NeoBiota 36: 1–16 (2017)
doi: 10.3897/neobiota.36.11418
http://neobiota.pensoft.net

DATA PAPER

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Plant parasitic nematode survival and detection to inform biosecurity risk assessment

Lee T. Aalders[1,3], Mark R. McNeill[2,3], Nigel L. Bell[1,3], Catherine Cameron[1]

1 *AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand* **2** *AgResearch, Lincoln, Private Bag 4749, Canterbury 8140, New Zealand* **3** *Better Border Biosecurity*

Corresponding author: *Lee T. Aalders* (lee.aalders@agresearch.co.nz)

Academic editor: *R. Shaw* | Received 7 December 2016 | Accepted 23 May 2017 | Published 26 June 2017

Citation: Aalders LT, McNeill MR, Bell NL, Cameron C (2017) Plant parasitic nematode survival and detection to inform biosecurity risk assessment. NeoBiota 36: 1–16. https://doi.org/10.3897/neobiota.36.11418

Abstract

Plant parasitic nematodes (PPN) are known to survive periods of desiccation, an ability that increases the risk of them surviving unintentional transport between countries. To investigate nematode survival in soil subject to prolonged storage, soil collected from a native forest and an organic orchard was stored separately in cupboards at ambient temperature for 36 months. Subsamples were taken at 0, 3, 6, 12, 13, 24 and 36 months to determine the presence of plant parasitic and total nematodes using a standard misting technique. *Pratylenchus* was used as a model to determine if PPNs that had been under prolonged storage were able to infect plant hosts at 13, 24 and 36 months.

Overall, the total number of nematodes recovered from stored soil declined over time, with differences in species diversity determined by molecular methods, related to soil origin. No PPN were recovered in soil stored beyond 13 months using the three-day misting technique. By comparison, *Pratylenchus* nematodes, using a baiting method, were found to successfully invade host plant roots (ryegrass and white clover) even after 36 months storage and were observed to produce offspring at 13 months. Baiting was not effective for *Pratylenchus* found in soil originally collected from the forest but was for orchard soil, a result attributed to the lack of suitable host plants for the *Pratylenchus* species found in forest soil.

This study demonstrated, that in protected environments, nematodes could survive for at least 36 months and were observed to produce offspring at 13 months. Baiting with a host plant was more sensitive in detecting nematodes than using the misting extraction technique, although this approach only works where the host plant is known. Without *a priori* knowledge of the nematode-plant host association, plant baiting may also produce false negatives. In the context of plant biosecurity and providing an accurate risk assessment in soil contaminants, the development of a generic test for PPN that induces nematodes in a resting stage to emerge and respond to a cue would enhance the probability of detection. However, as assessments at the border are often time limited, a molecular based bioassay that can be used to indicate the presence of multiple species of live PPN species may be a more feasible option for risk assessments.

Keywords
invasion pathways, soil risk, plant biosecurity, screening tool, international trade, molecular diagnostics, invasive pests

Introduction

Trade and tourism are important to the economic wellbeing of the world's global economy, but carry with it the real risk of introducing unwanted organisms that threaten the productive sectors of individual countries or regions because of production losses due to direct yield reduction or cost for pest control (Mack et al. 2000, Pimentel et al. 2000, Work et al. 2005, Hulme 2014). For natural habitats and native biota, invasive species can have both direct and indirect impacts through modification, displacement or eradication costs along with a general loss of biodiversity (Lee and Lee 2015). Impacts can therefore be economic, ecological and social with the impacts and costs prolonged for intractable invasive species. Plant parasitic nematodes (PPN) are estimated to cause billions of dollars (USD) of crop damage worldwide each year, many of which have known or potential phytosanitary importance (Singh et al. 2013).

Amongst PPNs there are three main types of parasitism, ectoparasitic, endoparasitic and semi-endoparasitic (Decraemer and Hunt 2006). For ectoparasites (e.g. *Paratylenchus*, Criconematidae), the nematodes remain within the soil feeding externally on plant roots. Two types of endoparasites feed within the roots; there are migratory ones such as *Pratylenchus* which do not form a permanent feeding site and can move in and out of the plant; and sedentary ones such as *Globodera* and *Heterodera* which form a permanent feeding site except for the infective second stage juveniles which are mobile within the soil. For semiendoparasites, such as *Helicotylenchus*, only the anterior portion of the nematode penetrates the root with the posterior portion remaining in the soil (Decraemer and Hunt 2006).

While their minute size and cryptic nature in plants and soil makes discovery more difficult when transported, the ability of many PPN species to survive periods of desiccation (Norton 1978), makes these nematodes a biosecurity issue as it increases the probability of establishment when inadvertently transported from one country to another via trade and tourism routes. PPNs have been detected in soil associated with shipments of imported seeds (Lal and Lal 2006), plant material (Tenente et al. 1996), contaminated footwear (McNeill et al. 2011), used machinery (Hughes et al. 2011, Aalders et al. 2012) and sea containers (Gadgil et al. 2000, McNeill et al. unpublished data).

In an effort to improve predictions on which PPN species will become invasive in a country or region before they arrive, a Pest Screening and Targeting (PeST) framework has been developed to provide a more structured and systematic approach for screening large numbers of species and identifying species likely to become invasive (Singh et al. 2015). PeST integrates heterogeneous information and data on species biogeography, biotic and abiotic factors to first determine a preliminary risk index. While species with better survival adaptations pose greater risks than those without the capability, a paucity of information on survival in transit represents an important knowledge gap when developing a pest risk profile for PPNs (Singh et al. 2015).

In this current study, soil was collected and stored in cupboards to mimic soil contaminants that may be stored in a protected environment (e.g. contaminated footwear, used equipment or camping gear) for a period of time before reuse. The study assessed not only nematode survival but the viability of nematodes recovered from soil that had been stored in a cupboard over a 36 month period. While the research commenced prior to the development of the PeST framework proposed by Singh et al (2015), this work provides a valuable contribution to our understanding of nematode survival over time. The hypothesis tested was that nematodes surviving long term desiccation would be able to subsequently invade plant roots ('baiting'), thus presenting a heightened biosecurity risk.

Methods

Collection and processing of soil

Soil was collected in late winter (23 August 2011) from two sites in the Canterbury region of New Zealand; a native forest reserve in Prices Valley, Banks Peninsula (S43.7669, E172.7140) and an organic orchard at Lincoln (S43.6508; E172.4559). At each site, a spade square soil sample (140 mm × 140 mm) was taken to a depth of 5 cm at three randomly chosen points within a 3 m radius of an arbitrarily designated central point. The soil sampled from these three locations at each site were treated separately throughout the experiment. Any vegetation was cut to ground level with scissors and loose litter was removed from the sample point prior to collection. The individual soil samples were mixed separately in a stainless steel tray and transferred to a plastic bag. The spade was cleaned with 70% ethanol between each site and location. Disposable laboratory gloves were worn at all times, and changed between sites. The work presented in this paper is part of a project published in McNeill et al. (2017), methods for collecting and processing the soil are the same as described in that paper.

In the laboratory, the soil was sieved (10 mm sieve) and a subsample taken for nematode counts and identification. The remaining soil from each site and sampling location (six individual soil sources) was divided amongst stainless steel steam trays (dimensions c. 400 × 200 × 50 mm (300 mm × 240 mm internal dimensions)), in which twenty × 4 mm drainage holes had been drilled into the base, then allocated to treatments (c. 700 g of soil per tray). The soil was spread roughly evenly onto the tray surface and gently pressed with a stainless steel pan to lightly compact the soil, resulting in a soil depth of approximately 40 mm (McNeill et al. 2017). Soil from all sites contained plant root material.

The uncovered tray was then placed within a cupboard situated indoors at ambient temperature at Lincoln (S43.6279, E172.4704). The soil in the trays was subsampled at 3, 6, 12, 13, 24 and 36 months. Approximately 75 g soil was collected from each tray using a stainless steel spoon and placed in a 100 ml plastic screw cap container. The spoon was cleaned with 70% ethanol between sampling each tray.

In addition to the above, the two original locations were resampled at 3, 6, 12, 13, 24 and 36 months. This was to monitor the natural population in relation to counts taken from the stored soil to ensure that any decrease in population was due to storage. As per the original sampling strategy, at the three locations within each of the two original sites, soil was collected using 20 × 25 mm diameter × 100 mm deep cores, hand crumbled and mixed.

Extraction methodology

There are a range of accepted nematode extraction techniques (Hooper 1986, Hooper and Evans 1993, Hunt and De Ley 1996). Techniques are classified as either active methods such as the Whitehead and Hemming tray and misting, or passive such as centrifugation and flotation - sieving (McSorley and Walter 1991, Hooper and Evans 1993, Hunt and De Ley 1996). The misting method was chosen because it provided the capability for high throughput of the large number of samples. The limitation was that the method would not have extracted nematode cysts, but was the most efficient and cost effective method available.

Nematode survival and viability

To determine nematode survival over the duration of the study, at each storage time, a 25 g soil subsample was placed in a mistifier funnel for extraction and misted for 30 sec every 5 min over 72 hours at a water temperature of 20 °C. The water from the mister flushes the nematodes through the soil and into a test tube where they are collected. For the original day zero samples 100 g of fresh soil was placed on to extraction trays (Bell and Watson 2001) and extracted over a 72 hr period. At the three month sampling time the two methods of extraction were compared and no significant difference found (data unpublished). For the 36 month bioassay, samples were extracted for an additional 48 hours, in the expectation that prolonged storage could result in poor physiological condition so that more time may be required to extract surviving nematodes. Counts were taken for all nematodes (bacterivores, fungivores, omnivores, predators and plant parasites); PPNs were identified to genera where possible.

The endoparasitic nematode *Pratylenchus* was the only nematode extracted from soil after 12 months, so at 13, 24 and 36 months, the ability of *Pratylenchus* to invade plant roots was tested using both white clover (*Trifolium repens* L.) and ryegrass (*Lolium* spp.) as bait plants. To determine viability, plastic pots (50 mm × 50 mm × 120 mm), were part filled with 140 g of oven dried sand and topped with 46 g of soil from each ca. 75 g sample. The six original sites were also sampled to check plant host suitability of the sown seed for the nematode species present. This resulted in 24 pots of cupboard soil and 12 pots of fresh soil collected from the original six sites. Each pot was sown with three nil-endophyte ryegrass *Lolium multiflorum* (cv. Moata for

2012) and *L. perenne* (cv. Samson for 2013 and 2014) and 6–8 white clover *Trifolium repens* (cv. Sustain) seeds. The pots were randomised, placed into two forestry crates, maintained in a 20°C controlled environment room with a light: dark photoperiod of 16: 8 hours and watered as required. The forestry crates enabled the pots to be held separately from each other and above the bench to avoid cross contamination.

Twenty four days post-sowing, the ryegrass and white clover seedlings were removed from each pot, gently washed to remove adhering soil and counted before the shoots and roots were separated and weighed. For each pot, ryegrass and white clover roots were stained using aniline blue (Rohan et al. 2006), to determine if *Pratylenchus* nematodes had infected the seedling roots.

Molecular identification of PPNs

DNA was extracted from single nematode specimens using the prepGEM™ tissue kit (ZyGEM Corporation Ltd, New Zealand) according to manufacturer's instructions.

DNA was amplified in 25 µl reactions using 1x buffer (Thermo Scientific Finnzymes), 0.2mM dNTPs, 0.3 µM of each primer, 0.2 mg /ml BSA and 0.5 units of Phusion Hot Start II Hi-Fi DNA polymerase (Thermo Scientific Finnzymes). Thermo cycling included an initial denaturing at 98 °C for 2 min, then 40 cycles of 98 °C for 10 sec, 57 °C for 30 seconds, and 72 °C for 60 °C, with a final extension step of 72 °C for 5 minutes. The product was purified using the GeneJET PCR Purification Kit (Thermo Scientific™). The fragments were sequenced by Massey Genome Service (Massey University, New Zealand) and cleaned using the computer programme Geneious™ 8.1.5 (Kearse et al. 2012). The sequences were compared to nematode sequences in the BLAST (Basic Local Alignment Search Tool) database (http://www.ncbi.nlm.nih.gov/blast.cgi).

Restriction fragment length polymorphism analysis (RFLPs) of the internal transcribed spacer (ITS) regions of ribosomal DNA was used to try and distinguish between the closely related *Heterodera* species to identify the *Heterodera* specimen isolated from the orchard soil in this study. Three reference sequences for each of *H. trifolii*, *H. schachtii* and *H. betae* were imported into Geneious to compare. *In silico*, the restriction enzyme *Msp*I generated a RFLP profile that showed the

Table 1. The primers used for sequencing of the plant parasitic nematodes.

Nematode taxa	Primer code	Amplified region of the rDNA gene	Reference
Criconematidae	SSU_F_07 / 18P	18S	(Blaxter et al. 1998)
Globodera / *Heterodera*	TW81 /AB28	ITS1 – 5.8S – ITS2	(Joyce et al. 1994)
Paratylenchus / *Pratylenchus* / *Helicotylenchus*/ *Rotylenchus*	D2A / D3B	D2 – D3 segment of the 28S	(De Ley et al. 1999)

sequence of this *Heterodera* nematode was not *H. schachtii*, but did not distinguish *H. trifolii* from *H. betae*. *H. trifolii* is widespread throughout New Zealand while *H. betae* has not been described from New Zealand.

Soil Moisture

To determine soil moisture at the 6 and 12 month bioassay, a separate 20 g sub-sample of soil was taken from each sample and oven dried at 80 °C for 48 hours. The availability of the remaining soil was limited at 13, 24 and 36 months, so soil moisture was determined using the 25 g of soil following processing in the mistifier funnel. As with the earlier samples, the soil was oven dried at 80 °C for 48 hours.

Temperature and humidity in the cupboards was measured using a Tinytag Ultra Temperature/Humidity logger (Gemini Data Loggers (UK) Ltd.).

Analysis

Data was analysed by split plot analysis of variance using GenStat (16th edition). Soil samples were the main plots and replicate trays the sub plots. Nematode data were log transformed to equalize the variance to better meet the normality assumptions of the analysis.

Results

Temperature and humidity in the cupboards averaged 12.5 °C (range 0.8–25.9 °C) and 76.9 % (38.4–100 %), respectively, over the course of the 36 month experiment. Soil moisture at the beginning of the experiment (day zero) was 34–38 % and 30–32 % for the forest and orchard soils respectively. At 13 months, the forest soil moisture ranged from 4.2–4.6 % compared to 3.3–3.5 % for the orchard soil ($P < 0.001$), with no significant change in moisture content from 13 to 36 months.

Total nematode numbers

The total number of nematodes extracted from the freshly collected forest and orchard soils was variable within each site (mean of 37.9 and 43.4/ g dry soil for forest and orchard, respectively), but with no significant difference between the two sites or the six different sampling times (Figure 1).

By comparison, for the stored samples, there was a difference between soil origin with the forest soil having significantly less nematodes than the orchard soil at 6, 12, 13 and 24 months ($P <0.001$) storage. After 36 months of storage, nematodes were only extracted from one sample and that was from orchard soil (1 of 12 trays) (Figure 1).

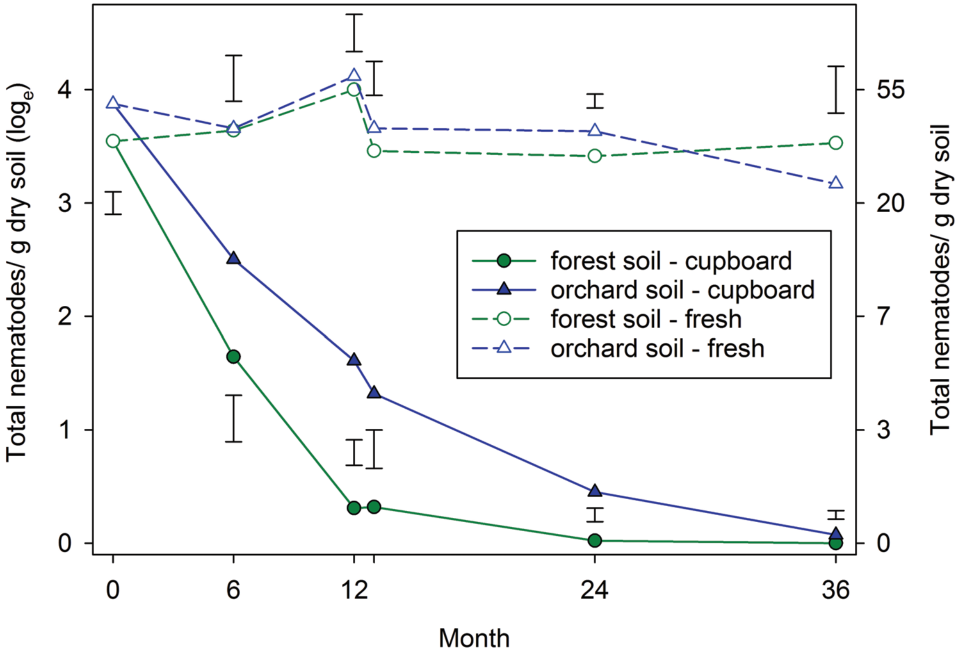

Figure 1. Mean total number of nematodes per gram of dry soil ($\log_e$ transformed) collected from either forest or orchard and stored in cupboards for up to 36 months or freshly collected from the original sites. Error bars are SEDs. Note: transformed data presented with back-transformed scale on right hand side for ease of conversion to actual numbers /g.

Plant parasitic nematodes

Fresh soil collected from the forest site contained the highest diversity of plant parasitic genera with a mean/g of dry soil of 1.9 *Pratylenchus*, 2.3 *Paratylenchus*, 0.3 *Globodera*, 0.3 *Helicotylenchus* / *Rotylenchus* and 0.1 for Criconematidae. By comparison, in the orchard soils, the plant parasitic genera consisted of 1.5 *Pratylenchus*, 1.8 *Paratylenchus* and 0.1 *Heterodera* spp. / g dry soil from the orchard site.

Over all sample times, *Pratylenchus* comprised 4.4% and 10.4% of the total nematode fauna in the forest and orchard soils, respectively. PPN populations were substantially larger in the fresh soil than were observed in stored soil (results not shown), especially so for the orchard samples.

Stored samples

Of the PPN taxa recovered at three months, *Pratylenchus* was the most common, found in seven of the twelve forest soil samples (58%) and in all of the orchard samples (12/12) (Table 2). Small numbers of *Paratylenchus*, *Globodera*, *Helicotylenchus* /*Rotylenchus* and Criconematidae were also recovered from the forest soil at three months.

Table 2. Age of soil from which plant parasitic nematode taxa were extracted using the three day misting technique, from 25 g of soil collected from either the forest or orchard location and stored in cupboards for 36 months.

	Months			
	3	**6**	**12 /13**	**24–36**
Forest				
Pratylenchus	Present	Present		
Paratylenchus	Present			
Globodera	Present			
Helicotylenchus / *Rotylenchus*	Present			
Criconematidae	Present	Present		
Orchard				
Heterodera	Present			
Pratylenchus	Present	Present	Present	
Paratylenchus		Present		

Heterodera were present in very low numbers from the orchard site, with 1–10 nematodes extracted at the 3 month sampling (3/12 samples) despite none being extracted from the original sample (day zero). At six months, with the exception of a single Criconematidae from the forest soil and two *Paratylenchus* nematodes from the orchard soil, *Pratylenchus* was the only plant parasitic nematode extracted (Table 2). At both the 12 and 13 month sampling, the only PPN recovered were *Pratylenchus* spp.

Thirteen months after soil had been placed into cupboards, *Pratylenchus* were the only PPN recovered using the misting technique, and then only from the orchard soil. Of those recovered, both female and juvenile stages were present.

The number of *Pratylenchus* recovered over time decreased substantially using a three day misting interval for extraction, with no specimens detected from soil stored for 24 and 36 months (Figure 2). An extra two days extraction time at 36 months did result in three *Pratylenchus* nematodes from one sample, all three of which were females.

Plant baiting

Sowing white clover and ryegrass seed resulted in *Pratylenchus* being recovered from more samples than with mistifier extraction at the 13, 24 and 36 month sampling intervals. At 13 months, *Pratylenchus* were found in four root samples (4 of 12 samples, 33%), but not in their respective misting samples. At 24 months, *Pratylenchus* were detected in five root samples (42%) and at 36 months in three samples (25%).

Reproductively mature *Pratylenchus* were evident in soil that had been stored for 13 months with eggs observed in white clover plant roots from two (c. 17%) of the stored orchard soil samples, demonstrating that not only could these nematodes survive in stored soil without a host plant but could also subsequently infect and reproduce in plant roots. No other PPN genera were detected using the plant baiting method.

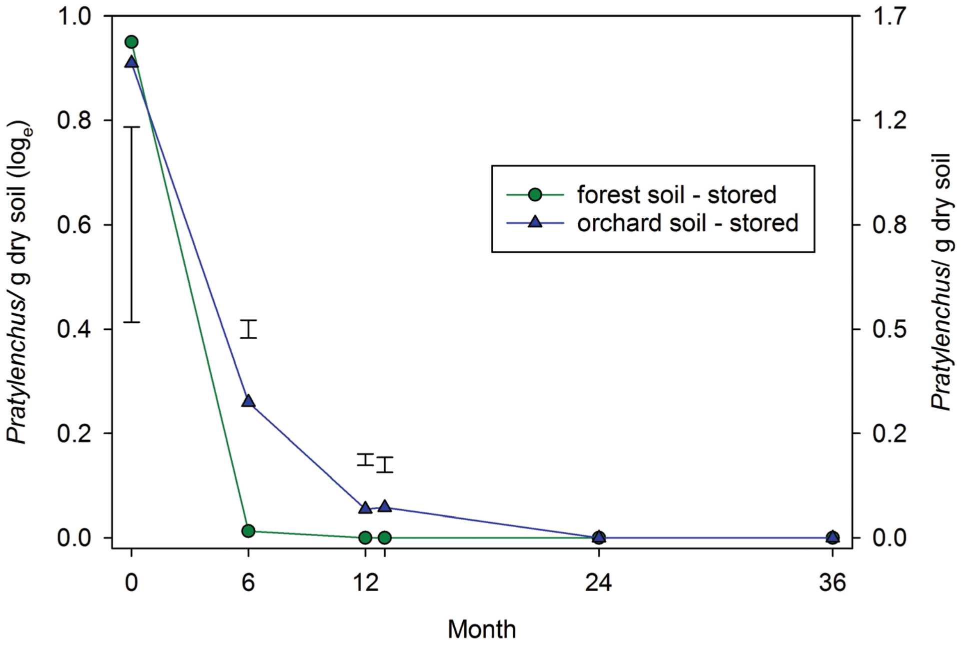

Figure 2. *Pratylenchus* per gram of dry soil (Log$_e$ transformed) collected from either forest or orchard and stored in cupboards for 36 months. Error bars are SEDs. Note: transformed data presented with back-transformed scale on right hand side for ease of conversion to actual numbers /g.

Molecular identification of PPNs

The *Pratylenchus* DNA sequences from the forest soil matched *P. bolivianus* from the NCBI database and specimens were preserved to be confirmed morphologically. *Pratylenchus* sequences from the orchard soil indicated the presence of at least four species: *P. crenatus*, *P. thornei*, *P. penetrans*, and an unidentified *Pratylenchus* that had a poor match to *Pratylenchus* currently in the database (Table 3).

Specimens found in the orchard soil at the start of the experiment were most commonly *P. penetrans* with the unknown *Pratylenchus* sp. also being isolated, while *P. crenatus* and *P. thornei* were only isolated once the soil had begun to desiccate (Table 4). Specimens *of P. crenatus* were isolated at six and 13 months after storage but not from the 36 month samples. *P. thornei*, along with the unidentified *Pratylenchus* sp., were isolated at 12 and 36 months post storage.

When comparing the number of *Pratylenchus* present in the roots of white clover grown in fresh soil collected from the two original sampling sites, there was a significant difference between the two locations at 13 months (P = 0.004), 24 and 36 months (both P<0.001). For ryegrass, the number of *Pratylenchus* present in the roots grown in fresh soil was significantly different (P<0.001), at all three sample times.

Fewer *Pratylenchus* were found in the white clover grown in the fresh forest soil samples with a mean, median and range of 5.2, 4 and 1–17, compared to the fresh orchard soil (130.7, 144 and 39–224, respectively).

Table 3. *Pratylenchus* specimens isolated and identified from orchard and forest soil using the closest matching BLAST reference (accessed August 2016).

***Pratylenchus* species**	**Soil source**	**BLAST reference**	**Match**
bolivianus[a]	forest	KP780256	99.9%
crenatus	orchard	KM580535	99.5%
penetrans	orchard	JX046990	99.9%
thornei	orchard	JX261954	99.9%
unidentified sp.	orchard	JX046999	92.0%

[a] to be confirmed morphologically

Table 4. *Pratylenchus* species extracted from orchard soil stored in cupboards and identified using D2/D3 primers for the 28S gene of rDNA.

Species	**Months**				
	3	**6**	**12 and 13**	**24**	**36**
P. penetrans	Present	Present			
P. crenatus		Present	Present		
P. thornei		Present	Present		Present[a]
Pratylenchus sp.		Present	Present		Present[a]

[a] five day extraction process

Table 5. Plant parasitic nematodes (excluding *Pratylenchus* spp.) isolated and identified from orchard and forest soil using the closest matching BLAST reference (accessed Aug 2016).

Plant parasitic nematode	**Soil origin**	**BLAST reference**	**Match**
Mesocriconema xenoplax	forest	KJ934180	96.3%
Rotylenchus conicaudatus	forest	HQ700698	93.8%
Globodera zelandica	forest	HQ260411	99.5%
Paratylenchus leptos	forest	KR270602	87%
Paratylenchus nanus	orchard	KF242196	100%
Heterodera trifolii[a]	orchard	LC030417	99.2%

[a]DNA sequencing did not give a distinction between *H. trifolii*, *H. schachtii* and *H. betae*.

Similar results were obtained for ryegrass growing in forest soil with a mean, median and range of 0.7, 0.5 and 0–3, respectively. This compared to a mean, median and range of 135.4, 115.5 and 24–291, respectively for orchard soils. For freshly collected forest soil, more *Pratylenchus* were recovered using the misting method than the baiting method.

For the forest soil, with the exception of *Globodera zelandica*, the PPNs were a poor match to the sequences found in the NCBI website (Table 5). Morphological and molecular identification found that the spiral nematodes observed in the samples were a mixed population consisting of both *Helicotylenchus* and *Rotylenchus* species. No *Helicotylenchus* specimens were sequenced.

For the *Heterodera* nematode extracted from the orchard soil, the DNA sequence did not give a clear distinction between *H. trifolii*, *H. schachtii* and *H. betae*. The sequence was compared to three reference sequences from NCBI of each species analysed in Geneious^{TM} using the restriction site *Msp*I. It produced a similar profile to *H. trifolii* and *H. betae* but not *H. schachtii*.

Discussion

This study has confirmed the hypothesis that not only are *Pratylenchus* species able to survive soil desiccation, but after prolonged storage are able to successfully reproduce on host plants.

The ability of nematodes to survive desiccation has been known for some time (Norton 1978). Nematodes that can achieve anhydrobiosis have been divided into two groups, slow-dehydration and fast-dehydration strategists (Womersley 1987). The majority of nematodes require a slow, controlled rate of water loss to achieve anhydrobiosis (Womersley et al. 1998). The soil in this study was stored in cupboards reducing air flow over the samples slowing the rate of desiccation, allowing any nematodes present that had the ability to survive water deficits to achieve anhydrobiosis. The survival of PPNs was greater in soil stored in cupboards than soil stored in sea containers (McNeill et al. 2017).

Nematodes have developed a number of means by which they can survive desiccation, which include survival stages such as eggs, cysts, and dauer larvae (Womersley et al. 1998; Wharton 2002). Nematodes from *Globodera* and *Heterodera* genera form cysts which can allow them to survive in the soil for many years, and some cyst species will not hatch unless stimulated by host root diffusates (Turner and Rowe 2006). This could potentially be the case for the *G. zelandica* and *Heterodera* juveniles that were extracted from the forest and orchard soil respectively that had been stored for three months. The other PPNs observed in this study, *Paratylenchus*, *Helicotylenchus*, *Rotylenchus* and Criconematidae also showed an ability to survive in stored soil albeit for a shorter period of time than *Pratylenchus*. Species from each of these genera have been found in previous studies to survive desiccation (Norton 1978). Other methods employed to slow the rate of water loss may include remaining in senescing plant tissue, swarming or forming aggregates and coiling (Womersley et al. 1998). Coiling has been observed in *P. penetrans* (Townshend 1984) and *P. thornei* (Glazer and Orion 1983). The soil in this current study included root fragments so it is possible they were a source of *Pratylenchus* nematodes able to withstand desiccation.

Pratylenchus species including *P. penetrans* and *P. thornei* have been recorded exhibiting anhydrobiosis (Glazer and Orion 1981; Townshend 1984; Townshend 1987; Anon 1997; Talavera et al. 1998; Ghaderi and Bideh 2011), but there is less information on the ability of *P. crenatus* to survive desiccation. Interestingly, *Pratylenchus crenatus* has been isolated from small quantities of soil associated with imported seed (Lal and Lal 2006). Survival of *P. penetrans* and *P. thornei* in air dried soil for up to 11 months has been previously recorded and listed in a review by Norton (1978). Talavera et al. (1998) found *P. thornei* was able to penetrate roots after 75 days of desiccation. The current study isolated *P. thornei* from soil stored in a cupboard for 36 months (1097 days), but as an unidentified *Pratylenchus* species was also found after 36 months of storage, it is unclear which of the two species infected perennial ryegrass roots in the "baiting" experiment. Townshend (1984) found *P. penetrans* in slowly dried soil could survive up to 770 days (25.3 months) and that their infectivity and reproduction at 207 days was not affected by anhydrobiosis. The current study did not however find *P. penetrans* in the soil beyond six months (180 days). Conversely, *P. crenatus* was detected in soil that had been stored for 13 months but not at 24 or 36 months. Of the known *Pratylenchus* species identified from this study all three have a broad host range, particularly *P. penetrans* (Castillo and Vovlas 2007; Singh et al. 2013). However, *Lolium perenne* and *L. multiflorum* are considered less favourable hosts (Kimpinski et al. 1984; Townshend et al. 1984) than legumes such as red (*Trifolium pratense* L.) (Willis et al. 1982; Kimpinski et al. 1984) or white clover (*T. repens* L.) (L. Aalders, unpublished data). The plant host preference of the unidentified *Pratylenchus* species isolated from the orchard soil has yet to be determined.

The *Pratylenchus* isolated from the forest soils and tentatively identified as *P. bolivianus,* was only detected up to six months. Both white clover and ryegrass proved to be unfavourable hosts for this *Pratylenchus* sp. with root infection rates considerably lower than numbers extracted from soil using the misting technique.

Pratylenchus crenatus, *P. penetrans* and *P. thornei* are each regulated pests for at least one country globally (Singh et al. 2013), and this study has shown that *P. crenatus* and an unidentified species of *Pratylenchus*, along with *P. penetrans* and *P. thornei*, can also survive prolonged periods of desiccation. According to a review by Jones et al. (2013), *Pratylenchus* are ranked third only to *Meloidogyne* and Heteroderidae (includes *Globodera* and *Heterodera*) nematodes as having the greatest impact on crops worldwide, and coupled with their ability to survive desiccation their status as a biosecurity risk is increased with more than 80 *Pratylenchus* species described (Siddiqi 2000).

The study showed that for disturbed soil stored in protected environments *Pratylenchus* nematode populations can survive prolonged storage for up to 36 months (1095 days) and that in the presence of a suitable host plant, 'baiting' was a more sensitive technique in detecting *Pratylenchus* spp. than the misting extraction technique. However, this study demonstrated that the approach only works if a suitable host plant is available. Without *a priori* knowledge of the PPN-plant host association, plant baiting may also produce false negatives. For other PPN, the lack of a suitable host plant meant that the mistifier extraction method was more accurate. Where the host plant was not known, this provided the best option to assess presence /absence, although this method may not extract cyst nematodes. Extraction using flotation /sugar centrifugation would have extracted cysts as well as vermiform stages but the technique was not feasible with the high numbers of soil samples. Furthermore, examining only the roots of bait plants for parasitic nematodes will only show those endo-parasitic species present, it cannot be used to assess external root feeding species, which would require that the soil surrounding bait plants is also checked.

In the context of plant biosecurity and providing an accurate risk assessment for soil contaminants, the development of a generic test for PPN that induces nematodes in a resting stage to emerge and respond to a cue would enhance the probability of detection. Having a better understanding of PPN survival in soil inadvertently transported with commodities, freight, used machinery or humans (e.g. footwear) is important in the development of both scientifically valid pest risk analysis as well as cost-effective management strategies (Colunga-Garcia et al. 2013, Singh et al. 2015, McNeill et al. 2017).

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors contribution

LA: Developed the research concept, led and carried out the extraction and identification of nematodes, contributed to manuscript writing. MM: Developed the research concept, carried out the soil sampling and contributed to manuscript writing, NB: Contributed to development of baiting technique, identification of nematodes and writing of the manuscript. CC: Analyzed data.

Acknowledgements

The research was funded by AgResearch via the Better Border Biosecurity research collaboration (www.b3nz.org). The authors thank Dr Barbara Barratt, Dr Scott Hardwick, Dr Alison Popay and Dr Michael Wilson (AgResearch) for reviewing the draft document.

References

Aalders LT, James TK, McNeill MR (2012) Excavators and dirt: assessing the quarantine risk posed by nematodes and seeds. New Zealand Plant Protection 65: 298 (poster abstract).

Bell NL, Watson RN (2001) Optimising the Whitehead and Hemming tray method to extract plant parasitic and other nematodes from two soils under pasture. Nematology 3: 179–185. https://doi.org/10.1163/156854101750236312

Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas WK (1998) A molecular evolutionary framework for the phylum Nematoda. Nature 392: 7175. https://doi.org/10.1038/32160

Castillo P, Vovlas N (2007) *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. Brill. https://doi.org/10.1163/ej.9789004155640.i-523

Colunga-Garcia M, Haack RA, Magarey RD, Borchert DM (2013) Understanding trade pathways to target biosecurity surveillance. NeoBiota 18: 103–118. https://doi.org/10.3897/neobiota.18.4019

De Ley P, Felix M-A, Frisse LM, Nadler SA, Sternberg PW, Thomas WK (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology 1: 591–612. https://doi.org/10.1163/156854199508559

Decraemer W, Hunt DJ (2006) Structure and Classification. In: Perry RNMM (Ed.) Plant Nematology. CABI PUBLISHING-C A B INT, Cabi Publishing, Wallingford 0x10 8de, Oxon, UK, 3–32. https://doi.org/10.1079/9781845930561.0003

Gadgil PD, Bulman LS, Crabtree R, Watson RN, O'Neil JC, Glassey KL (2000) Significance to New Zealand forestry of contaminants on the external surfaces of shipping containers. New Zealand Journal of Forestry Science 30: 341–358.

Ghaderi R, Bideh AK (2011) The effect of water stress on the survival of common cereal root-lesion nematodes (*Pratylenchus neglectus* and *P. thornei*), under laboratory, greenhouse, microplats and field conditions. Iranian Journal of Plant Pathology 47: e165-Pe177.

Glazer I, Orion D (1981) Anhydrobiosis a summer survival mechanism of *Pratylenchus thornei*. Nematropica 11: 82–83.

Glazer I, Orion D (1983) Studies on Anhydrobiosis of *Pratylenchus thornei*. Journal of Nematology 15: 333–338.

Hooper DJ (1986) Extraction of free-living stages from soil. In: Southey JF (Ed.) Laboratory methods for work with plant and soil nematodes. H M S O Books, Norwich, NR3 1PD, Norfolk, 5–30.

Hooper DJ, Evans K (1993) Extraction, identification and control of plant parasitic nematodes. In: Evans K, Trudgill DL, WJ M (Eds) Plant parasitic nematodes in temperate agriculture. CAB International, Wallingford, England, 1–59.

Hughes KA, Convey P, Maslen NR, Smith RIL (2010) Accidental transfer of nonnative soil organisms into Antarctica on construction vehicles. Biological Invasions 12: 875–891. https://doi.org/10.1007/s10530-009-9508-2

Hulme PE (2014) An Introduction to Plant Biosecurity: Past, Present and Future. In: Gordh G, McKirdy S (Eds) The Handbook of Plant Biosecurity. Springer Netherlands, 1–25. https://doi.org/10.1007/978-94-007-7365-3_1

Hunt DJ, De Ley P (1996) Nematodes in soils. In: Hall GS (Ed.) Methods for the examination of organismal diversity in soils and sediments. CAB International, Wallingford, UK, 227–240.

Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T, Manzanilla-López R, Palomares-Rius JE, Wesemael WML, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology 14: 946–961. https://doi.org/10.1111/mpp.12057

Joyce SA, Burnell AM, Powers TO (1994) Characterization of *Heterorhabditis* isolates by PCR amplification of segments of mtDNA and rDNA genes. Journal of Nematology 26: 260–270.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious

Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kimpinski J, Kunelius HT, Willis CB (1984) Plant Parasitic Nematodes in Temperate Forage Grass and Legume Species in Prince-Edward Island Canada. Canadian Journal of Plant Pathology 6: 160–164. https://doi.org/10.1080/07060668409501577

Lal R, Lal A (2006) Plant parasitic nematodes intercepted from seeds, soil clods and packing material under import quarantine. Journal of New Seeds 8: 49–60. https://doi.org/10.1300/J153v08n01_04

Lee WG, Lee DE (2015) New Zealand - a land apart. In: Stow A, Maclean N, Holwell GI (Eds) Austral Ark. Cambridge University Press, Cambridge, UK, 24–44.

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10: 689–710. http://dx.doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

McNeill M, Phillips C, Young S, Shah F, Aalders L, Bell N, Gerard E, Littlejohn R (2011) Transportation of nonindigenous species via soil on international aircraft passengers' footwear. Biological Invasions 13: 2799–2815. https://doi.org/10.1007/s10530-011-9964-3

McNeill MR, Phillips CB, Robinson AP, Aalders L, Richards N, Young S, Dowsett C, James T, Bell N (2017) Defining the biosecurity risk posed by transported soil: Effects of storage time and environmental exposure on survival of soil biota. Neobiota 32: 65–88. https://doi.org/10.3897/neobiota.32.9784

McSorley R, Walter DE (1991) Comparison of soil extraction methods for nematodes and microarthropods. Agriculture, Ecosystems & Environment 34: 201–207. doi:http://dx.doi.org/10.1016/0167-8809(91)90106-8

Norton DC (1978) Ecology of Plant-Parastic Nematodes. JohnWiley and Sons, New York, 268 pp.

Barker KR (1997) Opportunities for integrated management of plant-parasitic nematodes in the Near East. http://www.fao.org/docrep/V9978E/v9978e0c.htm [accessed]

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. Bioscience 50: 53–65. http://dx.doi.org/10.1641/0006-3568(2000)050[0053:EAECON]2.3.CO;2

Rohan TC, Bell NL, Lee NDF, Aalders LT (2006) Efficacy of root digestion and maceration in determining abundance of root-inhabiting nematodes. New Zealand Plant Protection 59: 166–171.

Said A, Subbotin SA, Moens M (2002) Identification of the beet cyst nematode *Heterodera schachtii* by PCR. European Journal of Plant Pathology 108: 497506

Siddiqi MR (2000) Tylenchida: parasites of plants and insects. (Ed 2) CABI Publishing, Wallingford, xvii + 833 pp. http://dx.doi.org/10.1079/9780851992020.0000

Singh SK, Ash GJ, Hodda M (2015) Keeping 'one step ahead' of invasive species: using an integrated framework to screen and target species for detailed biosecurity risk assessment. Biological Invasions 17: 1069–1086. https://doi.org/10.1007/s10530-014-0776-0

Talavera M, Valor H, Tobar A (1998) Post-anhydrobiotic viability of *Pratylenchus thornei* and *Merlinius brevidens*. Phytoparasitica 26: 293–299. https://doi.org/10.1007/BF02981443

Tenente RCV, Manso ESC, Mendes MAS, Marques ASAd, Filho EF (1996) Quarantine detection of nematodes and procedures for their eradication from vegetatively propagated materials imported by Brazil. Nematropica 26: 187191.

Townshend JL (1984) Anhydrobiosis in *Pratylenchus penetrans*. Journal of Nematology 16: 282–289. https://doi.org/10.4141/cjps84-050

Townshend JL (1987) Anhydrobiosis in *Pratylenchus penetrans* and *Tylenchorhynchus* new species in cultivated soils cropped to winter rye. Journal of Nematology 19: 164–171.

Townshend JL, Cline RA, Dirks VA, Marks CF (1984) Assessment of turfgrasses for the management of *Pratylenchus penetrans* and *Paratylenchus projectus* in orchards. Canadian Journal of Plant Science 64: 355–360.

Turner SJ, Rowe JA (2006) Cyst nematodes. In: Perry RN, Moens M (Eds) Plant Nematology. CABI Publishing, UK, 910–112. https://doi.org/10.1079/9781845930561.0091

Wharton DA (2002) Nematode Survival Strategies. In: Lee DL (Ed) The Biology of Nematodes. CRC Press, 389–412. https://doi.org/10.1201/b12614-17

Willis CB, Kimpinski J, Thompson LS (1982) Reproduction of *Pratylenchus crenatus* and *P. penetrans* on forage legumes and grasses and effect on forage yield. Canadian Journal of Plant Pathology 4: 169–174. https://doi.org/10.1080/07060668209501320

Womersley C (1987) A reevaluation of strategies employed by nematode anhydrobiotes in relation to their natural environment. Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists; 1987:89 ref. Society of Nematologists, Inc, Hyattsville, Maryland, 165–173.

Womersley CZ, Wharton DA, Higa LM (1998) Survival biology. In: Perry RN, Wright DJ (Eds) The physiology and biochemistry of free living and plant parasitic nematodes. CABI Publishing, Wallingford & New York, 271–302.

Work TT, McCullough DG, Cavey JF, Komsa R (2005) Arrival rate of nonindigenous insect species into the United States through foreign trade. Biological Invasions 7: 323–332. http://dx.doi.org/10.1007/s10530-004-1663-x

NeoBiota 36: 17–38 (2017)
doi: 10.3897/neobiota.36.13842
http://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Facilitation, competition and parasitic facilitation amongst invasive and native liana seedlings and a native tree seedling

Kris French[1], Sharon A. Robinson[1], Liza Smith[1], Eva Watts[1]

[1] *Centre for Sustainable Ecosystem Solutions, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia*

Corresponding author: *Kris French* (kris@uow.edu.au)

Academic editor: *B. Murray* | Received 25 April 2017 | Accepted 14 July 2017 | Published 14 August 2017

Citation: French K, Robinson SA, Smith L, Watts E (2017) Facilitation, competition and parasitic facilitation amongst invasive and native liana seedlings and a native tree seedling. NeoBiota 36: 17–38. https://doi.org/10.3897/neobiota.36.13842

Abstract

Lianas are prevalent in gaps and edges of forests where they compete intensely with trees, reducing growth and recruitment. Invasive lianas have the potential to be particularly harmful as the competitive advantage of the liana life history may be coupled with the more competitive qualities of invasiveness. However, in early stages of growth of lianas and native tree seedlings, facilitatory interactions or competitive interactions associated with soil nutrients may be more prevalent. We investigated interactions at the early stages of growth between native and invasive lianas with a common rainforest tree of temperate Australian rainforests under different light conditions. Invasive lianas, as a group, were not more competitive than native lianas in reducing growth of a native rainforest seedling. At this stage in the life cycle most lianas were as competitive as a conspecific seedling. However, one invasive liana, *Anredera cordifolia*, was particularly competitive and reduced biomass of tree seedlings. Light had little effect on growth of lianas nor on the impact of competition, however, specific leaf area differed between low and medium light conditions. Moderate light did improve growth in the rainforest tree seedling. When lianas were grown with a rainforest tree, three liana species overyielded, while one species was unaffected by growing with the tree seedling. Overyielding suggests a strong positive interaction with the neighbouring plant, mediated through belowground processes. We discuss the potential for these interactions to be facilitative, parasitic or competitive. We therefore show that interactions early in the life of rainforest species can be complex mixtures of interactions which are likely to influence the ability of lianas to dominate rainforests.

Keywords

forest edges, forest interior, interspecific competition, intraspecific competition, invasion ecology, relative growth rates, seedlings, SLA, temperate forests, vines

Introduction

Recently, the focus of competition as the most important plant-plant interaction has been questioned as acknowledgement of the role of positive interactions (facilitation) in influencing neighbouring plants has been established (Callaway and Walker 1997, Brooker et al. 2008; Montgomery et al. 2010, Wright et al. 2014). Gaining advantages from neighbouring plants may increase growth opportunities over and above those gained through competitive superiority, particularly in highly stressful environments (stress gradient hypothesis: Bertness and Callaway 1994, Callaway and Walker 1997, Holmgren et al. 1997). Over the life of a plant, the relative strengths of competition and facilitation vary (Wright et al. 2014; Paterno et al. 2016) and interactions are influenced by other biotic interactions (e.g. herbivory, Hamilton III and Frank 2001). Consequently, understanding community processes involves investigating interactions through the life cycle. Early growth of plants is likely to be an important life stage where interactions are likely to quickly influence the success of seedlings, although this stage has not been investigated widely.

Competition is often measured in the field as lower relative growth compared to the competing plant. However, competitive interactions that may be present are often confounded by species-specific differences in growth rates and resource use as well as a range of other positive and negative interactions amongst other species. Competition is a negative-negative interaction, whereby both species should do worse when growing with the other competitor than each does when growing alone. Competition is only possible when there is a limiting resource and few studies establish the limiting resource where competition is acting. In the field, measuring growth rates of individual plants growing alone is particularly difficult and thus identifying competition and the strength of competition is often not clear. Instead studies often focus on differentiating the relative differences between putative competitors. The outcome of these 'competitive interactions' in the field could therefore be caused by a range of other interactions which result in a difference in growth through better acquisition of non-limiting resources, rather than the result of competition with a neighbouring plant. Similarly facilitation is a positive interaction being experienced by at least one partner in the interaction compared to when growing alone and is usually associated with 'within guild' interactions (McIntire and Fajardo 2014). In the past, facilitation was traditionally associated with (+,0) or (+,+) interactions. What is difficult to tease apart in a field situation, is that while species might be facilitated, one species might still 'win' compared to the other species as a result of the strength of facilitation. Without adequate controls, this would be seen to be the result of competition. 'Winning' in a field situation, therefore, may be due to a number of reasons: competition for a limiting resource, better use of non-limiting resources, better facilitation or a combination of any of these. Changes in community structure are, therefore, a result of the relative advantages of facilitation versus competitive superiority that each species experiences when growing with each other (Montgomery et al. 2010). Laboratory experiments are, therefore, useful in teasing apart this complexity of interactions.

Lianas are considered to be strong competitors as they spend fewer resources on mechanical support allowing greater allocation to leaves, stem elongation and roots. They are also often considered to be structural parasites (Stevens 1987). In comparison to other growth forms, lianas have more leaves and a higher annual increment in new stem mass for a given aboveground mass (Wyka et al. 2013, Ichihashi and Tateno 2015), and increased photosynthetic capacity (Pasquini et al. 2015). Lianas influence tree regeneration in tropical rainforests (Schnitzer et al. 2014) through both aboveground and below-ground competition (Schnitzer et al. 2005; Toledo-Aceves and Swaine 2008; Lobos-Catalan and Jiminez-Castillo 2014). Our understanding of lianas in forests is largely associated with work in the neotropics (summarised in Paul and Yavitt 2011) with very little work done elsewhere. In southern hemisphere temperate rainforests, Lobos-Catalan and Jiminez-Castillo (2014) suggested that resource competition was less influential as nutrients are greater than in the neotropics. Apart from Lobos-Catalan and Jiminez-Castillo (2014), little work has been done to determine whether lianas in other forest types follow similar trajectories and have similar impacts as those in the tropics. Furthermore, variable use of terminology such as competition and parasitism to describe the same attributes is confusing, highlighting the need to be very clear in attributing the direction of the interaction in studies.

Exotic, invasive lianas have the potential to be particularly harmful to rainforest habitats as the 'competitive' advantage of the liana life history may be coupled with the more competitive qualities of invasiveness. Theoretically, the advantage of being invasive is associated with escape from co-evolved pathogens and predators in the native habitat (Enemy Release Hypothesis, Keane and Crawley 2002) which frees up resources to be used in growth and reproduction. For many invasive species, growth is improved in host habitats where species have evolved improved competitive strategies (Blossey and Notzold 1995). Invasive species have faster growth strategies resulting in higher specific leaf area (SLA) and assimilation rates (Leishman et al. 2007) which causes higher relative growth rates (James and Drenovsky 2007). There are some examples of equivalent patterns associated with invasive lianas. Leicht-Young et al. (2011) found that under stronger competition the invasive liana, *Celastrus orbiculatus*, had greater relative growth rates, biomass and survival than the native *Celastrus scandens*. Osunkoya et al. (2010) found that four invasive lianas had higher respiration load, higher light compensation points and higher SLA indicating better carbon economy, but similar relative growth rates to four native species in tropical Australia. In contrast, while an invasive liana in the US, *Cayratia japonica,* had faster growth compared to a native, *Parthenocissus quinquefolia*, another invasive (*Ampelopsis brevipedunculata*) did not (Emerine et al. 2013). Native and invasive liana species in temperate forests in Australia have similar allometry of growth characteristics (French et al. 2016).

When lianas initially establish, advantages usually associated with their growth form may be less relevant, as establishment at the ground layer is likely to be associated with low light conditions where seedlings of all species have low biomass. During this stage of the life cycle, below-ground resources may be more important in determining the outcome of species that are seeking to occupy a site. Furthermore, positive interactions with neighbours may facilitate early growth in seedling lianas and could neutralise or outweigh the negative effects of resource competition. Increases in positive plant-plant interactions during this early stage, relative to negative competitive interactions, may improve growth of plants over and above growth when plants are grown alone (known as overyielding) and may buffer high levels of seedling mortality which would be otherwise experienced. Seedling lianas are unlikely to smother seedling trees as there is strong selection to invest in seeking a taller plant to reach the canopy and escape the darker ground level rather than continuing to grow large amounts of biomass at this level in the forest. Accordingly, in the early stages of establishment, the growth rates of seedlings of lianas and trees is likely to be more strongly affected by below-ground resources (Toledo-Aceves and Swaine 2008) and positive plant interactions may become relatively more important. Being able to maintain high growth in low-light environments in the early stages of growth would be advantageous, however the relative importance of low light, facilitation and competition for resources on growth at this early stage is unknown. Toledo-Aceves and Swaine (2008) found that although changes in light availability (reflecting gaps and interior conditions) did not influence below-ground competition there was an overall positive effect on early growth in 3 species. However, Osunkoya et al. (2005) identified an interaction between below-ground competition and light availability for two tree species suggesting that competition may well change depending on the position of seedlings in the rainforest.

Gaps and edges of rainforests are often areas where lianas are particularly prevalent (Putz 1984, Schnitzer et al. 2000, Schnitzer and Carson 2001, Schnitzer et al. 2012) and are areas where they compete intensely with trees, reducing tree growth and recruitment (van der Heijden and Phillips 2009, Schnitzer and Carson 2010, Schnitzer et al. 2014). However, within forest interiors, resource limitation, particularly phosphorus, is important for both trees and lianas although lianas always outperform trees (Pasquini et al. 2015) and can suppress seedling growth and survival (Martinez-Izquierdo et al. 2016). Thus comparing competitive and facilitatory effects at the edges and interiors of rainforests may indicate the strength of competition, relative importance of facilitation and how effective lianas are in these early periods of growth.

We investigated plant-plant effects in low and moderate light conditions to simulate light conditions on rainforest edges and interiors. We compared two native and two invasive lianas grown with a common rainforest tree of temperate rainforests in Australia and predicted that both invasive lianas would be more competitive than native lianas with a better capacity to add biomass in competition with rainforest seedlings. We predicted that this interaction would be maintained in low and high light conditions. If facilitation occurred, then we predicted that lianas growing with another plant would show improved growth compared to when growing alone and that this effect would be greater for invasive than native lianas. If positive effects were evident in lianas, then native tree seedlings would also be positively (mutualism) or neutrally affected (commensalism) in line with the concept of facilitatory interactions.

Methods

Study species

Seedlings of *Guioa semiglauca* (F.Muell) Radlk. (Sapindaceae) were used in growth trials in pots in a shade house. *G. semiglauca* is a common tree up to 18 m used as a host by lianas in a range of rainforest communities along Eastern Australia (Harden et al. 2006). It can grow in both edges and understorey areas in rainforests. Two native and two invasive lianas were compared in the trial: the native species were *Cissus antarctica* Vent. (Vitaceae) and *Pandorea pandorana* (Andrews) Steenis. subsp. *pandorana* (Bignoniaceae) and the invasive species were *Araujia sericifera* Brot. (Apocynaceae) and *Anredera cordifolia* (Ten.) Steenis (Basellaceae). All species were chosen as all can be abundant in disturbed rainforests. *Cissus antarctica* is a robust tendril climber reproducing from berries and spreading clonally through numerous stems (Fairley and Moore 2010, Harden et al. 2007). *Pandorea pandorana* subsp. *pandorana* is found throughout Australia in a range of vegetation communities including rainforests. *Anredera cordifolia* is a succulent climber from South America which was first introduced to Australia in the early 1900's (Vivian-Smith et al. 2007). It is currently listed nationally as a Weed of National Significance. It primarily spreads vegetatively through smaller aerial tubers and extensive subterranean tuber networks (Swarbrick 1999). *Araujia sericifera* is a common stem twiner native to Peru and declared a noxious weed in many areas in eastern Australia. It produces numerous wind-dispersed seeds from large pear-shaped fruit (Harden et al. 2007, Pellow et al. 2009).

All native plants were bought commercially as tube stock. The exotic species were obtained from the field as seedlings (*Ar. sericifera*) or tubers (*An. cordifolia*) and grown in a glasshouse for approximately three months prior to the experiment. Plants were potted (12cm diam pots) in coarse river sand to facilitate final harvest of belowground biomass, and given 5 g of slow-release native fertiliser (Osmocote® native) at the beginning of the experiment. Lianas were supplied with wire and rope trellises in the same cardinal direction, hence pots were not rotated during the experiment. We accounted for this by randomly allocating pots to competition treatments within each of the light treatments (see below).

Experimental design

Plants were grown under experimental conditions over spring and summer from August 2011 to February 2012 (24 weeks). Seven replicate pots of each experimental condition were set up in a shade house. To measure maximum growth under no competition, control pots contained a single plant of each species (liana or host). Intraspecific competition was measured in pots that contained two individuals of a species and

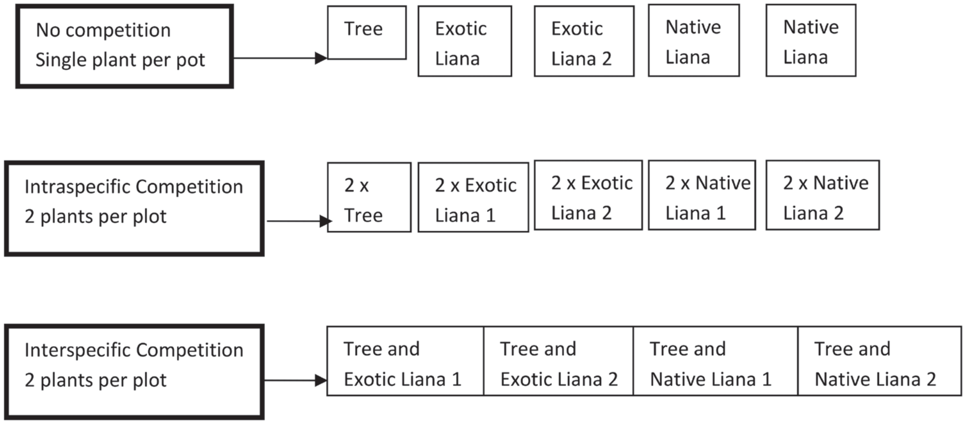

Figure 1. Experimental design showing set up of pot trials to measure maximum growth rate, intraspecific competition and interspecific competition of native and invasive lianas and a native host species. Seven replicates of each were grown in both medium and low light conditions.

interspecific competition was measured in pots that contained one individual of the host species, *G. semiglauca*, and one individual of a species of liana (Fig. 1).

For the experiment, the two light treatments were created by constructing two adjacent shadehouses using standard shadecloth. Plants were grown in either medium light (ML, 33% daytime PAR) or low light (LL, 7% daytime PAR) to simulate the available PAR in forest edges and interiors respectively. Measurements at various points in each shade house showed them to be an average of 33±2% and 7±1 % full PAR. Readings were made using two Spectrosense dataloggers attached to quantum sensors (Skye Instruments Ltd, Llandrindod Wells, Powys, UK).

Initial measurements of stem, leaf and belowground biomass were obtained from four randomly chosen plants of each species prior to placing in light treatments. Each plant was divided into stem, leaf and belowground portions, washed and oven dried at 60°C for five days before being weighed. Destructive measurements of specific leaf area (SLA = leaf area/ dry weight) were conducted after four weeks from five leaves of each species, using spare plants. Each leaf was labelled and its area measured with a Li-Cor leaf area meter (Model Li-3000A, Lincoln, Nebraska, USA), before being dried and weighed.

During the six months, all pots were watered daily via an automatic mist irrigation system and soil was maintained at field capacity. The lianas were allowed to climb freely onto trellises but were prevented from growing onto adjacent hosts by moving stems away from adjacent plants every few days. Aerial tuber production on *Anredera cordifolia* plants was monitored and recorded.

After 24 weeks, final measurements of leaf number were made before all plants were harvested and then biomass assessed (see below). Aerial tubers from *A. cordifolia* were removed before measurements to prevent them from falling off their stems. These were dried and weighed separately. No plants died during the experiment.

Analysis

For the host plant, we investigated changes in biomass by analysing accumulated biomass, above- and below-ground biomass, stem and leaf biomass. We also investigated effects on SLA and leaf number. For plants grown with a conspecific we chose a single plant randomly from each pot as the focal individual to be used for analysis. For comparisons between species we calculated relative growth rate per month using the following equation: $(\ln DW_f - \ln DW_i)$/no. months, where DW_i is the average dry weight of 4 plants sacrificed at the beginning of the experiment, DW_f is the weight of an individual seedling at the end of the experiment, and no. months is the amount of time, in months, over which plants were in the experiment (5.6 mo).

We undertook two different analyses to test questions about how lianas influence seedling trees. Using two factor ANOVAs, we investigated whether any of our measures of growth for *G. semiglauca* varied with competition or light level (JMP Pro 11). Secondly, we used a linear mixed effects model fitted using restricted maximum likelihood to investigate how changes in biomass of *G. semiglauca* varied with origin of the liana species and light levels. Species of liana were treated as random effects and nested within origin (exotic, native). Only interspecific competition treatments were included in this analysis.

For each liana species, we tested whether inter- or intra-specific competition influenced growth rates using a two factor ANOVA with competition and light level as fixed factors, comparing each liana species grown alone with those grown with another conspecific or with *G. semiglauca*. Tubers of *An. cordifolia* were analysed in two ways. Initially we undertook a nominal logistic model to investigate the probability of producing tubers associated with different competition and light levels and tested the effects using a likelihood test. Secondly, for those plants that produced tubers, we investigated whether dry biomass of tubers varied with competition or light using a two factor ANOVA. Finally, we compared differences in growth amongst the four liana species and *G. semiglauca* using an ANOVA on relative growth rates. We included light level as a factor.

As data fitted the assumptions of normality and homogeneity we did not transform any variables. Tukeys HSD multiple comparisons were used to determine where differences lay in significant factors in the ANOVAs. We used nominal logistic models on pairs of levels of competition when the overall nominal logistic model was significant for tuber production associated with competition, and corrected probability values to $\alpha = 0.017$ (a Bonferroni correction) to account for Type 1 errors.

Results

Effect of plant-plant interactions and light on native tree seedlings

Guioa semiglauca seedlings were not significantly affected by intraspecific competition (Table 1, Fig. 2) although plants grown with a conspecific grew to only 68% of the

Table 1. Summary of p values of ANOVA tests investigating impacts of competition and light on growth for the tree, *Guioa semiglauca*. Degrees of freedom of tests are in brackets. Multiple comparisons (Tukeys Test) show where differences lie. Pp = *Pandorea pandorana*, As = *Araujia sericifera, Ac* = *Anredera cordifolia.* ML = medium light (33% PAR), LL = low light (10% PAR).

		Factor	**p**	**Multiple comparison**
Competition	Total Biomass	**Competition (5,84)**	**0.005**	As,Pp>Ac. Others intermediate
		Light (1, 84)	**0.014**	ML>LL
		Light*competition (4,84)	0.217	
	Above ground biomass	**Competition (5,84)**	**0.004**	Pp,As, alone > Ac. Others intermediate
		Light (1, 84)	**0.012**	ML>LL
		Light*competition (4,84)	0.367	
	Below-ground biomass	**Competition (5,84)**	**0.017**	As>Ac. Others intermediate
		Light (1, 84)	**0.008**	ML>LL
		Light*competition (4,84)	0.244	
	Stem biomass	**Competition (5,84)**	**0.047**	As>Ac. Others intermediate
		Light (1, 84)	**0.004**	ML>LL
		Light*competition (4,84)	0.536	
	Leaf biomass	**Competition (5,84)**	**0.001**	Pp,As, alone > Ac. Others intermediate
		Light (1, 84)	0.051	
		Light*competition (4,84)	0.438	
	SLA	Competition (5,84)	0.186	
		Light (1, 84)	**0.003**	ML<LL
		Light*competition (4,84)	0.600	
	No. Leaves	Competition (4,70)	0.401	
		Light (1,70)	0.089	
		Light*competition (4,70)	0.776	
Competition (Effect of Origin)	Total Biomass	Light (1,2)	0.171	
		Origin (1,2)	0.814	
		Origin*Light (1,2)	0.643	
	Above ground biomass	Light (1,2)	0.173	
		Origin (1,2)	0.820	
		Origin*Light (1,2)	0.658	
	Below-ground biomass	Light (1,2)	0.172	
		Origin (1,2)	0.798	
		Origin*Light (1,2)	0.614	
	Stem biomass	Light (1,2)	0.172	
		Origin (1,2)	0.798	
		Origin*Light (1,2)	0.614	
	Leaf biomass	Light (1,2)	0.249	
		Origin (1,2)	0.755	
		Origin*Light (1,2)	0.524	
	SLA	**Light (1,2)**	**0.037**	ML<LL
		Origin (1,2)	0.609	
		Origin*Light (1,2)	0.059	
	No. Leaves	Light (1,2)	0.378	
		Origin (1,2)	0.109	
		Origin*Light (1,2)	0.220	

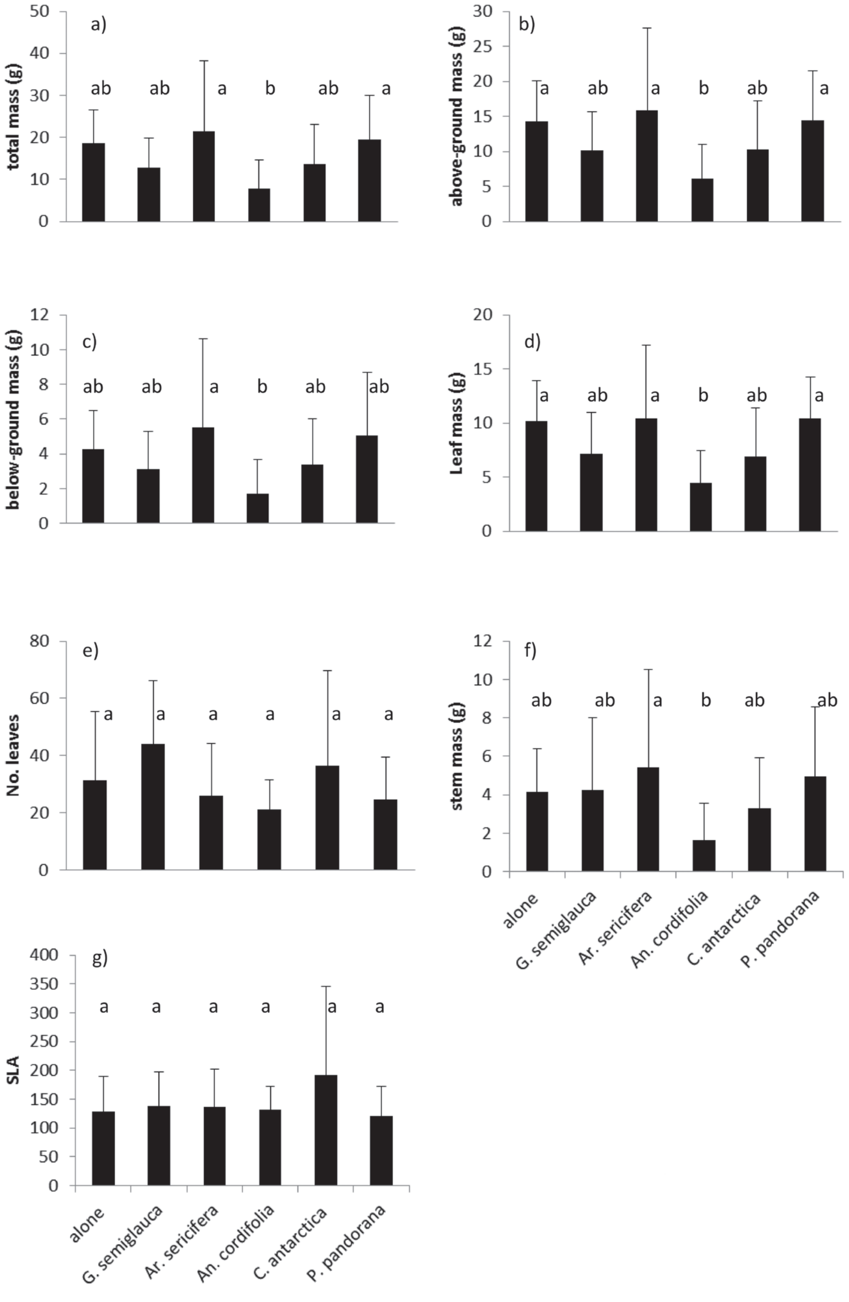

Figure 2. Average **a** total biomass **b** above-ground biomass **c** below-ground biomass **d** leaf biomass **e** number of leaves **f** stem biomass and **g** specific leaf area (SLA) of *Guioa semiglauca* seedlings grown under different competition treatments: alone, with another *G.semiglauca* (with conspecific), with two invasive lianas, *Araujia sericifera, Anredera cordifolia,* and two native lianas, *Cissus antarctica and Pandorea pandorana*. Light levels are pooled for each mean. Different letters represent significant differences between bars based on Tukeys test.

biomass of plants grown alone. Liana species differed in their ability to affect biomass of *G. semiglauca*. *Anredera cordifolia* reduced overall biomass of *G. semiglauca* to 42% of that when it was grown alone, while *Araujia sericifera* and *Pandorea pandorana* did not appear to impact biomass in *G. semiglauca* seedlings at all (Table 1, Fig. 2a). Where biomass of *G. semiglauca* was reduced by interspecific competition (with *An. cordifolia*) both above- and below-ground biomass accumulation appeared to be impacted although the biomass was often not different to growing alone or in intraspecific competition (Table 1, Fig. 2b, c). There was no evidence of facilitation with any of the lianas.

For *G. semiglauca,* a reduction in light influenced growth, reducing total biomass through reductions in both above-ground and below-ground biomass (Table 1, Fig. 3). Above-ground changes were most apparent with an increase in stem growth for plants grown in ML conditions. In deep shade (LL), overall growth was reduced to 70% of growth in the ML treatment. Below-ground biomass was more affected by LL and was reduced to 62% of growth in the ML conditions, while above ground biomass was reduced to only 71%.

The number of leaves produced was not affected by competition or light levels while SLA showed a typical increase in LL conditions (Table 1, Fig. 2e,f). In LL, SLA was 167 + 59 (s.d.) while in the ML treatment it was 116 + 93. The origin of the competing liana had no effect on any growth parameter of *G. semiglauca* (Table 1).

Effect of plant-plant interactions and light on biomass of lianas

Both invasive lianas showed similar patterns of biomass change in response to plant-plant interactions, although changes in biomass were only significant for *An. cordifolia* (Fig. 4, Table 2). For *An. cordifolia,* growth appeared to be facilitated by growing with *G. semiglauca* (Fig. 4). Growth increased nearly 4-fold from 6.0 + 3.0 g when grown alone to 22.6 + 7.9 g when grown with *G. semiglauca.* For *An. cordifolia,* growth alone was not different from growth with a conspecific. Increased growth with *G. semiglauca* was similar for both above-ground and below-ground biomass and was influenced by a doubling in the number of leaves produced (see Appendix 1).

In contrast, total biomass in *Ar. sericifera* was less influenced by plant-plant interactions. Again, facilitation was evident when this species was grown with *G. semiglauca* compared with a lower increase in biomass when grown with a conspecific. This increase could not be assigned to an increase in above- or below-ground biomass but appeared largely influenced by changes in overall leaf biomass (Table 2, Fig. 4).

The two native lianas were quite different in their responses to plant-plant interactions (Table 2, Fig. 4). Like both invasive species, *C. antarctica,* plants appeared to be facilitated by growing with *G. semiglauca* adding twice the biomass compared to when grown alone or with a conspecific (Fig. 4). Both below- and above-ground biomass were affected similarly. For *C. antarctica,* the interaction of competition type with light (Table 2) identified that plants grown alone put on biomass to similar levels to when grown with *G. semiglauca* at LL but did not compete as well under ML and had similar growth to plants grown with

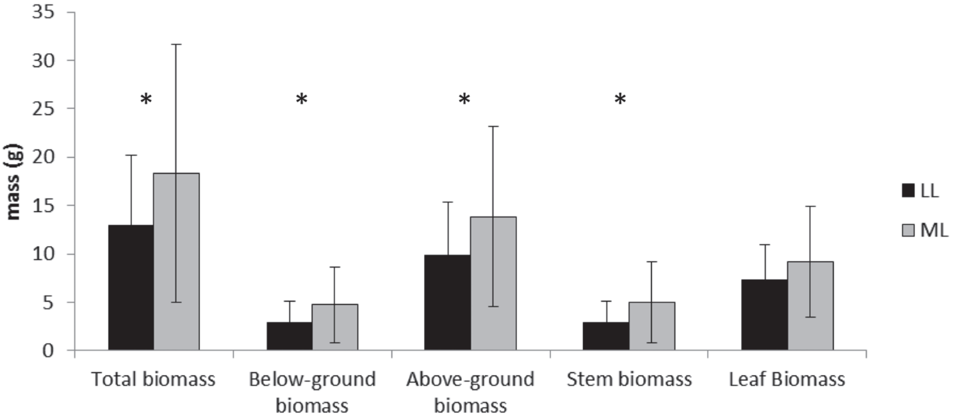

Figure 3. Average total, below-ground, above-ground, stem and leaf biomass of *Guioa semiglauca* seedlings grown under medium (ML) and low (LL) light conditions. Asterisks denote where significant differences lay.

Table 2. Probability values for ANOVA tests for effects of competition and light on accumulated dry biomass and leaf characteristics for 4 species of lianas. Degrees of freedom are shown next to the first species. Probabilities in bold represent significant differences at α = 0.05.

		Total Mass	Above ground mass	Below ground mass	Leaf mass	Stem mass	SLA	Leaf number
An. cordifolia	Competition (2,41)	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	0.420	**<.0001**
	Light (1,41)	0.896	0.671	0.977	0.080	0.615	**<.0001**	0.787
	Comp. × light (2,41)	0.212	0.271	0.432	0.907	0.125	**0.013**	0.557
Ar. sericifera	Competition	**0.023**	0.174	0.235	**0.029**	0.233	0.786	0.182
	Light	0.574	0.685	0.525	0.612	0.532	0.392	0.523
	Comp. x light	0.763	0.398	0.387	0.478	0.388	0.455	0.535
C. antarctica	Competition	**<.0001**	**<.0001**	**<.0001**	**0.006**	**<.0001**	**<.0001**	**0.083**
	Light	0.256	0.053	0.070	0.066	0.070	**0.020**	**0.039**
	Comp. × light	**0.011**	0.066	**0.036**	0.151	**0.036**	**<.0001**	0.276
P. pandorana	Competition	0.100	0.104	0.307	**0.044**	0.307	**0.004**	0.786
	Light	0.183	**0.031**	0.075	**0.023**	0.075	**0.004**	0.392
	Comp. x light	0.557	0.966	0.819	0.974	0.819	**0.007**	0.455

a conspecific (see Appendix 1). The improvement in biomass accumulation in control plants was largely attributed to a change in root biomass. For *P. pandorana*, competition with different species had little effect on growth. Individuals accumulated on average 19.3 + 10.4 g dry biomass over the experiment.

While light level had some moderate impacts on interactions for *C. antarctica,* light level alone did not influence biomass accumulation in lianas, with the exception of *P. pandorana* (Table 2). This species responded with an increase in leaf mass under ML conditions (av. ML, 7.8 + 3.7 g; LL, 5.5 + 2.8 g). Plants in LL had increased SLA

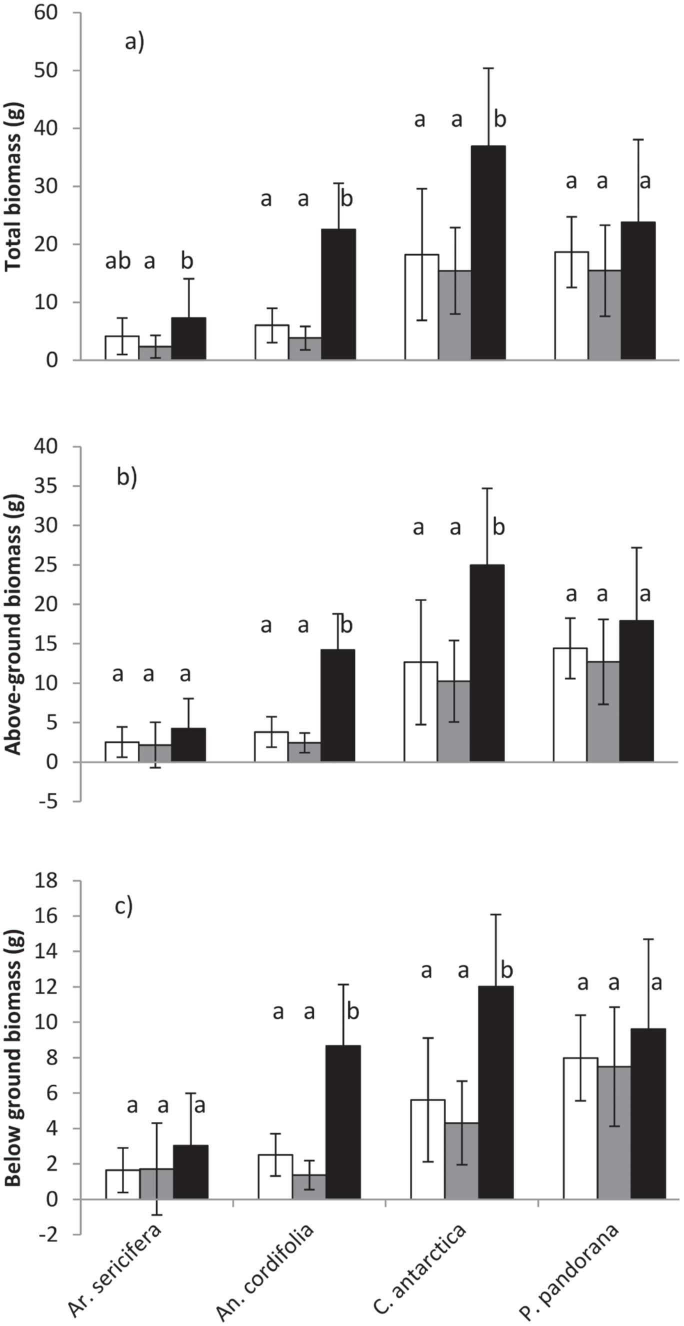

Figure 4. Average **a** total biomass **b** above-ground biomass **c** below-ground biomass of two invasive lianas, *Araujia sericifera, Anredera cordifolia,* and two native lianas, *Cissus antarctica and Pandorea pandorana* grown under different competition treatments: alone (white bars), with a conspecific (grey bars) and with *G. semiglauca* (black bars). Letters above each group of bars are results from Tukeys multiple comparison tests where different letters represent significant differences within each set of bars.

compared to those in ML, and SLA was not affected by growth in competition in either invasive species although it was in both native species (Table 2).

Anredera cordifolia developed tubers over the course of the experiment. The probability of tubers developing was influenced by plant-plant interactions (χ^2 = 14.78, p <0.001). When grown with *G. semiglauca*, 93% of plants produced tubers whereas 57% of plants produced tubers when grown alone, however these did not differ in the likelihood of producing tubers (χ^2 = 6.24, p = 0.013, α=0.017). When grown in competition with another *An. cordifolia,* 29% of plants produced tubers and the probability of producing tubers did not differ from plants grown alone (χ^2 = 2.37, p = 0.124, α=0.017), however there was a higher probability of producing tubers when growing with *G. semiglauca* than when growing with a conspecific (χ^2 = 14.77, p <0.001, α=0.017). For plants producing tubers, there was no difference in tuber biomass per plant amongst treatments ($F_{2,19}$ = 1.68, p = 0.212) or light environments ($F_{1,19}$ = 0.013, p = 0.910). On average plants accumulated 1.45 + 1.58 g dry biomass of tuber which amounted to 10% additional biomass when in competition with *G. semiglauca*, 59% when in competition with a conspecific and 38% additional biomass when grown alone.

Comparison of growth rates of lianas and *G. semiglauca*

Relative growth rates differed amongst species ($F_{4,120}$ = 7.17, p <0.0001). Under LL conditions *G. semiglauca* had the lowest relative growth rates when grown without competition with all lianas having relative growth rates about 10 times higher, however, this difference was not evident when plants were grown with a conspecific, with *G. semiglauca* having a higher relative growth rate (Fig. 5). There were no differences amongst species in the ML treatment ($F_{1,120}$ = 0.661, p = 0.418).

Discussion

Our prediction that invasive lianas would be more competitive than native lianas with a better capacity to add biomass in competition with rainforest seedlings was not supported and light had little effect on the responses. We found strong evidence of facilitation although not all lianas benefited. However, the facilitation of growth in lianas was coupled with a loss of growth in the rainforest seedling.

Are invasive or native lianas good competitors in rainforests?

The success of these invasive lianas in establishing in habitats is not based on an improved capacity to compete in early establishment, although for *An. cordifolia*, early competition may contribute to invasion success. The two invasive lianas did not show

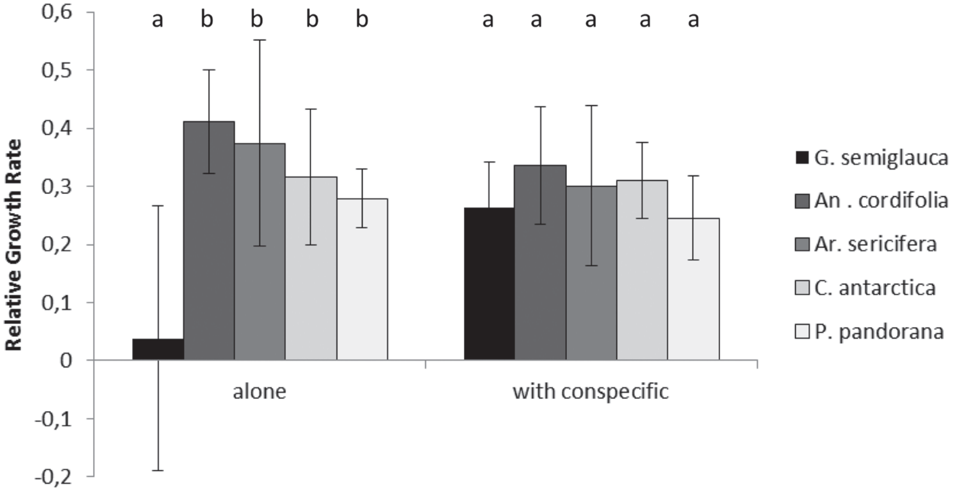

Figure 5. Average relative growth rate for seedlings of one rainforest tree and four species of lianas grown alone and with a conspecific. Letters above each group of bars are results from Tukeys multiple comparison tests where different letters represent significant differences within each set of bars.

consistent patterns in their effects on native seedling growth, suggesting that invasive lianas are not always more competitive than native lianas in reducing growth of a native rainforest seedling. However, no liana showed any positive effect on native tree seedlings, suggesting no facilitation. Exotic *An. cordifolia* had the capacity to reduce both above-ground biomass and leaf mass in the rainforest tree, however, the other exotic species, *Ar. sericifera,* had no impact on growth of the rainforest tree in these early stages. Native *P. pandorana* and *C. antarctica* did not influence growth. At this stage in the life cycle, most lianas were as competitive as a conspecific seedling for *G. semiglauca*. Osunkoya et al. (2010) identified a number of traits in invasive lianas which may provide them with competitive advantages but also noted that there were few differences in a range of traits associated with plasticity between native and invasive lianas. In temperate regions, our study suggests that competitiveness against a common rainforest tree seedling is not related to being invasive. Similar allometry has been found between these invasive and native lianas, identifying few differences in life history strategies between these two groups (French et al. 2016).

Light had no effect on growth of lianas nor on the impact of competition. As expected, plants that grew at low light increased their SLA through an increase in the area of leaf relative to the biomass of the leaf, however, all lianas grew equally well in both light treatments and there was no increase in the proportional effect of neighbouring plants on biomass. This suggests that these lianas grow equally quickly in both the interior and edges of rainforests and, in a similar way to lianas in tropical forests, have the capacity to impact on tree seedlings in gaps (Schnitzer and Carson 2010) and in understorey (Martinez-Izquierdo et al. 2016). While Toledo-Aceves and Swaine (2008) also found no interaction between competition and light, they did find that growth was enhanced with higher light in tropical rainforests. All lianas maintained high relative growth rates in different lighted and competition treatments and appeared quite resilient to these factors and able to successfully maintain growth despite these limiting resources. Similar relative growth rates between native and invasive lianas, including some of the same species as our study, were found in tropical areas of Australia (Osunkoya et al. 2010).

In contrast, the rainforest tree, *G. semiglauca,* showed improved growth under the higher light treatment, associated with both below-ground and above-ground increases, suggesting that it should show improved growth in gaps and along edges of rainforests. This species is clearly more light-limited in the interior of the rainforest although it is not restricted to edges in rainforests. If lianas are increasing in abundance in temperate rainforests, as they are in the neotropical rainforests (Schnitzer and Bongers 2011), then lianas are likely to impact on recruitment rates of this rainforest tree. While this is the first experiment to test the effect of such interactions in these temperate forests, there is the potential for a range of other tree species to be negatively affected.

Our results suggest that the invasive, *An. cordifolia* is a particularly strong competitor in rainforest environments and a serious invasive weed at early stages of growth. Three results particularly highlight this; overyielding in the presence of *G. semiglauca*, coupled with its strong negative effect on *G. semiglauca* and the increased growth of tubers while growing with the native tree seedling. Within 6 months, this plant had the capacity to spread in both edge-simulated light levels and interior-forest light levels through the release and dispersal of tubers.

As rainforest communities in temperate Australia are naturally patchy in distribution, edges are important sources of recruitment. Temperate rainforests are likely to be particularly affected by the predicted increase in drought and extreme temperatures in the future and they are already faced with significant threats from habitat clearing. If native and exotic lianas also increase in abundance, then the recruitment capacity at edges and within forests may well be hampered. There is much research to be done on how lianas may interact with rainforest trees within this future environment.

Variation in plant-plant interactions

When lianas were grown with a rainforest tree, rather than experiencing a decrease in biomass (relative to growing alone), three species had enhanced accumulation of biomass; both exotic species and the native *C. antarctica.* Overyielding in *An. cordifolia* and *C. antarctica* occurred in both above-ground and below-ground biomass and in tuber growth in *An. cordifolia.* For *Ar. sericifera,* overyielding could not be attributed to above- or below-ground biomass, as the magnitude of difference compared to plants grown alone was not as large. This suggests that positive plant interactions were far more influential on growth of these three liana species than for the other native liana species and the tree seedling.

While not often done (e.g. Montgomery et al. 2010, Dohn et al. 2013), we have measured both sides of the interaction in this experiment, and the positive effect was only seen for one of the participating species (the liana) with a strong negative effect for the native tree seedling. The interaction is, therefore, more associated with the directions of advantage associated with a parasitic interaction (+,-), a term not usually applied to plant-plant interactions where both species are physiologically independent. This is the first time such a plant-plant interaction has been reported to our knowledge; we have termed this, *parasitic facilitation.* Without measuring each individual species response to being grown alone, an understanding of the direction of the interaction would not be able to be distinguished from competition or facilitation, highlighting the complexity of interactions amongst species, and the difficulty of identifying true interactions without suitable controls.

The mechanism for *parasitic facilitation* is currently unknown but a number of possibilities can be identified. It is plausible that the parasitic-style interaction that is shown by the three species of liana, is mediated by some change in the soil environment rather than above ground. While facilitation was seen in Brazilian *Restinga* communities where shrubs facilitated the abundances of vines through providing trellises for initial growth (Garbin et al. 2012), we consider that in early stages of growth there was no facilitatory effect of structural support by the native seedling as we did not observe smothering or shading to any great extent. Our results may be associated with coupling through shared mycorrhizae (Simard and Durall 2004; Giovannetti et al. 2004, Walder et al. 2012) lending some weight to the idea that the plant-plant interaction is being mediated by a third taxa (mycorrhizal fungae). If this link is present, then the liana could be viewed as being parasitic on the symbiotic mycorrhizae, and the term *parasitic facilitation* is useful.

One other possibility is that *G. semiglauca* changes other soil microflora to enhance release of nutrients which benefit the lianas as well (Hooper and Vitousek 1998, Zak et al. 2003). In an example of this, Hamilton III and Frank (2001) showed that, when two species were grown together, defoliation increased root exudation of carbon in one species which increased N pools in the soil improving soil resources for neighbouring plants. Likewise, increases in nutrients by nitrogen-fixing species can enhance and cause overyielding in co-occurring crop species (Li et al. 2007). If lianas are competitively superior then they may gain greater access to these freed resources at the expense of the native seedling; perhaps more indicative of a parasitic interaction.

An alternative interpretation is that the liana may be clearly superior in gaining resources from the fungi, which could be viewed as highly asymmetric *resource competition* where the liana is better at using resources provided by the fungi, than the native seedling. There are a range of studies which have identified changes in mycorrhizal communities associated with invasive plants that influence neighbouring native species (e.g., Stinson et al. 2006; Zhang et al. 2010, Shannon et al. 2014). Marler et al.(1999) showed that the presence of mycorrhizae increased the negative effect of the invasive *Centaurea maculosa* on native bunchgrass, *Festuca idahoensis.* Competitive interactions usually result in negative effects whereby plants when grown without another plant do better than when grown with a plant. Given the overyielding identified in three of our species (a positive response), a competitive interaction is less accurate as a description, although resource limitation is at the base of our interpretation of the mechanism. It is clearly important to measure responses of both species to distinguish between negative-negative and positive-negative interactions. Using appropriate terminology will be an important factor in understanding plant-plant interactions. Distinguishing between facilitation, competition and other more complicated interactions such as parasitism is difficult experimentally, and confirms that describing accurately many plant-plant interactions is necessary to understand the underlying mechanism of invasion.

Acknowledgments

This project was funded by a grant from Rural Industries Research and Development Corporation (Australian Commonwealth Government).

References

Bertness MD, Callaway R (1994) Positive interactions in communities. Trends in Ecology and Evolution 9: 191–193. https://doi.org/10.1016/0169-5347(94)90088-4

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive non-indigenous plants: a hypothesis. Journal of Ecology 83: 887–889. https://doi.org/10.2307/2261425

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancourt P, Tielborger K, Travis JMJ, Anthelme F, Armas C, Coll L, Corcket E, Delzon S, Forey E, Kikvidze Z, Olofsson J, Pugnaire F, Quiroz CL, Saccone P, Schiffers K, Seifan M, Touzard B, Michalet R (2008) Facilitation in plant communities: the past, the present, and the future. J. Ecol. 96: 18–34.

Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78: 1958–1965. https://doi.org/10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2

Dohn J, Dembélé F, Karembé M, Moustakas A, Amévor KA, Hanan NP (2013) Tree effects on grass growth in savannas: competition, facilitation and the stress-gradient hypothesis. J. Ecol. 101: 202–209. https://doi.org/10.1111/1365-2745.12010

Emerine SE, Richardson RJ, Arellano C (2013) Porcelain berry (*Ampelopsis brevipedunculata*), Bushkiller (*Cayratia japonica*), and Virginia-crepper (*Parthenocissus quinquefolia*) in interspecific competition. Invasive Plant Science and Management 6: 99–104. https://doi.org/10.1614/IPSM-D-12-00008.1

Fairley A, Moore P (2010) Native plants of the Sydney district. Kangaroo Press, Kenhurst, Australia.

French K, Smith L, Watts E, Robinson S (2016) Invasive alien lianas have similar allometry to native lianas in temperate forest. Biol Invasions. https://doi.org/10.1007/s10530-016-1300-5

Giovannetti M, Sbrana C, Avio L, Strani P (2004) Patterns of belowground plant interconnections established by means of arbuscular mycorrhizal networks. New Phyt. 164: 175–181. https://doi.org/10.1111/j.1469-8137.2004.01145.x

Hamilton III EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Ecology 82: 2397–2402. https://doi.org/10.1890/0012-9658(2001)082[2397:CPSSMA]2.0.CO;2

Harden G, McDonald B, Williams J (2006) Rainforest trees and shrubs. Gwen Harden publishing, Nambucca Heads, N.S.W.

Harden G, McDonald B, Williams J (2007) Rainforest climbing plants: a field guide to their identification in Victoria, New South Wales and subtropical Queensland using vegetative features. Gwen Harden Publishing, Nambucca Heads, N.S.W.

Hooper DU, Vitousek PM (1998) Effects of plant composition and diversity on nutrient cycling. Ecol. Monogr. 68: 121–149. https://doi.org/10.1890/0012-9615(1998)068[0121:EOPCAD]2.0.CO;2

Holmgren M, Scheffer M, Huston MA (1997) The interplay of facilitation and competition in plant communities. Ecology 78: 1966–1975. https://doi.org/10.1890/0012-9658(1997)078[1966:TIOFAC]2.0.CO;2

Ichihashi R, Tateno M (2015) Biomass allocation and long-term growth patterns of temperate lianas in comparison with trees. New Phyt 207: 604–612. https://doi.org/10.1111/nph.13391

James JJ, Drenovsky RE (2007) A basis for relative growth rate differences between native and invasive forb seedlings. Rangeland Ecol. and Manage. 60: 395–400. https://doi.org/10.2111/1551-5028(2007)60[395:ABFRGR]2.0.CO;2

Leicht-Young SA, Latimerb AM, Silander Jr JA (2011) Lianas escape self-thinning: Experimental evidence of positive density dependence in temperate lianas *Celastrus orbiculatus* and *C. scandens*. Perspectives Plant Ecol Evol Syst 13: 163–172. https://doi.org/10.1016/j.ppees.2011.04.002

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. TREE 17: 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0

Leishman MR, Haslehurst T, Ares A, Baruch Z (2007) Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. New Phyt. 176: 635–643. https://doi.org/10.1111/j.1469-8137.2007.02189.x

Li L, Li S-M, Sun J-H, Zhou L-L, Bao X-G, Zhang H-G, Zhang F-S (2007) Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. PNAS 104: 11192–11196. https://doi.org/10.1073/pnas.0704591104

Lobos-Catalan P, Jiminez-Castillo M (2014) Different patterns of biomass allocation of mature and sapling host tree in response to liana competition in the southern temperate rainforest. Austral Ecol. 39: 677–685. https://doi.org/10.1111/aec.12131

Marler MA, Zabinski CA, Callaway RM (1999) Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80: 1180–1186. https://doi.org/10.1890/0012-9658(1999)080[1180:MIECEO]2.0.CO;2

Martinez-Izquierdo L, Garcia MM, Powers JS, Schnitzer SA (2016) Lianas suppress seedling growth and survival of 14 tree species in a Panamanian tropical forest. Ecology 97: 215–224. https://doi.org/10.1890/14-2261.1

McIntire EJB, Fajardo A (2014) Facilitation as a ubiquitous driver of biodiversity. New Phyt. 201: 403–416. https://doi.org/10.1111/nph.12478

Montgomery RA, Reich P B, Palik B J (2010) Untangling positive and negative biotic interactions: views from above and below ground in a forest ecosystem. Ecology 91: 3641–3655. https://doi.org/10.1890/09-1663.1

Osunkoya OO, Othman FE, Kahar RS (2005) Growth and competition between seedlings of an invasive plantation tree, *Acacia mangium*, and those of a native Borneo heath-forest species, *Melastoma beccarianum*. Ecol. Res. 20: 205–214. https://doi.org/10.1007/s11284-004-0027-4

Osunkoya OO, Bayliss D, Panetta FD, Vivian-Smith G (2010) Variation in ecophysiology and carbon economy of invasive and native woody vines of riparian zones in south-eastern Queensland. Austral Ecol. 35: 636–649. https://doi.org/10.1111/j.1442-9993.2009.02071.x

Pasquini SC, Wright J, Santiago L (2015) Lianas always outperform tree seedlings regardless of soil nutrients: results from a long-term fertilization experiment Ecology 96: 1866–1876. https://doi.org/10.1890/14-1660.1

Paterno GB, Siqueira Filh, JA, Ganade G (2016) Species-specific facilitation, ontogenetic shifts and consequences for plant community succession. J. Vege. Sc. 27: 606–615. https://doi.org/10.1111/jvs.12382

Paul GS, Yavitt YB (2011) Tropical vine growth and the effects on forest succession: A review of the ecology and management of tropical climbing plants. Bot. Rev. 77: 11–30. https://doi.org/10.1007/s12229-010-9059-3

Pellow B, Henwood M, Carolin R (2009) Flora of the Sydney Region (5th Edition), Sydney University Press, Sydney.

Putz FE (1984) The natural history of lianas on Barro Colorado Island, Panama. Ecology 65: 1713–1724. https://doi.org/10.2307/1937767

Schnitzer SA, Bongers F (2011) Increasing liana abundance and biomass in tropical forests: emerging patterns and putative mechanisms. Ecology Letters 14: 397–406. https://doi.org/10.1111/j.1461-0248.2011.01590.x

Schnitzer SA, Dalling JW, Carson WP (2000) The impact of lianas on tree regeneration in tropical forest canopy gaps: evidence for an alternative pathway of gap-phase regeneration. J. Ecol. 88: 655–666. https://doi.org/10.1046/j.1365-2745.2000.00489.x

Schnitzer SA, Kuzee ME, Bongers F (2005) Disentangling above- and below-ground competition between lianas and trees in a tropical forest. J. Ecol. 93: 1115–1125. https://doi.org/10.1111/j.1365-2745.2005.01056.x

Schnitzer SA, Mangan SA, Dalling JW, Baldeck CA, Hubbel SP et al. (2012) Liana abundance, diversity, and distribution on Barro Colorado Island, Panama. PLoS ONE 7: e52114. https://doi.org/10.1371/journal.pone.0052114

Schnitzer SA, Carson WP (2001) Treefall gaps and the maintenance of diversity in a tropical forest. Ecology 82: 913–919. https://doi.org/10.1890/0012-9658(2001)082[0913:TGATMO]2.0.CO;2

Schnitzer SA, Carson WP (2010) Lianas suppress tree regeneration and diversity in treefall gaps. Ecol. Letters 13: 849–857. https://doi.org/10.1111/j.1461-0248.2010.01480.x

Schnitzer SA, van der Heijden G, Mascaro J, Carson WP (2014) Lianas in gaps reduce carbon accumulation in a tropical forest. Ecology 95: 3008–3017. https://doi.org/10.1890/13-1718.1

Shannon SM, Bauer JT, Anderson WE, Reynolds HL (2014) Plant-soil feedbacks between invasive shrubs and native forest understory species lead to shifts in the abundance of mycorrhizal fungi. Plant Soil 382: 317–328. https://doi.org/10.1007/s11104-014-2158-x

Simard SW, Durall DM (2004) Mycorrhizal networks: a review of their extent, function, and importance. Can J Bot 82: 1140–1165. https://doi.org/10.1139/b04-116

Stevens GC (1987) Lianas as structural parasites: the *Bursera simaruba* example. Ecology 68: 77–81. https://doi.org/10.2307/1938806

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM et al. (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biol 4(5): e140. https://doi.org/10.1371/journal.pbio.0040140

Swarbrick JT (1999) Seedling production by Madeira vine (*Anredera cordifolia)*. Plant Protection Quarterly 14: 38–39.

Toledo-Aceves T, Swaine MD (2008) Above- and below-ground competition between the liana *Acacia kamerunensis* and tree seedlings in contrasting light environments. Plant Ecol. 196: 233–244. https://doi.org/10.1007/s11258-007-9347-0

van der Heijden GMF, Phillips OL (2009) Liana infestation impacts tree growth in a lowland tropical moist Forest. Biogeosciences 6: 2217–2226. https://doi.org/10.5194/bg-6-2217-2009

Vivian-Smith G, Lawson BE, Turnbull I, Downey P (2007) The Biology of Australian weeds 46. *Anredera cordifolia* (Ten.) Steenis. Plant Protection Quarterly 22: 2–10.

Walder F, Niemann H, Natarajan M, Lehmann MF, Boller T, Wiemken A (2012) Mycorrhizal networks: Common goods of plants shared under unequal terms of trade. Plant Phys. 159: 789–797. https://doi.org/10.1104/pp.112.195727

Wyka TP, Oleksyn J, Karolewski P, Schnitzer SA (2013) Phenotypic correlates of the lianescent growth form: a review. Annals Bot. 112: 1667–1681. https://doi.org/10.1093/aob/mct236

Wright A, Schnitzer SA, Reich PB (2014) Living close to your neighbors: the importance of both competition and facilitation in plant communities. Ecology 95: 2213–2223. https://doi.org/10.1890/13-1855.1

Zak DR, Holmes WE, White DC, Peacock AD, Tilman D (2003) Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology 84: 2042–2050. https://doi.org/10.1890/02-0433

Zhang Q, Yang R, Tang J, Yang H, Hu S et al. (2010) Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. PLoS ONE 5(8): e12380. https://doi.org/10.1371/journal.pone.0012380

Appendix I

Average (standard deviations) of treatment effects for different biomass accumulation measures and leaf characteristics for the five species (one host tree and 4 lianas) grown in competition treatments under two different light levels.

Origin	Species	Light leve	Competition	Total biomass	Below-ground Biomass	Above-ground Biomass	Leaf Biomass	Stem Biomass	No. Leaves	SLA	Relative growth rate
Native tree	*G. semiglauca*	medium	Alone	21.9 (9.6)	5.1 (2.9)	4.3 (1.8)	11.7 (4.4)	5.0 (2.9)	37.1 (23.2)	94.7 (10.4)	0.06 (0.29)
			G. semiglauca	11.1 (8.2)	2.9 (2.3)	2.5 (1.4)	7.0 (3.9)	5.0 (5.0)	30.3 (19.6)	93.9 (16.7)	0.27 (0.09)
			An. cordifolia	9.0 (8.9)	8.1 (6.2)	5.5 (3.7)	4.8 (3.8)	2.1 (2.6)	21.7 (11.1)	100.2 (10.1)	-
			Ar. sericifera	29.4 (20.4)	2.2 (2.6)	1.8 (1.6)	13.3 (8.2)	8.0 (6.2)	28.4 (21.0)	103.3 (13.1)	-
			C. antarctica	16.8 (10.6)	4.5 (2.6)	3.2 (2.0)	7.9 (5.4)	4.4 (2.6)	46.4 (45.0)	195.8 (216.4)	-
			P. pandorana	21.7 (10.8)	5.6 (4.1)	4.1 (1.8)	10.7 (3.9)	5.5 (4.1)	28.9 (14.4)	109.2 (54.6)	-
		low	Alone	15.3 (4.3)	3.4 (1.0)	3.1 (0.9)	8.6 (2.4)	3.3 (1.0)	25.6 (18.2)	163.4 (67.6)	0.02 (0.17)
			G. semiglauca	14.3 (5.7)	3.3 (2.2)	2.7 (1.6)	7.5 (4.0)	3.5 (2.0)	31.0 (9.1)	182.4 (51.9)	0.26 (0.08)
			An. cordifolia	6.7 (4.1)	2.9 (1.8)	2.7 (1.3)	4.2 (2.2)	1.2 (1.0)	20.7 (10.1)	165.1 (30.6)	-
			Ar. sericifera	13.3 (6.9)	1.3 (1.0)	1.4 (0.8)	7.5 (3.4)	2.8 (1.8)	23.1 (16.8)	171.5 (79.9)	-
			C. antarctica	10.5 (7.7)	2.3 (2.2)	2.1 (1.4)	6.0 (3.5)	2.2 (2.2)	26.4 (18.2)	189.5 (65.2)	-
			P. pandorana	17.3 (10.5)	4.5 (3.4)	3.3 (1.9)	10.2 (3.9)	4.4 (3.4)	20.3 (14.6)	132.2 (51.1)	-
Exotic liana	*An. cordifolia*	medium	Alone	4.6 (2.5)	2.0 (1.0)	2.9 (1.5)	0.8 (0.7)	2.0 (1.0)	33.6 (30.6)	250.4 (79.6)	0.37 (0.08)
			An. cordifolia	3.6 (1.6)	1.2 (0.7)	2.0 (0.6)	0.8 (0.6)	1.2 (0.7)	19.1 (13.2)	205.2 (39.9)	0.34 (0.07)
			G. semiglauca	24.5 (8.8)	9.8 (3.8)	15.0 (5.1)	5.2 (1.7)	9.8 (3.8)	92.1 (53.9)	237.9 (36.7)	-
		low	Alone	7.4 (2.9)	3.0 (1.3)	4.8 (1.9)	1.8 (1.5)	3.0 (1.3)	43.9 (15.1)	318.2 (65.8)	0.45 (0.08)
			An. cordifolia	4.1 (2.5)	1.5 (1.0)	2.9 (1.6)	1.4 (0.8)	1.5 (1.0)	30.0 (18.6)	423.1 (66.2)	0.33 (0.13)
			G. semiglauca	20.6 (7.0)	7.6 (2.9)	13.4 (4.3)	5.8 (1.9)	7.6 (2.9)	79.3 (43.2)	379.4 (76.7)	-
Exotic liana	*Ar. sericifera*	medium	Alone	4.4 (3.6)	2.1 (1.3)	2.7 (2.3)	0.9 (0.6)	1.8 (1.5)	19.3 (8.3)	299.1 (107.7)	0.36 (0.23)
			Ar. sericifera	1.8 (1.1)	2.6 (3.4)	3.2 (3.8)	0.5 (0.6)	2.6 (3.4)	13.4 (12.8)	338.9 (141.0)	0.29 (0.09)
			G. semiglauca	6.3 (4.4)	2.7 (1.9)	3.6 (2.5)	0.9 (0.6)	2.7 (1.9)	17.0 (12.5)	340.5 (133.2)	-
		low	Alone	3.8 (2.9)	1.5 (1.1)	2.4 (1.8)	0.9 (0.7)	1.5 (1.1)	32.4 (42.4)	480.7 (69.5)	0.38 (0.13)
			Ar. sericifera	2.9 (2.5)	0.8 (1.0)	1.2 (1.3)	0.4 (0.4)	0.8 (1.0)	9.6 (6.1)	525.2 (100.7)	0.32 (0.18)
			G. semiglauca	8.2 (8.8)	3.4 (3.8)	4.8 (5.0)	1.4 (1.1)	3.4 (3.8)	19.7 (14.1)	453.3 (104.6)	-

Origin	Species	Light leve	Competition	Total biomass	Below-ground Biomass	Above-ground Biomass	Leaf Biomass	Stem Biomass	No. Leaves	SLA	Relative growth rate
Native liana	*C. antarctica*	medium	Alone	9.6 (7.3)	3.0 (2.0)	6.7 (5.3)	3.7 (3.3)	3.0 (2.0)	35.7 (21.8)	116.9 (12.5)	0.23 (0.10)
			C. antarctica	18.1 (9.2)	3.9 (2.6)	9.3 (5.6)	5.4 (3.3)	3.9 (2.6)	58.3 (39.3)	141.3 (24.8)	0.33 (0.08)
			G. semiglauca	37.6 (16.0)	12.4 (4.7)	25.2 (11.8)	12.8 (8.3)	12.4 (4.7)	67.3 (47.9)	151.1 (55.6)	-
		low	Alone	26.9 (7.2)	8.3 (2.4)	18.7 (4.8)	10.4 (2.7)	8.3 (2.4)	73.9 (13.0)	213.4 (43.1)	0.40 (0.04)
			C. antarctica	12.8 (4.3)	4.7 (2.2)	11.2 (4.9)	6.5 (2.8)	4.7 (2.2)	57.6 (19.0)	220.1 (40.1)	0.29 (0.05)
			G. semiglauca	36.3 (11.7)	11.1 (6.2)	24.7 (8.1)	13.1 (4.6)	11.6 (3.6)	94.1 (38.1)	61.1 (34.7)	-
Native liana	*P. pandorana*	70	Alone	21.1 (5.9)	8.6 (2.7)	16.3 (3.3)	7.7 (1.4)	8.6 (2.7)	84.1 (20.7)	145.4 (12.7)	0.30 (0.04)
			P. pandorana	15.3 (7.5)	8.6 (3.3)	15.0 (4.2)	6.4 (2.8)	8.6 (3.3)	53.7 (43.9)	155.5 (21.5)	0.25 (0.07)
			G. semiglauca	27.8 (17.7)	11.1 (3.2)	20.4 (11.6)	9.3 (5.6)	11.1 (6.2)	79.9 (65.3)	182.4 (43.4)	-
		90	Alone	16.2 (5.7)	7.4 (2.1)	12.6 (3.6)	5.2 (1.6)	7.4 (2.1)	87.4 (47.6)	249.2 (16.9)	0.26 (0.05)
			P. pandorana	15.6 (8.8)	6.4 (3.3)	10.4 (5.8)	4.0 (2.6)	6.4 (3.3)	92.9 (28.9)	267.4 (47.6)	0.24 (0.08)
			G. semiglauca	19.8 (9.6)	8.1 (3.6)	15.4 (6.2)	7.3 (3.2)	8.1 (3.6)	76.0 (64.4)	453.3 (104.6)	-

NeoBiota 36: 39–55 (2017)
doi: 10.3897/neobiota.36.14953
http://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Comparing functional traits and abundance of invasive versus native woodwasps

Ann E. Hajek[1], Jacob C. Henry[1,3], Christopher R. Standley[2,4], Christopher J. Foelker[2,5]

1 *Department of Entomology, Cornell University, Ithaca, New York, USA 14853-2601* **2** *Department of Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, New York, USA 13210* **3** *Present address: Department of Forestry, Mississippi State University, Starkville, Mississippi, USA 39762* **4** *Present address: New York State Department of Environmental Conservation, 21 South Putt Corners Rd., New Paltz, New York, USA 12528* **5** *Present address: Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, Wisconsin, USA 53708*

Corresponding author: *Ann E. Hajek* (aeh4@cornell.edu)

Academic editor: *A. Roques* | Received 9 July 2017 | Accepted 25 September 2017 | Published 3 November 2017

Citation: Hajek AE, Henry JC, Standley CR, Foelker CJ (2017) Comparing functional traits and abundance of invasive versus native woodwasps. NeoBiota 36: 39–55. https://doi.org/10.3897/neobiota.36.14953

Abstract

Functional traits are useful for comparing the resource use of invasive and native species, with goals of identifying resource overlap to predict competitive interactions. The invasion of northeastern North America by the woodwasp *Sirex noctilio* has resulted in competition with the native congeneric *Sirex nigricornis* for suppressed and weakened pines. We compared sizes of adults, venom glands, fecundity, tree species use, voltinism and abundance of the invasive woodwasp *S. noctilio* with the native *S. nigricornis* in northeastern North American pines. Rearing adults from attacked pines showed that these species used the same tree species but *S. noctilio* were far more abundant, especially with increasing time since establishment. Adults of the invasive *S. noctilio* were larger than *S. nigricornis*, female *S. noctilio* had larger glands carrying phytotoxic venom in relation to body size, average-sized *S. noctilio* females carried more eggs, and *S. noctilio* developed faster than *S. nigricornis*. *Sirex noctilio* was the dominant woodwasp infesting suppressed pines in our study areas. We hypothesize that the future abundance of *S. nigricornis* could depend in part on the availability of wood for oviposition by this native that is not available or acceptable to the earlier-emerging *S. noctilio*.

Keywords

invasive species, functional traits, Siricidae, wood borers

Introduction

A major cause of global change is the pervasive introduction of alien species (Wardle et al. 2011; Simberloff et al. 2013) and the increase in global biotic homogenization is not predicted to diminish any time soon (Seebens et al. 2017). Once established in new locations, alien species with strong impacts can change ecosystems in many ways, including altering the biology and ecology of native competitors. The results from interactions between invasive and native competitors can range between coexistence, survival by differential use of resources that arise occasionally or periodically, and competitive displacement of the native species that can remain at low densities or become extinct (see Boivin et al. 2008).

The nature and extent of ecological impacts of alien species depend on the functional ecology of these species in the context of the structure, diversity, and evolutionary experience of the recipient community (Ricciardi et al. 2013). Evaluation of functional traits of invasives and co-existing natives is one means for understanding the effects of invasions, and especially the extent that an invasive is impacting native communities (Mouillot et al. 2013). Although impacts must be evaluated in the context of communities, functional traits such as rapid exploitation of resources and elevated fecundity have been associated with high-impact invasives (Ricciardi et al. 2013). Invasive species that have the greatest impact have also been suggested as being those introducing novel traits and becoming dominant members of communities (Rodriguez-Cabal et al. 2015; Callaway and Ridenour 2004).

Arthropods constitute the most diverse group of organisms (Pimm et al. 1995) and our understanding of the biologies and ecologies of the majority of native species in native ecosystems is frequently limited for many arthropod species prior to invasions. Yet, invasive arthropods have had and are presently having enormous impacts in forests (Liebhold et al. 1995; Gandhi and Herms 2010). Woodwasps are generally not considered forest pests and relatively little is known about their functional traits, although the aggressive invasive *Sirex noctilio* is a major exception. *Sirex noctilio* is not a pest where it is native in Eurasia and North Africa, but has caused economic damage in plantations of exotic pines (*Pinus* spp.) after introductions in the southern hemisphere (Slippers et al. 2015). Established populations of *S. noctilio* were first reported in northeastern North America in 2004 (Hoebeke et al. 2005) and this invasive now occurs in eight eastern US states (CERIS 2017) and two eastern Canadian provinces (de Groot et al. 2006, CFIA 2009) and it is expected to continue to spread. In the southern and northern hemispheres, *S. noctilio* typically oviposits in suppressed pines (Neumann and Minko 1981, Dodds et al. 2010; Ayres et al. 2014). However, populations can increase and, especially in overstocked stands and during droughts, *S. noctilio* can kill pines that are considered healthy (Madden 1988). *Sirex noctilio* kills pines by injecting an obligate symbiotic white rot fungus, *Amylostereum areolatum* (Ryan and Hurley 2012), and a phytotoxic venom (Bordeaux et al. 2014) in association with laying eggs into trees. Wood-boring *S. noctilio* larvae can only develop if the white rot fungus is present, as it serves as an external rumen for larvae (Thompson et al. 2014).

The establishment of *S. noctilio* in eastern North America is the first time that this invasive has invaded an area hosting native *Pinus* and *Sirex* communities, including the congeneric native pine-specialist *S. nigricornis* which has never been considered a pest in North America. *Sirex nigricornis* is associated with one of two fungal symbionts: either the same fungal symbiont as *S. noctilio* (*A. areolatum*) or *Amylostereum chailletii* (Hajek et al. 2013; Olatinwo et al. 2013). *Sirex nigricornis* can co-occur in pines with *S. noctilio* (Ryan et al. 2012a; Hajek et al. 2013). Most adults of *S. nigricornis* emerge to mate and oviposit approximately 1-2 months after *S. noctilio* (Ryan et al. 2012a; Foelker et al. 2016; Suppl. material 1: B). Both species can be highly variable in size which depends on sex, parasitism and larval developmental conditions (Ryan and Hurley 2012; Kroll et al. 2013; Haavik et al. 2016a), but in North American areas of sympatry, *S. noctilio* adults are usually slightly larger and carry more eggs than *S. nigricornis* (Haavik et al. 2016a; AEH and JCH unpublished data). While *S. noctilio* is more aggressive and can attack healthier trees, both species will oviposit in pines of poor vigor or that are recently dead (see Suppl. material 1: A). The resources needed by these species are ephemeral and often patchily distributed; in agreement, studies have shown that populations of *S. noctilio* are limited by the availability of acceptable pines (Haavik et al. 2016b). To investigate these two *Sirex* species in pines after establishment of *S. noctilio*, we compared the functional traits of *S. noctilio* and *S. nigricornis*. Here, we compare the sizes of venom glands of these two species in relation to female sizes, and their pine species use and voltinism. Using trees with evidence of *S. noctilio* infestation, we present the relative densities of the two *Sirex* species across time, beginning three years after the first detection of *S. noctilio* in the northeastern US. Our goal is to compare functional traits between the native and invasive to begin to understand the effects of the *S. noctilio* invasion on native siricid communities.

Materials and methods

Rearing procedures

Red pines (*Pinus resinosa*) and scots pines (*Pinus sylvestris*) with resin beads characteristic of *S. noctilio* attack (Dodds et al. 2010) were located from October to December in New York and Pennsylvania. These trees were often suppressed and patchily distributed. In spring, infested trees were felled, areas of trunks with resin beads were cut into bolts, and ends of bolts were coated in sealant (Waxlor, Willamette Valley Company, Eugene, Oregon, USA). For the majority of studies, 70 cm long bolts were placed in fiber barrels (91 cm high × 61 cm diam) covered securely with window screening, in an unheated barn. Once adult wasp emergence began (in early to mid-July), barrels were checked daily for two months and then subsequently 3–4 times per week. *Sirex* that emerged were placed in 29 ml clear plastic cups at 4°C. All *Sirex* emerging were identified using characters described in Schiff et al. (2012).

To cover the emergence periods for both *Sirex* species, checking for emergence finished in early November, and, at that time, barrels were completely checked for any additional specimens.

For studies comparing the abundance of *Sirex* species by tree species, *Sirex* were reared from 50 cm long bolts placed in horizontal cardboard rearing tubes. Bolts were spaced from tube sides using nails and were housed at ambient environmental conditions in a screened outdoor insectary. Glass emergence jars were fitted to the tube ends, oriented facing screened windows for a natural photoperiod, and emerging *Sirex* were collected daily.

Adult size and venom gland measurement

To quantify the sizes of emerging adults, we followed Madden (1974) and Nahrung (2016) in using the apical width of the prothorax. Measurements were made using digital calipers (Traceable Products, Webster, Texas, USA). Infection with the parasitic nematode *Deladenus siricidicola* negatively impacts adult size (Kroll et al. 2011; Haavik et al. 2016a), so we diagnosed whether each of the adults being measured was parasitized by these nematodes. Males and females were dissected alive under a dissecting microscope at 10× magnification. The abdomen was removed and cut lengthwise to open it, without damaging the venom glands in females, and presence of nematodes within the abdominal cavity and reproductive tissues was recorded. Eggs from females were counted at 63× magnification and venom glands were removed and immediately weighed on a microscope cover slip.

To compare sizes of *S. noctilio* adults with (n = 92) and without (n = 412) nematodes, we used randomly selected individuals emerging from barrels in 2012–2014. There were far fewer *S. nigricornis* emerging during this period and nematode infections were uncommon in *S. nigricornis*, so analyses included only *S. nigricornis* of both sexes without nematodes (n = 36).

To assess weights of venom glands, a total of 51 *S. noctilio* were randomly selected in 2014–2016 for dissection and 21 of these contained nematodes. Since few female *S. nigricornis* emerged from rearings from the northeast during 2014–2016, 30 *S. nigricornis* females from Arkansas and Louisiana that had been caught in panel traps were used for venom gland weights. We were concerned that these *S. nigricornis* from the southeast both had been flying before being trapped and were from a different geographic region. To test the accuracy of using southeastern *S. nigricornis* as replacements for northeastern *S. nigricornis*, we compared the relationship between numbers of eggs and body size for *S. nigricornis* females collected from traps in the southeastern vs. emerging from wood from the northeastern USA. Southeastern *S. nigricornis* carried the same number of eggs in relation to prothorax width as *S. nigricornis* emerging from wood in the northeastern US (t = -1.10; P = 0.2713), so we used venom gland weights from southeastern *S. nigricornis* for comparisons.

Table 1. First and second year emergence by *Sirex noctilio* and *Sirex nigricornis*.

	Location	2 years of emergence	Total Sirex emerging	Emergence year 1 (%)	Emergence year 2 (%)	% trees with 2nd year emergence
Sirex nigricornis	Warren County, NY	2013–2014	38	44.7%	55.3%	50.0%
Sirex noctilio	Warren County, NY	2013–2014	15	73.3%	26.7%	25.0%
	Tioga County, PA	2014–2015	1056	98.5%	1.5%	39.1%
	Tioga County, PA	2015–2016	709	89.1%	10.9%	42.9%

Tree species use

Use of *P. resinosa* versus *P. sylvestris* by *S. nigricornis* and *S. noctilio* was quantified using infested trees in northern New York State, with site information in Foelker et al. (2016; Table 1) (2010: 10 *P. resinosa*, 12 *P. sylvestris*; 2011: 22 *P. resinosa*, 10 *P. sylvestris*). Trees were harvested from 21 April – 24 May in 2010 and 31 March – 7 May in 2011 and *Sirex* were reared from trees as described above.

Voltinism

On 4 June 2013, 12 *Sirex*-infested *P. resinosa* were collected from a plantation on River Road, Warren Co., New York (43°31'59.7"N 73°49'30.8"W). Trees were cut into 219 70 cm long bolts and woodwasps were reared as described above, with barrels checked every 1–2 days to collect individuals very soon after emergence; females were never found to be ovipositing when collected this soon after emergence. In November 2013, barrels were thoroughly checked for dead *Sirex* so that any first year emergers were not mistaken for second-year emergers. Barrels were stored in an unheated barn over the winter and were checked for emergence throughout the 2014 flight season. This procedure was repeated for a third year, through the 2014–2015 winter and 2015 flight season. As a continuation to this study, in Tioga Co., Pennsylvania, in spring 2014 we harvested 23 infested *P. resinosa* and, in spring 2015, 29 *P. resinosa*. Wood from trees cut in 2014 and 2015 was maintained and emergence was checked for two years.

Densities and co-occurrence of *S. noctilio* and *S. nigricornis*

Pines with resin beads indicative of *S. noctilio* attack were harvested in central and northern New York State and north-central Pennsylvania and *Sirex* were reared from them. Even within the same region, it was rare that the exact same site was sampled more than one year. *Pinus resinosa* were harvested from plantations in Tioga Co., Pennsylvania yearly, from 2011 and 2013–2015, with 20–30 trees harvested each year. *Sirex* were also reared from mixtures of *P. resinosa* and *P. sylvestris* from natural forests in northern New York State and from mature plantations in central New York State. In 2007 only *P. sylvestris* was sampled as described in Long et al. (2009). Trees included in analyses were those from which either one or the other or both *Sirex* species emerged.

Data analysis

A general linear mixed model with year as a random effect was used to compare sizes of *Sirex*. To compare body size versus venom gland mass, the significance of difference between slopes was calculated (Cohen et al. 2003). Comparisons of body size across species, sex and nematode parasitism were conducted using mixed models with year as a random effect and least squares means were used for post hoc comparisons (SAS 2014). Because very few *S. nigricornis* were reared that were parasitized by nematodes, comparing body sizes for individuals that were parasitized versus not parasitized was only possible for *S. noctilio*. Tree species use analysis was conducted using a mixed model with (ln +1)-transformed densities by volume of wood for *S. nigricornis* versus *S. noctilio* (SAS 2014). *Sirex* species, tree height, tree diameter and tree species were explanatory variables, with tree nested within site and site as random effects. The interaction of *Sirex* species with tree species was initially included but was removed as it was not significant. Wilcoxon signed ranks tests were used to compare numbers of *S. noctilio* versus *S. nigricornis* adults emerging from trees in 2007–2014.

Results

Trait-based studies: Venom glands and *Sirex* size

Weights of venom glands increased with increasing body size (measured as prothorax width) (Fig. 1) but this relationship differed by species. The slope of the regressions of body size against venom gland weight for *S. nigricornis* without nematodes was less steep compared with *S. noctilio* without nematodes ($t = 4.527$; $df = 56$; $P < 0.0001$); this slope was close to twice as steep for *S. noctilio* compared with *S. nigricornis*. The weight of the venom glands for *S. nigricornis* females without nematodes also was significantly less than for *S. noctilio* with nematodes ($t = 3.3824$; $df = 47$; $P = 0.0015$). *Sirex noctilio* parasitism by nematodes did not significantly affect the relationship between the weight of the venom gland and body size, when compared with non-parasitized *S. noctilio* individuals ($t = 1.1141$; $df = 47$; $P = 0.2709$).

For collections across 2012–2014, on average non-parasitized *S. noctilio* females (prothorax width: 3.90 + 0.83 mm) were larger than *S. nigricornis* females (2.46 + 0.35 mm) ($t = 6.27$; $P < 0.0001$) and non-parasitized *S. noctilio* males (2.92 + 0.83 mm) were larger than *S. nigricornis* males (2.38 + 0.56 mm) ($t = 3.00$; $P = 0.0028$).

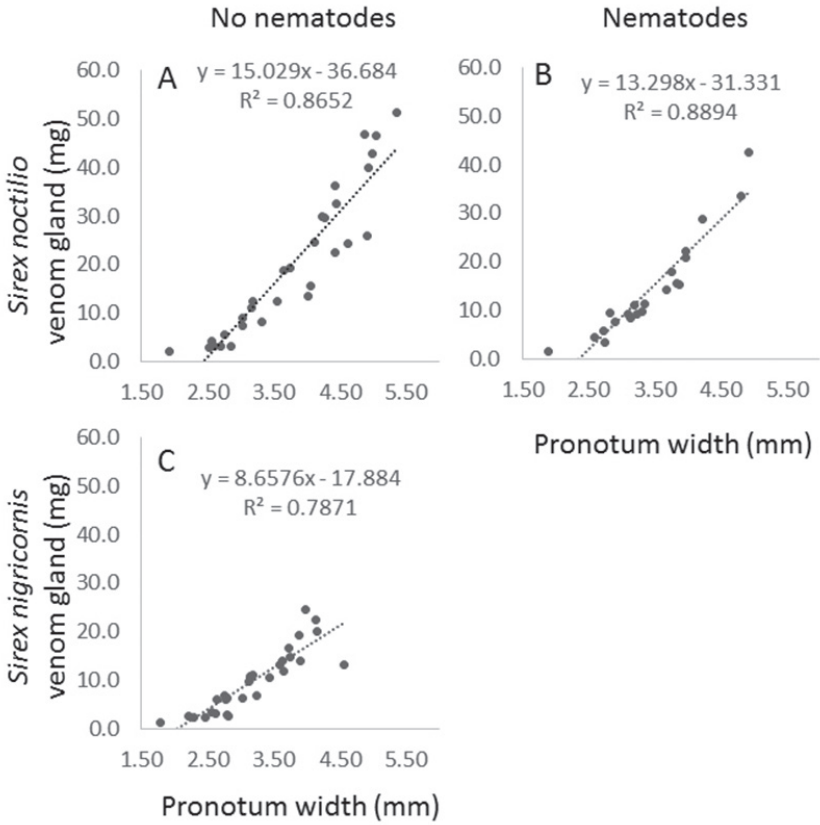

Figure 1. Relationships between venom gland mass and body size, measured as pronotum width, for *S. noctilio* and *S. nigricornis*. *Sirex noctilio* data were analyzed by presence or absence of parasitism by nematodes (*Deladenus siricidicola*) while numbers of *S. nigricornis* parasitized by nematodes were too low for analysis. **A** *Sirex noctilio* females without parasitism by nematodes **B** *S. noctilio* females parasitized by nematodes, and **C** *S. nigricornis* without nematode parasitism.

For male *S. noctilio*, nematode parasitism unexpectedly resulted in larger body sizes ($t = -2.62$; $P = 0.0091$) while this relationship was reversed for females ($t = 2.34$; $P = 0.0195$) (Fig. 2).

Trait-based studies: Tree species preference

Sirex densities by tree species did not differ between the two *Sirex* species ($F_{1,52.6} = 0.01$; $P = 0.9395$). The only main effect that was significant in the model was the comparison of densities of the two *Sirex* species ($F_{1, 56} = 32.11$; $P < 0.0001$; *S. nigricornis* density = 8.9 ± 2.6/m^3, *S. noctilio* density = 44.3 ± 11.5/m^3).

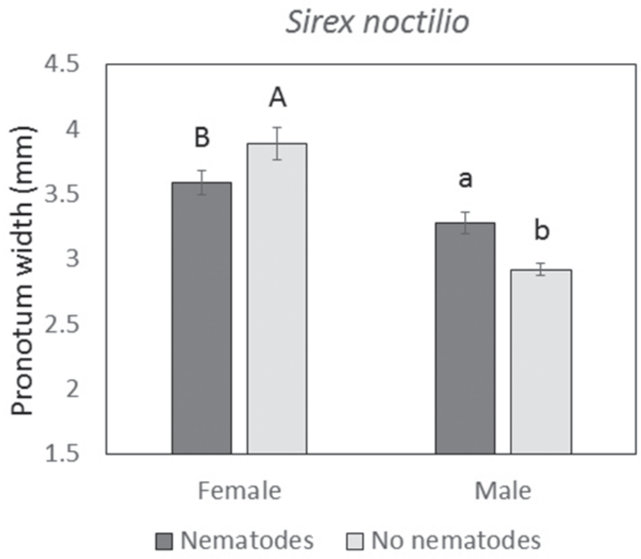

Figure 2. Mean body size (+ SE), measured as pronotum width, for *S. noctilio* males or females parasitized by the nematode *D. siricidicola* or not. Different letters (comparing either capital letters or comparing lower case letters) demonstrate significant differences within sexes.

Trait-based studies: Comparing voltinism

Nearly half of adult *S. nigricornis* emerged from wood during the first season in our rearings, over half emerged during year 2 (Table 1), and no *S. nigricornis* emerged during year 3. In contrast, emergence of *S. noctilio* adults primarily occurred in year 1 (Table 1). Numbers of *S. noctilio* emerging from wood in 2013–2014 were low but among these, 26.7% emerged during the second year. For wood harvested in 2014 or 2015 and reared for two years, from 1.5% to 10.9% of the *S. noctilio* emerged during year 2.

Densities and co-occurrence of *S. noctilio* and *S. nigricornis*

Between 2010 and 2015, *S. nigricornis* densities were lower than densities of *S. noctilio* in central New York State and north central Pennsylvania, where most pines that were sampled had been purposefully planted (Table 2). However, when sites in central and northern New York State were sampled within 2–3 years of the first reports of *S. noctilio* in those areas (see Table 2 footnotes), numbers of *S. nigricornis* emerging did not differ significantly from *S. noctilio*, although they were usually numerically lower. When *S. nigricornis* emerged, they more commonly co-occurred in the same trees as *S. noctilio* and rarely were the only *Sirex* species emerging from a tree. In 2015, no *S. nigricornis* emerged from infested material from north central Pennsylvania and during this study, densities of *S. nigricornis* from this same area were always low.

Table 2. Co-occurrence and densities of *S. noctilio* and *S. nigricornis* emerging from *P. sylvestris* and *P. resinosa* from New York and Pennsylvania, 2007-2015 (only including trees from which *Sirex* emerged).

	Total infested trees sampled	**Tree species**[a]	**Total trees with only *S. noctilio***	**Total trees with only *S. nigricornis***	**Total trees with both *Sirex* species**	**Mean *S. noctilio*/tree ± SE**	**Mean *S. nigricornis*/tree ± SE**	**P values**[b]
Central New York State[c]								
2007	6	S	3 (50%)	0 (0%)	3 (50%)	218.8 ± 64.9	13.2 ± 10.4	0.0625
2010	9	SR	6 (67%)	1 (11%)	2 (22%)	41.8 ± 9.8	1.6 ± 0.9	0.0117 *
2011	19	SR	12 (63%)	0 (0%)	7 (37%)	48.2 ± 14.9	5.5 ± 3.0	<0.0001 *
Northern New York State[d]								
2010	13	SR	3 (23%)	3 (23%)	7 (54%)	13.4 ± 4.8	20.2 ± 6.5	0.5532
2011	8	SR	4 (50%)	2 (25%)	2 (25%)	17.7 ± 5.7	3.7 ± 1.8	0.1641
North Central Pennsylvania[e]								
2011	19	R	15 (79%)	0 (0%)	4 (21%)	38.4 ± 10.3	0.7 ± 0.4	<0.0001 *
2013	17	R	12 (71%)	1 (6%)	4 (24%)	22.8 ± 6.0	1.6 ± 1.2	0.0001 *
2014	26	R	22 (85%)	0 (0%)	4 (15%)	52.5 ± 10.7	0.7 ± 0.5	<0.0001 *
2015	15	R	15 (100%)	0 (0%)	0 (0%)	22.8 ± 7.4	0.0 ± 0.0	<0.0001 *

[a] S = scots pine (*P. sylvestris*), R = red pine (*P. resinosa*), SR = mixture of scots and red pines.

[b] Wilcoxon ranked sign tests comparing numbers of *S. noctilio* versus *S. nigricornis* emerging from trees. Asterisks indicate significantly different results, with more *S. noctilio* than *S. nigricornis*.

[c] *S. noctilio* first collected in this area in 2004 and 2005 (Dodds and de Groot 2012).

[d] *S. noctilio* first collected in this area in 2008 (AEH unpubl. data).

[e] *S. noctilio* first collected in this area in 2008 (B. Regester, pers. comm).

Discussion

Comparing functional traits

The overall body sizes of *S. noctilio* as well as the sizes of their venom glands were significantly larger than the bodies and venom glands of the native *S. nigricornis*. The venom glands of *S. noctilio* are also larger than those of seven other European siricids (Spradbery 1977) and experimentation has shown that venom from *S. noctilio* had greater phytotoxic activity than venom of these European siricids (Spradbery 1973). While a key component of the venom in the *S. noctilio* gland has been characterized as noctilisin (Bordeaux et al. 2014), the identities of compounds in the venom glands of *S. nigricornis*, or glands of any other siricids, are not known (Wang et al. 2016). Regardless, the fact that the *S. noctilio* venom glands are larger (relative to adult body size) than venom glands known from other siricids is consistent with the fact that this is the most aggressive siricid, reported as able to kill relatively healthy pines where it is adventive (see Suppl. material 1: A), while other woodwasps are considered relatively benign secondary pests in forests (Schiff et al. 2012).

Haavik et al. (2016a) found that *S. nigricornis* carries more eggs per body size than *S. noctilio* and our studies have documented the same trend (AEH and JCH, unpublished data). However, the present study demonstrated that on average *S. noctilio* females with or without nematode infections were larger than *S. nigricornis* females and the same relationship holds for males. So, although *S. nigricornis* has the potential to carry more eggs than *S. noctilio*, when we calculated the numbers of eggs per female based on sizes of females that emerged from wood in our studies, using the regression equations in Haavik et al. (2016a), the number of eggs for average-sized *S. noctilio* female was 166 but 138 eggs would be produced by *S. nigricornis*. Thus, the larger body size of *S. noctilio* emerging from trees compensated so that on average females of the invasive carried more eggs than *S. nigricornis*. It was unexpected that while nematode-infected *S. noctilio* females were smaller than healthy females, nematode-infected males were larger than healthy males, an association also found by Haavik et al. (2016a).

The densities of the two *Sirex* species emerging from *P. resinosa* and *P. sylvestris* did not differ by tree species. Studies have found a trend of *P. sylvestris* being colonized more frequently by *S. noctilio* compared with *P. resinosa* (Dodds et al. 2010; Foelker 2016) and *S. nigricornis* was not included in those studies. Largely based on the southern hemisphere where many North American pines have been introduced, susceptibility of pine species to *S. noctilio* varies by tree species (Ryan and Hurley 2012; Nahrung et al. 2015). Our result was somewhat unexpected because *P. sylvestris* is native to Europe, where it is assumed to have coevolved with *S. noctilio* (Ayres et al. 2014). However, we do not know exactly where the genotypes of *S. noctilio* introduced to New York and Pennsylvania originated and, given the broad native geographic range of *S. noctilio*, it is possible that these genotypes of this woodwasp did not co-evolve with *P. sylvestris* (Boissin et al. 2012; Bittner et al. 2017). On the other hand, *P. resinosa* and *S. nigricornis* co-evolved in North America and in this case there was no preference for the native pine over the introduced pine.

Sirex noctilio and *S. nigricornis* also differed significantly based on the percentages of the populations emerging from wood after 1 vs 2 years. In our studies, *S. noctilio* mainly emerged in year 1; from 1.5–27.7% of *S. noctilio* emerged the second year (Table 2) while in Ontario, Ryan et al. (2012b) found 2.2% emergence during year 2. Second-year emergence of *S. noctilio* was 15–25% in New Zealand and Australia, including Tasmania (Morgan 1968; Taylor, 1978), 1.6% in Eurasia (Spradbery and Kirk 1978), and 24% in Galicia, Spain (Lombardero et al. 2016). In all cases, percent emergence for *S. noctilio* in the second year of rearing was less than that recorded for *S. nigricornis* in our study, for which close to 50% emergence occurred in both years 1 and 2. Differences in voltinism between *S. noctilio* and *S. nigricornis* could have large impacts on their population dynamics. Based on our results, future studies of *S. nigricornis*, at least in northeastern North America, should allow two years for emergence from infested wood.

Comparing abundance of *Sirex* species

In northeastern North America *S. noctilio* is now the most abundant woodwasp attacking pines (Long et al. 2009; Ryan et al. 2012a; Foelker et al. 2016). Unfortunately, there are no records of the population densities or tree use by *S. nigricornis* before *S. noctilio* arrived but now, use of suppressed pines by *S. nigricornis* is much less than use of this resource by *S. noctilio* (e.g., Table 2).

Sirex noctilio has a temporal advantage over *S. nigricornis* as many emerge 1–2 months before *S. nigricornis*, although there is overlap in emergence between these species in northeastern North America (Ryan et al. 2012a; Haavik et al. 2013; Hartshorn et al. 2016; Suppl. material 1: B). *Sirex noctilio* is found colonizing trees at higher densities than *S. nigricornis*. Therefore, each year *S. noctilio* will occupy what is considered as being their preferred resources (i.e., suppressed trees that are not yet dead; see Suppl. material 1: A) before *S. nigricornis* adults have emerged. Among all pines from which *Sirex* were reared, only *S. noctilio* emerged from 56% (across the 9 years of this study). However, after the invasive had been present for a few years in an area, when the two species both emerged from trees, numbers of *S. nigricornis* were lower than *S. noctilio* and we very rarely found only *S. nigricornis* emerging from a tree. Based on the low numbers of *S. nigricornis* emerging from infested trees, we hypothesize that population densities of *S. nigricornis* emerging could be low because when adults of the native species emerge, the transient resource of suppressed trees had already mostly been exploited. However, *S. nigricornis* could have an advantage in situations where pines unacceptable to *S. noctilio* for some reason could be available for use by *S. nigricornis* or when pines could become weakened (e.g., by lightning strikes) after the main flight of *S. noctilio* adults and before or during the *S. nigricornis* flight time. In addition, the fact that the transient resource of suppressed trees are often patchily distributed (e.g., Ayres et al. 2014) could lead to coexistence of these species via differential spatial distribution of use of suppressed pines.

Monceau et al. (2015) have shown that some degree of niche differentiation between native and invasive hornets can minimize competition. We did not sample recently dead trees in this study but experiments have shown that *S. nigricornis* will oviposit into wood from trees that have recently been cut, although oviposition was minimal as soon as 30 days after cutting the wood (Hartshorn 2012). We do not know to what extent *S. noctilio* will choose to oviposit in wood from recently cut trees but when siricids were reared from diverse types of wood sampled over eight years from Europe, Turkey and North Africa, *S. noctilio* only emerged from samples from standing trees and timber and never from fallen trees or wood on the ground (Spradbery and Kirk 1978). While niches of these two *Sirex* species probably differ to some extent relative to the health or condition of pines that are preferred or acceptable for oviposition and development, there is also niche overlap (see Suppl. material 1: A). Based on relative densities and traits, we hypothesize that after the invasion of *S. noctilio*, *S. nigricornis* could develop in suppressed trees less often than prior to the invasion and the native might now more frequently use recently fallen, dead trees, as *S. noctilio* would have already exploited the majority of standing suppressed pines. Alternatively, suppressed pines further weakened by *S. noctilio* attack could at times provide an increased resource for *S. nigricornis* populations. Knowledge about normal densities of *S. nigricornis* before invasion by *S. noctilio* is necessary to further understand the impact of the native on the invasive woodwasp. In addition, further studies investigating the health of pines associated with oviposition and development of these two *Sirex* species are needed to clarify the overlap in niches of these two species with regard to tree health.

Conclusions

In this study, we investigated aspects of the biology and ecology of these now-sympatric native and invasive siricids toward predicting the impact *S. noctilio* might be having on this congeneric native species also utilizing suppressed pines (Suppl. material 1: A). The invasive *S. noctilio* uses the same tree species as the native *S. nigricornis* but venom glands of *S. noctilio* are larger. Although *S. nigricornis* carries more eggs than *S. noctilio* per body size, *S. nigricornis* adults are significantly smaller than *S. noctilio*, resulting in greater fitness for *S. noctilio*. In other systems, fecundity has been shown to be an important functional trait for predicting success of invasives (e.g., Boivin et al. 2008; Capellini et al. 2015). In addition, the phenology of the invasive and its faster speed of development also allow *S. noctilio* to exploit common resources before *S. nigricornis.*

We cannot definitively answer to what extent the presence of *S. noctilio* results in more or less habitat in which *S. nigricornis* can develop. Little is known of the biology and ecology of *S. nigricornis* but we know that it will oviposit and develop in pines already attacked by *S. noctilio*. Attacks by *S. noctilio* could create more suppressed trees acceptable to *S. nigricornis* by pre-injecting the symbiont plus venom and thereby disabling tree defenses and thus creating more habitat for *S. nigricornis*. However, abundance data suggest that few *S. nigricornis* emerge from trees that they co-inhabit with *S. noctilio*. As an alternative, since *S. noctilio* mostly flies and oviposits before *S. nigricornis*, perhaps this invasive attacks the best of the transient resource of weakened trees that could potentially be used by either species and *S. noctilio* thus uses most of this resource before *S. nigricornis* adult females would have emerged to oviposit. The extent that co-occurring *S. nigricornis* and *S. noctilio* will compete for recently dead trees remains to be determined. Further data documenting the ecology and naturally occurring densities of *S. nigricornis* in the southeastern US, where pine forests are extensive, will assist with predicting to what extent *S. nigricornis* niches and abundance will be altered when *S. noctilio* spreads into this region.

Acknowledgments

We thank Brad Regester, Bill Laubscher and Tim Marasco, Pennsylvania DCNR Bureau of Forestry for assistance with infested wood from north central Pennsylvania, Jim Meeker, Wood Johnson, Jessica Hartshorn and Fred Stephen for providing *S. nigricornis* from Louisiana and Arkansas, and USDA APHIS for assistance with obtaining *S. noctilio*-infested wood in 2007. Francoise Vermeylen assisted with statistical analyses and we thank Flora Krivak-Tetley, David Harris, Stefanie Kroll, and Sandy Liebhold for assistance with this manuscript. This work was supported by USDA Forest Service Cooperative Agreements granted to AEH.

References

Ayres MP, Pena R, Lombardo JA, Lombardero MJ (2014) Host use patterns by the European woodwasp, *Sirex noctilio*, in its native and invaded range. PLOS ONE 9: e90321. https://doi.org/10.1371/journal.pone.0090321

Bittner T, Hajek AE, Haavik L, Allison J, Nahrung H (2017) Multiple introductions of *Sirex noctilio* (Hymenoptera: Siricidae) in northeastern North America based on microsatellite genotypes, and implications for biological control. Biological Invasions 19: 1431–1447. https://doi.org/10.1007/s10530-016-1365-1

Boivin T, Rouault G, Chalon A, Candau J-N (2008) Differences in life history strategies between an invasive and a competing resident seed predator. Biological Invasions 10: 1013–1025. https://doi.org/10.1007/s10530-007-9180-3

Boissin E, Hurley B, Wingfield MJ, Vasaitis R, Stenlid J, Davis C, de Groot P, Ahumada R, Carnegie A, Goldarazena A, Klasmer P, Wermelinger B, Slippers B (2012) Retracing the routes of introduction of invasive species: the case of the *Sirex noctilio* woodwasp. Molecular Ecology 21: 5728–5744. https://doi.org/10.1111/mec.12065

Bordeaux JM, Lorenz WW, Johnson D, Badgett MJ, Glushka J, Orlando R, Dean JFD (2014) Noctilisin, a venom glycopeptide of *Sirex noctilio* (Hymenoptera: Siricidae), causes needle wilt and defense gene responses in pines. Journal of Economic Entomology 107: 1931–1945. https://doi.org/10.1603/EC14151

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2: 436–443. https://doi.org/10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2

Canadian Food Inspection Agency (CFIA) (2009) Plant protection survey report: European wood wasp. http://www.inspection.gc.ca/plants/plant-protection/plant-pest-surveillance/2009-plant-protection-survey-report/eng/1337307704518/1337307793309 [accessed 11 Nov 2015]

Capellini I, Baker J, Allen WL, Street SE, Vendetti C (2015) The role of life history traits in mammalian invasion success. Ecology Letters 18: 1099–1107. https://doi.org/10.1111/ele.12493

Center for Environmental and Research Information Systems (CERIS), Purdue University (2017) Survey Status of Sirex woodwasp - *Sirex noctilio*. http://pest.ceris.purdue.edu/map.php?code=ISBBADA&year=2017 [accessed 26 May 2017]

Cohen J, Cohen P, West SG, Aiken LS (2003) Applied multiple regression/correlation analysis for the behavioral sciences (3rd edn). Lawrence Earlbaum Associates, Mahwah, NJ.

de Groot P, Nystrom K, Scarr T (2006) Discovery of *Sirex noctilio* (Hymenoptera: Siricidae) in Ontario, Canada. Great Lakes Entomologist 39(1/2): 49–53.

Dodds KJ, de Groot P (2012) Sirex surveys and management: challenges of having *Sirex noctilio* in North America. In: Slippers B, de Groot P, Wingfield MJ (Eds) The sirex woodwasp and its fungal symbiont: research and management of a worldwide invasive pest. Springer, Netherlands, 265–286. https://doi.org/10.1007/978-94-007-1960-6_19

Dodds KJ, de Groot P, Orwig DA (2010) The impact of *Sirex noctilio* in *Pinus resinosa* and *Pinus sylvestris* stands in New York and Ontario. Canadian Journal of Forest Research 40: 212–223. https://doi.org/10.1139/X09-181

Foelker CJ (2016) Beneath the bark: associations among *Sirex noctilio* development, bluestain fungi, and pine host species in North America. Ecological Entomology 41: 676–684. https://doi.org/10.1111/een.12342

Foelker CJ, Standley CR, Parry D, Fierke MK (2016) Complex ecological relationships among an assemblage of indigenous hymenopteran parasitoids, the exotic European woodwasp (*Sirex noctilio*; Hymenoptera: Siricidae), and a native congener. The Canadian Entomologist 148: 532–542. doi: https://doi.org/10.4039/tce.2016.6

Gandhi KJ, Herms DA (2010) Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biological Invasions 12: 389–405. https://doi.org/10.1007/s10530-009-9627-9

Haavik LJ, Meeker JR, Johnson W, Ryan K, Turgeon JJ, Alison JD (2013) Predicting *Sirex noctilio* F. and *S. nigricornis* F. (Hymenoptera: Siricidae) emergence using degree-days. Entomologia Experimentalis et Applicata 149: 177–184. https://doi.org/10.1111/eea.12119

Haavik LJ, Allison JD, MacQuarrie CJK, Nott RW, Ryan K, de Groot P, Turgeon JJ (2016a) Nonlethal effects of nematode infection on *Sirex noctilio* and *Sirex nigricornis* (Hymenoptera: Siricidae). Environmental Entomology 45: 320–327. https://doi.org/10.1093/ee/nvv223

Haavik LJ, Dodds KJ, Ryan K, Allison JD (2016b) Evidence that the availability of suitable pine limits non-native *Sirex noctilio* in Ontario. Agricultural and Forest Entomology 18: 357–366. https://doi.org/10.1111/afe.12167

Hajek AE, Nielsen C, Kepler RM, Long SJ, Castrillo L (2013) Fidelity among *Sirex* woodwasps and their fungal symbionts. Microbial Ecology 65: 753–762. https://doi.org/10.1007/s00248-013-0218-z

Hartshorn JA (2012) Effects of felled shortleaf pine (*Pinus echinata* Mill.) moisture loss on oviposition preferences and survival of *Sirex nigricornis* F. (Hymenoptera: Siricidae). M.S. thesis, University of Arkansas, Fayetteville.

Hartshorn JA, Haavik LJ, Allison JD, Meeker JR, Johnson W, Galligan LD, Chase KD, Riggins JJ, Stephen FM (2016) Emergence of adult female *Sirex nigricornis* F. and *Sirex noctilio* F. (Hymenoptera: Siricidae) coincides with a decrease in daily minimum and maximum temperature. Agricultural and Forest Entomology 18: 206–213. https://doi.org/10.1111/afe.12153

Hoebeke ER, Haugen DA, Haack RA (2005) *Sirex noctilio:* Discovery of a Palearctic siricid woodwasp in New York. Newsletter of the Michigan Entomological Society 50(1-2): 24–25.

Kroll SA, Hajek AE, Morris EE, Long SJ (2013) Parasitism of *Sirex noctilio* by non-sterilizing *Deladenus siricidicola* in northeastern North America. Biological Control 67: 203–211. https://doi.org/10.1016/j.biocontrol.2013.08.005

Liebhold AM, MacDonald WL, Bergdahl D, Mastro VC (1995) Invasion by exotic forest pests: a threat to forest ecosystems. Forest Science Monographs 30: 1–49.

Lombardero MJ, Ayres MP, Krivak-Tetley FE, Fitza KNE (2016) Population biology of the European woodwasp, *Sirex noctilio*, in Galicia, Spain. Bulletin of Entomological Research 106: 569–580. https://doi.org/10.1017/S0007485316000043

Long SJ, Williams DW, Hajek AE (2009) *Sirex* species (Hymenoptera: Siricidae) and their parasitoids in *Pinus sylvestris* in eastern North America. The Canadian Entomologist 141: 153–157. https://doi.org/10.4039/n08-068

Madden JL (1974) Oviposition behavior of the woodwasp, *Sirex noctilio* F. Australian Journal of Zoology 22: 341–351. https://doi.org/10.1071/ZO9740341

Madden JL (1988) *Sirex* in Australasia. In: Berryman AA (Ed.) Dynamics of forest insect populations. Plenum, New York, 407–429. https://doi.org/10.1007/978-1-4899-0789-9_20

Monceau K, Maher N, Bonnard O, Thiéry D (2015) Evaluation of competition between a native and an invasive hornet species: do seasonal phenologies overlap? Bulletin of Entomological Research 105: 462–469. https://doi.org/10.1017/S0007485315000280

Morgan FD (1968) Bionomics of Siricidae. Annual Review of Entomology 13: 239–256. https://doi.org/10.1146/annurev.en.13.010168.001323

Mouillot D, Graham NAJ, Villéger S, Mason NWH, Bellwood DR (2013) A functional approach reveals community responses to disturbances. Trends in Ecology & Evolution 28: 168–177. http://dx.doi.org/10.1016/j.tree.2012.10.004

Nahrung HF (2016) Sirex woodwasp *Sirex noctilio* (Hymenoptera: Siricidae): revisiting some past perceptions. Austral Entomology 56: 148–152. https://doi.org/10.1111/aen.12217

Nahrung HF, Ramsden M, Griffiths M (2015) Sirex woodwasp range expansion in Australia: performance and parasitism on two commercial pine species. Forestry (London) 89: 310–315. https://doi.org/10.1093/forestry/cpv039

Neumann FG, Minko G (1981) The *Sirex* wood wasp in Australian radiata pine plantations. Australian Forestry 44: 46–63. https://doi.org/10.1080/00049158.1981.10674289

Olatinwo R, Allison J, Meeker J, Johnson W, Streett D, Aime MC, Carlton C (2013) Detection and identification of *Amylostereum areolatum* (Russulales: Amylostereaceae) in the mycangia of *Sirex nigricornis* (Hymenoptera: Siricidae) in central Louisiana. Environmental Entomology 42: 1246–1256. http://dx.doi.org/10.1603/EN13103

Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The future of biodiversity. Science 269: 347. https://doi.org/10.1126/science.269.5222.347

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs 83: 263–282. https://doi.org/10.1890/13-0183.1

Rodriguez-Cabal MA, Gibbons TC, Schulte PM, Barrios-Garcia NM, Crutsinger GM (2015) Comparing functional similarity between a native and an alien slug in temperate rain forests of British Columbia. Neobiota 25: 1–14. https://doi.org/10.3897/neobiota.25.8316

Ryan K, Hurley BP (2012) Life history and biology of *Sirex noctilio*. In: Slippers B, de Groot P, Wingfield MJ (Eds) The sirex woodwasp and its fungal symbiont: research and management of a worldwide invasive pest. Springer, Netherlands, 15–30. https://doi.org/10.1007/978-94-007-1960-6_2

Ryan K, de Groot P, Nott RW, Drabble S, Ochoa I, Davis C, Smith SM, Turgeon JJ (2012a) Natural enemies associated with *Sirex noctilio* (Hymenoptera: Siricidae) and *S. nigricornis* in Ontario, Canada. Environmental Entomology 41: 289–297. http://dx.doi.org/10.1603/EN11275

Ryan K, de Groot P, Smith SM (2012b) Evidence of interaction between *Sirex noctilio* and other species inhabiting the bole of *Pinus*. Agricultural and Forest Entomology 14: 187–195. https://doi.org/10.1111/j.1461-9563.2011.00558.x

SAS (2014) Statistical analysis software, 9.4. SAS Institute Inc., Cary, North Carolina.

Schiff NM, Goulet H, Smith DR, Boudreault C, Wilson AD, Scheffler BE (2012) Siricidae (Hymenoptera: Symphyta: Siricoidea) of the Western Hemisphere. Canadian Journal of Arthropod Identification 29: 1–305. https://doi.org/10.3752/cjai.2012.21

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, et al. (2017) No saturation in the accumulation of alien species worldwide. Nature Communications 8: 14435. https://doi.org/10.1038/ncomms14435

Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, Garcìa-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. Trends in Ecology & Evolution 28: 58–66. https://doi.org/10.1016/j.tree.2012.07.013

Slippers B, Hurley BP, Wingfield MJ (2015) Sirex woodwasp: A model for evolving management paradigms of invasive forest pests. Annual Review of Entomology 60: 601–619. https://doi.org/10.1146/annurev-ento-010814-021118

Spradbery JP (1973) A comparative study of the phytotoxic effects of siricid woodwasps on conifers. Annals of Applied Biology 75: 309–320. https://doi.org/10.1111/j.1744-7348.1973.tb07980.x

Spradbery JP (1977) The oviposition biology of siricid woodwasps in Europe. Ecological Entomology 2: 225–230. https://doi.org/10.1111/j.1365-2311.1977.tb00885.x

Spradbery JP, Kirk AA (1978) Aspects of the ecology of siricid woodwasps (Hymenoptera: Siricidae) in Europe, North Africa and Turkey with special reference to the biological control of *Sirex noctilio* F. in Australia. Bulletin of Entomological Research 68: 341–359. https://doi.org/10.1017/S0007485300009330

Taylor KL (1978) Evaluation of the insect parasitoids of *Sirex noctilio* (Hymenoptera: Siricidae) in Tasmania. Oecologia 32: 1–10. https://doi.org/10.1007/BF00344686

Thompson BM, Bodart J, McEwen C, Gruner DS (2014) Adaptations for symbiont-mediated external digestion in *Sirex noctilio* (Hymenoptera: Siricidae). Annals of the Entomological Society of America 107: 453–460. http://dx.doi.org/10.1603/AN13128

Wang T, Zhao M, Rotgans BA, Ni G, Dean JF, Nahrung HF, Cummins SF (2016) Proteomic analysis of the venom and venom sac of the woodwasp, *Sirex noctilio*-Towards understanding its biological impact. Journal of Proteomics 146: 195–206. https://doi.org/10.1016/j.jprot.2016.07.002

Wardle DA, Bardgett RD, Callaway RM, Van der Putten WH (2011) Terrestrial ecosystem responses to species gains and losses. Science 332: 1273–1277. https://doi.org/10.1126/science.1197479

Supplementary material 1

A. Association between health of pines and response by *S. noctilio* and *S. nigricornis*; B. Emergence of *S. noctilio* and *S. nigricornis* from pines collected from New York and Pennsylvania in spring 2012

Authors: Ann E. Hajek, Jacob C. Henry, Christopher R. Standley, Christopher J. Foelker

Data type: Microsoft Word Document (.docx)

Link: https://doi.org/10.3897/neobiota.36.14953.suppl1

NeoBiota 36: 57–69 (2017)
doi: 10.3897/neobiota.36.21926
http://neobiota.pensoft.net

EDITORIAL

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Seven years of NeoBiota – the times, were they a changin'?

Ingolf Kühn[1,2,3], Petr Pyšek[4,5], Ingo Kowarik[6,7]

1 *Helmholtz Centre for Environmental Research – UFZ, Dept. Community Ecology, Theodor-Lieser-Str. 4, 06120 Halle, Germany* **2** *Martin Luther University Halle-Wittenberg, Geobotany & Botanical Garden, Am Kirchtor 1, 06108 Halle, Germany* **3** *German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany* **4** *Institute of Botany, Department of Invasion Ecology, The Czech Academy of Sciences, CZ-252 43 Průhonice, Czech Republic* **5** *Department of Ecology, Faculty of Science, Charles University, Viničná 7, CZ-128 44 Prague, Czech Republic* **6** *Technische Universität Berlin, Department of Ecology, Chair of Ecosystem Science/Plant Ecology, Rothenburgstr. 12, 12165 Berlin, Germany* **7** *Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstr. 34, 14195 Berlin, Germany*

Corresponding author: *Ingolf Kühn* (ingolf.kuehn@ufz.de)

Received 29 October 2017 | Accepted 30 October 2017 | Published 19 December 2017

Citation: Kühn I, Pyšek P, Kowarik I (2017) Seven years of NeoBiota – the times, were they a changin'? NeoBiota 36: 57–69. https://doi.org/10.3897/neobiota.36.21926

Background

During the NEOBIOTA conference 2010 in Copenhagen (see http://www.neobiota.eu/conferences for an overview of all conferences), the attendants decided to transform the serial of the European Group on Biological Invasions *Neobiota*, edited by Ingo Kowarik and Uwe Starfinger, into an international, open access journal. In the following year, *NeoBiota* was relaunched under the same name, but with an upper case 'B', by Pensoft Publishers. In the editorial of the first issue, a large group of co-editors claimed for openness in covering a broad range of issues in invasion science, including the intersections with applied and social sciences, and referring to different groups of taxa and geographical regions (Kühn et al. 2011). What happened since then? We think that it is now time to shortly reflect how the new *NeoBiota* journal has developed in the first years of its infancy – based on some data on the published papers, the addressed topics and the geographical background of our contributing authors.

First of all, we are pleased with the increasing visibility of *NeoBiota* – thankworthy to many papers by our esteemed authors that you, our readers, found interesting. Since the relaunch in 2011, we passed through two stages with respect to visibility in major bibliometric databases, namely ISI Web of Science (since 2017 Clarivate Analytics, http://www.webofknowledge.com) and Scopus (https://www.scopus.com/home.uri). We were scrutinised for the first five years by both companies. All papers published since 2015 by *NeoBiota* are now listed by Web of Science as well as Scopus. But also papers published before 2015 are well visible: up to October 2017, they were cited on average, more than 6 times in Web of Science, the more conservative of the two bibliometric databases recognised in this study.

Some early highlights

Indeed, a range of papers seems to have clearly raised timely scientific interest and hence contributed to get successfully listed in both bibliometric databases. For brevity, we just present some prominent examples (cited at least 15 times in Web of Science), starting with the two most cited *NeoBiota* papers. The first was on the support of major hypotheses in invasion biology by Jeschke et al. (2012). This was some sort of seminal work, leading also to further analyses on this topic (Jeschke 2014). The second was a conceptual framework on prioritising alien species for management (Kumschick et al. 2012), based on the approach introduced by Nentwig et al. (2010), that also had several follow-up papers (Kumschick et al. 2015, 2017, Kumschick and Richardson 2013).

Bridging the two aforementioned topics is the study of Colautti et al. (2014). They used hypotheses in invasion biology and improved tests of these by introducing a simple mathematical framework to quantify the invasiveness of species. Also the work of Atwood and Meyerson (2011) was based on favourite hypotheses in invasion biology. They argue that the lack of consensus across studies that test EICA (evolution of increased competitive ability; Blossey and Nötzold 1995) may be in part due to the lack of consistent definitions and varying experimental designs. They provide a design framework that will increase data harmony across future studies and will facilitate examinations of any potential selection pressure driving evolution in the invaded range. Humair et al. (2014) featured an essay on why experts disagree on common concepts and risk assessments. Gassó et al. (2012) modelled the potential distribution range of invasive plant species in Spain.

The work of Kowarik and von der Lippe (2011) on secondary wind dispersal of an invasive species in urban road corridors is one of a suite of related papers on dispersal of alien plant species along urban roads (von der Lippe and Kowarik 2007a, b, 2008) and was followed by another experimental study (von der Lippe et al. 2013). Saul et al. (2013) provided a seminal study that lead to a more detailed one (Saul and Jeschke 2015) on the role of ecoevolutionary experience in invasion success. The study of MacNeil et al. (2013) shows how analysing the functional response of alien gammarid species enhances understanding of the success or failure of invasions in the face of various resident predators. Lastly, checklists of alien species are used by many others, such as the inventory of invasive alien species in China (Xu et al. 2012).

Submissions before and after 2015

Here we analyse whether certain characteristics have changed for papers submitted to *NeoBiota* before getting listed on Web of Science and Scopus in 2015 and after getting listed. In particular, we will explore whether rejection rates, paper lengths, countries of authors and topics have changed. We considered all papers submitted to *NeoBiota* from 2011 until September 2017. Although looking hard, we did not find any publication trying this sort of analysis for other papers of new journals with sufficient time before and after being listed in the relevant bibliometric databases.

The number of submissions was rather stable (Figure 1), with slightly more papers submitted per year after being listed (not accounting for incomplete 2017). Until 2014, 162 papers were submitted to *NeoBiota* (i.e. before being listed) and 112 since 2015 until September 2017 (after being listed). In the prelisting phase, we accepted 89 papers, after that 52 (see Figure 2), resulting in a rejection rate of 45.1% and 53.6%, respectively. The difference is not significant, though ($\chi^2 = 1.59$, df = 1, p = 0.21). The length of the published papers did not change significantly (mean±standard deviation: 17.1 ±6.9 vs 19.0 ±8.1, t = -1.34, p = 0.18) between the two periods.

Although a lower number of papers were published so far in the second period, the number of individual authors of published papers increased from 168 to 191; the median number of authors increased only insignificantly (W = 2073, p = 0.3)

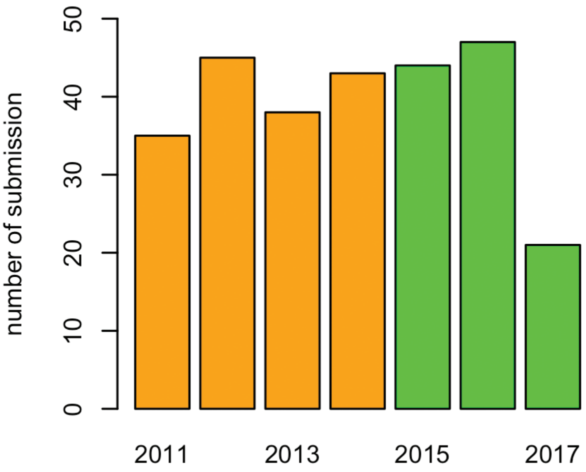

Figure 1. Number of papers submitted to *NeoBiota* between 2011 and September 2017, differentiated into those submitted before (orange) the journal got listed in Web of Science as well as Scopus, and those after (green).

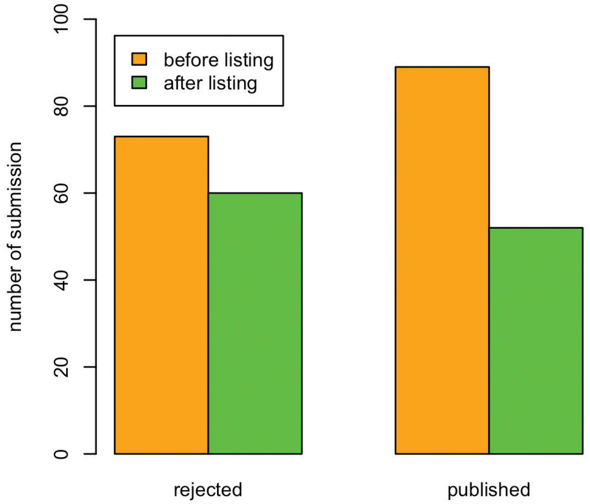

Figure 2. Number of papers submitted to *NeoBiota* that were accepted or rejected before and after being listed in Web of Science and Scopus in 2015.

from 3 to 4 per paper. Yet, there were some remarkable changes in the countries of the institutions the submitting authors were affiliated with (Figure 3, Table 1). In the years 2011–2014, especially submissions coauthored by researchers from Spain, but also Canada, China, Ecuador, Germany, The Netherlands, Switzerland, UK, USA were over-represented compared with the second period. In 2015–2017, especially Austria, New Zealand, South Africa were over-represented compared with the first period. These changes were significant ($\chi^2 = 133.7$, df = 38, $p < 0.0001$). Still, in terms of the total number of submissions we observe the geographical bias stated by Pyšek et al. (2008), i.e. Europe, North America and Australia are over-represented, many parts of Africa, Asia and South America are under-represented.

Topics covered

The topics covered by *NeoBiota* range across a variety of issues (Figure 4). Most prominent, among the papers rejected as well as accepted in both periods, is the term "plant", indicating a taxonomic bias (Pyšek et al. 2008). Modelling studies as well as distributional analyses are more represented in those papers that were rejected. This mirrors the availability of methods and data. The Global Biodiversity Information Facility GBIF (http://gbif.org) is a great source of information. Unfortunately, it does have many biases in occurrence records as well as taxa (Beck et al. 2014, Meyer et al. 2015, 2016). Similarly, MaxEnt (Elith et al. 2011) is an extremely powerful tool for distributional analyses, if properly used (Merow et al. 2013, Kramer-Schadt et al. 2013). Combining not revised GBIF data with standard settings of MaxEnt, though,

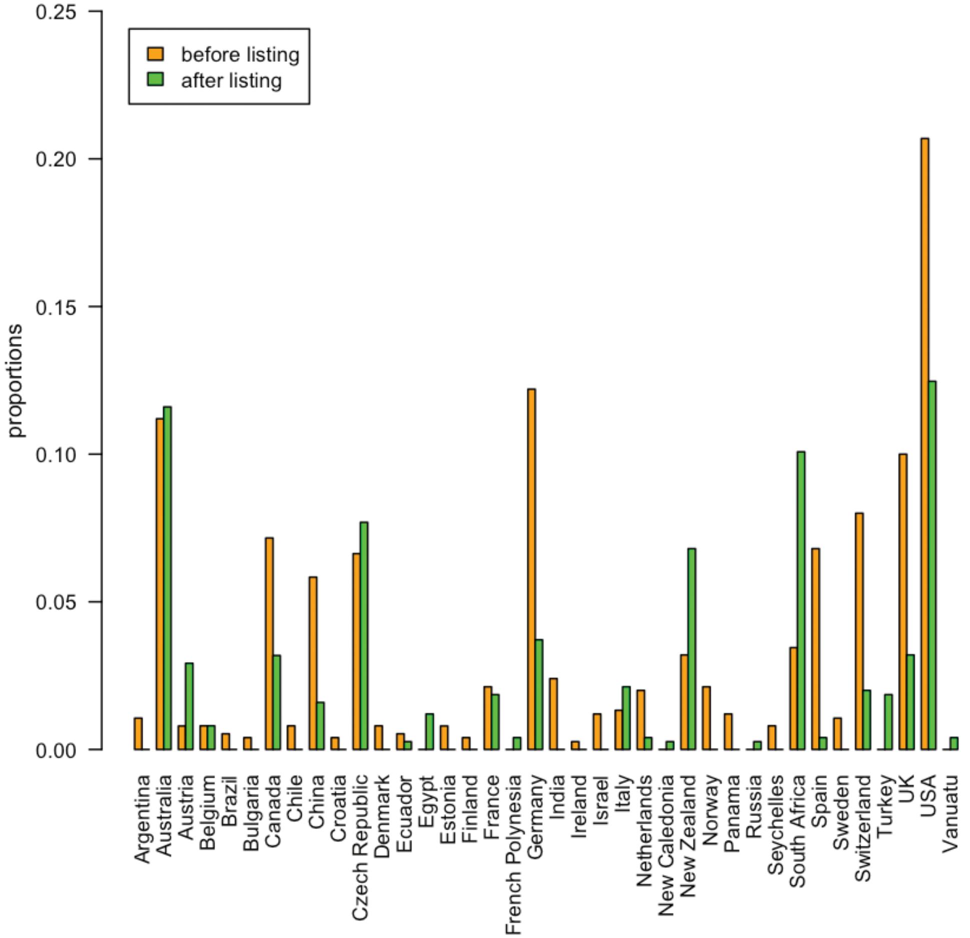

Figure 3. Proportional contribution to the total number of papers of countries in which the institutions of the submitting authors are located (multiple affiliations can result in multiple countries per author). Papers submitted to *NeoBiota* before (orange) and after (green) being listed in Web of Science and Scopus are shown.

leading to poor ecological results, can be a reason for an immediate rejection. But also successful distributional analyses were published on taxa that were not mainstream, until recently. Saltmarsh et al. (2016) published an analysis on the distribution and abundance of exotic earthworms in Alaskan forests. A combination of ecophysiological models with a correlative model to project coypu (*Myocastor coypus*) distribution under climate change was presented by Jarnevich et al. (2017). Tabak et al. (2015) modelled the distribution of Norway rats (*Rattus norvegicus*) on offshore islands in the Falkland Islands.

Also quite prominent in both periods were papers on risk assessment. This topic even made it into the so far (December 2017) only "highly cited" paper, i.e. among the top cited papers of their publication cohort: namely Kumschick et al. (2017),

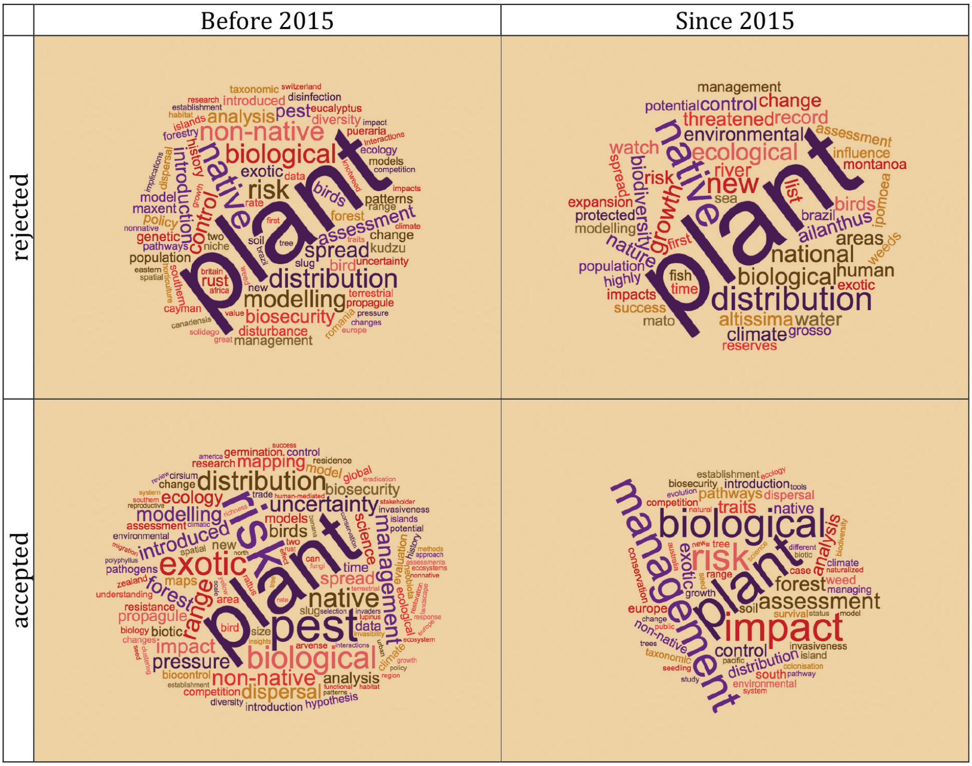

Figure 4. Word cloud (www.wortwolken.com) of words used in the title and provided in the keywords of those paper *submitted* before and after being listed in bibliometric databases in 2015 and of papers subsequently rejected or accepted. Words present in singular and plural were transformed into singular; only words with ≥3 occurrences are displayed, the terms *invasion*, *invasive*, *alien* and *species* were deleted.

Table 1. Geographical background of authors, illustrated by the number of countries of the institutions the submitting authors are affiliated with (multiple affiliations can result in multiple countries per author) submitted to *NeoBiota* before and after being listed in Web of Science and Scopus in 2015.

Countries	before 2015	since 2015
Argentina	4	0
Australia	28	29
Austria	3	11
Belgium	2	2
Brazil	2	0
Bulgaria	1	0
Canada	27	12
Chile	2	0
China	22	6
Croatia	1	0
Czech Republic	25	29
Denmark	2	0

Countries	before 2015	since 2015
Ecuador	2	1
Egypt	0	3
Estonia	3	0
Finland	1	0
France	8	7
French Polynesia	0	1
Germany	46	14
India	6	0
Ireland	1	0
Israel	3	0
Italy	5	8
Netherlands	5	1
New Caledonia	0	1
New Zealand	8	17
Norway	8	0
Panama	3	0
Russia	0	1
Seychelles	2	0
South Africa	13	38
Spain	17	1
Sweden	4	0
Switzerland	20	5
Turkey	0	7
UK	25	8
USA	78	47
Vanuatu	0	1

comparing different impact-assessment tools on alien amphibians. There were on one hand specific risk assessments for, e.g. a pest under climate change (Hong et al. 2015), or studies relating invasiveness and impact of *Cactaceae* (Novoa et al. 2016). On the other hand, there were also more general ones related to, e.g. the EU regulation on invasive species (Tanner et al. 2017), a complete set of biota, namely those of soil (McNeill et al. 2017), or the role of traits (Emiljanowicz et al. 2017). Other trait studies were also more prominent in the second phase: Buru et al. (2016) compared growth traits between abundant and uncommon forms of *Dolichandra unguis-cati* (Bignoniaceae), a non-native vine in Australia.

In the second period, 'management' and 'impact' became frequent topics, with a large overlap, resulting in jointly 18 papers published. Here we focus just on a few with more or less unusual topics or having more general implications. Nielsen and Fei (2015) explore the potential of utilizing the Analytic Hierarchy Process (AHP; Saaty and Vargas 2001), an information-driven tool to flexibly prioritise various invasion scenarios by incorporating a broad spectrum of management data. They tested the flexibility of the AHP management tool with two distinct invasion-stage-specific prioritisations for Amur honeysuckle (*Lonicera maackii*) and conclude that the flexible AHP tool could be useful for prioritizing management of exotic plant invasions. Laypersons' perceptions of invasive alien plant species and their attitudes towards their management were analysed by Lindemann-Matthies (2016) in Switzerland. Few participants could correctly identify pictures of alien species. Knowing a species, though, resulted in a higher positive attitude towards their management, but the perceived beauty of a species inhibited support of their management. Planted forests are a major source of invasive alien trees in Europe. Therefore Brundu and Richardson (2016) introduced the '*Code of Conduct on Planted Forest and Invasive Alien Trees*' relevant to stakeholders and decision makers in the 47 Member States of the Council of Europe. Panetta and Gooden (2017) review different management options for biodiversity, recognizing impact and action thresholds for invasive plants in natural ecosystems. They conclude that economic and ecological considerations are aligned when invaders are sustainably maintained at relatively low abundances.

Some highlights since 2015

NeoBiota always claimed to be open minded and aimed at facilitating scientific discussion (Kühn et al. 2011). We therefore always welcome papers raising scientific discourse. One of the most controversial papers probably was that of Hoffmann and Courchamp (2016). The authors argued that human-mediated invasions are part of the spectrum of species movements, not a unique phenomenon, because species self-dispersing into novel environments are subject to the same barriers of survival, reproduction, dispersal and further range expansion as those assisted by people. They proposed an all-encompassing framework of species range expansion, including alien species. This paper was challenged by Wilson et al. (2016), who state that invasion science now is not only a biological phenomenon, but that the human dimension of invasions is a fundamental component in the social-ecological systems in which invasions need to be understood and managed.

Other *NeoBiota* highlights published since 2015 that were well perceived, cover several different aspects: Using data from the DAISIE database (www.europe-aliens.org) (DAISIE 2009), Pergl et al. (2017) address whether established alien plants, mammals, freshwater fish and terrestrial invertebrates with known ecological impacts are associated with particular introduction pathways (release, escape, contaminant, stowaway, corridor and unaided; Hulme et al. (2008). Woodford et al. (2016) review problems arising from the management of biological invasions and argue that they can be either tame (with simple or obvious solutions) or wicked, where difficulty in appropriately defining the problem can make complete solutions impossible to find. On a similar topic Kuebbing and Simberloff (2015) surveyed land stewards of a major conservation NGO. Their results indicate that these managers are selective rather than profligate, targeting species that are having a demonstrable impact or are likely to do so. Another aspect of impacts of alien species are human health problems, reviewed by Schindler et al. (2015) for Europe.

Outlook

So far, *NeoBiota* seems to be well perceived by the invasions science community. We have found some differences regarding submissions before and after the listing of *NeoBiota* by Web of Science and Scopus. It would be interesting to see, how submission rate, rejection rate, involved countries and featured topics would change in the future, not only following recent advances in the scientific literature (Ricciardi et al. 2017), but also in response to increasing Scopus CiteScores or receiving an Web of Knowledge impact factor.

Despite the broad range of issues addressed by the previous contributions of 463 individual authors from 38 countries to *NeoBiota*, we are still short of papers covering social, legal or economic aspects. We thus strongly encourage further submissions also from these topical areas. Still we are confident that *NeoBiota* will gain an increasing role in all aspects related to the multi-disciplinary topics of invasion science and its interconnections with other disciplines.

Acknowledgements

We would cordially like to thank the team of Pensoft Publishers for their constant support, namely Lyubomir Penev (Managing Director) and Pavel Stoev (Editorial Director), further Teodor Georgiev (Technical Director), Plamen Pankov (Layout Manager), and Boriana Ovcharova (Editorial Secretary) providing underlying material to analyse the data presented in the editorial and for logistic help.

References

Atwood JP, Meyerson L (2011) Beyond EICA: understanding post-establishment evolution requires a broader evaluation of potential selection pressures. NeoBiota 10: 7–25. https://doi.org/10.3897/neobiota.10.954

Beck J, Böller M, Erhardt A, Schwanghart W (2014) Spatial bias in the GBIF database and its effect on modeling species' geographic distributions. Ecological Informatics 19: 10–15. https://doi.org/10.1016/j.ecoinf.2013.11.002

Blossey B, Nötzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology 83: 887–886. https://doi.org/10.2307/2261425

Brundu G, Richardson DM (2016) Planted forests and invasive alien trees in Europe: a Code for managing existing and future plantings to mitigate the risk of negative impacts from invasions. NeoBiota 30: 5–47. https://doi.org/10.3897/neobiota.30.7015

Buru JC, Dhileepan K, Osunkoya OO, Firn J (2016) Comparison of growth traits between abundant and uncommon forms of a non-native vine, *Dolichandra unguis-cati* (Bignoniaceae) in Australia. NeoBiota 30: 91–109. https://doi.org/10.3897/neobiota.30.8495

Colautti R, Parker JD, Cadotte MW, Pyšek P, Brown CS, Sax D, Richardson DM (2014) Quantifying the invasiveness of species. NeoBiota 21: 7–27. https://doi.org/10.3897/neobiota.21.5310

DAISIE (2009) Handbook of alien species in Europe. Springer, Berlin.

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ (2011) A statistical explanation of MaxEnt for ecologists. Diversity and Distributions 17: 43–57. https://doi.org/10.1111/j.1472-4642.2010.00725.x

Emiljanowicz LM, Hager HA, Newman JA (2017) Traits related to biological invasion: a note on the applicability of risk assessment tools across taxa. NeoBiota 32: 31-64. https://doi.org/10.3897/neobiota.32.9664

Gassó N, Thuiller W, Pino J, Vilà M (2012) Potential distribution range of invasive plant species in Spain. NeoBiota 12: 25-40. https://doi.org/10.3897/neobiota.12.2341

Hoffmann BD, Courchamp F (2016) Biological invasions and natural colonisations: are they that different? NeoBiota 29: 1–14. https://doi.org/10.3897/neobiota.29.6959

Hong SC, Magarey RD, Borchert DM, Vargas RI, Souder SK (2015) Site-specific temporal and spatial validation of a generic plant pest forecast system with observations of Bactrocera dorsalis (oriental fruit fly). NeoBiota 27: 37–67. https://doi.org/10.3897/neobiota.27.5177

Hulme PE, Bacher S, Kenis M, Klotz S, Kühn I, Minchin D, Nentwig W, Olenin S, Panov V, Pergl J, Pyšek P, Roques A, Sol D, Solarz W, Vilà M (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. Journal of Applied Ecology 45: 403–414. https://doi.org/10.1111/j.1365-2664.2007.01442.x

Humair F, Edwards PJ, Siegrist M, Kueffer C (2014) Understanding misunderstandings in invasion science: why experts don't agree on common concepts and risk assessments. NeoBiota 20: 1–30. https://doi.org/10.3897/neobiota.20.6043

Jarnevich CS, Young NE, Sheffels TR, Carter J, Sytsma MD, Talbert C (2017) Evaluating simplistic methods to understand current distributions and forecast distribution changes under climate change scenarios: an example with coypu (Myocastor coypus). NeoBiota 32: 107–125. https://doi.org/10.3897/neobiota.32.8884

Jeschke J, Gómez Aparicio L, Haider S, Heger T, Lortie C, Pyšek P, Strayer D (2012) Support for major hypotheses in invasion biology is uneven and declining. NeoBiota 14: 1–20. https://doi.org/10.3897/neobiota.14.3435

Jeschke JM (2014) General hypotheses in invasion ecology. Diversity and Distributions 20: 1229–1234. https://doi.org/10.1111/ddi.12258

Kowarik I, von der Lippe M (2011) Secondary wind dispersal enhances long-distance dispersal of an invasive species in urban road corridors. NeoBiota 9: 49–70. https://doi.org/10.3897/neobiota.9.1469

Kramer-Schadt S, Niedballa J, Pilgrim JD, Schroder B, Lindenborn J, Reinfelder V, Stillfried M, Heckmann I, Scharf AK, Augeri DM, Cheyne SM, Hearn AJ, Ross J, Macdonald DW, Mathai J, Eaton J, Marshall AJ, Semiadi G, Rustam R, Bernard H, Alfred R, Samejima H, Duckworth JW, Breitenmoser-Wuersten C, Belant JL, Hofer H, Wilting A (2013) The importance of correcting for sampling bias in MaxEnt species distribution models. Diversity and Distributions 19: 1366–1379. https://doi.org/10.1111/ddi.12096

Kuebbing SE, Simberloff D (2015) Missing the bandwagon: nonnative species impacts still concern managers. NeoBiota 25: 73–86. https://doi.org/10.3897/neobiota.25.8921

Kühn I, Kowarik I, Kollmann J, Starfinger U, Bacher S, Blackburn TM, Bustamante RO, Celesti-Grapow L, Chytrý M, Colautti RI, Essl F, Foxcroft LC, García-Berthou E, Gollasch S, Hierro J, Hufbauer RA, Hulme PE, Jarošík V, Jeschke JM, Karrer G, Mack RN, Molofsky J, Murray BR, Nentwig W, Osborne B, Pyšek P, Rabitsch W, Rejmánek M, Roques A, Shaw R, Sol D, van Kleunen M, Vilà M, von der Lippe M, Wolfe LM, Penev L (2011) Open minded and open access: introducing NeoBiota, a new peer-reviewed journal of biological invasions. NeoBiota 9: 1–12. https://doi.org/10.3897/neobiota.9.1835

Kumschick S, Bacher S, Dawson W, Heikkilä J, Sendek A, Pluess T, Robinson T, Kühn I (2012) A conceptual framework for prioritization of invasive alien species for management according to their impact. NeoBiota 15: 69–100. https://doi.org/10.3897/neobiota.15.3323

Kumschick S, Gaertner M, Vilà M, Essl F, Jeschke JM, Pyšek P, Ricciardi A, Bacher S, Blackburn TM, Dick JTA, Evans T, Hulme PE, Kühn I, Mrugała A, Pergl J, Rabitsch W, Richardson DM, Sendek A, Winter M (2015) Ecological impacts of alien species: quantification, scope, caveats, and recommendations. BioScience 65: 55–63. https://doi.org/10.1093/biosci/biu193

Kumschick S, Richardson DM (2013) Species-based risk assessments for biological invasions: advances and challenges. Diversity and Distributions 19: 1095–1105. https://doi.org/10.1111/ddi.12110

Kumschick S, Vimercati G, de Villiers FA, Mokhatla MM, Davies SJ, Thorp CJ, Rebelo AD, Measey GJ (2017) Impact assessment with different scoring tools: how well do alien amphibian assessments match? NeoBiota 33: 53–66. https://doi.org/10.3897/neobiota.33.10736

Lindemann-Matthies P (2016) Beasts or beauties? Laypersons' perception of invasive alien plant species in Switzerland and attitudes towards their management. NeoBiota 29: 15–33. https://doi.org/10.3897/neobiota.29.5786

MacNeil C, Dick J, Alexander ME, Dodd J, Ricciardi A (2013) Predators vs. alien: differential biotic resistance to an invasive species by two resident predators. NeoBiota 19: 1–19. https://doi.org/10.3897/neobiota.19.4839

McNeill MR, Phillips CB, Robinson AP, Aalders L, Richards N, Young S, Dowsett C, James T, Bell N (2017) Defining the biosecurity risk posed by transported soil: effects of storage time and environmental exposure on survival of soil biota. NeoBiota 32: 65–88. https://doi.org/10.3897/neobiota.32.9784

Merow C, Smith MJ, Silander JA (2013) A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. Ecography 36: 1058–1069. https://doi.org/10.1111/j.1600-0587.2013.07872.x

Meyer C, Kreft H, Guralnick R, Jetz W (2015) Global priorities for an effective information basis of biodiversity distributions. Nature Communications 6: 8. https://doi.org/10.1038/ncomms9221

Meyer C, Weigelt P, Kreft H (2016) Multidimensional biases, gaps and uncertainties in global plant occurrence information. Ecology Letters 19: 992–1006. https://doi.org/10.1111/ele.12624

Nentwig W, Kühnel E, Bacher S (2010) A generic impact-scoring system applied to alien mammals in Europe. Conservation Biology 24: 302–311. https://doi.org/10.1111/j.1523-1739.2009.01289.x

Nielsen AM, Fei SL (2015) Assessing the flexibility of the Analytic Hierarchy Process for prioritization of invasive plant management. NeoBiota 27: 25–36. https://doi.org/10.3897/neobiota.27.4919

Novoa A, Kumschick S, Richardson DM, Rouget M, Wilson JRU (2016) Native range size and growth form in Cactaceae predict invasiveness and impact. NeoBiota 30: 75–90. https://doi.org/10.3897/neobiota.30.7253

Panetta FD, Gooden B (2017) Managing for biodiversity: impact and action thresholds for invasive plants in natural ecosystems. NeoBiota 34: 53-66. https://doi.org/10.3897/neobiota.34.11821

Pergl J, Pyšek P, Bacher S, Essl F, Genovesi P, Harrower CA, Hulme PE, Jeschke JE, Kenis M, Kühn I, Perglová I, Rabitsch W, Roques A, Roy DB, Roy HE, Vilà M, Winter M, Nentwig W (2017) Troubling travellers: are ecologically harmful alien species associated with particular introduction pathways? NeoBiota 32: 1–20. https://doi.org/10.3897/neobiota.32.10199

Pyšek P, Richardson DM, Pergl J, Jarošík V, Sixtová Z, Weber E (2008) Geographical and taxonomic biases in invasion ecology. Trends in Ecology & Evolution 23: 237–244. https://doi.org/10.1016/j.tree.2008.02.002

Ricciardi A, Blackburn TM, Carlton JT, Dick JT, Hulme PE, Iacarella JC, Jeschke JM, Liebhold AM, Lockwood JL, MacIsaac HJ, Pyšek P, Richardson DM, Ruiz GM, Simberloff D, Sutherland WJ, Wardle DA, Aldridge DC (2017) Invasion science: A horizon scan of emerging challenges and opportunities. Trends in Ecololgy & Evolution 32: 464–474. https://doi.org/10.1016/j.tree.2017.03.007

Saaty T, Vargas L (2001) Models, methods, concepts, and applications of the analytic hierarchy process. Springer, Boston, MA.

Saltmarsh DM, Bowser ML, Morton JM, Lang S, Shain D, Dial R (2016) Distribution and abundance of exotic earthworms within a boreal forest system in southcentral Alaska. NeoBiota 28: 67–86. https://doi.org/10.3897/neobiota.28.5503

Saul WC, Jeschke J, Heger T (2013) The role of eco-evolutionary experience in invasion success. NeoBiota 17: 57–74. https://doi.org/10.3897/neobiota.17.5208

Saul WC, Jeschke JM (2015) Eco-evolutionary experience in novel species interactions. Ecology Letters 18: 236–245. https://doi.org/10.1111/ele.12408

Schindler S, Staska B, Adam M, Rabitsch W, Essl F (2015) Alien species and public health impacts in Europe: a literature review. NeoBiota 27: 1–23. https://doi.org/10.3897/neobiota.27.5007

Tabak MA, Poncet S, Passfield K, del Rio CM (2015) Modeling the distribution of Norway rats (Rattus norvegicus) on offshore islands in the Falkland Islands. NeoBiota 24: 33–48. https://doi.org/10.3897/neobiota.24.8433

Tanner R, Branquart E, Brundu G, Buholzer S, Chapman D, Ehret P, Fried G, Starfinger U, van Valkenburg J (2017) The prioritisation of a short list of alien plants for risk analysis within the framework of the Regulation (EU) No. 1143/2014. NeoBiota 35: 87–118. https://doi.org/10.3897/neobiota.35.12366

Uludag A, Aksoy N, Yazlik A, Arslan ZF, Yazmis E, Uremis I, Cossu TA, Groom Q, Pergl J, Pyšek P, Brundu G (2017) Alien flora of Turkey: checklist, taxonomic composition and ecological attributes. NeoBiota 35: 61–85. https://doi.org/10.3897/neobiota.35.12460

von der Lippe M, Bullock JM, Kowarik I, Knopp T, Wichmann M (2013) Human-mediated dispersal of seeds by the airflow of vehicles. PloS One 8. https://doi.org/10.1371/journal.pone.0052733

von der Lippe M, Kowarik I (2007a) Crop seed spillage along roads: a factor of uncertainty in the containment of GMO. Ecography 30: 483–490. https://doi.org/10.1111/j.2007.0906-7590.05072.x

von der Lippe M, Kowarik I (2007b) Long-distance dispersal of plants by vehicles as a driver of plant invasions. Conservation Biology 21: 986–996. https://doi.org/10.1111/j.1523-1739.2007.00722.x

von der Lippe M, Kowarik I (2008) Do cities export biodiversity? Traffic as dispersal vector across urban-rural gradients. Diversity and Distributions 14: 18–25. https://doi.org/10.1111/j.1472-4642.2007.00401.x

Wilson JRU, Garcia-Diaz P, Cassey P, Richardson DM, Pyšek P, Blackburn TM (2016) Biological invasions and natural colonisations are different: the need for invasion science. NeoBiota 31: 87–98. https://doi.org/10.3897/neobiota.31.9185

Woodford DJ, Richardson DM, MacIsaac HJ, Mandrak NE, van Wilgen BW, Wilson JRU, Weyl OLF (2016) Confronting the wicked problem of managing biological invasions. NeoBiota 31: 63–86. https://doi.org/10.3897/neobiota.31.10038

Xu H, Qiang S, Genovesi P, Ding H, Wu J, Meng L, Han Z, Miao J, Hu B, Guo J, Sun H, Huang C, Lei J, Le Z, Zhang X, He S, Wu Y, Zheng Z, Chen L, Jarošík V, Pyšek P, (2012) An inventory of invasive alien species in China. NeoBiota 15: 1–26. https://doi.org/10.3897/neobiota.15.3575