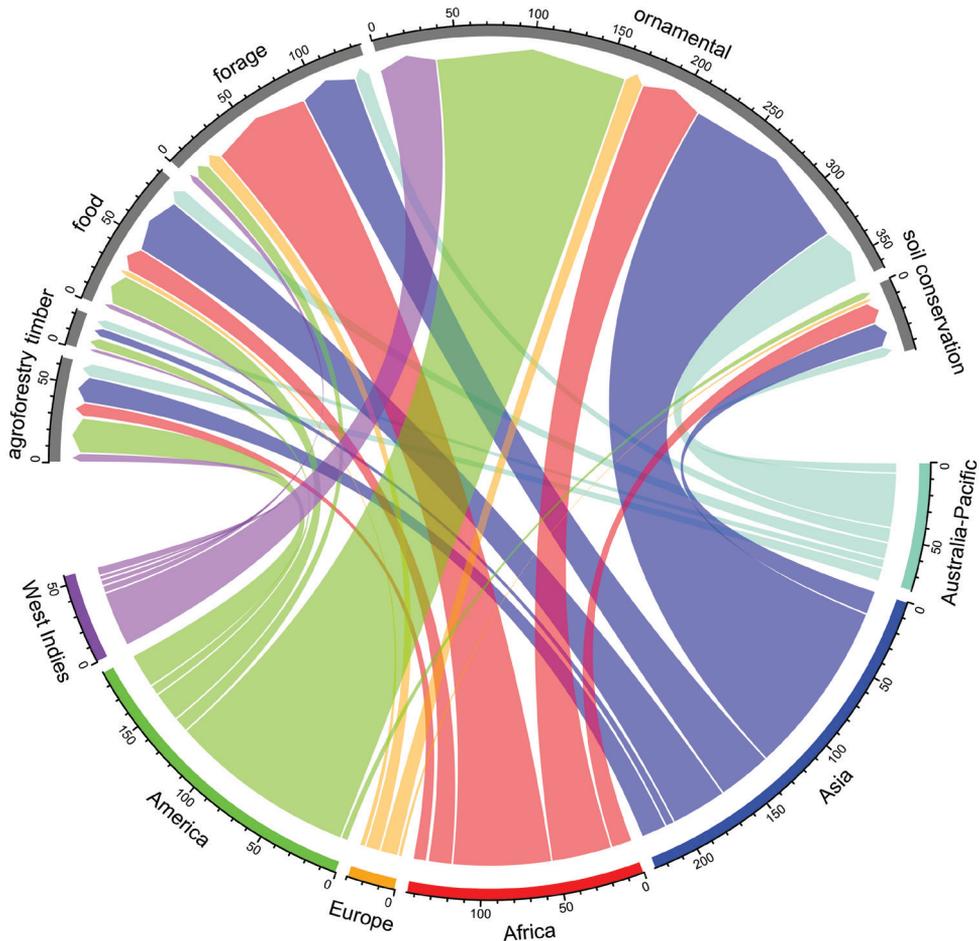


**Figure 4.** Temporal patterns of introduction of invasive alien species on Caribbean islands considering **a** the cumulative number of species that have become invasive plotted against the year of introduction **b** minimum residence time (invasive species are grouped by the number of years that they have been present on Caribbean islands) **c** cumulative number of species origination from each continent and **d** cumulative number of species introduced for different purposes.

food and forest products, most plant invaders in this region are species that were intentionally introduced for esthetic purposes as ornamentals. Indeed, we found that ornamental introductions have been leading the cumulative number of invasive species in the Caribbean over the last 200 years. Our results also showed that invasive species largely came from tropical and subtropical regions of Asia, South America, and Africa. The findings of this study highlight the role of introduction history attributes to explain invasion patterns and show that ornamental trade is the primary activity that has been facilitating the introduction and dissemination of invasive alien species in the region (see below).

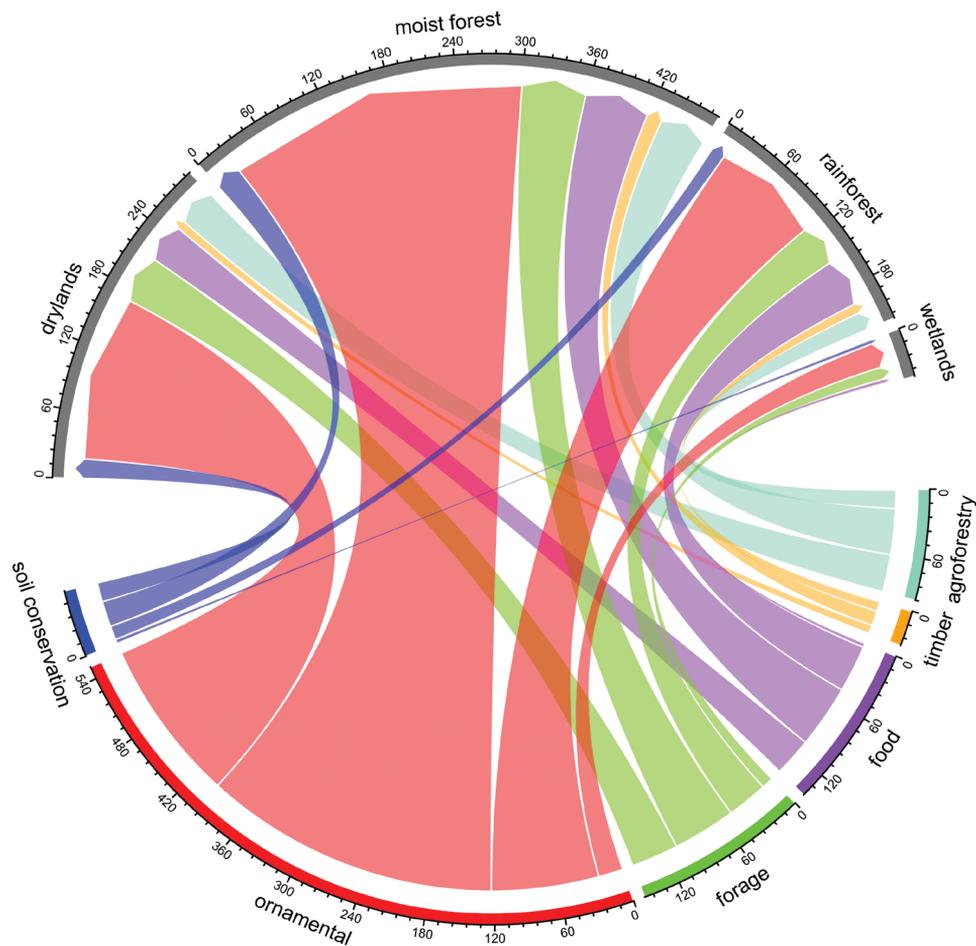
### Patterns in taxonomy and geographical origin

The taxonomic composition of the invasive flora in the Caribbean is quite diverse, but it is dominated by species belonging to large species-rich families such as Fabaceae, Poaceae, and Asteraceae, which as expected, are also highly diverse families across tropical regions (Stevens 2017). This result can be evaluated from multiple perspectives. One possibility could be that this outcome simply reflects a numeric response. These three plant families



**Figure 5.** Flows of invasive species between continent of origin and reason of introduction. This chord diagram shows the number of invasive alien species received on Caribbean islands from each continent by the different reasons of introduction. Colored outer sections indicate the number of species originating from each continent and the width of a chord represents the number of species that have been introduced for each different purpose.

are known for contributing disproportionately most to the global naturalized alien flora (Pyšek et al. 2017). However, a recent study has shown that across angiosperm plant families naturalization success is positively associated with their evolutionary history, implying that for families with high diversification rates and large geographic ranges the likelihood of becoming naturalized increases (Lenzner et al. 2020). Another possibility could be related to specific traits of members of these families that may result in higher adaptation to the new habitats (Pyšek and Richardson 2008; Kueffer et al. 2013; Otto 2018). For example, it is well known that species in the Poaceae and Asteraceae are well adapted to highly disturbed and ruderal environments, a life-history strategy known to promote the naturalization of alien plants (Guo et al. 2018). Similarly, members of the



**Figure 6.** Flows of invasive species between reason of introduction and habitat type invaded. This chord diagram shows the number of invasive alien species established in each habitat type resulting from each reason for introduction. Colored outer sections indicate the number of species introduced for each purpose and the width of a chord represents the number of invasive species that are invading each habitat type.

Fabaceae share the ability to fix atmospheric nitrogen, a trait that give them a competitive advantage in coping with anthropogenic habitats and nutrient-poor soils in their introduced range (Sprent 2009; Le Maitre et al. 2011; Rascher et al. 2011).

As expected, we found that continents with large tropical regions (Asia, South America, and Africa) dominate the geographic origin of invasive species in the Caribbean. This is consistent with previous studies showing that climatic similarity with the native region is an essential requirement for invasion success as such species are more likely to be pre-adapted to their new environments (Thuiller et al. 2005; Hayes and Barry 2008; Bellard et al. 2016; Cabra-Rivas et al. 2016). Another plausible explanation could be related to the apparent “high naturalization potential” intrinsic of spe-

cies native to geographical regions which share particular evolutionary histories that make them highly competitive and thus capable of invading elsewhere (Fridley and Sax 2014). For example, it has been recently shown that species native to Asia are over-represented as donors of naturalized plants worldwide and that they are most likely to become naturalized in other continents probably due to a “higher innate naturalization potential” of Asian species compared to plants from other regions (van Kluenen et al. 2020). Independently of the reasons explaining why species native to Asia, South America, and Africa are overrepresented on Caribbean islands, the relevance of this result is that plants introduced from these three continents should be a priority concern given that they appear in general capable of more rapid shift to invasiveness.

### Ornamentals lead the way

Our results revealed that historical factors related to the type, intensity, and frequency of introductions of alien species in the Caribbean are influencing the composition of the regional invasive floras. While all the reasons for introduction that we analyzed are contributing species to the pool of invasive species, our data clearly show that introduction for ornamental use is the major contributor of invaders. This is a pattern that have been previously reported for other regions (Reichard and White 2001; Dehnen-Schmutz et al. 2007; Lambdon et al. 2008; Zenni 2014) and a recent global comparison of the frequency of invasive plant species also showed that most invaders have originated from ornamental trade (Hulme et al. 2018). For the Caribbean region we also found that the cumulative number of invasive species has been steadily increasing during the last 200 years, but since 1850 this trend has been notably led by species introduced as ornamentals. This pattern could be explained by the increasing popularity of gardening and landscaping, both of which are associated with tourist development and the expansion of urban areas (see below). Such human activities create a demand for ornamentals and amenity species (Lambdon et al. 2008; Waugh 2009; van Kleunen et al. 2018). These results underscore the assertion that alien ornamentals are one of the major threats to the conservation of native floras and pose a significant environmental concern for the Caribbean region.

Plants commercialized as ornamentals are not randomly selected, and some of the biological traits that are desirable for the ornamental trade, such as rapid growth and establishment, production of large numbers of flowers, fruits and seeds, and easy propagation, are also traits that promote invasion (van Kleunen et al. 2018). Moreover, ornamentals have more opportunities for introduction and spread than other plant species as they are actively propagated and repeatedly planted generating high propagule pressure (Lockwood et al. 2009; Hulme et al. 2018; Gou et al. 2019; van Kleunen et al. 2020). Propagule pressure has been recognized as one of the main drivers of invasion success, and locations receiving heavy influx of propagules often have higher establishment rates and are more invaded than areas with fewer introductions (Cassey et al. 2018; Lenzner et al. 2020). For the Caribbean, long residence times and high propagule pressure appear to be key elements for the success achieved by alien ornamentals.

The overrepresentation of Asian and American ornamentals detected in the invasive flora of the Caribbean could be explained by the globalization of horticultural trade and the intercontinental fluxes of alien ornamental plants. The United States, the major provider of nursery products for the Caribbean region (Waugh 2009), has a nursery trade dominated by species imported from Asia and tropical America (especially Mexico, Central America and Brazil; Bradley et al. 2012), indicating how the geographical origin of ornamental nursery stocks in the US is influencing sources of naturalized and invasive plant species in recipient regions (Bradley et al. 2012; Hulme et al. 2018; van Kleunen et al. 2018). The overrepresentation of African forage species has a very different origin and could be explained by the establishment of human-maintained pasturelands to support livestock activity. Across tropical regions, the conversion of native forests to pasturelands has resulted in landscapes dominated by alien grasses which has had intense ecological and climatic consequences (D'Antonio and Vitousek 1992; Williams and Baruch 2000). The Caribbean region is not the exception. Agricultural experiment stations across the region worked together to improve primary productivity and nutritional quality of forage species. To this end, they introduced alien species, many from Africa which were presumably more resistant to grazing pressure (Sterns 1992; Fernandez-Prieto 2013).

### Implications for management and conservation

Caribbean islands have undergone profound social and economic changes transitioning from an economy based largely on agriculture and exploitation of natural resources (e.g., logging and mining) to one more based on tourist development. This conversion has led to the removal and alteration of much of the original vegetation and has contributed to major changes on terrestrial habitats and a significant reduction in biodiversity (Dixon et al. 2001). While the abandonment of agriculture has led to forest regeneration, the increasing demand for tourism facilities and the expansion of commercial and residential development is contributing to the degradation and reduction of natural habitats and thus facilitating the introduction and establishment of potentially invasive species (Parés-Ramos et al. 2008; Timms et al. 2013; Walters 2016; Rojas-Sandoval et al. 2020). Due to the fragile conservation status of Caribbean natural habitats, the fact that invasive species are occurring in all the habitat types analyzed in this study represents an additional level of vulnerability. Moreover, the socio-political complexity of this region (that comprises independent nations as well as British, Dutch, French and U.S. overseas territories), with many competing units and scales of governance, hinders the implementation of effective actions to address the conservation issue of invasive species (Vaas et al. 2017).

Currently, the tourism industry (including transportation, lodging, and amenities) is the dominant economic force in the Caribbean (Palmer 2009) and is the major consumer of ornamental horticulture products throughout the region (Waugh 2009). Tourism facilities (e.g., resorts and vacation homes) often have large green areas and thus consume large quantities of gardening products, including live plants (Waugh 2009). Similarly, the current expansion of urban areas is also facilitating the natu-

ralization and invasion of alien plants in the Caribbean region. For example, about two-thirds of the ornamentals cultivated in domestic gardens in urban areas in Puerto Rico are introduced alien species and, in agreement with our results, most of them are also alien ornamentals native to Asia and tropical America (Vila-Ruiz et al. 2014; Rojas-Sandoval and Acevedo-Rodríguez 2015; Melendez-Ackerman and Rojas-Sandoval 2021). These results are relevant because gardens often act as “reservoirs” of potential invasive species since many ornamentals have high naturalization success and thus represent a greater risk of escaping and becoming invasive (Guo et al. 2019).

## Conclusions

Caribbean forests are among the most heavily utilized, disturbed, and least preserved ecosystems across the tropics. This study provides evidence that historical factors related to the type, intensity, and frequency of human-mediated introductions of alien species have been influencing the composition of invasive plant species in the Caribbean during the last 200 years. These factors are important for understanding current patterns of invasions, but they are also crucial for planning adequate management actions for the control and prevention of current and future invasions. Our results clearly identified the drivers and sources that contribute most to the pool of invasive species in the Caribbean. We also showed that introduced ornamentals are successfully invading all major habitats, exacerbating conservation issues and threatening the diverse native flora of the Caribbean. Therefore, effective biosecurity actions to regulate ornamental trade and importations from Asia, America and Africa regions should become a management priority. Due to the complexity of the problem, reducing intentional introductions of alien species through the ornamental pathway will require the cooperation of the tourism industry, landscapers, garden owners, and nursery vendors. Additionally, Caribbean states should strengthen their biosecurity protocols and implement and enforce effective management strategies to address the problem of invasive species.

## Data availability statement

The databases that we used are all publicly available and the references for all the sources consulted are provided in the Supplementary Materials. The data used in this study were deposited in the FigShare Digital Repository <https://figshare.com/s/36971893d1fdf9b43a77>

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## Supplementary material I

### Supplementary materials

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Data type: tables and figures

Explanation note: **Appendix S1**. List of sources consulted to compile the initial list of invasive alien plant species on Caribbean islands. **Appendix S2**. List of resources consulted to complement the dataset of invasive alien plant species on Caribbean Islands with information on taxonomic family, continent of origin, life-form, habit, reason of introduction, and habitats invaded. **Figure S1**. Map of the Caribbean islands included in this study showing the number of invasive alien plant species reported for each island. **Figure S2**. Continent of origin of invasive plant species on Caribbean islands showing the percentage of species with tropical and temperate origin. **Figure S3**. Number of invasive alien species occurring in each habitat type across Caribbean islands. **Figure S4**. Flows of invasive plant species among continent of origin and the habitat types invaded. **Figure S5**. Flows of invasive plant species among life-forms and the habitat types invaded.

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Link: <https://doi.org/10.3897/neobiota.64.62939.suppl1>

