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Non-native spiders change assemblages of Hawaiian forest fragment kipuka over space and time

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Abstract

We assessed how assemblages of spiders were structured in small Hawaiian tropical forest fragments (Hawaiian, *kipuka*) within a matrix of previous lava flows, over both space (sampling kipuka of different sizes) and time (comparison with a similar study from 1998). Standardized hand-collection by night was carried out in May 2016. In total, 702 spiders were collected, representing 6 families and 25 (morpho-)species. We found that the number of individuals, but not species richness, was highly correlated with the area of sampled forest fragments, suggesting that kipuka act as separate habitat islands for these predatory arthropods. Species richness was significantly lower in the lava matrix outside the kipuka compared to the kipuka habitats, although there was no statistical difference in species composition between the two habitats, largely because of similarity of non-native species in both habitats. Over the last 20 years, the abundance of non-native spider species substantially increased in both kipuka and lava habitats, in marked contrast to the vegetation that has remained more intact. With endemicity of terrestrial arthropods reaching over 95% in native forests, non-native predatory species present a critical challenge to the endemic fauna.

Keywords

Araneae, diachronic study, island

Introduction

Kipuka are small tropical forest fragments surrounded by recent lava flows on Hawaiian islands. Many of these kipuka are rich ecosystems that have been previously investigated for their importance for native passerine birds, plants (trees and ferns) and picture-winged flies (e.g. Muir and Price 2008). Due to their spatial arrangement in a volcanic matrix, kipuka can be considered as islands within islands (Tielens et al. 2019), and thus consequently important, and original, to test classical assumptions regarding the combined effects of area and isolation on species assemblages.

The kipuka system might also be used to examine whether such particular habitats retain a strong biotic resistance to non-native species. In Hawaii, due to the remote localization of this archipelago, there are well known impacts of non-native species, with most of these studies focusing on changes in ecosystem structure (e.g. Krushelnycky and Gillespie 2008). Indeed, multiple ecological impacts of non-native flora and fauna threaten communities world-wide, and can be potentially even damaging to island systems (Vitousek 1988). Although some non-native species are reported from kipuka (e.g., rats and other mammals), kipuka appear to be less vulnerable to non-native than other (lowland) habitats, with, for example, non-native plant species almost entirely absent from kipuka (Flaspohler et al. 2010). To the best of our knowledge, no study has yet assessed how non-native spiders colonized kipuka, although they represent a major group of predators in this habitat, potentially reducing native populations.

In this study, we examined how assemblages of spiders are structured over space (e.g., comparisons across kipuka as well as lava matrix), but also potentially changed over time (in comparison to a previous study during the 1990s in the same study site: Vandergast and Gillespie 2004). We tested the general hypothesis suggested by observations of relatively intact native biota (Krushelnycky and Gillespie 2008, Flaspohler et al. 2010), that kipuka function as islands with strong biotic resistance to arthropod non-native species. More precisely, our expectations are that 1) abundance and species richness increase with increasing kipuka area, 2) species composition and richness differ between kipuka habitat and the surrounding areas and 3) despite an overall increase in non-native spider species over the last decades, the increase is less in kipuka habitats than outside.

Materials and methods

Study site and collecting

Fieldwork was conducted on the youngest and largest island of the Hawaiian Archipelago, Hawaii or the Big Island. The Kipuka system investigated consists of forest fragments surrounded by an 1855–1856 lava flow originating from Mauna Loa Volcano (19°40'01"N, 155°20'56"W). These forest fragments grow on 3,000–5,000 years

BP soil (Vitousek 2004), range in age from approximately 750–1,500 year BP and were most likely connected before the 1855–1856 flow that currently surrounds them (Trusdell and Lockwood 2017). These kipuka occur within the State of Hawaii Forest Reserve System (Upper Waiakea Forest Reserve), along the Daniel K. Inouye Highway (formerly Saddle Road; mile marker 16–18, Kaumana Trail).

The forest fragments' plant species composition is relatively constant across forest fragments (Raich et al. 1997) and is categorized as mesic to wet *ohia* (*Metrosideros polymorpha,* Myrtaceae) forest (Jacobi and Warshauer 1990). Soil characteristics are also quite constant across fragments (Raich et al. 1997) but soil nutrient conditions may vary (Vannette et al. 2016). Surrounding these fragments, the lava matrix is a rough textured patchwork of undulating mounds of smooth lava and free chunks of very angular pieces, extremely porous where the vegetation is sparser and poorer than those of the fragments (for a detailed description of both habitats, see Vandergast and Gillespie 2004; Flaspohler et al. 2010; Vaughn et al. 2014).

Vegetation-dwelling assemblages of spiders were collected in May 2016 in the kipuka fragments and outside, in the surrounding lava, of five small (<1 ha) and five large forest patches (2–11 ha) along the Saddle Road (mile marker 16–18: Kaumana trail). The influence of kipuka area on both species richness and abundance of spiders was tested using linear regressions. Kipuka area was estimated using airborne LiDAR (Light Detection and Ranging) technology as reported by Flaspohler et al. (2010).

Field collection involved beating vegetation at night, the best time for collecting spiders. Each kipuka and surrounding lava was sampled by 2 people for a total of 30 min, a time determined following initial sampling. Individuals were collected separately in ethanol for identification to species or morpho-species (i.e., spider family) level at the lab. During identification, specimens were checked against voucher specimens obtained/used in the previous study (Vandergast and Gillespie 2004). All specimens are stored at the University of Rennes, France.

Data analysis

The influence of kipuka size on both species richness and abundance of spiders was tested using linear regressions. Paired T-tests and Permanova (1000 permutations) were used to test for differences in species richness and composition, respectively, between the two habitat types. Non-metric multi-dimensional scaling ordination was used to visualize spider assemblages in the two habitats. ‘Indigeneity status' was assessed using multiple bibliographic sources (see details in Vandergast and Gillespie 2004), combined with the expertise of one of the authors (RG). The proportion of native vs non-native species was compared between kipuka and lava habitats in the same area in 2016 as had been compared in 1998 (from Vandergast and Gillespie 2004) using χ^2 tests. To allow a direct comparison of kipuka and lava habitats with previous sampling we pooled the two kipuka sampling categories of “core" and “edge" reported in the earlier study.

Results

In total, 702 spiders belonging to 6 families and 25 (morpho-) species were collected (see details in Table 1).

Kipuka size had a large effect on the number of individuals (linear regression, Y = 5.58X(ha) +27.4 , $R^2 = 0.93$, $p < 0.001$, 9 df), but no effect on species richness (linear regression, $R^2 = 0.12$, NS, 9 df). Species richness of spiders was significantly higher in kipuka habitats compared to the surrounding lava (Mean±SE: 10.8 ± 0.6 *vs.* 6.8 ± 0.6 respectively; Paired T-test, t = 6.34, 9 df, $p < 0.001$), although there was no statistical difference in species composition between the two habitat types (Permanova: F1,8 = 0.06, p = 0.349, Fig. 1). The proportion of non-native spider species increased significantly between 1998 and 2016, in both kipuka and lava habitats (χ^2 tests, $\chi^2 = 67.53$, 1 df, $p < 0.001$ and $\chi^2 = 4.34$, 1 df, $p < 0.037$ respectively), with a higher increase in kipuka habitats (see Fig. 2). This increase in both habitat types was due to both an increase in the number of non-native species and a particular increase in the number of individuals of few species (e.g. *Steatoda grossa*: Table 1).

Table 1. Number of individuals, status (Na = Native, Nn = Non-native, *: species not found in 1998, according to Vandergast and Gillespie 2004) and code of spider taxa collected inside and outside the kipuka.

Family	Genus	Species	Status	Nind (kipuka)	Nind (lava)	Code
Araneidae	*Cyclosa*	sp.	Na	21	51	Cycsp
	Neoscona	sp1	Nn	42	1	Neosp1
		sp2	Nn*	23	31	Neosp2
Linyphiidae	*Agyneta*	sp.	Nn*	3	0	Agysp
Miturgidae	*Cheiracanthium*	sp.	Nn*	1	13	Chesp
Philodromidae	*Pagiopaplus*	sp.	Na*	4	0	Pagsp
Tetragnathidae	*Tetragnatha*	*acuta*	Na	56	85	Tetac
		anuenue	Na	43	44	Tetan
		brevignatha	Na	26	0	Tetbr
		hawaiiensis	Na	21	0	Tetha
		quasimodo	Na	27	6	Tetqu
		spp.	Na	2	0	Tetsp
Theridiidae	*Argyrodes*	sp.	Na	1	0	Thesp7
	Ariamnes	spp.	Na*	59	1	Argspp
	Steatoda	*grossa*	Nn*	11	0	Stegr
	Theridion	*grallator*	Na	20	0	Thegr
		sp1	Na	23	35	Thesp1
		sp2	Na	8	2	Thesp2
		sp3	Na	1	0	Thesp3
		sp4	Na	4	2	Thesp4
		sp5	Na	4	6	Thesp5
		sp6	Na	2	3	Thesp6
Thomisidae	*Mecaphesa*	sp1	Na*	1	3	Mecsp1
		sp2	Na*	4	10	Mecsp2
	Synaema	sp.	Na*	1	1	Synsp

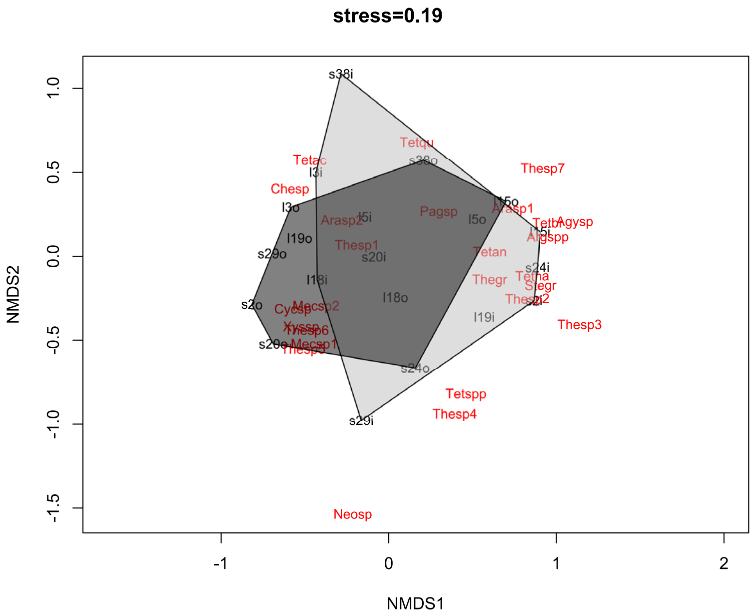

Figure 1. Non-metric multi-dimensional scaling ordination of spider assemblages from kipuka habitats (grey ellipsoid) and surrounding lava (black ellipsoid). Species are in red and sampling stations in black. Stress = 0.189. For spider taxa code, see Table 1.

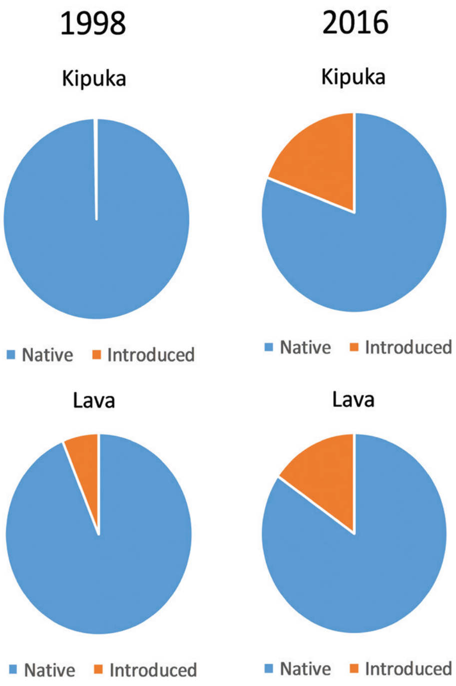

Figure 2. Relative abundance of native (blue) and non-native (orange) species in kipuka habitats and surrounding lava, in 1998 (430 individuals) and in 2016 (702 individuals).

Discussion

The total number of individuals across all spider species was strongly associated with the area of sampled forests, suggesting that kipuka act, at least partly, as separate habitat islands for the spiders. Yet, as recently observed for entire arthropod communities in the same area (Tielens et al. 2019), spider species richness was not a function of kipuka size. Indeed, spider species in kipuka habitats tend to be found in all such habitats (Vandergast and Gillespie 2004, Vandergast et al. 2004). These two results altogether suggest that kipuka act as islands for native species in a hostile matrix. Yet, the abundance of several native and non-native species was found to be high in both kipuka habitats and surrounding lava, leading to overlapping, and statistically indistinguishable, assemblage compositions (see also Tielens et al. 2019). Fragment size is known to be a strong determinant of the structure of the forest in the kipuka (Vaughn et al. 2014) with larger fragments having greater average height, larger diversity of height and lower edge to core habitat being thus less affected by the matrix overall. Kipuka edges are also known to house different species from the kipuka interiors (Vandergast and Gillespie 2004). For example, more native *Drosophila* are found in large kipuka while more non-native *Drosophila* and other insects are found in small kipuka (Mueller 2015). Patch size, on top of influencing habitat quality, is also known to act on migration rates, and the linear relationships between kipuka area and number of spider individuals can also be the result of immigration-emigration dynamics (Hambäck and Englund 2005).

There was an important increase in the proportion of non-native species over time, while no native species disappeared between the two sampling periods. Several new species like *Cheiracanthium* sp. and *Steatoda grossa* were detected in this study, i.e. they were not recorded in the same study site twenty years ago. Other species have an unclear biogeographic and taxonomic status, e.g. *Agyneta* sp., which shows that more intensive studies are still needed on the Hawaiian archipelago (Gertsch 1973). The kipuka we sampled belong to the State of Hawaii Forest Reserve System (Upper Waiakea forest reserve), an area subject to limited anthropogenic disturbance for the last 160 years, but strategically located along the Daniel K. Inouye Highway that was reconstructed in 2008 and 2011. Thus, the increase of non-native species is likely associated with previous construction activity and more road traffic along the renovated highway. Non-native arthropods in Hawaii respond more quickly to disturbance than their endemic counterparts (Gillespie et al. 2008) and are known to follow pathways of human activity (Krushelnycky and Gillespie 2008). Interestingly, the increase in non-native spiders was actually higher in kipuka habitats compared to the outside lava, rejecting our hypothesis of a stronger biotic resistance to non-native species in intact forest fragments, and in marked contrast to the relatively intact flora of these habitats. Kipuka offer many micro-habitats for newcomers, for example, bark of *Metrosideros polymorpha* where *Steatoda grossa* are often found in numbers, as well as complex edge habitats where one species of non-native spiders was found previously (Vandergast and Gillespie 2004).

In conclusion, kipuka function as habitat islands within islands, with many island-like features such as a characteristic biota, unique microclimate, and biological associations with increasing area. Kipuka, like other island-shaped habitats, also appear to be vulnerable to non-native species, especially as compared to the surrounding lava. Until now native species of spiders do not seem to have been affected by these newcomers, but spiders can have a large impact on prey species because they are ecologically dominant and generalist predators in a large variety of habitats (Pekár and Toft 2015), including tropical forests (Coddington et al. 2009). In Hawaii, with a large number of endemic species (Howarth 1990, Eldredge and Evenhuis 2003), non-native spiders are a critical challenge for management. The impact of these new generalist predators on the structure and functioning of kipuka is currently investigated using meta-barcoding and NGS techniques. We are also planning to look at other spider associates, including parasite occurrence in spiders (see Vandergast and Roderick 2003), to see how the associated community has changed together with increased non-native species.

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RESEARCH ARTICLE

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The economic cost of control of the invasive yellow-legged Asian hornet

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Abstract

Since its accidental introduction in 2003 in France, the yellow-legged Asian hornet *Vespa velutina nigrithorax* is rapidly spreading through France and Europe. Economic assessments regarding the costs of invasive species often reveal important costs from required control measures or damages. Despite the rapid invasion of the Asian yellow-legged hornet in Europe and potential damage to apiculture and pollination services, the costs of its invasion have not been evaluated yet. Here we aimed at studying the costs arising from the Asian yellow-legged hornet invasion by providing the first estimate of the control cost. Today, the invasion of the Asian yellow-legged hornet is mostly controlled by nest destruction. We estimated that nest destruction cost €23 million between 2006 and 2015 in France. The yearly cost is increasing as the species keeps spreading and could reach €11.9 million in France, €9.0 million in Italy and €8.6 million in the United Kingdom if the species fills its current climatically suitable distribution. Although more work will be needed to estimate the cost of the Asian yellow-legged hornet on apiculture and pollination services, they likely exceed the current costs of control with nest destruction. It could thus be worth increasing control efforts by aiming at destroying a higher percentage of nests.

Keywords

biological invasions, IAS, Invasive alien species, yellow-legged hornet, impact

Introduction

Invasive species are one of the greatest threats to biodiversity and ecosystem functioning (Bellard et al. 2016) and part of global environmental change (Simberloff et al. 2013; Lewis and Maslin 2015). As globalisation keeps increasing, so does the amount of successful invasions (Seebens et al. 2017). Besides their negative impact on biodiversity and ecosystems, invasive species are also very costly to the global economy (Marbuah et al. 2014; Bradshaw et al. 2016). Indeed, invasive species can be very costly to goods and services such as agriculture (Paini et al. 2016), forestry (Aukema et al. 2010), aquaculture, tourism, recreation and infrastructure (Su 2002), but also to human health (Gubler 1998). Categorising and estimating these costs is not an easy task, so frameworks have been developed to categorise them, especially in ecology (Bradshaw et al. 2016). Bradshaw et al. (2016) suggest a framework that categorizes costs of species invasions into prevention, damage and response costs, but also into goods and services, human health, ecosystem processes and ecology. They estimated that "invasive insects cost a minimum of US$70.0 billion per year globally" in goods and services, "while associated health costs exceed US$6.9 billion per year", although these estimates are believed to be much underestimated (Bradshaw et al. 2016). Amongst the invasive species for which no cost has been estimated yet, the yellow-legged hornet *Vespa velutina nigrithorax*, the invasive sub-species of the Asian hornet, is considered an important threat to both biodiversity and apiculture and the importance of the damage it causes is regularly invoked in the media.

Vespa velutina nigrithorax is an Asian hornet native to China that invaded South Korea in 2003 and France in 2004. The species was first identified in 2003 in the southern part of South Korea (Kim et al. 2006). Introduced from China, it invaded most of the peninsula at an approximate rate of 10-20 km per year and became more abundant than other native *Vespa* species (Choi et al. 2012). The invasive hornet was then introduced into Japan: in Tsushima Island in 2012 (Ueno 2014) and Kyushu Island in 2015 (Minoshima et al. 2015). In France, *V. velutina nigrithorax* was first observed in south-western France in 2004 (Haxaire et al. 2006) after its accidental introduction from China (Arca et al. 2015). It spread rapidly, colonising most of France at an approximate rate of 60-80 km per year (Rome et al. 2015; Robinet et al. 2016) and progressively invading other European countries: Spain in 2010 (López et al. 2011), Portugal (Grosso-Silva and Maia 2012) and Belgium (Rome et al. 2013) in 2011, Italy in 2012 (Demichelis et al. 2014), Germany in 2014 (Witt 2015) and, finally, the UK where it was first recorded on 20 Sept 2016 (Budge et al. 2017). The rapid spread of the species in France and Europe is not necessarily a consequence of human-mediated dispersal, indicating that the species can rapidly spread on its own (Robinet et al. 2016), although human-mediated dispersal is not uncommon (Bertolino et al. 2016). Both climate and land-use have been shown to influence the spread of *V. velutina nigrithorax* (Villemant et al. 2011; Bessa et al. 2016; Fournier et al. 2017).

The yellow-legged hornet is believed to have several negative consequences on apiculture, biodiversity and, thus, on human well-being. Indeed, within its native and invasive range, *V. velutina nigrithorax* actively feeds on honeybees (Tan et al. 2007;

Monceau et al. 2013, 2014; Arca et al. 2014; Choi and Kwon 2015). Besides, the species could also have a negative impact on ecosystems by feeding on wild insects (Beggs et al. 2011) and contributing to the current global decline of pollination services and honey production (Villemant et al. 2011; Arca et al. 2014; Rortais et al. 2017). Given that nests are often found in urban areas (Franklin et al. 2017; Fournier et al. 2017), stings to humans are possible. Although multiple stings can be dangerous for humans, very few cases have been reported so far (de Haro et al. 2010), but the size of the hornet and its reputation for aggression make its presence dreaded and nest destruction systematically requested when the nest is close to human habitations or human activities. All of these negative impacts of the yellow-legged hornet invasion are likely to have an important economic cost, although such costs have not yet been estimated. Besides these potentially high cost, controlling the species in the already invaded areas and preventing the species further spread also have an economic cost that has not been estimated either.

The control of *V. velutina nigrithorax* invasion is mainly undertaken by nest destruction and bait trapping (Monceau et al. 2014), although neither of these methods are sufficient to achieve eradication even in a limited area when the yellow-legged hornet population is already too dense (Beggs et al. 2011). Several attractants have been used for bait trapping (Kishi and Goka 2017) but their efficiency is very limited as baits catch individuals rather than colonies. Moreover, they do not target *V. velutina nigrithorax* exclusively (Monceau et al. 2012). A previous study concluded that the most efficient strategy for controlling the yellow-legged hornet invasion remains to identify its presence early in new areas (with the help of predictions) and locate the nests for their systematic destruction (Robinet et al. 2016). In this study, we aimed at providing the first cost estimates for the control of the yellow-legged hornet invasion associated with nest removal. As these costs are not readily available for the entire invaded area, we did so by identifying potential correlates of the cost of nest destruction and extrapolated its total cost in the already invaded area, as well as in its potential invaded area.

Methods

The economic costs of invasive insects can be divided into three main categories: costs related to the prevention of invasion, the cost of fighting the invasion and the costs of the damage caused by the invasion (Bradshaw et al. 2016). There is no simple relationship between these cost categories. As the invasion is already underway, the costs related to the prevention of the invasion are non-existent. The costs of the damage caused by the invasion will be addressed in another study, as they require very specific data and methods. The main identifiable cost of fighting the invasion of yellow-legged hornets is the cost of nest destruction and will be the focus of this study. This first step, when combined with a subsequent estimation of damage costs, will allow the assessment of cost effectiveness, return on investment and similar indicators which will be useful indicators for decision-making frameworks for the use of funds for control programmes.

Data gathering regarding the cost of nest destruction

Estimating the average price of destroying a yellow-legged hornet nest would, in principle, be possible by surveying the many businesses providing such a service. However, as our aim is to estimate the total cost of nest destruction in the entire invaded range yearly, we also needed to know the total number of nests being destroyed each year. It seemed quite testing to gather such data exhaustively within a large enough spatial unit to then make reliable extrapolations. Therefore, we chose to focus our effort on identifying cities and departments subsidising nest destructions, as they were likely to have data, such as the number of nests destroyed and the total amount it costs them yearly. Indeed, given the rapid spread of the yellow-legged hornet, the administration of some French cities and departments decided to subsidise the destruction of the yellow-legged hornet in order to fight off the invasive species and the mechanism of the subsidy obviously encourages all the actors to be recognised by these administrations. To identify such cities and departments, we ran an internet search (using google.fr) with the key words "subvention", "destruction", "nid" and "frelon asiatique" or "*vespa velutina*" (i.e. "subsidy", "destruction", "nest" and "Asian hornet"). All cities and departments, identified as subsidising the yellow-legged hornet nest destruction, were then contacted to obtain data regarding the total yearly cost of nest destruction, as well as the number of nests that were destroyed.

Extrapolating the cost of nest destruction spatially

To take into account invaded areas with no subsidy of nest destruction, we aimed at spatially extrapolating this cost by identifying potential correlates of the cost of nest destruction. As potential correlates, we chose to investigate the surface area and the human population size of the spatial unit for which we were able to gather cost information. As we could only gather a reduced dataset, potential correlations were investigated through simple models – a linear model and a log-log linear model: for each potential correlate, we fitted the two following models (1) $y \sim x$ and (2) $\log(y) \sim \log(x)$.

Spatial extrapolation to countries other than France, need to be adjusted to *per capita* GDP (in purchase power parity terms), i.e. to the cost of living in a given country. To do that, we gathered the 2015 *per capita* GDP (PPP) of all countries and calculated their ratio to the one of France. The spatially extrapolated cost in a given country is then adjusted by multiplying it by this ratio.

However, if the yellow-legged hornet is rapidly spreading, we must limit our spatial extrapolation to areas it currently occupies and to climatically suitable areas it could likely invade in the next few years. As we aim at providing information useful for managers and decision-makers now, we will not account here for climate change of the next decades. We thus need to predict the potential distribution of the yellow-legged hornet.

Modelling the potential distribution of the yellow-legged hornet

Presence data of the yellow-legged hornet in its native and invaded ranges

Presence data of the yellow-legged hornet from the native Asian range was obtained by gathering information on museum specimens, published records and hornet sampling performed in China (Villemant et al. 2011). As for the invaded range in Europe, data from the French part of the invaded range came from the INPN database that aggregates all validated French records (https://inpn.mnhn.fr/). To this French database, we added the recent locations reported in other European countries (Spain, Portugal, Italy, Belgium and Germany) (López et al. 2011; Rome et al. 2013; Porporato et al. 2014; Witt 2015; Goldarazena et al. 2015; Bertolino et al. 2016). Overall, we obtained 10,395 records in the European invaded range observed from 2004.

Climate data

We used the same eight climatic variables as in previous studies for the niche modelling of the yellow-legged hornet (Villemant et al. 2011; Barbet-Massin et al. 2013). We considered: (1) annual mean temperature, (2) mean temperature of the warmest month, (3) mean temperature of the coldest month, (4) temperature seasonality, (5) annual precipitation, (6) precipitation of the wettest month, (7) precipitation of the driest month and (8) precipitation seasonality. The seasonality is the coefficient of variation of the monthly means. Current data were downloaded from the worldclim database (Hijmans et al. 2005) (http://www.worldclim.org/) as 2.5 arc-min grids (subset of the 19 bioclim variables). These data are interpolations from observed data representative of current climatic conditions.

Climate suitability modelling

Climate suitability of the yellow-legged hornet was modelled by running eight different modelling techniques implemented within the *biomod2* package (3. 3-7 version) (Thuiller et al. 2009) in R (R Core Team 2015): three regression methods (GLM, GAM and MARS), two classification methods (CTA and FDA) and three machine learning methods (ANN, BRT and RF). As no absence data were available for the species, pseudo-absences were randomly drawn (Barbet-Massin et al. 2012) from the South-East part of Asia and from Europe. We used 10,000 random pseudo-absences, with the total weight of presences being equal to the total weight of pseudo-absences (Barbet-Massin et al. 2012). As results might depend on the choice of pseudo-absences, models were replicated three times (with different pseudo-absences selection) (Barbet-Massin et al. 2012). To obtain a consensus distribution, we used an ensemble forecast technique (Marmion et al. 2009): the consensus distribution was calculated as the average of all distributions across modelling techniques and pseudo-absences replicates. Model predictive accuracy was evaluated through cross validation by splitting the data into training data (70%) and evaluation data (30%). The data split for cross validation was repeated five times.

Results

Through our data search, we were able to obtain data on total cost of nest destruction (as well as the number of nests being destroyed) for 10 administrations (two departments and eight cities, Fig. 1). Human population was found to be a strong predictor of the total cost of nest destruction, better so than the surface of the area studied (Table 1). The linear model was better than the log-log linear model, so it was selected for further extrapolation. Spatial extrapolation of potential cost of nest destruction given the population was then realised, based on a gridded population of the world (Center for International Earth Science Information Network – CIESIN – Columbia University 2016) and adjusted to *per capita* GDP (PPP) (Table 1). This spatially extrapolated cost was only applied where the climate is suitable for the yellow-legged hornet. The predicted climate suitability is a continuous value (from 0 to 1). A 0.5 threshold is frequently applied to transform the continuous suitability into binary output (suitable vs. non suitable). However, the yellow-legged hornet is unlikely to be at equilibrium in its invaded area, so we chose a less conservative threshold of 0.3 as the predicted climate suitability might be underestimated. Climate suitability below 0.3 was forced to 0. Not all climatically suitable areas have been invaded yet (Figure 2). To obtain a potential spatial cost of nest destruction in all areas suitable for the yellow-legged hornet, we can multiply the hornet climate suitability by the spatially extrapolated cost. This is the estimated yearly cost once the hornet has established. In Europe, the main yearly costs, once the hornet has colonised all its climatically suitable distribution, are estimated for France (€11.9M), Italy (€9.0M) and the United Kingdom (€8.6M) (Fig. 3 and Table 1). In Japan and South Korea, where the species has already been observed, the total yearly cost of nest destruction is estimated at €19.5M and €11.9M respectively (Fig. 3 and Table 1). If the species has been accidently introduced into the countries that have not yet been invaded, the yearly cost of nest destruction could be important in some countries, such as the USA (€26.9M), Australia (€3.6M), Turkey (€3.5M), Argentina (€2.6M) and Brazil (€1.8M) (Fig. 3 and Table 1). All these estimated costs are contingent on successful invasions.

In France, the hornet is already successfully spreading at a very fast rate and we know which year each department was invaded. So, we estimated the yearly cost of nest destruction since the start of the invasion, by only considering costs within invaded departments each year (a department was considered as successfully invaded when the tenth individual was observed). In 2006, only two years after the hornet was first observed in France, three departments were already invaded and the cost of nest destruction was estimated at €408k (Fig. 4). Since then, the estimated yearly costs have been increasing by ~€450k each year (Fig. 4), as the hornet keeps spreading and invades new departments. Overall, we estimated €23M as the cost of nest destruction between 2006 and 2015. If this temporal trend can be extrapolated for the next few years (i.e. if the hornet keeps spreading at a similar rate), we expect the yearly cost of nest destruction to reach an estimated value of €11.9M (given all suitable areas are invaded) by 2032.

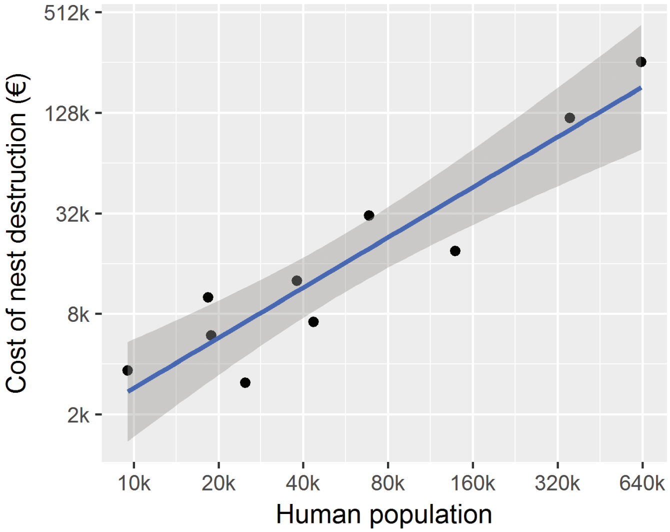

Figure 1. Relationship between population and the cost of nest destruction. The blue line represents the selected linear model (model 3 in Table 1). The darker grey area represents the confidence interval of the regression curve. Note that both axes are logarithmic.

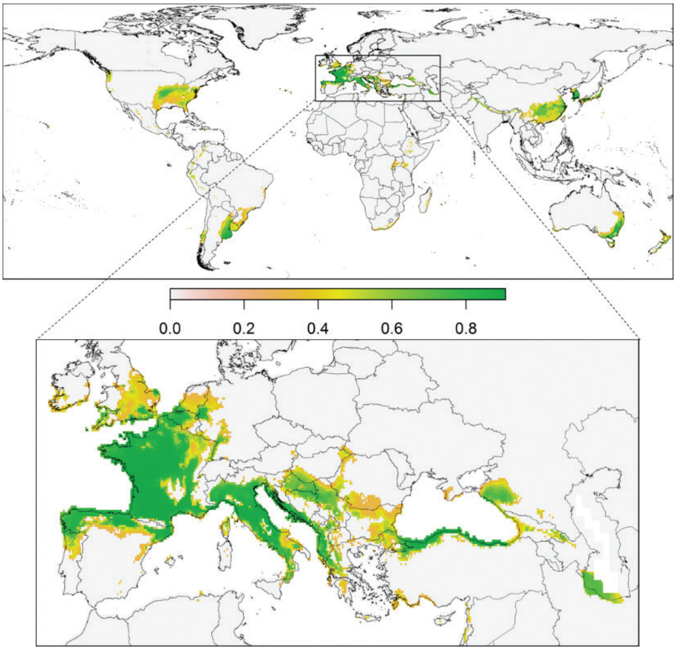

Figure 2. Consensus climate suitability of the yellow-legged hornet predicted from species distribution modelling. The climate suitability can be interpreted as a probability of having a suitable climate. The mean cross-validation TSS (respectively AUC) of all models considered to compute the consensus is 0.90 ± 0.07 (respectively 0.97 ± 0.03).

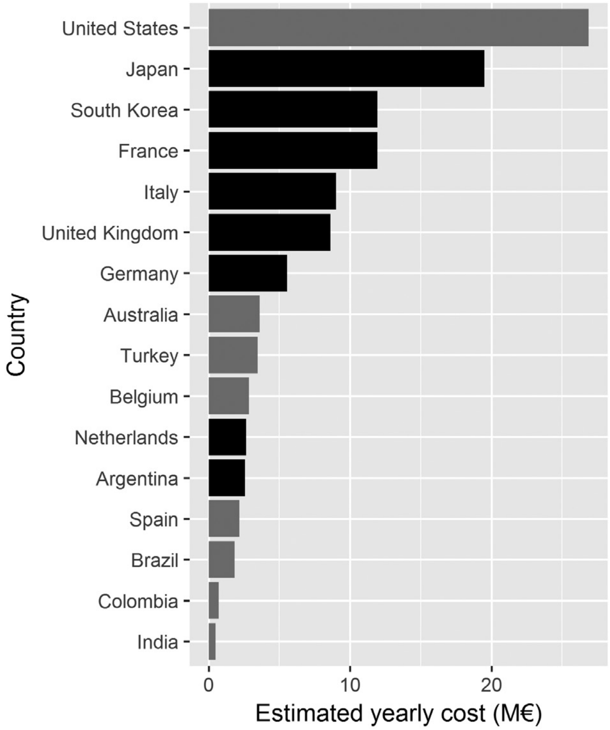

Figure 3. Estimated yearly cost of nest destruction if climatically suitable areas are fully invaded. Bars are coloured in black if the species is already invading the country and in grey for countries where the species has not established yet.

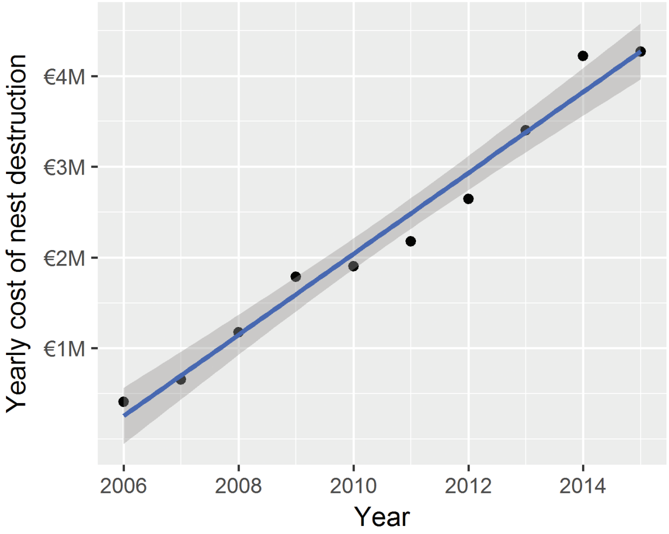

Figure 4. Estimated yearly cost of nest destruction in France since the start of the invasion given the yearly invasive range. The darker grey area represents the confidence interval of the regression curve. The increase results from the spread of the species.

Table 1. Results of the four models tested the potential influence of population and surface on the cost of nest destruction.

Model	Intercept	Slope	R^2	F	df	p-value
Cost~Population	$-6.49e^3$	0.39	0.97	303.2	8	$1.2e^{-7}$
Cost~Surface	8767	28.6	0.88	61.32	8	$5.09e^{-5}$
log(Cost)~log(Population)	-1.23	0.99	0.86	54.3	8	$7.82e^{-5}$
log(Cost)~log(Surface)	6.93	0.58	0.82	40.95	8	$2.09e^{-4}$

Discussion

As of today, nest removal remains the main strategy for efficiently controlling the yellow-legged hornet population. Indeed, even though European parasitic flies or nematodes can infect *V. velutina nigrithorax* (Darrouzet et al. 2014; Villemant et al. 2015), they seem to have a limited impact on the species colony survival (Villemant et al. 2015). Besides, intraspecific competition was shown to be unlikely as a potential mechanism for population regulation (Monceau and Thiery 2017), so there is no indication that the rapid spread of the species in Europe will lessen if control strategies do not improve and are not reinforced. Climate change may, on the contrary, worsen the invasion in the near future (Barbet-Massin et al. 2013) and, therefore, the overall economic costs. Nest removal thus currently remains the main strategy for controlling the spread and the population density of the yellow-legged hornet and we suggest it should be maintained or intensified (see below). It could also be combined with trapping individuals with more selective traps and more selective attractant, in order to make the control more efficient (Robinet et al. 2016). Successful case studies with *Vespula* wasps suggest the possibility of toxic baiting for the control of *V. velutina nigrithorax* (Kishi and Goka 2017), but further research is needed. As of today, the effort put into nest removal is not sufficient to prevent the spread of the species. Indeed, it has been estimated that only an average of 30-40% of detected nests have been destroyed each year in France (Robinet et al. 2016). The number of nests being destroyed does not result from a control strategy aiming at destroying all or a given percentage of detected nests, but rather from nests being destroyed because of their being potentially harmful to human (nests close to human habitations) or beekeeping activities (nests close to beehives). However, enforcing a control strategy that would aim at doubling the number of nests destroyed – thus potentially doubling the estimated yearly cost of nest destruction, to €23.8M if the cost per unit of control is constant- could reduce the spread (rate of dispersal) of the species by 17% and its nest density by 29% (Robinet et al. 2016). Further destroying 95% of the detected nests – thus tripling the estimated yearly cost of nest destruction, to €35.7M – could reduce the species' spread by 43% and its nest density by 53%. Our study thus provides the first estimates of the costs for nest destruction following the yellow-legged hornet invasion. These results can further be used to estimate the costs/benefits of different control strategies involving nest removal. If a more systematic nest destruction is considered for better control of the yellow-legged hornet invasion, public awareness campaigns need to be raised and nest removal could be required by a country's regulation. Furthermore, in order to reach a higher percentage of nests being detected and localised, new detection techniques need to be implemented.

As our cost estimates rely on scarce data, they therefore have to be interpreted with caution. Although our data were concentrated in western France, there is no reason to believe that the population – cost correlation would differ in another region. Despite a low amount of data, we were able to detect a strong correlation between the cost of nest destruction and human population within a given spatial unit. The cost of destroying a nest can vary significantly with local circumstances; but the quality of this correlation tends to show that, for a minimum area, the aggregate cost is not affected by this variability (there is no spatial correlation of the cost heterogeneity). Given the standard error of the correlation coefficient estimate, the confidence interval around extrapolation estimates should be ~10% of the extrapolated estimate. For example, the confidence interval for the estimated €11.9M yearly cost in France is €11.2M-€12.6M. The population – cost correlation, found by the authors, is not that surprising, given that the yellow-legged hornet was shown to favour urban and anthropised habitats (Franklin et al. 2017; Fournier et al. 2017). Besides, a nest is most likely to be destroyed if it is close to human habitations or activities, so it seems logical that larger numbers of nests are destroyed in areas with higher population density.

For a better understanding of the costs/benefits of different potential control strategies, it will also be important to compare the costs of nest removal strategies with the economic costs due to the negative impacts of the yellow-legged hornet, such as a potential decrease in the beekeeping activity or a decrease in pollination services or health costs. If the health costs are not currently available, the apiculture revenue was €135M in France in 2015. Given that half of France is currently invaded by the yellow-legged hornet, approximately 50% of this revenue can be at risk from the yellow-legged hornet. If the invasive species were to cause a 5% decrease in honey production, there would be an associated yearly cost of €3.3M. This is a broad estimate that would require data regarding the spatial distribution of honey production and the impact of the yellow-legged hornet on honey production to be refined. The yearly pollination services to agriculture were estimated at €2 billion in France (Gallai et al. 2009), so, if the yellow-legged hornet were to cause a 5% decrease in pollination services over half the territory, there would be an associated yearly cost of €50M. Obviously further research is needed to refine these estimates and, in particular, to assess the percentage of honey production and pollination services affected, but comparing it to the yearly €11.9M of nest destruction gives an idea about the order of magnitude of relative costs of damage and damage prevention. If more stringent control measures aiming at tripling the number of nests being destroyed were to be implemented, they would still be less costly than the cost of potential damage to apiculture and agriculture if the yellow-legged hornet causes more than a 5% decrease in honey production and in pollination services.

Estimates of costs associated with surveillance or prevention would also be very informative. Indeed, given the potential high costs associated with the yellow-legged hornet invasion to goods and services and given how difficult and costly it can be to control it once well established, preventing the species introduction into new countries will likely be less costly. We thus advise monitoring efforts to target areas projected as climatically suitable, especially on islands such as the UK and Japan (Robinet et al. 2016). Indeed, if the yellow-legged hornet were only observed a few times in the UK, a rapid nationwide colonisation is possible, even from a single invasive site (Keeling et al. 2017) and control would be less cost-effective than invasion prevention for other regions in the country. Moreover, various records in new areas took place in the vicinity of train station, port and airport cargo areas (e. g. northern Parisian suburb train freight station in 2009 and airport in 2011, near Viana do Castelo port, Portugal in 2011, Burela port in Galicia, Spain 2012, near Bristol port, UK in 2016) suggesting that commercial transport also plays a significant role for long-distance spread and, above all, for the creation of new foyers of dissemination and its impact on the spread of the invasive hornet must not be neglected. Monitoring efforts should, therefore, strongly focus on commercial and human transport crossroads. Other countries, such as the US, Australia, Turkey and Argentina, appear to be climatically suitable for the species, even if the yellow-legged hornet has not yet been observed there. Given their distance to the native and current invasive range of the species, it is unlikely that the species will disperse in these countries on its own. However, given the estimated costs of damage related to nest destruction alone, it is worth implementing surveillance programmes to prevent human-mediated dispersal in these countries in order to avoid the high economic impacts of the yellow-legged hornet if the species were to establish there.

Our study provides the first estimates of economic costs resulting from the yellow-legged hornet. We were able to estimate the cost of nest destructions – used to control the spread of the species and limit its presence close to human habitations and activities – and extrapolate these costs to all areas which are climatically suitable for the species. Although more studies will be needed to estimate other costs related to the yellow-legged hornet invasion (in particular, the cost of its impact on apiculture and pollination), the destruction of nests already cost €23M in France alone and a further €11.9M each year at least, with a likely increase as the species keeps spreading.

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Advancing research on alien species and biological invasions

On the RIP: using Relative Impact Potential to assess the ecological impacts of invasive alien species

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Abstract

Invasive alien species continue to arrive in new locations with no abatement in rate, and thus greater predictive powers surrounding their ecological impacts are required. In particular, we need improved means of quantifying the ecological impacts of new invasive species under different contexts. Here, we develop a suite of metrics based upon the novel Relative Impact Potential (RIP) metric, combining the functional response (consumer *per capita* effect), with proxies for the numerical response (consumer population response), providing quantification of invasive species ecological impact. These metrics are comparative in relation to the eco-evolutionary baseline of trophically analogous natives, as well as other invasive species and across multiple populations. Crucially, the metrics also reveal how impacts of invasive species change under abiotic and biotic contexts. While studies focused solely on functional responses have been successful in predictive invasion ecology, RIP retains these advantages while adding vital other predictive elements, principally consumer abundance. RIP can also be combined with propagule pressure to quantify overall invasion risk. By highlighting functional response and numerical response proxies, we outline a user-friendly method for assessing the impacts of invaders of all trophic levels and taxonomic groups. We apply the metric to impact assessment in the face of climate change by taking account of both changing predator consumption rates and prey reproduction rates. We proceed to outline the application of RIP to assess biotic resistance against incoming invasive species, the effect of evolution on invasive species impacts, application to interspecific competition, changing spatio-temporal patterns of invasion, and how RIP can inform biological control. We propose that RIP provides scientists and practitioners with a user-friendly, customisable and, crucially, powerful technique to inform invasive species policy and management.

Keywords
biological control, ecological impacts, functional response, invasive alien species, numerical response, propagule pressure, relative impact potential metric, risk assessment

Introduction

In recent decades, the tourism, agriculture, aquaculture, horticulture and pet trades, among others, have been boosted by new globalised transport networks (Hulme 2009, Seebens et al. 2018), facilitating novel pathways for invasive alien species (IAS; herein invasive species) to spread and establish (Zieritz et al. 2016, Seebens et al. 2019). Indeed, the number of confirmed invasive species per country has risen around 70% since 1970 (IPBES 2019). The combination of species introductions with changing climate (Seebens et al. 2015, Gallardo et al. 2018) and other anthropogenic impacts (MacDougall and Turkington 2005, Didham et al. 2007) is aiding species establishment and spread, with the number of invasive species unlikely to saturate in the near future (Seebens et al. 2017). While the effects of invasive species can be benign (invasiveness is not correlated with impact: Ricciardi and Cohen 2007), many invasive species have severe ecological consequences that drive negative economic, social and health implications (Laverty et al. 2015a,b). Assessing and predicting such ecological impacts are crucial to prevent and mitigate invasions (Simberloff et al. 2013). Note that here, we define ecological impact as changes in populations of affected species, principally through consumption of living resources by animals (e.g. predator-prey) and interspecific competition for limited resources (especially in plants - see Dick et al. 2017a,b,c and "Understanding and predicting competition with RIP" section below). Further, predicting which future species are likely to exert ecological impacts, and how such impacts are likely to change under different abiotic and biotic contexts, are vital objectives for the conservation of biodiversity worldwide (Dick et al. 2017a, IPBES 2019).

The management of invasive species is challenging, with certain high-profile failed management programmes (Bergstrom et al. 2009, but see Simberloff 2009). Successful prediction of impact is vital to successful prevention, which is deemed the most effective invasive species management (Piria et al. 2017) and one that may avoid unpredictable indirect effects, such as trophic cascades (Jackson et al. 2017). Aichi Biodiversity Targets of the Convention on Biological Diversity focus on invasive species prevention, requiring a list of priority invasive species and preventative measures by 2020. EU legislation (Regulation No 1143/2014), alongside the list of IAS of Union Concern (Regulation No 2016/1141), and subsequent updates (Regulation Nos 2017/1263, 2019/1262), add further impetus to impact prediction, requiring all member states to develop evidence-based lists of invasive species likely to impact biodiversity and ecosystem services.

Here, we develop a suite of metrics based on the *per capita* effects and abundances of species (Dick et al. 2017c) that can quantify the spectrum of benign through to severely damaging invasive species. We outline the background to these metrics, the development of their key components, namely *per capita* effects (principally the functional response), the consumer population response (i.e. the numerical response), incorporation of risk (i.e. propagule pressure) and suitable proxies for these three elements. We then demonstrate the versatility of the metrics across abiotic and biotic contexts, including the prediction of climate change effects on invasive species impacts, how native species and established invaders can offer biotic resistance, how evolution can affect impact, aspects of interspecific competition, spatio-temporal patterns of invasion and impact, and the assessment of biological control agent efficacy.

Background and development of invasive species ecological impact metrics

Numerous studies have failed to find traits of species, spanning diverse taxonomic and trophic groups, that reliably predict invader ecological impact (Hayes and Barry 2008, Ricciardi et al. 2013, Dick et al. 2014; but see Valdovinos et al. 2018, Fournier et al. 2019 for recent studies on specific taxa). While some have found traits that predict invasiveness (Hui et al. 2016), there is no correlation between invasiveness (i.e. establishment and spread) and ecological impact (Ricciardi and Cohen 2007). Parker et al. (1999) proposed the impact (I) of an invader as the product of its range (R), abundance (A) and its *per capita* effect (E):

$$I = R \times A \times E \tag{1}$$

Following this "Parker-Lonsdale" equation, Dick et al. (2013, 2014) proposed the classic metric of the functional response (see Fig. 1) could be used as a universal *per capita* effect in invasion ecology. Functional responses typically describe the rate of prey capture by a predator in relation to prey density (Solomon 1949, Holling 1959), however, they can be applied to any consumer/resource interaction (see Dick et al. 2017a,b,c), including herbivores (Farnsworth and Illius, 1996), microbial communities (Graves et al. 2016) and nutrient uptake rates by plants (Tilman 1977,

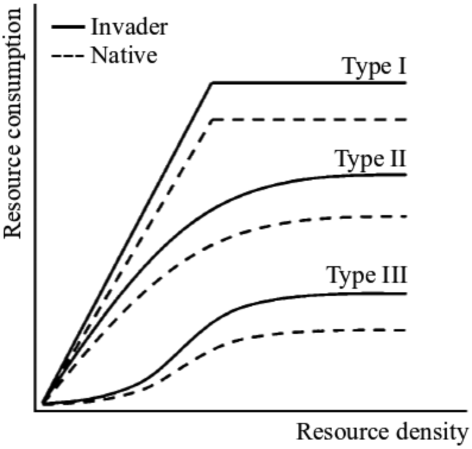

Figure 1. Type I, II and III functional responses and hypothetical invader/native comparisons (see Dick et al. 2014).

Rossiter-Rachor 2009). Indeed, the concept of functional responses in plants was the basis of the "functional resource-utilisation responses" proposed by Tilman (1977). By focusing on resource sequestration rate, functional responses quantify the strength of primary ecological interactions exhibited by any species, and hence can potentially unify invasion ecology across trophic and taxonomic groups (Dick et al. 2017a,b). Indeed, resource acquisition is at the core of the majority of longstanding hypotheses in invasion ecology (Catford et al. 2009, Ricciardi et al. 2013).

Comparing the functional responses of native and invader consumers can highlight differences in the strength of consumer/resource interactions (Fig. 1), with implications for population stability of the prey or other resources (Holling 1959, Dick et al. 2014). Type I functional responses are typical of filter feeders (Jeschke et al. 2004) with a directly proportional increase in resource consumption with availability; Type II responses are hyperbolic and inversely density-dependent; and Type III responses are sigmoidal, with low consumption at low resource densities. Type II responses may have a destabilising effect due to high proportional resource consumption at low resource densities, while Type III responses may have a stabilising effect due to low proportional consumption at low resource densities (Dick et al. 2014). Type III may arise when consumers switch to more abundant resources (Van Leeuwen et al. 2007) or when habitat complexity offers refuge for prey (Alexander et al. 2012).

The comparative functional response approach (Fig. 1), whereby the impacts of invasive species are compared with analogous native species as eco-evolutionary baselines (Dick et al. 2017c), have proved a reliable tool for explaining the ecological effects of existing invasive species and predicting the impacts of new, emerging and indeed potential future invaders under a wealth of different contexts (Dick et al. 2014, 2017a, b, c, Crookes et al. 2018, Howard et al. 2018, Hoxha et al. 2018). Such contexts include dissolved oxygen levels (Laverty et al. 2015a), habitat complexity (Wasserman et al. 2016), temperature regimes (Zamani et al. 2006), water chemistry gradients (Kestrup et al. 2011), higher order predators (Barrios-O'Neill et al. 2014) and parasites (Laverty et al. 2017b). Such studies are not limited to the laboratory either, with numerous examples of functional responses estimated from field studies (Goss-Custard et al. 2006, Smout et al. 2014). Further, Penk et al. (2017) illustrated the utility of this approach, providing a framework for its application across a range of novel organisms. Finally, the comparative functional response approach can be applied to asking which of several actual or potential invaders will have more or less impact, with several studies indicating that higher functional responses (i.e. maximum feeding rates) do indeed predict higher ecological impact. For example, the killer shrimp (*Dikerogammarus villosus*) has consistently higher maximum feeding rates than the less ecologically damaging demon shrimp (*Dikerogammarus haemobaphes*: Bovy et al., 2014), with a similar effect being noted for the invasive golden apple snail relative to other introduced snails (Xu et al. 2016). With regards to plants, it is notable that resource utilization curves, which are essentially functional responses, can be used comparatively to understand the dynamics of plant invasions (e.g. Rossiter-Rachor et al. 2009). Most recently, the utility of determining functional responses of multiple populations of the same invasive species was shown by Howard et al. (2018) and Boets et al. (2019), with functional responses derived within and between geographical populations tracking actual impacts in the field. Further, a recent study of multiple populations of the invasive crayfish *Faxonius limosus* shows context-dependencies of functional response parameters (Grimm et al. 2020), and the authors caution that risk assessments of invasive species should, where possible, be based on multiple population estimates of functional responses. This recognition of population level as opposed to species level differences is now a burgeoning question and we encourage more research in this area.

Although the comparative functional response approach has been successful in characterising ecologically damaging invasive species by itself (e.g. Dick et al. 2013, Alexander et al. 2014, Dick et al. 2014, 2017a,b,c), it may provide limited quantification of total ecological impact as the functional response is only one of the three components of the aforementioned Parker-Lonsdale equation (Eq. 1: Parker et al. 1999). Since "range" is dependent on time since invasion, and is not necessarily a species characteristic (Kumschick et al. 2013), the ecological impact of a species can be viewed as a function of its *per capita* effects and some measure of the number of individuals involved (Dick et al. 2017c, Pearse et al. 2019). The latter is often measured as the consumer population numerical response, but often more simply as consumer abundance (Dick et al. 2017c). Assessment of both functional response and numerical response (or proxies) is crucial. For example, Fig. 2 illustrates that high ecological impact will result when both the functional and numerical responses of an invader are high, but impact may also result when a few individuals demonstrate particularly high functional responses, or when the functional responses of invaders are similar to natives but invader numerical response is very high. Note also from Fig. 2 that high invader numerical responses alone do not necessarily equate to high, or indeed any noticeable impact if there are low levels of inter-specific interactions, or no detectable interaction (see Dick et al. 2017c; Fig. 2). Indeed, this method proposes that relatively low functional and/or numerical responses can be used to identify low impact or relatively

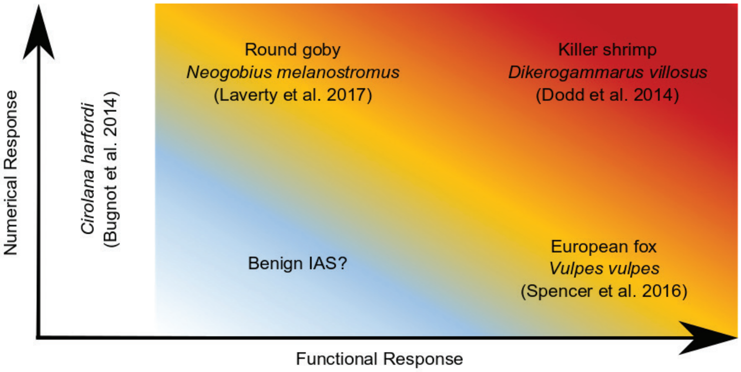

Figure 2. Ecological impact heat map of the combination of functional and numerical responses of invasive species compared to trophically analogous equivalents, with ecological impact increasing from bottom left to top right (see text for details).

benign invaders (Fig. 2). Here, we thus propose that Relative Impact Potential (RIP, see below), which incorporates functional responses and numerical responses into a ratio for invader and native analogues, offers a way to identify the range of impacts of invasive species illustrated in Fig. 2.

The Relative Impact Potential metric for quantification of invasive species ecological impacts

The combination of functional and numerical responses is consistent with the idea of the total response (TR) of a consumer (Holling 1959):

$$TR = FR \times NR \tag{2}$$

Unlike the functional response, the rather nebulous numerical response has proven difficult to derive due, for example, to time lags in consumer population responses (see Dick et al. 2017c, Laverty et al. 2017a), resulting in the need for more simplistic and pragmatic proxies for the numerical response that capture consumer reproduction, aggregation and resource assimilation (Dick et al. 2017c). Consequently, Dick et al. (2017c) proposed that the ”Impact Potential” (IP) of an invader can be represented as the product of the functional response (FR) and a chosen proxy for the numerical response (NR_{proxy}), such as field abundance/density (AB: see Dick et al. 2017c, Laverty et al. 2017a), giving an equation that blends Eq.1 and Eq. 2:

$$IP = FR \times NR_{proxy} \tag{3}$$

e.g.

$$IP = FR \times AB \tag{4}$$

An IP value by itself offers limited insight, but we relate the IP of an invasive species to the IP of a trophically analogous native (the baseline, or co-evolved relationship), giving the "Relative Impact Potential" of the invader (henceforth, RIP) as:

$$\text{RIP} = \left(\frac{\text{FR invader}}{\text{FR native}}\right) \times \left(\frac{\text{NRproxy of invader}}{\text{NRproxy of native}}\right) \tag{5}$$

In Eq. 5, the functional response may be the maximum feeding rate, that is, the curve asymptote, or $1/h$ (where h is the handling time parameter: Dick et al. 2017c), or, where this is not possible, the consumer feeding rate with an over-abundance of resource supply. However, the latter will lack the benefits of the functional response method, which gives the extrapolated maximum feeding rate ($1/h$) and resolution of potentially destabilising Type II versus stabilising Type III functional responses (see Fig. 1). When the resulting RIP value is < 1, this predicts the invader will have less impact than the trophically analogous native; when RIP = 1, we predict invader impact no different from that of the native; and when RIP is > 1, we predict the invader will have a greater impact than the native (Dick et al. 2017c). The native comparator thus allows the degree of ecological impact of the invader to emerge, and RIP values do indeed correlate positively with actual ecological impacts of invasive species in the field (Dick et al. 2017c, Laverty et al. 2017a, Kemp et al. 2018). The choice of native comparator(s) can be guided by assessment of those native species that are trophically similar to the invader and found in the invader's new range, with multiple native comparators useful if available (see Dick et al. 2017c). For example, comparing invasive *Dikerogammarus villosus* with both *Gammarus pulex* and *G. duebeni celticus* gives coverage of almost all of the UK/Ireland and many European mainland freshwaters (Bollache et al. 2008), and Rossiter-Rachor et al. (2009) successfully compared the invasive gamba grass with two locally abundant and analogous native grasses.

RIP lends itself to data collection by experiment and/or survey, or information from already available/published results. Single estimates of the functional response and the numerical response proxy may be used in the RIP equation; alternatively, means, standard errors, variances, standard deviations or confidence intervals can allow the incorporation of uncertainty into RIP. To do this, it is assumed that the observed functional response and numerical response proxy are samples from underlying distributions of values (see Dick et al. 2017c). With both measures being positive, a log-normal form for both underlying distributions is used, giving the probability density function (pdf) for the RIP measure given the four input pdfs (two numerators, two denominators):

$$f(\text{RIP}) = \left(\frac{f(\text{ FR invader })}{f(\text{ FR native })}\right) \times \left(\frac{f(\text{ NRproxy of invader })}{f(\text{ NR proxy of native })}\right) \tag{6}$$

where $f()$ = the pdf.

As an example, we have functional responses for the Ponto-Caspian invasive amphipod *D. villosus* (killer shrimp) and the native analogue *G. duebeni* towards *Asellus* *aquaticus* prey (Bollache et al. 2008). Abundances were taken from Berezina & Duris (2008) and Dick (1996) respectively, giving RIP as:

$$\text{RIP}=\left(\frac{\text{FR }D.villosus}{\text{FR }G.\,duebeni}\right)\times\left(\frac{\text{AB }D.villosus}{\text{AB }G.\,duebeni}\right)=\left(\frac{22}{16}\right)\times\left(\frac{230.5}{31.6}\right)=10.03$$

We can then use the pdf, f (RIP), and report RIP and the confidence intervals (80% and 60%) and the probability that RIP is greater than 1, or any other figure (e.g. >10; see Dick et al. 2017c). By using the means and SDs from the worked example above [i.e. mean (SD) = 22(3.5), 230.5(23.3), 16(1.7), 31.6(5.4)], we get:

$$f(\text{RIP})=\left(\frac{f(\text{FR }D.\,villosus\,)}{f(\text{FR }G.\,duebeni)}\right)\times\left(\frac{f(\text{AB }D.\,villosus)}{f(\text{AB }G.\,duebeni)}\right)$$

$$\text{RIP}=10.44\begin{pmatrix}80\%CI=4.36-51.8\\ 60\%CI=7.98-12.7\\ P\text{RIP}>1=100\%\\ P\text{RIP}>10=50.8\%\end{pmatrix}$$

The result can also be visualised using “RIP biplots”, with maximum feeding rate on the x-axis, and the numerical response proxy on the y-axis (e.g. Laverty et al. 2017b; Cuthbert et al. 2018b, c). Using an example from Laverty et al. (2017a; Fig. 3), scanning diagonally from the origin to the top right of the plot, the invasive top mouth gudgeon *Pseudorasbora parva* has a greater ecological impact than the native bitterling *Rhodeus amarus*. Here, the numerical response proxy is fish field abundance but alternatives may be available (see below).

The other classic functional response parameter “*a*”, the attack rate, is an alternative to the maximum feeding rate. This quantifies the initial gradient of the functional response curve, and gives insights into the critical impact a consumer exerts at low resource densities (Dick et al. 2014). Cuthbert et al. (2018c) show the consistency of the maximum feeding rate and attack rate as functional response measures for two copepod species, *Macrocyclops albidus* and *Megacyclops viridis*, at three different temperatures. However, Dickey et al. (2018) reveal a noticeable difference between these two metrics for two turtle species, with *Trachemys scripta troostii* demonstrating a high maximum feeding rate but a low attack rate, and *Kinosternon subrubrum* demonstrating the reverse. However, a solution to the dilemma of whether to use *a* or *h* has emerged, with the unified metric of FRR, the Functional Response Ratio, which is simply *a*/*h* (Cuthbert et al. 2019).

There may be difficulties in determining functional and numerical responses, for example, practicalities surrounding consumer and resource supply, or ethical issues. Hence, we now review proxies for both.

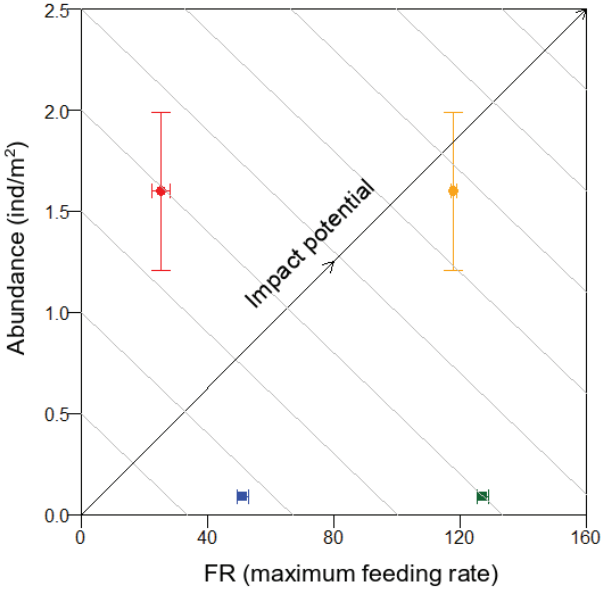

Figure 3. RIP biplot from Laverty et al. (2017a): invasive top mouth gudgeon *Pseudorasbora parva* (red and orange circles representing prey type 1 and 2 respectively), has a greater ecological impact than the native bitterling *Rhodeus amarus* (blue, prey type 1, and green, prey type 2, squares).

Choosing appropriate functional response proxies

In some situations (e.g. large-bodied species in the wild) functional response experiments may prove difficult. For example, the functional responses of deer species are poorly described (but see Illius et al. 2002), but there are proxies in the literature, such as absolute daily intake rate (e.g. Newman et al. 1998). Intake per metabolic body mass ($kg^{0.75}$) is a proxy (Drożdż 1979) that allows comparison of trophic analogues differing in body size (e.g. Reeves' muntjac, *Muntiacus reevesi*, versus native British deer species). For plants (e.g. Rossiter-Rachor et al. 2009), there are a number of metrics from resource uptake curves that are analogous to functional response metrics, such as V_{max} (maximum uptake rate over time) and K_m (substrate concentration at 50% maximum uptake rate).

As per Dick et al. (2017c), offering an over-abundance of a resource in experiments could suffice as a functional response proxy, however, highly informative aspects such as curve type will not then be available (see Fig. 1) and such an over-abundance may lead to unrealistically high consumption rates. On the other hand, low consumption rates can emerge from gregarious prey defence behaviours (e.g. the postulated Type IV functional response: Jeschke and Tollrian 2000). Consequently, where possible, we strongly recommend performing full functional response experiments/surveys, with a range of resource availability, to maximise information.

Choosing appropriate numerical response proxies

Population abundance/density are backed theoretically and in practice as suitable proxies for the numerical response (Dick et al. 2017c, Laverty et al. 2017a). These can be derived in numerous ways, such as through monitoring programmes, and from estimates of consumer densities from non-native ranges. In most cases, relative abundance is a sufficient proxy, with the effort required to determine absolute abundance often better spent on extra sampling (Hayes et al. 2007). One example of a fisheries technique directly proportional to abundance is Catch Per Unit Effort (CPUE; Maunder and Langley 2004), and there are numerous other techniques for estimating abundance and density for both open and closed populations (e.g. Seber 1986, Hayes et al. 2007; Suppl. material 1: Table S1).

Where abundance/density data are not suitable, for example, due to large body size differences among species, biomass is a suitable proxy. For example, the invasive sharptooth catfish *Clarias gariepinus* reaches lengths of 148 cm (Clay 1984), whereas the native trophic analogue river goby, *Glossogobius callidus*, reaches only 12 cm (Greenwood 1994, Alexander et al. 2014). The benefit of using biomass instead of abundance for this example is demonstrated in Fig. 4, which gives a more realistic demonstration of impact for *C. gariepinus*. Further, for plant numerical response proxies, estimates of individuals per unit area proved useful in calculating RIP values for invasive gamba grass in comparison to native grass species (Dick et al. 2017c), and other measures such as biomass and percentage coverage could be utilised.

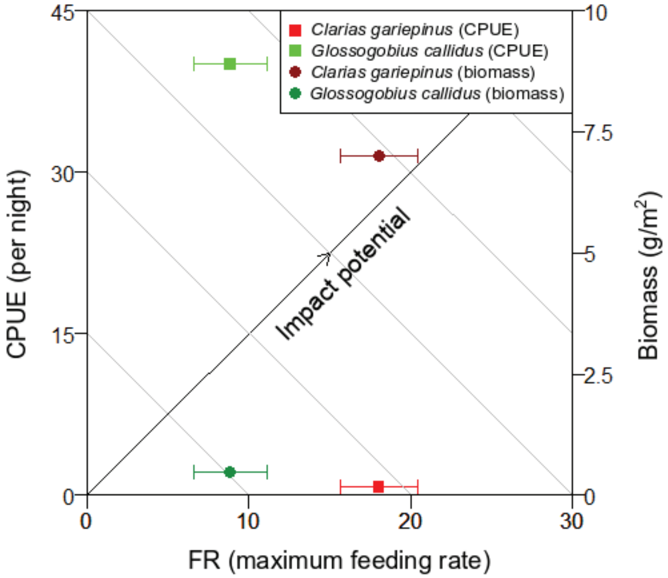

Figure 4. Comparison of impact derived from use of Catch Per Unit Effort and biomass, whereby CPUE gives a misleading impact assessment of the extralimital predator. CPUE data were taken from Bokhutlo et al. (2016) and Richardson et al. (2006), with unpublished biomass data from O.L.F Weyl taken from Dick et al. (2017c).

Often there is no known invasion history of a species, which will become increasingly common as new source pools of invaders are linked to human transport (Seebens et al. 2018). In such contexts, basing the RIP upon the abundance/density of the species in its native range, where it has co-evolved with natural enemies, could be misleading and it may be more prudent to use numerical response proxies based on other life history traits. For example, fecundity can be a key determinant of invasive species establishment (Grevstad 1999) and persistence (Pöckl 2007). While many highly fecund species never become invaders, and a large number of invaders have low fecundity, fecundity can strongly affect population size (Parvulescu et al. 2015). Thus, fecundity may be a pragmatic proxy for the numerical response (see Cuthbert et al. 2018b, Dickey et al. 2018). For example, in the abundance and fecundity impact biplot for two *Gammarus* species (Fig. 5), the invader *G. pulex* is compared with native *G. duebeni celticus*. We size-matched the two species by using the average size of the native (10–11mm: Hynes 1954), and took the corresponding mean number of eggs for the invader at that size (Hynes 1955). Abundance figures are from Kelly et al. (2006). The resulting biplots are consistent (Fig. 5), with *G. pulex* showing greater impact across both proxies of the numerical response, in line with actual field impacts. We outline further numerical response proxies in Suppl. material 1: Table S1.

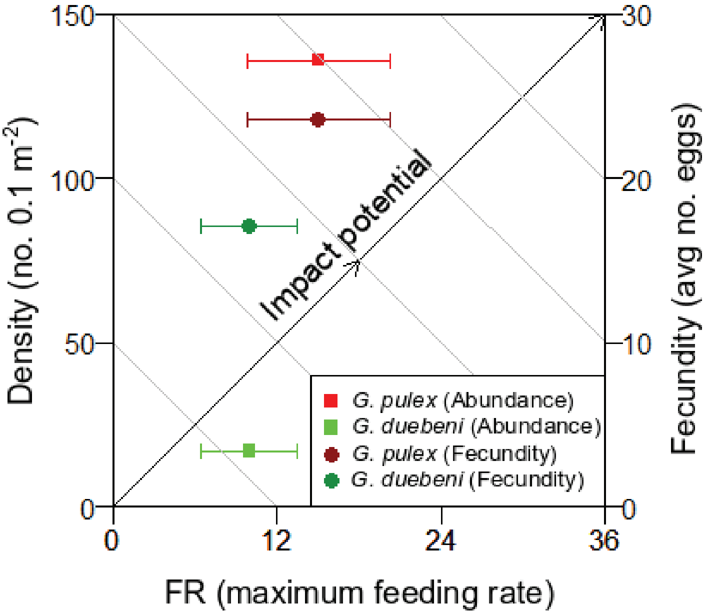

Figure 5. Comparing abundance and fecundity as numerical response proxies for the invader amphipod *G. pulex* and native *G. duebeni*. Functional response data taken from Laverty et al. (2015a), abundance taken from Kelly et al. (2006), and fecundity data taken from Hynes (1955).

Incorporating risk into RIP: propagule pressure proxies and Relative Invasion Risk, RIR

Invasive species success can be heavily dependent on propagule pressure, that is, the number, frequency and viability of individuals introduced (Briski et al. 2012). Management prioritisation thus needs to balance the likely ecological impacts of an invasive species with its risk of arriving, establishing and spreading. Dickey et al. (2018), using terrapins in the pet trade, combined the RIP metric with the multiplier "Pet Propagule Pressure" (PPP) to calculate Invasion Risk (IR: Eq. 7):

$$\mathrm{IR} = \mathrm{FR} \times \mathrm{NR} \times \mathrm{PPP} \tag{7}$$

PPP took two forms: one assessed availability of the species across 20 pet stores; the other surveyed classified advertisement websites for unwanted pets. Three dimensional triplots (i.e. x-, y- and z-axes) visualise relative invasion risk i.e. RIR (R script available therein). Dickey et al. (2018) also proposed other proxies for propagule pressure, such as live wildlife import and export data (US Law Enforcement Management Information System), and studies assessing survivability in ship ballast water (Gollasch et al. 2000). Surveys that account for the role of horticulture in plant invasions (e.g. Bayón and Vilà 2019) and some invertebrate invasions (Cannon et al. 1999) could also offer valuable proxies of propagule pressure. We therefore propose that combining such proxies of propagule pressure alongside functional and numerical responses offers an effective three-pronged assessment and prioritisation method that assesses overall invasion risk.

RIP in predicting invasive species impacts due to climate change

The past four years have been the hottest on record (2015–2018: NOAA 2019), and such changing environmental conditions will affect the establishment and impact of invading species (Kelley 2014, Iacarella et al. 2015a, Laverty et al. 2017b). Resultant changes in species range, phenology and physiology (see Bellard et al. 2012) may thus influence both functional and numerical responses. For example, temperature often influences functional responses (Englund et al. 2011) in a number of ways, affecting metabolism (especially for ectotherms: Gillooly 2001) and digestion efficiency (Pavasovic et al. 2004). Numerical responses will also be affected with, for example, many reptile species exhibiting temperature dependent sex determination (Laloe et al. 2014). Phenological shifts can also influence food availability and hence growth and abundance (Visser and Both 2005), potentially increasing the impact disparity between native and invader (Lediuk et al. 2014).

We thus propose that, for any invader, the effect of temperature increases (or other abiotic variables) on its ecological impact can be assessed by Eq. 8 as:

$$\mathrm{RIP} = \left(\frac{\text{FR of invader at high temp}}{\text{FR of invader at low temp}} \right) \times \left(\frac{\text{AB of invader at high temp}}{\text{AB of invader at low temp}} \right) \tag{8}$$

where 'high temp' could be the mean environmental temperature projected from climate models. Note that abiotic variables such as temperature may not affect functional response and numerical response proxies linearly (e.g. feeding parameters can show hump-shaped responses to temperature: Englund et al. 2011), and this RIP formula compares snapshots of impact at the study temperatures of choice.

The rate of reproduction of the prey (or other resource e.g. plant growth and reproduction) will also likely be affected by the same temperature rise, thus either decreasing or increasing impact. For example, if reproduction by the prey increases at higher temperatures (e.g. Sutcliffe and Carrick 1981), then impact will be reduced. Alternatively, a prey species already close to its thermal tolerance may be stressed by temperature rise and thus actually reduce its reproductive allocation (Dhillon and Sharma 2009), increasing impact. We thus propose a modifier to Eq. 8 that we call the "Resource Reproductive Qualifier" (RRQ), defined as the reciprocal of the fraction or proportion to which reproduction changes with temperature (or other variable: Eq. 9):

$$\text{RRQ} = 1/\left(\frac{\text{Reproductive output of prey at the higher temperature}}{\text{Reproductive output of prey at the lower temperature}}\right) \tag{9}$$

For example, if a prey species doubles its reproductive output at the higher temperature, then this will halve the RIP value as it is multiplied by ½; alternatively, a prey species that halves its reproductive output at a higher temperature will double the RIP value (i.e. multiply RIP by 1/0.5=2). Eq. 8 thus becomes:

$$\text{RIP q} = \left(\frac{\text{FR of invader at high temp}}{\text{FR of invader at low temp}}\right) \times \left(\frac{\text{AB of invader at high temp}}{\text{AB of invader at low temp}}\right) \times \text{RRQ} \tag{10}$$

For example, South et al. (in prep) demonstrate that lionfish *Pterois volitans* exert higher predation upon shrimp *Palaemonetes varians* at 26 °C (max. feeding rate of 8.34 ± 0.65 SE) than at 22 °C (4.34 ± 0.55 SE) and that lionfish have greater abundances at the higher temperature (28.80 ± 1.75 SD ha^{-1}: Kulbicki et al. 2012 vs 21.20 ± 5.1 SD ha^{-1}: Whitfield et al. 2007), thus:

$$\text{RIP} = \left(\frac{8.34}{4.34}\right) \times \left(\frac{28.8}{21.2}\right) = 2.611$$

However, their prey is likely to increase in abundance by 5% between the two temperatures, meaning RRQ is:

$$\text{RRQ} = 1/\left(\frac{1.05}{1}\right) = 0.952$$

Since more prey means the impact exerted lessens, this leads to a reduced RIP of:

$$\text{RIPq} = 2.611 \times 0.952 = 2.486$$

However, we can see that the increased prey abundance due to temperature increase does not offset the increased feeding rate and abundance of the predator, leading to maintenance of an RIP value > 1.

RIP can thus be adapted with RRQ to include context dependencies like temperature, but also associated climate change conditions such as ocean acidification (Uthicke et al. 2013) and freshening (Casties et al. 2015), providing new predictive metrics for the vast array of climate change consequences for invasive species impacts.

RIP as a measure of biotic resistance

Functional and numerical responses of resident species towards invasive species may provide biotic resistance (see also Twardochleb et al. 2012, Cuthbert et al. 2018c). For example, high functional responses of native and naturalised *Gammarus* species towards invasive prey *Crangonyx pseudogracilis* explain the field patterns of presence/absence of the invader (MacNeil et al. 2013, Cuthbert et al. 2018c). Using RIP, we propose here a powerful biotic resistance metric that can: (1) determine which native/naturalised species exhibit greater biotic resistance; and (2) predict the influence of abiotic factors on the strength of such biotic resistance (br). Thus, to assess which of two resident species better resists an invader:

$$\mathrm{RIPbr} = \left(\frac{\mathrm{FR\ of\ native1}}{\mathrm{FR\ of\ native2}}\right) \times \left(\frac{\mathrm{AB\ of\ native1}}{\mathrm{AB\ of\ native2}}\right) \tag{11}$$

Taking the functional response data of MacNeil et al. (2013) with non-native *C. pseudogracilis* prey, and the *Gammarus* spp. abundance data of Kelly et al. (2006), the RIPbr for the naturalised *G. pulex* relative to the native *G. duebeni* is:

$$\mathrm{RIPbr} = \left(\frac{\mathrm{FR}\ G.\ pulex}{\mathrm{FR}\ G.\ duebeni}\right) \times \left(\frac{\mathrm{AB}\ G.\ pulex}{\mathrm{AB}\ G.\ duebeni}\right) = \left(\frac{11.7}{9.1}\right) \times \left(\frac{136}{17}\right) = 10.29$$

Therefore, resistance to the non-native *C. pseudogracilis* prey by the naturalised *G. pulex* is stronger than by native *G. duebeni* due to higher *per capita* feeding rate and abundance.

One possible issue of using functional response data to infer biotic resistance is the use of a single prey species, unlikely in the wild where alternative prey will occur. We thus suggest functional response experiments feature the target invasive prey and additional native prey, coupled with experiments that explore the other classic ecological concept of prey "switching" or "frequency dependent predation" (Murdoch 1969). Prey switching, or lack thereof, has strong implications for the stability of prey populations and the biotic resistance that predators can exert upon invasive species. For example, Cuthbert et al. (2018c) assessed predation by the native amphipod *G. duebeni celticus* upon native mayfly larvae *Baetis rhodani* and invasive *C. pseudogracilis*. They noted similar Type II destabilising functional responses upon both prey species when offered separately. However, when both prey species were offered simultaneously, the predator did not exhibit prey switching, and instead consumed disproportionately less of the invader, indicating a lack of biotic resistance. This matches field patterns, where the invader successfully colonises diverse communities, counter to the idea that high community diversity leads to high biotic resistance (Howeth 2017, Cuthbert et al. 2018c).

RIP and the effect of evolution on invasive species impact

There is a notable lack of evolutionary theory for invasive species (Colautti and Lau 2015), but RIP could determine the drivers and consequences of adaptive evolution and thus help long-term decision-making. Siemann and Rogers (2001) highlighted that invasive species in receiving environments may be different from those in native ranges. In addition, Shine (2012) highlighted that differences in traits of a species can be evident when comparing the invasion front and long-colonised areas. Alleles coding for enhanced dispersal, aggression and rapid resource consumption are likely to accumulate within the invasion front, while alleles coding for slower dispersal will be confined in the long-colonised areas (Phillips et al. 2006, Shine 2012). Thus the dispersal process and selection may lead to differences in behaviour that increase impact on native species at invasion fronts (the Invasion Front Hypothesis: Iacarella et al. 2015b). In support of this hypothesis, front line *Hemimysis anomala* have higher attack rates (Iacarella et al. 2015b) and front line *Orconectes limosus* have greater clutch sizes (Parvulescu et al. 2015). Comparing front line and long-established populations with RIP could thus improve invasive species risk assessments by explicitly incorporating spatio-temporal variation in impact. We therefore propose:

$$\mathrm{RIP} = \left(\frac{\text{FR of frontline pop.}}{\text{FR of long established pop.}}\right) \times \left(\frac{\text{AB of frontline pop.}}{\text{AB of long established pop.}}\right) \tag{12}$$

There are, however, a very limited number of studies of functional and numerical response changes with range expansion, and we can only encourage collection of data to populate Eq. 12 to test these ideas. We discuss other aspects of RIP in spatio-temporal contexts below.

Finally, the use of RIP in the evolutionary context could assist with a still relatively untested conservation technique, genetic backburning (Phillips et al. 2016). This involves moving long-established individuals ahead of the front line individuals, and slowing the progression of the invasion. RIP could test the ecological outcomes of such efforts.

Understanding and predicting competition with RIP

Interspecific competition can reduce the abundances of interacting species and drive species exclusions and coexistence (Connell 1961, Tilman 1977, Schoener 1983, Oyugi et al. 2012). The patterns of resource use by, and the population densities of, interacting species determine these outcomes. Hence RIP, by capturing both, could help to elucidate the role of competition in invasions. Tilman (1977) explicitly included the role of "functional resource-utilisation responses" in determining interspecific competition "winners" and "losers", a concept very close to the "functional response" as described by Holling and others. However, Tilman's examples were from plants and referred to competition over abiotic resources (e.g. Tilman 1977, 1982, 2004), while Holling and subsequent animal-focused researchers have viewed functional responses as determining impacts on living resources, especially prey (e.g. Holling 1959, 1966, Schoener 1974). While functional responses are implicit in competition theory (e.g. Abrams 1980), their routine measurement as determinants of interspecific competition outcomes is lacking in the literature, especially for animals. We contend this is due to what we call the "Competition Spectrum" (Fig. 6), whereby the role of differential use of shared and limiting resources in driving interspecific competition varies across trophic and taxonomic groups. In particular, plants compete for resources that are discrete, unique in the sense of not having equivalents (such as nitrogen), meaning that competing species cannot switch to analogous resources (Fig. 6). At the other extreme, a generalist predator has many potential prey items of some energetic and nutritional equivalence, and can switch between prey analogues, such that reduction of one prey item by a competitor (e.g. invader) could lead to little or no effect on interspecific competition (Fig. 6). Further, motility to find and utilise alternative resources increases from left to right in Fig. 6, thus decreasing the utility of functional responses in elucidating competition (i.e. potential competitors can reduce overlap in time and space). Along this spectrum lie, for example, filter feeders that can only utilise certain species/sizes of resource, with limited switching, and specialist predators that can at least move to new resource-rich areas and have some switching opportunities.

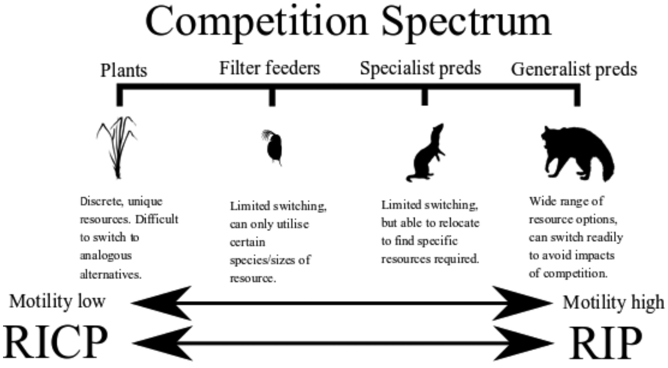

Figure 6. The Competition Spectrum, outlining how differential use of shared and limiting resources drives interspecific competition, with outcomes varying across trophic and taxonomic groups. For example, plants compete for resources lacking equivalents, preventing competing species from switching to analogous resources, while generalist predators have many relatively equivalent potential prey items and the reduction of one prey item by an invader could lead to little or no effect on interspecific competition. We propose that RIP (Relative Impact Potential) will be most useful towards the right, whereas the same metric might better be named RICP (Relative Inter-specific Competitive Potential) to the left.

This spectrum perhaps explains why animal ecologists have simply not used functional responses in competition studies, while plant ecologists have done so for decades (see also Dick et al. 2017a). On the other hand, plant ecologists have not embraced the use of functional responses to explain and predict the identities of invader plants (but see resource use efficiency concept of Funk and Vitousek 2007); however, as pointed out by Dick (2017a, c), damaging invader plants may be identifiable from their higher maximum "feeding rates" (i.e. nutrient and other non-living resource uptake rates) compared to natives (e.g. Rossiter-Rachor et al. 2009). We propose that RIP as originally developed to assess impact on prey populations will be most useful towards the right of Fig. 6, whereas the same metric might better be named the Relative Inter-specific Competitive Potential to the left of Fig. 6. Thus, for example, we could assess the Relative Inter-specific Competitive Potential (RICP) as:

$$\mathrm{RICP} = \left(\frac{\mathrm{FRplant\ 1}}{\mathrm{FRplant\ 2}}\right) \times \left(\frac{\mathrm{ABplant\ 1}}{\mathrm{ABplant\ 2}}\right) \tag{13}$$

For example, using the uptake rates of NH_4^+ of two grass species, the invasive *Andropogon gayanus* and the native *Eriachne triseta* (Rossiter-Rachor et al. 2009), and taking their respective abundance data from Parr (2010), we find the Relative Inter-specific Competitive Potential as:

$$\mathrm{RICP} = \left(\frac{\mathrm{FR}\ A.\ gayanus}{\mathrm{FR}\ E.\ triseta}\right) \times \left(\frac{\mathrm{AB}\ A.\ gayanus}{\mathrm{AB}\ E.\ triseta}\right) = \left(\frac{11.6}{4.5}\right) \times \left(\frac{38.3}{5.1}\right) = 19.36$$

This large RICP value is congruent with the much greater general impact of the invasive species than the native analogue, particularly in terms of out-competing native plants.

Alternatively, it may be that the less commonly used functional response metric of attack rate offers greater insights into competition, since this captures the ability to effectively consume resources at low resource densities, reflective of Tilman's R* theory (Tilman 1982). Thus, calculating RIP with attack rates and abundances may better predict the degree of competition between species. We thus propose that Relative Inter-specific Competitive Potential could unify the plant-animal dichotomy in invasion science.

Investigating spatio-temporal patterns of invasion using RIP

RIP as originally formulated assumed complete replacement of the native by the invader, for example, the invasion of *G. pulex* leading to the replacement of *G. duebeni celticus* by intraguild predation (Kelly et al. 2006). However, there are often lag phases between invasive species arriving and exerting impact (Coutts et al. 2018), with further

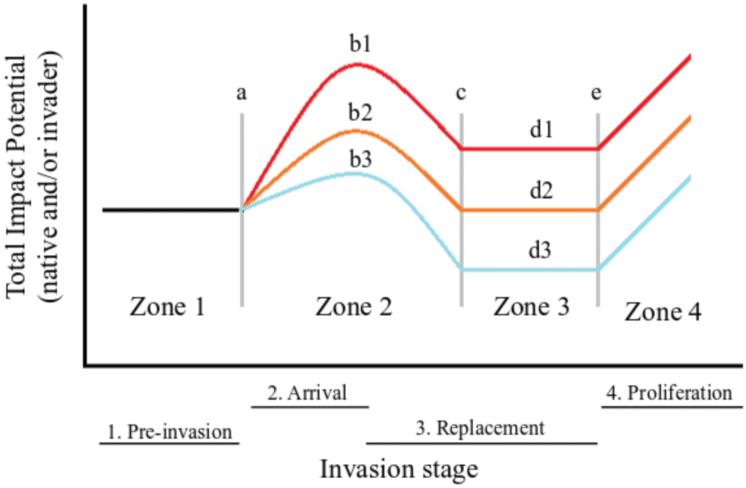

Figure 7. Conceptual spatio-temporal patterns of invasion impact across four invasion stages. In Zone 1, the "Pre-invasion" baseline impact is driven by the native species before the invader arrives, and at point "a" the invasion takes place. In Zone 2, additional impact is exerted by the "Arrival" of the invader, that is, impact is driven by invader and native combined, up to a temporary impact peak, which might vary in magnitude, denoted "b1–b3" in Zone 2. Following these peaks, impact declines as the invader replaces the native, with the point of complete "Replacement" denoted "c". In Zone 3, with only the invader now present, the impact level may remain higher than the native species baseline. Further, in Zone 4, after point "e", "Proliferation" of the invader may occur with consequent heightened impact. This scheme does not assume all stages will occur (e.g. partial replacement may persist) but outlines all likely scenarios.

time before total (or partial) species replacement. Hence, we outline four main zones in the fluctuation of invasive species impact potential over the course of an invasion (Fig. 7). In Zone 1 of Fig. 7, the "Pre-invasion" baseline impact is driven solely by the native species, and at point "a" the invasion takes place. In Zone 2, additional impact is exerted by the "Arrival" of the invader, up to a temporary impact peak, which might vary in magnitude, denoted "b1-b3" in Zone 2. Following these peaks, impact declines as the invader replaces the native, with the point of complete "Replacement" denoted "c". In Zone 3, with only the invader now present, the impact level may be higher ("d1"), or similar to ("d2"), or lower than ("d3") the native species baseline. Further, in Zone 4, after point "e", "Proliferation" of the invader may occur with consequent heightened impact. We can quantify the changes in total impact (Zone 2, 3 or 4) against the Zone 1 baseline, giving us Relative Total Impact Potential (RTIP). This is calculated by dividing the total impact potential of the invader and trophically analogous native species by the Pre-invasion baseline impact:

$$\mathrm{RTIP} = \left(\frac{(\mathrm{FRnative} \times \mathrm{ABnative}) + (\mathrm{FRinvader} \times \mathrm{ABinvader})}{(\mathrm{FRnative} \times \mathrm{ABnative})} \right) \tag{14}$$

In a hypothetical example:
Zone 2 (point b1, native + invader)

$$\mathrm{RTIP} = \left(\frac{(10 \times 100) + (20 \times 200)}{(10 \times 100)} \right) = 5$$

An alternative scenario could result from one-sided intraguild predation, whereby the invader consumes the native and converts native abundance into its own. In this situation, the presence of the native species may lead to a greater abundance of invader than if the native had been extirpated:

$$\mathrm{RTIP} = \left(\frac{(10 \times 80) + (20 \times 250)}{(10 \times 100)} \right) = 5.8$$

Most studies fail to account for these potential changing impacts of an invader over time, and while there is a need to study the often acute initial effects of the invader, subsequent effects also need focus (Strayer et al. 2006).

RIP application to biological control

Biocontrol agent selection targeting native or invader pests has commonly examined the functional responses of agents toward target organisms (Van Driesche and Bellows 2011, Cuthbert et al. 2018a,b). However, assessments of functional responses alone have frequently failed to forecast or explain biocontrol agent success in the field due to omission of context dependencies and a disregard for associated numerical responses of agents (Fernández-Arhex and Corley 2003). The application of RIP offers a holistic metric to assess and predict the comparative potential impact of biocontrol agents, denoted Relative Control Potential (Cuthbert et al. 2018b,d). The coupling of *per capita* effects (i.e. functional response) and proxies such as field abundance or fecundity estimates in the Relative Control Potential metric facilitates a rapid assessment of agent potential. Levels of uncertainty can also be projected using the pdf approach (see above), with biplots further enabling the clear illustration of comparative impact potential (Cuthbert et al 2018a,b,c). Moreover, Relative Control Potential can compare the impact of both native and non-native biocontrol agents under differing environmental contexts, reducing the potential for harmful effects commonly associated with 'classical' biocontrol agent releases (Simberloff and Stiling 1996). Non-native biocontrol agents are often unreliable and ecologically damaging (e.g. Azevedo-Santos et al. 2016), yet native analogues that exert similar impact levels may be available and should be preferentially selected. Relative Control Potential (Cuthbert et al. 2018b) is thus proposed as:

$$\mathrm{RCP} = \left(\frac{\text{FR of agent A}}{\text{FR of agent B}} \right) \times \left(\frac{\text{NR proxy of agent A}}{\text{NR proxy of agent B}} \right) \tag{15}$$

Proxy selection for this metric can additionally be adjusted to suit the nature of biocontrol in respect to the method of release. Inoculative agent releases that seek to induce self-sustaining populations from a single introduction may be best to incorporate fecundity estimates, whilst temporary, inundative releases may be better suited to apply a proxy such as agent longevity. For example, Cuthbert et al. (2018b) compared the functional responses of two predatory cyclopoid copepods *Macrocyclops albidus* (agent A) and *Megacyclops viridis* (agent B) towards larvae of the West Nile virus vector mosquito *Culex pipiens*. Field abundance data for the two copepods originating from the same site (Tinson and Laybourn-Parry 1986) were integrated alongside attack rate (*a*) estimates from the functional responses, allowing comparison between the two species using RCP (Eq. 15):

$$\mathrm{RCP} = \left(\frac{1.98}{1.77}\right) \times \left(\frac{6727}{562}\right) = 13.39$$

Here, the Relative Control Potential value is substantially above 1, and thus *M. albidus* (agent A) is a much more efficacious agent of target mosquito prey than *M. viridis* (agent B). This corroborates with the demonstrated effectiveness of *M. albidus* in biocontrol applications aiming to reduce mosquito populations (Marten and Reid 2007).

To exemplify the influence of context dependency on biocontrol agent efficacy using Relative Control Potential, Cuthbert et al. (2018b) integrated functional response maximum feeding rates (1/*h*) of the same two copepods (*M. albidus*, agent A; *M. viridis*, agent B) across a temperature gradient (12–20 °C). Fecundity data for the two copepod species across matched temperatures from Laybourn-Parry et al. (1988) were then used to compare agents across temperatures:

$$\mathrm{RCP}_{12} = \left(\frac{15.88}{13.99}\right) \times \left(\frac{7.55}{8.28}\right) = 1.04$$

$$\mathrm{RCP}_{20} = \left(\frac{30.42}{33.25}\right) \times \left(\frac{12.66}{6.70}\right) = 1.73$$

Here, at 12 °C (RCP_{12}), efficacies between agent A and agent B are relatively similar; however, as temperature increases to 20 °C (RCP_{20}), differential efficacies in favour of agent A emerge. Thus, environmental context dependencies which alter the efficacy of biocontrol agents can be explicitly integrated into the Relative Control Potential metric.

Future challenges

The Relative Impact Potential (RIP) metric addresses the lack of consistent quantification and representation of "ecological impact" in invasion ecology. Indeed, research has often focused on only one of the three components of the Parker-Lonsdale equation (Parker et al. 1999), and as a result ignored the "total response". What RIP offers is a standardised, user-friendly means of quickly calculating the impacts of established invaders, potential invaders, relocated natives, and pests, relative to each other and trophically analogous equivalents. There is potential to account for a range of abiotic and biotic conditions over spatio-temporal scales, and to study the vast suite of mechanistic hypotheses within the invasion ecology literature. A challenge now is to groundtruth these metrics with real world examples, such as the positive relationship found between RIP and actual field impacts shown by Dick et al. (2017c).

We also recognise that RIP has to this point assumed linearity by assessing impact as the product of *per capita* effects and the numerical response (or proxy). We have hence assumed intraspecific interactions are neutral, rather than antagonistic or synergistic. We also note similarities with the "Density-Impact curve", which assesses non-linear effects of invasive species abundance with economic impact (Yokomizo et al. 2009). Currently, there are conflicting theories on whether antagonistic or synergistic interactions best facilitate invasion spread. For example, aggression towards conspecifics is thought to facilitate spread (e.g. aggressive individuals inhabiting the range frontier: Groen et al. 2012), while a lack of aggression towards conspecifics may facilitate coexistence in high densities in the invaded range (e.g. Argentine ant, *Linepithema humile*: Suarez et al. 1999). Calls for "bivariate FR approaches", i.e. functional response experiments with differing numbers of predators as well as prey, have thus been made (Médoc et al. 2013). We thus recognise that functional responses as derived from multiple predator experiments, revealing neutral, antagonistic or synergistic effects, must be conducted and such data incorporated into RIP metrics.

Until now, quantitative evaluations of impact have not been satisfactorily included in risk assessments (Blackburn et al. 2014, Dick et al. 2014). For example, Gallardo et al. (2016) required that scores be assigned based on the likelihood and magnitude of ecological impact. Uncertainty amongst assessors meant there were large standard deviations for invasive impact evaluation scores, suggesting a need for a more objective system. Blackburn et al. (2014) also encountered issues, presenting a risk assessment classification scheme heavily reliant on assessing the impact of invaders based on invasion history, which will be increasingly unavailable with new invasions. RIP offers a quantifiable measure of impact that avoids these pitfalls and removes the subjectivity inherent in horizon scans. By comparing non-native species to trophically analogous natives, the most potentially impactful invaders could be prioritised by RIP, and the addition of proxies for propagule pressure could highlight overall risk (Dickey et al. 2018). While certain impacts would not be covered by RIP, such as hybridisation, spread of disease and bio-fouling, expert opinion would still be required, allowing a rounded description of impact built upon quantitative foundations. RIP and its other derived metrics above thus require new data across a range of taxonomic and trophic groups, necessitating new and imaginative data collection methods (see also Dick et al. 2014). By providing a user-friendly method of calculating impact, as well as offering succinct, intuitive means of displaying the results (e.g. via biplots, Laverty et al. 2017a, and triplots, Dickey et al. 2018), we also propose that RIP could go some way towards closing the knowledge gap between scientists and managers/practitioners, readily informing prioritisation and control (Matzek et al. 2014).

Currently, successful implementation of RIP for real-world decision making is constrained by the lack of data on functional and numerical responses and their proxies. However, with university research laboratories and dedicated research facilities worldwide (e.g. CABI), and databases such as FoRAGE (Functional Responses from Around the Globe in all Ecosystems), there are growing opportunities to compile functional and numerical response data across a wide range of taxa, trophic levels and ecosystems (Dick et al. 2017a). This should lead to a readily accessible capacity to aid policy decisions and intervention. Once in place, RIP metrics offer considerable promise for aiding the management of invasive species and pests, and we call for their usage as a vital component of risk assessments and horizon scans, thus facilitating the assessment and prioritisation of invaders as required by EU legislation and global biodiversity targets.

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Supplementary material 1

Table S1. Outline of different numerical response proxies available, guidance for their use and the advantages and disadvantages of each

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RESEARCH ARTICLE

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The impact is in the details: evaluating a standardized protocol and scale for determining non-native insect impact

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Abstract

Assessing the ecological and economic impacts of non-native species is crucial to providing managers and policymakers with the information necessary to respond effectively. Most non-native species have minimal impacts on the environment in which they are introduced, but a small fraction are highly deleterious. The definition of 'damaging' or 'high-impact' varies based on the factors determined to be valuable by an individual or group, but interpretations of whether non-native species meet particular definitions can be influenced by the interpreter's bias or level of expertise, or lack of group consensus. Uncertainty or disagreement about an impact classification may delay or otherwise adversely affect policymaking on management strategies. One way to prevent these issues would be to have a detailed, nine-point impact scale that would leave little room for interpretation and then divide the scale into agreed upon categories, such as low, medium, and high impact. Following a previously conducted, exhaustive search regarding non-native, conifer-specialist insects, the authors independently read the same sources and scored the impact of 41 conifer-specialist insects to determine if any variation among assessors existed when using a detailed impact scale. Each of the authors, who were selected to participate in the working group associated with this study because of their diverse backgrounds, also provided their level of expertise and uncertainty for each insect evaluated. We observed 85% congruence in impact rating among assessors, with 27% of the insects having perfect inter-rater agreement. Variance in assessment peaked in insects with a moderate impact level, perhaps due to ambiguous information or prior assessor perceptions of these specific insect species. The authors also participated in a joint fact-finding discussion of two insects with the most divergent impact scores to isolate potential sources of variation in assessor impact scores. We identified four themes that could be experienced by impact assessors: ambiguous information, discounted details, observed versus potential impact, and prior knowledge. To improve consistency in impact decision-making, we encourage groups to establish a detailed scale that would allow all observed and published impacts to fall under a particular score, provide clear, reproducible guidelines and training, and use consensus-building techniques when necessary.

Keywords

environmental impact, expert opinion, impact assessment, joint fact-finding, non-native species management, policy-making, uncertainty

Introduction

Globally, anthropogenic, abiotic, and biotic threats increasingly affect the structure and function of forest ecosystems (Millar and Stephenson 2015). Of these threats, non-native species may cause considerable changes to the environments in which they are introduced, including ecological, economic, social, and cultural impacts (Jeschke et al. 2014). These impacts can be viewed as negative when there are undesirable effects or positive when they provide beneficial ecosystem services or economic value (Schlaepfer et al. 2011; Kumschick et al. 2012). Frequently, impacts must be assessed in the absence of sufficient published or otherwise available empirical data (Murray et al. 2009). One approach for estimating impact when empirical information is sparse (e.g., impacts on unclassified ecosystem services; Roy et al. 2018) is through surveys of expert opinion that consider the 'wisdom of the crowd' (e.g., observations, unpublished or preliminary datasets; Aspinall 2010; Gale et al. 2010; Thompson et al. 2013; Roy et al. 2014). However, it remains unclear how reliable expert opinion is.

In particular, consensus among experts may be difficult to achieve (Giannetti et al. 2009; Humair et al. 2014; González-Moreno et al. 2019). Further difficulty may occur when stakeholder groups and experts have different perspectives regarding the impact of non-native species. Disagreements and uncertainty among expert assessors, and between stakeholders and experts, may affect decision-making and resource allocation (Kumschick et al. 2012; Van Der Wal et al. 2015; Kumschick et al. 2015). For example, decision-makers may use information that is not necessarily based on taxon-specific scientific evidence, but rather broad ecological principles based on legal or regulatory considerations found in procedural manuals and technical guides developed by regulatory agencies (Fleischman and Briske 2016). This lack of taxon-specific, science-based evidence in the decision-making process may complicate the development and implementation of effective biosecurity policies, including surveillance and intervention strategies (Green et al. 2015).

Although disagreements may arise, impact assessments perform a crucial role in biosecurity programs for management of non-native species (Perrings et al. 2005; Hulme 2011). Many scales and assessment protocols have been developed to assess the impacts of non-native species on local or regional economies and societies. While new protocols, such as the INvasive Species Effects Assessment Tool (INSEAT; Martinez-Cillero et al. 2019), are being developed, some researchers are now evaluating the efficiency and efficacy of other long-standing impact assessment protocols to develop more robust, accurate, and consistent protocols. For example, González-Moreno et al. (2019) summarized and evaluated consistency in 11 commonly used protocols developed and applied in Europe, and found considerable inconsistency among assessors. Difficulties in creating and utilizing these standardized scoring systems and impact assessment protocols may include: 1) disagreement in how impact should be evaluated; 2) differences among the diverse array of introduced species and their typical and maximum impacts; 3) the extent to which species are broadly distributed versus limited to cultivated systems; and 4) differential impacts for unclassified ecosystem services and various socio-economic sectors (Humair et al. 2014; Roy et al. 2018). Consequently, experts often do not provide consistently defined impacts of studied organisms (Jeschke et al. 2014).

To help remedy inconsistency and disagreement among assessors, standard impact scoring systems (Kumschick et al. 2012; Blackburn et al. 2014; Roy et al. 2018) with seven to ten-points are suggested because they are more reliable and better measure an assessor's true evaluation (Preston and Colman 2000). Some impact scoring systems and assessment protocols have been developed in a way that can only be used by assessors with a high level of expertise as they require specialized knowledge about the species in question (González-Moreno et al. 2019). Other researchers argue that a diverse group of experts with broader knowledge should complete the assessments (e.g., Murray et al. 2009; Hemming et al. 2018a,b) to achieve accurate and consistent decisions. Additionally, structured protocols can help reduce biases and improve accuracy and transparency, and discussions can help resolve disagreements (Hemming et al. 2018a,b; González-Moreno et al. 2019; Osunkoya et al. 2019). This method of resolving conflicting assessments by allowing the assessors to openly discuss available data and the research used to draw conclusions is known as joint fact-finding (Matsuura and Schenk 2016). Even with disagreements, the aggregated scores of a group tend to be closer to the true value than the score provided by any individual within the group (Roy et al. 2014).

Impact scores were recently used to categorize non-native forest insects that specialize on conifers (Mech et al. 2019a). During this project, a group of scientists (the "High-Impact Insect Invasion" working group; HIWG) collaborated to create a detailed nine-point scale of impact, but only one assessor was responsible for determining the impact score for the 58 non-native conifer-specialists currently in North America. These scores were eventually used as the basis for a statistical model that will be used to predict the impact of non-native conifer-specialists that have not yet become established in North America (Mech et al. 2019a). The purpose of our study was to evaluate whether the impact scale used in Mech et al. (2019a) is detailed enough for multiple people with different levels of expertise to reach the same impact score. We examined how level of expertise, uncertainty, and disagreement may affect impact assessment of non-native conifer-specialist insect species. Specifically, the objectives of the study were to: 1) evaluate the level of consensus among individual assessments of non-native insect impacts; 2) measure correlation among level of prior expertise, impact score, and assessor level of uncertainty; 3) assess the points of agreement and disagreement to determine which types of insects are the most difficult to assess with consensus; and 4) explore how experts can use joint fact-finding, a form of consensus-building, to identify sources of highly divergent impact scores and achieve consensus in decision-making using a case study of two insect species with highly divergent impact scores.

Materials and methods

Assessor group

In 2016, the HIWG, composed mainly of the co-authors of this paper, convened to examine the drivers of non-native insect invasions (Mech et al. 2019a) and develop a model to predict future high-impact, non-native, phytophagous insect species in natural ecosystems in North America. The group of scientists had different specialties (Suppl. material 1: Table S1) and diverse backgrounds (e.g., ethnic, cultural, age, stage of scientific career), with many having long-standing research experience in invasion ecology. Fifteen members of the 2016 HIWG participated in this project to determine whether the impact scores used in the analyses would be the same regardless of which working group member conducted the assessment.

Impact scoring system

The HIWG designed an original nine-point scale (Fig. 1) to classify the impacts of non-native insects already in North America (Mech et al. 2019a). We designed an original

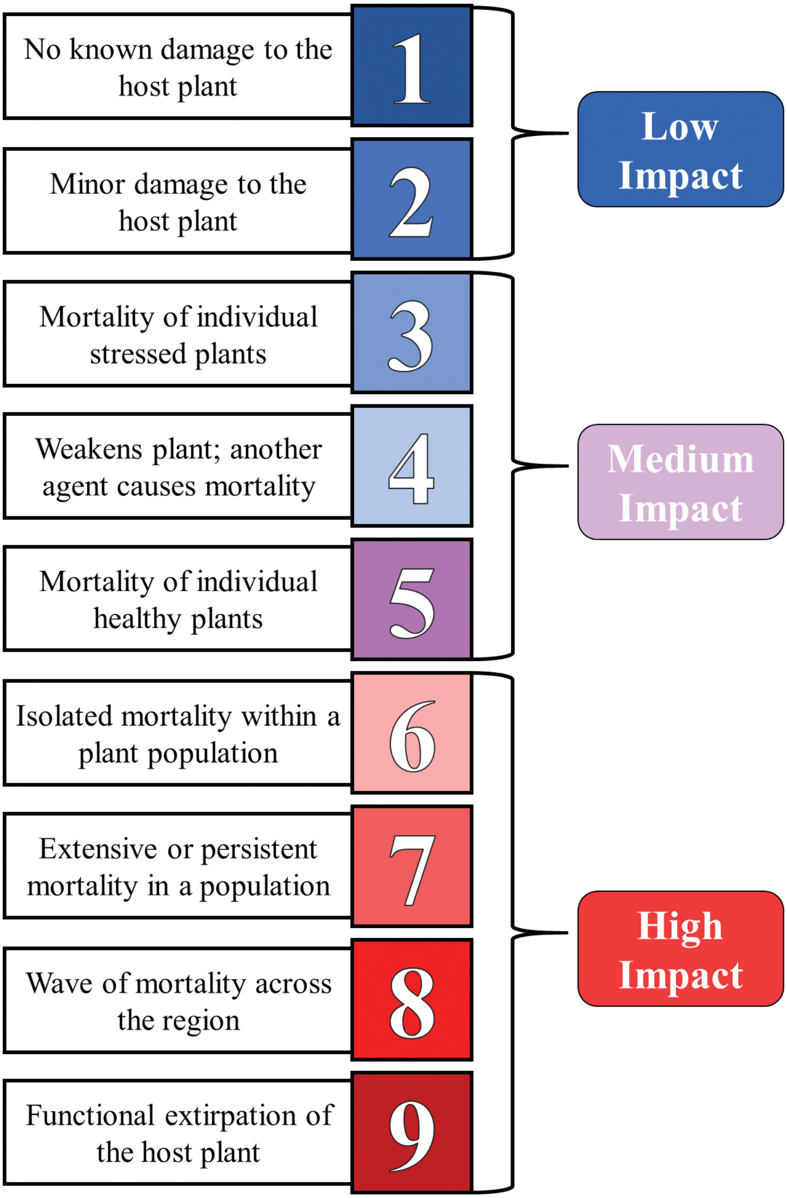

Figure 1. Impact scoring system (1–9) established by the HIWG for non-native, phytophagous insects, where 1 indicates no damage and 9 indicates severe damage (also found in Mech et al. 2019a).

scale because other impact scales were considered too general (e.g., EPPO-EIA, which addresses impacts of non-native plants and invertebrates overall), too specific (e.g., only addresses species within a particular feeding guild or region), or too complex (e.g., Kumschick et al. 2015, the generic impact scoring system) for the primary purposes of the project (i.e., Mech et al. 2019a). Our original impact scale ranged from 1–9, with one being the lowest and nine being the highest possible impact (Fig. 1). The HIWG determined that insects in levels 1–2 can be considered low impact species on a ternary impact scale (i.e., low, medium, or high), since they have no or minor (e.g., leaf or needle loss, foliage discoloration, twig dieback, cone drop) documented damage to their host plant. Insects in levels 3–5 can be considered medium impact species, since they cause mortality to individual host plants, and insects in levels 6–9 can be classified as high-impact because they cause mortality within a population of host plants (Fig. 1). The details in this scale were included with the goal that any description of impact in the literature would be able to fall under one of these scores (i.e., little need for interpretation).

Impact assessment

The HIWG initiated their research by conducting a pilot study on the 58 non-native, conifer-specialist insect species (i.e., restricted to feeding on one or more of the three conifer families in North America: Cupressaceae, Pinaceae, and Taxaceae) currently in North America (Mech et al. 2019a; Suppl. material 1: Table S2). For each non-native insect included in Mech et al. (2019a), one initial assessor conducted a comprehensive search of the peer-reviewed and gray literature (e.g., university and federal government websites, other credible online resources) to find any and all descriptions of impact. Gray literature was only referenced when publications were lacking, which typically occurred with insects that caused little to no damage. For each insect included in the study, the assessor identified the highest impact the insect had on trees native to North America. This information on the highest observed impact was used to determine impact score for each insect, and was used to create the models developed in Mech et al. (2019a).

For this study, we were interested in evaluating the impact scale used in Mech et al. (2019a), so we also focused on non-native, conifer-specialist insects in North America. For each conifer-specialist insect, assessors were provided with the list of references that described the host damage used to determine the impact scores used in Mech et al. (2019a). Of the 58 conifer-specialist insects that were originally identified in the pilot study, 17 insect species were excluded from our study because they received an impact score of one. This meant there was no documented damage and, therefore, no references were provided. The remaining 41 conifer-specialist insects (Suppl. material 1: Table S2; Fig. 2) were randomly assigned to three new assessors for impact scoring. In total, each insect was assessed by four assessors, including the original assessor who assessed the impacts for Mech et al. (2019a). The HIWG provided a diverse group to participate in the assessment (as suggested in Turbé et al. 2017 and Hemming et al. 2018a, b).

For each insect, the three new assessors were provided the same list of references as the initial assessor. The new assessors did not have access to the impact score assigned by the initial assessor to avoid bias. The references provided for each insect were mostly exhaustive, but for well-studied species (e.g., hemlock woolly adelgid [*Adelges tsugae* Annand]), references that were representative of the damage repeatedly found in published articles were selected in lieu of providing all impact literature. No publications

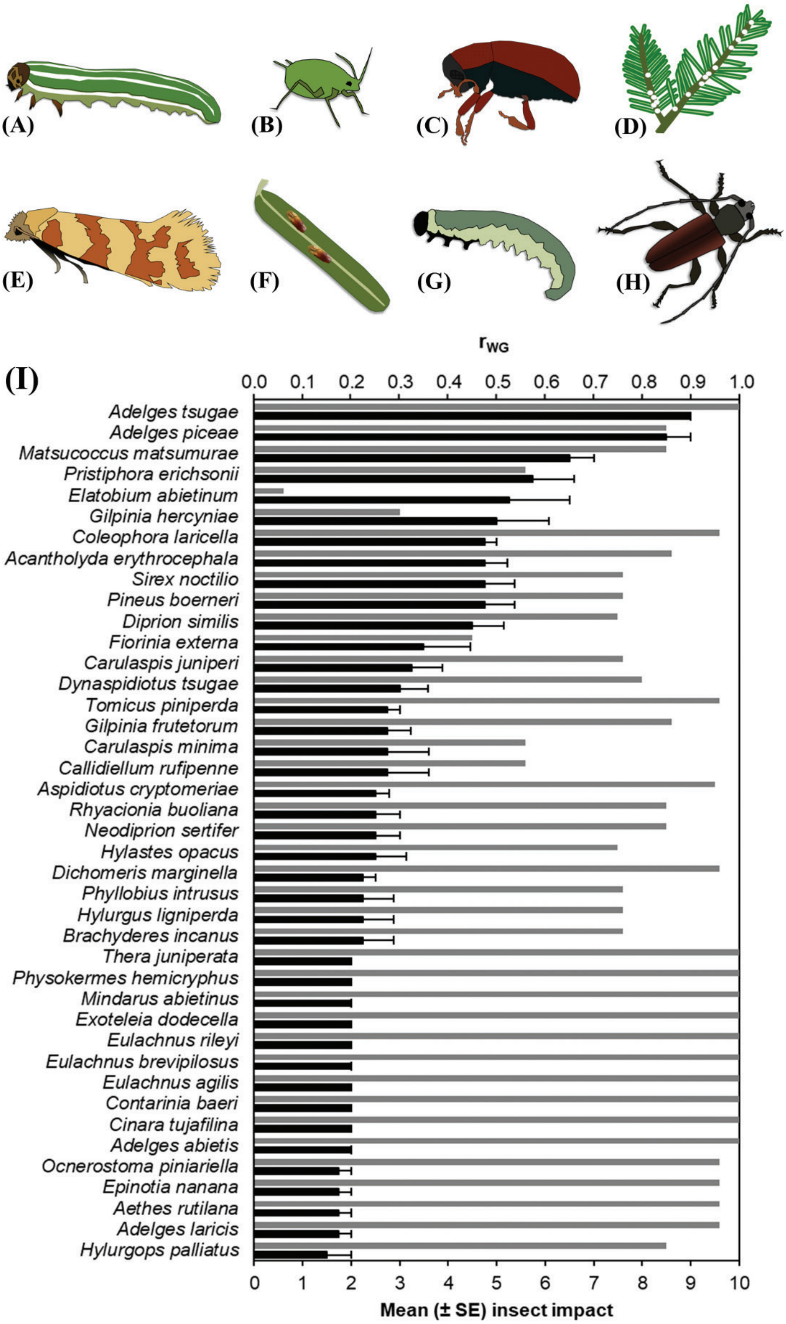

Figure 2. Examples of non-native, conifer-specialist insects, including (**A**) European spruce sawfly (*Gilpinia hercyniae*), (**B**) spruce needle aphid (*Elatobium abietinum*), (**C**) lesser spruce shoot beetle (*Hylurgops palliatus*), (**D**) hemlock woolly adelgid (*Adelges tsugae*), (**E**) pale juniper webworm (*Aethes rutilana*), (**F**) elongate hemlock scale (*Fiorinia externa*), (**G**) larch sawfly (*Pristiphora erichsonii*), and (**H**) Japanese cedar longhorned beetle (*Callidiellum rufipenne*) with the (**I**) mean (± SE) insect impact score (black bars) and within-group interrater agreement index (r_{WG}, gray bars) for all 41 conifer-specialist insects assessed in this study.

or websites, other than the ones provided, could be used by the assessors. Further, assessors were advised to not use their existing knowledge to evaluate impact and base their impact score solely on the information provided in the references.

Prior to completing the impact assessment exercise, assessors were provided with a sample score sheet that was developed by the first author. The score sheet included directions on how to assess impact and self-assign their level of expertise and uncertainty for each insect (Suppl. material 1: Table S3). Assessors were directed to select the highest applicable impact value based on their interpretation of the references. If a reference cited the impact of the insect on a conifer outside of North America, even if the conifer was native to North America, the assessors were instructed to disregard that information and only focus on the impacts that occurred in North America. For each insect, the assessors, including the initial assessor, self-reported their level of expertise on the insect they were assessing (scale of 1–5, from no to high expertise), as well as the level of uncertainty about their impact score decision (scale of 1–5, from low to high uncertainty) (Suppl. material 1: Table S3). During a conference call, assessors were trained to conduct an impact assessment using a sample insect not included in this study, and were given the opportunity to discuss any questions or concerns (approach also implemented by González-Moreno et al. 2019). Once all assessors were trained, score sheets with randomly assigned insects (from the list of 41 conifer-specialist insects; Suppl. material 1: Table S2) were sent to each assessor. Completed score sheets were assessed for completeness and then compiled into one spreadsheet with masked assessor identities.

Statistical analyses

Descriptive statistics were calculated for impact score and assessor levels of expertise and uncertainty for each insect, with all means reported ± 1 SE. A power function analysis was used to determine the required number of assessments per species. To evaluate the overall level of consensus among assessors, we calculated Krippendorff's alpha (Kα), a coefficient used to measure agreement among observers (Krippendorff 2017). To calculate Kα, we used the *kripp.alpha* function in the IRR (Interrater Reliability) package in R v.3.4.0 (R Core Team 2017; Gamer et al. 2012). Kα ranges from 0 to 1, with higher values indicating stronger agreement. In general, any values above 0.70 are thought to indicate high agreement (LeBreton and Senter 2008). To quantify agreement among the ordinal impact scores for each insect, we used the within-group inter-rater agreement index of r_{WG},

$$r_{WG} = 1 - \frac{s_X^2}{\sigma_E^2},$$

where s_x^2 is the observed variance among the impact scores from the four assessors, and σ_E^2 is the expected variance in the case of no consensus among assessors (LeBreton and Senter 2008).

When assessors are in perfect agreement, the index r_{WG} equals one, and any disagreement will cause the r_{WG} index to approach zero. Like Kα, $r_{WG} = 0.70$ is the traditionally accepted threshold that demarcates high versus low assessor agreement, whereby any values ≥ 0.70 indicate high agreement among assessors (LeBreton and Senter 2008). We used r_{WG} values to determine which insects were the most difficult to assess.

Spearman's rank correlation tests were conducted to measure the correlations between assessor levels of expertise and uncertainty. To measure whether expertise and uncertainty influence assignment of impact scores, we calculated the coefficients of variation for insect impact score, level of expertise, and level of uncertainty using the four assessor scores and ratings for each insect. We then conducted Spearman's rank correlation tests using the coefficients of variation for level of expertise and impact score and level of uncertainty and impact score, respectively.

Joint fact-finding meeting

Following the completion and compilation of all assessments, assessors met in person for a joint fact-finding session in August 2017 to identify potential sources of variation for insects with highly divergent impact scores. For our joint fact-finding discussion (Matsuura and Schenk 2016), we selected two conifer-specialist insects with the most divergent impact scores (i.e., lowest r_{WG} values): European spruce sawfly (*Gilpinia hercyniae* Hartig; Fig. 2A) and spruce needle aphid (*Elatobium abietinum* Walker; Fig. 2B). Since only four assessors evaluated these insects, references for the two species were provided to the group to read in preparation for the discussion. During this meeting, members reflected on the variance among impact scores for both insects and identified potential sources of uncertainty in the assessment of these insects.

Results

Mean impact scores ranged from 1.5 ± 0.5 for lesser spruce shoot beetle (*Hylurgops palliatus* Gyllenhal; Fig. 2C) to 9.0 ± 0.0 for hemlock woolly adelgid (Fig. 2D) (Table 1; Fig. 2). Although we removed 17 species that had an impact score of one (i.e., no documented damage) before the assessment, 12 of the remaining 41 insects that were evaluated had at least one assessor who scored the impact level as one. As a result, five insects (e.g., pale juniper webworm [*Aethes rutilana* Hübner; Fig. 2E]), had a mean impact score < 2. The coefficient of variation for impact score ranged from 0 to 67%, with 11 insects (27% of the insect species evaluated) having no variation in assessed impact scores (Fig. 2I; Fig. 3). The coefficient of variation peaked for insects with medium impact (levels 3–6), with less variation in extreme impact scores (i.e., high or low impact). We determined that, with four assessments per species, differences were readily evident among the 41 insects ($F_{40,123} = 11.49$, $P < 0.0001$), and SE for species-specific estimates was approximately 0.53 on the nine-point scale of impact (Suppl. material 1: Fig. S1). The 95% CI with four assessors was ± 1.69 units on the nine-point scale.

Table 1. Summary of descriptive statistics (mean ± SE) for the self-assessed level of expertise (range of 1–5, in which 1 is no expertise and 5 is high expertise), impact level (range of 1–9, in which 1 is no documented damage and 9 is functional extinction of the host plant), and self-assessed level of uncertainty (scale of 1–5, where 1 is low uncertainty and 5 is high uncertainty) for each insect species assessed in this study.

Conifer-specialist Insect Species	Mean ± SE Expertise	Mean ± SE Impact	Mean ± SE Uncertainty
Acantholyda erythrocephala	1.50 ± 0.50	4.75 ± 0.48	2.75 ± 0.25
Adelges abietis	2.75 ± 0.75	2.00 ± 0.00	1.50 ± 0.29
Adelges laricis	2.75 ± 0.85	1.75 ± 0.25	1.75 ± 0.25
Adelges piceae	3.75 ± 0.95	8.50 ± 0.50	2.00 ± 0.70
Adelges tsugae	4.75 ± 0.25	9.00 ± 0.00	1.50 ± 0.29
Aethes rutilana	2.00 ± 0.41	1.75 ± 0.25	1.75 ± 0.48
Aspidiotus cryptomeriae	2.75 ± 0.48	2.50 ± 0.29	2.00 ± 0.41
Brachyderes incanus	2.00 ± 0.00	2.25 ± 0.63	2.50 ± 0.65
Callidiellum rufipenne	1.50 ± 0.29	2.75 ± 0.85	2.25 ± 0.75
Carulaspis juniperi	2.50 ± 0.87	3.25 ± 0.63	2.50 ± 0.96
Carulaspis minima	1.75 ± 0.25	2.75 ± 0.85	3.00 ± 0.71
Cinara tujafilina	2.25 ± 0.25	2.00 ± 0.00	1.75 ± 0.25
Coleophora laricella	2.00 ± 0.41	4.75 ± 0.25	1.75 ± 0.48
Contarinia baeri	2.00 ± 0.41	2.00 ± 0.00	2.25 ± 0.25
Dichomeris marginella	2.00 ± 0.71	2.25 ± 0.25	2.75 ± 0.75
Diprion similis	2.25 ± 0.63	4.50 ± 0.65	2.50 ± 0.29
Dynaspidiotus tsugae	2.25 ± 0.25	3.00 ± 0.58	3.00 ± 0.71
Elatobium abietinum	2.00 ± 0.41	5.25 ± 1.25	1.75 ± 0.25
Epinotia nanana	1.25 ± 0.25	1.75 ± 0.25	2.25 ± 0.48
Eulachnus agilis	2.00 ± 0.41	2.00 ± 0.00	2.25 ± 0.75
Eulachnus brevipilosus	2.00 ± 0.41	2.00 ± 0.00	2.75 ± 0.85
Eulachnus rileyi	1.75 ± 0.25	2.00 ± 0.00	2.50 ± 0.65
Exoteleia dodecella	2.00 ± 0.41	2.00 ± 0.00	2.25 ± 0.48
Fiorinia externa	2.75 ± 1.03	3.50 ± 0.96	2.75 ± 0.75
Gilpinia frutetorum	2.50 ± 0.50	2.75 ± 0.48	2.25 ± 0.63
Gilpinia hercyniae	2.25 ± 0.48	5.00 ± 1.08	3.00 ± 0.58
Hylastes opacus	2.75 ± 0.75	2.50 ± 0.65	1.75 ± 0.48
Hylurgops palliatus	1.75 ± 0.25	1.50 ± 0.50	1.75 ± 0.48
Hylurgus ligniperda	1.50 ± 0.29	2.25 ± 0.63	2.50 ± 0.29
Matsucoccus matsumurae	2.50 ± 0.65	6.50 ± 0.50	2.75 ± 0.48
Mindarus abietinus	2.00 ± 0.41	2.00 ± 0.00	2.25 ± 0.63
Neodiprion sertifer	2.75 ± 0.85	2.50 ± 0.50	2.00 ± 0.41
Ocnerostoma piniariella	2.00 ± 0.41	1.75 ± 0.25	2.25 ± 0.48
Phyllobius intrusus	2.00 ± 0.41	2.25 ± 0.63	2.25 ± 0.48
Physokermes hemicryphus	2.00 ± 0.41	2.00 ± 0.00	2.25 ± 0.25
Pineus boerneri	3.25 ± 0.85	4.75 ± 0.63	2.50 ± 0.65
Pristiphora erichsonii	2.50 ± 0.87	5.75 ± 0.85	2.00 ± 0.41
Rhyacionia buoliana	3.25 ± 0.48	2.50 ± 0.50	1.75 ± 0.25
Sirex noctilio	2.50 ± 0.96	4.75 ± 0.63	2.00 ± 0.41
Thera juniperata	2.00 ± 0.41	2.00 ± 0.00	2.25 ± 0.48
Tomicus piniperda	3.00 ± 0.71	2.75 ± 0.25	1.50 ± 0.29

The r_{WG} index to assess within-group variation for each species varied from 0.06–1.00, with 85% (35 of 41) of the insects having a $r_{WG} \geq 0.70$ and 27% (11 out of 41) having a $r_{WG} = 1.00$ (Fig. 2I). The 11 species with perfect agreement (those with no variation) had a mean impact of 2, except hemlock woolly adelgid, which had a mean

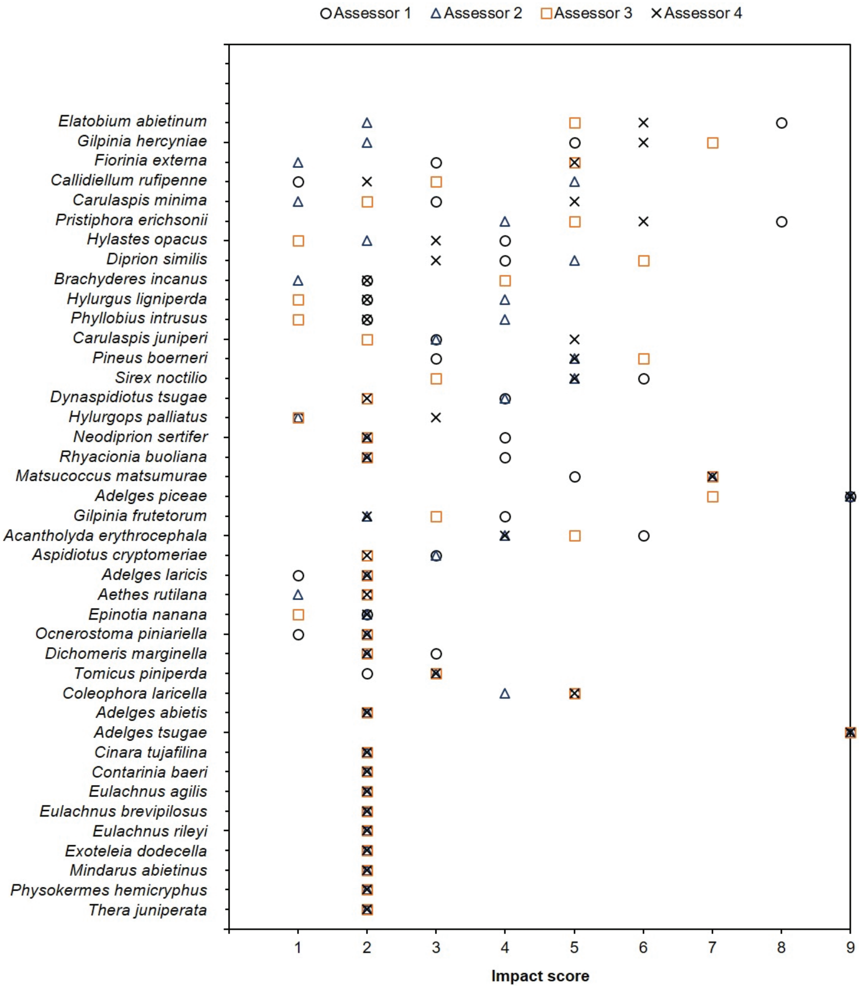

Figure 3. Insect impact scores assigned by each of the four assessors for each insect. Insects with the most disagreement are at the top of the figure, whereas insects with the most consensus are at the bottom of the figure.

impact of 9 (Fig. 3). As with the coefficient of variation, insects with a medium impact tended to exhibit the most divergence in assessed values among experts ($r_{WG} < 0.70$; Fig. 4). The mean impact score of the six species (15% of those in the sample) generating the most disagreement ($r_{WG} < 0.70$) ranged from 2.75–5.75 (Figs 3, 4). These include elongate hemlock scale (*Fiorinia externa* Ferris; Fig. 2F), European spruce sawfly, larch sawfly (*Pristiphora erichsonii* Hartig; Fig. 2G), Japanese cedar longhorned beetle (*Callidiellum rufipenne* Motschulsky; Fig. 2H), minute cypress scale (*Carulaspis minima* Borchsenius), and spruce needle aphid. For all 41 insect impact assessments, $K\alpha$ was 0.55.

The mean self-assessed level of expertise ranged from 1.25 ± 0.25 (novice; no expertise) for European spruce needle miner (*Epinotia nanana* Treitschke) to 4.75 ± 0.25 (expert; high expertise) for hemlock woolly adelgid (Table 1; Suppl. material 1: Fig.

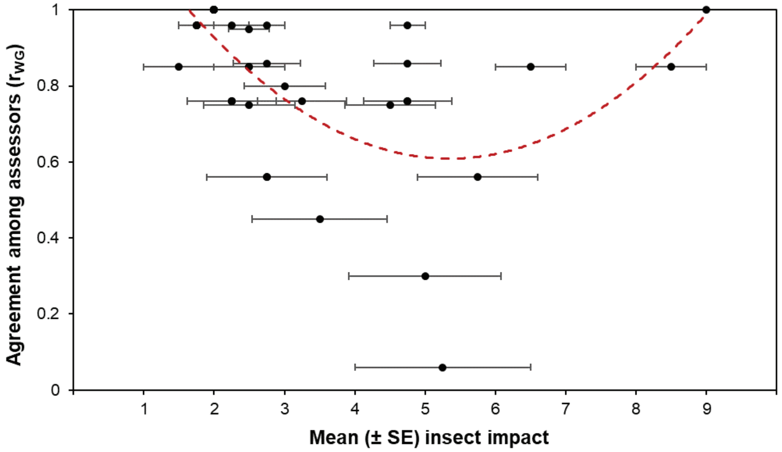

Figure 4. Within-group inter-rater agreement (r_{WG}) values (0–1, with 0 indicating no agreement and 1 indicating perfect agreement) for each mean (± SE) insect impact (1–9, with 1 indicating low impact and 9 indicating high impact insect species) with a trendline shown in red.

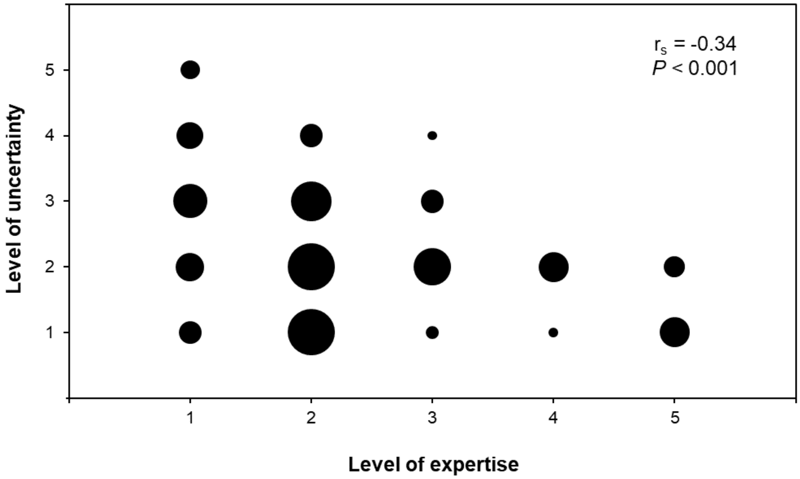

Figure 5. Significant, positive correlation between level of expertise (scale of 1–5, from no to high expertise) and level of uncertainty (scale of 1–5, from low to high uncertainty) with bubbles that are proportional to the number of overlapping data points.

S2). The overall mean level of expertise for all 41 insects that were assessed was 2.3 ± 0.6 (advanced beginner; low expertise). The mean self-assessed level of uncertainty ranged from 1.5 ± 0.3 (no uncertainty) for eastern spruce gall adelgid, European pine shoot borer (*Tomicus piniperda* L.), and hemlock woolly adelgid to 3.0 ± 0.7 (moderate uncertainty) for minute cypress scale and shortneedle conifer scale (*Dynaspidiotus tsugae* Marlatt) (Table 1). The overall mean level of uncertainty for all 41 insect assessments was 2.2 ± 0.5 (low uncertainty). The levels of expertise and uncertainty were

Table 2. Common themes that emerged from the joint fact-finding discussion on variation in non-native, conifer-specialist insect impact scores and reflection on problems that the assessors encountered when making their assessments.

Theme	Description
Ambiguous information	Information in the literature was vague, lacking, incorrect, or unconvincing. Often, very little information was provided on the impacts of generally low impact species. Misinterpretation of the ambiguous information provided in the references may have resulted in an under- or over-estimated impact score.
Discounted details	The assessor unintentionally overlooked details because s/he did not thoroughly read the provided literature. Alternatively, the assessor may have intentionally disregarded details.
Observed vs. potential impact	Some references provided understated or overexaggerated impacts not supported by empirical data or observations. The assessor did not find it acceptable to assign a lower or higher impact when the species had rarely achieved that potential.
Prior knowledge	A more specialized assessor had previous knowledge about the insect. Consequently, s/he had more insight than what was provided in the references and/or disagreed with the content in the references based on personal experiences with the insect.

negatively correlated ($r_s = -0.34$, $P < 0.001$, Fig. 5), whereas the correlations between the coefficients of variation for level of expertise and impact score ($r_s = -0.05$, $P = 0.77$) and level of uncertainty and impact score ($r_s = 0.11$, $P = 0.49$) were not significant.

The joint fact-finding discussion on European spruce sawfly and spruce needle aphid allowed the working group to constructively reflect on the variation in insect impact scores and identify potential sources of uncertainty. The joint fact-finding meeting also provided a forum to discuss problems that assessors encountered when assigning impact scores for other insects included in this study. Four common themes emerged from the discussion: ambiguous information, discounted details, observed vs. potential impact, and prior knowledge (Table 2). The group discussed and resolved divergent impact scores, concluding the meeting with participant agreement that both the European spruce sawfly and spruce needle aphid should be assigned level 6 on the nine-point impact scale.

Discussion

Impact assessment protocols for non-native insect species

For this study, we evaluated the efficacy of a detailed nine-point impact scale (Fig. 1) that was developed to assess impacts of non-native insects in forests. Our decision to only have four assessors score each insect rather than every assessor score each insect was supported by the results of our power function analysis (Suppl. material 1: Fig. S1). Employing four assessments per insect species allowed us to evaluate many species while still having reasonable precision in the species-specific estimates.

We found 11 of the 41 non-native, conifer-specialist insects assessed had perfect agreement among assessors, 24 had a high level of agreement, and only six elicited a low level of agreement. Although the Krippendorff's alpha indicated a moderate level of consensus, the fact that most insects had a high or perfect level of agreement indicated a generally high consensus among assessors. All insects with low agreement among assessors were scored within or on the margin of the medium impact range, whereas the insects with perfect or high agreement among assessors fell near the extremes of their respective impact range. This pattern indicates that divergence in agreement peaked in insects with a medium impact score, perhaps highlighting the challenges associated with determining impact for species that are neither truly benign (low-impact) nor undeniably catastrophic (high-impact). Our use of standardized information may have contributed to this pattern, as this limited the information assessors used to make their assessment. The initial assessor endeavored to select the most comprehensive and accurate references available, but published information can be vague, inaccurate, or misinterpreted. Although we advised assessors to not use their prior knowledge, some assessors had specialized expertise to use when the literature was deficient, while others disagreed with what was written. The joint fact-finding discussions improved understanding and ultimately led to consensus about these medium-impact species. Following the discussions and reassessment, there was no variability in which impact level (low, medium, or high) all 41 insects should be.

This pattern of highly divergent impact scores may also result from intraspecific variation in impact. For this assessment, we considered a taxonomic definition of impact (Colautti and MacIsaac 2004; i.e., a species manifests the same level of impact throughout its invaded region). However, a medium score could reflect regional variation in impact. For example, one population may have natural enemies that limit impact, whereas another population does not. Regional variation in impact score may also reflect differences in stakeholder perceptions, as individuals living in urban areas may perceive impact to be higher, whereas people in rural areas may perceive impact to be lower (Kumschick et al. 2012; Jeschke et al. 2014). Although we advised assessors to select the highest impact score supported by the information in the provided literature, some assessors may have overlooked details about intraspecific variation in impact or assigned an average score that considered the impacts in all of the regions.

Higher variation among medium impact species highlights the importance of having a robust impact scoring system. Although a few impact assessment scoring systems have multiple levels with detailed descriptions from which to choose (e.g., Ricciardi and Cohen 2007; D'hondt et al. 2015; Nentwig et al. 2016), most impact assessment protocols employ an impact scale with three to five levels (e.g., Kenis et al. 2012; Martinez-Cillero et al. 2019). Overall, the generally high level of consensus in our assessment may be attributed in part to our clearly defined impact scoring system.

Assessor expertise and uncertainty

In this study, the overall self-assessed expertise level was low, with most insects eliciting an expertise level below three (moderate expertise). The only species that elicited a moderate-high to high self-assessed expertise (> level 3 on the expertise scale) were high impact species: balsam woolly adelgid (*Adelges piceae* Ratzeburg), hemlock woolly adelgid, and pine woolly aphid (*Pineus boerneri* Annand). In a pool of assessors, one would expect to have more assessors with expertise on high- than low-impact insect species because high-impact species generate more research funding and publicity in the academic community (e.g., more peer-reviewed publications) and the general public (e.g., more outreach and awareness efforts) than low-impact species. All three species are high-profile insects with widespread documentation, research, and public reporting, such that even non-specialist scientists may be acquainted enough with these species to rate their expertise level as high. High self-assessed levels of expertise might also be elicited from other high-impact species not included in this study.

Uncertainty is often of concern when assessing impact. It is important for assessors to consider the available information and determine the potential impact that the non-native species has or will have with accuracy and consistency to efficiently allocate resources to management and biosecurity strategies (Andersen et al. 2004). In our study, the level of self-assessed uncertainty was low, with all insects eliciting a self-assessed uncertainty level of ≤ 3. In other words, most assessors were confident in their decisions. This confidence could be attributed, in part, to our simple, yet clearly defined impact scoring system, which reduced the need for complex interpretation and guessing. Achieving consistent decision with certainty is often difficult. In situations where assessors have uncertainty not eliminated with appropriate elicitation and consensus-building techniques (e.g., lack of data or uneven evidence base), it has been suggested that assessors should quantify and communicate their true level of uncertainty to decision-makers for use in the decision-making process (Aspinall et al. 2010; Turbé et al. 2017; Vanderhoeven et al. 2017). Assessors can abide by the precautionary principle (Kriebel et al. 2001) and consider the species a higher risk until more information can be collected to indicate otherwise (Vanderhoeven et al. 2017).

Most studies that address expertise and expert opinion also address uncertainty (e.g., Murray et al. 2009; Vanderhoeven et al. 2017; Roy et al. 2018; González-Moreno et al. 2019) because the two variables can be closely associated. We observed a negative correlation between these two variables (Fig. 5), indicating assessors with high levels of expertise were more certain than assessors with lower levels of expertise. This pattern may be expected if experts generally have more prior knowledge, making them more certain. However, our assessors self-assigned fairly low levels of expertise and uncertainty, which is seemingly inconsistent with the negative correlation we observed. Many assessors rated themselves as “low expertise-no uncertainty” and “low expertise-low uncertainty” rather than “no expertise” (Fig. 5), which may have contributed to the negative correlation.

We observed no associations between the coefficient of variation for impact score and the coefficients of variation for the levels of expertise and uncertainty, as both correlations were non-significant. This suggests that expertise and uncertainty may not influence the interpretation of non-native insect impact. In other words, assessors interpreted the same information and arrived at similar conclusions regardless of specific expertise. This is a good indication that the goal of the HIWG for designing the detailed impact scale was met–the same conclusions would most likely be met regardless of which group member did the assessing. It is worth noting that although assessors varied in their self-reported expertise, all are trained ecologists with experience interpreting ecological literature and may be considered “experts” as defined by Krueger et al. (2012).

Collaborative discussion promotes assessor consensus

Consensus-building and other participatory techniques are increasingly cited in the environmental impact assessment literature (e.g., Hemming et al. 2018b; González-Moreno et al. 2019; Osunkoya et al. 2019). Social scientists have long used approaches such as the Delphi technique, a process that uses iterative structured questionnaires and group communication to evaluate expert knowledge (e.g., Mukherjee et al. 2015), and general discussion (e.g., Hemming et al. 2018b; González-Moreno et al. 2019; Osunkoya et al. 2019) such as joint fact-finding (e.g., Matsuura and Schenk 2016). However, these techniques are still new to studies of biological invasions. Through our consensus-building discussion, we were able to identify four common themes regarding problems encountered by assessors when making their assessments (Table 2).

The first theme, *ambiguous information*, was a common problem encountered by the initial assessors as they sorted through the provided literature, much of which was vague or lacking. This problem was especially acute for species categorized as low impact, some of which were scored as level one, indicating that the new assessor read no information regarding impact, whereas the initial assessor documented at least minor damage. We determined that many of these errors were due to ambiguous language in the references (e.g., Jeschke et al. 2014) that may have led to misinterpretation of the information. Consensus-building discussions among expert assessors may help alleviate this problem.

The second theme that emerged regarded *discounted details*. Some of the sources referenced were lengthy and detailed, while others were more anecdotal and lacked sufficient detail for rigorous evaluation. An assessor that does not carefully read a reference in its entirety may overlook important details about impacts or the assessor may disregard some statements altogether. For example, an assessor may discount a specific older source because subsequent controlled experiments failed to replicate it. This source of variation may be alleviated if an assessor expresses concerns to the other expert assessors during discussion.

The third theme that emerged focused on *observed versus potential impacts*. Some references discussed potential impacts not yet supported by empirical data or observations and the assessor did not find it appropriate to assign a score based solely on this interpretation of potential. Our assessments were based on documented impacts rather than potential for future impacts (e.g., under predicted global climate change scenarios or once new hosts were accessed). Other impact assessment protocols, such as Sandvik et al. (2019), have established criteria for quantifying invasion potential of non-native species in all taxonomic groups. As with previous themes, this issue can be addressed through rating scale clarification and assessor consensus.

The final theme focused on variation from *prior knowledge*. In some cases, an assessor had more insight than provided in the references, but their perception differed little from the reference. In other scenarios, the assessor had experimental results or insight that did not support or failed to replicate the reference information, so they chose to base their score accordingly. Such decisions can contribute variation, whether or not the assessor incorrectly rejects correct information. This scenario highlights the value of strict, standardized guidelines, and consensus-building techniques (Hemming et al. 2018b; González-Moreno et al. 2019; Osunkoya et al. 2019) that generate alternative perspectives guiding the group to a more uniform consensus.

Additional consensus was achieved through our joint fact-finding activity. The open dialogue among assessors facilitated achievement of consensus because assessors were able to critically evaluate ambiguous statements and, since some members of the group had prior knowledge that they used to inform their decisions, provide background knowledge based on experience not documented in the literature. Turbé et al. (2017) found that applying a similar joint fact-finding approach, along with clear guidelines and closed-ended questions, considerably improved outcomes. Other studies have also successfully used discussion groups to address uncertainty and disagreement and to make final decisions on environmental impacts (e.g., Hemming et al. 2018b; González-Moreno et al. 2019).

Conclusions

As written, the protocol and detailed, nine-point impact scale provided by the HIWG has the potential to result in a lack of consensus, particularly with medium-impact insect species. However, we found that adding joint fact-finding can alleviate any potential discrepancies in impact scoring. We demonstrate that consensus among diverse expert assessors can be achieved for invasive species decision-making and management. When empirical data are lacking for specific species, decision-makers may use broad ecological principles (Fleischman and Briske 2016) for management decisions, which is not ideal. To aid in the decision-making process, experts can first work independently to use rapid risk assessment techniques (e.g., Alves da Rosa et al. 2017) to characterize the impacts of the target species, after which consensus-building techniques can be used to reduce uncertainty and variation in impact scores (Turbé et al. 2017; Vanderhoeven et al. 2017; Hemming et al. 2018a,b; González-Moreno et al. 2019; Osunkoya et al. 2019). Reliable assessments based on vetted scientific evidence bolstered by diverse expert opinion and transparency about uncertainty (Vanderhoeven et al. 2017) will benefit decision-makers and managers tasked with allocating finite resources to manage the many threats confronting global ecosystems.

Data accessibility

All of the references used for this impact assessment are archived in the U.S. Geological Survey ScienceBase Catalog (Mech et al. 2019b). Suppl. material 1: Table S4 includes the level of expertise, impact score, and level of uncertainty assigned by the four assessors for each of the 41 conifer-specialist insects included in this study.

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Supplementary material 1

Supplementary tables and figures

Authors: Ashley N. Schulz, Angela M. Mech, Craig R. Allen, Matthew P. Ayres, Kamal J.K. Gandhi, Jessica Gurevitch, Nathan P. Havill, Daniel A. Herms, Ruth A. Hufbauer, Andrew M. Liebhold, Kenneth F. Raffa, Michael J. Raupp, Kathryn A. Thomas, Patrick C. Tobin, Travis D. Marsico

Data type: statistical data

Explanation note: **Table S1.** List of the 15 participating assessors with their affiliation at the time of the 2016 HIWG meeting and their area(s) of expertise. **Table S2.** List of 58 conifer-specialist insect species and their nine-point (1–9 impact scale), ternary (1 = low, 2 = medium, 3 = high), and binary (0 = low, 1 = high) impacts as assessed by the initial assessor. Conifer-specialist insect species highlighted in gray were not included in the impact assessment, since they had no documented damage on their host plants in North America. **Table S3.** Directions on how to complete the impact assessment for non-native, conifer-specialist insects in North America, including scales for assessment of expertise and uncertainty. Developed by A.N. Schulz. **Table S4.** Level of expertise, impact score, and level of uncertainty assigned by the four assessors for each of the 41 conifer-specialist insects included in this study. **Figure S1.** Expected 95% confidence intervals for species-specific estimates of impact for 3–11 assessors per conifer-specialist insect species. **Figure S2.** Box plot depicting the distribution of assessor expertise among conifer-specialist insect species.

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RESEARCH ARTICLE

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Trait differentiation between native and introduced populations of the invasive plant *Sonchus oleraceus* L. (Asteraceae)

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Abstract

There is growing evidence that rapid adaptation to novel environments drives successful establishment and spread of invasive plant species. However, the mechanisms driving trait adaptation, such as selection pressure from novel climate niche envelopes, remain poorly tested at global scales. In this study, we investigated differences in 20 traits (relating to growth, resource acquisition, reproduction, phenology and defence) amongst 14 populations of the herbaceous plant *Sonchus oleraceus* L. (Asteraceae) across its native (Europe and North Africa) and introduced (Australia and New Zealand) ranges. We compared traits amongst populations grown under standard glasshouse conditions. Introduced *S. oleraceus* plants seemed to outperform native plants, i.e. possessing higher leaf and stem dry matter content, greater number of leaves and were taller at first flowering stage. Although introduced plants produced fewer seeds, they had a higher germination rate than native plants. We found strong evidence for adaptation along temperature and precipitation gradients for several traits (e.g. shoot height, biomass, leaf and stem dry matter contents increased with minimum temperatures, while germination rate decreased with annual precipitations and temperatures), which suggests that similar selective forces shape populations in both the native and invaded ranges. We detected significant shifts in the relationships (i.e. trade-offs) (i) between plant height and flowering time and (ii) between leaf-stem biomass and grain yield between native and introduced plants, indicating that invasion was associated with changes to life-history dynamics that may confer competitive advantages over native vegetation. Specifically, we found that, at first flowering, introduced plants tended to be taller than native ones and that investment in leaf and stem biomass was greater in introduced than in native plants for equivalent levels of grain yield. Our study has demonstrated that climatic conditions may drive rapid adaption to novel environments in invasive plant species.

Keywords
Agricultural weed, Common sowthistle, ecological trade-offs, plant trait differentiation, Rapid trait evolution

Introduction

Introduced plant species are a threat to native biodiversity (Hejda et al. 2009; Pyšek et al. 2012) and drive considerable economic costs associated with their management and reduced agricultural yields (Hoffmann and Broadhurst 2016). According to a recent review, about 4% of vascular plant species have become naturalised beyond their native range (van Kleunen et al. 2018), with the highest densities of naturalised plant species recorded for Pacific Islands (van Kleunen et al. 2015; Essl et al. 2019). There are several mechanisms by which alien plants can become invasive after introduction to a novel range. An understanding of why some plants become invasive is essential to prioritise their management. For example, interactions between the introduced propagules and the resident plant species of the novel environment may account for invasion success (Catford et al. 2009). Escape from specialist enemies (e.g. herbivores and pathogens) in the introduced range may enhance the survivorship and competitive performance of alien plants, as proposed by the Enemy Release Hypothesis (Darwin 1859; Keane and Crawley 2002). Damage induced by natural enemies are then reduced in the introduced range compared to the native range (e.g. Meijer et al. 2016).

Adaptation to novel environmental conditions, through rapid evolution resulting in phenotypic changes (Oduor et al. 2016; van Kleunen et al. 2018), may also account for successful plant invasion (Prentis et al. 2008; Colautti and Lau 2015; Stutz et al. 2018). Several recent studies have shown that alien plants can undergo rapid evolution through trait adaptation to novel selection pressures (e.g. Molina-Montenegro et al. 2018; van Boheemen et al. 2019a; Lustenhouwer et al. 2019; Brandenburger et al. 2019a, b). Rapid trait adaptation may be driven by several processes; for example, the Evolution of Increased Competitive Ability (EICA) hypothesis posits that there is an evolutionary shift from costly defensive abilities to competitive performance in introduced populations, due to the absence of co-introduced specialist enemies (Blossey and Notzold 1995). Despite evidence for differences in several growth and defence traits between native and introduced populations (e.g. Felker-Quinn et al. 2013; Colautti and Lau 2015; Rotter and Holeski 2018; van Boheemen et al. 2019b), some meta-analyses have reported that the EICA hypothesis is only partially supported (Felker-Quinn et al. 2013; Rotter and Holeski 2018).

Other traits relating to plant phenology (Turner et al. 2014) and resource acquisition efficiency (e.g. specific leaf area, leaf and stem dry matter content, carbon to nitrogen ratio of seeds and leaves, Wright et al. 2004; Grassein et al. 2010)) have also been shown to rapidly change following introduction as a result of competition for novel resources (Gioria and Osborne 2014; Lustenhouwer et al. 2019). Many recent studies have shown that climate niche envelopes occupied by invasive plants in their introduced ranges can differ substantially to native ranges and exposure to novel climatic regimes may select for divergent traits of alien plant populations (Early and Sax 2014; Moran and Alexander 2014; van Boheemen et al. 2019a). Indeed, rapid adaptive evolution has been observed along latitudinal and longitudinal gradients in response to variations in temperature and precipitation (e.g. Callaway and Maron 2006; van Kleunen and Fischer 2008; Colautti et al. 2009; Alexander et al. 2012). However, it remains poorly understood at global scales whether the degree to which trait differences between native and introduced ranges are modulated by novel climate regimes and whether such traits confer fitness benefits (and thus invasion potential) to the introduced plant populations.

Classical functional ecological theory posits that plant growth, reproduction and defence may be traded-off or partitioned along competition, stress and disturbance gradients (e.g. Reekie and Bazzaz 1987; Stearns 1989; Edward and Chapman 2011), especially when resources (e.g. nutrients) are limiting (Grime 2006). It has been suggested that these trade-offs and their response to changing environmental factors, play a crucial role in invasion success of alien plants (Beckmann et al. 2009; Forrest and Miller-Rushing 2010; Colautti et al. 2010, 2017; Anderson and Gezon 2015). For example, some introduced species can overcome trade-offs in growth and reproduction by producing large numbers of flowers in dense populations under high competition (Lambrecht-McDowell and Radosevich 2005; Beckmann et al. 2009; Weiner et al. 2009). Trade-offs can also occur between timing of flowering and plant size at time of reproduction. Plant phenology is highly constrained by environmental selection pressures (e.g. climate, resources, disturbances) (Forrest and Miller-Rushing 2010). Reproductive effort, as measured by flower production, can often be determined by a "time-size" trade-off that balances the advantages of early reproductive maturity versus greater growth prior to fruit production (Bolmgren and Cowan 2008). Earlier flowering implies the allocation of fewer resources to maternal plant growth, resulting in smaller plants at flowering (Vile et al. 2006). The resources saved in this manner are instead invested in offspring development (Bolmgren and Cowan 2008). However, there is little knowledge about how trade-offs between key functional traits associated with growth and reproduction vary between native and introduced populations of introduced species or their contribution to invasion success.

The aim of this study was to investigate differences in plant functional traits associated with growth, resource acquisition, reproduction, phenology and defence between native and introduced populations of the common sowthistle, *Sonchus oleraceus* L. (Asteraceae) across temperature and precipitation gradients. This species is a herbaceous plant native to Europe (Gleason and Cronquist 1991), North Africa and West Asia (Peschken 1982) that was introduced to the islands of Oceania (i.e. Australia and

New Zealand) in the late 18th century (Boulos 1974; Prebble 2008). It is now the most widely distributed plant species around the globe (present on 48% of emerged land) (Pyšek et al. 2017).

Field-based trait measurements can determine how plants respond to environmental change *in situ* but cannot discriminate between phenotypically plastic versus genetic responses to local conditions (Montesinos and Callaway 2018; Brandenburger et al. 2019a). For invasive species, adaptive genetic responses in their introduced ranges can be identified using common garden experiments, whereby seeds sourced from native and introduced locations are propagated and grown under standard conditions (e.g. Stutz et al. 2018). Such experiments can control the confounding effects of phenotypically plastic responses to novel environmental conditions observed in the field (Hierro et al. 2005). In this study, we examined the variation in 20 traits associated with growth, resource acquisition, reproduction, phenology and defence between native and introduced populations of *S. oleraceus* using a common garden experiment. The specific objectives were to assess whether (1) the traits of *S. oleraceus* plants differ between native and introduced populations, (2) variation in traits is related to climatic conditions and (3) there has been a shift in trade-offs between reproduction and growth between native and introduced populations. We hypothesised that introduced *S. oleraceus* plants would display enhanced growth, more efficient resource use and higher reproductive output, as well as delayed maturity and weaker physical defences, compared with plants from the native range. We also predicted that offspring traits would vary along temperature and precipitation gradients experienced by parent plants, but we had no *a priori* expectation about the direction of these relationships or whether they would vary between native and introduced populations.

Materials and methods

Biological study system

Sonchus oleraceus is an annual, or occasionally biennial species, that has expanded across most of Australia, becoming established in more than 4.3 M ha of crops (cereals and cotton) and fallow land in south-eastern Queensland and northern New South Wales, in particular, where it causes an estimated annual loss of AUD $ 6.3 M (Walker et al. 2005; Osten et al. 2007; Llewellyn et al. 2016). *Sonchus oleraceus* is a ruderal species found primarily in disturbed, open habitats, such as gardens, crops and fallows, including roadsides (Hutchinson et al. 1984). It has been suggested that the spread of *S. oleraceus* in Australia has been favoured by the expansion of no-till agricultural practices (Chauhan et al. 2006) and the emergence of resistance to some herbicides, such as glyphosate (Boutsalis and Powles 1995; Adkins et al. 1997; Cook et al. 2014; Meulen et al. 2016).

Collection of seeds

Seeds were collected from 2016 to 2018, from 14 field populations across two geographic ranges: the native range in the Western Palaearctic (Europe and North Africa) and the introduced range in Oceania (Australia and New Zealand) (Figure 1). We are aware that this sampling does not entirely cover the native area of *S. oleraceus,* but populations were collected from a diverse range of climatic and geographic contexts. Furthermore, preliminary genetic analyses on European (including populations from Great Britain) and Australian populations indicated that the Australian populations were genetically more similar to populations from Southern Europe than to those from Great Britain (CSIRO 2018). One flower head (i.e. capitulum), containing fully ripe seeds (i.e. achenes) with a well-developed pappus, was sampled from each of 14 separate mature *S. oleraceus* plants within each of the 14 populations (i.e. 196 flower heads in total). Seeds were stored for up to two years in paper bags at a constant room temperature (~22 °C) with silica gel until sowing.

Within the native range, a collection permit was obtained for Andalusia (Spain) (ID: 64oxu764FIRMAF+xU9RItQJeLhEPV, 05/12/2017). No specific permission was required for seed sampling at other sites in Europe and North Africa or for seeds collected in Australia and New Zealand. No specific authorisation was required to introduce seeds into France.

Cultivation of plant material

Plant propagation and common garden growth experiments were performed in a glasshouse at the CSIRO European laboratory in Montpellier, France. In March 2018, eight seeds from each flower head (i.e. 1,568 in total) were sown on moistened Whatman® filter paper on a substrate of vermiculite in a Petri dish. Seeds were maintained in

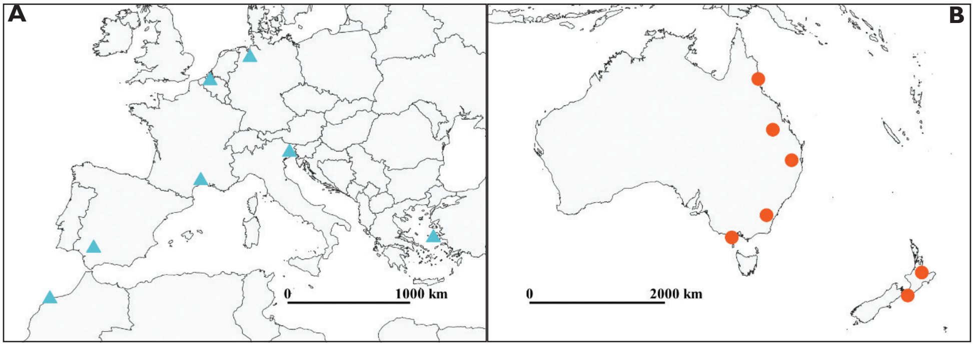

Figure 1. Maps of the collection locations for *Sonchus oleraceus* seeds across (**A**) the native range in Europe and North Africa (blue triangles) and (**B**) the introduced range in Australia and New Zealand (orange circles).

a growth room at a temperature of 25 °C/20 °C (day/night) to stimulate germination. Seven days after sowing, three seedlings per flower head were planted in a single pot (upper diameter ~ 16 cm, height ~ 19 cm) filled with 1.15 kg of nursery-grade soil ("Terreau à mottes Neuhaus, Humin-Substrat N2", ratio of N:P:K = 14:16:18). Pots were transferred to a glasshouse with a minimum night-time temperature of ~15 °C and maximum daytime temperature of ~32 °C. Pots were arranged in a standard Latin square design, such that plants derived from each source population were present once in each row and once in each column. Twelve days after planting, two seedlings were removed from each pot, leaving a single target plant, which was used for growth and functional trait measurements. All plants were watered two to three times per week, with equal volumes of tap water (i.e. between 100 and 400 ml). All plants were sprayed with a sulphur solution (Sulfostar, BASF) every two weeks, to control powdery mildew infestation. Pots were redistributed within the glasshouse at random every three days, to account for variability in light exposure.

Measurement of plant traits

We measured 20 traits (from five categories: growth, resource acquisition, reproduction, phenology and defence) at different stages of plant development on 194 replicates (two plants died during the experiment) between March and July 2018.

Growth traits. We first determined the height of each *S. oleraceus* plant when the first flower bud appeared, measured as the distance (cm) between the soil surface and the first cauline leaf at the base of the inflorescence. As described by Bolmgren and Cowan (2008), we considered this vegetative height measurement to correspond to the point at which investment in the growth of the maternal plant body switched to investment in reproductive output. We ended the experiment after ~80 days when each of the 194 plants had produced seeds from at least five flower heads and begun senescing. At this point, we measured maximum shoot height (cm) from the base of the soil to the top of the tallest flower head and above-ground biomass (g) after oven-drying at 70°C for 72 h. After 80 days, the phenological stage might have differed slightly between plants, raising questions about the influence of the phenological stage on maximum shoot height and above-ground biomass. Although significant for dry biomass, no strong correlation was found between these two traits and days to flowering (dry biomass: $R^2 = 0.024$, $F_{(1,188)} = 4.706$, $p\ value = 0.031$ and shoot height: $R^2 = 0.019$, $F_{(1,189)} = 3.819$, $p\ value = 0.052$), demonstrating their independence from plant phenology and validating their use for plant comparisons.

Resource acquisition traits. Basal leaves (forming a rosette) capture light and synthesise chemical energy to support the growth of stem, cauline leaf and reproductive tissues (Cici et al. 2009). We therefore used the basal leaves to assess leaf resource acquisition (associated with light interception and photosynthate assimilation). The largest basal leaf on each plant was sampled when the first flower bud emerged. Each leaf was placed in a test tube filled with deionised water and was stored in the dark for 24 h at 4°C. We measured the fresh weight (g) and surface area (cm^2) of each rehydrated leaf with an Epson Perfection V550 Photo digital image scanner, processing the images obtained with WinFOLIA software. Leaf dry mass (g) was then determined by drying the leaves in an oven at 70°C for 72 h. Specific leaf area (SLA) was calculated as the ratio of leaf surface area to dry mass (m^2/kg; Pérez-Harguindeguy et al. 2013). Leaf dry matter content (LDMC) was calculated as the ratio of dry mass to rehydrated fresh mass (mg/g; Pérez-Harguindeguy et al. 2013). Leaf thickness (μm) was estimated with the equation proposed by Vile et al. (2005): $[1/(SLA \times LDMC)]$, which provides a good approximation for thickness in laminar leaves (Pérez-Harguindeguy et al. 2013). We estimated the overall potential of the plant for light interception and photosynthate assimilation by counting the total number of leaves produced by the time the first flower bud had emerged. At the end of the experiment, we also calculated LDMC for the first cauline leaf located at the base of the inflorescence and stem dry matter content (SDMC, mg/g) for a 5 cm-long piece of stem. Carbon-to-nitrogen ratio (C:N) was measured on the basal leaves and seeds of glasshouse plants at the end of the experiment with an elemental analyser (model EA 1108; Carlo Erba Instruments, Milan, Italy), after the plant materials had been dried at 70°C for 72 h.

Reproductive traits. We first calculated the viability of field-collected seeds as the proportion of the seeds sown that germinated at three and six days after sowing. We chose to measure germination at two time points, as we had no preconceived notions about potential differences in germination rates between the two ranges. On average, 75-80% of the seeds had germinated after six days (Suppl. material 2, Table S2). At this time point, the seeds that had not germinated tended to become soft, discoloured and started to rot, indicating that they were not viable (see Edwards et al. 2019). Over the course of the experiment, we counted the total number of flower heads longer than 5 mm per plant. The mean number of seeds per flower head and mean seed mass (μg) per plant were estimated from the mean dry weight (μg) of 100 seeds for five flower heads. We also determined the seed dispersal potential (termed the seed dispersal window), as the difference (cm) between the highest and lowest flower heads on each inflorescence per plant. Previous studies have shown that plant height during seed production is strongly related to seed dispersal distance (Thomson et al. 2011). However, we found that flower heads were widely spread across inflorescences, so a single measurement of the highest flower head would not adequately represent the breadth (or window) of dispersal opportunities for the plant.

Phenological traits. We measured two phenological traits associated with the timing of key reproductive stages: we counted the number of days until development of the first flower bud (longer than 5 mm) and the number of days until the emergence of the first fully-open flower head.

Defence traits. We characterised investment in defence against generalist herbivores (Hanley et al. 2007), by visually recording the number of trichomes in two 1 cm^2 quadrats (one on the upper surface and one on the lower surface) at the distal tip of a single basal leaf per plant.

Climatic data for the original locations of the populations

We evaluated the effects of two climate variables on each of the 20 functional traits considered: mean minimum temperature of the coldest month and mean annual precipitation, calculated from 1970 to 2000 (Supplementary material S1). Data were retrieved from WorldClim with the 'raster' package (Hijmans et al. 2019) and R software (R Core Team 2018). We selected these two variables, based on the extensive overlap of values between native and introduced regions and the absence of interdependence between them ($R^2 = 0.004$, $F_{(1/194)} = 0.127$, $p\ value = 0.723$). We also tested mean maximum temperature of the warmest month, but we did not retain this variable for the analysis because it was correlated with the other selected variables (maximum temperature and precipitation: $R^2 = 0.23$, $F_{(1/194)} = 57.96$, p value < 0.001; maximum temperatures and minimum temperatures: $R^2 = 0.18$, $F_{(1/194)} = 45.19$, p value < 0.001).

Trade-offs between traits associated with growth and reproduction

We investigated differences between native and introduced populations in two resource allocation trade-offs related to growth and reproductive effort: *i.e.* relationships (1) between time to flowering and vegetative height at first flower bud and (2) between grain yield and leaf-stem biomass. Grain yield represents an aggregate measure of reproductive effort (Donald and Hamblin 1976; Unkovich et al. 2010). Estimates of grain yield and leaf-stem dry biomass were obtained as follows:

1) Grain yield (g) = seed mass (g) * number of seeds per flower head * number of flower heads per plant
2) Leaf-stem dry biomass (g) = total above-ground dry biomass (g) – grain yield (g)

Statistical analyses

As a first step, we performed a phylogenetic principal component analysis (PCA) incorporating the 20 traits to explore the multidimensional distribution of individual plants from native and introduced ranges, based on the entire suite of traits. The phylogenetic PCA was used, because it accounts for the non-independence of plants derived from the same source population (Revell 2009). We used the R package phytools (function *phyl.pca*) (Revell 2012) for the analysis. To perform this phylogenetic PCA, we provided, as an additional dataset, a phylogenetic tree depicting the relationships amongst individuals derived from the same source population (i.e. the tree is composed of 14 clades, each containing 14 individuals, simply coded as 14 vectors of the 14 plant identification numbers and was built using *read.tree* and *compute.brlen* functions). We excluded data for 24 plants from the analysis due to incomplete measurements for some leaf traits as a result of the samples being damaged (i.e. we analysed *n* = 170 plants). The missing data were evenly distributed between the populations and concerned ten traits (days to flowering, SLA, thickness, basal LDMC, SDMC, shoot height, dispersal window, number of seeds, seed mass and seed and leaf C:N ratios). As an overall approach to distinguishing plants between specimen areas of origin, we performed tests with individual coordinates on the first and second dimensions of the PCA. As the coordinates of dimension 1 were not normally distributed, we analysed it with a Mann-Whitney non-parametric test. The coordinates of dimension 2 were approximately normally distributed and were therefore analysed with a one-way parametric ANOVA.

We used mixed models to test for differences in each plant trait between native and introduced ranges. Linear mixed models (LMM) were used for continuous data, such as masses and lengths. For C:N ratios of leaves, data were log-transformed to meet the requirement of a normal distribution of residuals. Generalised linear mixed models (GLMM) were used for discrete variables, such as counts (Poisson distribution), percentages (binomial distribution) and duration (Gamma distribution). Range of origin was considered as a fixed factor, whereas population of origin within each range was considered as a random categorical predictor variable. For each trait, the bench, on which the plants were placed in the greenhouse, was tested as a random factor, but was subsequently removed from the model as it was found to have no effect, confirming the successful randomisation of the experiment. Previous research by Hutchinson et al. (1984) and Widderick et al. (2010) showed that the half-life of mature *S. oleraceus* achenes is up to 3 years under dry storage conditions, with no effect on seed viability or "germinability". To confirm this assumption, we tested the effect of year-of-seed collection on germination rate by including this variable as a covariate predictor in models (Suppl. material 1, Table S1). No significant effect of year of collection was observed (germination rate after 3 days: LRTχ^2 = 0.69, *df* = 2, *p value* = 0.71, germination rate after 6 days: LRTχ^2 = 0.36, *df* = 2, *p value* = 0.83), so this covariate was removed from the models for further traits tested.

We then accounted for the possible influence of climatic conditions on trait differences between native and introduced populations using a second series of mixed models that included the two climate covariates: mean minimum temperature of the coldest month and mean annual precipitation. The interaction of range with each of the covariates was also considered, as traits might respond differently to climate between ranges. For both series of mixed models (with and without climatic covariates), the significance of each main effect or interaction was assessed in a stepwise manner, using likelihood ratio tests (LRTs). The proportion of the variance explained by each full model (i.e. R^2 values) is reported (Nakagawa and Schielzeth 2013). When using LRTs, since it is not meaningful to test the significance of main effects that are included in significant interactions ($p < 0.5$), the main effects were not tested but were retained in the model as recommended by Floyd and Gurevitch (1997).

We finally investigated whether the trade-offs between flowering time and vegetative height at time of reproduction and between leaf/stem biomass and grain yield differed between native and introduced plant populations. We performed two analyses of covariance (ANCOVA) for each of the trait combinations, considering the predictor variable and range as fixed factors and population of origin as a random covariate. We accepted the hypothesis (i.e. that the trait associations differ between native and introduced ranges), based on significant interaction terms in each model along with different slopes of regression lines. Interactions were tested by comparing two different models (with and without the interaction term) in LRTs. Both trade-offs are plotted to illustrate the correlation patterns by range.

All analyses were performed with the software R (R Core Team 2018). The "nlme" package (function *lme*) was used for linear mixed models and the "lme4" package (function *glmer*) was used for generalised linear mixed models. For mixed models, R^2 was obtained with the "piecewiseSEM" package (function *rsquared*).

Results

Difference in *S. oleraceus* traits between native and introduced ranges without taking climatic covariates into account

The first two principal component axes of the phylogenetic PCA accounted for 34.53% of the variance (Figure 2). Axis 1 was inversely correlated with phenological traits (days to first flower bud and days to flowering), height at first flower bud and leaf C:N ratio. Axis 2 was weakly positively correlated with SLA of basal leaves and inversely correlated with biomass, SDMC and LDMC of cauline leaves. The confidence ellipses (Figure 2) revealed a large overlap between native and introduced plants, although there appeared to be some differentiation between a few specimens along Axis 2. No significant differences between the two ranges was observed for the first component co-ordinates ($W = 3069$, p value = 0.12), but a significant difference between the two ranges was detected for the second component (F value = 8.37, $df = 1$, p value < 0.01). Introduced plants tended to have a higher biomass, SDMC and LDMC of cauline leaves and a lower SLA of basal leaves. However, the low proportion of the variation, accounted for by the first two dimensions, highlighted the need to test the effect of range for each trait independently.

Regarding growth traits (Figure 3, Supplementary materials S2, S3), vegetative height at first flower bud and the above-ground dry biomass of the plant were both significantly higher for introduced than for native plants ($LRT\chi^2 = 3.857$, $df = 1$, p value = 0.049 and $LRT\chi^2 = 4.885$, $df = 1$, p value = 0.027, respectively). For resource acquisition traits, significantly higher values were observed for introduced plants for the number of leaves at first flower bud and SDMC ($LRT\chi^2 = 9.687$, $df = 1$, p value = 0.002 and $LRT\chi^2 = 7.955$, $df = 1$, p value = 0.005, respectively). None of the other traits differed significantly between the two ranges (all p values > 0.05).

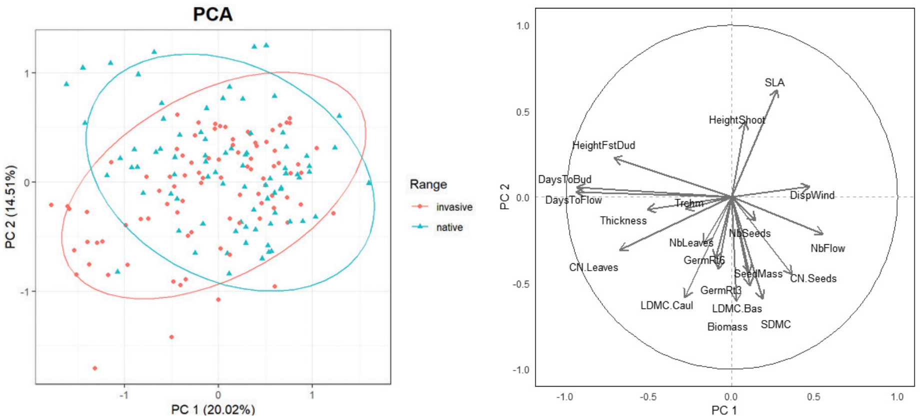

Figure 2. Phylogenetic principal component analysis (PCA) plot, based on individual values for 20 traits measured in 14 populations of *Sonchus oleraceus* from the native (Europe and North Africa, blue triangles) and introduced (Australia and New Zealand, orange circles) ranges. The 95% confidence ellipses, defined by the centre of gravity of each range, are represented. The first two components account for 34.53% of the total variance. On the right, is presented the correlation circle on the 20 variables represented by the two principal components (*HeightFstBud*: vegetative height at first bud, *HeightShoot*: total shoot height, *Biomass*: biomass, *SLA*: SLA, *LDMC.Bas*: LDMC of basal leaf, *LDMC.caul*: LDMC of cauline leaf, *Thickness*: leaf thickness, *NbLeaves*: number of leaves, *SDMC*: SDMC, *CN.leaves*: C:N ratio of leaves, *CN.seeds*: C:N ratio of seeds, *GermRt3*: rate of germination at three days, *GermRt6*: rate of germination at six days, *NbFlow*: number of flower heads, *NbSeeds*: number of seeds, *DispWind*: seed dispersal window, *SeedMass*: seed mass, *DaysToBud*: number of days to bud formation, *DaysToFlow*: number of days to flowering, *Trchm*: leaf trichome density).

Interactive effects of climate and range of origin on plant trait variation

The conclusions drawn after adjustment for bioclimatic covariates were different from those for the previous analysis (Table 1). For growth traits, a significant interaction was observed between range and minimum temperature for vegetative height at first flower bud (Figure 4). The height of the introduced plants increased with increasing minimum temperature, whereas no such relationship was observed for native plants. Total shoot height was significantly influenced by minimum temperature (increasing with increasing minimum temperature), but no significant differentiation was observed between ranges (Table 1). The above-ground dry biomass did not differ significantly between ranges, although it was significantly positively associated with minimum temperature across both ranges (Table 1).

Regarding resource acquisition traits, LDMC of cauline leaves, number of leaves and SDMC differed significantly between native and introduced plants (Table 1). Cauline LDMC was ~10% higher for introduced plants, as were the number of leaves by ~38% and SDMC by ~10%. Cauline LDMC and SDMC were also positively influenced by minimum temperature (Table 1). A significant interaction between range and minimum temperature was detected for seed C:N ratio (Figure 4), which increased with increasing temperature for introduced plants, but decreased with in-

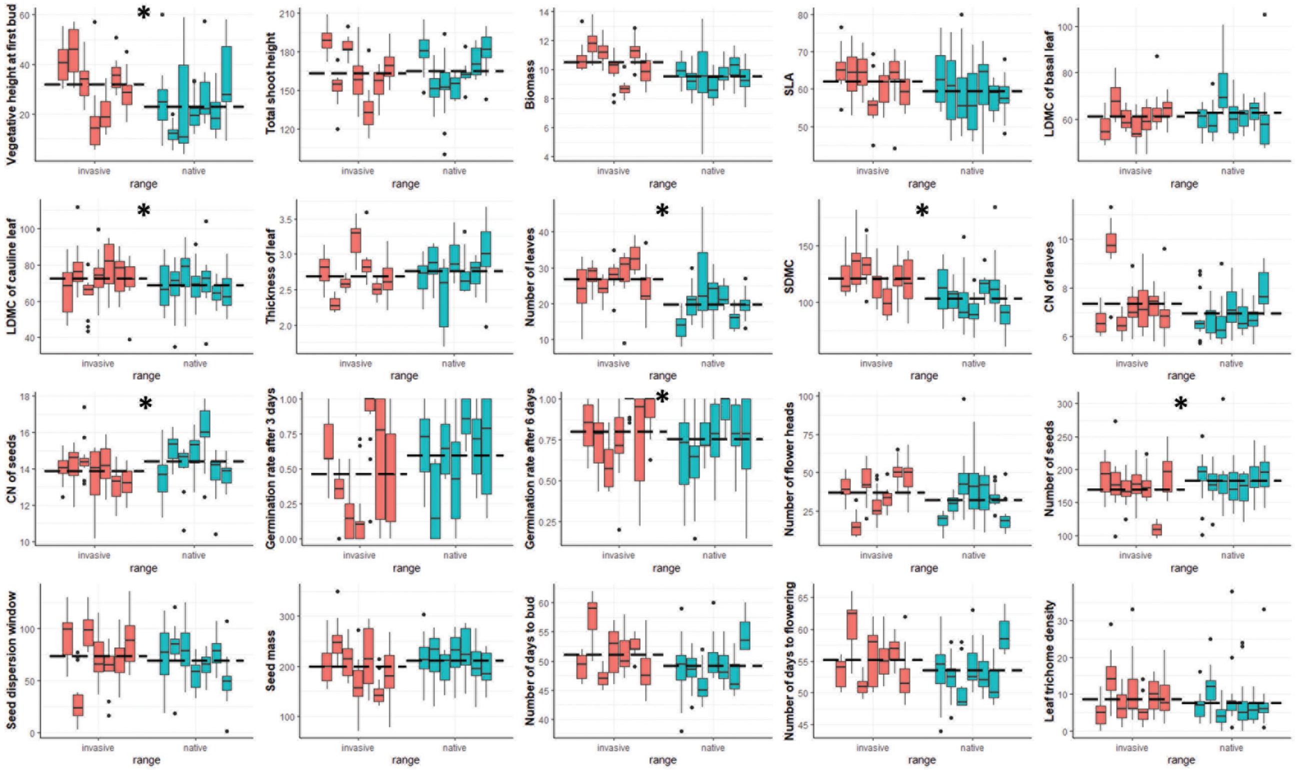

Figure 3. Box plots for twenty traits measured in native (Europe and North Africa, blue) and introduced (Australia and New Zealand, orange) *Sonchus oleraceus* plants, grown under standardised conditions. Growth: vegetative height at first bud (cm), total shoot height (cm), biomass (g). Resource acquisition: SLA (m^2/kg), LDMC of basal and cauline leaves (mg/g), leaf thickness (µm), number of leaves, SDMC (mg/g), C:N ratio of leaves, C:N ratio of seeds. Reproduction: rate of germination (0 to 100%) at three and six days, number of flower heads, number of seeds, seed dispersal window (cm), seed mass (µg). Phenology: number of days to bud formation, number of days to flowering, defence: leaf trichome density ($trichomes/cm^2$). The dashed horizontal lines through boxplots indicate the mean for each range. Asterisks indicate a significant difference between ranges (as main effect or in interaction with one of the climatic variables).

Table 1. Results of mixed models assessing the effect of range (native: Europe and North Africa, introduced: Australia and New Zealand), two climatic covariates (mean minimum temperature of the coldest month and mean annual precipitation) and their interaction (the population within a range being considered as a random factor), for 20 plant traits measured on *Sonchus oleraceus* under standardised conditions.

Response variable	**Main effect**			**Interaction**		***R²***	
Trait	Range	Minimum temperatures	Annual precipitation	Range: Min. temp.	Range: An. Prec.		
	LRTχ^2 (*df*) *p*	*LRT*χ^2 (*df*) *p*	*LRT*χ^2 (*df*) *p*	*LRT*χ^2 (*df*) *p*	*LRT*χ^2 (*df*) *p*	R^2m	R^2c
Growth							
Vegetative height at first bud	nt	nt	0.20 (1) *0.66*	4.04 (1) ***0.04*** *	0.20 (1) *0.66*	0.20	0.42
Total shoot height	1.11 (1) *0.28*	6.54 (1) ***0.01*** *	0.01 (1) *0.92*	2.80 (1) *0.09* •	0.16 (1) *0.69*	0.21	0.52
Biomass	1.26 (1) *0.26*	12.04 (1) ***<0.001*** ***	3.78 (1) *0.05* •	0.65 (1) *0.42*	0.17 (1) *0.68*	0.31	0.39
Resource acquisition							
SLA	0.74 (1) *0.39*	3.18 (1) *0.07* •	0.56 (1) *0.45*	0.21 (1) *0.65*	1.33 (1) *0.25*	0.06	0.09
LDMC of basal leaf	0.30 (1) *0.58*	1.45 (1) *0.23*	0.89 (1) *0.34*	0.43 (1) *0.51*	1.31 (1) *0.25*	0.06	0.23
LDMC of cauline leaf	6.14 (1) ***0.01*** *	4.11 (1) ***0.04*** *	1.13 (1) *0.25*	2.91 (1) *0.08* •	0.88 (1) *0.34*	0.06	0.07
Leaf thickness	0.01 (1) *0.93*	3.77 (1) *0.05* •	0.14 (1) *0.71*	0.08 (1) *0.77*	0.05 (1) *0.83*	0.12	0.41
Number of leaves	8.99 (1) ***<0.01*** **	2.28 (1) *0.13*	0.71 (1) *0.40*	0.23 (1) *0.63*	2.75 (1) *0.10*	0.33	0.52
SDMC	4.34 (1) ***0.04*** *	0.54 (1) ***<0.01*** **	2.07 (1) *0.15*	0.19 (1) *0.66*	0.37 (1) *0.54*	0.26	0.32
C:N ratio of leaves	1.17 (1) *0.28*	0.98 (1) *0.32*	0.01 (1) *0.92*	0.22 (1) 0.64	0.11 (1) *0.74*	0.06	0.40
C:N ratio of seeds	nt	nt	0.13 (1) *0.71*	4.84 (1) ***0.03*** *	0.15 (1) *0.69*	0.16	0.27
Reproduction							
Germination rate at 3 days	0.23 (1) *0.63*	1.90 (1) *0.17*	5.46 (1) ***0.02*** *	1.48 (1) *0.22*	0.94 (1) *0.33*	0.11	0.52
Germination rate at 6 days	11.30 (1) ***<0.001*** ***	10.77 (1) ***<0.001*** ***	11.25 (1) ***<0.001*** ***	0.31 (1) *0.58*	0.65 (1) *0.42*	0.07	0.20
Number of flower heads	0.25 (1) *0.62*	0.03 (1) *0.86*	0.09 (1) *0.76*	0.36 (1) *0.55*	1.32 (1) *0.25*	0.02	0.46
Number of seeds	nt	0.35 (1) *0.56*	nt	0.86 (1) *0.35*	5.21 (1) ***0.02*** *	0.54	0.66
Seed dispersal window	0.06 (1) *0.80*	3.06 (1) *0.08* •	0.19 (1) *0.67*	0.42 (1) *0.52*	1.16 (1) *0.28*	0.09	0.37
Seed mass	0.03 (1) *0.87*	0.02 (1) *0.89*	1.86 (1) *0.17*	1.72 (1) *0.19*	1.67 (1) *0.20*	0.05	0.22
Phenology							
Number of days to bud	0.93 (1) *0.34*	1.15 (1) *0.28*	0.05 (1) *0.82*	0.13 (1) *0.72*	0.14 (1) *0.71*	0.13	0.29
Number of days to flowering	0.85 (1) *0.36*	1.10 (1) *0.29*	0.04 (1) *0.84*	0.04 (1) *0.84*	0.16 (1) *0.69*	0.12	0.28
Defence							
Leaf trichome density	0.05 (1) *0.82*	1.49 (1) *0.22*	3.59 (1) *0.06* •	0.05 (1) *0.83*	1.37 (1) *0.24*	0.15	0.32

χ^2, chi-squared test statistic; *df*, degrees of freedom; nt, not tested, because of significant interaction term ($p < 0.05$).
The significance of a term is indicated by symbols: •, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.
R^2m, R^2 marginal (variance explained by fixed factors only); R^2c, R^2 conditional (variance explained by fixed and random factors).

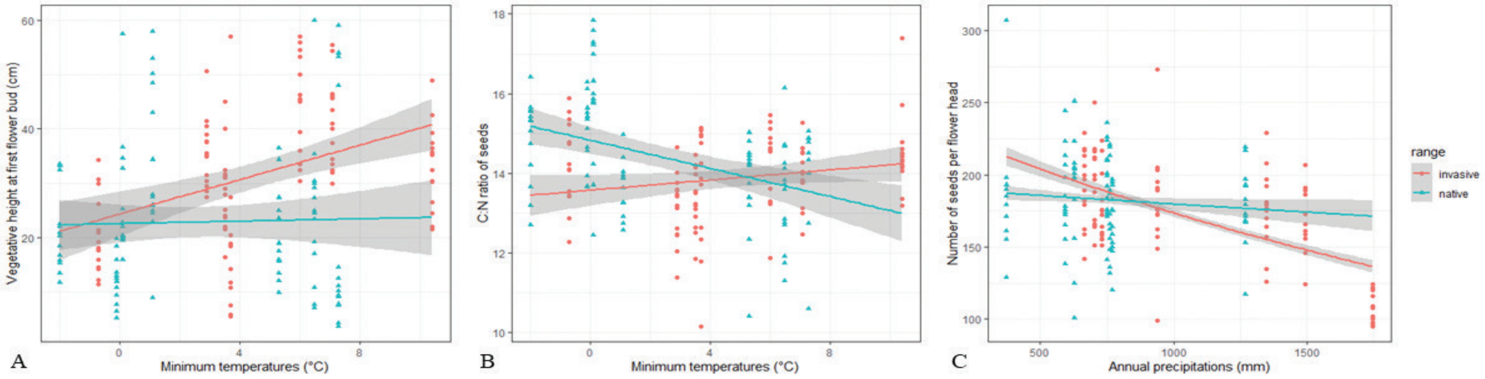

Figure 4. Significant interactions between range of origin (native: Europe and North Africa, introduced: Australia and New Zealand) and climatic conditions (mean minimum temperature of the coldest month and mean annual precipitation) for A) Vegetative height at first flower bud (*LRT*χ^2 = 4.04, *df* = 1, *p value* = 0.04), B) Carbon-to-nitrogen ratio of seeds (*LRT*χ^2 = 4.84, *df* = 1, *p value* = 0.03) and C) Mean number of seeds per flower head (*LRT*χ^2 = 5.21, *df* = 1, *p value* = 0.02).

creasing temperature for native plants. None of the other resource acquisition traits responded significantly to range or bioclimatic covariates.

Amongst reproduction traits, the rate of germination after three days was negatively influenced by annual precipitation but did not differ between ranges (Table 1). However, the rate of germination after six days differed significantly between native and introduced plants, being ~28% higher for introduced plants and was negatively influenced by temperature and precipitation (Table 1). For the number of seeds per flower head, a significant interaction was observed between range and precipitation (Figure 4), with introduced plants tending to display a faster decrease in seed production with increasing precipitation than native plants. None of the other reproductive traits responded significantly to the factors tested.

No significant difference between ranges or influence of climatic conditions was detected for phenological traits (number of days to bud formation and number of days to flowering) and the defence trait (leaf trichome density) (Table 1).

Shifts in trade-offs between native and introduced ranges

The overall relationship between the number of days to flowering and vegetative height at first flower bud was strongly significant (*LRT*χ^2 = 112.74, *df* = 1, *p value* < 0.001, Figure 5.A). However, the gradient of the relationship differed between native and introduced plants, as indicated by the significant interaction with range in the ANCOVA (significant interaction, *LRT*χ^2 = 12.35, *df* = 1, *p value* < 0.001). For plants producing their first bud before 55 days, introduced plants tended to be taller than native ones. This difference ceased to be significant for plants that flowered later (indicated by overlapping standard errors and intersecting regression lines, Figure 5.A).

Overall, there was also a very strong negative association between grain yield and leaf/stem biomass (*LRT*χ^2 = 19.33, *df* = 1, *p value* < 0.001); however, this relationship differed strongly between native and introduced plants (significant interaction term, *LRT*χ^2 = 6.81, *df* = 1, *p value* < 0.01). For low grain yield (below about 1.75 g per plant), introduced plants invested more resources than native plants in leaf and stem tissues. For grain yields greater than 1.75 g, this difference was no longer significant (overlapping standard errors and intersecting regression lines, Figure 5.B).

Discussion

When considering the full suite of traits, we found that there were only moderate differences in *S. oleraceus* populations between native and introduced ranges (as illustrated by the PCA). Native and introduced plants differed for seven of the 20 traits considered. Three of the seven significant traits were associated with resource acquisition, with higher values obtained for introduced plants (higher leaf and stem dry matter content, larger number of leaves). Climatic conditions significantly influenced nine

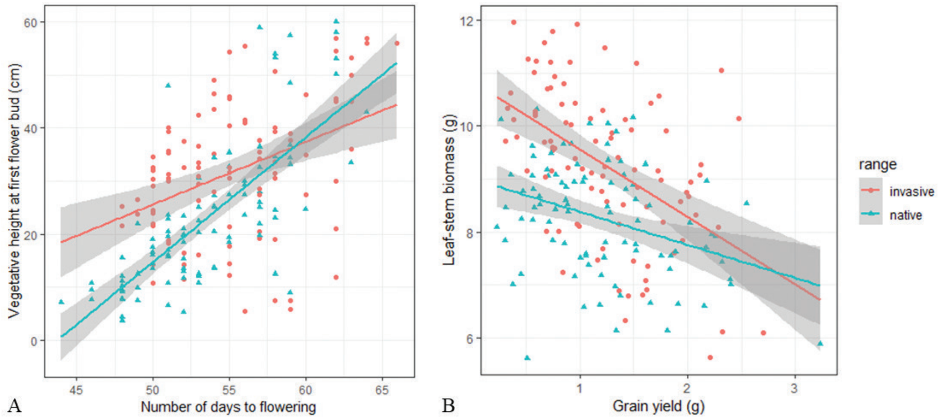

Figure 5. Significant shift in trade-offs between native and introduced populations. **A** Relationship between vegetative height at first flower bud (cm) and number of days to flowering for *Sonchus oleraceus* populations in the native range (West Palearctic, blue) and in the introduced range (Oceania, orange). Regression estimates for native plants: $y = 2.35\,x – 102.95$ and for introduced plants: $y = 1.18\,x - 33.43$. The interaction is significant (*LRT*χ^2 = 12.35, *df* = 1, $p < 0.001$). The shaded area represents the standard error of the mean. **B** Relationship between leaf-stem biomass (g) and grain yield (g) for *Sonchus oleraceus* populations in the native range (West Palearctic, blue) and in the introduced range (Oceania, orange). Regression estimates for native plants: $y = -0.63\,x + 9.01$ and for introduced plants: $y = -1.27\,x + 10.84$. The interaction is significant (*LRT*χ^2 = 6.81, *df* = 1, $p < 0.01$). The shaded area represents the standard error of the mean.

of the 20 traits considered either as a main effect or in interaction with range. Shoot height, biomass, LDMC and SDMC increased with minimum temperatures, while germination rate decreased with annual precipitations and temperatures. The height of the introduced plants increased with increasing minimum temperature, whereas no such relationship was observed for native plants. Seed C:N ratio increased with increasing temperature for introduced plants, but decreased with increasing temperature for native plants. Introduced plants tended to display a more rapid decrease in seed production with increasing precipitation than native plants.

Divergence in growth and resource acquisition traits between native and introduced populations

We found that, across all populations (i.e. when the climate experienced by the plant's ancestors was not considered), native and introduced populations differed in terms of vegetative height at first flower bud, biomass, number of leaves and stem dry matter content, with significantly higher values obtained for introduced than for native plants. These results are consistent with those reported for *Centaurea maculosa* (larger and more competitive introduced plants) (Ridenour et al. 2008) and *Silene latifolia* (larger plants in introduced populations) (Blair and Wolfe 2004). However, after considering climatic covariates in the models, we found strong evidence that some trait divergences for *S. oleraceus* were mediated by climate variation within the native and introduced ranges. We found that minimum temperature had a significant positive effect on several traits across all populations, including plant shoot height, biomass, LDMC of cauline leaf and SDMC. These repeatable trait clines for each range indicate that adaptation to similar selective factors has occurred in native and invaded ranges. Latitudinal gradients in phenological traits are common observations in various geographically widespread plant species (Colautti et al. 2010; Li et al. 2015). For example, it has been shown that SLA of the invasive species *Ambrosia artemisiifolia* had a similar latitudinal cline in the native and the introduced ranges (van Boheemen et al. 2019a). Our results for *S. oleraceus*, in addition to previous studies (e.g. Colautti et al. 2009; Cripps et al. 2010; Alexander et al. 2012), highlight the need to consider climatic variables when investigating phenotypic divergence between native and introduced ranges for invasive plant species, since temperature, precipitation or photoperiod constitute important selective forces driving rapid trait adaptation.

Differential responses to climatic conditions between native and introduced plant populations were also detected for some traits. Specifically, significant interactive effects were observed between range and minimum temperature on vegetative height at first flower bud and seed C:N ratio. Trait values increased with increasing minimum temperature for the introduced plants, whereas no such relationship was observed for native plants. Similarly, a study by van Boheemen et al. 2019a found a differential response to latitudinal cline between native (North American) and introduced (European and Australian) populations for maximum height of the plant *Ambrosia artemisiifolia*. When considering vegetative height at first flower bud for *S. oleraceus*, it could be expected that such trait-climate relationships would be stronger for native populations due to long-term evolutionary adaptations to prevailing environmental selection forces. However, as has been shown in some recent studies (e.g. Smith et al. 2020), genetic structure amongst introduced plant populations is often weaker than amongst native populations due to multiple introductions (whereby propagules are sourced from multiple sites across the native range), resulting in subsequent genetic admixture and expression of novel genotypes. Such novel genotype expression in introduced *S. oleraceus* populations might have resulted in the observed divergence in phenotypic responses to novel climate. Even if no such genetic changes happened during *S. oleraceus* invasion, it is likely that introduced plants grow under novel competitive and environmental (e.g. soil) conditions that may release them from the usual constraints on development and demographic processes. For instance, in the native range, positive effects of temperature on plant height might be offset by commensurate increases in competitive pressures from co-evolved neighbouring plants, yet such competitive pressures on growth may be diluted with increasing temperature if *S. oleraceus* is better able to ‘tolerate’ competition with its novel neighbours in the introduced range (see discussion by Golivets and Wallin 2018). Although our study has clearly shown patterns of trait divergence between native and introduced plant populations across climate clines, future research on population genetic structure, coupled with climate niche and competition modelling, would be required to untangle the mechanisms underpinning such observed patterns (van Boheemen et al. 2019a).

Moreover, even when climate variation was accounted for in the models, we found that the range of origin had a significant effect on cauline leaf dry matter content, number of leaves and stem dry matter content for *S. oleraceus*, indicating that the differences in phenotype between ranges could not be attributed solely to climatic conditions. Similarly, for the introduced *Solidago gigantea*, environmental differences and latitude only explained a small proportion of the total variation observed between the two ranges (Jakobs et al. 2004). The greater number of leaves in introduced *S. oleraceus* populations suggests a greater ability for light interception and photosynthate assimilation. Higher LDMC of cauline leaf and SDMC also indicate that introduced plants invest in a longer life cycle, as dry matter content has been found to be positively related to leaf life span and negatively related to growth rate (Niemann et al. 1992; Ryser 1996; Pérez-Harguindeguy et al. 2013). A higher LDMC has also been observed for the introduced plant *Centaurea stoebe* in North America (Henery et al. 2010). Surprisingly, no significant differences were found for SLA, LDMC or for the thickness and C:N ratio of basal leaves between native and introduced *S. oleraceus* populations. The only significant difference was for the LDMC of cauline leaves. Although we found only moderate trait divergence between native and introduced ranges for *S. oleraceus*, our data still suggest that introduced populations may be evolving a strategy favouring competitive performance of mother plants (i.e. higher leaf and stem dry matter content, larger numbers of leaves) over reproductive output.

There may be several reasons for these observed trait differences. First, maternal effects on plant traits cannot be completely excluded in this study, since the plants were not cultivated in standardised conditions before the experiment. Maternal plants may have experienced variable environmental conditions that influenced growth and resource acquisition traits in the first-generation offspring. However, some studies on different plant species suggest that maternal effects tend to mainly affect early developmental stages and are less pronounced later in the life cycle (Roach and Wulff 1987; Bischoff and Müller-Schärer 2010). Hence, maternal effects might not be responsible for the large differences observed for the latter resource acquisition traits (number of leaves, LDMC of cauline leaf and SDMC). Second, it is possible that populations have indeed undergone rapid post-introduction evolution, as evidenced by trait differences for plants grown under uniform environmental conditions reflecting genetic changes. Rapid evolution is frequently invoked as a reason for phenotypical divergence, for example, in *Leucanthemun vulgare* (Stutz et al. 2018), *Centaurea diffusa* (Turner et al. 2014) and *Arctotheca populifolia* (Brandenburger et al. 2019a, b). In our study, we considered climate as one of the main selective forces shaping trait evolution, but other factors, such as habitat characteristic or soil condition, may also be involved. Third, genetic drift, due to founder effects, could have induced the observed trait differences between native and introduced *S. oleraceus* populations (Bossdorf et al. 2005; van Kleunen et al. 2018). It is possible that the introduced populations were composed of genotypes from the native range already well-adapted to the prevailing climate and other environmental conditions of the novel range. Furthermore, the likelihood of a bridgehead effect (Bertelsmeier and Keller 2018) and the possibility that European populations selected for comparison did not actually originate from the native area of the plant could be questioned. *Sonchus oleraceus* has been partly characterised genetically (CSIRO 2018). Preliminary analyses showed that diversity in Australia was lower than that in Europe and that Australian samples most likely derived from southern Europe and northern Africa. Considering the extensive occupation of Australia by European descendants since the late 18th century (Cook and Price 1971; van Klinken et al. 2013), a European origin of the plant is the most likely hypothesis. However, to better address this hypothesis, deeper molecular studies would be necessary.

Moderate differences in reproductive but not in phenology and defence traits between native and introduced populations

For reproductive traits, only a few differences were observed between ranges and these differences were contrary to those expected. The main observations concerned the rate of germination after six days. Both climatic covariates, minimum temperature and precipitation, influenced this germination trait. Variations in germination rate along precipitation and temperature gradients are also frequently observed (Gillard et al. 2017; Molina-Montenegro et al. 2018; Yuan and Wen 2018). For example, an increase in germination rate with temperature has been observed in two introduced *Ludwigia* species (Gillard et al. 2017) and three introduced weeds from the Asteraceae (Yuan and Wen 2018). Similarly, an increase in germination rate along a rainfall gradient was shown for *Taraxacum officinale* (Molina-Montenegro et al. 2018). Moreover, for *S. oleraceus*, the rate of germination after six days was significantly higher for introduced plants in models that included climatic covariates. A greater ability to germinate is considered to be an essential life history trait for invasiveness, allowing early access to nutrients, water and space and reducing competition at early stages of establishment (Pyšek and Richardson 2007; Dickson et al. 2012; Gioria et al. 2018). Rapid adaptation of seed germination traits has been shown for the introduced species *Plantago virginica*, which has contributed to its invasion success in China (Xu et al. 2019). Besides climatic conditions, agricultural practices and habitat management could be potential factors of selections in invasive ruderal plant species (Tecco et al. 2010). The reduction of tillage in recent decades, preventing seed burial, may have favoured higher rates of *S. oleraceus* seed germination. Indeed, two studies (i.e. Chauhan et al. 2006; Widderick et al. 2010) have shown that the germination of *S. oleraceus* seeds is greatly decreased by burial at a depth of at least 2 cm.

A significant interaction between range and annual precipitation was also observed for the number of seeds, with introduced plants tending to display a more rapid decrease in seed production with increasing precipitation than native plants, indicating contrasting responses to environmental conditions between native and introduced populations. Similarly, differential trait responses to environmental gradients between native and introduced populations has been observed for reproductive output in the alien plant *Ambrosia artemisiifolia* (van Boheemen et al. 2019a). One possible explanation for our observation is that *S. oleraceus* adapts to lower drought stress in the introduced range by decreasing its investment in the production of offspring in favour of the establishment of longer-lived mother plants (Jackson and Koch 1997; Grime 2006).

Contrary to our hypotheses, we found no differences in other reproductive traits, phenology or defences against generalist herbivores between native and introduced plants. Release from specialist enemies is thought to lead to strong evolutionary changes within a few generations (Agrawal et al. 2012). Resistance to specialist herbivores would be expected to decrease, whereas resistance to generalist herbivores should increase in introduced populations (Zhang et al. 2018). We have no evidence for these patterns in *S. oleraceus*, as no change in structural defences against generalist herbivores (i.e. leaf trichomes density) was observed. Measurements of the actual damage caused by herbivores in both ranges and the use of a wider range of defence traits, such as secondary metabolite loads, would be required to test this hypothesis more comprehensively (Felker-Quinn et al. 2013).

Shift of the trade-offs between growth and reproduction between native and introduced populations

We found an overall significant positive relationship between vegetative height and number of days to flowering. This likely represents a trade-off between growth and reproductive effort, whereby investment in vegetative tissues, related to growing tall, results in delayed onset of flower production, i.e. short plants flower earlier than tall plants. This relationship is commonly observed for herbaceous plants (Vile et al. 2006; Bolmgren and Cowan 2008; Garnier et al. 2016); however, our study is one of the first to explicitly demonstrate that the pattern of these trade-off relationships can change when a species is introduced to a novel range. A significant difference in the trade-off between vegetative height at first bud and number of days to flowering indicated that introduced plants tended to be taller when they produced their first flowers compared with native plants. Growing taller when initiating flower production may confer competitive benefits for introduced plants relative to neighbouring plants in terms of light acquisition (King 1990; Westoby et al. 2002). Furthermore, for herbaceous plants, being taller at the reproductive stage may improve efficiency of pollination and seed dispersal (Donnelly et al. 1998; Lortie and Aarssen 1999; Soons et al. 2004; Carromero and Hamrick 2005; Thomson et al. 2011).

The overall strong negative relationship between stem-leaf investment and reproductive output confirmed that there is a trade-off between allocation to growth and reproduction, such that larger plants tended to invest relatively less in reproductive output than smaller plants. Trade-offs in growth and reproduction are commonly observed for ruderal plant species (Grime 2006), i.e. short-lived plants growing in marginal, highly disturbed environments with fluctuating resource availabilities. This trade-off is due to the limited carbon budget that the plant can allocate towards either one of these two strategies under high competition or environmental stress (Reekie and Bazzaz 1987; Stearns 1989; Edward and Chapman 2011). We demonstrated that this growth-reproduction trade-off in *S. oleraceus* differed significantly between native and introduced populations, with a greater investment in plant growth found in introduced compared with native plants for equivalent levels of resource allocated to reproduction. This may represent a fitness benefit for introduced plants along two key niche axes that may explain invasion success (Felker-Quinn et al. 2013). Introduced plants tended to be larger than native plants, suggesting increased competitive abilities and the maintenance of a relatively high grain yield with increasing leaf and stem biomass may favour invasibility through propagule pressure and dispersal opportunities. Similar results (ability to increase population density while sustaining a similar proportion of flowers as native plants) were found for the introduced populations of *Achillea millefolium* in New Zealand (Beckmann et al. 2009) conferring an advantage on introduced plants over native populations.

Conclusion

Our study found that the introduced *S. oleraceus* populations in Australia and New Zealand seem to outperform native populations, by having higher leaf and stem dry matter content, larger number of leaves, greater vegetative height at the early flowering stage, smaller number of seeds and higher germination rate. Shifts in trade-offs for plant height at time of reproduction vs. flowering time and leaves/stems biomass vs. grain yield were observed, suggesting that an ability to adapt life-history traits may also contribute to the invasion success in *S. oleraceus*. We found strong evidence for repeated adaptation to local temperatures and precipitation. When comparing model results with and without climatic covariates, climatic conditions were partly responsible for the observed differences. However, a clear effect of range of origin was observed for some traits, implying a role for other selective factors, such as habitat characteristics, in plant rapid evolution between ranges (Tecco et al. 2010; Colautti and Lau 2015).

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Supplementary material 1

Table S1. Location and climatic data for the 14 *Sonchus oleraceus* populations used for offspring comparisons under standardised conditions

Authors: Melodie Ollivier, Elena Kazakou, Maxime Corbin, Kevin Sartori, Ben Gooden, Vincent Lesieur, Thierry Thomann, Jean-François Martin, Marie Stéphane Tixier

Data type: species data

Link: https://doi.org/10.3897/neobiota.55.49158.suppl1

Supplementary material 2

Table S2. Mean (± standard error) values for 20 traits assessed for native (Europe and North Africa) and invasive (Australia and New Zealand) populations of *Sonchus oleraceus* under standardised conditions

Authors: Melodie Ollivier, Elena Kazakou, Maxime Corbin, Kevin Sartori, Ben Gooden, Vincent Lesieur, Thierry Thomann, Jean-François Martin, Marie Stéphane Tixier

Data type: species data

Link: https://doi.org/10.3897/neobiota.55.49158.suppl2

Supplementary material 3

Table S3. Results of mixed models assessing the effect of range (native: Europe and North Africa, introduced: Australia and New Zealand), population within range being considered as a random factor, for 20 plants traits measured on *Sonchus oleraceus* under standardised conditions

Authors: Melodie Ollivier, Elena Kazakou, Maxime Corbin, Kevin Sartori, Ben Gooden, Vincent Lesieur, Thierry Thomann, Jean-François Martin, Marie Stéphane Tixier

Data type: statistical data

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RESEARCH ARTICLE

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In-season leaf damage by a biocontrol agent explains reproductive output of an invasive plant species

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Abstract

One of the biggest challenges in classical biological control of invasive weeds is predicting the likelihood of success. *Ambrosia artemisiifolia*, a North American plant species that has become invasive in Europe, causes economic losses due to health problems resulting from its huge amount of highly allergenic pollen and as a weed to agricultural crops resulting from high seed densities. Here we assessed whether the pollen and seed output of the annual *A. artemisiifolia* (at the end of the season) is related to in-season abundance of, or damage by, the accidentally introduced biological control agent *Ophraella communa*. We monitored the growth and leaf damage of individually labelled *A. artemisiifolia* plants at four locations in Northern Italy and recorded abundance of different *O. communa* life stages at regular intervals. We found that the in-season level of leaf damage by *O. communa* consistently helped to explain seed production in combination with plant volume and site throughout the season. Feeding damage, plant volume and site also explained pollen production by *A. artemisiifolia* six weeks before male flower formation. At three out of four sites, plants with more than 10% leaf damage in mid-June or early July had a very low likelihood of seed formation. Leaf damage proved to be a better explanatory variable than *O. communa* abundance.

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* These authors contributed equally to the manuscript.

Our results suggest that the monitoring of the in-season leaf damage can help to project the local impact of *O. communa* on *A. artemisiifolia* at the end of the season and thus inform management regarding the needs for additional measures to control this prominent invader.

Keywords

Ambrosia artemisiifolia, biological invasions, classical biological control, common ragweed, herbivory, *Ophraella communa*

Introduction

It is now well established that plant species that are introduced into areas outside their native range and become invasive can wreak serious impact on nature and human well-being (Vilà et al. 2011). One option to mitigate the negative impacts of invasive alien species is classical biological control, i.e. the use of specialist natural enemies from the native range to reduce invader densities below an economic and ecological threshold or to slow down their spread (Müller-Schärer and Schaffner 2008). Classical biological control of invasive alien plant species has been implemented worldwide for more than 120 years (Winston et al. 2014). The method is used either alone or in combination with other weed management practices (Müller-Schärer and Collins 2012, Lake and Minteer 2018).

One of the biggest challenges in classical biological control of weeds is predicting the likelihood of success, and thus the necessity for considering additional management practices (Schwarzländer et al. 2018). Once established, biological control agents need to reach high densities to impact the target plant to such an extent that vital rates, and ultimately its population growth rate, are negatively affected (Jamieson et al. 2012). In cases of successful biological control of invasive alien plant species, population densities of the introduced biological control agents often reach numbers several orders higher than observed within their native range (Müller-Schärer and Schaffner 2008). However, whether biological control agents are able to build up high densities depends, among others, on the weather and climate conditions they encounter in the new range (Weed and Schwarzländer 2014; Mills 2018). Including biological control in integrated weed management presupposes an understanding of the likely abundance of the biological control agent and its impact on the target weed in a given region or year.

Predicting the impact of herbivore abundance on plants has a long history in crop pest forecasting (Magarey and Isard 2017), where it is used to support decision making regarding the scheduling of pest management interventions. For example, Lemic et al. (2016) found a strong positive correlation between the number of noctuid moths caught in pheromone traps and damage to sugar beet crops, which can be used to inform the timing of insecticide application to keep damage under an economical threshold level. This principle is also used, among many others, in the management of codling moth in apple orchards (Rather et al. 2018), of western corn rootworm in maize (Kos et al. 2014) and of navel orangeworm in almond plantations (Rosenheim et al. 2017). Thus, economic threshold levels are used in pest predictive models to decide when to apply pesticides. Along the same lines, a threshold level could be used in biological control programmes to inform weed managers whether or not additional control measures are required to achieve pre-defined management objectives. While the use of threshold levels in integrated weed management seems intuitive, there are only a few examples in classical biological control of weeds where in-season parameters related to herbivore abundance or damage have been identified that would allow informed management decisions. For example, Ding et al. (2006) estimated the number of larvae of the leaf beetle *Galerucella birmanica* necessary to control growth and reproduction of water chestnut, an invasive weed in several continents. Similarly, Häfliger et al. (2006) showed a negative linear relationship between attack rates of the stem boring noctuid *Archanara geminipuncta* early in the season and the performance of the invasive reed *Phragmites australis* towards the end of the season.

Ambrosia artemisiifolia L. (*Asteraceae*) is one of the most notorious plant invaders in Europe (Essl et al. 2015, Müller-Schärer et al. 2018). Originating from North America, it has been considered a noxious weed in Europe since the early 1920s (Csontos et al. 2010). In some parts of the invaded range, it causes serious yield losses in spring-sown crops such as maize, soy bean, and sunflower (e.g. Kőmives et al. 2006). Even more importantly, it produces large amounts of highly allergenic pollen annually, which causes substantial medical costs and reduced quality of life among the allergic population (Smith et al. 2013, Mouttet et al. 2018, Schaffner et al. in press). Current management tools for *A. artemisiifolia* in Europe comprise mechanical and chemical control, which are applied in agricultural environments and along roadsides (Milakovic and Karrer 2016, Lommen et al. 2018c). Due to their costs, impracticality, and/or their negative impact on the natural environment, these tools are unsuitable for most other habitat types where common ragweed thrives, such as wasteland, riversides, or on small patches of ruderal land. As both the spread and health impact of common ragweed in Europe are likely to increase with the changing climate (Storkey et al. 2014, Hamaoui-Laguel et al. 2015, Sun et al. 2017), and the number of persons sensitised to *A. artemisiifolia* pollen is rising (Lake et al. 2016), alternative methods such as biological control will be important to help to control this invasive weed (Gerber et al. 2011). Biological control of *A. artemisiifolia* has already been implemented in Australia and China (Palmer et al. 2010, Zhou et al. 2014). In China, biological control of common ragweed is based on joint mass releases of the deliberately introduced noctuid moth *Epiblema strenuana* Walker and the accidentally introduced leaf beetle *Ophraella communa* LeSage (Zhou et al. 2014).

In Europe, *O. communa* was found for the first time in Northern Italy in 2013, probably also due to an accidental introduction (Bosio et al. 2014, Müller-Schärer et al. 2014). Since then, the beetle has become widely established in Northern Italy and has been spreading all over the Po Plain (Augustinus et al. 2015, Lommen et al. 2017b). In its current range in Europe, the beetle can complete up to four generations per year, which can result in complete defoliation and impaired reproduction of *A. artemisiifolia* plants (Bosio et al. 2014, Müller-Schärer et al. 2014). Since the first sighting of high abundances of *O. communa* in Northern-Italy in 2013, yearly airborne ragweed pollen counts in the region have decreased by 80%. As this drop in airborne pollen counts could not be explained by land use change or meteorological factors, it is most likely attributable to feeding damage by *O. communa* (Bonini et al. 2015a, Bonini et al. 2015b). At the field plot level, Lommen et al (2018b) showed a negative effect of the presence of *O. communa* on the density of *A. artemisiifolia* seeds produced. However, the observed effect of *O. communa* on *A. artemisiifolia* plants varied considerably, both at the spatial and temporal scale (Lommen and Augustinus, unpublished data).

In Northern Italy, the yearly peak of *O. communa* population size is only reached at the time when the first flower buds are produced. Identifying earlier, in-season indicators that are related to the level of biological control at the end of the season could help to project whether in a particular season or location *O. communa* damages *A. artemisiifolia* to such an extent that it prevents plants from reproduction, i.e. from producing pollen (which impacts human health) or seeds (which impacts long-term population dynamics and crop yield).

Here we report on a field experiment to assess whether abundance of or damage by *O. communa* during the season is related to *A. artemisiifolia* reproduction at the end of the season. We followed individually labelled *A. artemisiifolia* plants in four locations in Northern Italy during the summer of 2016 to answer the following questions: (1) what is the in-season variation in a) in-season survival of *A. artemisiifolia*, b) the number of *O. communa* individuals of, and leaf damage caused by *O. communa* on individual *A. artemisiifolia* plants , and (2) what is the effect of in-season *O. communa* abundance or leaf damage on *A. artemisiifolia* reproduction at the end of the season?

Material and methods

Study species

Ambrosia artemisiifolia is an annual plant that has invaded areas in all continents except Antarctica (Csontos et al. 2010, Essl 2015). The monoecious plant produces racemes with male flower heads that release highly allergenic pollen. The wind-pollinated female flowers are located in clusters in the leaf axils, with each flower producing one single seed. The seeds can remain viable in the soil for up to 40 years (Toole and Brown 1946). In Northern Italy, the first plants emerge in early April but they can emerge anytime until late summer, e.g. after soil disturbance or heavy rains. They form male flowers by mid-August, producing pollen in August and September (Bonini et al. 2015a), while female flowers are formed in early September, and produce seeds from mid- to late September (Fogliatto et al. 2019, Lommen et al. 2018b).

Ophraella communa is a multivoltine leaf beetle which overwinters at the adult stage and lays eggs in egg batches in spring. The beetle then goes through three larval stages, which feed on the green parts of the host plant. It then pupates and starts mating shortly after emergence from the lightly woven cocoon. Adults feed on green parts of the plant as well. In Northern Italy, the beetle can complete up to 4 generations per year (Mouttet et al. 2018). In southern China, where climatic conditions appear to be particularly suitable, *O. communa* concludes five generations in a year (Meng et al. 2007).

Study sites

We selected three former crop fields and one meadow with natural populations of both *A. artemisiifolia* and *O. communa* in the Po Plain of the Italian Piedmont and Lombardy regions (see Suppl. material 1). The sites were in different successional stages, thus allowing us to include variation in interspecific competition and ragweed growth rate, which likely affect biocontrol impact. One field site (Magnago) had been converted from woodland to grassland 4 years prior to the experiment. Another site (Unito) was a former crop site that had not been used for agriculture for the past two years and was dominated by grasses. Two other crop sites (Magenta, Busto Arsizio) were in agricultural use until very recently and were still dominated by early-succession weeds. The Busto Arsizio site was tilled and prepared for maize production (as grown adjacent) by a local farmer two weeks before commencing the experiment. The preparations included application of fertilizer, but no application of pre-emergence herbicides. The size of the study plots in each site ranged from 125–400 m^2. To ensure that enough plants were available for the experiment, we disturbed the plots in April by mowing and raking to encourage recruitment of *A. artemisiifolia* from the soil seedbank.

Selection of plants

The study plants were selected between 13 and 18 June 2016, when *A. artemisiifolia* was between the 4- and the 12-leaf stage. We maximised the variation in initial size of *A. artemisiifolia* by randomly measuring plants at each site for 10 minutes and separating them into three equally numbered size classes (small, medium, large). We then laid transects of 20 m length through the study plots and selected 20 plants per size class along this transect, with an as homogeneous distribution over the site as possible. Minimum distance between selected plants was 50 cm and the maximum distance away from the transect was 2 m. Plants were individually marked with an aluminium label around the stem and a bamboo stick.

Timing of the study

We decided to start our experiment in mid-June to exclude background seedling mortality from the dataset, since seedling establishment can vary considerably within and among sites (Rothrock et al. 1993). Furthermore, *A. artemisiifolia* has a long germination period (Kazinczi et al. 2008) and possesses high variability in germination rate and onset even among individuals of a population (Fogliatto et al. 2019). Thus, starting our experiment in mid-June allowed us to include plants that had germinated later and thus to cover a larger range in plant size.

Insecticide treatment

In order to increase intra-site variation in abundance of and damage by *O. communa*, two subplots of approximately 5 m long along the transect were selected at random for insecticide application. The two subplots contained in total 12 labelled plants (4 plants per size class) per site. These subplots were sprayed twice a month with insecticides, alternating between contact and systemic insecticides. We used Lambda-Cyhalothrin in a dosage of 20g/ha (Syngenta KarateZeon) as contact insecticide, and a combination of Acetamiprid in a dosage of 100g/ha (Sipkam EPIK), and Deltamethrin in a dosage of 20g/ha (Bayer DecisEVO) as systemic insecticides. Insecticides were applied at a spray volume of 1000L/ha using a backpack sprayer. Previous studies revealed that there is no direct effect of this insecticide treatment on the measured plant parameters (Lommen et al. 2018a). The rest of the study plots were sprayed with the equivalent amount of water with the same intervals.

Measurements

Plant survival and size, *O. communa* abundance and leaf damage caused by *O. communa* were assessed on individual plants six times ("censuses") at three-week intervals from mid-June until mid-September 2016 (see exact dates in Suppl. material 2). To assess plant survival, plants were scored as 'alive' as long as parts of their above-ground biomass were green; for example, completely defoliated plants were scored alive when parts of the main stem or lateral shoots were still green. To monitor plant size, we measured height (from the ground to the highest point when the plant was held erect) and width (at the widest span) of the plants and used these values to calculate volume, using the following formula:

$$Volume = height * \pi * (width/4)^2$$

To assess the abundance of *O. communa* on individual plants, we counted the number of *O. communa* egg batches, larvae >5 mm long (larger L2 and L3 larvae), and the number of adults on each labelled plant at each census. We disregarded egg batches with less than 5 eggs, because laboratory experiments indicated that eggs from small egg batches are mostly unfertilised (Augustinus, unpublished data). As small larvae are difficult to find since they can hide in buds and flowers, we did not count these to minimize observer errors. In addition, we measured leaf damage per plant by estimating the percent leaf area removed by *O. communa* from the total leaf area if the plant was intact (plants without leaves were given a value of 100% area removed). We did not score damage that was clearly not caused by *O. communa* (e.g. with traces of snail mucus). However, we never observed other leaf-chewing insect herbivores on *A. artemisiifolia* than *O. communa*, and rarely found traces of molluscs.

To estimate levels of plant competition early in the season, we assessed percent bare soil in a 50×50cm square around each marked plant in early July. A square frame of 50×50cm was laid around a plant and the fraction of that surface covered by bare soil, when projecting the vegetation onto the ground, was estimated by at least two persons and the average taken. Stones or dead leaf material were scored as bare soil as well. In late August, we measured the summed length of all racemes per plant as a proxy for pollen production (Lommen et al. 2018b). Seed formation was assessed between 19 and 25 September 2016 by counting the number of seeds and female flowers (each flower gives rise to a single seed) produced per plant before seed rain.

Statistical analysis

To compare the change in leaf damage over time between sites, we conducted a repeated measures ANOVA with damage as response variable, site as fixed variable, and census as random effect. The fit of the residuals was evaluated graphically, and we took the square root of damage to obtain a better fit.

Because of the highly zero-inflated nature of our data, we applied a hurdle approach to analyse the effect of *O. communa* numbers on male (i.e. pollen) and female (i.e. seeds) *A. artemisiifolia* reproduction by first using presence/absence of racemes (pollen-bearing structures) in late August, and of seeds in mid-September to assess the probability of male and female reproduction, respectively. In a second step, we analysed the quantity of male and female reproduction conditional on the probability of reproduction (i.e. only using plants that did produce), using raceme length (as a proxy for the number of pollen produced), and numbers of seeds as response variables, respectively.

In the first part of the hurdle approach, we assessed the effect of *O. communa* on likelihood of raceme or seed formation in separate analyses by formulating generalised linear models for each of the first four (for raceme formation) or five (for seed formation) censuses. As fixed effects we included site, the natural logarithm of plant volume, as well as none or one of the four *O. communa*-related variables (number of eggs per plant, number of pupae per plant, number of adults per plant, and percent leaf damage) in each model, as these were inter-correlated. We produced models with and without an interaction term for the *O. communa*-related variable and plant volume, and with and without percent bare soil. We compared all resulting 18 models for each response variable at each census and selected the model with the lowest conditional Akaike information criterion (AICc) value, which penalizes models with more parameters (Burnham and Anderson 2002). To assess the fit of each model, we added Nagelkerke's pseudo r-squared (Tables 1, 2). We displayed the effect size of the explanatory variables by plotting the odds ratios for all explanatory variables of the best performing model per census (Figures 4, 5).

In the second step of the hurdle approach, we assessed the effect of *O. communa* on total raceme length or number of seeds of those plants that did produce racemes or seeds, respectively. We formulated a set of linear models for the natural logarithm of raceme length and number of seeds, assuming a Gaussian distribution of the response variable. We chose to use a Gaussian distribution over a Poisson distribution since it reduced AICc values of the fitted models by more than 5000 for every case. As fixed effects we included the natural logarithm of volume and site and added none or one of the four *O. communa*-related variables. To prevent overparameterization, we did not include bare soil and interactions with plant volume in these models, since the sample size of plants that successfully formed racemes and seeds was too low to include more than three fixed effects.

All analyses were conducted in R version 3.5.1 (2018–07–02) --"Feather Spray" (2018). Data were prepared using the readxl (Wickham and Bryan 2016) and reshape (Wickham 2007) packages, models were formulated in lme4 (Bates et al. 2015), model fits explored in DescTools (Signorell 2017) and MuMIn (Bartoń 2013), comparisons of damage levels between sites were conducted in agricolae (De Mendiburu 2019) and figures produced in ggplot2 (Wickham 2009) , sjPlot (Lüdecke 2018) and gridExtra (Auguie 2016) packages.

Results

Ophraella communa abundance and damage

Until late July, we found less than one egg batch, larva or adult of *O. communa* per plant (Fig. 1). The number of egg batches increased in early August but declined again in late August; after that, we found no more egg batches. Larvae and adult counts peaked in late August, with larval counts averaging 5 individuals per plant in late August (Fig. 1).

Plant volume steadily increased until late August, and decreased or stayed stable thereafter (Suppl. material 4). The few plants with no leaf damage during the course of the experiment were exclusively plants treated with insecticides (Fig. 2). Plants that were not treated with insecticides showed a moderate but continuous increase in percent leaf damage by *O. communa* until early August, reaching an average of approximately 20–50% leaf damage across all sites (Fig. 2). Between early and late August, the percent of leaf tissue damaged rapidly increased to approximately 85%. Plant mortality occurred throughout the experiment, but was most severe between late August and the beginning of September (Fig. 3), before natural mortality due to senescence occurred. Percent leaf damage varied significantly among sites (repeated measures ANOVA,

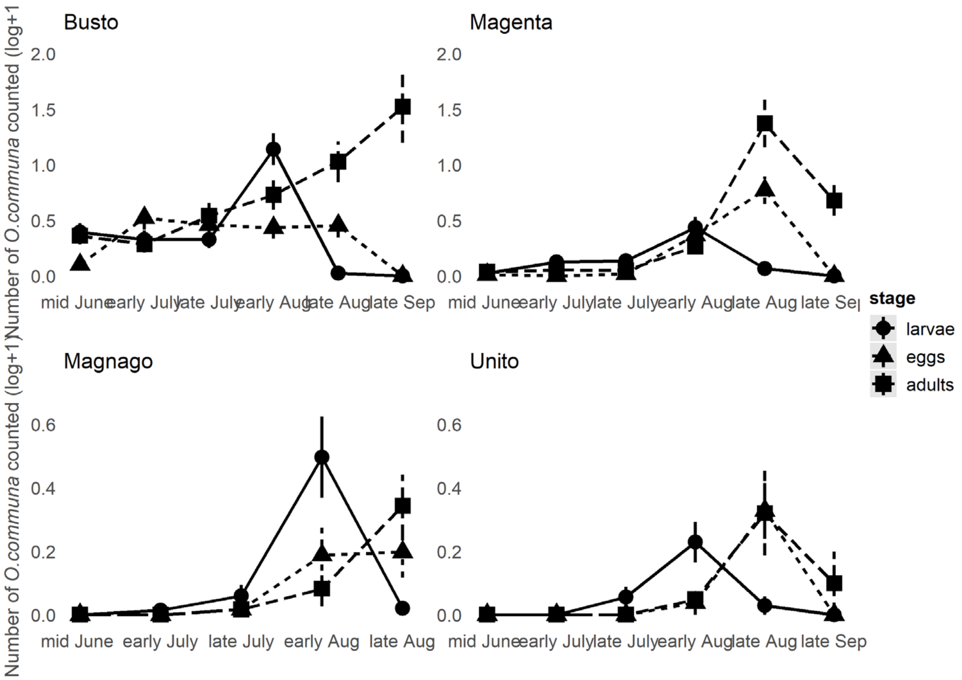

Figure 1. Average number of *O. communa* individuals per plant during the experiment in the four different sites. Different life stages are marked with different lines and symbols. Vertical lines indicate the standard error.

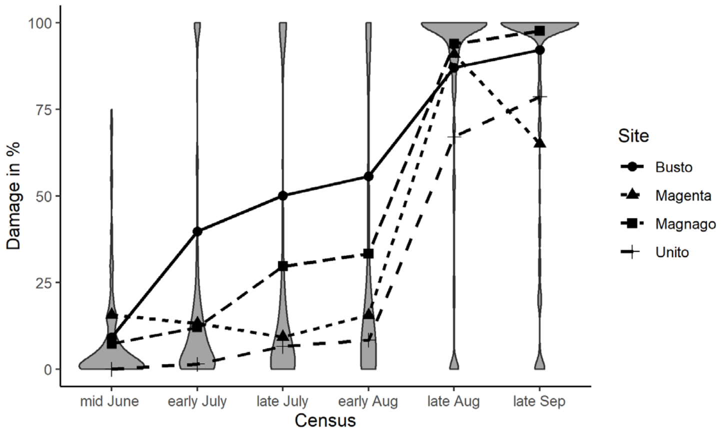

Figure 2. Violin plot of *Ambrosia artemisiifolia* leaf damage by *O. communa* feeding. The lines indicate the mean of the leaf damage scored on living plants in the different sites. The distribution of the damage measurements is shown with the grey shapes. Only damage of plants which were not treated with insecticides are displayed.

$H = 147.33$, $df = 3$, $p < 0.01$) , with Busto Arsizio exhibiting the highest percent leaf damage until early August and Unito the lowest percent leaf damage throughout the whole study period (Fig.2).

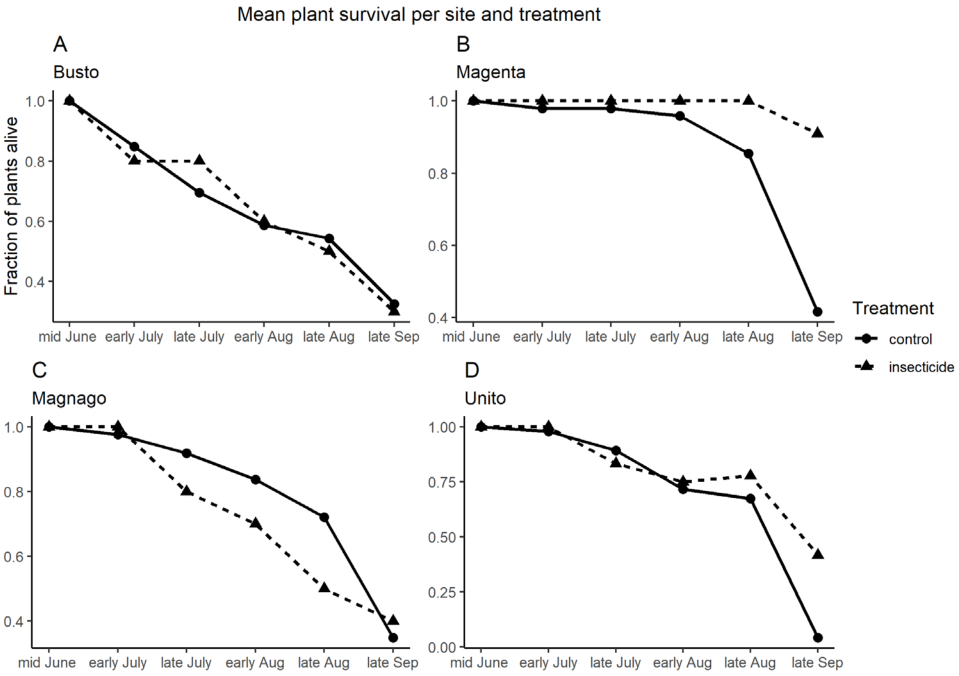

Figure 3. Fraction of plants exposed to *O. communa* and those treated with insecticides alive over time during the experiment.

Effect of *O. communa* on *A. artemisiifolia* reproduction

Models with the lowest delta AICc values (compared to the best performing model) for successful raceme formation included *O. communa* abundance parameters measured in early August (number of adults), and models with the lowest delta AICc values for raceme length of the plants that successfully formed racemes included *O. communa* abundance parameters measured in late July (number of egg batches) and early August (number of adults; see Table 1).

In the model with the lowest delta AICc value for successful raceme formation in late July, we found a positive relationship between leaf damage in percent and successful raceme formation (Fig. 4). In the models for raceme length, the number of egg batches per plant in late July was positively related to raceme length, and number of adults per plant was positively related to raceme length (Table 1).

The selected models for successful seed formation included *O. communa* abundance parameters measured in early July (number of adults per plant), early August (number of adults, larvae and egg batches per plant) and late August (number of adults and larvae per plant) (Table 2). Successful seed formation could best be explained by models including the number of adults per plant in early July, the number of adults, number of larvae or number of eggs per plant in early August, and the number of adults or larvae per plant in late August. In all cases, the number of *O. communa* had a negative effect on successful seed formation. Five out of six models had a lower AICc

Table 1. Delta AICc values (upper number) compared to the model with the lowest AICc value, pseudo r-squared (second number), odds ratio for the *O. communa* related factor (third number), and confidence interval of the odds ratio for the *O. communa* related factor (lowest number) for models showing correlation between chance of successful raceme formation (left part of table) and total raceme length of raceme-producing plants (right part of table) and explanatory factors at different censuses. Models, where the confidence interval of the odds ratio for the effect size of the *O. communa* related factor does not cross 0, are shaded. Models including interactions with volume and the explanatory factor are marked with '*'. Corresponding p-values can be found in Suppl. material 3.

Factor	Probability of raceme formation dependent on factor				Raceme length dependent on factor …			
	Mid-June	Early July	Late July	Early August	Mid-June	Early July	Late July	Early August
No *O. communa* parameter	59	58	46	6.2	13	16	8.1	1.5
	0.40	0.40	0.37	0.40	0.724	0.737	0.738	0.713
# egg batches	59	60	48	8.2	15	18	3.5	1.5
	0.41	0.40	0.37	0.40	0.72	0.74	0.74	0.72
	0.74	0.88	0.92	1.03	1.11	1.15	1.55	1.06
	(0.48, 1.15)	(0.58, 1.34)	(0.52,1.65)	(0.88, 1.22)	(0.70, 1.75)	(0.86, 1.55)	(1.12, 2.13)	(0.98, 1.16)
# larvae	61	60	47	7.8	14	18	10	2.7
	0.40	0.40	0.37	0.40	0.73	0.74	0.74	0.72
	0.82	1.01	0.74	1.13	1.82	1.08	1.20	1.06
	(0.25, 2.72)	(0.78, 1.32)	(0.42, 1.30)	(0.81, 1.57)	(0.70, 4.73)	(0.91, 1.28)	(0.76, 1.91)	(0.95, 1.19)
# adults	60	60	48	4.5*	15	16	10	**0**
	0.41	0.40	0.37	0.43	0.73	0.75	0.75	0.73
	0.85	1.05	0.95	24.61	1.13	1.25	1.09	1.13
	(0.58, 1.25)	(0.65, 1.70)	(0.73, 1.23)	(0.97, 624.27)	(0.72, 1.77)	(0.93, 1.67)	(0.91, 1.30)	(1.00, 1.28)
% leaf damage	59	58	39*	**0***	15	18	11	2.6
	0.41	0.41	0.43	0.46	0.73	0.74	0.74	0.72
	0.97	0.98	1.02	1.06	1.01	0.99	1.01	0.98
	(0.94, 1.01)	(0.96, 1.01)	(1.00, 1.04)	(0.96, 1.17)	(0.97, 1.04)	(0.97, 1.02)	(0.98, 1.03)	(0.95, 1.01)

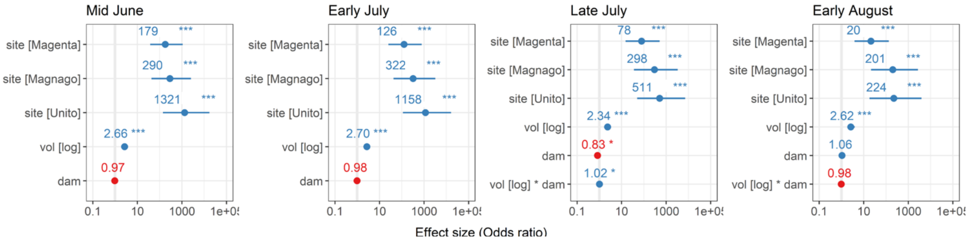

Figure 4. Odds-ratios of effect size of explanatory variables of the models with the lowest AIC per census, explaining successful raceme formation. Red dots/values <1 indicate that the effect is negative, blue dots/values >1 indicate that the effect is positive. The factor "site" with the corresponding site name in square brackets show the effect size of site compared to Busto. Plant volume "vol" (in cm^3) is log-transformed for the analysis, leaf damage in percent is abbreviated with "dam". In models with interaction between leaf damage in percent and volume, the effect size of this factor is described as "vol[log]*dam".

when an interaction term with volume and the number of *O. communa* individuals was included (see Table 2, Figure 5). Including percent bare soil did not increase any model fit. In contrast to the models for successful seed formation, none of the models

Table 2. Delta AICc values (upper number) compared to the model with the lowest AICc value, pseudo r-squared (second number), odds ratio for the *O. communa* related factor (third number), and confidence interval of the odds ratio for the *O. communa* related factor (lowest number) for models showing correlation between chance of successful seed formation (left part of table) and total seeds produced (right part of table) and explanatory factors at different censuses. Models, where the confidence interval of the odds ratio for the effect size of the *O. communa* related factor does not cross 0, are shaded. Models including interactions with volume and the explanatory factor are marked with '*'. Corresponding p-values can be found in Suppl. material 3.

Factor	Probability of seed formation dependent on…					Number of seeds produced dependent on…				
	Mid-June	Early July	Late July	Early August	Late August	Mid-June	Early July	Late July	Early August	Late August
No *O. communa* parameter	69	70	63	63	51	9.0	9.0	10	1.2	9.5
	0.16	0.17	0.16	0.21	0.16	0.85	0.86	0.85	0.86	0.85
# egg batches	66*	66*	61	28*	51*	9.5	12	13	1.1	13
	0.21	0.23	0.16	0.32	0.19	0.85	0.86	0.85	0.85	0.85
	1.79	0.00	1.30	0.03	0	1.19	1.06	1.16	0.91	1.04
	(0.83, 3.87)	(0, 4.51)	(0.76, 2.23)	(0.00, 0.44)	(0, inf)	(0.95, 1.49)	(0.85, 1.33)	(0.77, 1.73)	(0.82, 1.01)	(0.15, 6.99)
# larvae	60*	71	57	28*	26*	12	11	13	4.4	13
	0.26	0.18	0.17	0.32	0.40	0.84	0.86	0.85	0.83	0.85
	0.00	1.17	1.31	0.00	0.00	1.08	1.08	1.05	1.07	1.00
	(0, inf)	(0.90, 1.54)	(0.79, 2.18)	(0, 0.79)	(0, 0.27)	(0.54, 2.16)	(0.92, 1.25)	(0.71, 1.55)	(0.68, 1.69)	(0.77, 1.30)
# adults	68*	64	58	28*	31*	12	11	10	3.9	12
	0.19	0.23	0.17	0.31	0.36	0.84	0.86	0.87	0.84	0.85
	0.20	2.74	1.22	0	0.14	0.95	1.15	1.12	1.04	1.02
	(0, 109.11)	(1.15, 6.53)	(0.90, 1.65)	(0, 0.58)	(0.03, 0.74)	(0.75, 1.20)	(0.91, 1.45)	(0.97, 1.30)	(0.93, 1.16)	(0.95, 1.09)
% leaf damage	56*	59*	48*	1.9*	**0**	12	6.8	12	3.2	**0**
	0.29	0.27	0.30	0.51	0.57	0.83	0.88	0.86	0.84	0.90
	0.40	0.68	0.50	0.06	0.93	1.02	0.98	0.99	0.96	0.98
	(0.19, 0.83)	(0.51, 0.92)	(0.31, 0.80)	(0.01, 0.35)	(0.89, 0.98)	(0.87, 1.19)	(0.96, 0.99)	(0.97, 1.01)	(0.88, 1.04)	(0.97, 0.99)

with the lowest AICc values for number of seeds produced by successfully reproducing plants included an *O. communa* abundance parameter (Table 2).

Effect of percent leaf damage by *O. communa* on *A. artemisiifolia* reproduction

Including percent leaf damage by *O. communa* in models for successful raceme formation generated the models with the lowest AICc values for late July and early August, and including percent leaf damage by *O. communa* measured in late July generated the respective model with the lowest AICc value for raceme length of plants that successfully formed racemes (see Table 1). In those cases where damage reduced the probability of raceme formation, an interaction term with volume increased the fit of the models. The significance of the leaf damage x plant volume interaction term in late July for the probability of raceme formation could be explained when focusing on plants

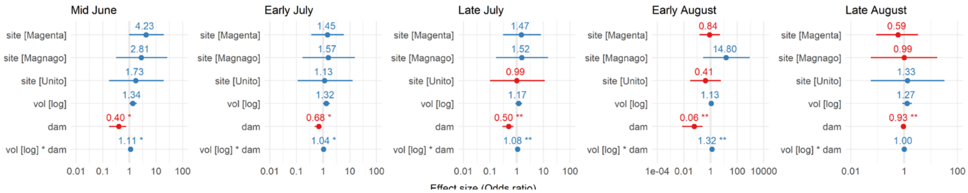

Figure 5. Effect size of explanatory variables of the models with the lowest AIC per census, explaining successful seed formation. Red dots/values <1 indicate that the effect is negative, blue dots/values >1 indicate that the effect is positive. The factor "site" with the corresponding site name in square brackets shows the effect size of site compared to Busto. Plant volume "vol" (in cm^3) is log-transformed for the analysis, leaf damage in percent is abbreviated with "dam". In models with interaction between leaf damage in percent and volume, the effect size of this factor is described as "vol[log]*dam".

with higher percent leaf damage. Plants with 1–33% leaf damage were on average smaller than plants with 34–66% leaf damage (see Figure 6A).

With regard to the models for successful seed formation, all models with the lowest AICc values calculated from mid-June to late August included percent leaf damage by *O. communa*. Of the *A. artemisiifolia* plants with more than 10% leaf damage in mid-June (19.7% of all plants), none produced seeds at the end of the season (Table 2). Likewise, only 4% of the plants with more than 10% leaf damage in early August produced seeds at the end of the season. In contrast, 14.5% and 40% of the plants with no leaf damage in mid-June and early August, respectively, produced seeds at the end of the season. Models including percent leaf damage measured in early July and late August also resulted in the respective models with the lowest AICc values for the number of seeds produced by successfully reproducing plants (Table 2). In particular, including percent leaf damage in models for successful seed production generated the lowest AICc values for all censuses from mid-June, when average leaf damage was approximately 5%, to the end of August, when almost all plants were defoliated to 80–100% (Table 2, Fig. 2).

In general, models for the successful formation of racemes and seeds that included percent leaf damage had lower AICc values than those that included *O. communa* abundance parameters (Tables 1, 2). For successful raceme formation, the effect size of sites was much higher than other explanatory factors. In contrary, the effect size of site was comparable to damage, volume or damage*volume interactions for successful seed formation (Figures 4, 5). For the plants that successfully formed seeds, we found that models including damage in early July and late August explained the number of seeds produced best, and plants with more damage produced less seeds or racemes (see Table 2). Including percent bare soil did not increase any model fits.

Interaction of *O. communa* abundance or damage with plant volume

In 18 out of 20 cases, the models including an interaction of plant volume and *O. communa* abundance or damage improved the model fit for successful seed formation, and in the two cases where *O. communa* abundance or damage improved the models

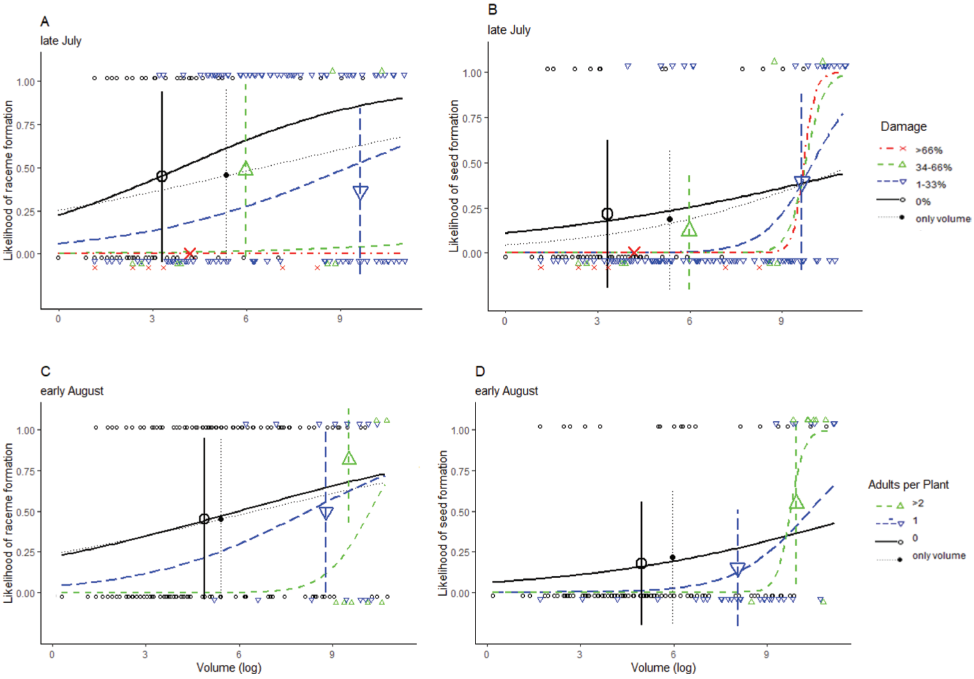

Figure 6. Likelihood of successful raceme (**A, C**) and seed formation (**B, D**) dependent on plant volume. In **A** and **B** the data are presented for four damage classes and in **C** and **D** for three *O. communa* infestation classes (0,1, or ≥2 adults per plant). The large symbols give median volume and mean probability of raceme or seed formation, respectively, together with their associated standard errors. "Only volume" shows the average values without consideration of damage or abundance classes.

for successful raceme formation, the model included an interaction of abundance or damage with plant volume. To explore the nature of these interaction terms, we displayed the interactions graphically, splitting the data into groups (by level of damage or abundance) and plotted the probability of successful raceme formation against the log of plant volume (Fig. 4). We chose late July and early August as time points for exploration, since the models with the lowest AICc values for both successful raceme and seed formation contain an interaction term with volume at these points in time, and the relationship between adult abundance and successful raceme formation was positive in late July, contrary to all other cases. Overall, leaf damage decreased and the number of adult beetles increased with plant volume, except for plants with 0% leaf damage, which were generally very small in size. The chance of raceme formation increased with plant size and decreased with percent leaf damage and the number of adults per plant.

Explained variation

The models with the lowest AICc values within one census also had the highest pseudo R-squared values. *Ophraella communa* presence and/or damage explained

Table 3. Number of plants with a certain % leaf damage producing seeds at the end of the season. Given are the number of plats within a certain damage category producing seeds / total number of plants within this damage category.

% Damage	Mid-June	Early July	Late July	Early August	Late August	Late September
0	12/83	8/59	14/66	14/35	11/18	9/14
1–10%	22/92	22/103	12/76	19/90	7/11	7/9
11–20%	0/23	4/29	6/20	2/22	0/2	4/8
21–30%	0/8	0/7	0/7	0/4	2/2	2/4
31–40%	0/4	0/3	2/3	0/4	3/3	0/3
41–50%	0/1	0/2	0/2	0/3	1/3	0/1
51–60%	0/3	0/4	0/3	0/4	2/3	0/1
61–70%	0/3	0/0	0/0	0/3	2/4	1/4
71–80%	0/1	0/1	0/1	0/1	4/5	0/6
81–90%	0/0	0/1	0/2	0/2	2/17	1/5
91–100%	0/0	0/4	0/3	0/3	1/89	5/61

the likelihood of seed formation better than the likelihood of raceme formation. Including leaf damage increased the pseudo-R^2 value of models for successful seed formation much more (max. 40%) than for raceme formation (max. 6%). In contrast, including *O. communa* abundance parameters hardly improved the pseudo-R^2 value of models of seed numbers (max. 4%) or raceme length (max. 2%). For probability of both raceme and seed formation, models had much lower AICc values and higher pseudo R-squared values from early August on. In general, *O. communa* induced leaf damage and abundance explained more variation the closer it was assessed to the flowering time.

Discussion

Our study provides evidence that the level of in-season leaf damage by *O. communa*, in combination with plant volume and site, helps to explain final seed production. Six weeks before flowering, leaf damage by *O. communa* together with plant volume is correlated to pollen production by *A. artemisiifolia* at the end of the season. Explanatory power of models improved over the season. Models including leaf damage had generally higher explanatory power than models including *O. communa* abundance parameters. For successful raceme formation, experimental sites had a much higher explanatory power than leaf damage, but for seed formation, explanatory power of leaf damage was similar to explanatory power of site, with lower variation. This offers possibilities to use in-season leaf damage for developing impact forecast models, which help informing management whether biological control is likely to successfully reduce seed production of this invasive alien plant species in a given region or year, or whether complementary management interventions should be considered to achieve long-term population decrease.

In-season *Ophraella communa* abundance and damage and plant mortality

The peak in *O. communa* abundance in early August coincides with the expected timing of the fourth and last generation in this region (Mouttet et al. 2018). We did not observe population growth of *O. communa* until late July, probably due to the relatively low densities of the different life stages and considerable variation within censuses. The lack of egg batches from September onwards is most likely due to a photoperiod-induced diapause in reproduction, as described for *O. communa* in Japan (Tanaka and Murata 2017).

While damage increased significantly in August at all sites, there was considerable variation in average leaf damage among sites (Table 2). In particular, average damage in Busto Arsizio increased to > 25% within the first three weeks of the study and was at least 25% higher than the site with the next highest average damage in early August (Magenta; Fig. 2). The high average damage in Busto Arsizio can be explained by the observations that small plants were defoliated quickly and died earlier than the extraordinarily large plants (> 2m) on this ex-arable site, while the large plants also out-shaded the smaller ones (Fig. 3). Intraspecific competition between *A. artemisiifolia* plants has been shown to have a negative effect on leaf area and aboveground biomass (Patracchini et al. 2011), and herbivory could amplify these effects by reinforcing competitive interactions (Crawley 1983).

It should be noted that our study did not cover the very first months of the growing season of *A. artemisiifolia*. In Northern Italy, gravid *O. communa* females that have overwintered start laying eggs on *A. artemisiifolia* seedlings as soon as the temperature is high enough for the beetle to fly (Bosio et al. 2014). At the study sites, first egg batches on *A. artemisiifolia* seedlings were observed from early April onwards (H. Müller-Schärer, unpublished results). Mortality of plants in spring was not covered by our experimental set-up, but is likely to further increase the impact of *O. communa* on *A. artemisiifolia* at the population level (Lommen, unpublished results).

Significant impact on target weed populations is only expected with high densities of biological control agents (Myers and Sarfraz 2017, McEvoy 2018), and the outcome of our experiment supports this notion. Defoliating *A. artemisiifolia* plants up to 90% by clipping leaves did not influence reproductive traits under laboratory conditions (Gard et al. 2013). Similarly, Lommen et al. (2017a) found that 90% defoliation of adult plants by *O. communa* in laboratory experiments did not reduce pollen production, as long as the racemes were not attacked. In our study, average leaf damage by *O. communa* only reached more than 90% in late August, which coincided with increased plant mortality.

Effect of in-season *O. communa* abundance and damage on *A. artemisiifolia* raceme and seed production

With regard to the probability of both raceme and seed formation, *O. communa* leaf damage appears to be a better explanatory variable than *O. communa* abundance, since

AICc values were lower for models including damage than those including abundance for all census dates. This could be due to the behaviour of the beetle; *Ophraella communa* adults are highly mobile (Yamanaka et al. 2007) and larvae have been observed to regularly move between plants under laboratory and outdoor conditions (Stéphanie von Bergen, personal communication). So, while leaf damage reflects the cumulative effect of the feeding activity of the beetle over time, beetle abundance represents rather a certain point in time. In weed biocontrol, impact assessments often focus on the abundance of biological control agents that are required to control a certain number of plants. For example, the impact of different life stages of *Zygogramma bicolorata* on *Parthenium hysterophorus* (Shabbir et al. 2016), or the estimation of *O. communa* impact on *A. artemisiifolia* in China (Guo et al. 2011, Chen et al. 2013) were estimated in number of adults per plant. This may be useful information when the herbivore load per plant is augmented by mass-releasing biological control agents, but it remains difficult to estimate absolute abundances of insects in field settings (Fowler and Witter 2017). Furthermore, the per capita impact may vary depending on the plant size (Biere et al. 2017), shading conditions (Muth et al. 2008), nutrient composition of the plant leaves (Zehnder and Hunter 2009), or the amount of induced defence compounds in the plants (Burghardt and Schmitz 2015). Our results suggest that for the leaf beetle *O. communa*, and probably other mobile insect herbivores, in-season damage levels may provide a more robust and easier-to-quantify variable for projecting potential impact on reproductive output of *A. artemisiifolia* at the end of the season. Similarly, early season feeding damage of the bug *Bagrada hilaris* on broccoli was also found to be a reliable and accurate variable for monitoring this pest in broccoli fields (Palumbo and Carrière 2015).

All but one model in which *O. communa* abundance explained the probability of reproductive organ formation contained an interaction with plant volume. Plant volume influences the response of the plant to abundance of herbivores or herbivore-induced leaf damage. In line with Lommen et al. (2018b), volume was positively correlated with probability of reproductive organ formation (see Figure 4, 5).

In general, we found more adults on bigger plants, probably explained by a positive effect of plant volume on adult beetle abundance, rather than a positive effect of *O. communa* abundance on plant size. Caged experiments with varying plant sizes and number of adults could shed some additional light on the potentially interacting effects of plant volume and *O. communa* impact.

The models for the likelihood of seed formation generally had higher pseudo R-squared values than the models for the likelihood of raceme formation (Tables 1, 2), indicating a larger amount of variation in the models for successful raceme formation remaining unexplained. Experimental site had a much higher effect size than damage or volume to explain chance of raceme formation, but for the chance of seed formation size effects of site and volume, damage or volume*damage interactions were comparable (see Figure 4, 5). This means that the effect of volume and leaf damage could be used to explain successful seed formation, but for successful raceme production, other factors might be more important. We observed that the mortality between late August (sampling time for racemes) and late September (sampling time for seed formation) increased in the plants that were not treated with insecticides compared to those treated in three of the four sites (Fig. 3). We can explain why models for successful seed formation have less unexplained variation than models for raceme formation, if we assume that plant mortality is damage-dependent, but only obvious in late September. In that case, raceme formation would be more influenced by other factors that are not included in our model.

Our study provides evidence that the window of impact by *O. communa* on reproductive output of *A. artemisiifolia* is relatively narrow (see Fig. 2). Similarly, two biological control agents released to control *Clematis vitalba* in New Zealand only induced disease symptoms late in the season, resulting in no significant impact on plant growth and only minor reduction of area covered by the invasive weed (Paynter et al. 2006), and the impact of two biological control agents of *Parthenium hysterophorus* had different levels of impact between 1996 and 2000 depending on weather conditions (Dhileepan 2003). Thus, in regions or years with less favourable climatic conditions, the population peak of *O. communa* may be delayed or reduced to an extent that the impact of this biological control agent on the reproductive output of *A. artemisiifolia* is considerably impaired.

Arthropod demography is strongly influenced by climate, especially temperature, where an increase often results in quicker population growth. Since overall damage is strongly dependent on the number of generations, and as these are expected to increase with temperature in species with a multivoltine life cycles, damage is also expected to increase in a warming climate in the future (Möller et al. 2017). However, if rising temperatures leads to reduced relative humidity, climate change may also have a negative effect on population build-up of *O. communa*. Laboratory studies revealed that relative humidity of less than 50% during the warmest time of the day significantly reduced egg hatching rates of this biological control agent (Augustinus and Sun et al. 2020). Since biocontrol agent impact is dependent on high population densities (Myers and Sarfraz 2017, McEvoy 2018), both temperature and humidity should be considered for models predicting *O. communa* impact on *A. artemisiifolia* (Augustinus and Sun et al. 2020).

Implications for *A. artemisiifolia* management

Our findings that average leaf damage from mid-June onwards explained a significant amount of variation in the likelihood of seed formation indicates that *O. communa* feeding has a direct detrimental effect on female reproduction in *A. artemisiifolia*. Moreover, while the negative effect of leaf damage on the likelihood of pollen production only was significant in the census made in late July, *O. communa* exclusion experiments conducted in the same area revealed that *O. communa* reduces pollen production per unit area by 82% (Lommen et al. unpublished results). These findings are in line with an observed 80% decrease in airborne ragweed pollen counts in the Milano region since the establishment of *O. communa* (Bonini et al. 2015a, Bonini et al. 2015b), also during the year in which this study was conducted.

Hence, our findings suggest that percent leaf damage in mid-June or early July could be used as an indicator for the likelihood that *O. communa* significantly reduces reproductive output of *A. artemisiifolia* at the end of the season (see Suppl. material 5). At three out of four sites, plants with more than 10% leaf damage in mid-June or early July had a very low likelihood of seed formation. The plants with more than 10% leaf damage in early July that produced seeds were all large plants (91–181 cm high in early July) growing at the Busto Arsizio site. At this site, where leaf damage had a less pronounced negative effect on seed and raceme formation, plants were far taller than at most other sites in Northern Italy. Apparently, plants with such an extraordinarily high volume are able to collect enough reserves to survive and produce seeds successfully before the stark increase of damage by *O. communa* in early to late August, while smaller plants cannot compensate for the damage caused by the beetle. Developing an *O. communa* damage forecasting model based on average leaf damage and plant volume in early summer may be used to support decision making regarding the scheduling of additional common ragweed management interventions. When population build-up is predicted not to be early and high enough to prevent flowering, mass releases through mass-rearing, as practiced in China (Guo et al. 2011, Chen et al. 2013), could be envisaged. Also, as *A. artemisiifolia* can form dense stands on fallow crop fields (Lehoczky et al. 2013, Ottosen et al. 2019), prospective management methods could be additional mowing, ploughing or mulching of the fields just before male flower formation. Decreasing these stands mechanically would result in less pollen production in a direct way, by removing these plants from the flowering part of the *A. artemisiifolia*, and indirectly by forcing *O. communa* to other *A. artemisiifolia* plants that are less accessible for mechanical control. Timing of mowing or herbicide applications along linear transport infrastructures (railways, roads) might also be a cost-efficient means of managing *A. artemisiifolia*, but at least three mowing interventions are needed (Lommen et al. 2018c). Experiments combining mowing regimes and beetle presence on *A. artemisiifolia* are needed to better clarify the joint effect on the control of this species.

Conclusion

This study provides evidence that the level of in-season leaf damage by *O. communa* helps to explain the impact of this biological control agent on seed and – to a lesser extent – pollen production by *A. artemisiifolia* at the end of the season. Leaf damage measured as early as mid-June partially explains, in combination with plant volume, the likelihood of reproductive output of *A. artemisiifolia* at the end of the season. For example, none of the plants with more than 10% leaf damage in mid-June formed seeds at the end of the season. It should be noted, though, that at extreme sites where *A. artemisiifolia* plants grow 2 m and taller (such as at Busto Arsizio), impact of *O. communa* may be largely explained by plant volume, rather than by average leaf damage in early summer. Our results suggest that in-season assessment of leaf damage and plant volume could be used to develop predictive models for *O. communa* impact on *A. artemisiifolia* seed production, similar to the approach used in crop pest forecasting.

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Supplementary material 1

Study sites

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Data type: species data

Link: https://doi.org/10.3897/neobiota.55.46874.suppl1

Supplementary material 2

Census dates

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Data type: measurement

Link: https://doi.org/10.3897/neobiota.55.46874.suppl2

Supplementary material 3

p-values for *O. communa*

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Data type: statistical data

Link: https://doi.org/10.3897/neobiota.55.46874.suppl3

Supplementary material 4

Figure S1. Mean plant volume ± se of *A. artemisiifolia* plants measured during the experiment in the four experimental sites

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Data type: statistical data

Link: https://doi.org/10.3897/neobiota.55.46874.suppl4

Supplementary material 5

Figure S2

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Explanation note: Likelihood of *A. artemisiifolia* seed formation dependent on *O. communa* leaf damage in early July. The different line types show the different responses between the sites.

Link: https://doi.org/10.3897/neobiota.55.46874.suppl5

Supplementary material 6

Successful raceme formation

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Explanation note: Summaries of selected glms, with successful raceme formation depending on different *Ophraella communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*, per census.

Link: https://doi.org/10.3897/neobiota.55.46874.suppl6

Supplementary material 7

Raceme length

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected linear models, with raceme length depending on *O. communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*.

Link: https://doi.org/10.3897/neobiota.55.46874.suppl7

Supplementary material 8

Damage ~ abundance

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected glms, with leaf damage depending on *Ophraella communa* abundance parameters.

Link: https://doi.org/10.3897/neobiota.55.46874.suppl8

Supplementary material 9

Successful seed formation

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected glm(m)s, with successful seed formation depending on *Ophraella communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*.

Link: https://doi.org/10.3897/neobiota.55.46874.suppl9

Supplementary material 10

Number of seeds

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected lms, with number of seeds produced dependent on *Ophraella communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*.

Link: https://doi.org/10.3897/neobiota.55.46874.suppl10