Predators, plants, and empires: the logistics of insect invasions

by

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Abstract

Non-native insects have established throughout the world, facilitated by human activity. Due to their potential to cause ecological and economic damage, a variety of biosecurity and pest management measures are used to prevent new introductions and manage existing populations. These efforts rely on an understanding of the biological invasion process and the risks posed by specific species to specific regions. To better understand some of these processes and risks, I aimed to (1) test hypotheses relating to the relative overabundance of European non-native insects established in North America and Australasia, and (2) predict host suitability and biotic resistance in North America and Europe for the European spruce bark beetle (*Ips typographus* L.) and the North American spruce beetle (*Dendroctonus rufipennis* Kirby), respectively. Regarding my first research goal, I identified that the introduction of European plants, much of which occurred under colonialism, is the most likely explanation for the asymmetrical exchange of insects between Europe, North America, and Australasia. These plant introductions likely facilitated accidental introductions of associated insects and provided suitable hosts for later insect arrivals. Regarding my second research goal, I determined that *Ips typographus* would likely find abundant and suitable hosts in North America, but its pheromone blend would attract generalist bark beetle predators. Consequently, low-density founding populations of *Ips typographus* in North America may experience high mortality from predation. I determined that *D. rufipennis* may find Norway spruce (the dominant spruce species in Europe) to be a suboptimal host, possibly experiencing reduced reproductive success. On the other hand, it is likely to avoid some predation and competition by producing a pheromone blend that is

inconspicuous to European predators or other bark beetles. My results help resolve the mystery of why Europe has been such a dominant 'exporter' of non-native insects, while providing new evidence of the large-scale facilitation of insect invasions through introductions of exotic plants. My results also provide new information on the potential suitability of the biotic environments of North America and Europe to non-native spruce bark beetles, which should aid the improvement of invasive species risk assessments.

Dedication

This thesis is dedicated to my parents, who inspired me to study biology by encouraging my countless childhood hobbies, among them insect collecting and nature photography, which I continue to practice decades later. As an early indication of my future in entomology, I once proudly captured a dozen or so rather large and lively grasshoppers, placed them into a heart-shaped plastic container, and attempted to offer them to my mother as a gift. However, the lid of the container was tinted a dark red, so to aid my mother in her appreciation for the contents of the container, I removed the lid. This incident occurred inside the house. And still my parents supported me.

Acknowledgements

This thesis would not have been possible without the excellent guidance of my supervisory committee (Jason Addison, Stephen Heard, Deepa Pureswaran, and Jon Sweeney), funding and in-kind support, and the efforts of numerous lab and field technicians who are mentioned by name in the acknowledgements for chapters two, three, and four. The field experiments, in particular, involved a considerable amount of manual labor (setting up and taking down experiments, collecting samples), taxonomic expertise, and tedious counting of thousands of insects. To all those named, and to unnamed friends and family that kept me in good spirits (particularly during COVID shutdowns), thank you for your support!

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Chapter 1 – Introduction

The movement of species into novel habitats has been a natural process since long before human civilization. However, human activity has dramatically facilitated the dispersal of species at an unprecedented speed and geographical scale (Ricciardi, 2007). International trade has been identified as the most important factor in the spread of non-native species (Westphal et al., 2008). The rates of establishment of non-native plants and insects have increased substantially since WWII, alongside similar increases in global trade openness (Bonnamour et al., 2021). Whether this represents a universally negative outcome has been vigorously debated; non-native populations may be viewed as either 'good' or 'bad' depending on context, with impacts ranging from subtle to profound (Shackelford et al., 2013). Nevertheless, the global accumulation of non-native species is a concern due to the potential for negative impacts on ecosystems and economies. Non-native species (or, more precisely, their populations) that can spread rapidly and/or cause serious negative impacts are often called 'invasive species', though there is no consensus over the precise definition of the term (Davis, 2009). Invasive species can have negative impacts, including biodiversity loss and changes to ecosystem function. Consequently, many biologists advise a precautionary approach towards invasive species management (Dahlstrom et al., 2011; Shackelford et al., 2013). However, different invasion pathways and species present different risks. The finite resources available for the management of invasive species are typically prioritized through invasive species risk assessments. To be useful, these risk assessments require evidence-based information on the possible impacts a species may have, and the processes behind its dispersal, establishment, and spread (Vanderhoeven et al., 2017).

The emergence of a new non-native species is a cyclical process of introduction (also termed dispersal or arrival), establishment, and (optionally) spread. In the introduction stage, propagules (i.e., individuals, eggs, seeds, etc.) are transported from an existing population towards some destination. The magnitude of arrivals at a given location is known as 'propagule pressure'. Introduction often fails – propagules frequently die during transport – and decreasing the likelihood of successful introductions is an important goal for biosecurity efforts. In the establishment phase, propagules that successfully arrived in a new habitat must persist for long enough to reproduce. This requires that introduced individuals locate suitable environments, resources necessary to reach reproductive maturity, and mates (if applicable), all the while avoiding mortality. The success or failure of a species to establish is influenced both by the suitability (or 'invasibility') of its new habitat, and by the species' intrinsic biological traits (or 'invasiveness'). Finally, spread beyond the point of introduction, e.g., the establishment of additional populations, is often considered an important requirement for a non-native species to be deemed as 'invasive'. Spread can also be thought of as additional introductions and establishments over smaller spatial scales. Prior to spread, eradication of a nascent population may be possible, but is often prohibitively difficult and expensive for well-established populations (Davis, 2009).

Globally, insects are one of the most numerically represented groups of non-native species. Out of 100 invasive species selected by experts as the most impactful on biodiversity and human activity, 15 are terrestrial insects – second only to terrestrial plants with 32 species (Lowe et al., 2000). Non-native insects have caused losses to agriculture and forestry products, the displacement of native species, changes to ecosystem functioning, the spread of disease, and more (Bradshaw et al., 2016; Kenis et

al., 2009). As a group, insects likely owe their success as invaders in part to their small size, often cryptic coloration, and proclivity towards hiding inside of plants and plant products (National Research Council, 2002). However, some insects are better invaders than others, and this leads to the question of whether successful invaders possess specific traits not possessed by less successful invaders. Such traits may include associations with live plant imports or wood packaging materials, which promotes accidental transport; asexual reproduction and sib-mating, which help insects avoid mate-finding failure; strong dispersal abilities (or the opposite in some cases, e.g., if staying in one place aids in mate-finding); and a lack of host specificity, which broadens the availability of suitable host plants (Brockerhoff and Liebhold, 2017). As each species differs in its traits, which further interact with the biotic and abiotic environment, invasive species risk assessments should ideally be based on species- and region-specific information (Vanderhoeven et al., 2017).

In chapter two of this thesis, I (and co-authors)[1] sought to better understand the reasons for an historical overabundance of European insects that have established into North America, relative to the opposite, touching on many of the invasion processes discussed above. I combined datasets of dated non-native insect discoveries and historical trade values exchanged between Europe, North America, and Australasia in order to test three hypotheses regarding asymmetrical establishments. These hypotheses were that differences in the rate of establishment of non-native species could be driven

[1] Rylee Isitt, Andrew M. Liebhold, Rebecca M. Turner, Andrea Battisti, Cleo Bertelsmeier, Rachael Blake, Eckehard G. Brockerhoff, Stephen B. Heard, Paal Krokene, Bjørn Økland, Helen Nahrung, Davide Rassati, Alain Roques, Takehiko Yamanaka, and Deepa S. Pureswaran. In submission for peer-review and publication. I conducted the data analyses, produced the figures and tables, wrote the first draft, and incorporated edits from co-author feedback.

by (1) differences in source species pool sizes, (2) differences in propagule pressure (using import values as a proxy), and (3) differences in habitat 'invasibility' based on the niche diversity of host plants (using plant species richness as a proxy). My analyses led me to reject the role of differential source species pools sizes, and to partially reject the role of differential propagule pressure (as measured by trade value). I found that the most compelling explanation for the relatively large numbers of European non-native insects in North America and Europe was the introduction and establishment of European plants into those regions, most of which were likely introduced via European colonial efforts. These European plants likely facilitated insect introductions both by increasing propagule pressure (insect 'stowaways' in live plant imports), and by providing suitable hosts to insects that arrived later.

In chapters three and four of this thesis, I (and co-authors) [2] conducted field experiments to predict the habitat suitability (invasibility) of North America and Europe to the European spruce bark beetle (*Ips typographus* L.) and North American spruce beetle (*Dendroctonus rufipennis* Kirby), respectively. These are 'aggressive' bark beetles that can outbreak into and kill live spruce, and thus have the potential for major impacts as non-native species. However, despite introductions of *I. typographus* into North America (Haack, 2006), and of *D. rufipennis* into England (Lieutier, 2004), neither is known to have established beyond its native continent. In my field

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[2] Rylee Isitt, Bjørn Økland, Paal Krokene, Jon Sweeney, Stephen B. Heard, Deepa S. Pureswaran. Chapter 3, *Colonization of novel spruce hosts by European and North American spruce bark beetles*, is published in *Forestry* under a different title, *Successful colonization of novel spruce hosts by European and North American spruce bark beetles can favor trans-Atlantic range expansion*. Chapter 4, *Using semiochemicals to predict biotic resistance and facilitation of introduced species*, is published in *Biological Invasions*. For both chapters, I conceived of and carried out the experiments (with input and assistance from co-authors and lab/field technicians), performed data analyses, wrote the first drafts of the papers, and revised the papers based on co-author and peer-reviewer feedback.

experiments, I investigated the potential for semiochemically-mediated interactions between the bark beetles and potential predators, competitors, or facilitators in a non-native habitat. I did this by deploying *I. typographus* pheromone lures in Canada, and *D. rufipennis* pheromone lures in Norway. These lures were placed in multiple funnel traps, allowing me to identify and count the ecologically relevant insect species that arrived, and draw conclusions from comparisons with suitable control treatments. I also conducted 'host use' field experiments, again in New Brunswick and Norway, to compare the beetles' performance (attack density, brood density, and reproductive success) between native and non-native host trees. From these field experiments, I found that *I. typographus* would likely attract generalist predators of bark beetles via its pheromone blend, and thus would likely experience predation pressure that may inhibit establishment. However, *I. typographus* also performed nearly as well in North American black spruce [*Picea mariana* (Mill.) BSP] and white spruce [*Picea glauca* (Moench) Voss.] as in its native host, Norway spruce [*Picea abies* (L.) Karst.], suggesting an abundance of suitable hosts for *I. typographus* in North America. In contrast, *D. rufipennis* appeared 'semiochemically inconspicuous' in that the synthetic pheromone lures did not attract possible predators. While this may allow it to escape predation in Europe, it also did not perform as well in Norway spruce as it did in its preferred North American host (white spruce). Consequently, the two bark beetle species both have points for and against their potential holarctic spread. However, given that Norway spruce still seemed potentially suitable to *D. rufipennis*, and that North American spruce have been planted in Europe, I proposed that it may pose the greater risk of establishment due to the possibility of enemy escape.

My analyses and field experiments provide examples of why it is important to consider regional- and species-specific risks in the formulation of invasive species risk assessments. Herbivorous insects comprised 58% of the records in the insect establishment database (see chapter two), and most insects exhibit a degree of host-specificity (Forister et al., 2015). This further interacts with the different plant hosts available in different regions, both native and non-native. When also considering regional differences in climate, biotic resistance, and species' traits that influence invasiveness, the ability to make global generalizations at higher taxonomic levels appears to be very limited. I expect that my analyses in chapter two will contribute to the knowledge of why some regions receive more non-native insects than others, and I expect that my experiments in chapters three and four will facilitate improved invasive species risk assessments for *I. typographus* and *D. rufipennis*.

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Chapter 2 – Drivers of asymmetrical insect invasions between three world regions

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Abstract

The geographical exchange of non-native insects can be highly asymmetrical, with some world regions 'exporting' or 'importing' more species than others. Several hypotheses have been proposed to explain such asymmetries, including differences in propagule pressure, environmental features in recipient regions, or biological traits of invaders. We tested aspects of these hypotheses in the context of the exchange of non-native insects between North America, Europe, and Australasia. Europe was the dominant exporter of non-native insect species between the three regions, with most of this asymmetry arising prior to 1950. The European dominance could not be explained by differences in import value, source species pool sizes, or native plant richness in the recipient regions. We identified that the introduction of non-native plants, driven in part by European colonization, best explains the asymmetrical exchange of non-native insects between our focal regions.

Introduction

Biological invasions can transform ecosystems, with serious ecological, economic, and cultural consequences worldwide. Non-native insects have been implicated in displacing native species, altering the composition of ecological communities, damaging economically important plants including trees and food crops, vectoring diseases, and more (Bradshaw et al., 2016; Kenis et al., 2009). Biosecurity measures of various kinds, such as phytosanitation and surveillance programs, are implemented in many countries to minimize new non-native insect establishments; these measures are informed in part by knowledge of the most likely invasion pathways and the taxa that pose the greatest risk (Nahrung et al., 2023). Overall risk associated with the invasion of an individual insect species is determined both by the probability that the species becomes established and the damage that it is likely to cause once established. In turn, a species' establishment probability is affected both by the likelihood that it is transported to the recipient region and the probability that arriving populations successfully sustain themselves (Blackburn et al., 2011).

An intriguing aspect of invasion risk is that some regions "export" disproportionately more non-native insects during biotic exchange than others. For example, considerably more phytophagous forest insects have invaded North America from Europe than the reverse (Niemelä and Mattson, 1996). The question of why such asymmetries occur has fascinated ecologists for decades, with several mutually compatible hypotheses offered (Niemelä and Mattson, 1996; Vermeij, 1991, 1996; Visser et al., 2016):

1) *The source species pool size hypothesis*: the number of invaders from a region should be proportional to the number of species in that region

(Vermeij, 1991). Using simulations, Seebens et al. (2017) showed that the total number of species that will eventually establish into a region is determined mostly by the size of the pool of potential invaders. However, they also note that the pool of potential invaders is a subset of the total species pools and depends on many interacting factors including species distributions and abundances, environmental suitability, and propagule pressure. Thus, species richness in a donor region is an imperfect proxy for the size of the pool of potential invaders coming from that region.

2) *The propagule pressure hypothesis*: differences in the rate of establishments between regions are driven by differences in arrival rates caused by differences in the magnitude of trade or other invasion vectors (Liebhold et al., 2017; Vermeij, 1991, 1996). International trade dominates as a driver of propagule pressure, though it is partly counteracted by biosecurity measures (Liebhold et al., 2017). Propagule pressure may be the most important determinant of establishment success, as high propagule pressure is likely required to overcome environmental and demographic stochasticity, and Allee effects such as mate-finding failure (Brockerhoff and Liebhold, 2017). Recent literature suggests that trade patterns correlate well with the establishment rates of non-native insect species (Lantschner et al., 2020; Levine and D'Antonio, 2003; MacLachlan et al., 2021). In addition, events such as world wars and accelerating globalization, which affected arrival rates, have also left their mark on temporal patterns of species establishments (Bertelsmeier et al., 2017; Bonnamour et al., 2021). However, as trade increases, the per-shipment probability of transporting new species does not

scale linearly. Additional shipments do not bring entirely new assemblages of species, but rather 'samples' of species pools that have previously been sampled from. Species that are abundant in a donor region are more likely to be transported to a recipient region and become established earlier than less abundant species, leading to a decline over time in the number of novel species that are introduced to the recipient region. Consequently, effects of increasing trade may be attenuated by the gradual exhaustion of donor species pools and the saturation of invaders in the recipient region (Levine and D'Antonio, 2003; Liebhold et al., 2017).

3) *The recipient environment hypothesis*: differences in the environments of recipient regions (e.g., climate, ecology, or existing biota) may promote or inhibit invasion. The lack of suitable climates or host plants may explain many failed establishments of introduced insects. Furthermore, approximately 75% of herbivorous insects exhibit a degree of host-specificity (Forister et al., 2015), thus successful establishment may depend on the phylogenetic similarity of potential host plants between the native and non-native habitats (Brockerhoff and Liebhold, 2017). Diversity of both native and non-native host plants appears to be a strong driver of insect invasions (Liebhold et al., 2018). The role of non-native plants in facilitating the establishment of insects is particularly noteworthy given that European colonization promoted the worldwide establishment of European plants and increased the similarity of floral composition between empires and their colonies (Lenzner et al., 2022).

4) *The invader quality hypothesis*: differences in the biological traits of insects native to some regions may make them better at invading or competing than those native to other regions (Vermeij, 1991, 1996). For example, life history traits such as asexual reproduction (e.g., parthenogenesis) and sib-mating are likely to reduce mate-finding failure in incipient populations, strong dispersal abilities may help individuals find suitable host plants, and species that are effective at colonizing disturbed habitats may be particularly effective invaders (Brockerhoff and Liebhold, 2017).

In their influential paper, Niemelä and Mattson (1996) favored the recipient environment and invader quality hypotheses to explain a relative overabundance of European phytophagous insects in North America compared to the converse. They proposed the existence of a 'European crucible' resulting from repeated and extensive glaciations which pushed species into highly isolated refugia. The European crucible may have led to the extinction of many plant genera (particularly of trees), reducing the availability of suitable niches for phytophagous insects. Furthermore, repeated isolation and release from refugia may have made European species superior competitors in disturbed and fragmented habitats, possibly increasing their ability to invade novel habitats (Niemelä and Mattson, 1996). However, aspects of Niemelä and Mattson's European crucible hypothesis are controversial; when non-native insects from all world regions are taken into consideration, Europe has approximately as many established non-native forest insects as does North America and does not appear to be particularly resistant to invasion (Roques et al., 2020). This suggests that the asymmetry in non-native forest insects exchanged between Europe and North America may be a consequence of the specific invasion pathways and histories between these two regions.

We considered the above hypotheses in the context of asymmetric exchange of all non-native insect taxa between three world regions that engage in significant anthropogenic interactions and exchange of species: North America, Europe, and Australasia (limited to Australia and New Zealand in our analyses). Our goal was to test specific predictions arising from the above hypotheses. However, due to the difficulty of quantifying the 'invasiveness' of regional source species pools, we only indirectly address the invader quality hypothesis as a possible explanation for variation that cannot be convincingly attributed to other causes. Our predictions were:

1. As per the source species pool size hypothesis, donor regions with greater richness of native insect species will export proportionally more insects to recipient regions.
2. As per the propagule pressure hypothesis, differences in trade volume, using inflation-corrected import values as a proxy, will drive differences in the exchange of non-native insects between region pairs among Europe, North America, and Australasia.
3. As per the recipient environment hypothesis, regions with greater native or non-native plant richness (tested separately) may provide greater niche diversity, and thus facilitate the establishment of non-native insects.

Additionally, we sought to determine if the relative overabundance of European forest insects in North America noted by Niemelä and Mattson (1996) extended across all taxa, if the similar overabundance of European insects in New Zealand noted by Edney-Browne et al. (2018) extended to Australia, and if any temporal changes have occurred in these asymmetries that may further elucidate their causes.

By drawing on recent literature and combining datasets of annual discoveries of non-native insects and annual import values, we have confirmed that disproportionate numbers of European insects have established into North America and Australasia, across all taxa. However, our analyses do not support the notion that differential import values or native plant diversity in the recipient regions are shaping these asymmetries. Instead, we propose that the disproportionate invasion of European insects into North America and Australasia can be explained by the introduction of non-native plants, likely facilitated by European colonization, which increased propagule pressure of associated insects and changed floral composition such that European insect invaders were more likely to find suitable host plants.

Methods

Experimental Design

We tested three hypotheses of possible drivers of differences in the number of insect establishments across each of the six directional routes between Europe, North America, and Australasia. These hypotheses proposed that the number of insects exchanged between donor and recipient regions were proportional to: (1) the richness of insect species in the donor region, as a proxy for invader pool size; (2) inflation-corrected import values flowing from donor to recipient region, as a proxy for propagule pressure; and (3) plant species richness (both native and non-native) in the recipient region, as a proxy for niche availability.

To test hypotheses (1) and (3), we compiled regional estimates of native insect richness (Arnett, 2000; Atlas of living Australia, 2022; de Jong et al., 2014; New Zealand organisms register, 2022), native vascular plant richness (van Kleunen et al.,

2015), and non-native vascular plant richness (Chapman, 2009; de Lange et al., 2017; Euro+Med Plantbase, 2021; Ulloa et al., 2017), then compared these estimates to the numbers of non-native insects established over each of the six routes. We calculated the coefficients of variation (CVs; standard deviation divided by the mean) between routes in the numbers of non-native insects, and their proportions to donor species pools and native and non-native plants (separately) in the recipient regions. If donor species pools or plant richness in the recipient regions were drivers of asymmetries in insect establishments over our routes, then the CVs for the proportions of non-native insects to these potentially explanatory factors should be markedly reduced compared to the CV for non-native insect counts.

To test hypotheses (2) and to investigate temporal changes in establishment rates, we constructed non-linear Poisson process models that predicted the annual rates of establishment of non-native insects over each of the six routes. In these models, the number of annually established insects flowing from donor to recipient regions was proportional to annual import values and (optionally) inversely proportional to the numbers of already-established non-native insects. Additionally, the models contained an 'annual establishment rate' parameter (r) that captured unexplained variation in the number of non-native insect species per unit of import value. If differences in import values fully explained the route-wise asymmetries in non-native insect establishments, then we would expect no significant differences between routes in the value of r. By incorporating estimates of discovery lag, these models also helped us to address concerns over records of first discovery being poor proxies for the timing of establishments (Costello and Solow, 2003).

Datasets and world regions

Insect establishment data were based on the International Non-native Insect Establishment database (Turner et al., 2021), supplemented by several other online datasets (GBIF, 2022; Liebhold et al., 2021; Mally et al., 2022; Nahrung and Carnegie, 2020; Seebens et al., 2017). We used an automated taxonomic cleaning script (Blake and Turner, 2021) using the GBIF (GBIF, 2022) API to standardize species names (merge synonyms and correct misspellings).

We worked with a subset of the establishment database that allowed us to compare non-native insect discoveries between donor and recipient regions. The chosen regions were North America (NA), Europe (EU), and Australia and New Zealand combined into an Australasian region (AU). Note that there were minor mismatches in the spatial extents of these regions depending on context. For example, as a donor region, Australasia included Papua New Guinea, but as a recipient region, it only included Australia and New Zealand because we did not have non-native insect discovery records for Papua New Guinea.

For all analyses except the regional counts of non-native insects reported in Table 2.1, we excluded discovery records in which: (1) species had native ranges spanning multiple biogeographic regions (e.g., Holarctic or cosmopolitan species); (2) the native ranges and establishment regions were the same (indicating species spread within these regions); (3) the establishment was limited to "indoors" (e.g., greenhouses); or (4) the establishment was a result of intentional introduction. This left us with a dataset of 2,324 non-native insect discovery records across the six pairwise routes between North America, Europe, and Australasia. For the regional counts of non-native insects reported in Table 2.1, we only excluded intentional and indoor-only establishments.

Trade data were obtained from the TradeHist database (Fouquin and Hugot, 2016), modified with modern ISO-3 country codes. The TradeHist database describes the annual value of trade goods from 1827–2014 in British pounds sterling (not corrected for inflation) flowing from origin to destination countries. We corrected all trade values for inflation relative to 2020 based on the annual percent change of the UK retail prices index (Office for National Statistics). We grouped the origin and destination countries into the same regions as above (North America, Europe, and Australasia), with some minor unavoidable differences where national borders did not follow biogeographic boundaries. From these groupings of countries, we created a subset of the TradeHist database representing the six directional pairwise routes between North America, Europe, and Australasia by summing annual trade value across all countries within each region. Records of trade between countries within each of the resulting biogeographic regions were dropped.

We obtained estimates of species richness for native vascular plants, non-native vascular plants, and native insects in our focal regions from a variety of literature sources (see citations in Table 2.1). However, we could not find an estimate of native plant richness for the combined geographical area of Australia and New Zealand. Because endemism of native plants in New Zealand is estimated to be greater than 80% (McGlone et al., 2001) we used the sum of native plant richness in Australia and New Zealand, based on the assumption that relatively few species would be common to both regions. The total numbers of discovered non-native insects in each region were calculated as the sums of unique non-native insect species binomials in each region's discovery records, after excluding intentional and indoor-only introductions but prior to any further exclusions.

Statistical Analyses

We used *G*-tests (log-likelihood ratio goodness-of-fit tests) to test the null hypothesis of equal species richness of plants and insects, both native and non-native, between our three regions.

To investigate simple spatial asymmetries in non-native insect discoveries, we tallied the number of first discoveries of non-native insects for each of the six directional pairwise routes between North America, Europe, and Australasia. We investigated possible explanations based on plant and insect richness by calculating the proportions of non-native insect discoveries to (i) native insect richness in the donor community, (ii) native plant richness in the recipient community, and (iii) the richness of non-native plants which established from the donor to the recipient. To compare the variation between these proportions and non-native insect discoveries, we calculated the coefficients of variation (CV) separately for each metric. We did not use formal statistical tests due to the low sample size of six routes.

To further investigate temporal patterns of non-native insect establishments among the six routes, accounting for lags between species establishments and discoveries, we used a Poisson process modelling approach modified from Costello et al. (Costello et al., 2007) and Morimoto et al. (Morimoto et al., 2019). These models estimated the lag between establishments and discoveries, predicted the annual establishments necessary to fit to observed discoveries given the lag estimates, and (optionally) modelled reductions in the per-unit-import rate of non-native insect establishments due to the saturation of finite species pools. Because the Poisson-process models depend on dated discovery data, we excluded discovery records with unspecified dates. This left us with 1,872 dated records (~80% of the dataset).

We omitted an intercept term in our models, forcing them to account for all establishments as a function of imports. We modelled the saturation of establishments as a non-linear (squared) probability of establishment based on the assumption that the invader pool will be rapidly depleted of its more numerous and/or best invaders. These modifications were necessary to produce good fits to our data – initial attempts to use the same models as in Morimoto et al. (Morimoto et al., 2019) resulted in nonsensical parameter estimations and poor fits in most cases. Our full model was as follows:

$$\lambda_t = r v_t s_t$$

$$s_t = \left(1 - \frac{d_{c,t}}{d_{sat}}\right)^2$$

$$N_t \sim \text{Poisson}\left(\delta_t = \sum_{j=1827}^{t} \lambda_j \, p_{j,t}\right) \qquad (2.1)$$

$$p_{j,t} = \pi(1-\pi)^{t-j},$$

where:

λ_t is the predicted number of new non-native establishments in year t,

r is the number of species established per billion pounds sterling (prior to saturation),

v_t is the value of imports (2020 billion pounds sterling) in year t,

s_t is the per-species probability of establishment in year t,

$d_{c,t}$ is the (observed) cumulative species discovered by year t,

d_{sat} is the number of discoveries after which new establishments cease (saturate),

N_t is the actual number of non-native discoveries in year t,

δ_t is the predicted number of non-native discoveries in year t,

$p_{j,t}$ is the probability that a species which established in year j will be discovered in year t,

and π is the annual probability of discovery.

The cumulative sum of discoveries ($d_{c,t}$) was calculated by summing the number of annual discoveries from the first year of records (1827) to year t, inclusive. We used the sum of discoveries instead of establishments for modelling the saturation of species pools because discovery sums could be easily calculated from the original data. The main drawback to this technique was that it slightly complicated the interpretation of the saturation parameter (d_{sat}): rather than being a predicted maximum number of established species, it was the predicted number of discoveries at which point the maximum number of established species had been reached.

All analyses were performed in R 4.1.0 (R Core Team, 2021). We fit the models to observed annual discoveries (N_t) for each combination of donor and recipient region, minimizing the maximum likelihood as described by Morimoto et al. (Morimoto et al., 2019):

$$L(r, d_{sat}, \pi) \propto \prod_{j=1827+\tau}^{t} \exp(-\delta_j)\delta_j^{N_j}, \qquad (2.2)$$

where τ =20 as "preservation years" to prevent fitting the model to species that established prior to 1827 (the first year of discovery records in our database) but were discovered after 1827. Without these "preservation years", δ_t (the predicted number of discoveries in year t) may be underestimated near the start of the dataset because there

will be a lack of prior years of predicted establishments from which to model the lagged discoveries (Morimoto et al., 2019). We also used a reduced model which omitted the saturation effect from Eq. (2.1), effectively making s_t a constant with a value of 1. We then removed the associated parameter (d_{sat}) from Eq. (2.2). This "without saturation" model was otherwise identical to the full model.

For parameter estimation, we set lower and (in a few cases) upper bounds on each parameter using the 'L-BFGS-B' method (Byrd et al., 1995). We bounded the rate of establishments (r) to ≥ 0.005 non-native species per billion pounds sterling, and the annual probability of discovery (π) to between 0.0125 and 0.95 (corresponding to 1.05 – 80 years of discovery lag). For the saturation term (d_{sat}), we set the lower bound to the cumulative number of dated discoveries, which varied for each of the six routes (1121 for EU to NA, 205 for NA to EU, 349 for EU to AU, 70 for AU to EU, 74 for NA to AU, and 53 for AU to NA), with no upper bound. We fit both the full and reduced models (the latter lacking the saturation term) to each establishment route and selected the one with the lowest Akaike information criterion (AIC) value.

The *R* function *optim* was employed for all the parameter estimation in Poisson process models (R Core Team, 2022). The confidence intervals are approximately calculated using the inverse of the Hessian matrix evaluated at the last iteration in the optimization process. For parameters with lower or upper bounds, we truncated the confidence intervals to the parameter estimation boundaries.

Observed annual discoveries and model predictions of annual establishments and discoveries were plotted as cumulative curves versus cumulative import value, based on Levine and D'Antonio (Levine and D'Antonio, 2003).

Results

Regional differences in cumulative discoveries, plant richness, and species pools

Species counts of vascular plants and insects, both native and non-native, differed significantly between North America, Europe, and Australasia (all $p < 0.001$; Table 2.1). Counts of non-native plants and insects are particularly variable between regions and follow similar patterns: Europe has approximately half as many non-native plants as North America or Australasia, 57% fewer non-native insects than North America, and 33% fewer non-native insects than Australasia (Table 2.1).

Table 2.1. Approximate numbers of described native and non-native vascular plants and insects in North America (NA, north of Mexico), Europe (EU), and Australasia (AU). Counts of non-native insects are from all world regions in our dataset (excluding intentional and indoor-only introductions). All other counts are obtained from literature or taxonomic databases (see citations). We used log-likelihood ratio goodness-of-fit tests (G-tests) to assess the equality of counts between regions for each category.

	Vascular plants		Insects	
	Native	Naturalized non-native (extra-continental)[1]	Native	Non-native (all origins)
North America	15,447[2]	3,513 in total (1,839 from EU 364 from AU)	87,107[3]	3,364
Europe	16,000[4]	1,883 in total (759 from NA 163 from AU)	94,000[5]	1,439
Australasia	~ 21,826* (19,324 in Australia[6], 2,502 in New Zealand[7])	3,371 in total (1,322 from EU 775 from NA)	81,667[8,9]	2,146 (1,004 in Australia, 1,543 in New Zealand)
G ($df = 2$)	1,363 ($p < 0.001$)	601 ($p < 0.001$)	955 ($p < 0.001$)	813 ($p < 0.001$)

[1] van Kleunen et al., 2015
[2] Ulloa et al., 2017
[3] Arnett, 2000
[4] Euro+Med Plantbase, 2021
[5] de Jong et al., 2014
[6] Chapman, 2009
[7] de Lange et al., 2017
[8] Atlas of living Australia, 2022
[9] New Zealand organisms register, 2022
* Assuming few native species are common to both Australia and New Zealand due to high endemism of plants in New Zealand (McGlone et al., 2001).

European insects accounted for 80% of the discoveries of non-native insects exchanged between Europe, North America, and Australasia. The bidirectional exchange of species between Europe and North America was highly asymmetrical, with 6.1 times more European insects discovered in North America than the converse. Nearly the same relationship was seen between Europe and Australasia, with 5.8 times as many European insects discovered in Australasia than the converse (Fig. 2.1a).

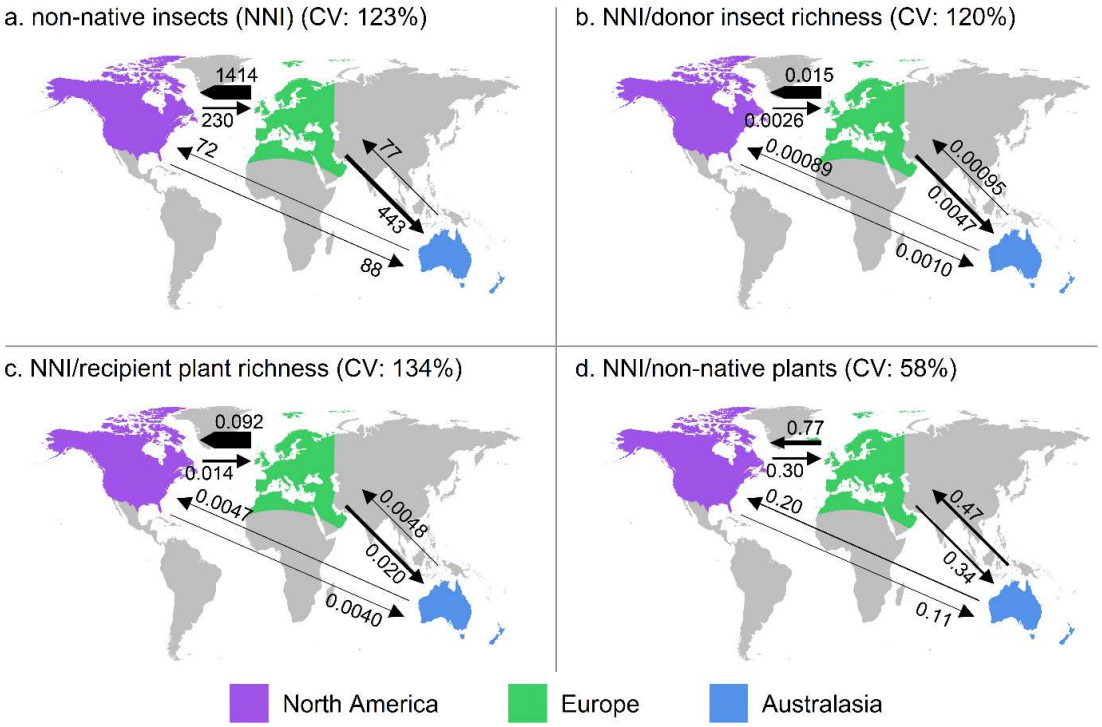

Figure 2.1. Counts of discovered non-native insects exchanged between North America, Europe, and Australasia (a) and the proportions of these counts to the richness of native insects in the donor regions (b), the richness of native plants in the recipient regions (c), and the number of non-native plants established from the donor to the recipient regions (d). Proportions were calculated from the values in Fig. 2.1a and Table 2.1. Arrow widths are proportional to the counts/proportions. CVs are the coefficients of variation (standard deviation divided by the mean) for each of the six counts/proportions per panel.

The relative magnitudes and coefficients of variation for non-native insect discoveries were similar regardless of whether they were based on simple counts of non-native insects, their proportions to donor species pool sizes, or their proportions to

recipient native plant richness. In contrast, expressing non-native insect discoveries as proportions of the numbers of non-native plants established over the same routes reduced the apparent asymmetries between donor and recipient pairs, and consequently reduced the coefficient of variation (Fig. 2.1). This indicates a close correspondence between the numbers of non-native insect and plant species exchanged over each of the six routes.

Per-unit-import establishments of European insects into North America began to slow in the 1950s, indicated by highly convex plots of cumulative establishments/discoveries versus cumulative imports. The best model included a saturation effect. By 1950, approximately 75% of non-native insects from Europe had already established into North America (Fig. 2.2a). In contrast, ~ 25% of establishments from North America into Europe had occurred by 1950, with only a slight indication of convexity in the rate of per-unit-import establishments (Fig. 2.2b).

The patterns of cumulative establishments and discoveries between Europe and Australasia resembled those between Europe and North America (compare Figs. 2c, d with Figs. 2a, b). Cumulative plots of establishments and detections of European insects in Australasia are strongly convex, with the best model including a saturation effect. As with the model of European insects into North America, approximately 75% of insect establishments from Europe into Australasia had occurred by 1950 (Fig. 2.2c). Establishments and discoveries of Australasian insects into Europe showed no effects of saturation, with both observations and models of non-native species exhibiting a linear relationship to imports (Fig. 2.2d).

Spatiotemporal analyses and Poisson process models

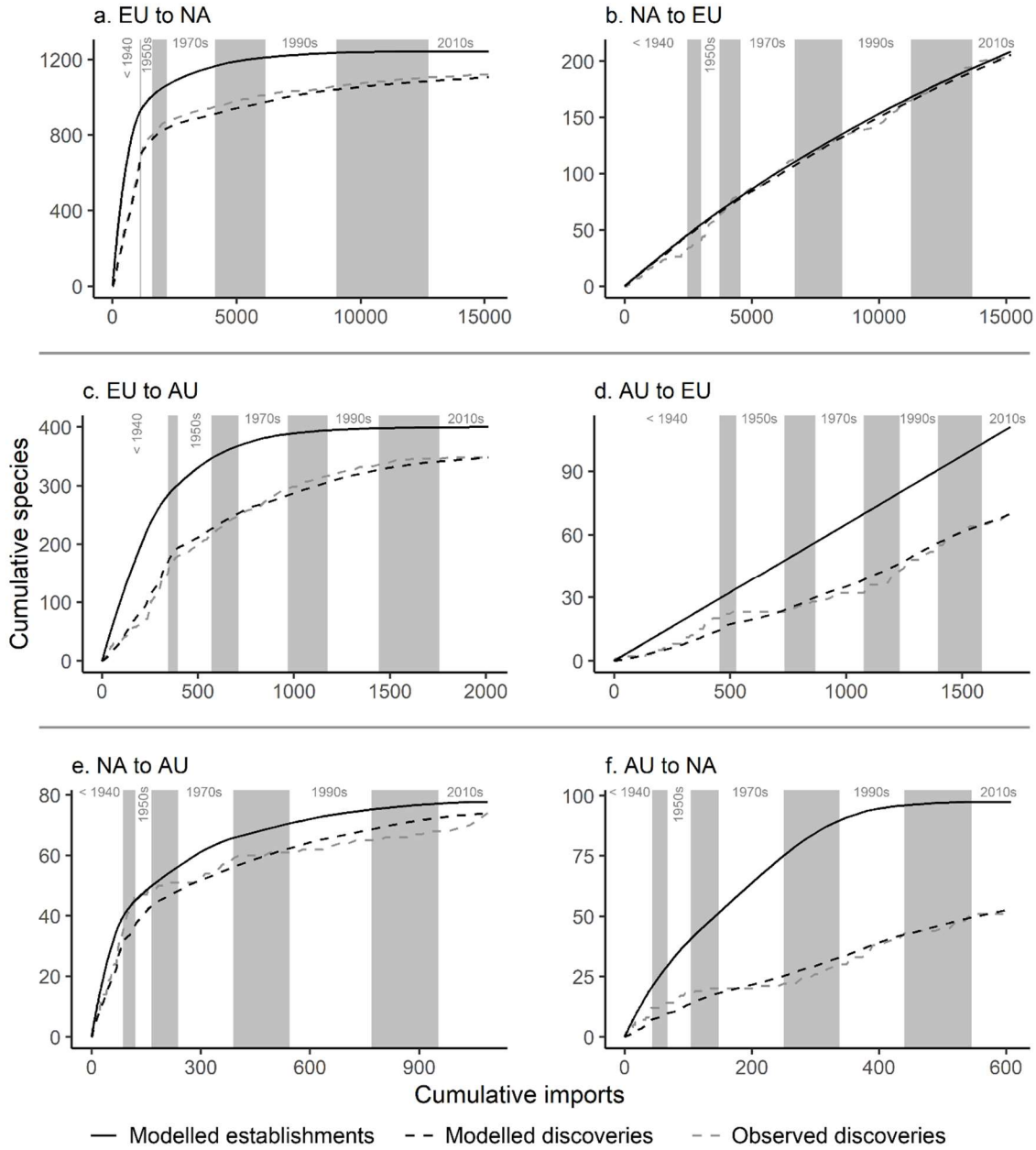

Figure 2.2. Cumulative discoveries (observed and modelled) and establishments (modelled) of non-native insects exchanged between Europe (EU), North America (NA), and Australasia (AU) versus cumulative import value (inflation-corrected to 2020 British pounds sterling), 1827–2014. Alternating background shading indicates decade boundaries, with shading omitted prior to the 1940s for clarity.

Establishments and discoveries of non-native insects between North America and Australasia were within the same order of magnitude in both directions (Fig. 2.2e, f). There was evidence of saturation in the exchange of non-native insects in both directions between North America and Australasia, though less so from Australasia to North America.

The predicted numbers of non-native insect establishments per unit of import value (annual establishment rate, *r*) was significantly different from Europe to North America and from Europe to Australasia versus the directionally opposite routes (Table 2.2).

Table 2.2. Parameters and 95% confidence intervals of Poisson-process models of establishments and lagged discoveries of non-native insect species exchanged between Europe (EU), North America (NA), and Australasia (AU). All models included a parameter for imports (*r*, the number of annual establishments per billion pounds sterling) and lag (π, the annual probability of discovery of established species). Models including an additional term for saturation (a decrease in establishment probability as the cumulative number of discoveries approaches d_{sat}) were selected for most invasion routes, with model selection based on Akaike information criterion (AIC) values.

Route	Best model (ΔAIC of next-best model)	Annual establishment rate, *r* (95% CI)	Discoveries at maximum establishments, d_{sat} (95% CI)	Annual discovery probability, π (95% CI)	Lag years (95% CI)
EU to NA	Imports + saturation + lag (2118)	1.58 (1.40 – 1.77)	1121 (1089-1153)	0.0277 (0.0345 – 0.0208)	36.1 (29.0 – 48.0)
NA to EU	Imports + saturation + lag (6.08)	0.0194 (0.0144 – 0.0245)	701 (290 – 1114)	0.499 (1 – 0)	2.0 (1.00 - ∞)
EU to AU	Imports + saturation + lag (251)	1.212 (0.825 – 1.60)	366 (312 – 419)	0.0245 (0.0386 – 0.0103)	40.9 (25.9 – 96.7)
AU to EU	Imports + lag (2.0*)	0.0647 (0.0173 – 0.112)	n/a	0.0259 (0.0690 – 0)	38.5 (14.5 – ∞)
NA to AU	Imports + saturation + lag (99.6)	0.771 (0.448 – 1.09)	76 (68-83)	0.0721 (0.141 – 0.00354)	13.9 (7.11 – 283)
AU to NA	Imports + saturation + lag (8.37)	0.621 (0 – 2.23)	53 (1.60-104)	0.0153 (0.0598 – 0)	65.5 (16.7 – ∞)

* Although this low ΔAIC could be considered "substantial evidence" for both the full and reduced model (Burnham and Anderson, 2004), the d_{sat} parameter estimate in the full model greatly exceeded the number of insect species in the donor region, thus the full model effectively lacked saturation and was not ecologically appropriate.

Discussion

Considerably more insects have invaded North America and Australasia from Europe than in the opposite directions (Fig. 2.1). This concurs with the previously observed overrepresentation of tree-feeding insects from Europe in North America (Niemelä and Mattson, 1996), and with non-native insects from the western Palearctic (i.e., Europe) being overrepresented in New Zealand (Edney-Browne et al., 2018). Our results demonstrate that these asymmetries are consistent across all insect taxa, including non-phytophagous insects.

Asymmetries in establishments between different routes may arise from differences in the size of donor species pools, and thus the numbers of potential invaders (Vermeij, 1991). The numbers of described native insects differed significantly in size between Europe, North America, and Australasia (Table 2.1), but these differences were small compared to the differences in cumulative establishments across the various routes between the three regions. We also caution that small differences in described species richness may be a consequence of differential scientific effort, and not necessarily a true reflection of the ecological community. When we expressed the numbers of non-native insect discoveries exchanged over each route as proportions to the richness of native insects in the donor regions, the resulting coefficient of variation was not noticeably different from that of unadjusted insect discoveries (Fig. 2.1a, b). We are confident in rejecting the hypothesis of differences in source species pool sizes as a major factor driving the asymmetrical exchange of non-native insects between Europe, North America, and Australasia.

After a non-native species establishes, there is typically a time lag until it is discovered (Essl et al., 2011). Differential discovery lags may lead to asymmetries in

cumulative discoveries (but not establishments) between regions. Our models attempted to account for this by estimating the lag between establishment and discovery, allowing us to compare predicted establishment curves among invasion routes. According to these models, the maximum rates of establishment per unit of import value (*r*) of European insects into North America and Australasia were 81 and 19 times greater, respectively, than the establishment rates into Europe from these regions (Table 2.2). Europe and North America have similar climates and floral composition (Cox, 2001) and a longer period of colonial contact than between Europe and Australasia (Engerman and Sokoloff, 2013), and these factors likely facilitated the relatively greater exchange of insects between Europe and North America.

The rates of non-native insect establishments between our focal regions have shifted markedly over time. While the overall rate of global establishments of non-native species has not slowed (MacLachlan et al., 2021; Seebens et al., 2017), our results show that establishments of European insects in North America and Australasia per unit of import value have drastically decreased since 1950 (Figs. 2a, c). Similarly, Levine and D'Antonio (2003) noted a decline in the rate of accumulation of exotic species per unit of imports in the United States and attributed this to a reduction in the per-ship probability of introducing a species due to the exhaustion of local source species pools. MacLachlan et al. (2021) estimated rates of non-native Hemiptera establishments in the USA over time and found that the risk of species establishment per unit of imports declined from 1850 to 2000, attributing this either to species pool depletion or improved biosecurity effectiveness. Forecasted accumulations of Asian and European bark beetles (Scolytinae) in the USA suggested that the rate of establishments per value of imports will slow over time (Liebhold et al., 2017). There were also declines in the rates of non-

native insects exchanged between North America and Australasia, though to a lesser extent (Fig. 2.2e, f). For North American species established in Australasia, this saturation again starts in the 1950s. For Australasian species established in North America, the evidence for saturation is less convincing, as it is not evident in cumulative discoveries and may be an artifact of an overestimated discovery lag. Discoveries of North American and Australasian species in Europe do not exhibit similarly declining trends and models show only weak or no effects of saturation (Figs. 2b, d). As a result of these trends, the exchange of non-native species per unit of import value between Europe, North America, and Australasia has equalized considerably in recent decades. Most of the asymmetries in discoveries between our focal regions thus accumulated prior to 1950. Although this appears to defy global trends which show accelerating rates of plant and insect invasions after World War II (Bonnamour et al., 2021), this is largely a consequence of different ways of measuring establishment rates: as a function of time, or as a function of import value. Declining establishments per unit of import value do not necessarily translate into declining annual establishments, as import values have increased exponentially over time (Liebhold et al., 2017). Should these trends continue, the imbalance of established European insects in North America and Australasia versus the reciprocal exchange will diminish over time. Seebens et al. (2021) also predicted that the rate of non-native arthropod establishments in North America and Australasia would plateau (NA) or slow (AU) through to 2050, while the rate of establishments in Europe would accelerate.

Although we have modelled the declines in per-unit-import establishments as a gradual exhaustion of source species pools, it is also likely that biosecurity measures have contributed. International biosecurity regulations, specifically phytosanitary

measures, began in earnest in the latter half of the 20th century (Allen et al., 2017). With plant-feeding insects making up 58% of all non-native insect species in our dataset, strengthened phytosanitary measures applying to pathways including live plants, wood, and crops have almost certainly led to contemporary reductions in per-unit-import rates of establishments. Historically, Europe has had relatively weak phytosanitary measures, while Australia and New Zealand have maintained strict phytosanitary policies for many decades (Eschen et al., 2015). The lack of obvious saturation in the establishments of North American and Australasia insects into Europe may be partly due to Europe's historically weaker biosecurity. However, differential biosecurity is unlikely to have played a major role in creating the considerable asymmetries we observed in non-native insect establishments and discoveries prior to 1950.

International trade is considered the single most important pathway for unintentional introductions of insects (Brockerhoff and Liebhold, 2017), and greater trade activity generally results in greater propagule pressure of non-native species. Existing literature identifies a positive correlation between import value and the establishment of non-native species (Lantschner et al., 2020; Levine and D'Antonio, 2003; MacLachlan et al., 2021; Seebens et al., 2017). Similarly, our models provided excellent fits of historical inflation-corrected import values to temporal changes in non-native insect establishment rates (after accounting for gradual depletion of source pools). However, the modelled establishment rates (r), which represent the maximum rates of establishments per unit of imports prior to any depletion of source pools, differ significantly between the Europe to North America route and its converse, and between the Europe to Australasia route and its converse (Table 2.2). These significant differences indicate that even after accounting for differences in trade values and invader

pools, large asymmetries between routes remain unexplained by the models. Thus, contrary to our prediction, and despite the important role of trade in facilitating the establishment of non-native species, we must look to other explanations, such as unaccounted propagule pressure, differences in regional 'invasibility', and differences in the 'invasiveness' of regional taxa.

Differences in 'invasibility' between regions may be driven by differences in the niche diversity available to invaders; plant diversity in the recipient community does, for example, appear to be a strong driver of insect invasion (Liebhold et al., 2018). Niemelä and Mattson (1996) proposed that a history of extensive glaciations may have reduced niche diversity in Europe by leading to extinctions of plant genera. At the time of their publication, Niemelä and Mattson noted approximately 18,000 species of vascular plant in North America (north of Mexico) and 12,000 in Europe, suggesting that this made Europe less invasible to insects (Niemelä and Mattson, 1996). However, Europe has been fairly heavily colonized by insect invaders from regions other than North America, particularly the Asian Palearctic (Roques et al., 2020), suggesting that Europe is not strongly resistant to invasion. Additionally, more recent estimates of plant richness show very similar estimates between Europe and North America (Table 2.1). We find it unlikely that relatively small differences in native host plant richness could lead to such unequal niche opportunities for arriving insects, as observed relationships between plant and insect richness often follow linear or nearly linear relationships (Basset et al., 2012; Procheş et al., 2009; Zhang et al., 2016). When we expressed total discoveries of non-native insects as their proportions to the richness of native vascular plants in the recipient regions, the asymmetry between reciprocal routes, as measured by coefficients

of variation, increased slightly (Fig. 2.1c). This is contrary to predictions that regions with greater native plant diversity would facilitate non-native insect establishments.

Europe stands apart from North America and Australasia in having a long imperial history, including the colonization of the latter two regions. This colonization promoted both deliberate and accidental introductions of European plants (Lenzner et al., 2018). Introductions of exotic plants by colonial powers accelerated in the 19th and early 20th centuries and have left a lasting legacy on the global flora (Lenzner et al., 2022). This is noteworthy, because non-native plant diversity may be a stronger driver of insect invasions than native plant diversity (Liebhold et al., 2018). North America and Australasia each have nearly twice as many extra-continental non-native plant species as Europe (van Kleunen et al., 2015) (Table 2.1). Second to temperate Asia, Europe is the dominant source of non-native plants worldwide, and a considerable proportion of naturalized non-native plants in North America (52%) and Australasia (39%) are native to Europe (Table 2.1). These patterns are consistent with the observed asymmetries in discoveries of non-native insects between North America, Europe, and Australasia. When cumulative discoveries of non-native insects between donor and recipient regions were expressed as proportions to the number of non-native plants exchanged between the same donor and recipient regions, the asymmetries between invasion routes are considerably reduced (Fig. 2.1a, d).

The import of European plants into its colonies in North America and Australasia would have promoted the establishment of European insects via two distinct means. First, the import of live plants would have increased the propagule pressure of insects associated with those plants. In the USA, for example, live plant imports may have facilitated the establishment of approximately 70% of damaging non-native forest

insects (Liebhold et al., 2012). Second, naturalization of introduced European plants would have provided a landscape containing suitable hosts even to highly host-specific European insects. As a large proportion of herbivorous insects exhibit some degree of host specificity (Forister et al., 2015), naturalized non-native plants likely play an important role in facilitating establishments of non-native insects. In Australia, for example, nearly 90% of non-native insect pests and pathogens were associated with non-native plants, only half were associated with native plants, and those associated with native plants were more likely to be polyphagous (Nahrung and Carnegie, 2020). The establishment of herbivorous insects from a given region may also be facilitated by the naturalization of host plants from a different region, particularly if those host plants are phylogenetically similar to the insects' native hosts (Brockerhoff and Liebhold, 2017). In the context of our focal regions, Australasia contains a unique native flora, whereas Europe and North America are united into the Holarctic floral kingdom (Cox, 2001). Thus, the naturalization of Holarctic plants into Australasia, regardless of their specific origin, may have facilitated both European and North American insect establishments.

We have documented strong asymmetries in the rates of discoveries of non-native insects between Europe, North America, and Australasia. These asymmetries were strongest prior to 1950 and favored Europe as the dominant source of non-native insects between our focal regions. Our results have allowed us to largely rule out differences in source species pool sizes, overall trade volume (using trade value as a proxy), and native plant diversity as causes of these asymmetries. Although we cannot rule out the possible role of regional differences in insect invasiveness, the introduction of exotic plants driven in large part by European colonization is the most compelling explanation for Europe's dominance as an 'exporter' of non-native insects. This

represents both a source of propagule pressure of European insects that was not adequately represented by comparisons of trade value, and changes to floral composition which have facilitated subsequent establishments of European insects. We also cannot rule out other factors not addressed here, such as differences in establishment probability driven by biotic resistance (e.g., native predators and competitors), differences in propagule pressure driven by the specific types of trade goods exchanged between regions, or the effect of establishments originating from non-native populations ('bridgeheads'). Regardless, we believe our results to be an important step forward in understanding the factors that drive international patterns of non-native species establishments.

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Chapter 3 – Colonization of novel spruce hosts by European and North American spruce bark beetles

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Abstract

The European spruce bark beetle *Ips typographus* and the North American spruce beetle *Dendroctonus rufipennis* can outbreak and cause high mortality of spruces on their native continents. Both species have been inadvertently transported beyond their native ranges. With similar climates and the presence of congeneric spruce hosts in Europe and North America, there is a risk that one or both bark beetle species will become established into the non-native continent. An introduced population of bark beetles faces many challenges, but an important prerequisite for establishment is the presence of suitable host trees. We tested the suitability of non-native versus native hosts by exposing bolts of Norway spruce (*Picea abies*), black spruce (*P. mariana*), and white spruce (*P. glauca*) to beetle attacks in the field in Norway and Canada. We quantified attack density, brood density, and reproductive success of *I. typographus* and *D. rufipennis* in the three host species. We found that *I. typographus* attacked white and black spruce at comparable densities to its native host, Norway spruce, and with similar reproductive success in all three host species. In contrast, *D. rufipennis* strongly preferred to attack white spruce (a native host) but performed better in the novel Norway spruce host than in black spruce, a suboptimal native host. Our results suggest that *I. typographus* will find abundant and highly suitable hosts in North America, while *D. rufipennis* in Europe may experience reduced reproductive success in Norway spruce.

Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a widely distributed group of phytophagous beetles that most frequently feed on the phloem of host trees. Most species attack stressed or dead trees and are ecologically beneficial decomposers,

but some species can outbreak into living trees and cause considerable economic losses to forestry and agriculture (Raffa et al., 2015).

Two such species are the European spruce bark beetle (*Ips typographus* L.) and the North American spruce beetle (*Dendroctonus rufipennis* Kirby), which can cause widespread mortality of spruce (*Picea* spp.) in Europe and North America, respectively (Boucher and Mead, 2006; Hlásny et al., 2019). The first new infestations of the year begin in the spring or summer, following the dispersal of the previous year's brood. *Dendroctonus rufipennis* has an obligate winter diapause (univoltine or semivoltine), with a two-year life cycle being typical. In contrast, *I. typographus* may have multiple generations per year if temperatures are favorable (Schebeck et al., 2017). Both species produce aggregation pheromones to attract conspecifics, which facilitates mate-finding and, during outbreaks, high-density 'mass attacks' that exhaust the defenses of healthy trees (Krokene, 2015).

Some bark beetles have become invasive species beyond their native ranges. For example, *Dendroctonus valens* LeConte, a minor pest of pines in North and Central America, established into China in the 1980s and has since become a serious pest of Chinese red pine (*Pinus tabuliformis* Carr.) (Sun et al., 2013). In its native range, *D. valens* is a host generalist, attacking at least 22 different species of pine, and this broad host range may have facilitated the beetle's host shift to *P. tabuliformis*. Other species of bark beetles are known to attack only one or a few host species; for example, *Dendroctonus jeffreyi* Hopkins is a specialist on Jeffrey pine and *Dendroctonus simplex* LeConte is a specialist on eastern larch (Kelley and Farrell, 1998). Because host specificity can inhibit the establishment or spread of bark beetle populations into novel habitats (Brockerhoff and Liebhold, 2017), it is important to know if the new habitat

contains suitable hosts when evaluating whether a species of bark beetle is likely to establish.

The European *I. typographus* has been repeatedly intercepted in North American ports (Haack, 2006), and the North American *D. rufipennis* has been intercepted in England (Lieutier, 2004). While both species remain contained (as of this writing) to their respective continents, several factors could encourage transcontinental establishment. Climates in Europe and North America are similar enough to allow both species to establish large invasive ranges across the Atlantic (Bentz et al., 2019; Godefroid et al., 2016). *Ips typographus* and *D. rufipennis* may even facilitate each other's establishment and subsequent outbreaks through cross-attraction of *D. rufipennis* to the aggregation pheromone blend of *I. typographus*, promoting joint mass attacks on less susceptible hosts (Isitt et al., 2022; Økland et al., 2009). What remains less certain, particularly for *D. rufipennis*, is whether the beetles will find suitable hosts in the non-native range.

To date, there are no published studies on the ability of *D. rufipennis* to colonize and successfully reproduce in European spruce species. The reciprocal question of whether *I. typographus* can reproduce in North American spruce species has been partially answered through experiments where *I. typographus* adults were caged onto the bark of different spruce species. These experiments suggested that *I. typographus* is capable of colonizing and reproducing in North American spruces but may experience slightly to moderately reduced reproductive success and brood quality in some host species (Flø et al., 2018; Økland et al., 2011). However, there is a need to assess the colonization and reproductive success of *I. typographus* in North American spruce under more natural conditions where the beetles locate and colonize spruce species in the field.

Such natural colonization could lead to assortative mating according to preferences for specific spruce species and may influence reproductive success.

We conducted two field experiments to test for differences in infestation density, brood density, and reproductive success of *D. rufipennis* and *I. typographus* in cut bolts of white spruce [*Picea glauca* (Moench) Voss.; native to North America and a preferred host for *D. rufipennis*], black spruce [*Picea mariana* (Mill.) BSP; native to North America and a suboptimal host for *D. rufipennis*], and Norway spruce [*Picea abies* (L.) Karst.; native to Europe and a preferred host for *I. typographus*]. Our experiments took advantage of introduced non-native spruce in Norway (white and black spruce) and New Brunswick, Canada (Norway spruce), allowing us to conduct these field experiments with native bark beetle populations. Results from prior experiments (Flø et al., 2018; Økland et al., 2011) informed our working hypothesis that *I. typographus* would attack all three species to similar extents, but with reduced reproductive success in black and white spruce. For *D. rufipennis*, we expected overall lower performance in Norway versus white spruce due to the beetle's host specificity; its preferred hosts (white, Engelmann, and Sitka spruce and their hybrids) occupy a monophyletic clade and are more distantly related to other spruce species than to each other (Holsten and Werner, 1990; Lockwood et al., 2013).

Methods

Our two field experiments compared the attack density, brood density, and reproductive success of *I. typographus* (in Norway) and *D. rufipennis* (in Canada) in white, black, and Norway spruce.

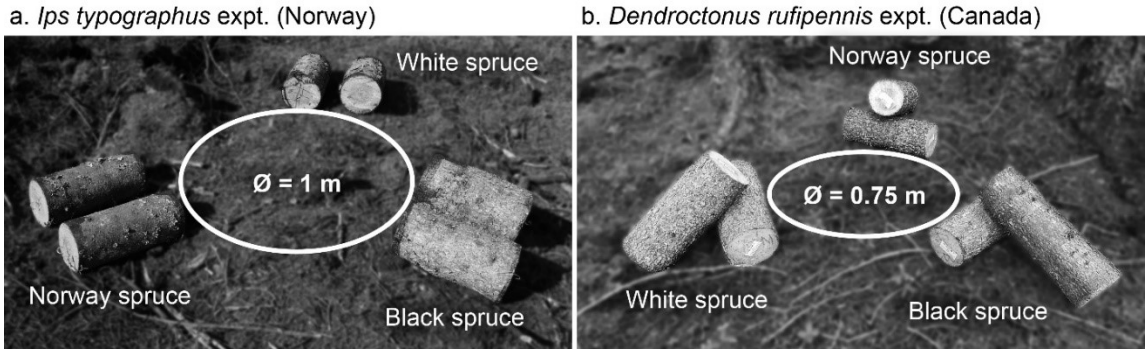

Figure 3.1. Example block configurations for bark beetle host-use experiments. Placement of host species were randomized for each block.

Ips typographus *host-use experiment (Ås, Norway)*

Ten trees each of white, black, and Norway spruce ranging from 14–22 cm (mean 17.4 cm) in diameter at breast height were felled near Prestebakke, Norway (58.992°N, 11.545°E) on April 22, 2018, and transported to the Norwegian Institute of Bioeconomy Research (NIBIO) in Ås, Norway. Two adjoining and approximately 40 cm long bolts were cut from the lower bole of each tree. This resulted in 30 pairs of bolts, where each pair was treated as a single replicate. The cut ends of each bolt were sealed with paraffin wax to prevent desiccation.

We set up the experiment on May 7, 2018, in a clearcut in a privately owned forest near Ås (59.657°N, 10.817°E). We used a clearcut instead of a forest interior to avoid spill-over of beetle attacks onto standing trees. The spruce bolts were divided evenly across 10 experimental blocks in a randomized block design, with each block receiving one replicate treatment (two 40 cm bolts) each of white, black, and Norway spruce. The three pairs of bolts were arranged around the perimeter of a 1 m circle, with the two matching bolts placed directly beside each other and 120° from the other pairs (Fig. 3.1a). The placement of the spruce treatments was randomized within each block.

We distributed the 10 blocks evenly along a 135 m linear transect, with each block separated from its neighbors by 15 m relative to the block centers. Semiochemical-based trapping experiments often use 10–15 m of separation between blocks (Borden et al. 1996, Dodds et al. 2015, Lindgren et al. 2012); on this basis, we assumed that our blocks were suitably independent.

To attract *I. typographus* into the area but not directly to the bolts, we placed commercial Ipslure pheromone dispensers (releasing methylbutenol, cis-verbenol, and ipsdienol; KjemiKonsult ANS, Jar, Norway) on wooden stakes at the midpoints between the blocks, including at either end of the transect (11 dispensers in total, separated from block centers by 7.5 m).

The bolts were left in the field for three weeks to allow beetles to attack and colonize them. We chose a three-week duration to prevent the beetle brood from developing to maturity and emerging in the field, which was a potential concern given the favorable conditions for rapid brood development (warm weather, with bolts in direct sun). On May 31, 2018, the bolts were moved from the clearcut to a partially enclosed insectary (ambient temperature) and hung from the roof joists using screw hooks and twine. We measured bolt sizes (length and diameter) and counted all entrance holes of the expected shape/size for *I. typographus* to allow us to calculate attack density (entrance holes per m^2 of bark), brood density (emerged beetles per m^2 of bark), and relative reproductive success (emerged beetles per entrance hole). Because entrance holes were sometimes hidden underneath loose bark scales, we used putty knives to remove any loose scales prior to counting. We then enclosed the bolts in loose-fitting sleeve cages with plastic funnels and collecting cups at the bottom. Emerged beetles dropped into the collecting cups and were removed and counted for each block and

spruce host treatment combination every 1–5 days. Emergence began in late June and ended in early October. A small proportion of beetles (~ 6%) did not emerge and spent the winter in the bolts. They were extracted for a different experiment and not included in our counts.

We also observed and quantified considerable numbers of the six-toothed spruce bark beetle *Pityogenes chalcographus* (L.) emerging from the bolts. Due to the opportunistic nature of these data, we did not obtain estimates of initial attack density for *P. chalcographus* and were limited to comparisons of brood density between host species. *Pityogenes chalcographus* are considerably smaller than *Ips typographus*, and it is unlikely that its entrance holes were counted as *Ips typographus* entrance holes.

Dendroctonus rufipennis *host-use experiment (Fundy National Park, New Brunswick, Canada)*

The host-use experiment in Canada was similar in design to the one in Norway but was slightly more compact due to the smaller site.

Ten trees each of white, black, and Norway spruce were felled in the Acadia Research Forest (Canadian Forest Service; 46.014°N, 66.344°W) on May 24, 2019. Approximately 100 cm of the lower bole was taken from each tree and cut into two ~ 50 cm sections. The bolts were briefly stored at the Atlantic Forestry Centre (AFC, Fredericton, New Brunswick), where they were sealed at both ends with paraffin wax.

We set up the field experiment on June 7, 2019, in the interior of a small spruce stand in Fundy National Park (45.566°N, 64.984°W), where there was evidence of recent spruce beetle attacks on standing trees. The bolts were placed into 10 experimental blocks using a randomized block design. Each block received one treatment replicate (two adjoining 50 cm bolts) of white, black, and Norway spruce.

Unlike the clearcut site in Norway, the Fundy National Park site was shaded by forest canopy and the ground was generally mossy and damp. To keep at least one bolt of each treatment replicate dry, we arranged the pairs in a T, with the end of one bolt placed onto the middle of the other. This also served to provide beetles with better access to the underside of the upper bolt. We spaced the treatments in each block evenly around the perimeters of 0.75 m circles (Fig. 3.1b). The placement of spruce species was randomized within each block.

The blocks were placed 10 m apart at their centers along two parallel transects, with the transects also separated by 10 m. To attract *D. rufipennis* to the site, we placed 12 Atlantic *D. rufipennis* lures (releasing frontalin, seudenol, and spruce terpenes; Synergy Semiochemicals Corp., Burnaby, BC, Canada) on wooden stakes equidistant between all blocks (5 m from adjacent block centers), including at either end of each transect.

We left the bolts in the field for two months to allow beetles to attack and colonize the bolts. On August 9, 2019, the bolts were moved to a greenhouse pod at AFC to expedite beetle development. We counted entrance holes of the appropriate size/shape for *D. rufipennis* and measured bolt dimensions (length and diameter). The greenhouse pod was maintained at approximately 6 °C above ambient temperature. Logged greenhouse temperatures were captured for the month of August and ranged between 15–33 °C (minimum night-time and maximum day-time temperatures, respectively).

We tracked development of the beetles by peeling back small sections of outer bark and stapling them back into place in between checks (every 1–2 weeks). We noted mostly late instar larvae in mid-August, and by late October 2019 the bolts contained sclerotized adults. In the lab, *D. rufipennis* must be exposed to approximately 70 days of

cold temperatures (≤ 4 °C) to satisfy adult reproductive diapause requirements (Bleiker and Willsey, 2020) and prompt synchronized emergence when subsequently warmed (Bleiker and Meyers, 2018). We therefore moved the bolts outdoors on November 6, 2019, covering them with a tarp, to subject the beetles to natural winter temperatures. This allowed us to hold beetles in diapause and rapidly emerge them in groups (two blocks at a time).

In mid-February 2020, we brought bolts from the first two experimental blocks to a rearing facility at AFC to initiate emergence. The facility was maintained at 21 °C with always-on artificial lighting. We contained the bolts and beetles in plexiglass emergence cages and collected emerged beetles every 1–2 days until emergence ceased (after approx. 3 weeks), counting the numbers of emerged beetles for each block and host treatment combination. COVID pandemic measures began after the emergence of beetles from the first two experimental blocks, but before subsequent blocks were brought indoors for emergence. The shutdown of most on-site activities at AFC required us to move all overwintering bolts for the remaining eight blocks into a -4 °C walk-in freezer in late March, to prevent unmonitored emergence and loss of beetles while we secured alternative arrangements. In early July, we obtained indoor rearing space at the University of New Brunswick (Fredericton). This space was not climate-controlled (20–30 °C depending on ambient temperatures) and had a combination of outdoor light from windows and room lights which were always kept on. We again brought bolts indoors for emergence, two to four experimental blocks at a time. Beetles typically finished emerging in less than three weeks, but we kept bolts caged for four weeks to catch late-emerging beetles. In all cases, this four-week period was sufficiently long that no beetles

had emerged for at least three days prior to removing the bolts from the cages. The emergence of beetles from all blocks was completed by early October 2020.

A noticeably low number of emerging *D. rufipennis* even from the preferred host (white spruce) prompted us to peel the bark from many of the bolts to investigate possible causes. Specifically, we looked for the presence or absence of larval galleries tunneling outwards from the parental galleries. This helped us determine whether the low reproductive success was due to mate finding failure or brood mortality.

In addition to *D. rufipennis*, we also observed and counted emerged *Polygraphus rufipennis* (Kirby) bark beetles. The opportunistic nature of these data meant that we did not measure initial attack densities for this species and were limited to comparisons of brood density.

Statistical analyses

Bark surface area and counts of entrance holes and emerged beetles were summed for the two bolts constituting each replicate. Brood density (emerged beetles per m^2 of bark) was calculated for all combinations of beetle species and bolt replicates. For *I. typographus* and *D. rufipennis*, attack density (entrance holes per m^2 of bark) and relative reproductive success (emerged beetles per entrance hole) were also calculated. Replicates with no beetle attacks (zero entrance holes) were dropped from calculations of relative reproductive success. While parental beetles may have re-emerged after their initial attacks, they are likely to have exited through their original attack holes and left little impact on subsequent measurements of attack density. Even if some parental beetles exited through new boreholes, we have no reason to suspect that this is a host-specific effect that would influence relative differences in attack density, brood density,

or reproductive success. Relative reproductive success should be interpreted with some care, however; it is influenced not only by differences in the number of offspring per mating event, but also by gallery abandonment and mate-finding failure. Nevertheless, it is indicative of true differences in reproductive output per unit of attack density arising from a combination of pre- and post-mating effects.

We used permutational multivariate ANOVAs (PERMANOVAs; Anderson, 2001) on Euclidean distance matrices with 10,000 permutations to determine if there were any significant effects of host tree species on the attack density, brood density, or relative reproductive success of *I. typographus* and *D. rufipennis*. Because Euclidean distance is not scale invariant, we scaled each variable to its root-mean-square. Multivariate ANOVAs (MANOVAs) provided nearly identical results, but our data violated assumptions of multivariate normality.

If the PERMANOVAs indicated a significant effect of tree species on the performance of *I. typographus* or *D. rufipennis*, we followed up with separate ANOVAs for each of the three response variables. In most cases (where data were balanced), we compared attack density, brood density, and reproductive success using multiple two-way ANOVAs (type I SS with the factors 'block' and 'host tree species'), one for each combination of beetle species and response variable. Block number was included in each model as a random factor. The models were: $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$, where Y_{ij} was beetle attack density, brood density, or relative reproductive success in host species i and block j, μ was the grand mean, τ_i was the ith host effect, β_j was the jth block effect, and ε_{ij} was the random error. For these models, all response variables were square root transformed to satisfy assumptions of residual normality and equal variances. For *P. chalcographus* and *P. rufipennis* there was only one response variable (brood density), so we did not

run multivariate tests for these species and proceeded directly to two-way ANOVAs as described above.

Due to an unbalanced design that resulted from dropping uncolonized bolt replicates, we used a slightly different ANOVA design to compare the relative reproductive success of *D. rufipennis* across host tree species. Because many blocks were no longer complete, we dropped the random block factor from the model. We used a one-way ANOVA of the form $Y_i = \mu + \tau_i + \varepsilon_i$, where Y_i was the relative reproductive success in host species i, μ was the grand mean, τ_i was the ith host effect, and ε_i was the random error. Because a square root transformation was inadequate to satisfy model assumptions, we used a two-parameter Box-Cox transformation ($\lambda_1 = 0.102$, $\lambda_2 = 0.0000343$) to satisfy assumptions of normality and equal variances (Box and Cox, 1964).

For models where host species was a significant effect ($\alpha < 0.05$), we used post-hoc tests to compare attack density, brood density, or relative reproductive success between all pairwise combinations of host tree species. We used the emmeans function of the emmeans R library (Lenth, 2022) for the post-hoc tests, with Tukey's method of adjusting for multiple comparisons. All statistical analyses were performed in R 4.1.2 (R Core Team 2021).

Results

Table 3.1. ANOVAs testing the effect of host tree species (black, white, and Norway spruce) on attack density (entrance holes per m^2), brood density (emerged beetles per m^2) and relative reproductive success (emerged beetles per entrance hole) of different bark beetle species. *Ips typographus* is absent due to the non-significant effect of host tree species on overall beetle performance according to a multivariate test (PERMANOVA).

Response variable / beetle species	**Test stat. (between host species)**	***P***
Attack density		
Dendroctonus rufipennis	$F_{2,18} = 43.5$	< 0.001
Brood density		
Dendroctonus rufipennis	$F_{2,18} = 15.8$	< 0.001
Pityogenes chalcographus	$F_{2,18} = 21.7$	< 0.001
Polygraphus rufipennis	$F_{2,14} = 2.30$	0.137
Relative reproductive success		
Dendroctonus rufipennis	$F_{2,20} = 4.72$	0.021

There was no significant effect of host tree species on the performance (attack density, brood density, or reproductive success) of *I. typographus* (PERMANOVA *Pseudo-*$F_{2,27} = 0.69$, $P = 0.61$). There was a significant effect of host tree species on the performance (attack density, brood density, or reproductive success) of *D. rufipennis* (PERMANOVA *Pseudo-*$F_{2,27} = 6.66$, $P < 0.001$).

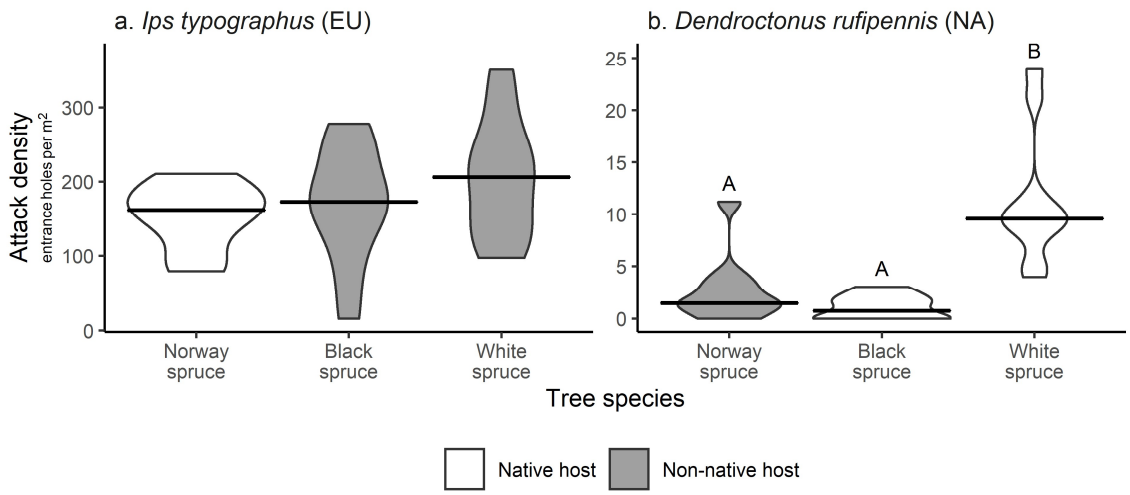

Figure 3.2. Attack density (number of entrance holes per m^2 of bark) of *Ips typographus* and *Dendroctonus rufipennis* in cut bolts from native and non-native spruce species in Norway and Canada. Horizontal lines indicate the median of each group. The native range of each beetle species is given in the headings: EU = Europe/Palearctic and NA = North America/Nearctic. Letters above violin plots in (b) indicate significance groups, with different letters indicating significant differences in attack density between tree species ($\alpha < 0.05$), based on ANOVA post-hoc pairwise comparisons using Tukey's method of correcting for multiple comparisons. The lack of significance groupings in (a) indicates that the effect of tree species was not significant. n = 10 for all combinations of beetle and tree species.

Ips typographus attacked all bolts regardless of tree species and the attack density did not vary significantly between native and non-native spruce species (Fig. 3.2a). In contrast, *D. rufipennis* showed a significant preference for white spruce (native host) over black spruce (native host) and Norway spruce (non-native host) (Fig. 3.2b). However, *D. rufipennis* attack density was slightly higher in Norway spruce than in black spruce, and this difference was nearly significant ($P = 0.07$). *Dendroctonus rufipennis* attacked all 10 white spruce replicates, eight Norway spruce replicates, and five black spruce replicates.

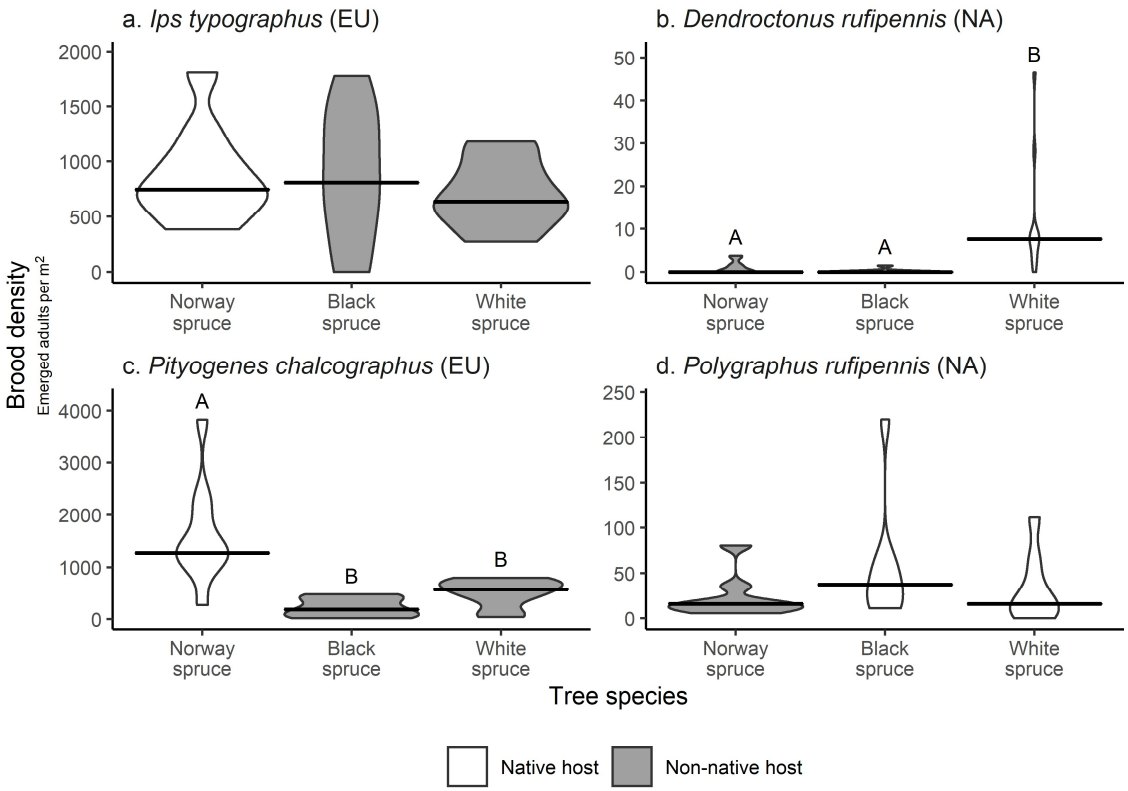

Figure 3.3. Brood density of bark beetles (emerged adults per m^2 of bark) in cut bolts from native and non-native spruce species in Norway and Canada. Horizontal lines indicate the median of each group. The native range of each beetle species is given in the headings: EU = Europe/Palearctic and NA = North America/Nearctic. Letters above violin plots indicate significance groups, with different letters within each subplot indicating significant differences in brood density between tree species ($\alpha < 0.05$), based on ANOVA post-hoc pairwise comparisons using Tukey's method of correcting for multiple comparisons. The lack of significance groupings in (a) and (d) indicates that the effect of tree species was not significant. For *I. typographus*, *D. rufipennis*, and *P. chalcographus*, n = 10 for all tree species. For *P. rufipennis*, n = 6 for all tree species.

Dendroctonus rufipennis and *P. chalcographus* were the only beetle species that showed significantly different brood densities between different host tree species (Table 3.1). In both cases, brood density was highest in a native host (white spruce for *D. rufipennis*, Norway spruce for *P. chalcographus*). Nevertheless, small numbers of *D. rufipennis* and moderate numbers of *P. chalcographus* also emerged from the non-native hosts. *Ips typographus* had a high brood density in all hosts (Fig. 3.3).

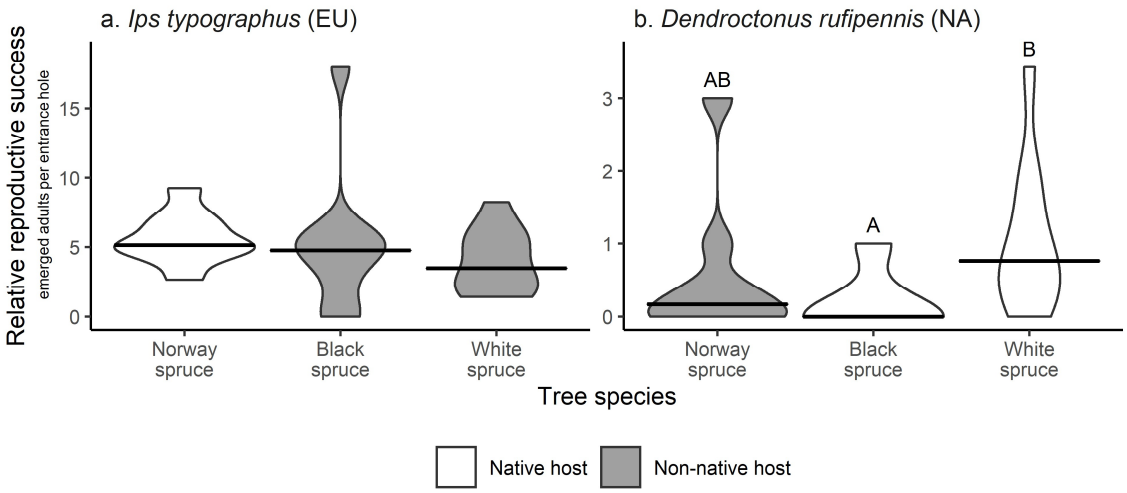

Figure 3.4. Relative reproductive success (emerged adults per entrance hole) of *Ips typographus* and *Dendroctonus rufipennis* in cut bolts from native and non-native spruce species in Norway and Canada. Horizontal lines indicate the median of each group. The native range of each beetle species is given in the headings: EU = Europe/Palearctic and NA = North America/Nearctic. Letters above violin plots in (b) indicate significance groups, with different letters indicating significant differences in relative reproductive success of beetles between tree species ($\alpha < 0.05$), based on ANOVA post-hoc pairwise comparisons using Tukey's method of correcting for multiple comparisons. The lack of significance groupings in (a) indicates that the effect of tree species was not significant. For *I. typographus*, n = 10 for all three tree species. For *D. rufipennis*, n = 8 (Norway spruce), 5 (black spruce), and 10 (white spruce).

Relative reproductive success of *I. typographus* was relatively high and did not vary significantly between tree species. *Dendroctonus rufipennis* exhibited greater reproductive success in white spruce than in black spruce ($P = 0.03$), while its reproductive success in Norway spruce was not significantly different from that in either white or black spruce (Fig. 3.3.4).

The reproductive success of *D. rufipennis* was low even in its preferred host (white spruce). Only two white spruce replicates produced > 10 emerged beetles. Peeling the bark from several bolts (post-emergence) showed that many *D. rufipennis* galleries lacked larval tunnels and thus had failed to produce viable brood. No live un-emerged adults were found under the bark.

Discussion

Ips typographus is likely to find abundant and immediately suitable hosts in North America, and thus may establish into North America under the right circumstances. Several additional factors may further promote the establishment of *I. typographus* in North America: suitable climate (Bentz et al., 2019), possible facilitation by *D. rufipennis* (Isitt et al., 2022; Økland et al., 2009), as well as suitability of North American spruce species to *I. typographus'* fungal associates and attraction of the beetles to allospecific fungal associates (Tanin et al., 2021). Yet despite being intercepted in North American ports 465 times from 1949–2008 (Liebhold et al., 2017b), *Ips typographus* has not yet established, and various explanations have been suggested for this failure (Kausrud et al., 2012).

One factor that may inhibit the establishment of *I. typographus* in North America is its reduced reproductive success in North American spruce species. Prior experiments involving caging of *I. typographus* onto spruce bolts have found that the reproductive output and offspring quality (body mass) of *I. typographus* were slightly lower in non-native spruce hosts versus Norway spruce (Flø et al., 2018; Økland et al., 2011). Our findings are generally consistent with these prior experiments; the reproductive success of *I. typographus* was slightly (but not significantly) lower in black spruce (median of 4.77) and white spruce (median of 3.48) versus Norway spruce (median of 5.13). However, the magnitude of these differences is small enough that we suspect that other factors will exert more influence on the process of establishment. Such factors may include the presence of several North American predators that are pre-adapted to locate and handle *I. typographus* (Isitt et al., 2022), and Allee effects such as mate-finding

failure (Contarini et al., 2009) or the inability to muster a mass attack against resistant hosts (Liebhold and Tobin, 2008).

In contrast to *Ips typographus*, *D. rufipennis* showed clear host preferences and differences in reproductive success between white, black, and Norway spruce. This is consistent with expectations based on its apparent host specificity in its native range: its preferred hosts of white, Engelmann, and Sitka spruce (and their hybrids) are more phylogenetically similar to each other than to other spruce species (Lockwood et al., 2013). *Dendroctonus rufipennis* preferred to attack native white spruce over native black spruce or non-native Norway spruce and experienced the greatest reproductive success in white spruce. However, we suspect that a low population density of *D. rufipennis* in our experimental stand led to Allee effects from mate-finding failure that exaggerated the differences in reproductive success between spruce species. Many beetles failed to produce offspring (no visible larval galleries), likely due to the inability of the pioneering females to attract males. We suspect that this reduced the reproductive success unequally between the tree species, with a stronger effect in Norway and black spruce due to a greater risk of mate-finding failure at progressively lower attack densities. Additionally, fewer pioneering females in Norway and black spruce would have produced less concentrated aggregation pheromone plumes, possibly reducing the secondary attraction to these hosts. When attack density, number of attacked replicates, brood density, and reproductive success are considered together, non-native Norway spruce appears to be a more attractive and suitable host to *D. rufipennis* than black spruce, a suboptimal native host.

Reduced reproductive success of *D. rufipennis* in Norway spruce may inhibit its establishment into Europe, but this may be offset by other factors promoting its

establishment. *Dendroctonus rufipennis* is likely to find a suitable climate in Europe (Godefroid et al., 2016), and it is attracted to the pheromone blend of *I. typographus*, which may enable facilitation between the two species (Isitt et al., 2022; Økland et al., 2009). Competitive displacement is another potential outcome of an association between two bark beetle species (Rankin and Borden, 1991), but the simulations by Økland et al. (2009) suggest that when both species are 'aggressive' (tree-killing), as is the case for *D. rufipennis* and *I. typographus*, their interactions are more likely to be facilitative. *Dendroctonus rufipennis* may also escape some predation and competition by being 'semiochemically inconspicuous' in Europe (Isitt et al., 2022).

Even if *D. rufipennis* should struggle to establish in European spruce species, it might invade European landscapes via introduced hosts. North American spruce species, including Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Lutz spruce (*Picea x lutzii* Little), have been introduced into plantations in Europe (Øyen and Nygaard, 2020). Sitka and Lutz spruce are both highly suitable hosts for *D. rufipennis* (Werner and Illman, 1994). These introduced North American spruces may be at greater risk of colonization by *D. rufipennis* than native European spruce species. Such 'catch-ups' of insect herbivores with their co-evolved host plants have been observed in South African tree plantations (Crous et al., 2017). More generally, the introduction of non-native plants appears to facilitate the establishment of non-native insects (Liebhold et al., 2018; Nahrung and Carnegie, 2020). Should *D. rufipennis* first establish in introduced North American spruces, it may later shift to novel hosts such as Norway spruce. The fact that some *D. rufipennis* chose to attack Norway spruce when white spruce bolts were less than a meter away suggests that the beetles have enough existing variation in host preferences to permit such host shifts.

In addition to our two focal species, we obtained brood density measurements for two other noteworthy bark beetle species: *Pityogenes chalcographus* (in Norway) and *Polygraphus rufipennis* (in New Brunswick). *Pityogenes chalcographus* is a European bark beetle known for attacking stands of young Norway spruce and has occasionally been considered a serious pest. It was previously found to attack several non-native species, including the North American Sitka spruce, but at lower density and with lower reproductive success than in Norway spruce (Bertheau et al., 2009). Our results concur: *P. chalcographus* strongly preferred to attack Norway spruce over black and white spruce (Fig. 3.3c). *Polygraphus rufipennis* is a North American bark beetle known to attack many conifer species, though it prefers spruce species (Bright, 1976). It can cause tree mortality, particularly in black spruce that has been weakened by spruce budworm defoliation (Bowers et al., 1996). The beetle's flexibility in host use appears to extend to Norway spruce, in which brood density was not significantly different from that in black or white spruce (Fig. 3.3d). The establishment of *P. rufipennis* into Europe may therefore be of potential concern.

Our experiments were restricted to quantifying attacks and reproductive success on cut bolts, and for this reason there may be doubts as to how far we can extrapolate our results. Beetle attacks on cut bolts will not induce a defensive response by the tree (production of oleoresin), and the volatiles emitted from cut bolts are likely to differ markedly from live trees. However, in the context of the establishment of non-native bark beetles, newly introduced populations will likely be small and reliant on stressed or downed trees. Therefore, we believe that using cut bolts is an appropriate method to assess the suitability of stressed/downed host material to incipient bark beetle populations.

To our knowledge, ours is the first experiment to assess host choice and reproductive success of *D. rufipennis* in non-native spruce. Although our field experiment was complicated by low attack densities across all host species, our results suggest that Norway spruce is of intermediate suitability to *D. rufipennis*, between the native hosts white and black spruce. We have further confirmed the suitability of North American white and black spruce as hosts for *I. typographus*. Finally, we have shown that both *I. typographus* and *D. rufipennis* will attack novel, non-native spruce hosts even under nearly natural attack conditions that require active host selection by the beetles.

With host availability being only one of many factors influencing establishment success, it is difficult to determine whether *I. typographus* or *D. rufipennis* present a high risk of establishment in North America or Europe, respectively. Our results suggest that both species have the potential to encounter suitable hosts in the range of the other, though *I. typographus* may suffer less from reduced reproductive success in non-native spruces. *Ips typographus* may have an additional edge over *D. rufipennis* in the form of a greater selection of possible hosts, due to a greater diversity of spruce species in North American than in Europe (Brockerhoff and Liebhold, 2017). As both species can outbreak and kill trees in their native ranges and may be even more damaging in a non-native range due to enemy release and naïve hosts (Liebhold et al., 2017a), we recommend that both species be considered high risk as potentially invasive species.

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Chapter 4 – Using semiochemicals to predict biotic resistance and facilitation of introduced species

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Abstract

Invasive species are leading causes of biodiversity loss and economic damage. Prevention and management of invasions require risk assessments based on ecological knowledge for species of potential concern. Interactions between introduced species and heterospecifics in the recipient community may affect the likelihood of establishment through biotic resistance and facilitation and are therefore important predictors of invasion risk. Experimentally exposing one species to another to observe their interactions is not always safe or practical, and containment facilities offer artificial environments which may limit the number of species and the types of interactions that can be tested. To predict biotic resistance and facilitation in a more natural setting, we deployed traps with pheromone lures in the field to mimic the presence of two potentially invasive spruce bark beetles, the European *Ips typographus* (tested in eastern Canada), and the North American *Dendroctonus rufipennis* (tested in Norway). We identified and counted possible predators, competitors, and facilitators that were captured in the traps. In eastern Canada, possible predators and competitors responded strongly to *I. typographus* lures, suggesting the potential for considerable biotic resistance. In Norway, *D. rufipennis* lures prompted little response by predators or competitors, suggesting that *D. rufipennis* may experience reduced biotic resistance in Europe. *Dendroctonus rufipennis* was also attracted to *I. typographus* pheromone, which may encourage facilitation between these species through cooperative mass attack on trees. Our findings will inform invasive-species risk assessments for *I. typographus* and *D. rufipennis* and highlight useful methods for predicting interactions between species that rely heavily on semiochemical communication.

Introduction

Invasive species are a major cause of ecological and economic damage in terrestrial ecosystems and threaten to drive many species to extinction (McNeely 2001). With respect to insects, invasive species management and prevention efforts are largely aimed at taxa that negatively impact agriculture, forestry, or human health. Bark beetles (Curculionidae: Scolytinae) are one such group, including several species (particularly in the genera *Ips* and *Dendroctonus*) that can cause considerable tree mortality (Bright 1976, Økland et al. 2011). Bark beetles may be transported via plant material and are frequently intercepted at ports around the world (Haack 2006, Kirkendall and Faccoli 2010). Invasive bark beetles may damage or kill host trees, displace native species, introduce pathogens, and reshape the ecology of the recipient community. Furthermore, invading bark beetles may escape predation or encounter naïve host trees that lack co-evolved defenses (Lee et al. 2007, Clark et al. 2014). For example, the red turpentine beetle (*Dendroctonus valens* LeConte) is a minor pest of pines in its native range of North and Central America, but has become invasive in China, where it has caused widespread tree mortality. This has been attributed in part to naïve hosts, a lack of natural enemies, and mutualistic associations with fungal pathogens (Sun et al. 2013).

The European spruce bark beetle, *Ips typographus* L., and the North American spruce beetle, *Dendroctonus rufipennis* Kirby, are particularly noteworthy due to known instances of inadvertent transport and the potential for damaging impacts. These are widely distributed bark beetles in their respective continents that can cause extensive mortality of spruce trees (*Picea* spp.) (Boucher and Mead 2006, Hlásny et al. 2019). While neither has yet established across the Atlantic, they have been intercepted beyond

their native ranges (Lieutier 2004, Haack 2006), and *I. typographus* has recently established in England (EPPO 2021).

Ips typographus and D. rufipennis live in close association with their host trees, where they feed on the phloem tissue. New infestations typically begin in the spring or summer when adults disperse in search of mates and new host trees. Eggs are laid in galleries in the phloem, and the larvae feed outward from the parental galleries until they pupate. Teneral adults continue to feed until sexually mature and ready to disperse. *Ips typographus* may have multiple generations per year if temperatures are favorable, while adult winter diapause appears to be obligate for *D. rufipennis*, limiting it to one generation per year. Both species prefer to infest stressed or downed trees at low population densities but may attack and kill healthy trees at high densities (Schebeck et al. 2017).

To successfully establish in a new habitat, both *I. typographus* and *D. rufipennis* must find a suitable ecological niche and proliferate despite Allee effects that inhibit population growth at low densities (Taylor and Hastings 2005). This means finding suitable host trees and persisting despite biotic resistance from host defenses, competitors, predators/parasitoids, and pathogens (Mattson et al. 2007). Should they cross the Atlantic, *I. typographus* and *D. rufipennis* appear capable of overcoming at least some of these challenges. Climates appear broadly suitable for both species across the Holarctic (Godefroid et al. 2016, Bentz et al. 2019), and both species can complete their life cycle in non-native spruce species (Økland et al. 2011, Flø et al. 2018, Isitt et al. unpublished data). Understanding how *I. typographus* and *D. rufipennis* may interact with novel heterospecifics (such as predators and competitors) is also crucial for

predicting the success of introduced populations, and for obvious reasons this cannot be studied by intentional introductions of the beetles into non-native habitats.

Chemical communication is ubiquitous among insects and can be used to indirectly assess interactions between non-native species and their novel community. Chemical odors emitted by one individual and responded to by another, known as "semiochemicals", facilitate many intra- and interspecific interactions. *Ips typographus* and *D. rufipennis* produce aggregation pheromones to attract conspecifics, which allows them to quickly "mass attack" healthy trees, a strategy for exhausting host tree defenses (Krokene 2015). An anti-aggregation pheromone is later produced which discourages the further arrival of beetles and reduces intraspecific competition (Werner and Holsten 1995, Sun et al. 2006). *Dendroctonus rufipennis* appears to locate host trees in part by following the scent of volatile oleoresin components (Pureswaran and Borden 2005), although it is uncertain if *I. typographus* also locates hosts in the same manner (Kalinová et al 2014). Predators may likewise find their bark beetle prey by cueing on the pheromones (functioning as "kairomones" in this context) produced by *I. typographus* and *D. rufipennis*, or by locating likely host trees by scent (Bakke and Kvamme 1981, Poland and Borden 1997). Additionally, it is possible for multiple species of bark beetle to be cross-attracted to a single pheromone blend, leading to close associations between them (Gara and Holsten 1975, Smith et al. 1990). Because of the importance of semiochemicals in mediating ecological interactions between insects, we propose that synthetic semiochemicals can be powerful tools for predicting interactions between native and non-native species. By deploying synthetic pheromones to mimic the presence of a non-native species, in this case either *I. typographus* or *D. rufipennis*, we can observe the response of potential predators, competitors, or facilitators. From these

observations, we can make more informed predictions about the establishment risk of introduced populations.

Using this approach, we carried out two trapping experiments in the field to quantify the response of ecologically relevant bark beetles and bark beetle predators to the synthetic pheromone blends of *I. typographus* (in North America) and *D. rufipennis* (in Norway), as well as to host tree odors. Beetles caught in traps baited with synthetic pheromone lures and host tree volatiles were identified and counted, and these counts were compared with control treatments to determine if the pheromone lures and/or host odors were attractive to native species. Attraction of a native species to the synthetic pheromone blends of non-native *I. typographus* or *D. rufipennis* was assumed to indicate a likely interaction, resulting in predation, competition, or facilitation depending on the natural history of the species in question. A lack of attraction was assumed to indicate a degree of "semiochemical inconspicuousness", making the non-native beetle less apparent to native predators and competitors. We propose that similar methods can be applied beyond our study species, and possibly also to non-insect organisms.

We predict that North American taxa will respond to *I. typographus* pheromone, because it contains components (cis-verbenol, trans-verbenol, ipsenol, and ipsdienol) also present in the pheromone blends of many North American *Ips* spp. (Symonds and Elgar 2004). In contrast, we predict that few European taxa will recognize and respond to *D. rufipennis* pheromone because, apart from *Dendroctonus micans*, there are no congeners in Europe for *D. rufipennis* (Grégoire 1988). Its aggregation pheromone, therefore, broadly differs from European bark beetle pheromones.

Methods

We conducted two trapping experiments using synthetic beetle pheromone lures and host odors (Table 4.1) to quantify responses of predatory beetles, bark beetles, and ambrosia beetles to the simulated presence of non-native spruce bark beetles in Canada and Norway. The experiment in New Brunswick, Canada assessed responses of beetles to Ipslure®, a synthetic analog of the *I. typographus* aggregation pheromone blend. A similar experiment in Ås, Norway quantified responses of beetles to a synthetic *D. rufipennis* aggregation pheromone blend. To mimic the odor of trees under attack by beetles, we included synthetic host volatiles (ethanol and (–)-α-pinene) with all pheromone lures. We also included separate host-odor-only ("Host") treatments to determine if insect response was due solely to the host volatiles. The enantiomeric ratio of α-pinene is highly variable in white and interior spruce (Grant et al. 2007, Pureswaran et al. 2004), but Norway spruce generally exhibits an excess of (–)-α-pinene (Lindström et al. 1989). Because racemic α-pinene release devices were not available from our supplier, we chose the (–)-enantiomer as the most suitable host odor for both experiments.

Table 4.1. Technical specifications and suppliers for semiochemical components used in Canada and Norway. PE = polyethylene.

Component	Load	Release rate	Purity	Release device	Product	Supplier
(−)-α-pinene	15 mL	120-130 mg/day @ 25 °C	100%	PE bottle	P/N 3153	Synergy Semiochemicals, Burnaby, BC, Canada
Ethanol	8 mL	16 mg/day @ 20 °C	95%	PE pouch	P/N 3344	
Frontalin, racemic	250 µL	1.5 mg/day @ 20 °C	97.50 %	400 µL PE centrifuge tube	P/N 3065	
Seudenol, racemic	250 µL	5 mg/day @ 20 °C	>= 95%	PE bubble cap	P/N 3006	
MCOL, racemic	250 µL	5 mg/day @ 20 °C	> 95%	PE bubble cap	P/N 3247	
2-methyl-3-buten-2-ol	1500 mg	22 mg/day @20-21 °C *	98%	PE pouch	Ipslure ®	KjemiKonsult ANS, Jar, Norway
(S)-cis-verbenol	70 mg	1 mg/day @20-21 °C *	97%			
Ipsdienol, racemic	15 mg	0.22 mg/day @20-21 °C *	98%			

We identified trapped insects of ecological relevance (predatory beetles and bark and ambrosia beetles) to species or sometimes to genus (depending on availability of keys and taxonomic expertise). We counted taxa separately for each individual trap, representing each combination of experimental block and semiochemical treatment. Identification was based on Barr (1962), Bright (1976), Anderson (2002), Opitz (2002), and Majka (2006) for New Brunswick; Duffy (1953), Tottenham (1954), Bakke and Kvamme (1993), Jordal and Knížek (2007), Kvamme and Lindelöw (2014), and Klimaszewski et al. (2018) for Norway; plus local taxonomic expertise and voucher specimens (stored at NIBIO in Ås, Norway and at the Atlantic Forestry Centre in Fredericton, New Brunswick). Uncommon taxa (those with zero median counts across all treatments) were excluded from our results.

Ips typographus *community response experiment (New Brunswick, Canada)*

This experiment took place in Acadia Research Forest, New Brunswick (46.0122°, -66.3254°), in the interior of a mixed red spruce (*Picea rubens* Sargent) and black spruce (*Picea mariana* (Mill.) B.S.P.) forest. We arranged treatments in a randomized block design, with 30 m between adjacent traps. On May 15, 2017, we hung 21 12-funnel Lindgren multiple-funnel traps (Chemtica International, Costa Rica) from rebar poles along two parallel transects, with 12 traps (four blocks) approximately 20 m from and parallel to an access road, and the remaining 9 traps (three blocks) a further 30 m into the forest. This design exceeded the 10–15 m spacing typical of beetle trapping experiments in North America (Borden et al. 1996, Dodds et al. 2015, Lindgren et al. 2012) and ensured that adjacent traps were suitably independent. The seven blocks each contained one replicate of each of three different treatments: a control (no host odors or pheromone lures), host kairomone ("Host"; α-pinene and ethanol), and the commercial Ipslure® pheromone lure plus host kairomone ("Ipslure + Host"; methylbutenol, cis-verbenol (4,6,6-trimethylbicyclo[3.1. 1]hept-3-en-2-ol), ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), α-pinene, and ethanol). Semiochemical release devices were hung using twist-ties inside the 6th funnel from the top. Collection cups were filled with concentrated sodium chloride solution as a killing agent and preservative, plus a few drops of liquid dish detergent to reduce surface tension. We collected insects from the traps every second week for ~2.5 months, starting on June 1 and ending on August 11. We stored collections at -20 ° C until they could be processed for identification, and thereafter at room temperature in 75% ethanol.

Dendroctonus rufipennis *community response experiment (Ås, Norway)*

This experiment took place in a private clearcut near Ås, Norway (59.6426°, 10.8003°). We used a clearcut to maintain a forest-free buffer around our traps, preventing beetle attacks from spilling over into nearby standing trees. Spillover attacks were a greater concern in Norway than in Canada because the experiment in Norway was carried out in a privately owned forest. Forty "BEKA" free-standing 10-funnel traps (NoveFella, Norway) were arranged into 10 experimental blocks placed along a transect running parallel to the forest edge, with four traps in the corners of each 10 x 10 m block. We were constrained by the size of the clearcut but considered 10 m separation between traps to be adequate given that the effective attraction radius for *I. typographus* to its aggregation pheromone blend is 3–10 m (Schlyter 1992). Block centers were separated from each other and from the forest edge by 30 m. We randomized the treatment placement within block 1 and rotated this arrangement clockwise by 90 degrees for each subsequent block. The four treatments were the same as the *I. typographus* experiment (controls, "Host", and "Ipslure + Host"), plus a combination of *D. rufipennis* pheromone lure and host kairomone ("DRlure + Host"; frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane), seudenol (3-methyl-2-cyclohexen-1-ol), MCOL (1-methyl-2-cyclohexen-1-ol), α-pinene, and ethanol). The *D. rufipennis* lure was a combination of the "Atlantic" spruce beetle lure and MCOL release devices (Synergy Semiochemicals Corp., Burnaby, BC, Canada). We chose the Atlantic lure to complement the sister experiment in New Brunswick and added MCOL to account for recent characterizations of eastern *D. rufipennis* pheromone blends (Isitt et al. 2020). The Ipslure + Host treatment was included as a positive control to ensure the arrival of native bark beetles and predators to at least one treatment combination. Semiochemical

release devices were hung approximately 30 cm down into the perforated central support columns of the traps.

To prevent predation and escape, we modified the BEKA trap cups to allow the use of 200 mL of concentrated propylene glycol as a killing agent and preservative. The experiment was set up on May 11, 2018. We initially collected catches twice a week, then slowed to once every two weeks. However, due to the large volume of insects captured, particularly of *I. typographus*, we sorted and counted only catches for May 14, May 17, and June 5. Catches were preserved and stored as for the New Brunswick experiment.

Statistical analyses

For each experiment, we compared community composition (counts of ecologically relevant taxa summed across collection dates) among semiochemical treatments using permutational multivariate analysis of variance (PERMANOVA; Anderson 2017) with 9999 permutations, including block as a random factor. If PERMANOVA showed a significant difference among treatments, we used multiple two-way ANOVAs (type I SS) to test for effects of the semiochemical treatment (fixed factor) and block number (random factor) on the counts of individual taxa. The ANOVA model was $Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$, where Y_{ij} is the taxon count in treatment i and block j, μ is the grand mean, τ_i is the ith treatment effect, β_j is the jth block effect, and ε_{ij} is the random error. Taxa counts were square-root transformed prior to ANOVA to improve residual normality. We corrected for multiple comparisons using the Holm-Bonferroni method. For taxon-specific ANOVAs that showed a significant effect of treatment, we used Tukey's post-hoc tests ($\alpha = 0.05$) to compare counts between all pairwise treatment

combinations. All statistical analyses were performed in R 4.1.0 (R Core Team 2021) using vegan 2.5-7 (Oksanen et al. 2020) and emmeans 1.6.1 (Lenth et al. 2021).

Results

Ips typographus *community response experiment (New Brunswick, Canada)*

From New Brunswick collections we quantified four predatory clerid beetles, one possibly predatory nitidulid beetle, and 10 spruce-inhabiting bark and ambrosia beetles. Community composition varied significantly among semiochemical treatments (PERMANOVA, Pseudo-$F_{(2, 18)} = 10$, $P < 0.001$). Twelve of 15 taxa showed differences in abundance among treatments (taxon-specific ANOVAs; Table 4.2).

Table 4.2. Results of taxon-specific ANOVAs testing for an effect of three semiochemical treatments on the counts of spruce-associated bark and ambrosia beetles (Curculionidae) and bark beetle predators (Cleridae, Nitidulidae) captured in multiple-funnel traps in Canada (n = 7 for each treatment). The semiochemical treatments consisted of: "Control" (no semiochemical amendment), "Host" (synthetic conifer host kairomone blend of α-pinene and ethanol), and "Ipslure + Host" (host kairomone blend plus the commercial *Ips typographus* lure containing methylbutenol, cis-verbenol, and ipsdienol).

Family	**Species**	**Ecological role**	$F_{(2,12)}$	P
Cleridae	*Madoniella dislocata*	Predator (Majka 2006)	24.2	< 0.001
	Thanasimus dubius		85.9	< 0.001
	Thanasimus undatulus		104	< 0.001
	Zenodosus sanguineus		1.3	0.31
Nitidulidae	*Epuraea* spp.	Possible predator (Kenis et al. 2004)	51.5	< 0.001
Curculionidae	*Cryphalus ruficollis*	Bark beetle (Bright 1976)	52	< 0.001
	Crypturgus borealis		3.23	0.15
	Dendroctonus rufipennis		174	< 0.001
	Dryocoetes affaber		26.1	< 0.001
	Dryocoetes autographus		10.6	0.011
	Ips borealis		257	< 0.001
	Orthotomicus caelatus		100	< 0.001
	Polygraphus rufipennis		5.76	0.053
Curculionidae	*Gnathotrichus materiarius*	Ambrosia beetle (Bright 1976)	8.3	0.0022
	Trypodendron lineatum		36	< 0.001

Among predators, the clerids *Madoniella dislocata* Say and *Thanasimus dubius* F. responded significantly to the combination of Ipslure® and host kairomone relative to the host kairomone alone, and we saw the same result for the possibly predatory nitidulid *Epuraea*. The clerids *Thanasimus undatulus* Say responded equally to both treatments that included the host kairomone, while *Zenodosus sanguineus* Say responded equally to all treatments including controls (Fig. 4.1).

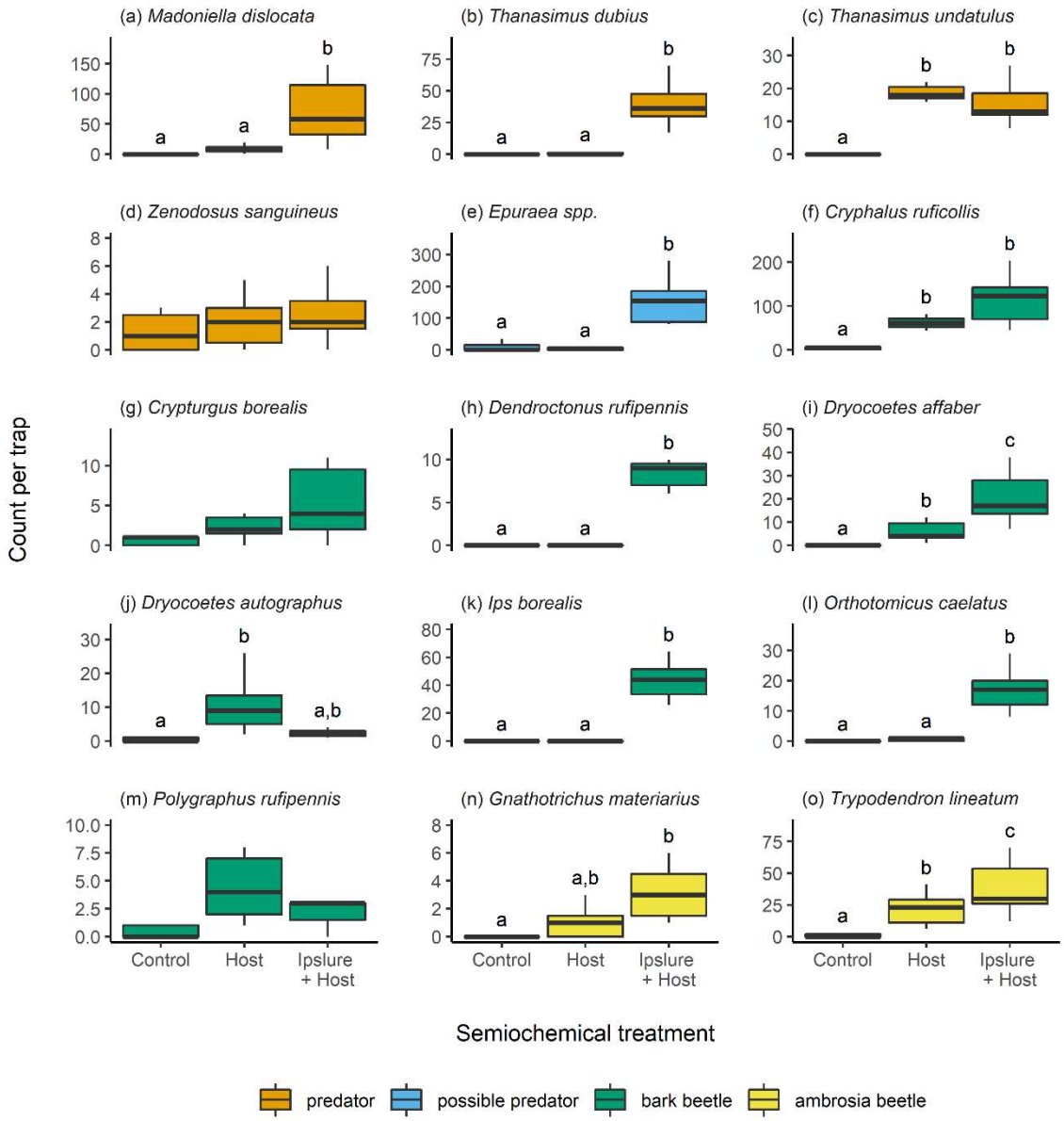

Figure 4.1. Counts of bark beetles and associated predators captured in multiple-funnel traps deployed in New Brunswick, Canada (n=7 for each treatment). "Control" treatments did not receive a semiochemical amendment. "Host" treatments received a synthetic conifer host kairomone blend (α-pinene and ethanol). "Ipslure + Host" treatments received the host kairomone blend further amended by the commercial *Ips typographus* lure (Ipslure®). Boxplot whiskers extend from the first and third quartiles to the most extreme sample values within 1.5 × interquartile range. Different letter designations above the boxplots indicate statistically significant differences (α = 0.05) according to Tukey's HSD tests following ANOVA.

The bark beetles *D. rufipennis*, *Dryocoetes affaber* Mannerheim, *Ips borealis* Swaine, and *Orthotomicus caelatus* Eichhoff responded significantly to the Ipslure +

Host treatment above all others. *Cryphalus ruficollis* Hopkins and *Crypturgus borealis* Swaine showed the same pattern, but the greater response to the Ipslure + Host treatment was not statistically significant. The ambrosia beetles *Gnathotrichus materiarius* Fitch and *Trypodendron lineatum* Olivier were also caught in greater numbers in traps amended with Ipslure® versus the host kairomone alone, but this was statistically significant only for *T. lineatum*. The bark beetle *Dryocoetes autographus* Ratzeburg responded significantly to the host kairomone, whereas the addition of Ipslure® seemed to suppress this attraction. *Polygraphus rufipennis* Kirby responded similarly to *D. autographus*, but the pattern was not statistically significant (Fig. 4.1).

Dendroctonus rufipennis *lure response experiment (Ås, Norway)*

From Norwegian collections, we quantified four predatory clerids and staphylinids and eleven bark and ambrosia beetles. Community composition differed among semiochemical treatments (PERMANOVA, Pseudo-$F_{(3, 36)} = 36.8$, $P < 0.001$). Seven of 16 taxa showed differences in abundance among treatments (taxon-specific ANOVAs; Table 4.3).

Table 4.3. Results of taxon-specific ANOVAs testing for an effect of four semiochemical treatments on the counts of spruce-associated bark and ambrosia beetles (Curculionidae) and bark beetle predators (Staphylinidae, Cleridae) captured in multiple-funnel traps in Norway (n = 10 for each treatment). The semiochemical treatments consisted of: "Control" (no semiochemical amendment), "Host" (synthetic conifer host kairomone blend of α-pinene and ethanol), "Ipslure + Host" (host kairomone blend plus the commercial *Ips typographus* lure containing methylbutenol, cis-verbenol, and ipsdienol) and "DRlure + Host" (host kairomone blend plus a commercial *Dendroctonus rufipennis* lure containing frontalin, seudenol, and MCOL).

Family	**Species**	**Ecological role**	$F_{(3,27)}$	P
Staphylinidae	*Nudobius lentus*	Predator (Weslien 1992)	0.21	~ 1
	Placusa depressa		197	< 0.001
	Quedius spp.		2.6	0.53
Monotomidae	*Rhizophagus ferrugineus*	Predator (Weslien 1992)	5.21	0.052
Cleridae	*Thanasimus formicarius*	Predator (Bakke and Kvamme 1993)	38.4	< 0.001
Curculionidae	*Crypturgus cinereus*	Bark beetle (Bakke and Kvamme 1993, Haack 2001, Jordal and Knížek 2007)	22	< 0.001
	Crypturgus hispidulus		0.15	~ 1
	Crypturgus pusillus		0.22	~ 1
	Crypturgus subcribrosus		10.6	< 0.001
	Dryocoetes autographus		0.84	~ 1
	Hylastes spp.		2.97	0.40
	Ips duplicatus		259	< 0.001
	Ips typographus		267	< 0.001
	Orthotomicus spp.		0.33	~ 1
	Pityogenes chalcographus		44.4	< 0.001
Curculionidae	*Trypodendron lineatum*	Ambrosia beetle (Bright 1976)	0.89	~ 1

No predatory beetle responded positively to the combination of host kairomone and *D. rufipennis* pheromone blend relative to the host kairomone alone (*Thanasimus formicarius* L. showed *reduced* attraction to the DRlure + Host treatment). Two taxa, *T. formicarius* and *Placusa depressa* Mäklin responded significantly to the combined Ipslure® and host kairomone treatment over all other treatments (Fig. 4.2).

Only one bark beetle (*Crypturgus subcribrosus* Eggers) responded positively to DRlure + Host treatment relative to the host kairomone alone. Several taxa (*Crypturgus cinereus* Herbst*, Ips duplicatus* Sahlberg*, I. typographus,* and *Pityogenes chalcographus*

L.) showed a significant response to the Ipslure + Host treatment when compared to the host kairomone treatment (Fig. 4.2).

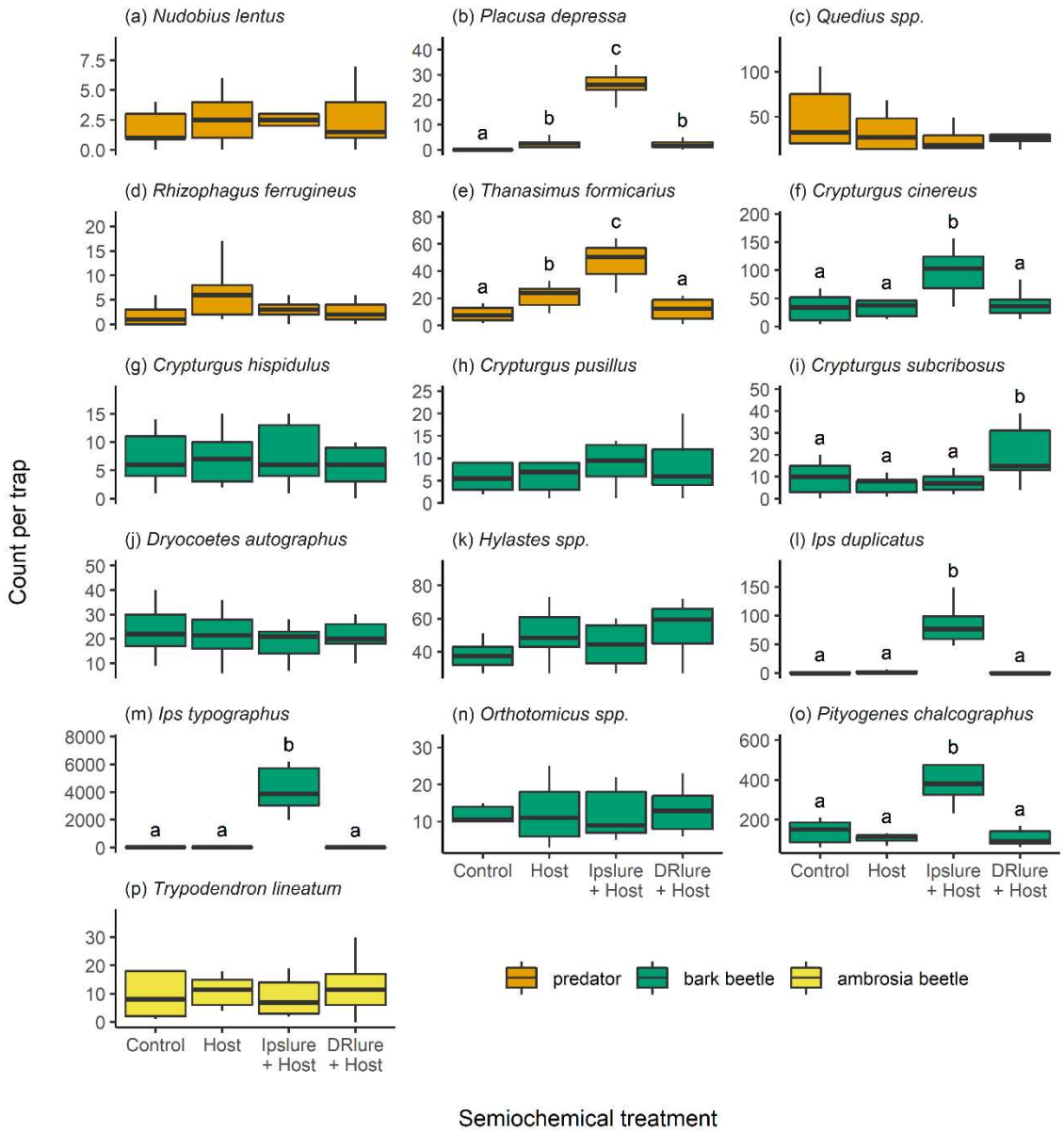

Figure 4.2. Counts of bark beetles and associated predators captured in multiple-funnel traps deployed in a clear-cut near Ås, Norway (n=10 for each treatment). "Control" treatments did not receive a semiochemical amendment. "Host" treatments received a synthetic conifer host kairomone blend (α-pinene and ethanol). "Ipslure + Host" treatments received the host kairomone blend further amended by the commercial *Ips typographus* lure (Ipslure®). "DRlure + Host" treatments received the host kairomone blend further amended by a commercial *Dendroctonus rufipennis* lure. Boxplot whiskers extend from the first and third quartiles to the most extreme sample values within 1.5 × interquartile range. Different letter designations above the boxplots indicate statistically significant differences (α = 0.05) according to Tukey's HSD tests following ANOVA.

Discussion

Our study allows us to make predictions about the relative magnitude of positive and negative heterospecific interactions (Table 4.4) that may affect the early establishment success of non-native spruce bark beetles in Europe and North America. These interactions can be broken down into two categories: biotic resistance (e.g., predation and competition) from the recipient community that inhibits successful establishment, and facilitative interactions that promote establishment.

Table 4.4. Summary of factors that may increase (🡅) or decrease (🡇) the likelihood of establishment of invasive populations of *Ips typographus* and *Dendroctonus rufipennis* into North America or Europe, respectively.

	***Ips typographus* into North America**	***Dendroctonus rufipennis* into Europe**
Climate suitability	🡅 Yes (Bentz et al. 2019)	🡅 Yes (Godefroid et al. 2016)
Host availability	🡅 Yes (Økland et al. 2011, Flø et al. 2018)	⇧ Likely, possibly suboptimal (Isitt et al. unpublished data)
Biotic resistance	🡇 Likely on par with native *Ips* spp.	🡅 Likely less than native species
Facilitation of mass attack by native species	🡅 Likely	🡅 Likely

Biotic resistance

There were clear differences in the responses of the recipient biology communities in Canada and Norway to the pheromone blends of non-native *I. typographus* and *D. rufipennis*, respectively. *Ips typographus* is likely to be more semiochemically conspicuous in North America than *D. rufipennis* would be in Europe.

In New Brunswick, Canada, the staphylinid predators *M. dislocata* and *T. dubius* were attracted to Ipslure®, while *T. undatulus* responded predominantly to host kairomone. North American *Epuraea* species were also strongly attracted to Ipslure®. Although the ecology of these species is unknown, larvae of European congeners are predators of bark beetles (Kenis et al. 2004). Because there are several native North

American *Ips* species, we expect North American predators to be preadapted to finding and handling *I. typographus* prey. Consequently, *I. typographus* may experience similar predation pressures to native *Ips* in North America.

Several spruce-inhabiting bark and ambrosia beetles in North America responded to Ipslure®. *Ips borealis* and *Orthotomicus caelatus* probably responded to the ipsdienol component, which is also produced by North American *Ips* species (Symonds and Elgar 2004) and (likely) *O. caelatus* (Phillips et al. 1989). *Dryocoetes affaber* and *D. rufipennis* were also attracted to Ipslure®, and *Cryphalus ruficollis* and *Crypturgus borealis* showed similar (but non-significant) responses. Trees under attack by *I. typographus* in North America would thus likely be colonized by numerous native species, resulting in both negative (competitive) and positive (facilitative) interactions.

In contrast, our results suggest that if *D. rufipennis* were introduced into Europe, very few heterospecifics would be attracted to its pheromone blend. The only native *Dendroctonus* species in Europe is *D. micans* (Grégoire 1988). Its gregarious larvae produce a short-range aggregation pheromone consisting of *trans*-verbenol, *cis*-verbenol, verbenone, and myrtenol, whereas adults do not mass-attack hosts and are not known to produce pheromone (Grégoire et al. 1981). Verbenene, a component of *D. rufipennis* pheromone, resembles these molecules structurally, but the *D. rufipennis* pheromone blend would be otherwise novel in Europe. The lack of response by European beetles to *D. rufipennis* pheromone may therefore be due to limited co-evolution with species producing similar pheromone blends. Some predation may still occur from species such as *Nudobius lentus*, *Quedius* spp., *Rhizophagus ferrugineus*, and *Thanasimus formicarius*, which all showed some response to the control traps (the shape of the trap is intended to resemble a tree) and might arrive at trees under attack by *D.*

rufipennis. *Rhizophagus grandis* is a known predator of *D. micans* in Europe and has been found to respond to novel *Dendroctonus* prey (Grégoire et al. 1991). However, we did not capture any individuals of this species.

Only two heterospecifics in Norway responded significantly to *D. rufipennis* pheromone. The clerid predator *T. formicarius* was either repelled by the *D. rufipennis* pheromone lure, or its attraction to the host kairomone was nullified by the addition of the lure. The bark beetle *Crypturgus subcribrosus* was attracted to the *D. rufipennis* lure but does not compete with *I. typographus* (Weslien 1992) and thus is unlikely to compete with *D. rufipennis*. *Crypturgus subcribrosus* is closely related to Nearctic *Crypturgus* species (Jordal and Knížek 2007), and its response to the *D. rufipennis* pheromone blend may be a remnant of ancestral co-evolution in North America.

Because North American predators and competitors are attracted to its aggregation pheromone, we predict that *I. typographus* would face greater biotic resistance in North America than *D. rufipennis* would experience in Europe. This may make *I. typographus* less likely to establish in North America by pushing founding populations below their Allee threshold. The main competitor of *I. typographus* in North America may be *D. rufipennis*, which has similar habits and hosts. However, *I. typographus* may be able to reduce this competition by infesting spruce hosts that are suboptimal for *D. rufipennis*. One such option is black spruce, which appears to be a suitable host for *I. typographus* (Økland et al. 2011, Flø et al. 2018), is widely distributed across North America, and is seldom attacked by *D. rufipennis*.

Facilitation between heterospecifics

Biotic interactions can also be facilitative, potentially favouring invasion by raising populations above their Allee threshold. For some bark beetles, facilitation may result from a need for rapid, cooperative colonization (mass attacks) of live host trees. Tree-killing bark beetles such as *I. typographus* and *D. rufipennis* may either colonize a stressed, dead, or dying tree, or attack a healthy tree in sufficient numbers to exhaust the tree's defenses (Krokene 2015). Failure to meet the attack threshold in healthy trees leads to high brood mortality, but success opens up an abundant, high-quality resource that can reduce interspecific competition (Raffa et al. 2008).

In simulations, Økland et al. (2009) showed highly facilitative interactions among bark beetle species. Although competitive exclusion may also occur, facilitation appears to be more likely between 'aggressive' (tree-killing) species that need to overcome attack thresholds for colonizing living trees. Species with lower population density, such as recently introduced species, would especially benefit from this facilitation, piggybacking on native species in heterospecific mass attacks to exploit abundant but less susceptible hosts. Our results, which show attraction of *D. rufipennis* to the aggregation pheromone of *I. typographus*, suggest the possibility of strong interactions between them. Both species disperse from overwintering sites in the spring, with similar flight temperatures (14.5 °C for *D. rufipennis*, Holsten and Werner 1987; 16.5 °C for *I. typographus*, Mezei et al. 2017). If either species is introduced into the range of the other, facilitation may promote the establishment of the newcomer; but this effect might be stronger for *D. rufipennis* invading Europe (where it would encounter abundant *I. typographus*). If either species should establish in the other's range, this facilitation could also lead to simultaneous outbreaks of the two species.

While the number of *D. rufipennis* captured in Ipslure®-baited traps was only moderate, we interpret this as a strong response. An unrelated experiment conducted in the same study area in New Brunswick simultaneously failed to attract spruce beetles to felled white spruce (Isitt et al. unpublished data), consistent with a low local population density of *D. rufipennis*. Additionally, *D. rufipennis* is likely to respond more strongly to the natural pheromone blend of *I. typographus* than to a synthetic lure. Ipslure® contains racemic ipsdienol, which inhibits attraction of *D. rufipennis*, whereas *I. typographus* produce pure (–)-ipsdienol which does not inhibit attraction (Kohnle et al. 1991, Poland and Borden 1998).

Facilitation between exotic and native bark beetles may have already been observed in nature. In China, the invasive *Dendroctonus valens* is attracted to the pheromone of the native *Hylastes parallelus* Chapuis, and the two species have become common associates within their shared host, *Pinus tabuliformis* Carrière. Lu et al. (2007) propose that these species jointly attack their host trees, and that this may have promoted the *D. valen*s invasion by facilitating their aggregation. With both theoretical and observational support for the possibility of facilitation between bark beetles, the attraction of *D. rufipennnis* to *I. typographus* lures is concerning as it may increase invasion and outbreak risks for both species.

Conclusion

We have shown how *in-situ* semiochemical-based assays can be used to identify behavioral responses to potential invaders by native species. These responses may shape the tri-trophic niches of potentially invasive phytophagous insects, and thus their likelihood of successful invasion. Our approach allows responses to be assessed across

numerous species in broad taxonomic groups depending on trap design, with no risk of introducing potentially invasive insects.

The evolutionary histories of the potential invader and recipient community may result in very different outcomes for different invading species and recipient communities. Our study provides a compelling example of this: *Ips typographus* pheromone elicited a strong response from heterospecifics in eastern Canada, but *Dendroctonus rufipennis* pheromone deployed in Norway did not (Table 4.4). Coevolution with other *Ips* species in North America has likely pre-adapted North American predators and other heterospecifics to responding to Ipslure®, while the absence of pheromone-producing *Dendroctonus* in Norway made reciprocal pre-adaptation unlikely. Although the beetle communities in our sites are unlikely to be representative of all North America or Europe, the clear patterns that we see across numerous broadly distributed species suggests that we should expect similar results in other locations.

Our approach can be extended beyond the study of phytophagous insects. For example, plants produce scents that attract insect herbivores, predators, and pollinators (e.g., Metcalf and Kogan 1987, Drukker et al. 2000). Chemical extracts from potentially invasive plant species could be deployed in the field to assess responses by native insects, informing us about ecological interactions that could influence the plant's establishment. Semiochemical communication is also prevalent among fish (Sorensen and Johnson 2016) and their parasites (Mordue and Birkett 2009), which may enable similar studies for potentially invasive fish.

Of course, there are other major factors influencing establishment success that a study like ours cannot assess. Frequency and magnitude of introduction, habitat

suitability, and intrinsic biological traits may all strongly influence establishment (Carlton and Ruiz 2005, Hayes and Barry 2008). Thus, field experiments like ours may be especially useful in combination with other studies, such as climate suitability simulations, host-use experiments, and vector analyses. A major advantage of semiochemical-based trapping experiments, though, is that they can provide data on interactions across many taxa simultaneously, without requiring that those be identified or selected in advance. Studies like ours will improve invasive species risk assessments, advance our understanding of the evolution of pheromone systems, and help identify promising biocontrol agents for use against already-invasive species.

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Chapter 5 – Discussion

My work has led to new insights into the factors influencing the global exchange of non-native insects, and into the potential for future invasions. This involved a combination of modelling and field experiments.

In chapter two, *Drivers of asymmetrical insect invasions between three world regions*, I have attempted to elucidate the causes behind an old mystery: why has North America received many more non-native insects from Europe than vice versa? To do so, I tested three hypotheses: (1) differences in the size of donor species pools, (2) differences in propagule pressure via international trade (using value of trade goods as a proxy), and (3) differences in the niche diversity of host plants, using plant species richness as a proxy. My Poisson process models have shown that propagule pressure (using import value as a proxy) and donor species pool sizes are excellent predictors of temporal changes to rates of establishment of non-native species between a given donor and recipient region. That propagule pressure should be a good predictor of establishment rates is not surprising, as it is considered the most important factor in the biological invasion process over the long term (Brockerhoff and Liebhold, 2017). However, the ability of my models to explain the temporal trends does not allow them to explain differences between routes in the overall rates of establishment per unit of import value. Rather, they can only confirm that these differences exist. As a striking example of this, the initial rate of establishment of European insects into North America per unit of import value was 81 times the rate of establishments of North American insects into Europe. These analyses allowed me to rule out differences in source species pool sizes and import values as explanations for the regional asymmetries, but left open

the possibility of differences in propagule pressure that were not captured by import values (e.g., differences in the types of goods exchanged). Further analyses showed a close correspondence between the number of non-native insects and non-native plants exchanged between donor and recipient regions. Many of these plant imports/establishments were likely a result of European colonialism that has changed worldwide floral composition (Lenzner et al., 2022). I proposed that the import and establishment of non-native plants directly facilitated the establishment of non-native insects in two ways. First, live plant imports were a major pathway for introductions of non-native insects (Liebhold et al., 2012). This is a form of propagule pressure that may not have been captured in my gross comparisons of trade values. Second, non-native plants may have facilitated the establishment of non-native insects by providing suitable hosts to more host-specific species, thus increasing the invasibility of the habitat (Brockerhoff and Liebhold, 2017). Although I cannot rule out possible differences in insect invasiveness between Europe, North America, and Australasia, my results allow me to add some quantitative evidence in favor of the propagule pressure and invasibility hypotheses, while further ruling out differences caused by donor species pool sizes. My models may also enable future studies of other invasion routes or taxonomic groups. Future research may attempt to find a proxy for propagule pressure that omits irrelevant trade goods (e.g., focuses on live plants and untreated wood imports), as this may increase the explanatory power of the models. Additionally, a database of dated non-native plant establishments might be compiled and used to directly investigate causal links to subsequent establishments of associated insects, though compiling such a database and the network of associations between insect and plant species would be a considerable undertaking.

In chapters three and four, I conducted field experiments on two species of spruce bark beetles that have not yet established beyond their respective continents, with the goal of identifying factors that may promote or inhibit their trans-Atlantic range expansion. These were the North American spruce beetle, *Dendroctonus rufipennis* Kirby, and the European spruce bark beetle, *Ips typographus* L. Previous studies have shown that there are likely suitable abiotic environments for both species across much of the temperate Northern hemisphere (Bentz et al., 2019; Godefroid et al., 2016). My goals were to add further knowledge concerning the suitability of the *biotic* environment, specifically as it applies to biotic resistance (i.e., from predation), facilitation, and the availability of suitable host trees. First, I investigated whether *I. typographus* and *D. rufipennis* would find suitable hosts in North America and Europe, respectively. I did this by placing cut bolts of white spruce, black spruce, and Norway spruce into field experiments in New Brunswick and Norway and allowed beetles to attack and colonize the bolts. In comparing the attack density, brood density, and reproductive success of *I. typographus* and *D. rufipennis* between the three spruce species, I determined that *I. typographus* appears to be a host generalist (performed well in all three species), while *D. rufipennis* was more of a host specialist (performed best in white spruce, significantly worse in black spruce, and intermediately in Norway spruce). This suggests that *Ips typographus* will find suitable hosts in North America, while *D. rufipennis* may find suboptimal (if still usable) hosts in Europe that may decrease the ability of the species to successfully establish. Although the beetles' preferences may change when outbreaking into live trees (whereas cut bolts offer stressed/dead material only), introduced populations are unlikely to be large enough to outbreak. Thus, I suspect that incipient populations of bark beetles will depend mostly on stressed/dead

material until well-established (a possible exception being a facilitation between *I. typographus* and *D. rufipennis*, discussed below). The low population density of *D. rufipennis* in my New Brunswick host choice experiment might have biased the results in favor of white spruce, due to very high rates of mate-finding failure in the less well colonized species. Repeating the experiment under a higher population density may resolve this issue. Next, I investigated the community of possible predators, competitors, and facilitators that might interact with both bark beetle species in hypothetical non-native habitats. I did this by deploying synthetic pheromone lures – *I. typographus* pheromone in Canada, and *D. rufipennis* pheromone in Norway – to see what ecologically relevant species were attracted to them. Although indirect, these experiments allowed me to study the response of relevant species *in situ*, with no risk of accidental introductions of non-native species. I found that the pheromone blend of *I. typographus* would likely attract a variety of North American predators and competitors. Further, many of the responding predators were generalist predators, including of North American *Ips* species. Thus, enemy release seems unlikely for *I. typographus* in North America. The evidence for top-down regulation of bark beetle populations by predators is varied. For some species, mortality from predation may be as high as 60% (Reeve, 1997), but for outbreaking species such as *I. typographus*, population dynamics appear mostly driven by the availability of host material and its effect on intraspecific competition (Marini et al., 2013). However, this evidence comes from already established populations. Recently introduced populations are likely to experience low densities and reduced intraspecific competition. Consequently, the relative impact of predation may be much greater. In contrast, no relevant predators or competitors responded to the pheromone blend of *D. rufipennis* in Norway. This 'semiochemical

inconspicuousness' may allow *D. rufipennis* to escape some direct predation in Europe. I also found that *D. rufipennis* is attracted to the pheromone blend of *I. typographus*, setting up the possibility of facilitation through a multi-species 'mass attack' against healthy trees. As both species have similar flight temperature thresholds (Holsten and Werner, 1987; Mezei et al., 2017), it is likely that they could attack the same host trees simultaneously. Such interactions could also lead to competitive displacement, but this appears less likely given that *I. typographus* and *D. rufipennis* are both 'aggressive' (tree-killing) bark beetles, due to the additional resources (living trees) made available through a joint mass-attack (Økland et al. 2009).

Taken together, the results from chapters three and four are somewhat at odds with each other – *I typographus* may face strong biotic resistance in North America, but will find abundant and suitable hosts, whereas *D. rufipennis* may escape some predation in Europe, but may find suboptimal hosts. Consequently, I find it difficult to say which establishment scenario poses the greatest risk, but enough *D. rufipennis* attacked Norway spruce bolts that its ability to evade semiochemical detection in Europe may give it the edge over *I. typographus* in North America. These results further the species- and region-specific knowledge of risk factors that may promote the establishment of these two species and may be used to produce improved invasive species risk assessments. My results highlight the need for much more research of this type, as two species which might be similarly described as "aggressive spruce bark beetles" may pose very different risks when looked at more closely.

While chapter two aimed to explain large-scale patterns in invasion rates across all insect taxa, these models are not suited to explaining the invasion patterns of particular species. Chapters three and four represent the opposite approach: investigating

the risk factors for establishment of two specific species, at relatively smaller geographic scales, and grounded in species-specific knowledge of chemical ecology and life histories. A proper understanding of the risks posed by a given species requires both scales of knowledge, for the spread of any species is heavily influenced by global trade patterns, environmental suitability, and national/international policy.

A common theme throughout my results relates to host availability and specificity and serves as a cautionary note to forest managers and policy makers. Just as the historical introduction of European plants into North America and Australasia may have paved the way for a glut of insect invaders, so too might modern introductions of non-native plants. For example, the apparent host specificity of *D. rufipennis* may not inhibit its establishment into Europe if white, Sitka, or Engelmann spruce (or their hybrids) continue to be planted there. Once established into non-native spruce in Europe, populations of *D. rufipennis* could conceivably build up, outbreak, and shift into Norway spruce.

I introduced this thesis with a description of how humans have dramatically increased the magnitude and spatial scale of biological invasions (Ricciardi, 2007). My analyses and field experiments demonstrate this further by contributing evidence for the role of colonial history, international trade, and the import and establishment of exotic plants in promoting unintentional establishments of often damaging non-native insects. I hope that my results will lead to some additional hesitancy in the further introduction of non-native plants, particularly if few efforts have been made to conduct risk assessments on the species that may inadvertently follow them.

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