

Light and propagule pressure affect invasion intensity of *Prunus serotina* in a 14-tree species forest common garden experiment

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Abstract

Experiments testing multiple factors that affect the rate of invasions in forests are scarce. We aimed to assess how the biomass of invasive *Prunus serotina* changed over eight years and how this change was affected by light availability, tree stand growth, and propagule pressure. The study was conducted in Siemianice Experimental Forest (W Poland), a common garden forest experiment with 14 tree species. We investigated aboveground biomass and density of *P. serotina* within 53 experimental plots with initial measurements in 2005 and repeated in 2013. We also measured light availability and distance from seed sources. We used generalized additive models to assess the impact of particular predictors on *P. serotina* biomass in 2013 and its relative change over eight years. The relative biomass increments of *P. serotina* ranged from 0 to 22,000-fold. The success of *P. serotina*, expressed as aboveground biomass and biomass increment, varied among different tree species stands, but was greater under conifers. Total biomass of *P. serotina* depended on light and propagule availability while biomass increment depended on the change in tree stand biomass, a metric corresponding to tree stand maturation. Our study quantified the range of invasion intensity, expressed as biomass increment, in a forest common garden experiment with 14 tree species. Canopy cover was the most important variable to reduce susceptibility to invasion by *P. serotina*.

Even a modest decrease of overstory biomass, e.g. caused by dieback of coniferous species, may be risky in areas with high propagule pressure from invasive tree species. Thus, *P. serotina* control may include maintaining high canopy closure and supporting natural regeneration of tree species with high leaf area index, which shade the understory.

Keywords

Allometric equations, biomass, invasion dynamics, light availability, natural regeneration, tree species effect

Introduction

Although invasive trees and shrubs are a worldwide problem (Richardson and Rejmánek 2011; Rejmánek and Richardson 2013), their impacts and mechanisms of ecological success are insufficiently understood. Their longevity and the long duration of their lag period (Kowarik 1995; Lonsdale 1999), as well as the physically large dimensions of invasive woody species, make research more challenging (Richardson and Rejmánek 2011). Invasive tree and shrub species are often classified as “transformers” (Richardson et al. 2000), due to the ability of these species to modify habitats. Invasive trees modify light conditions, usually decreasing light availability (Knight et al. 2008; Mueller et al. 2016) and increase rate of soil nutrient cycling (Binkley and Valentine 1991; Allison and Vitousek 2004; Reich et al. 2005). Due to this potential, invasive species of trees and shrubs are a serious threat to the environment (Richardson 1998; Richardson and Rejmánek 2011; Hawkins et al. 2015; Aerts et al. 2017). On the other hand, these properties of trees also highlight the potential role of native tree species in shaping ecosystem invasibility, by creating regeneration niches for invaders (Knight et al. 2008; Jagodziński et al. 2018) or closing them, by providing unfavorable conditions (e.g. González-Muñoz et al. 2014; Nyssen et al. 2016; Dyderski and Jagodziński 2018).

There are three crucial elements shaping invasion success: ecosystem invasibility, propagule pressure, and species invasiveness (Lonsdale 1999; Richardson et al. 2000). In the case of forest ecosystems, the level of resources (e.g. light, water, nutrients) is relatively constant compared to other ecosystems, due to the low frequency of disturbances within a single forest patch (Herben et al. 2016). Furthermore, differences in the availability of these resources across sites with similar soil and climate are determined mainly by tree species composition (see previous paragraph) and tree density (Jagodziński and Oleksyn 2009). However, disturbances in forests, regardless of their spatial scale, are usually connected with increased resource availability (Verheyen et al. 2003; Chmura and Sierka 2007). Although forest ecosystems usually return to a dynamic equilibrium state after disturbance (Gunderson 2000; Johnstone et al. 2016), the pulse of resource availability is crucial for success of invasive species (Davis et al. 2000). However, establishment of alien species also requires the presence of propagules, and the capacity of alien species to spread is crucial for determination of invasiveness (Richardson et al. 2000). The quantity of available propagules determines propagule pressure, which may be approximated by the number of mature plants (Vanhellemont et al. 2009) and the distance to the nearest

propagule source (Deckers et al. 2005; Pairon et al. 2006a; Jagodziński et al. 2015) or area of alien species cultivation (Pyšek et al. 2009).

Few studies of invasive trees and shrubs have quantified the density and size of different life stages during the initial stages of invasion. Because seedlings are more vulnerable to environmental conditions (e.g. drought, frosts or herbivory) than adult trees (Niinemets and Valladares 2006), their survival and recruitment into adult life phases is important for shaping future tree stand composition (Baraloto et al. 2005). Moreover, there is a lack of long-term studies which focus on the dynamics of species invasion and provide a quantitative assessment of the process.

Due to different invasion metrics used in fully controlled experiments and those used in field assessments, there is a gap in transferability of results between these two types of studies. Experiments in fully controlled conditions have measured invader success as relative growth rate or biomass (e.g. Grotkopp et al. 2010). In contrast, field studies usually measure alien species presence/absence, cover, or density (e.g. Pauchard et al. 2009; Jarošík et al. 2011; Kowarik et al. 2013; Woziwoda et al. 2014). Thus, field studies that quantify the invasive potential of alien species using the metrics of invasion success which are used in experiments, e.g. biomass, would provide discernment between findings due to ecological processes and those due to differences in measurement techniques. Biomass may provide a better measure of ecological success, reflecting space filled by the species, and is also a proxy of plant fitness (Younginger et al. 2017).

We took advantage of the invasion of *Prunus serotina* Ehrh. into an experimental forest common garden comprised of different tree species. The wide range of ecological characteristics of the cultivated species, similar soil parent material, climate and site history, and presence of alien species, compromise an ideal experimental design for invasion ecology studies (Knight et al. 2008; Jagodziński et al. 2018). The results would facilitate better recognition of the threats and management vulnerabilities of particular invaders.

Our study addresses the following questions: (1) how did invasion intensity, expressed as total biomass of *P. serotina*, change over eight years? and (2) how was this change connected with changes in light availability, tree stand maturation and seed source availability?

Methods

Studied species

One of the most common invasive species of trees and shrubs in Europe is *P. serotina* (black cherry). It is a tree species from the Rosaceae family, with a natural range in the eastern part of North America, where it occurs in a wide range of habitats (Burns and Honkala 1990). Due to its high-quality timber and aesthetic values, *P. serotina* was introduced to Europe in the 17th century, first as an ornamental shrub in gardens, and then as a timber tree (Starfinger et al. 2003; Godefroid et al. 2005). In the 20th

century it was widely used as soil improver, due to its high-quality leaf litter (Starfinger et al. 2003; Godefroid et al. 2005; Horodecki and Jagodziński 2017; Horodecki et al. 2019). It has been reported in 22 European countries (Klotz 2007). This species limits growth of native species beneath its canopy (Robakowski and Bieliniś 2011; Halarewicz and Źohnierz 2014; Aerts et al. 2017; Hamm et al. 2017). Due to the extent of its distribution, as well as the length and expense required for its eradication, in forest management in Belgium and the Netherlands *P. serotina* is treated as a target species in silviculture, due to its valuable timber (Starfinger et al. 2003; Nyssen et al. 2016). Black cherry seeds are transported by birds and mammals up to 600 m (Deckers et al. 2008; Jagodziński et al. 2015), however, densities of its seedlings are highest in the nearest neighborhood of maternal trees (Pairon et al. 2006b). It can start fruiting at ages of 7–10 years (in full light), while the peak of fruit production is at ages of 30–100 years (Burns and Honkala 1990; Starfinger et al. 2003).

Black cherry was most frequently introduced in intermediate-fertile habitats of mixed-coniferous sites, forests of poor site quality with *Quercus* spp., and in *Pinus sylvestris* stands where it occurs the most frequently (Godefroid et al. 2005; Zerbe and Wirth 2006). Because it has been planted mainly on less-fertile, sandy soils (Muys et al. 1992; Starfinger et al. 2003; Nyssen et al. 2016) *P. serotina* is less frequent on more fertile sites. However, within plantations of coniferous species on habitats of fertile deciduous forests, *P. serotina* can reach densities up to 25,660 ind. ha⁻¹, including 20,690 individuals up to 0.5 m tall (Jagodziński et al. 2015). One of the most important factors influencing invasion success of black cherry is light availability (Closset-Kopp et al. 2007; Chabrerie et al. 2008; Vanhellemont et al. 2009). Seedlings of *P. serotina* can grow slowly in unfavorable light conditions, waiting for overstory tree mortality and gap creation. This suggests that *P. serotina* may be a passenger rather than a driver of disturbance in forest ecosystems (Chabrerie et al. 2008).

Study area

The study was conducted in the Siemianice Experimental Forest in Poland (51°14.87'N, 18°06.35'E, elev. 180 m a.s.l.). The mean annual temperature is 8.2 °C, the mean annual precipitation is 579 mm and the growing season (considered as the number of days with mean temperature ≥ 5 °C) length is 213 days (Reich et al. 2005). The common garden experiment consists of 53 plots (0.04 ha each), with monocultures of 14 tree species: *Abies alba* Mill., *Acer platanoides* L., *A. pseudoplatanus* L., *Betula pendula* Roth., *Carpinus betulus* L., *Fagus sylvatica* L., *Larix decidua* Mill., *Picea abies* (L.) Karst., *Pinus nigra* Arn., *P. sylvestris* L., *Pseudotsuga menziesii* Franco, *Quercus robur* L., *Q. rubra* L., and *Tilia cordata* Mill. (Fig. 1). All species are native to the European temperate zone, except *P. nigra* (Mediterranean), *P. menziesii* (Pacific shore of USA) and *Q. rubra* (eastern USA). The plots were established in 1970 and 1971 and were arranged in two blocks, differing in initial soil fertility, which was modified after 30 years of different tree species cultivation (Szymański 1982; Reich et al. 2005). Nine

annual height increments of 32.0 ± 8.9 cm (Closset-Kopp et al. 2007), the first individuals may have appeared in or before ca 1998, based on the height of the largest individual. In 2005, there were no fruiting *P. serotina* specimens in the nearest neighborhood (up to 50 m) of the experimental plots, but fruiting individuals were present up to 200 m from the site (Knight et al. 2008). We decided to use a threshold of 50 m as ca 80% of *P. serotina* fruits fall beneath canopy of parental trees (Pairon et al. 2006b) and density of *P. serotina* regeneration decreases strongly further than 50 m from the seed source (Jagodziński et al. 2015). For that reason, we considered propagule pressure in 2005 as constant for each study plot. In 2013, we found 132 fruiting specimens of *P. serotina*, but they were grouped mostly south and south-east of the experimental system (Fig. 1). Nevertheless, neither distance from the nearest fruiting *P. serotina* nor the number of fruiting trees within 50 m differed statistically significantly ($p > 0.05$) among plots with different tree species. For that reason, we treated proxies of propagule pressure measured in 2013 (distance from the nearest fruiting tree and the number of fruiting trees within a 50 m radius) as variables expressing the difference in propagule pressure between the two study dates.

Data collection

In 2005 and in September 2013, all specimens of *P. serotina* were investigated within all 53 experimental plots. We measured the root collar diameter (RCD) and height (H) of each tree and we determined its location within the plot with 0.25 m accuracy. Following Knight et al. (2008), we chose 1 mm RCD as a threshold to distinguish current-year from second-year or older specimens. The two size classes were distinguished due to low survival of seedlings after the first year (Dyderski and Jagodziński under review).

Biomass, as a function of both the dimensions and the density of plants, directly results from of invasive species growth, thus providing an approximation of invasion success. To assess the biomass of *P. serotina* within the experimental plots we harvested a subsample 59 trees (from a total population of 2339) for biomass estimation. We randomly selected 58 trees to harvest from the database of all measured trees, ordered by increasing RCD and H, plus the tallest tree, which was chosen to ensure coverage of the entire range of dimensions ($n = 59$; Suppl. material 2, Fig. S1). The selection was based on division of the dataset into equal intervals across increasing RCD and height. Then, we preselected trees with particular dimensions within particular study plots and in field we cut the first encountered tree with a given dimension. Model trees were harvested, oven-dried at 65 °C to constant mass and weighed with an accuracy of 0.001 g. Aboveground biomass (AB) of model trees ranged from 0.014 to 26546.52 g. Based on tree dimensions and masses, we developed allometric equations to estimate aboveground biomass (AB; [g]) of *P. serotina* within plots. For smaller trees (up to 10 mm RCD, $n = 52$) $AB = 0.3066 \cdot (RCD^2H)^{1.0165}$, with RMSE = 0.4 g and R^2 of the linearized model, i.e. transformed into $\ln(AB) = a + b \cdot \ln(RCD^2H)$ form, was 0.997.

For larger trees (10–126 mm RCD, $n = 7$) $AB = 0.3576 \cdot (\text{RCD}^2\text{H})^{0.9445}$, with RMSE = 7.8 g and the R^2 of linearized model was 0.999. Models were chosen based on Akaike's Information Criterion (AIC; Suppl. material 2, Table S2). We applied these models to the results of the inventory from 2013 to obtain information about biomass in 2013.

To assess propagule pressure, we used a GPS receiver to map fruiting trees up to 50 m from each plot, as the probability of occurrence of *P. serotina* natural regeneration is highest within this distance (Jagodziński et al. 2015). We also recorded fruiting *P. serotina* within the study plots. This data allowed calculation of two proxies of propagule pressure: distance to the closest seed tree, which may be more important in cases of longer dispersal distances, and the number of fruiting trees within a 50 m radius (see rationale in study area description). We also investigated tree stand structure of the overstory, by measurements of diameter at breast height of all trees within the plots (Suppl. material 1, Table S1). For each plot, we calculated tree stand biomass using allometric equations specific to each species (Suppl. material 3, Table S3) in 2013 and in 2005. To quantify light availability, we measured the canopy openness index (diffuse non-interceptance; DIFN) each month during the growing season (June–August) of 2005 and 2013 using a LAI-2000 and LAI-2200 plant canopy analyzers (Li-Cor Inc., Lincoln, NE, USA) following the methods used in Knight et al. (2008). We took 20 measurements at six locations near the center of each plot at the height of 0.5 m above ground. DIFN indicates the fraction of open-field diffusive solar radiation reaching the forest floor.

Data analysis

We calculated relative increments of biomass for tree stands and for *P. serotina* as $(B_{2013} - B_{2005})/B_{2005}$, where B_{2005} – biomass in 2005 and B_{2013} – in 2013. We used relative increments to account for the effect of initial biomass from population dynamics within the study period. Tree stand biomass change was used as a proxy for tree stand maturation, describing increments of tree quantity in the ecosystem. In the case of negative values, biomass change may reflect quantity of trees destroyed during disturbances. Tree stand maturation is also connected with decrease of light availability (Hamm et al. 2017). Although biomass change mainly expressed tree stand productivity, in our case, when some tree stands were damaged, this variable expressed the quantity of damage (in cases of negative values of biomass). To analyze impacts of tree stand maturation (expressed as the change in tree stand biomass), light availability (DIFN) and propagule pressure (distance to the nearest fruiting tree and number of fruiting trees up to 50 m) on biomass crop and biomass relative change of *P. serotina* we used generalized additive mixed models (GAMM), describing the additive interactions between the analyzed predictors. As mixed-effects models, GAMM also allowed us to handle study design by including random effects describing study block (Fig. 1). GAMMs were prepared using the *mgcv* package (Wood 2011). We compared GAMMs using Akaike's Information Criterion to choose the best fit model. Differences between tree stands

in *P. serotina* biomasses and densities were tested using Kruskal-Wallis tests, due to the small number of samples and lack of normality. All analyses were conducted in R software (R Core Team 2017).

Results

Changes of *P. serotina* density within experimental plots

Within the 53 experimental plots, the number of *P. serotina* specimens increased from 556 in 2005 to 2339 in 2013. Density of *P. serotina* in experimental plots in 2013 ranged from 0 ind. ha⁻¹ (in a plot with *A. alba* and a plot with *Q. rubra*) to 7895 and 8471 ind. ha⁻¹ in plots with *P. abies* (Suppl. material 4, Table S4). Overall density of *P. serotina* populations increased statistically significantly in plots with *L. decidua*, *P. abies*, *P. menziesii*, and *Q. robur* (Fig. 2). The differences were from double (*L. decidua*) to almost 23-fold (*P. abies*). For seedlings < 1 mm RCD, the difference between 2005 and 2013 inventories was significant only beneath *P. menziesii*, and for older trees (with RCD > 1 mm), differences in density were statistically significant in plots with *L. decidua*, *P. menziesii* (5-fold), and *Q. robur*. The differences between the two study dates in mean overall density were statistically significant for all coniferous species grouped together (315.2 ± 87.5 in 2005 and 1668.6 ± 438.7 in 2013; $p < 0.001$) and all deciduous species grouped together (216.9 ± 48.2 in 2005 and 565.2 ± 179.8 in 2013; < 0.05).

Changes in experimental tree stands and propagule availability

During the study period, almost all tree stands increased their biomass, with the exception of *P. abies* stands (-13.9 ± 4.7 Mg ha⁻¹), where mortality exceeded growth (Suppl. material 4, Fig. S2a–c). Growth dynamics were species dependent ($p < 0.001$) – the highest biomass increment was in stands of *Q. rubra* (85.0 ± 14.8 Mg ha⁻¹), *A. pseudoplatanus* (81.4 ± 14.7 Mg ha⁻¹), *F. sylvatica* (80.4 ± 9.4 Mg ha⁻¹), and *P. menziesii* (71.2 ± 3.3 Mg ha⁻¹), and the lowest in *C. betulus* (7.7 ± 2.2 Mg ha⁻¹). Nevertheless, the highest biomass increment was found in plots near the experiment's border located near the paths. Tree growth caused decreased light availability in 39 of 53 plots, although light availability increased in *P. abies* and *P. menziesii* tree stands (Suppl. material 4, Fig. S2d–f). Change in light availability differed among tree species ($p < 0.001$): the largest increases were in stands of *P. abies* (0.038 ± 0.007) and *P. menziesii* (0.010 ± 0.005), and the largest decreases were in stands of *P. nigra* (-0.070 ± 0.019), *Q. robur* (-0.071 ± 0.008), *P. sylvestris* (-0.080 ± 0.028), *L. decidua* (-0.103 ± 0.019) and *B. pendula* (-0.110 ± 0.017). In 2005 there were no fruiting *P. serotina* specimens within 50 m of the plots, but some were present up to 200 m away (Knight et al. 2008). In 2013, only six plots were farther than 50 m from the nearest fruiting *P. serotina* and most of the plots were located near at least five fruiting trees (Suppl. material 4, Fig. S3).

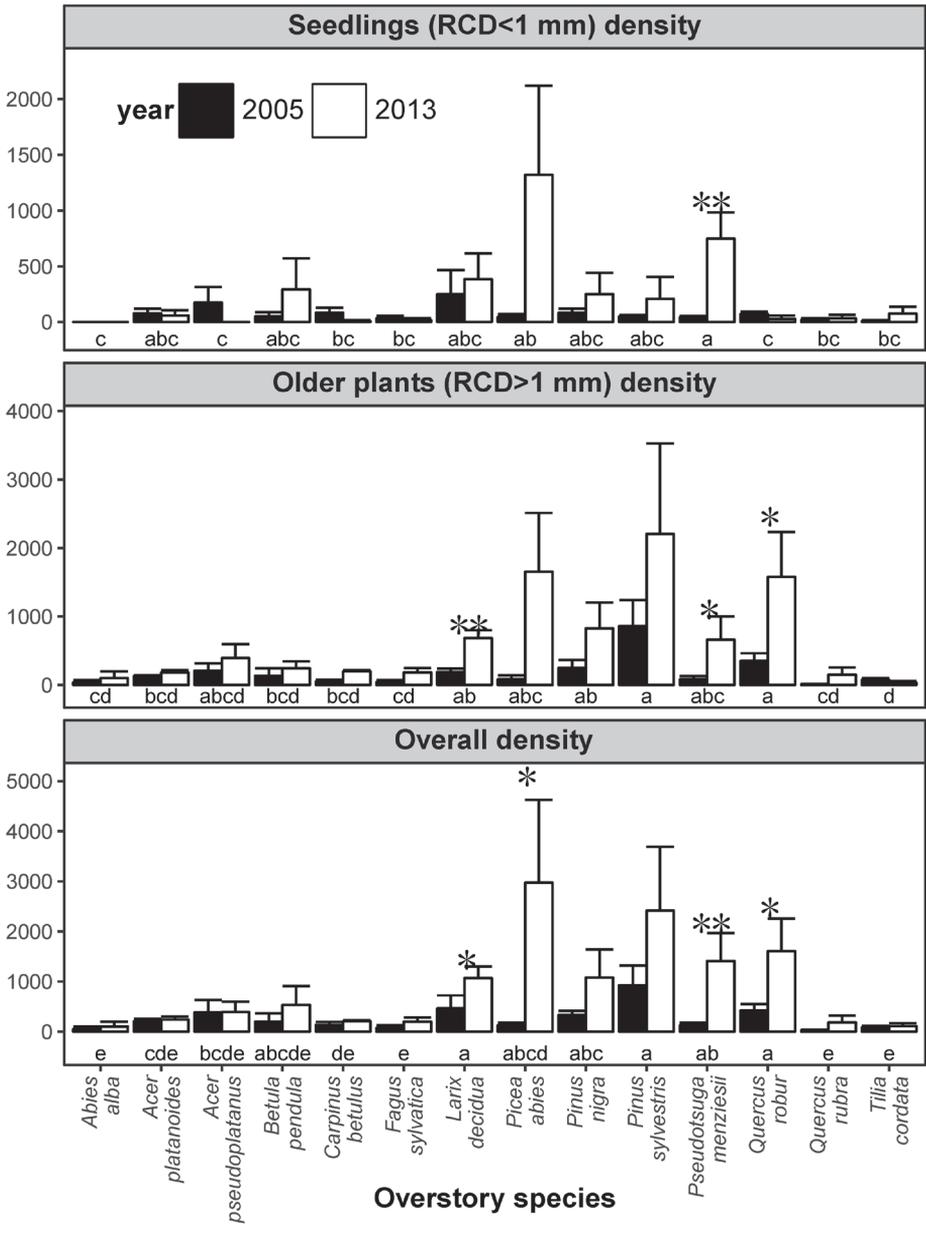


Figure 2. Mean +SE density (ind. ha⁻¹) of *Prunus serotina* within experimental plots of 14 tree species in 2005 and in 2013. Asterisks mark significance levels of differences between inventories in 2005 and 2013 (* – $p < 0.05$, ** – $p < 0.01$), based on Student’s t-test. Letters under bars represent significance of differences among tree species in 2013, based on Kruskal-Wallis tests; there are no statistically significant differences between values marked by the same letter.

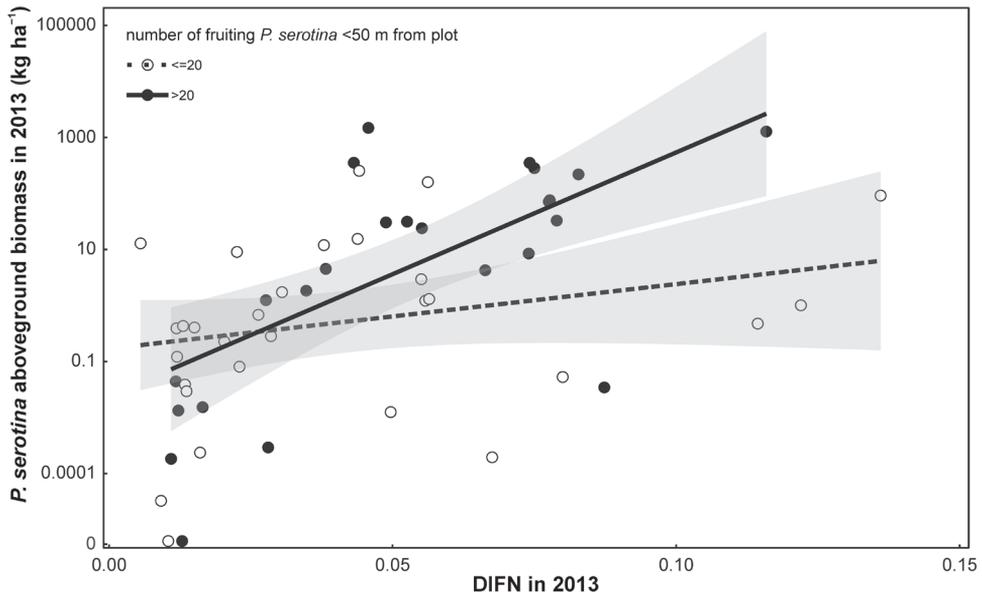


Figure 3. Relationship between light availability, expressed by DIFN, and aboveground biomass of *P. serotina*, in two classes of propagule pressure, expressed by the number of fruiting *P. serotina* trees up to 50 m from the plot (open dots and dashed line : ≤ 20 ; solid dots and line : > 20 ; $p < 0.001$, $R^2 = 0.32$). Threshold of 20 trees was chosen according to median and mean for all plots (18 and 18.49, respectively). Modification given by class of propagule pressure was statistically significant ($p < 0.01$). Note log-transformation of the y axis. Shading around regression lines indicate range of model SE.

Aboveground biomass of *P. serotina*

Biomass of *P. serotina* increased in almost all plots (except six; Suppl. material 4, Fig. S3). In 2005 total biomass varied from 0 to 144.4 kg ha⁻¹ (*P. sylvestris*) and in 2013 from 0 to 1485.6 kg ha⁻¹ (*P. sylvestris*). Biomass in 2013 differed among tree species stands ($p < 0.01$): the highest was in *P. sylvestris* (922.0 ± 464.0 kg ha⁻¹), *P. nigra* (143.00 ± 107.3 kg ha⁻¹), and *L. decidua* (112.6 ± 66.0 kg ha⁻¹), and the lowest in *T. cordata* (0.005 ± 0.004 kg ha⁻¹) and *Q. rubra* (0.006 ± 0.005 kg ha⁻¹). Relative increment of *P. serotina* biomass differed among tree stand species ($p < 0.01$): the highest values occurred in stands of *P. abies* ($742,453.1 \pm 390,415.3\%$), *F. sylvatica* ($155,700.5 \pm 93,531.8\%$), and *B. pendula* ($129,014.4 \pm 125,740.1\%$), and the lowest occurred in *Q. rubra* ($1,150.5 \pm 668.4\%$), *A. platanooides* ($570.1 \pm 309.8\%$), *A. alba* ($306.1 \pm 306.1\%$), and *T. cordata* ($-39.3 \pm 39.3\%$). Analysis of the factors responsible for invasion success has shown that total biomass of *P. serotina* in 2013 depended on propagule pressure (number of fruiting *P. serotina* up to 50 m and minimal distance to fruiting *P. serotina*) as well as light availability ($R^2 = 0.696$; Table 1, Figs 3, 4). Relative increment of *P. serotina* biomass depended on the change in tree stand biomass ($R^2 = 0.415$; Fig. 4).

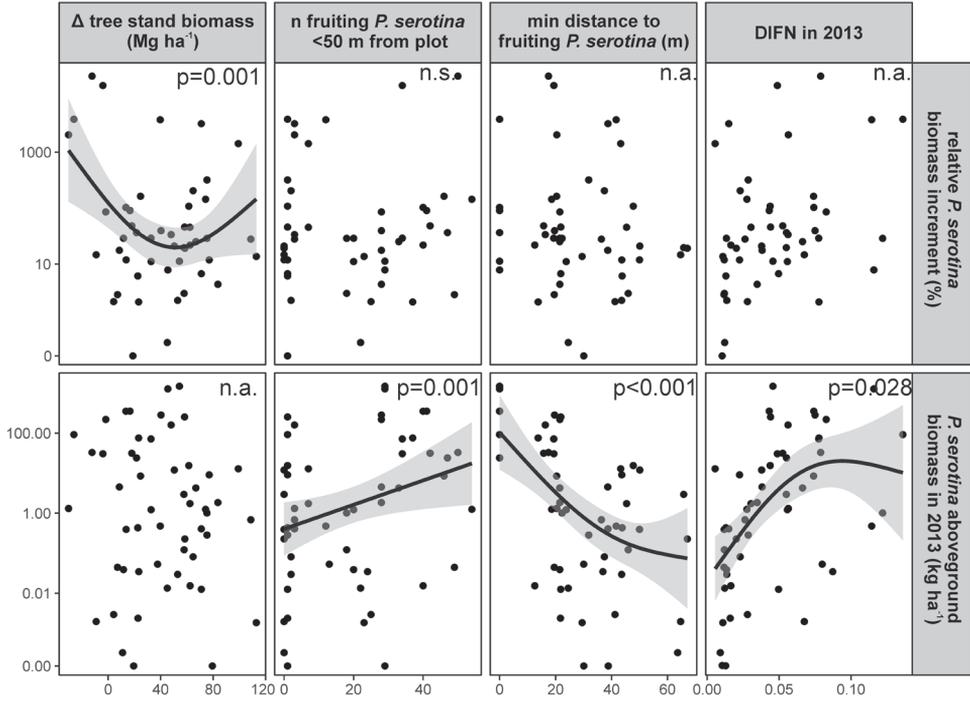


Figure 4. Responses of percentage increment of *Prunus serotina* biomass and total biomass in 2013 to the particular predictors. Lines were fitted using General Additive Models (see Table 1 for model parameters), grey area indicates SE of model, n.s. – not significant, n.a. – significance level not applicable as considered variable was not included in the final model (Table 1). Note the logarithmic scale of the Y axis.

Table 1. General Additive Models describing predictors of percentage increment of *Prunus serotina* biomass and total biomass in 2013. The best model was chosen according to AIC. AIC of null model refers to model with an intercept only, to express a final model inertia; edf – estimated degree of freedom, Ref. df – reference degree of freedom, used for F test for *p*-value computation

Estimated variable	Percentage increment of <i>P. serotina</i> biomass				Biomass of <i>P. serotina</i> in 2013			
	Estimate	SE	t	p	Estimate	SE	t	p
Parametric coefficients:								
(Intercept)	1032.700	401.300	2.573	0.014	91.070	20.720	4.396	<0.001
Approximate significance of smooth terms:	edf	Ref.df	F	p	edf	Ref.df	F	p
change of tree stand biomass	3.456	4.282	3.316	0.001	–	–	–	–
number of fruiting <i>P. serotina</i> up to 50 m	1.095	1.182	3.134	0.090	5.191	6.249	4.608	0.001
min distance to fruiting <i>P. serotina</i>	–	–	–	–	3.426	4.064	15.952	<0.001
DIFN in 2013	–	–	–	–	5.469	6.520	2.633	0.028
random effect (block)	<0.001	1.000	0.000	0.659	<0.001	1.000	0.000	0.768
Model parameters	R²	Deviance explained	AIC	AIC of null model	R²	Deviance explained	AIC	AIC of null model
	0.254	31.9%	1018.2	1027.7	0.696	77.9%	700.9	748.2

Discussion

We found the highest intensities of invasion, expressed as relative biomass change, within plots with low *P. serotina* density in 2005. In these plots, invasion accelerated later, which was indicated by the high proportion of seedlings compared to older plants (Fig. 2). In both 2005 and 2013, we found higher densities of older *P. serotina* in plots invaded earlier, where biomass of *P. serotina* was the highest. In both 2005 and 2013 the highest total biomass was in plots with *P. sylvestris*. Within these plots the presence of older trees, with higher height and diameter increments, could have decreased light availability, causing decreased seedling survival (Knight et al. 2008; Dyderski and Jagodziński under review) or lengthened persistence in a “seedling” stage (Mulligan and Munro 1981; Closset-Kopp et al. 2007). Beneath *P. sylvestris*, most *P. serotina* biomass was concentrated in old, often fruiting individuals, responsible for high densities of seedlings, connected with high propagule pressure. However, due to poor seedling recruitment to the shrub layer, the density of older plants was low, similar to the mechanism described by Godefroid et al. (2005). A similar pattern was observed in the plots with *B. pendula*, *L. decidua*, and *P. nigra*. Our study also suggests that disturbance, which led to decreases of tree stand biomass and canopy closure on some plots, acted as a pulse of increased light availability. This window of opportunity allowed rapid *P. serotina* invasion, consistent with the Fluctuating Resource Availability Theory of Invasibility (Davis et al. 2000). However, the relationship between biomass of *P. serotina* and light availability was strongly modified by propagule pressure (Fig. 3). The compound effect of propagule pressure and invasibility is known from earlier conceptual studies (Davies et al. 2005). Our other field study (Dyderski and Jagodziński 2018) also confirmed the superior role of propagule pressure over invasibility.

The species-dependent pattern of invasion success resulted from different life history traits of particular species. One of them is growth dynamics, shown by differences in biomass and biomass increment of overstory trees. At the same age, some of them may exhibit different growth stages, due to different costs of growth, connected with specific stem density (Díaz et al. 2016). Moreover, tree species create different light availability due to different branching architecture and foliage morphology (Breuer et al. 2003; Knight et al. 2008; Pretzsch 2009). Earlier invasion was found in the stands of pioneer tree species, where canopy closure and reopening due to self-thinning occurred earlier (Szymański 1982; Jagodziński and Kałucka 2008; Pretzsch 2009). This pattern is consistent with observations that forests dominated by pioneer species are more invasible (Zerbe and Wirth 2006) or that late-successional tree species limit *P. serotina* invasion (Godefroid et al. 2005; Chabrerie et al. 2008; Jagodziński et al. 2015). Starfinger et al. (2003) claim that presence of dense thickets of *P. serotina* in *P. sylvestris* forests results from planting it as a soil improver in these poor habitats. In our study *P. serotina* spontaneously colonized experimental plots, without being intentionally planted. This indicates that although introductions increase initial propagule pressure, intentional introduction into poor habitat was not crucial for invasion success of *P. serotina* in the study site.

Higher total biomass and increment of *P. serotina* in stands of coniferous species may also result from habitat modification by these species, i.e. higher nutrient leaching and acidification (Binkley and Valentine 1991). This effect of tree species is independent of modifying light availability. In our plots coniferous species had lower soil and litter pH, lower calcium content, and higher acidity, which resulted from different elemental contents of foliage (Reich et al. 2005; Mueller et al. 2012). These factors decrease abundance of understory vegetation (Chmura 2013), which competes with naturally established regeneration of invasive species (Knight et al. 2008; Jagodziński et al. 2018). Thus, coniferous tree stands, especially when planted on fertile sites, are more invulnerable due to negative tree species effects on understory vegetation cover (Zerbe and Wirth 2006; Jagodziński et al. 2015).

Studies on populations of *P. serotina* reveal that the proportion of seedlings and specimens in the herb layer (< 50 cm height) is very high. In Rogów Arboretum, within a sample of 20,843 specimens, 76.7% of them were < 50 cm height (Jagodziński et al. 2015). In Compiègne forest, seedlings comprised 72.1% of a sample of 4994 specimens (Closset-Kopp et al. 2007). As presence of *P. serotina* in the shrub layer limits development of the herb layer (Godefroid et al. 2005; Halarewicz and Żołnierz 2014, but see Chabrierie et al. 2008), it also limits the growth of its own offspring. Therefore, we may observe either low or high densities of young *P. serotina* under tree stands where the species fruits. However, in both scenarios *P. serotina* biomass is low. The high density of seedlings and low biomass of *P. serotina* is connected with shade intolerance of older *P. serotina* specimens (Burns and Honkala 1990). Therefore, mere consideration of density does not reflect invasion dynamics unless seedlings and older trees are considered separately. In contrast, biomass is a function of both density and dimensions of plants, and thus, it increases with increase of both parameters. For that reason, biomass assessment may yield a more accurate understanding of invasion dynamics than density alone.

Our study confirmed the sit-and-wait strategy of *P. serotina* (Closset-Kopp et al. 2007) over a longer time interval by showing the potential for quick increase of biomass after rapid increase of light availability caused by the windthrow. Persistence of the seedling bank and waiting for the window of opportunity allows this species to colonize forest ecosystems which are usually less invulnerable due to the low frequency of disturbances (Herben et al. 2016). Our study confirms observations of Chabrierie et al. (2008), who found *P. serotina* as a passenger rather than a driver of disturbances. A similar pattern of emergence after dieback of the overstory trees was observed for *Sorbus aucuparia* in mountain *P. abies* forests (Żywiec et al. 2013). *P. serotina* in its native range is a species able to dominate secondary succession after disturbances caused by windthrow, logging, or fire (Mulligan and Munro 1981).

Similar to Vanhellefont et al. (2009), we found that colonization of uninvaded areas is a combined effect of long-distance dispersal events in combination with windows of opportunity. However, our study had more emphasis on the role of propagule pressure, which is expressed by distance to the seed source and number of fruiting trees. The roles of the distance from the propagule source and invader density, modified by

type of tree stand, was confirmed in our previous study (Jagodziński et al. 2015). Long-distance dispersal, mostly bird-mediated (Deckers et al. 2008; Dylewski et al. 2017), comprises only 20% of seeds and the remaining 80% fall in the vicinity of parent trees (Pairen et al. 2006b). This high input of seeds in the invaded sites is responsible for persistence of the seedling bank every year. The subset of bird-mediated seeds is responsible for colonizing new sites, and in the broader perspective it is responsible for expansion of the invaded range. Propagule pressure is one of the most relevant factors driving invasion potential of alien woody species in Europe (Pyšek et al. 2009), and similar to Vanhellemont et al. (2009), we found that low levels of it limit invasiveness of *P. serotina*, even in invulnerable plots (Fig. 3).

Our study, for the first time, quantified the range of an invader's intensity, expressed as biomass increment, in a forest common garden experiment with 14 tree species. Relative biomass increments of *P. serotina* ranged from 0 to 22,000-fold in eight years. This highlights the urgent need for monitoring even small populations of *P. serotina*, as this species has the ability for sudden outbreaks. Noticing even small but stable populations of *P. serotina* that have not reached the dimensions allowing reproduction will provide time for local eradication, which can lower the high cost of *P. serotina* control (Reinhardt et al. 2003).

Our results also recommend prioritized risk assessment for *P. serotina*, as was stated by Carboneras et al. (2018). Success of *P. serotina* varied among different tree species. Total biomass depended on light and propagule availability, while biomass increment depended on disturbances, described by changes in tree stand biomass. Invasion intensity was driven by disturbance, i.e. dieback of trees, especially *P. abies*, caused increases in light availability, which increased invasibility of these stands. In conjunction with propagule pressure, disturbance allowed growth and survival of numerous *P. serotina* seedlings. Our findings suggest that propagule pressure and disturbance intensity, expressed in biomass change, should be considered together to understand the susceptibility of forest stands to invasion and to develop methods of control for invasive trees and shrubs (Brundu and Richardson 2016). Even modest disturbances, including vulnerability to dieback in succession of coniferous species, may be risky in areas with high propagule pressure from invasive trees. Thus, silvicultural methods for *P. serotina* control may include maintaining high canopy closure and supporting natural regeneration of tree species with high leaf area, which shade the understory. This result is consistent with recommendations of Nyssen et al. (2016), who highlighted the importance of life history traits of the invaded species habitats in shaping invasibility.

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Supplementary material 1

Parameters of tree stands on experimental plots in the Siemianice Experimental Forest

Authors: Andrzej M. Jagodziński, Marcin K. Dyderski, Paweł Horodecki, Kathleen S. Knight, Katarzyna Rawlik, Janusz Szmyt

Data type: measurement

Explanation note: **Table S1.** Parameters of tree stands on experimental plots in the Siemianice Experimental Forest.

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Link: <https://doi.org/10.3897/neobiota.46.30413.suppl1>

Supplementary material 2

Procedure for modelling the allometric relationships between *P. serotina* dimensions and biomass

Authors: Andrzej M. Jagodziński, Marcin K. Dyderski, Paweł Horodecki, Kathleen S. Knight, Katarzyna Rawlik, Janusz Szmyt

Data type: measurement

Explanation note: **Table S2.** Summary of models of *P. serotina* biomass. Parameters of the best model were bolded. **Figure S1.** Distribution of sampled trees compared with root collar diameter and height of all investigated trees.

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Link: <https://doi.org/10.3897/neobiota.46.30413.suppl2>

Supplementary material 3

Allometric equations determining aboveground biomass of particular tree species presented on sample plots

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Data type: measurement

Explanation note: **Table S3.** Allometric equations determining aboveground biomass of particular tree species presented on sample plots. Equations adopted were established for habitat conditions similar to those of this study. Abbreviations: DBH – diameter at breast height; NA – not available.

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Supplementary material 4

Changes of *P. serotina* density, biomass and environmental parameters included in models

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Data type: measurement

Explanation note: **Table S4.** Changes of *P. serotina* leaf area index, density, biomass and environmental parameters included in models. Abbreviations: Δ – difference 2013–2005; $\% \Delta$ – percent difference (2013–2005)/2005; LAI – leaf area index; DIFN – diffusive non—interceptance (flight availability expressed as a fraction of the open sky). **Figure S2.** Aboveground biomass of planted tree species [Mg ha^{-1}] within tree stands: (a) – in 2005; (b) – in 2013; (c) – difference between 2013 and 2005; and light availability level – DIFN: (d) – in 2005; (e) – in 2013; (f) – difference between 2013 and 2005; **Figure S3.** aboveground biomass of *P. serotina* [kg ha^{-1}]: (g) – in 2005 (h) – in 2013, (i) – difference between 2013 and 2005 and propagule pressure around the experimental plots: (j) – minimal distance from fruiting specimens [m] and (k) – number of fruiting *P. serotina* trees up to 50 m from the plot. Black dots in (j) and (k) indicate fruiting specimens of *P. serotina*.

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Eco-physiological performance may contribute to differential success of two forms of an invasive vine, *Dolichandra unguis-cati*, in Australia

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Abstract

Invasive plant species are hypothesized as being more efficient at resource acquisition and use, resulting in faster growth than co-occurring non-invasive plant species. Nonetheless, some findings suggest that trait differences between invasive and non-invasive species are context dependent. In this study, two forms of an invasive vine species, *Dolichandra unguis-cati*, were used to test the context-dependent hypothesis. *Dolichandra unguis-cati* is a weed of national significance in Australia with two different forms: the ‘long pod’ (LP) and ‘short pod’ (SP). The two forms have different levels of distribution on the eastern Seaboard of the continent, with the SP form occurring extensively in both States of Queensland and New South Wales while the LP form is found only in isolated sites in South-East Queensland. This study examines whether differences in eco-physiological performance could be responsible for differential success of the two forms. A partially factorial experiment was set up in controlled conditions where potted plants of both forms were grown under two levels of light, water and nutrient resources (high and low) for 15 months. We measured several traits that are known to correlate with plant performance and resource use efficiency (RUE). The SP form exhibited higher values of carbon assimilation, RUE, number of subterranean tubers and leaf nitrogen than the LP form. However, the LP form produced greater biomass than the SP form,

with the difference driven mainly by high resource conditions. The LP form displayed significantly higher phenotypic integration (number of traits significantly correlated) than the SP form in response to all treatments while the SP form exhibited higher phenotypic integration than the LP form in response to high resource conditions only. The SP form displayed traits that are well suited for successful colonization, possibly explaining its increased success in Australia, while the LP form possessed traits of opportunistic plants. Overall, we find that the two forms of the weedy vine deploy different carbon economies in response to resource conditions, which is evidence of the context-dependent trait hypothesis.

Keywords

Car's claw creeper, disturbance, functional traits, resource use efficiency, invasiveness, Bignoniaceae

Introduction

A key component of invasion ecology is to understand traits that enable introduced species to colonize and thrive in novel environments (Richardson and Pyšek 2006; Reichmann et al. 2016). Comparative trait-based frameworks involving congeneric introduced invasive and native species are often used to compare and understand the traits that may lead to varying levels of success (Daehler 2003; Bradley et al. 2010; Finerty et al. 2016; Hui et al. 2016). Although trait-based studies have advanced the state of knowledge of how functional traits drive plant community assemblages, results have been equivocal (Palacio-López and Gianoli 2011; Oduor et al. 2016). For example, several pairwise studies found that invasive and non-invasive species had comparable traits under similar environmental conditions (Meiners 2007; Palacio-López and Gianoli 2011; Jo et al. 2016). A review of plant ecological strategies revealed that significant trait differences are sometimes detectable within a species, indicating intraspecific trait variability (Funk et al. 2016). Thus, while there may be differences between some invasive and congeneric non-invasive species, this pattern is not universal (Leishman et al. 2010; Parker et al. 2013). These conflicting outcomes of trait-based pairwise studies may indicate context-dependence of trait differences (Leishman et al. 2010; Leffler et al. 2014; Li et al. 2016). Therefore, comparisons of multiple forms/varieties of an introduced species that both occupy similar niches but exhibiting different levels of invasion success could yield informative insights into invasion success (Kolar and Lodge 2001).

Invasive plants often display faster growth strategies than non-invasive species in the same environments (Davies et al. 2000; Firn et al. 2012). They explore limiting resources more efficiently than co-occurring species in the same environment (Vitousek et al. 1996). Invasive species display higher carbon assimilation rates (A_{max}) and higher relative growth rates (RGR) than non-invasive species (Pattison et al. 1998; Grotkopp et al. 2002; Feng et al. 2007; van Kleunen et al. 2010b). Significantly higher values for these traits and other resource use efficiency (RUE) indices including water use efficiency (WUE) and photosynthetic nitrogen use efficiency (PNUE) are directly linked with faster growing plants (Wright et al. 2004; Firn et al. 2012).

Other studies have demonstrated that invasive species show higher trait integration than non-invasive ones (Osunkoya et al. 2014; Luo et al. 2015). Trait integration (or

trait coordination) is an estimation of the number of trait pairs that are significantly correlated in their response to changes in environmental conditions. The higher the number of correlated trait pairs, the more integrated or coordinated a species is considered to be (Pigliucci 2003). More integrated traits may allow organisms to adapt better to the environment (Waite and Levin 1993; Godoy et al. 2012).

Many studies have tried to identify the key traits that explain successful colonization and establishment by invasive plants, but results have shown that context matters more than any one trait and disturbance seems to frequently matter too (MacDougall et al. 2014). Disturbed plant communities are more susceptible to invasion because of unused resources e.g. pulses in light gaps and nutrient load (Davis et al. 2000). In addition, most invading species have heightened capabilities for niche pre-emption (Baruch and Goldstein 1999; Stratton and Goldstein 2001), even in low resource environments such as canopy understories and arid environments (Abrams 1983; Stubbs and Bastow Wilson 2004; Schwillk and Ackerly 2005). Invasive species that are adapted to low resource environments often show traits associated with resource conservation such as slow growth and high RUE (Funk and Vitousek 2007; Firn et al. 2012; Ens et al. 2015). Thus, mechanisms allowing successful colonization of low resource habitats will be different from those driving invasion of high resource habitats (Funk et al. 2013). These two ends of a continuum of invasiveness strategy agree with the leaf economic spectrum (LES), which proposes a trade-off in the traits held by plant species growing in productive versus unproductive environments (Wright et al. 2004).

Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) (Bignoniaceae) is an invasive vine in Australia. Two morphologically distinct forms of this species occur in Australia ('long' and 'short' pod – in reference to the average length of fruit pods produced by each form) (Shortus and Dhileepan 2011). The two forms of *D. unguis-cati* show different prevalence or abundance. The short pod (SP) form is more abundant, occurring extensively in the states of Queensland and New South Wales (Dhileepan 2012), while the long pod (LP) form occurs in ~15 isolated sites in southeast Queensland (Liz Snow, personal communication, 07/03/2016). Where the LP form is present, it usually co-occurs with the SP form (Dhileepan 2012). The cause of variation in abundance and prevalence between the two forms is not known, but we hypothesised that differences in eco-physiological responses to environmental resources could offer a potential explanation (Buru et al. 2016a, 2016b). The SP form exhibited higher RGR, stem length, specific leaf area (SLA) and tuber abundance than the LP form in glasshouse conditions (Buru et al. 2016a). However, in a field study it was shown that the LP form accumulated more biomass and had higher RGR than the SP form (Taylor and Dhileepan 2012). The more abundant SP form has rapid and higher germination rates and a greater prevalence of poly-embryonic seeds (40%) than the less abundant LP form (Buru et al. 2014, 2016b).

To understand the eco-physiological mechanisms that underpin colonization success, it is vital to investigate potential links between plant growth and physiology, including photosynthetic traits (Osunkoya et al. 2010b). In this study, we compared eco-physiological traits of the two forms of *D. unguis-cati* to gain a better understanding

of the mechanistic reasons for the SP form being more successful in its invaded range than the LP form. We measured and compared photosynthetic capacity and biomass accumulation under varying environmental resources of water, light and nutrient (low vs. high) and examined shifts in these traits in response to changes in resource conditions. Specifically, we examined traits related to carbon gain including maximum carbon assimilation (A_{max}), transpiration, C: N ratio, total biomass, SLA, LDMC and tuber density. We correlated assimilation rates, SLA, resource use efficiency traits and tuber abundance with total biomass to ascertain whether physiological performance resulted in differences of fitness and growth in the two forms. We hypothesised that SP, the more abundant form of *D. unguis-cati* in Australia, would show higher photosynthetic capacity, higher RUE and perform better under disturbance scenarios of high nutrients and light levels than the less abundant LP form.

Methods

Study species

Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) (Bignoniaceae) is a native of the Greater and Lesser Antilles, Mexico, South and Central America, Argentina and Trinidad and Tobago (Gentry, 1976). This species was introduced into Australia for ornamental purposes from South America in the 1800s (Downey and Turnbull 2007). *Dolichandra unguis-cati* presents a serious threat to native biodiversity, especially in riparian and rainforest plant communities as a structural parasite (Raghu et al. 2006; Dhileepan 2012; Ewers et al. 2015). Where there is standing vegetation, it smothers tree canopies, and the biomass can build to a point where it causes the collapse of canopy structures (Batianoff and Butler 2003). In the absence of vertical support, it readily grows along the ground, forming dense mats that preclude recruitment, growth and germination of indigenous understory vegetation. This growth pattern transforms natural habitats into monospecific stands, resulting in loss of floral biodiversity and changes in soil biota and physico-chemical properties (Osunkoya et al. 2009). As a result, this invasive vine is classified as an ecosystem transformer. *Dolichandra unguis-cati* regenerates both asexually (vegetatively), by production of subterranean tubers, as well as sexually, with production of numerous papery seeds (Downey and Turnbull 2007; Osunkoya et al. 2009). The two forms of *D. unguis-cati* that occur in Australia have been informally referred to as long pod (LP) and short pod (SP) based on their average (\pm SE) fruit length at maturity (LP form: 70.024 ± 2.35 cm; SP form: 30.089 ± 8.96 cm). The LP and SP forms have been shown to carry an average of 120 ± 10.67 and 60.89 ± 23.17 seeds per pod at maturity, respectively. Seeds of both forms are two-winged, papery and flattened/oblong in shape, 10–18 mm long, 4.2–5.8 mm wide (Shortus and Dhileepan 2012). These two forms appear to prefer similar habitats, although there is general lack of research on the ecology of this species (Osunkoya et al. 2009).

Experimental design

This study was carried out in temperature-controlled glasshouse and shade-house facilities at the Queensland Department of Agriculture and Fisheries (DAF) in Brisbane, Australia. During the 15-month experiment, average temperature during the warmer months (October–April) ranged from 18 °C to 35 °C, and between 10 °C and 23 °C during the cooler months (May–September). Relative humidity ranged between 50–70% during this study.

Fruits containing seeds of the LP and SP forms were collected from various sites around the greater Brisbane area in South East Queensland (SEQ) and parts of New South Wales (NSW), Australia. Seeds of both forms were germinated in plant growth chambers, (model ADAPTIS A1000; Conviron Ltd., USA). For further germination details see Buru et al. (2016a). After two weeks of germination, seedlings were transferred into small 0.8 L plastic pots (dimensions: Diameter = 200mm, Height = 190mm) filled with locally available commercial potting mix (Osmocote) to establish the plants. Plants were watered every two days for two months without addition of extra nutrients. After two months of growth, plants of both forms were transferred into bigger 13.5 L plastic pots (dimensions: Diameter = 300 mm, Height = 290 mm) filled with a multi-purpose potting mix containing a wetting agent and trace elements (Osmocote). After two weeks in the bigger pots, these plants were then subjected to different treatments as described below.

Light x nutrients experiments

A factorial experiment of light and moisture was set up in a shade-house. For each form of *D. unguis-cati*, growth and physiology under two light resource levels were investigated i.e., (a) High light (HL) in which plants received ~35–40% of full sun (870–1100 $\mu\text{mol m}^{-2} \text{s}^{-1}$); and (b) Low light (LL) in which plants received ~1–2% of full sun (25–50 $\mu\text{mol m}^{-2} \text{s}^{-1}$). LL conditions were achieved by creating a shade using 2–3 layers of locally available Coolaroo 1.83m Charcoal 90% shade cloth. These two light levels were chosen as approximations of irradiance levels encountered in disturbed habitats and open spaces (HL), and in rainforest understories (LL) where *D. unguis-cati* usually occurs. Light levels were measured using a LICOR 6400 portable photosynthesis system (LICOR, Inc., Lincoln, NE). Both forms were grown under HL and LL conditions with plants in each light level receiving either of two nutrient regimes. High nutrient (HN) condition was obtained by adding granules of a slow-release all purpose fertiliser (Osmocote, NPK 21:2:6 plus trace elements) to the growth medium every two weeks. No additional fertilizer was added to the growth media to create the low nutrient (LN) condition. The two nutrient levels (HN, LN) were chosen to mimic habitats of high nutrients (e.g., following fertilizer discharge) and low nutrient pulses respectively. All plants were watered to pot capacity by the addition of ~300 ml of water every two days using an automated watering system. The combinations of treatments were as follows: HLHN, HLLN, LLHN and LLLN.

Water x nutrients experiments

These experiments were set up in a temperature-controlled glasshouse facility with temperatures ranging from 22–28 °C during the experiment. The mid-day photosynthetically active radiation (PAR) in the glasshouse was 800–1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. These light conditions are comparable to the HL conditions described above for the light x nutrient experiment. Two water regimes were applied, reflecting the riparian and non-riparian environments where *D. unguis-cati* occurs: (i) a well-watered or high water (HW) condition in which soil moisture level was maintained at 100% pot capacity by the addition of ~ 300 ml of water every two days, and (ii) a low water (LW) condition in which moisture was maintained at 5% pot capacity by adding ~ 15 ml of water once every two weeks. Pot water capacity was determined at the beginning of the experiment by filling four replicate 13.5 L plastic pots with the commercial potting mix (Osmocote Multi-Purpose). The potting mix was oven dried using a Thermolite Scientific + 6100 Model oven for 48 hours at 80 °C and weighed to determine dry weight (DW). The potting mix was then saturated with water and excess water allowed to drain freely for 2–3 hours until no more water drained out. The pots were weighed again to determine saturated weight (SW). Pot capacity was calculated as the difference between SW and DW (Frosi et al. 2013). The two water treatment levels were further factored by two levels of nutrient application as described above. Thus, the combinations of treatments were as follows: HWHN, HWLN, LWHN and LWLN.

Data collection

The light and water treatments, at two nutrient levels, were replicated seven (7) times for both the LP and SP forms. Thus, there were 112 total number of plants for this experiment. Physiological and growth traits were measured at the end of the experiment after 15 months of plant growth.

Physiological traits

Assimilation rates were measured using an open-path portable gas exchange system (LI-6400; LICOR, Inc., Lincoln, NE, USA). For each treatment, five replicates (plants) of each form were randomly selected, and for each plant, two recently matured leaflets were identified and tagged for measurements. Photosynthetic rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) were measured at a constant CO_2 concentration of 400 $\mu\text{L L}^{-1}$. The relative humidity within the leaf chamber ranged between 50–65% while the temperature was kept at 23–25 °C. To investigate the response of the leaflets to changes in PAR, instantaneous assimilation A and transpiration measurements were taken at 50, 500, 1500 and 2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaves were kept at the respective PAR levels for ~ 10 minutes until they were stabilised. From the primary physiological data collected, the instantaneous water-use efficiency (WUE) was calculated as follows:

WUE = A_{sat}/E , where A_{sat} was assimilation rate at 2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$

Leaf chlorophyll content (measured in SPAD units) was estimated using a chlorophyll meter (Konica-Minolta SPAD-502, Spectrum Technologies, IL, USA). The same leaves tagged for physiological measurements were used to determine chlorophyll content, taking three random measurements from each leaf. Physiological data could not be obtained for HWLN and LWLN combinations because the plants under these treatments developed very few leaves.

After measurement of physiological data, the tagged leaflets were harvested, weighed (fresh weight) and photographed against a graduated background using an IPAD camera (Apple Inc., CA, USA) for leaf area estimation. The open access software, Image J 1.47v (www.imagej.nih.gov/ij) was used to calculate the leaf area (cm^2) from the images. The harvested leaflets were thereafter dried at 65 °C for 72 hours, and their dry weight measured. The data collected were used to estimate specific leaf area (SLA = leaf area/leaf dry mass) and leaf dry matter content (LDMC = leaf dry mass/leaf fresh mass).

The dried leaf samples were analysed for total carbon (C) and nitrogen (N) concentrations using Plant CN Dumas combustion method (Jung et al. 2003). All chemical components of this study were analysed at the Chemistry Centre, Queensland Department of Science Information, Technology and Innovation (DSITI), Brisbane, Australia. After collection of the leaf chemical data, the following parameters were calculated:

Photosynthetic nitrogen use efficiency (PNUE) =
 $= A_{\text{max}}/\text{leaf N}$, where assimilation rate was at 2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$

Leaf Carbon to leaf Nitrogen ratio (C: N) = Leaf C / leaf N

Leaf Chlorophyll to leaf Nitrogen ratio (Chl: N) = Leaf Chl / leaf N

Growth and biomass traits

At the end of the experiment (i.e. at 15 months growth), all plants were harvested and separated into above-ground (shoots) and below-ground (roots and tubers) tissues. The number of subterranean tubers per plant was recorded per treatment and per plant form. All plant tissues were dried at 65 °C for three weeks before weighing to determine total dry mass (g) and shoot/root ratio.

Statistical analyses

All data were tested for normality and homoscedasticity using the Shapiro-Wilks test. Data that violated the ANOVA assumptions of normality and homogeneity of variance were either \log_{10} transformed (A_{max} , WUE), square-root transformed (shoot/root

ratio) or Box-Cox power transformed (basal stem density [BSD], number of tubers, root and shoot dry mass, total dry mass, and all leaf traits). Values presented in this paper were back-transformed data, unless otherwise stated.

Mean differences for all traits were analysed using a two-way analysis of variance (ANOVA + an error structure of replicate/leaf number/or treatment) with treatments (HLHN, HLLN, LLHN, LLLN, HWHN, HWLN, LWHN, LWLN) and plant form (LP or SP) as fixed effects. When significant differences were detected, a Tukey LSD post-hoc test was performed to check differences between specific means. Pearson correlation coefficients were generated to determine the linear association among traits and how they compare between the LP and SP forms, and they were also used to test for the extent of trait integration within each of the two forms. A multivariate method of principal components analysis (PCA) based on Euclidian distances was used to explore how the two forms were separated by traits on an ordination space. Principal components smaller than 15% were discarded. All statistical tests were conducted using R version 3.1.0 (R Development Core Team 2014) and graphics were created using SPSS (version 22.0; IBM SPSS Statistics; Armonk, NY, US).

Results

Biomass production and resource allocation patterns

Biomass production and allocation traits were not consistent within each form in response to high and low resources as shown by significant interactions between form and treatment for many of the traits examined ($F_{1,7} = 3.184$, $P < 0.005$; Table 2). The two forms differed significantly in biomass accumulation in the HLHN and LWHN treatments (Fig. 1a, b). Under the HLHN scenario, the LP accumulated more biomass, suggesting opportunist traits or a specialised micro-climate for this form. On the other hand, the SP form accumulated more biomass in the LWHN treatment, which suggests resource substitution. Overall (i.e., across treatments), SP and LP total biomass levels differed significantly at the end of the 15 months growth period ($F_{1,7} = 8.124$, $P < 0.006$; Table 1), with the LP individual plants showing more biomass. Light intensity, amount of nutrient and water supplied to the plants all had significant effects on all biomass allocation traits of the two forms ($F_{1,7} = 12.195$, $P < 0.0001$), and was of the order: HLHN > HWHN > HLLN > LWHN > LLHN > LLLN = LWLN (Table 2). Both the SP and LP forms accumulated more biomass in response to light x nutrient treatments than in response to water x nutrients treatments (Fig. 1a, b).

The SP form developed more tubers than the LP form in high light (HLHN and HLLN) and high nutrient (HWHN and LWHN) conditions ($F_{1,7} = 46.459$, $P < 0.001$; Fig. 1c, d; Table 1 and 2). However, the LP form showed significant differences in response to light x nutrients (HLHN > HLLN = LLHN > LLLN) and when both water and nutrients were abundant (HWHN > HWLN = LWHN = LWLN) (Fig. 1c, d; Table 2). There were no significant differences in total biomass and tuber development between the two forms under the most stressful conditions of low light, low water and

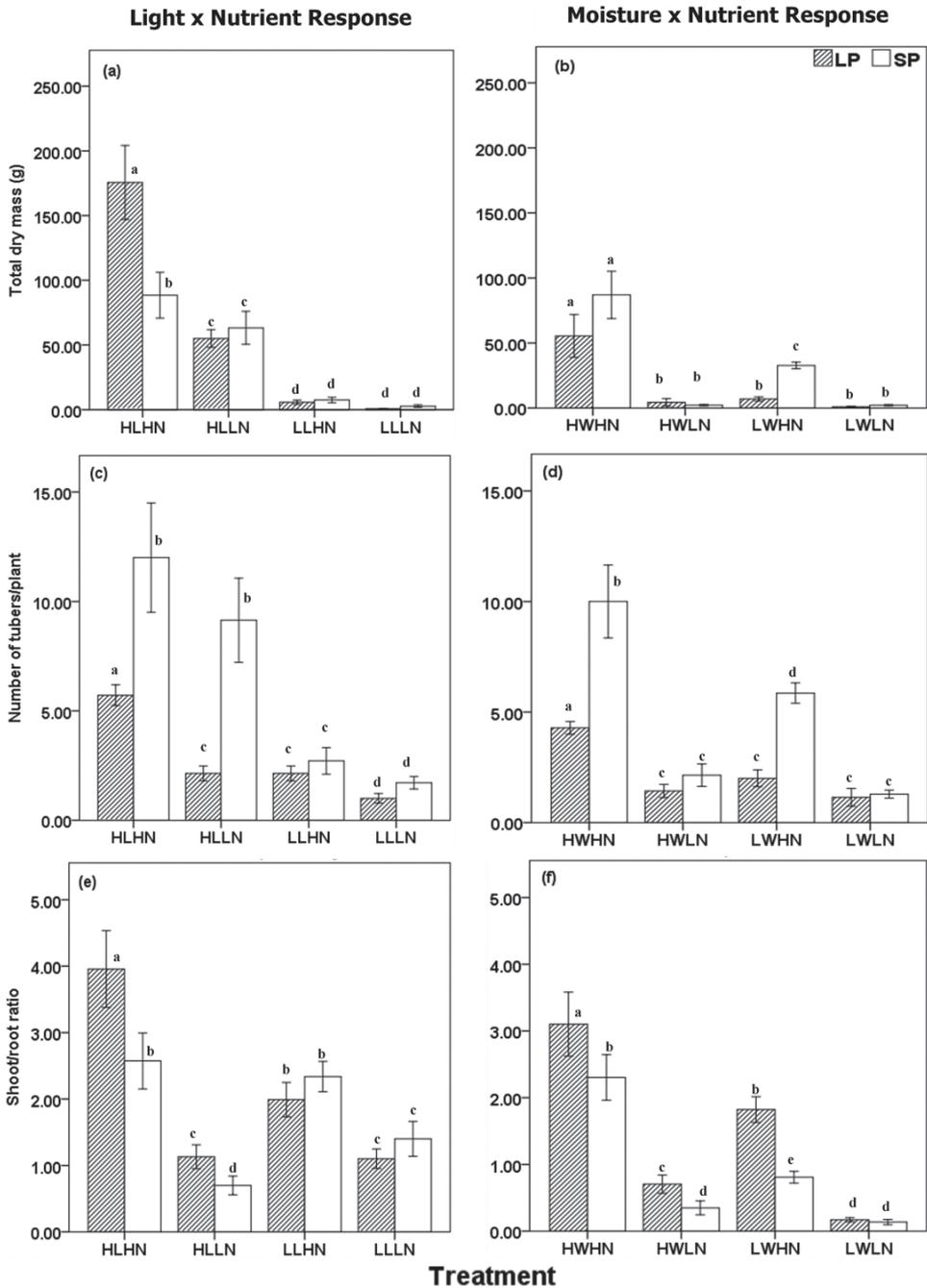


Figure 1. Trait response of the LP and SP forms to varying levels of light, water and nutrient conditions. Total biomass accumulated (**a, b**); Average number of tubers per plant (**c, d**); Shoot/root ratio (**e, f**). The legend in the graph (**b**) applies to all graphs. Graphs on the left (**a, c, e**) show traits responses to light x nutrient experiments and those on the right (**b, d, f**) show trait responses to water x nutrients experiments. Bars represent standard error of the mean (SEM). Differences across treatments are denoted by letters.

Table 1. Mean (\pm SE) growth, physiological and leaf chemical traits of both forms, long pod (LP) and short pod (SP) of *D. unguis-cati*. Summary ANOVA refers to F- and P-values of a two-way ANOVA (+ an error structure) of log transformed performance traits and physiological traits for both forms, with a fixed effects structure of form (LP and SP) and treatments (HLHN, HLLN, LLHN, LLLN, HWHN, LWHN). NS = not significant.

Traits	Form		Summary ANOVA		Direction of difference
	LP	SP	F-value	P-value	
Total biomass (g)	38.11 \pm 0.25	35.75 \pm 5.89	8.12	0.0060	LP>SP
Leaf area (cm ²)	26.27 \pm 2.14	11.74 \pm 0.80	54.52	0.0001	LP>SP
SLA (cm ² g ⁻¹)	3.95 \pm 0.19	4.08 \pm 0.27	0.45	0.60	NS
LDMC (mg g ⁻¹)	296.94 \pm 14.11	293.04 \pm 9.88	0.16	0.694	NS
No. of tubers plant ⁻¹	2.48 \pm 0.24	5.61 \pm 0.69	46.46	0.0001	LP<SP
Root dry mass (g)	11.35 \pm 2.13	15.23 \pm 2.54	2.54	0.122	NS
Shoot dry mass (g)	26.76 \pm 6.76	20.90 \pm 3.90	0.01	0.912	NS
*Shoot/root ratio (SRR)	1.78 \pm 0.19	1.31 \pm 0.15	1.99	0.20	NS
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	3.48 \pm 0.27	3.97 \pm 0.34	4.07	0.05	LP<SP
WUE ($\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$)	4.03 \pm 0.21	4.53 \pm 0.31	30.29	0.001	LP<SP
PNUE ($\mu\text{mol mol s}^{-1}$)	1.27 \pm 0.15	1.21 \pm 0.08	0.14	0.71	NS
C (g m ⁻²)	43.82 \pm 0.26	43.28 \pm 0.24	6.28	0.018	LP>SP
N (g m ⁻²)	3.45 \pm 0.26	3.73 \pm 0.19	5.31	0.03	LP<SP
C: N	14.63 \pm 1.38	12.37 \pm 0.82	7.29	0.01	LP>SP
Chl. (SPAD units)	41.00 \pm 0.85	57.24 \pm 0.03	58.52	0.0001	LP<SP
Chl: N	13.29 \pm 0.38	15.74 \pm 0.77	1.60	0.22	NS

*Root biomass includes tuber biomass

low nutrients: biomass (LLL: $P = 0.365$; LWL: $P = 0.424$) and tuber abundance (LLL: $P = 0.856$; LWL: $P = 0.836$) (Fig. 1a-d).

Response of leaf traits to light, water and nutrient availability

Overall, both the SP and LP forms differed in terms of SLA depending on treatments ($F_{1,4} = 257.845$, $P < 0.0001$; Table 2). Both forms invested significantly less biomass in their leaves in the LLHN condition compared to the HLHN condition (Table 2), indicating the importance of light intensity in determining resource allocation strategies.

Across treatments, the LP form accumulated significantly higher total leaf carbon (C) than the SP form ($F_{1,4} = 6.282$, $P < 0.018$). Conversely, the SP form showed higher area based total leaf nitrogen (N) ($F_{1,4} = 5.310$, $P < 0.03$) (Table 1). Thus, the LP form exhibited significantly higher C: N ratio ($F_{1,4} = 7.289$, $P < 0.02$) than the SP form (Tables 1 and 2). However, variations in leaf nutrient concentrations were best explained by treatment and its interactions with form (Table 1). Total leaf N concentrations were significantly higher in HLHN than HLLN treatments, but there was no significant difference in leaf N between LLHN and LLLN for either forms (Table 2). This pattern indicates a strong effect of light intensity in determining resource acquisition and use efficiency.

Table 2. Mean trait performance and summary of ANOVA of the SP and LP forms in different light, water and nutrient treatments. *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$; NS, not significant. Treatments: HL, high light; LL, low light; HN, high nutrient; LN, low nutrient; HW, high water; LW, low water.

Form	Treatment	N, g m ⁻²	C, g m ⁻²	C:N	Chl, spad units	Chl: N	A _{max} , μmol m ⁻² s ⁻¹	WUE, μmol CO ₂ mol ⁻¹ H ₂ O	PNUE, μmol mol s ⁻¹	SLA, cm ² g ⁻¹	LDMC, mg g ⁻¹	Total dry mass,g	No. of tubers	S/R ratio	BSD, cm
SP	HLHN	4.27	43.74	10.24	62.24	14.59	5.79	5.68	1.36	4.63	354.89	105.08	15.0	2.94	3.14
LP	HLHN	4.14	44.76	10.87	42.01	10.19	5.41	4.36	1.33	3.83	341.39	140.41	6.00	3.91	5.53
SP	HLLN	2.21	42.47	19.47	41.02	18.69	2.50	4.49	1.10	3.67	394.25	69.23	10.0	0.55	3.30
LP	HLLN	1.87	42.72	22.96	33.51	17.82	3.67	4.83	1.94	7.35	458.48	60.95	2.20	0.95	5.25
SP	LLHN	3.96	42.95	10.83	59.36	14.99	3.78	3.35	0.96	5.74	308.15	7.97	2.75	2.47	2.03
LP	LLHN	4.08	42.66	10.46	55.04	13.49	2.44	2.94	0.59	4.67	332.58	4.55	2.33	1.91	2.79
SP	LLLN	3.42	42.13	12.34	60.09	17.59	4.60	3.24	1.35	-	-	2.99	1.33	1.55	1.71
LP	LLLN	2.64	44.05	19.38	52.42	23.00	3.16	3.20	1.38	-	-	1.33	1.00	0.86	1.77
SP	HWHN	4.41	44.40	10.07	61.80	13.99	5.55	5.10	1.26	3.32	361.79	87.04	8.60	1.68	4.59
LP	HWHN	4.27	44.56	10.64	34.47	7.86	3.75	3.88	0.89	3.85	363.97	41.74	4.20	3.16	3.99
SP	HWLN	-	-	-	-	-	-	-	-	-	-	2.19	2.14	0.35	1.53
LP	HWLN	-	-	-	-	-	-	-	-	-	-	4.40	1.43	0.70	1.78
SP	LWHN	4.28	44.68	10.45	61.14	14.30	2.07	4.68	0.49	4.34	405.94	30.93	6.00	0.782	2.73
LP	LWHN	-	-	-	-	-	2.45	4.52	-	4.54	334.41	6.93	2.00	1.82	2.43
SP	LWLN	-	-	-	-	-	-	-	-	-	-	2.16	1.29	0.14	1.24
LP	LWLN	-	-	-	-	-	-	-	-	-	-	0.97	1.14	1.58	0.97
Summary of ANOVA															
Form		*	*	NS	***	*	*	***	NS	NS	NS	**	***	NS	**
Treatment		***	**	**	*	***	***	***	*	***	***	***	***	NS	***
Form x Treatment		.	.	*	NS	NS	**	*	*	***	*	**	*	NS	***
Direction of form difference		LP<SP	LP>SP	LP=SP	LP<SP	LP<SP	LP<SP	LP<SP	LP=SP	LP=SP	LP=SP	LP>SP	LP>SP	LP=SP	LP>SP

Carbon assimilation rate and resource use efficiency in response to variations in light, water and nutrients

The SP form showed a significantly higher rate of carbon assimilation (A) than the LP when compared across all resource treatments ($F_{1,5} = 4.067$, $P < 0.05$) (Table 1). The significant interaction effect of form x treatment suggests that response patterns of two forms were not consistent under changing environmental resources ($F_{1,5} = 4.499$, $P < 0.001$; Table 2). The two forms were not different in carbon assimilation (A) in the HLHN condition. The SP form had slightly higher A in low light (LLHN and LLLN), whereas the LP form had slightly higher A under HLLN (Fig. 2a; Table 2).

The SP form showed a significant shift in carbon assimilation in response to light levels as there was nearly a two-fold difference in A between low light (LLHN) and high light (HLHN) conditions ($P < 0.0001$; Fig. 2a) when nutrient level was high. In contrast, in the LP form, A only increased by a factor of 0.5 from low light (LLHN) to high light (HLHN) ($P < 0.001$). When nutrient level was low, the SP form still increased its A two-fold from high light (HLLN) to low light (LLLN) conditions ($P < 0.0001$) while there was no significant change in A in the LP form ($P > 0.05$; Fig. 2a; Table 2). This trend suggests a better plasticity and hence acclimation for the SP form to a decreasing light condition. A was measured for only two treatments under the moisture experiments, i.e. HWHN

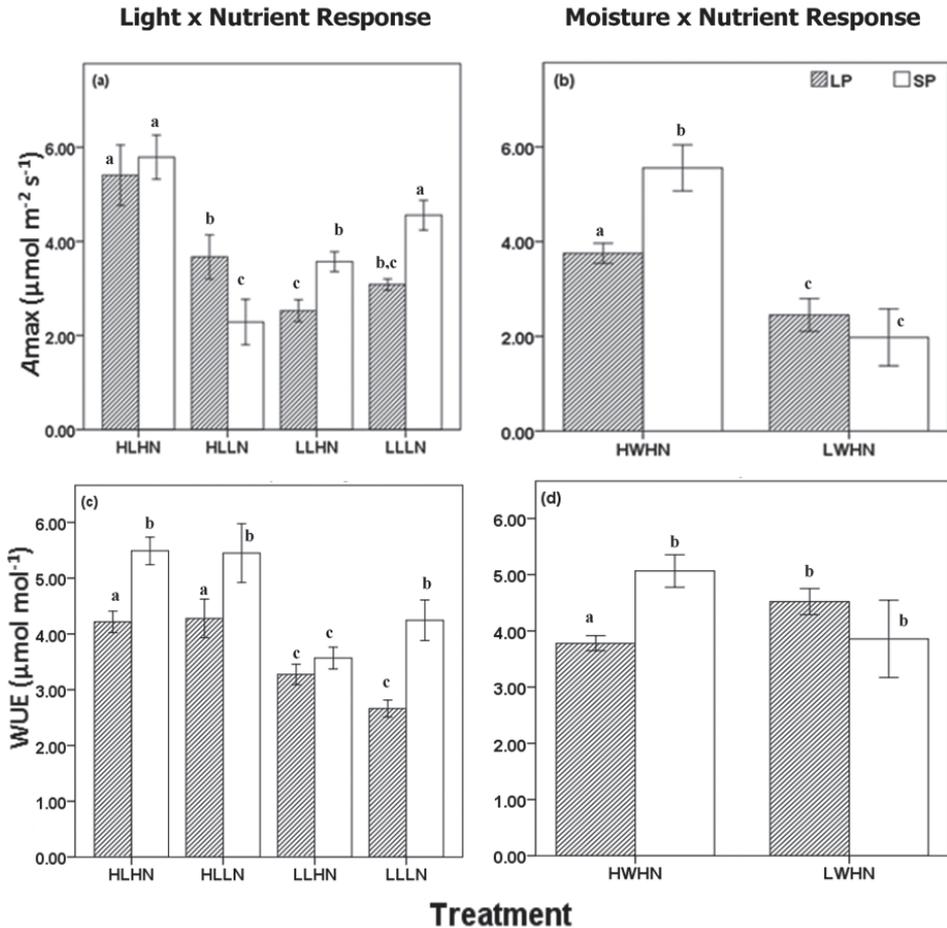


Figure 2. Carbon assimilation rates and water use efficiency of the LP and SP forms in response to light, water and nutrient resources. Maximum carbon assimilation, A_{max} (**a**, **b**); water use efficiency, WUE (**c**, **d**). The legend in the graph **b** applies to all graphs. Graphs on the left (**a**, **c**) show traits responses to light x nutrient experiments and those on the right (**b**, **d**) show trait responses to water x nutrients experiments. Bars represent standard error of the mean (SEM). Differences across treatments are denoted by letters.

and LWHN. The response did not vary significantly between the SP and LP forms (Fig. 2b) under LWHN but did so under HWHN (Table 2). In both forms A declined significantly with a reduction in water availability (from HWHN to LWHN) but the SP form had significantly higher A with abundant water and nitrogen (HWHN) (Fig. 2b).

The SP form showed higher water use efficiency (WUE) than the LP form in response to different treatments ($F_{1,5} = 30.294$, $P < 0.001$; Table 1). Water loss in the SP form was more restrained in the order $\text{HLLN} > \text{HLHN} > \text{LLL N}$. This resulted in significantly higher WUE for SP in these treatments than LLHN ($P < 0.01$; Fig. 2c). This is so because both A and WUE were large under the HLHN and LLLN conditions (Fig. 2a, c), i.e., HLLN is the only treatment where WUE in the SP form did not follow the same pattern as for A . WUE in the LP form was significantly higher in high light than in low light in

the order HLHN = HLLN > LLHN = LLLN ($P < 0.001$; Fig. 2c; Table 2). WUE in the SP form did not differ significantly between water treatments (HWHN and LWHN) ($P > 0.05$) but WUE for the LP form was significantly lower in the HWHN treatment than the LWHN treatment (Fig. 2d; Table 2). There was no difference between LP and SP in terms of photosynthetic nitrogen use efficiency (PNUE) ($F_{1,7} = 0.138$, $P < 0.712$; Table 1). Both forms exhibited lower A and RUE (WUE, PNUE) in stressful conditions (LL, LN and LW) than non-stressful conditions (HL, HN and HW) (Fig. 2; Table 2). Physiological data could not be obtained for treatments receiving HWLN and LWLN because the plants under these treatments developed few leaves. This suggests the importance of nutrient availability (N) in leaf development for both forms of the invasive vine.

Differences in trait coordination and trait correlations between the LP and SP forms

Across treatments, all performance traits examined changed in response to changes in biomass accumulated, although the trends were not significant for the SP form in terms of assimilation rate (A) and leaf chlorophyll content. It is instructive to see that at a given plant biomass, higher trait values were obtained for the SP form relative to the LP form (Fig. 3; Table 2). It is also interesting that SLA, a trait that facilitates photosynthetic capture, was significantly linked to carbon assimilation and biomass accumulation only in the SP form, but not in the LP form (Table 3; Fig. 3c).

Considering all possible bivariate relationships for the traits measured in the study (i.e. 45 pairwise comparisons), the number of significant correlations were higher for the LP form (23) than the SP form (17) (Table 3). To test whether there was a difference in level of trait integration or coordination between the two forms in response to high resources, eco-physiological traits were correlated with SLA and total biomass (two important performance and/or fitness traits) for (a) high light intensity (HL) and (b) enhanced nutrients (HN) scenarios separately. Under high light conditions, more traits were significantly correlated with biomass gained for the SP form (5 out of 12) than the LP form (3 out of 12). Two physiological traits, A and leaf N were significantly linked to SLA in the SP form while only one trait (PNUE) was linked to SLA in the LP form (Suppl. material 1: Table S1).

In the high nutrient scenario, more traits (4) were correlated with SLA in the SP form (biomass gained (negative), A_{\max} (positive), WUE (negative) and total leaf N (negative)). In contrast, only two traits (leaf N and leaf Chl.) were positively associated with SLA in the LP form. In the same high nutrient condition regardless of light and moisture condition, a slightly higher number of traits (compared to the high light scenario) were linked to biomass gained for the LP form (5 out of 12), but more traits were significantly correlated with total biomass for the SP form (7 out of 12) in the high nutrient scenario (Suppl. material 1: Table S2). Thus, it is safe to conclude that there was more trait integration in the SP form than in the LP form under high resource conditions of light and nutrients (Suppl. material 1: Table S1 and Table S2), while there was more trait integration in the LP than SP form when considering all the resource conditions investigated in the study (Table 3). Hence, context is key when considering the trait integration in the two forms of *D. unguis-cati*.

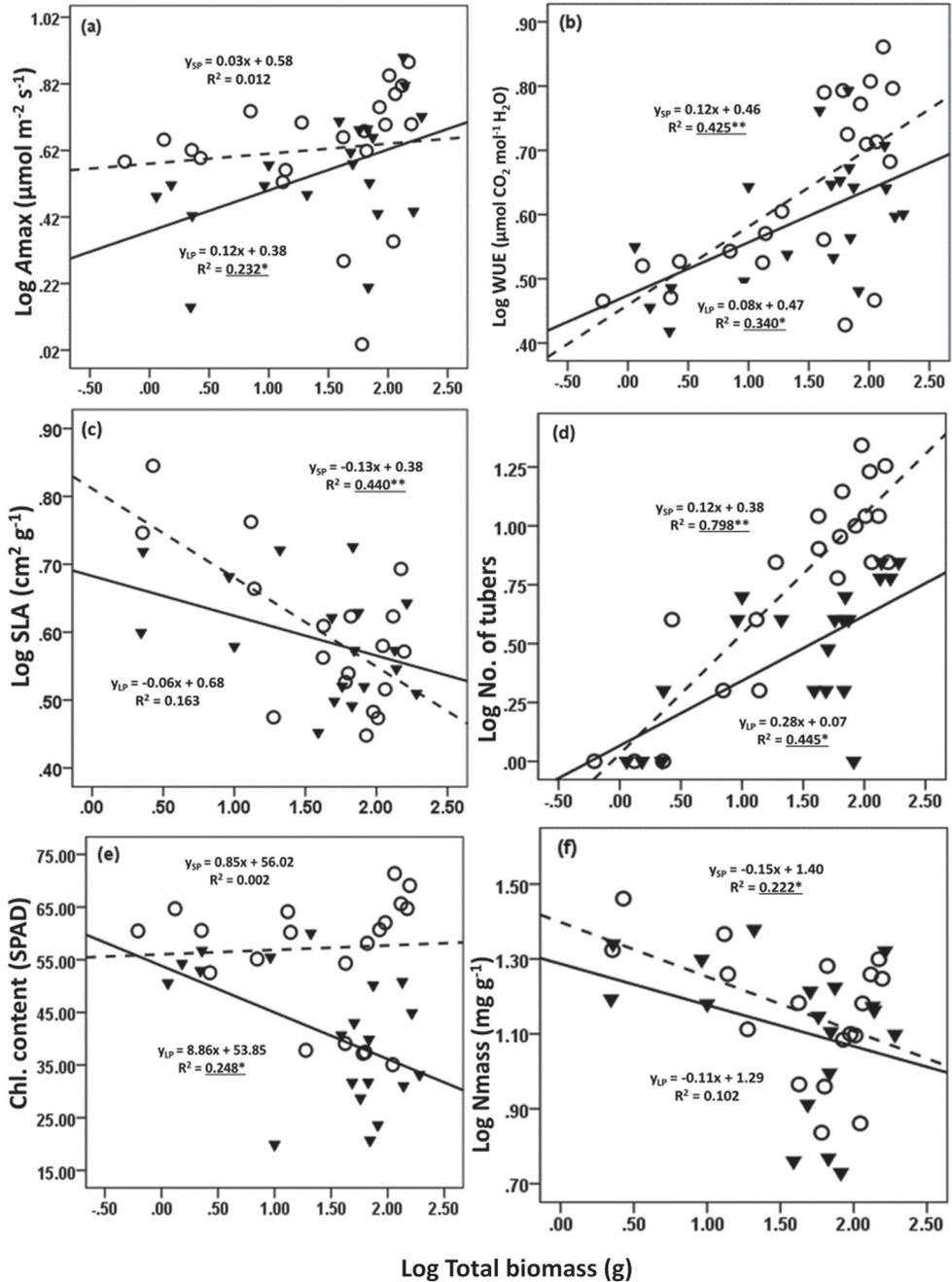


Figure 3. Trait relationships across light, water and nutrient regimes between total biomass accumulated versus A_{max} (a), WUE (b), SLA (c), number of tubers (d), chlorophyll content (e) and leaf N concentration (f). The LP form is represented by triangles (\blacktriangle) and a solid line (—) while the SP form is represented by open circles (\circ) and dotted lines (---). Significant relations ($P < 0.05$) are shown by underlined R^2 values, **, $P < 0.0001$; *, $P < 0.05$.

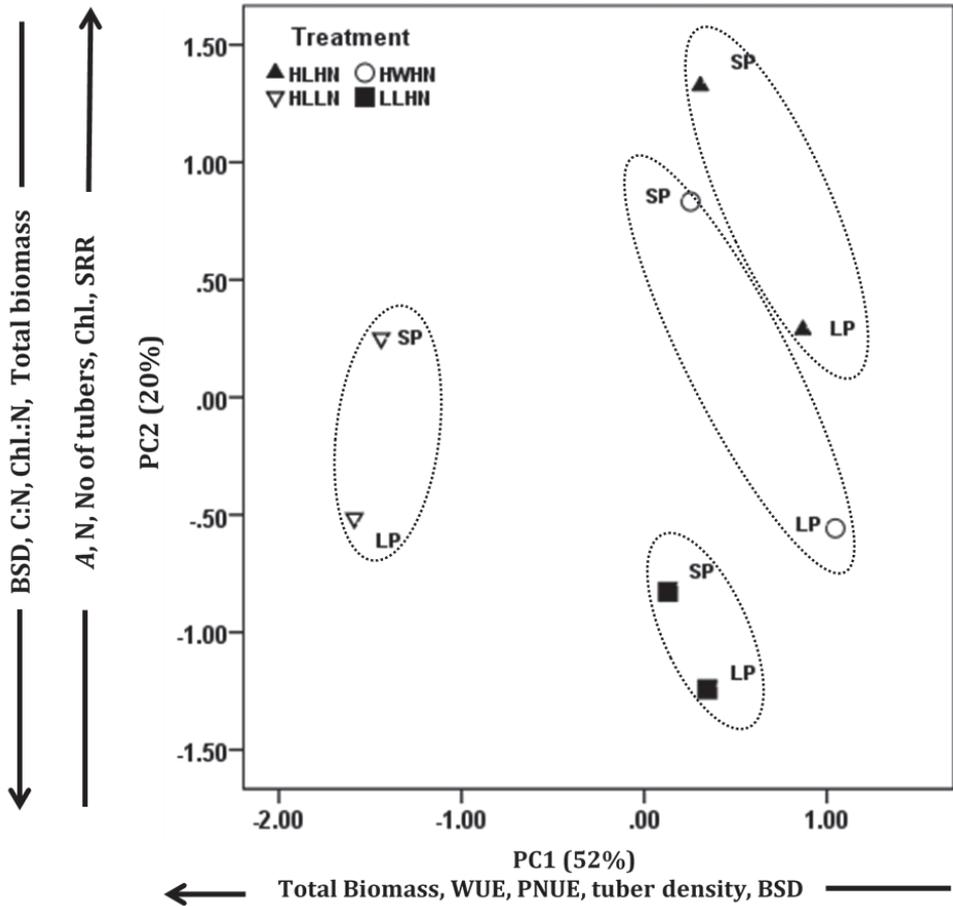


Figure 4. Principal component analysis of LP and SP across four treatments (HLHN, HWHN, HLLN, and LLHN) based on 13 eco-physiological traits projected on the first two axes. The traits on each axis are the main drivers of the variation explained by that axis. The percentage of the variance explained by each principal component is shown in brackets. There was no determination of leaf chemistry for treatments that are not included in this PCA because of insufficient leaf materials. Dotted lines connect the LP and SP forms under similar treatments for the sake of comparison.

A graphical representation of a principal component analysis (PCA) of the LP and SP forms based on 10 traits under four resource treatments (HLHN, HLLN, HWHN and LLHN) is shown in Figure 4. The remaining treatments (LLLN, LWHN, HWLN and LWLN) were not included because not all physiological and chemical traits were measured in those treatments. The PCA shows that the first two axes explained 72% of the total variation in the data. The first axis explained 52% of the data variation and was strongly correlated with RUE traits (WUE, PNUE), SLA and total biomass. The second axis explained 20% of the total variation in the data and was strongly linked to C: N ratio, shoot/root ratio, assimilation rate (A) and total leaf N per mass (Fig.

Table 3. Matrix of Pearsons correlation coefficients (r) for functional traits of the LP and SP forms (SP shown in brackets). Data have been pooled across light, water and nutrient treatments. Significant correlations (P<0.05) are shown by bold font and asterisks (**, P < 0.001; *, P < 0.05); n = 18–20.

	SLA	Total biomass	No of tubers	WUE	PNUE	N	C:N	Amass	A _{max}	Chl.
	LP (SP)	LP (SP)	LP (SP)	LP (SP)	LP (SP)	LP (SP)	LP (SP)	LP (SP)	LP (SP)	LP (SP)
SLA	1									
Total biomass	0.403(-0.663 ^{**})	1								
No of tubers	-0.073(-0.519)	0.667^{**} (0.893 ^{**})	1							
WUE	-0.383(-0.424)	0.583^{**} (0.652 ^{**})	0.474^{**} (0.515 [*])	1						
PNUE	-0.570^{**} (-0.213)	0.352(0.163)	0.069(0.161)	0.688^{**} (-0.094)	1					
N	0.588^{**} (0.684 ^{**})	-0.319(-0.471 [*])	0.404(-0.383)	-0.472^{**} (-0.120)	-0.736^{**} (0.097)	1				
C: N	-0.237(-0.083)	-0.018(0.039)	-0.519^{**} (0.037)	0.318(-0.183)	0.657^{**} (-0.293)	-0.924^{**} (-0.782 ^{**})	1			
Amass	-0.107(0.392)	0.426(-0.142)	0.715^{**} (-0.069)	0.515^{**} (-0.117)	0.552^{**} (0.659 ^{**})	0.157(0.812 ^{**})	-0.215(-0.781 ^{**})	1		
A _{max}	-0.533^{**} (-0.099)	0.482^{**} (0.111)	0.593^{**} (0.109)	0.595^{**} (0.069)	0.679^{**} (0.821 ^{**})	-0.126(0.521 [*])	-0.108(-0.785 ^{**})	0.898^{**} (0.877 ^{**})	1	
Chl.	0.564^{**} (0.027)	-0.498^{**} (0.045)	-0.254(-0.097)	-0.380(0.421)	-0.401(0.314)	0.568^{**} (0.446)	-0.310(-0.569 ^{**})	0.034(0.529)	-0.239(0.545)	1

4; Suppl. material 1: Table S3). The two forms of *D. unguis-cati* clustered together on the first axis, meaning that traits correlated with this axis have low explanatory power in differentiating the two forms. Separation on the first axis only increases slightly for treatments other than HLLN, but especially for HLHN and HWHN (Fig. 4). However, the two forms were significantly separated along the second axis (see the separation shown by dotted shapes comparing the two forms under similar experimental treatments). The separation on the second axis was strongly driven by varying responses to treatments of HWHN, HLHN and HLLN as noted for shoot-root allocation pattern, leaf N content, C: N ratio, root tuber abundance and *A*. In all cases, the SP form exhibited a higher combination of the above-named traits compared to the LP form (Suppl. material 1: Table S3).

Discussion

The two forms of *D. unguis-cati* in Australia were found to display significant differences in traits suggesting they likely occupy different positions in the LES (also see Penuelas et al. 2010; Wright et al. 2004), with the SP form positioned more towards the faster growing, high return on investment end. The observed stronger trait correlation in the SP form implies that this form exhibits a higher level of phenotypic integration than the LP form. High phenotypic integration (Luo et al. 2015) or coordination (Osunkoya et al. 2014) is a trait associated with invasiveness. Phenotypic integration is considered a phenomenon that could constrain non-adaptive phenotypic plasticity in plants (Pigliucci 2003), thereby increasing fitness in heterogeneous environments (Wanderley et al. 2016). As the two forms clearly separated along the 2nd axis in the ordination space of a PCA, our study shows that the two forms have varying patterns of resource acquisition, biomass allocation and carbon assimilation in response to treatments.

The LP form accumulated more biomass when grown under high light and high nutrient resource conditions while the SP form did so under low resource conditions. This suggests that the LP form exhibits traits of an opportunistic invader that effectively exploits extra resources in the environment while the SP form does not. The theory of fluctuating resource availability holds that species that can exploit excess resources have a higher chance to successfully colonize disturbed habitats (Davis et al. 2000). From a performance perspective, our results partly concur with Taylor and Dhileepan (2012) that the LP form has the potential for further spread, particularly under disturbed conditions where increased resource conditions such as light and soil nutrients is common. However, adaptation to low resources has also been shown to be a trait of some invasive species (Funk and Vitousek 2007). Thus, our results imply that the SP form has potential to perform better than the LP form in low resource habitats like undisturbed forest understories. This could partly explain the current extensive distribution of this form when compared to the LP form.

Biomass production and allocation patterns in response to resources

This study supports the context-dependent hypothesis of trait differences (Leffler et al. 2014) as shown by significant interactions of form and treatment in explaining biomass accumulation and tuber development differences between the SP and LP forms. Because biomass production is closely linked to RGR in plants (Malhi et al. 2015), it is often used as an indicator of performance (Luo et al. 2015) or fitness (Osunkoya et al. 2010a). Species with a propensity to be invasive may have similar carbon capturing strategies with less invasive species, but could exhibit trait differences in response to disturbances (Leishman et al. 2010). In our study, the LP form accumulated more biomass than the SP form in response to resources (Table 1 and 2), but this was largely driven by the HLHN condition, suggesting an opportunistic strategy for this form or a plant that has a specialised micro-climate. The SP form developed more biomass than the LP under resource poor conditions of LWHN, which indicates resource substitution by the SP form (Wright et al. 2001; Taylor and Eamus 2008). This finding is in agreement with Taylor and Dhilepan (2012) who reported that the LP accumulated more biomass than SP in a field experiment. This implies that the LP form performs better when growing in disturbed sites with high light and nutrient availability (e.g. river banks) (Davis et al. 2000; Melbourne et al. 2007), whereas the SP form can persist in both stressed (e.g. under canopies) and resource rich conditions (e.g. along exposed river banks). This buttresses the observed greater distribution of the SP form relative to the LP form in SE Australia because it is more capable of preempting and occupying varying niches of resource-rich and poor habitats.

Interestingly, another study showed that SP accumulated more biomass under low nutrient scenarios (Buru et al. 2016a), a trend also supported in this study (see Fig. 1a, treatment HLLN). This context-dependence of trait differences between the SP and LP forms and the significant differences of carbon assimilation between the two forms could explain their differential levels of invasiveness observed in the field (see also Burns 2004; Daehler 2003; Drenovsky et al. 2008). Other studies (reviewed in Funk et al. 2016) have demonstrated that different varieties of a species can exhibit significant trait differences, which is consistent with our findings.

The SP form developed a significantly higher number of tubers than the LP form (Fig. 3), which could have contributed to its reduced shoot/root ratio (Table 1 and 2). This is in agreement with Buru et al. (2016a) who found the SP form to produce more tubers earlier in its development (3–5 months following germination) than the LP form. Tubers act as a sink or storage organs for moisture and photo-assimilates, and they may also regenerate, producing new plants (Janeček and Klimešová 2014; Orthen 2001; Schubert and Feuerle 1997). Apart from seed germination (Vivian-Smith and Panetta 2004), *D. unguis-cati* propagates vegetatively through tubers (Downey and Turnbull 2007; Osunkoya et al. 2009). Horizontal stems and branches trailing along the ground develop adventitious roots at nodes (Vaughn and Bowling 2011), which penetrate the soil and develop more tubers (Osunkoya et al. 2009). If new plants regenerating at the nodal tubers are severed from the mother plant, they grow independently as

genets (Osunkoya et al. 2009). Tubers can also remain dormant for extended periods below-ground as a stress tolerance strategy (Orthen 2001). Thus, this finding of greater linkage between tuber density and biomass gained for the SP form suggests a greater niche pre-emption strategy leading to domination of invaded landscapes by this form (Ashton et al. 2010).

Leaf-level traits response to light, water and nutrients

Although the LP form is known to have broader leaves than the SP form (Shor-tus and Dhileepan 2011; Boyne et al. 2013), our study indicates that the SP form invested more biomass in leaf tissues as indicated by similar specific leaf area (SLA = LA/Leaf dry mass) between the two forms. Heavy investment in constructing leaf tissue (thicker leaves) (Lambers and Poorter 1992) is a trait often associated with slow growing plants (van Kleunen et al. 2010a). High specific leaf area (SLA) facilitates greater capture of light and is often associated with invasive species (but see Garcia-Serrano et al. 2005; Osunkoya et al. 2010a,b). Higher SLA indicates thinner leaves which are cheaper to produce quickly when compared to thicker leaves for the same surface area (Poorter and Remkes 1990). With thinner and broader leaves, the LP form appears to perform better than the SP form in this regard. If we consider the SP form to be the more successful colonizer than the LP form based on current abundance and distribution, then our study does not associate SLA with colonization success for this invasive species.

Developing thicker leaves by the SP form could be a strategy to compensate for less surface area by way of increasing photosynthetic apparatus (palisade parenchyma). Indeed, the SP form has significantly thicker palisade mesophyll tissue than the LP form (JC Buru, unpublished data). This trend also follows suit with our other findings that this form accumulates more biomass in undisturbed conditions where resources are lower. Thicker mesophyll tissues are known adaptations to low light conditions (Chabot and Chabot 1977). The most likely vines to be successful colonizers are those that are adaptable to low light conditions (Baars and Kelly 1996). These are traits exhibited by the SP form.

Physiological responses to light, water and nutrient resources

We found differences in carbon economy between the two forms with the SP form exhibiting higher assimilation rates (A) and WUE than the LP form. In the low nutrient scenario, carbon assimilation was two-fold higher under low light than high light for the SP form. This was accompanied by a greater leaf N concentration at the low light level. The leaf economic spectrum suggests that high A needs more leaf N to drive rapid growth (Wright et al. 2004). Thus, the observed higher A under low light conditions could be a strategy by the SP form to increase growth to reach greater

heights for more light acquisition. Faster growing plants have a larger demand for nutrients (Luo et al. 2015), therefore low C: N ratios found for the SP form may be a consequence of a higher N need.

The LP and SP forms were found to use resources in similar ways, at least under same and/or fluctuating light, water and nutrient resources, as no significant difference was found in their photosynthetic nitrogen use (PNUE). When light conditions were considered separately, a negative relationship between PNUE and biomass gained was obtained for the LP form, an indication of less RUE in this form. However, considering nutrient conditions separately, both forms show a positive relationship between PNUE and biomass gained. Correlation coefficient (r) values were greater in the SP form suggesting that at a given PNUE, a higher biomass was always attained for the SP form relative to the LP form, also indicating less RUE in the LP form.

Previous studies have found non-native invasive species to have higher RUE than native non-invasive congeners (Firn et al. 2012; Funk and Vitousek 2007). In the high light scenario, only the SP form showed positive (albeit marginally significant) relationship between PNUE and biomass gained, while in the LP form the relationship was not significant. This is yet again an indication of better RUE by the SP form than the LP form (Osunkoya et al. 2010b). However, Funk (2008) argues that traits such as PNUE and WUE may not correlate with fitness measures on a short time scale or may reflect a context-dependence of traits differences. Considering all resource conditions, the SP form exhibited better WUE than the LP form (Fig. 3). However, because this trait was significantly associated with biomass accumulation in both forms, it does not necessarily explain their difference in prevalence.

Trait coordination and ordination in response to resources

The traits measured in this study were correlated for each form to assess the extent of covariance among them, which gives an indication of phenotypic integration (Luo et al. 2015; Osunkoya et al. 2014). Consistent with the context-dependence theory, we found significant differences in trait correlations across resource conditions, with the LP form showing significantly higher integration than the SP form (Table 3). This relationship could indicate greater phenotypic integration for the LP form when considering all possible interactions. We thus reject our hypothesis that the SP form would exhibit more trait coordination than the LP form. However, it is interesting that SLA, a trait that facilitates photosynthetic capture was significantly linked to carbon assimilation and biomass accumulation only in the SP form, but not in the LP form.

There was a significant shift of trait integration in favour of the SP form in response to high light and nutrients resources, a result similar to findings of Osunkoya et al. (2010b) while working on a suite of invasive vs. native vines in Queensland including *D. unguis-cati*. This means that the SP form exhibited a higher level of phenotypic integration than the LP form in response to elevated resources only. Some previous studies have suggested that when traits respond to environmental

fluctuations in a coordinated fashion, it enhances plant performance (Reich et al. 2003; van Kleunen and Fischer 2005). A well-coordinated response to environmental heterogeneity enables plants to adapt better to abiotic changes in their habitat (Luo et al. 2015; Osunkoya et al. 2010b; Osunkoya et al. 2014). Based on this argument, the SP form could be expected to perform better than the LP form under similar environmental conditions.

Management implications of study findings

As both forms of *D. unguis-cati* were found to thrive in high resource environments, care must be taken to ensure that disturbances are minimised, especially along sensitive habitats like riparian corridors that the weed invades. Effluent discharge into creeks and riparian habitats of QLD should be monitored and minimised as this might encourage proliferation of this species, especially the opportunistic LP form (see Davis et al. 2000). Current control and management options of both forms include chemical, mechanical and biological control strategies (Dhileepan et al. 2013).

The biological control agents that have been released to control this weedy vine include a leaf mining beetle, *Hylaeogena jureceki* and leaf sucking tingid, *Carvalhotingis visenda* (Dhileepan et al. 2010; Dhileepan et al. 2013). These agents have shown evidence of success in controlling the populations of both the LP and SP forms (Dhileepan et al. 2013). Their feeding behaviour significantly reduce foliage and thus minimise photosynthetic capacity of the weed. Thus, concerted efforts must be prioritised to continually release these agents in large numbers to reduce the rate of shoot growth for both forms, thus keeping their populations within acceptable limits. We suggest that biological control agents that attack tubers in combination with the agents currently in use would be appropriate for this species, especially for the SP form which was found to produce significantly higher number of tubers than the LP form in this study (also see Raghu et al. 2006).

Conclusion

Overall, the results provide support for the context-dependent hypothesis (Leffler et al. 2014). This is at odds with some of our hypotheses in the Introduction section, as we had expected that the SP form would exhibit higher values for most of the traits we measured. The LP form performed better than the SP form when grown under high resource conditions (e.g. high light, high nutrient) whereas the SP form performed well in both high and low resource conditions. Both forms obviously underperformed at low resources, but the negative effects of such stressed conditions were more pronounced on the LP form than the SP form. This indicates that the LP form exhibits traits of an opportunistic plant that is likely to be restricted to disturbed areas characterised by high resource pulses, whereas the SP form can cope more with habitats of both low and high resources. This might explain the greater prevalence of the SP form in Queensland, Australia.

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Supplementary material I

Supplementary tables

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Data type: occurrence

Explanation note: The Supplementary Material for this article consists of three Tables showing traits coordination under high light and high nutrients (Table S1 and S2) and PC loadings for traits used in the PCA that is shown in Figure 4 (Table S3).

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Establishing surveillance areas for tackling the invasion of *Vespa velutina* in outbreaks and over the border of its expanding range

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Abstract

The yellow-legged hornet *Vespa velutina* is an invasive alien species in many areas of the world. In Europe, it is considered a species of Union concern and national authorities have to establish surveillance plans, early warning and rapid response systems or control plans. These strategies customarily require the assessment of the areas that could be colonised beyond outbreaks or expanding ranges, so as to establish efficient containment protocols. The hornet is spreading through a mix of natural diffusion and human-mediated transportation. Despite the latter dispersion mode is hardly predictable, natural diffusion could be modelled from nest data of consecutive years. The aim of this work is to develop a procedure to predict the spread of the yellow-legged hornet in the short term in order to increase the efficiency of control plans to restrain the diffusion of this species. We used data on the mean distances of colonial nests between years to evaluate the probability of yellow-legged hornet dispersal around the areas where the species is present. The distribution of nests in Italy was mainly explained by elevation (95% of nests located within 521 m a.s.l.) and distance from source sites (previous years' colonies; 95% within 1.4–6.2 km). The diffusion models developed with these two variables forecast, with good accuracy, the spread of the species in the short term: 98–100% of nests were found within the predicted area of expansion. A similar approach can be applied in areas invaded by the yellow-legged hornet, in particular beyond new outbreaks and over the border of its expanding range, to implement strategies for its containment. The spatial application of the models allows the establishment of buffer areas where monitoring and control efforts can be allocated on the basis of the likelihood of the species spreading at progressively greater distances.

Keywords

Asian yellow-legged hornet, invasive species, control plans, monitoring, nest distance, predictive models

Introduction

Implementing cost-effective management plans for invasive alien species requires the development of tools that can improve the performance of control activities. A control plan should foresee different stages, including assessment of feasibility, implementation, monitoring and evaluation of the results (Braysher 1993, Bertolino et al. 2005). Monitoring is a necessary step to both verify whether control activities are effective and to provide feedbacks to improve management strategies (Braysher 1993, Bertolino and Viterbi 2010). Prioritisation to support a cost-effective allocation of resources is part of decision-making in species management (McGeoch et al. 2016). When the goal of the management plan is the containment of a species, it is necessary to evaluate where the species is most likely to spread in the short term, in order to better localise control activities. This requires assessing which areas should be surveyed and the intensity of the monitoring activity that should be allocated in each area (Hauser and McCarthy 2009).

Modelling procedures are customarily used to predict the spatial dynamics of invasive species dispersal over time. Models are built by fitting empirical data into mathematical functions or using field data to simulate population dynamics to be spatially projected (Sharov and Liebhold 1998, Gilbert et al. 2004, Shatz et al. 2016). While these procedures represent a powerful tool to provide information to improve management strategies, they require good knowledge about the ecology and dispersal abilities of the target species and are mainly used for simulations at large scales (Hastings et al. 2005).

The yellow-legged hornet (*Vespa velutina* Lepeletier, 1836) is a social wasp, native to tropical and subtropical areas of Indo-China (Archer 1994, 2012). The species established itself in non-native countries such as France (Haxaire et al. 2006), South Korea (Choi et al. 2012) and Japan (Ueno 2014). From France, the species spread to neighbouring countries (Grosso-Silva and Maia 2012, Rome et al. 2013, Bertolino et al. 2016). In Italy, the yellow-legged hornet was detected for the first time in Liguria in 2012 (Demichelis et al. 2014); afterwards the hornet started to spread in this region mainly along the coastline (Porporato et al. 2014, Bertolino et al. 2016) and, in 2017, the species had colonised an area of at least 1,110 km² (Lioy et al. 2018). In Europe, the species is considered invasive, both for its expansion capabilities at European scale (Fournier et al. 2017, Robinet et al. 2017, Barbet-Massin et al. 2018) and the impacts that it could produce by preying on honey bees and native insects (Beggs et al. 2011, Monceau et al. 2013, 2014). Although the presence of the species is not considered a problem for human-health (De Haro et al. 2010), by frequently establishing colonial nests in urban areas, the yellow-legged hornet could generate social impacts due to citizens' perception of fear of possible stings, which could lead to thousands of phone calls from people asking for destruction of the nests (Liu et al. 2015, Tabar et al. 2015, Sumner et al. 2018). Moreover, the management of phone calls and the maintenance of control activities lead to significant economic costs (Robinet et al. 2017). For these

reasons, attempts to control this species have been undertaken in many countries since its early stage of invasion (Monceau et al. 2014, Bertolino et al. 2016, Rodríguez-Flores et al. 2018). Its recent inclusion in the European list of invasive alien species of Union concern (Reg. EU 1141/2016) requires Member states to implement surveillance protocols and control strategies.

The colony of the yellow-legged hornet is initiated by a single inseminated queen that builds a primary nest after overwintering, thus producing the first workers. Afterwards, during the warm season, they enlarge the primary nest or build a secondary nest; with time, nests grow up to a sphere of about 50–100 cm in diameter, containing several thousands of hornets. From September onwards, reproductive animals emerge and mate; in late autumn or winter, all nests die, while newly-mated queens search for a place where they can overwinter and, the following year, they start a new cycle (Archer 2012, Monceau et al. 2014, Rome et al. 2015). As for many other arthropods, invasions may proceed in smooth advances of the main front or in jumps. In the first case, species spread by natural dispersal of animals, giving rise to a diffusion-like process (Suarez et al. 2001). Conversely, jumps usually occur when the dispersal is human-mediated (Hastings et al. 2005, Homans and Horie 2011). In the case of the yellow-legged hornet, this happens usually by the accidental movement of goods (e.g. straw, soil, timber) that contain dormant overwintering queens or by active adults travelling as hitchhikers on vehicles, though long-distance active dispersal could not be excluded in many cases (Marris et al. 2011, Bertolino et al. 2016, Robinet et al. 2017). Human-mediated transportation is hardly predictable and therefore only a large scale monitoring system could allow the rapid finding of new sites of invasions. On the contrary, the natural dispersal could be forecast with observational data of presence recorded year by year. Distances covered by yellow-legged hornets to establish new colonies are not known. Although queens are considered efficient flyers, published studies that demonstrate in the field the flying abilities of new founder queens to disperse from their original colony and create their own colonies are, however, still lacking. Population spread rate has been estimated in some countries and values are non-consistent, suggesting that spread rate could be different case-by-case, for example, depending on environmental and morphological characteristics of the invaded area. Robinet et al. (2017) estimated a mean spread rate of the population of 78 km/year (range between 75–112 km/year) in France, Bertolino et al. (2016) a mean spread rate of 18 km/year in Italy and Choi et al. (2012) a diffusion of 10–20 km/year in South Korea. Sauvard et al. (2018) tested the flying abilities of workers in laboratory conditions throughout flight mill experiments; they demonstrated that workers are able to fly on average from 10 km to 30 km per flight test. This does not mean that workers in the field actually keep these flying values, since, in natural conditions, they are not forced to fly up to their maximum limit. It is likely that queens are also efficient flyers, but is not probable that queens in dispersion will travel to their maximum flight limit, but will probably stop to build their new colonies where they find a suitable spot (cost-benefit behaviour).

Habitat suitability and the possible spread of the yellow-legged hornet in Europe have already been modelled at large scales with different approaches (Ibáñez-Justicia and

Loomans 2011, Villemant et al. 2011, Fournier et al. 2017, Robinet et al. 2017, Keeling et al. 2017). Some of these models have recently been validated and the prediction has proved to be adequate for real occurrence data (Barbet-Massin et al. 2018). However, if large scale modelling (i.e. European level) allows understanding long-term potential distribution of the species, their use in control activities is limited, since control plans are developed locally based on nest dynamics and distribution. A detailed description of yellow-legged hornet nest dynamics has been reported and modelled for a municipality in France (Franklin et al. 2017, Monceau and Thierry 2017); however, the scenario of Andernos, in which the species has established a viable population and reached high-density values, could be different from new invaded areas of other European countries.

Though the fast spread of the yellow-legged hornet in Europe clearly shows that control activities have been generally ineffective, modelling scenarios indicate that increasing the percentage of removed nests could slow down the spread rate (Robinet et al. 2017). Currently, control plans for the yellow-legged hornet are based on finding and destroying the maximum number of nests, ideally all, present in the managed area before the dispersal of the new queens later in the year. Therefore, an efficient monitoring system must be established to locate colonial nests. This should consider not only the present known range of the species, but also an external buffer zone where it is likely that founder queens could disperse and establish new colonies in the short term. Customarily, the monitoring effort is high at the front of a species expansion and decreases with the distance. How fast it decreases is often connected with the species spread rate and human-resource availability. In the case of *V. velutina*, however, an optimal allocation of the effort could be established with information on the likelihood of nests being built at progressive distances from the frontline. With this information, the monitoring effort in an area could be calibrated with the likelihood of dispersal, increasing the cost-efficiency of the monitoring scheme.

The aim of this study is to create an adaptive predictive model of expansion for the yellow-legged hornet, which could be applied in any new invaded areas to both predict the hornet natural expansion and to allocate the available monitoring and control resources, based on species colonisation probabilities. We used data on the mean distances of colonial nests between years to infer the likelihood that queens will naturally spread the year after at a certain distance from the invasion front. This approach allows modelling species spread with no need for taking account of local characteristics (e.g. environmental characteristics, climatic conditions, carrying capacity) in the perspective of establishing early warning and rapid response systems for this species in new invaded areas.

Methods

The western part of Liguria, where many nests are discovered every year, is the main Italian district colonised by the yellow-legged hornet (Bertolino et al. 2016). The species has been detected in this area since 2012: *ì* a male was trapped in Loano at about 70 km from the French border (Demichelis et al. 2014), but no nests were detected in

the following 5 years in this area; *ii*) one hornet was trapped in Ventimiglia at about 2 km from the French border. First nests were discovered in 2013 (Porporato et al. 2014) in some municipalities near France (5 nests in the cities of Dolceacqua, Vallecrosia and Bordighera). The species has also been observed in eastern Liguria, Piedmont, Lombardy, Veneto and Tuscany, but here, observations were scanty and only few nest were reported (Liroy et al. 2018). Therefore, the main colonised area of Liguria has been selected as the study area for the development of the predictive model.

The analysis is based on verified nest positions collected during four years (2014–2017), considering both nests discovered in spring during the foundation phase, which represents a small proportion of the data (2–3% of the total nests discovered in each year) and developed nests discovered later in the season (data available as Suppl. material 6; 2013 nests were not included due to the small dimension of the sample size). Since nests are difficult to detect, in particular before the fall of the leaves, a great effort was dedicated in creating an enlarged monitoring network, including multiple sources of information. Nests were reported by: *i*) citizens and beekeepers; *ii*) firefighters, civil defence teams and local authorities that received reports from citizens; *iii*) a network of more than 1,000 beekeepers with 1,638 monitoring stations established in a wider area of Liguria and Piedmont (Suppl. material 5). Nests were also actively searched for by monitoring teams of the LIFE STOPVESPA project involved in field survey. These teams were *i*) verifying the reported nests, *ii*) verifying the presence of hornets in apiaries and searching for nearby nests and *iii*) actively monitoring the environment, searching for nests also with the use of binoculars. The teams were also active during autumn and the beginning of winter; this allowed the detection of additional nests that might have been hidden by tree leaves in the previous months. The teams' activity was fundamental to discover nests further away from urban areas and not frequented by people. Dissemination activities with hunters and fishermen allowed the involvement of people who frequented different environments, increasing the possibilities to detect nests in natural areas or riverbeds. Data were aggregated by year and analysed with R and QGIS (QGIS Development Team 2015, R Core Team 2015).

For each year, the area, colonised by the yellow-legged hornet, has been estimated by a range analysis, with the kernel method of the R's package ADEHABITATHR (Calenge 2006). The limits of the estimated ranges of each year were used as a starting point to evaluate the areas at different likelihood of colonisation in the subsequent year. Outlier nests, located in Liguria distant from the main colonised area, were treated as potential further source of diffusion in addition to the border of the expanding range.

In a natural diffusion process, queens which found new colonial nests in one year originated from nests of the year before (source sites). The set of these measures can be used as a forecast of distances where the nests could be found the following year. Accordingly, a nearest-neighbour analysis was used to estimate the distances between nests of each year from source sites of the previous years. We then used these measures to develop a probability model of the distances where queens could establish their nests in the following year. From the estimated distances, a probability plot was constructed respectively for years 2015, 2016 and 2017. A non-linear regression analysis was used

to estimate the equations with the best fitting for the data. These equations were used in QGIS to build the model: *i*) a grid with 100 m × 100 m cells was overlapped to the area outside the yellow-legged hornet's range of a single year; *ii*) the distances between the centroids of each cell and the nearest source sites was calculated and the species probability of colonisation for each cell of the grid was estimated according to the previous equation on nests distances from sources. This process was repeated for each year, to create predictive models for years 2016, 2017 and 2018.

Yellow-legged hornet's nests in Italy are not distributed with uniformity along the elevation (Fig. 1). Therefore, the ranges estimated applying the predictive models were clipped at three different altitudes (700, 900 and 1,200 m a.s.l.), thereby producing three different scenarios for each year. The criteria that guided the selection of these limits are: 99% of the nests were found within 700 m a.s.l.; only one nest was discovered at 906 m a.s.l. in Piedmont (Porporato et al. 2014); adult hornets have been reported up to 1,200 m a.s.l.

The predictive models for years 2016 and 2017 were validated comparing the probabilities of colonisation associated with the position of nests (i.e. position of the nest found in that year) for their respective years against the probabilities associated to pseudo-presence data, which are points randomly positioned in the areas of colonisation predicted by the models. A ROC analysis (Fielding and Bell 1997) that allowed the calculation of the area under the ROC function (AUC) was used for the validation procedure (Sing et al. 2005).

To further evaluate the importance of elevation and distance of nests from source sites when modelling the yellow-legged hornet expansion, a generalized linear model (GLM) with binomial distribution and logit link function was used to compare the variables associated with 1,130 points of presence (nests' positions) and 1,130 random points of pseudo-absence. Five variables (one species-dependent and four environmental) were selected as explanatory variables of the GLM: *i*) distance of nests from source sites (nests of the previous year), which is the species-dependent variable that has been hypothesised as the main explanatory variable; *ii*) elevation upon the sea level; *iii*) surface aspect, grouped in the eight corresponding factors of 45° each (north, north-east, east and so on); *iv*) distance between nests and water resources; *v*) land cover (Regione Liguria 2015, 1:10.000). Nine macro-categories were identified for the land cover variable, on the basis of main environmental characteristics of the study area: urbanised, agricultural, woodlands, riparian areas, coastal areas, alpine grasslands, vineyards and olive groves, greenhouses, other environments. GLM results were compared with AIC in order to select the best model. Climate conditions were not considered because they do not change considerably in short distances, while data on carrying capacity, according to habitat suitability, are not available.

Results

The nearest-neighbour analysis highlighted that nests of the yellow-legged hornets were mostly located within short distances from source sites: 50% of nests were found within 203–668 m from nests of the previous years and 95% within 1.4–6.2 km

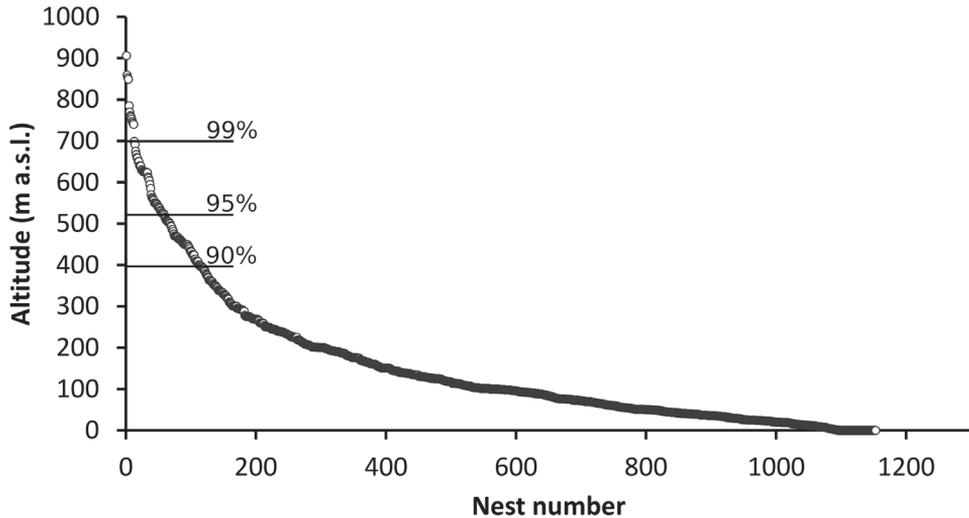


Figure 1. Distribution of yellow-legged hornet nests along the altitude gradient: most of the nests are at low altitude, 90% of them within 396 m, 95% within 521 m and 99% within 699 m a.s.l. Nests were discovered up to 906 m a.s.l.

Table 1. Maximum distance of nests from source sites (nests of the previous years) grouped in proportion intervals for years 2015, 2016 and 2017.

Proportion of nests (%)	Distance from source sites (m)		
	2015	2016	2017
50	668	411	203
75	1,852	864	450
90	3,222	1,637	924
95	6,211	2,633	1,372
100	10,912	11,162	3,513

(Table 1). Few nests were found at greater distances from source sites, up to about 11 km in 2015–2016, but only at 3.5 km in 2017.

The probability of finding yellow-legged hornet nests over the limits of its colonisation range consequently decreases rapidly with increasing distances from source sites (Fig. 2). The trends were explained by logarithmic functions (2015: $R^2 = 0.97$; $F_{1,230} = 7504$; $p < 0.001$; 2016: $R^2 = 0.94$; $F_{1,484} = 7738$; $p < 0.001$; 2017: $R^2 = 0.92$; $F_{1,411} = 4330$; $p < 0.001$).

The spatial application of the probabilistic models, developed to predict the expansion of the yellow-legged hornet in 2016, is reported in Fig. 3 for the three altitudinal ranges. Similar maps for 2017 and 2018 are reported in Suppl. materials 1 and 2, respectively. For each model, the amount of area at different level of probability of colonisation has been estimated in probabilities' intervals (Table 2 for 2016 and Suppl. materials 3 and 4 for 2017 and 2018).

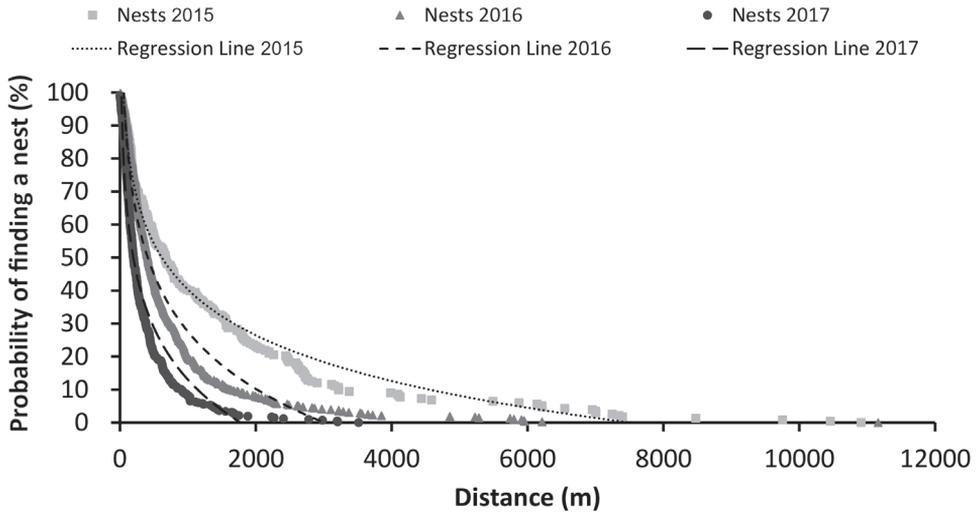


Figure 2. Nests distances from source of diffusion of the previous years: the distance of nests from a possible source of diffusion is given on the x-axis, while the probabilities to find a nest on the y-axis. The lines represent the logarithmic regression models of the data (regression line 2015: $y = -0.2 \ln(x) + 1.785$; $R^2 = 0.97$; regression line 2016: $y = -0.25 \ln(x) + 2.0057$; $R^2 = 0.94$; regression line 2017: $y = -0.227 \ln(x) + 1.6967$; $R^2 = 0.92$).

Table 2. Predictive models of year 2016: areas to be monitored for each probabilities range of colonisation by the yellow-legged hornet. The areas of the three elevation scenarios are reported: A) 700 m a.s.l.; B) 900 m a.s.l.; C) 1,200 m a.s.l.

Probabilities range (%)	Area A (km ²)	Area B (km ²)	Area C (km ²)
90–100	0.04	0.04	0.08
80–90	0.07	0.10	0.16
70–80	0.21	0.23	0.33
60–70	0.30	0.38	0.68
50–60	1.15	1.32	2.16
40–50	3.50	4.04	5.91
30–40	13.97	15.03	19.77
20–30	59.67	68.02	81.47
10–20	220.48	258.38	296.23
0–10	232.61	263.37	283.05
Total	532.00	610.91	689.84

The predictive models for years 2016 and 2017 have been tested with the position of nests actually discovered in those years. Of the nests located in 2016 outside the range of the previous year, 98% were included in the predicted areas of expansion of the two scenarios at 900 m and 1,200 m a.s.l. and all the nests in 2017 were included in the predicted areas of the three scenarios. The analysis of the area under the ROC function highlights a difference between probabilities associated with nests' position

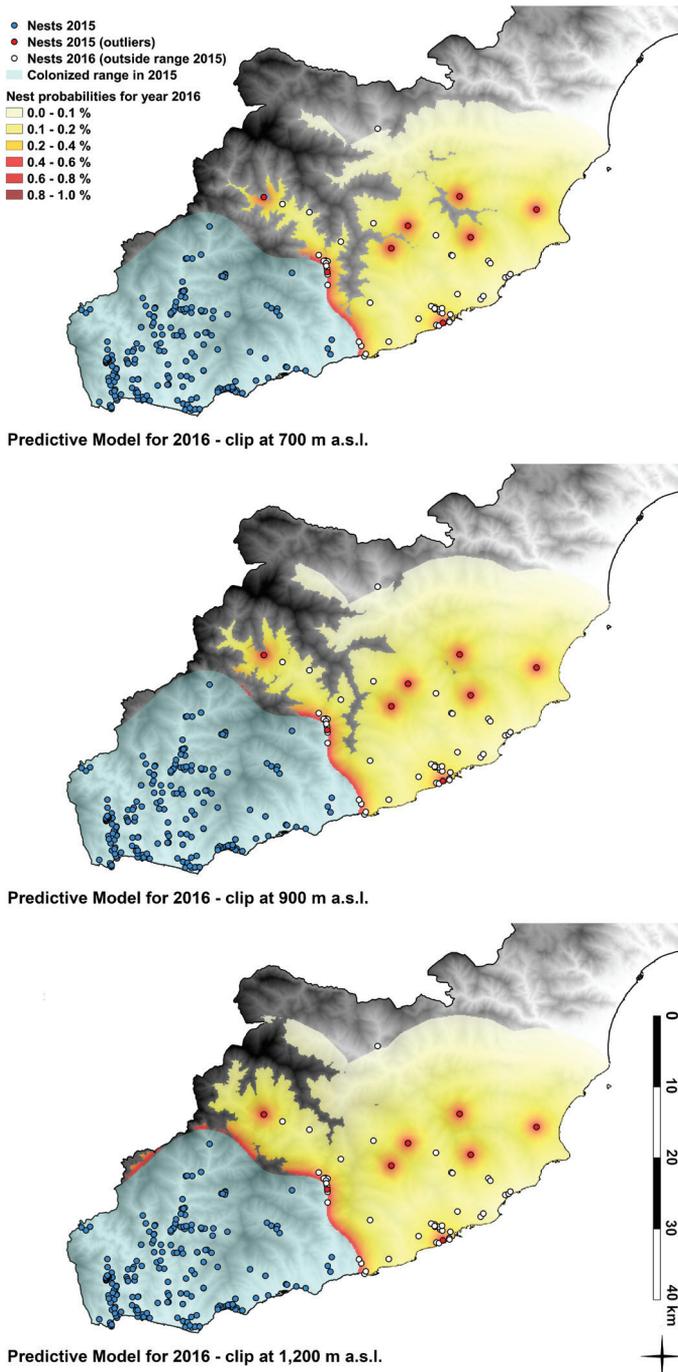


Figure 3. Predictive model of expansion for year 2016 clipped at three different altitude thresholds (700 m, 900 m and 1,200 m a.s.l.). Blue dots indicate nests of year 2015 inside the continuous range, red dots nests of 2015 outside the continuous range. For 2016, only nests outside the 2015 range are reported (white). Coloured areas from red to light yellow indicate progressively less probability of colonisation in 2016.

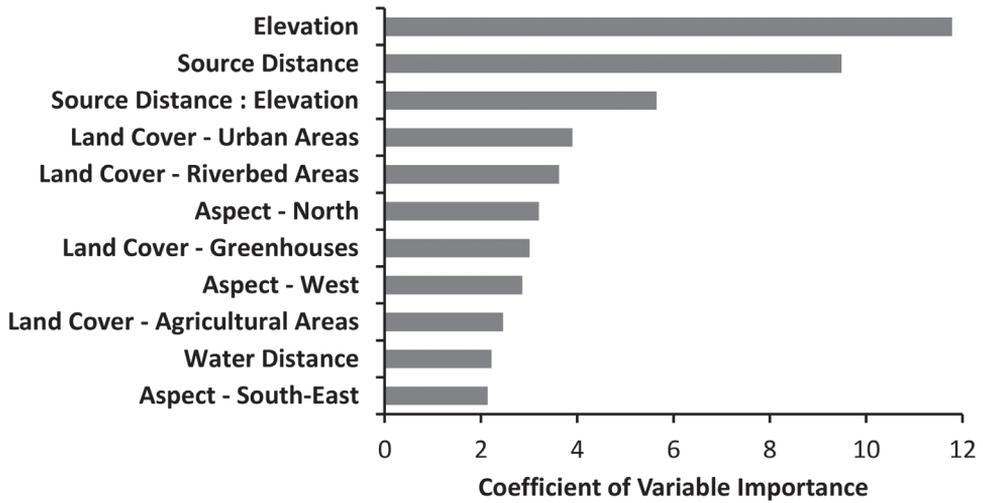


Figure 4. Coefficient scores for the explanatory variables of the GLM analysis on presence/pseudo-absence data: elevation, source distance and their interaction are the variables that contribute more in explaining spatial distribution of nests.

and probabilities associated with pseudo-presence data, therefore each model predicts quite well the spread of the yellow-legged hornet (2016: $AUC_{700\text{ m}} = 0.78$; $AUC_{900\text{ m}} = 0.78$; $AUC_{1200\text{ m}} = 0.77$; 2017: $AUC_{700\text{ m}} = 0.88$; $AUC_{900\text{ m}} = 0.88$; $AUC_{1200\text{ m}} = 0.88$).

The GLM analysis, which better explains the presence of hornet colonies in relation to species-dependent and environmental variables, takes into account all the considered explanatory variables and the interaction between the elevation and the distance between nests and source sites (Nagelkerke's pseudo- $R^2 = 0.60$). The variables that contribute more to the model are elevation, source distance and the interaction between these two variables (Fig. 4).

Discussion

The effective management of spreading invasive species requires the development of monitoring systems able to detect new areas colonised by the species in the short term, in order to timely extend control activities. We developed a system to evaluate the probability of yellow-legged hornet dispersal around the area where the species is present, with a progressively lower likelihood of colonisation by the species at increasing distances. The model was built with GIS software and a database with coordinates of nests located in each year. Measures of the distances of nests found in one year from a possible source of diffusion (nests of the previous year) were used to build likelihood percentages of spread at progressive distances in the subsequent year. Comparison of nest locations with pseudo-presence data confirmed that both altitude and distance from possible source sites were main factors explaining the distribution of nests. Fur-

thermore, our predictive models were tested in two years with real data (i.e. locations of nests found during control activities). In 2016 and 2017, 98–100% of yellow-legged hornet nests were found within the predicted area of expansion, supporting the validity of our modelling approach. With this method, data routinely collected during monitoring and control activities of yellow-legged hornet populations could be used as a feedback to increase the effectiveness of management strategies, allocating the available resources in relation to the probabilities of spread in the short term.

Of the nests reported in Liguria, more than a half were located within 1 km from nests of the previous year, about 90% within few kilometres (0.9–3.3 km) and nearly all within 11 km. These data indicate that new queens, despite their probable great flying ability, mostly build new colonies at short distances from their nests of origin and only few nests will be located at greater distances, due to natural diffusion on long distances or more probably to human mediated transportation. These reduced distances are in accordance with the spread of the species in Italy (18.3 ± 3.3 km/year, Bertolino et al. 2016), which is much lower than in France (78 km/year, Robinet et al. 2017). This means that local characteristics may drive species distribution and expansion; consequently, control approaches should be adaptive to local nest distributions that are a proxy of local characteristics.

The data on nests' distribution collected in these years in Italy suggest that nests are not randomly distributed in the study area, but follows aggregative patterns. This is normal in spreading populations, where areas firstly colonised by the species act as source sites for nearby areas, which are at lower densities. This is the contest where our modelling technique can be used to improve control strategies. On the contrary, areas colonised over many years by the yellow-legged hornet, such as the municipality of Andernos in France, have different local nest dynamics and, after the initial phase of invasion, nests became randomly distributed (Monceau and Thiery 2017). In this French municipality, the species reached a very high density in 2014 of 12.26 nests per km² with an average distance to the nearest nest of 153 m (95% confidence interval 143–163 m). This contest of high densities is completely different from the scenarios of new outbreaks or spreading populations. In the case of established populations, a control strategy that aims to limit or reduce the impact of the species should be developed. In case of new outbreaks or spreading populations, the control strategies should foresee the development of early warning and rapid response systems for early detection of nests or containment plans, as suggested by the EU (Reg. EU 1143/2014) or as performed after the invasion of Majorca in the Balearic Islands (Leza et al. 2018) or Great Britain (Defra 2017). For example, the contingency plan developed for Great Britain requires the establishment of demarcated areas (buffer areas) nearby the sites of invasion after the presence of the yellow-legged hornet has been confirmed. The early warning and rapid response approach supports the need to develop a predictive model of expansion in the short term using data collected locally: the protocol here proposed can be easily adapted and used to increase the efficiency of the monitoring activity. Intensive monitoring and control activities in a buffer area around the range of the species or new invasion outbreaks, allocated considering the different likelihood

of colonisation, might therefore allow cost-effective use of the available resources. In this regard, the situation in Liguria is ideal for developing a control strategy that foresees the identification of buffer areas to monitor with different intensity, because the species is spreading mainly through a corridor along the coastline from West to East, with the sea to the South and mountains that might act as a partial barrier to the North (Bertolino et al. 2016). These characteristics could constrain the spread of the yellow-legged hornet, thus reducing the areas that should be covered and increasing the possibility for effective monitoring. Therefore, morphologic characteristics of the environment should be considered when exporting this approach in other European areas, since monitoring and control effectiveness could be maximised by the presence of limiting factors or could be reduced by their absence.

Arthropods may jump long distances when the dispersal is human-mediated (Hastings et al. 2005, Homans and Horie 2011). An important implication of the possibility for a species to cross long distances is that it can overcome barriers, established to contain the species within the present range. For instance, nests of the yellow-legged hornet have been recorded in Europe, tens and even hundreds of kilometres away from the invasion front, thus suggesting an accidental human transportation of founders (Rome et al. 2009, Bertolino et al. 2016, Robinet et al. 2017). In 2016, only one nest was found in Veneto at about 270 km from the invaded areas in Italy, while, in 2017, some adults were observed at 140 and 170 km, respectively in the eastern part of Liguria and northern Tuscany (Lioy et al. 2018). In previous years, animals and nests were found at several tens and up to 150 km from possible sources of diffusion (Bertolino et al. 2016). Identifying natural dispersal from human-mediated transportation is not always easy. However, even considering some of long distance reports as resulting from natural dispersal would not change the validity of our simulation. In fact, we were interested in building an information system that could help plan the yearly optimal allocation of the monitoring effort, covering an area of possible expansion from the continuous range of the species. Of course, a comprehensive management strategy also requires the development of plans to find and manage sub-populations found even at considerable distances from the expansion front. This is what is usually foreseen in the surveillance protocol of an early warning and rapid response system (Britton et al. 2010, Homans and Horie 2011), a protocol that has been established in Italy by the development of a wide monitoring network with the collaboration of beekeepers (Suppl. material 5). Ideally, such surveillance system should allow the location of yellow-legged hornet nests, established from long-distance dispersal or human-mediated transportation of queens. In case of detection of new propagules, our data-informed process could help in establishing an intensive monitoring network to locate and destroy nests before a new invasion starts, as well as with the use of new technologies as the tracking of hornets with harmonic radars (Milanesio et al. 2016, 2017) or radio-telemetry (Kennedy et al. 2018).

An aspect that must be considered is the bias in nest detection, since tree leaves often hide *V. velutina* colonies. For this reason, a wide monitoring network has been developed, as well as for areas not colonised by the species and for nearby regions and

multiple sources of information have been considered (citizens, beekeepers, firefighter teams, monitoring teams, ...). Monitoring teams also continued to work in the field during autumn and winter, detecting nests that might have been previously covered by tree canopies.

The method here proposed allows the assessment of the proportion of landscape that should be surveyed over the front of the spreading range of an invasive social insect species and the intensity of the monitoring activity allocated at progressive distances. It only requires the availability of nest locations in successive years, which are a proxy of other local (either climate or environmental) characteristics, and can be improved by increasing the efficiency of data collection. This approach is different from other modelling techniques, such as climatic or habitat models widely used for invasive species (Beaumont et al. 2009, Di Febbraro et al. 2016), including the yellow-legged hornet (Ibáñez-Justicia and Loomans 2011, Villemant et al. 2011, Balmori 2015, Fournier et al. 2017, Keeling et al. 2017, Robinet et al. 2017). In fact, these models estimate the areas that could be invaded in the future, comparing climatic or habitat characteristics of such areas with niche requirements of the species, but their use in short-term management strategies is limited. This is because many of these models extrapolate the parameters from other areas with different characteristics or because they are produced at large scales, while species management is usually implemented at more local scales. These approaches are extremely important when the aim is to understand the consequences of invasion in the long term and at European level. Instead, our method estimates the likelihood of colonisation of new areas by the species in the short term, from one year to another and for the studied population, important information that could be used to improve the efficiency of local management plans for the yellow-legged hornet and other similar species that build colonial nests.

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Supplementary material 1

Predictive model of expansion (2017)

Authors: Simone Lioy, Aulo Manino, Marco Porporato, Daniela Laurino, Andrea Romano, Michela Capello, Sandro Bertolino

Data type: statistical data

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Supplementary material 2

Predictive model of expansion (2018)

Authors: Simone Lioy, Aulo Manino, Marco Porporato, Daniela Laurino, Andrea Romano, Michela Capello, Sandro Bertolino

Data type: statistical data

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Supplementary material 3

Areas to be monitored for the predictive models of year 2017

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Data type: statistical data

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Supplementary material 4

Areas to be monitored for the predictive models of year 2018

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Supplementary material 5

Monitoring network developed by LIFE STOPVESPA project in Liguria and Piedmont regions (Italy)

Authors: Simone Lioy, Aulo Manino, Marco Porporato, Daniela Laurino, Andrea Romano, Michela Capello, Sandro Bertolino

Data type: statistical data

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Supplementary material 6

Database of *Vespa velutina* nests discovered in Liguria region (Italy) in the period 2013–2017

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Data type: statistical data

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Assessment of social perception of an invasive parakeet using a novel visual survey method

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Abstract

The perceptions of the general public regarding invasive alien species (IAS) are important in the prevention of future invasions and the success of management programmes. Here we use a novel visual method to investigate the perception of a charismatic IAS, the rose-ringed parakeet, across different stakeholders in Seville, Spain. Respondents were asked to select images of 10 bird species they would like to have present in their surroundings, out of 20 available images, including the parakeet and three other non-natives. This makes the survey easy, fast to take and attractive to potential participants, while prior and potentially biasing information of survey goals is minimised. Although more than 95% of the respondents recognised the parakeet, at least up to family level, only 34.8% selected it. Selection rates were even lower for three other IAS and even more so when the status of non-native species was indicated next to the images, suggesting that a social norm against IAS may be established. To validate our novel visual approach, we also assessed perception via a traditional questionnaire and the results of the two survey methods coincided. Finally parakeet selection differed importantly amongst pre-defined sectors of the public and people who had prior experience with the parakeet selected it less frequently (e.g. farmers, park managers). These results highlight the importance of studying different stakeholders to get the full picture when considering IAS management programmes. Our new visual survey method can thus serve as an excellent and user-friendly tool to study people's perceptions regarding charismatic IAS and facilitate well-informed and sensible decision-making.

Keywords

Invasive species, urban ecology, wildlife management, public attitudes, *Psittacula krameri*, rose-ringed parakeet

Introduction

Invasive alien species (IAS) are recognised as being one of the major threats to biodiversity and represent a globally significant and rapidly growing economic cost (Tollington et al. 2017). Effective policy and management responses to the multiple threats posed by IAS are thus essential. However, taking action can be constrained by public objection, especially for charismatic species. Different groups of people can have considerably different social and ethical values, perceptions and knowledge about an ecological issue such as IAS, often resulting in conflict about whether active management such as removal or eradication is appropriate (García-Llorente et al. 2008, Webb and Raffaelli 2008). Public attitudes differ with respect to the type of species in question and the type of management proposed (Fraser 2006). This variation can be related to socio-demographic factors, for example, the types of job people have or the social group they belong to (Vanderhoeven et al. 2011) and people's awareness of impacts (Bremner and Park 2007, Sharp et al. 2011). In addition, social benefits (such as hunting) and other cultural associations can influence attitudes (White et al. 2011). There are also moral arguments surrounding the distinction between native and non-native species (Simberloff 2003) and the potential conservation benefits/detriments of each (Schlaepfer et al. 2011) that can influence people's attitudes. Other factors influencing social perception of invasive species include the taxonomic group the species belong to (Fraser 2006), the type of impact they cause (Fulton et al. 2004) and the severity of their impacts (Reiter et al. 1999).

It is increasingly recognised that the issue of management of invasive non-native species is as much a social issue as it is a scientific one (Reaser 2001, Verbrugge et al. 2013), encompassing political and human factors. Politics and society are part of the management of nature and the support of the general public can be important, since carrying out management may go against the interests of some citizens or pressure groups, which can have negative consequences for the success of the management actions (Bertolino and Genovesi 2003, Crowley et al. 2019). In the case of IAS, the attitude and involvement of the public can be relevant, both for prevention of future invasions and for the success of control or eradication, if needed. However, assessments of the attitudes and perceptions of the general public toward IAS is not often part of the deployed methodology in the management of a biological invasion (Campbell et al. 2015, Dawson et al. 2015). This may, in part, be because invasion biologists focus on compiling evidence on impacts and spread of IAS and on understanding invasion mechanisms. Such information is essential for promoting informed policy, but even persuasive evidence may not be sufficient in influencing values, attitudes and behaviour of people (Clayton and Myers 2009, Courchamp et al. 2017). In addition, conservation or invasion biologists, who are interested in implementing policies to manage IAS, may lack information on the application of suitable and easy methods to collect such social data. They may also be concerned by the outcomes of such analysis, as it can highlight complexities and trade-offs, but these considerations can be essential in prompting effective management and policy.

In this study, we set out to gather information on the perception of the public regarding one charismatic non-native species, the rose-ringed parakeet (*Psittacula krameri*)

in the metropolitan area of Seville (Spain). This parakeet has been introduced from Asia and Africa, establishing approximately 90 populations in 10 European countries (Pârâu et al. 2016). Several studies already demonstrated that rose-ringed parakeet can have both ecological and socio-economic impacts, notably outcompeting local species, damage to agriculture and noise pollution near roost site, but their impacts are often restricted to certain populations and some ecoregions (Turbé et al. 2017). Specifically in Seville, there is strong evidence that parakeets attack, displace and even kill an endangered bat species, the Giant noctule (*Nyctalus lasiopterus*), due to competition over tree cavities for roosting and reproduction (Hernández-Brito et al. 2018). Similar concerns exist with respect to the vulnerable Lesser kestrel (*Falco naumanni*, Hernández-Brito et al. 2014). Evidence from Seville and other Mediterranean countries also demonstrate that parakeets can cause economic and social impacts, as they feed heavily in agricultural areas and roost in large groups in urban localities, producing localised pollution by noise and faeces. On the other hand, the parakeet is a colourful and potentially attractive bird that may add a feeling of wildlife and something exotic to the urban parks where it spends much of its time. Indeed, plans to reduce its population via active management have attracted fierce opposition and, because of this, management has yet to be undertaken. Hence, we hypothesised that the perception of the parakeet could vary considerably amongst different segments of the public, depending on their specific exposure to the parakeet.

Written questionnaires are a common way to study such queries, but the wording and complexity of questions and response bias towards positive normative answers could have large effects on the results (Filion 1981, Paulhus 1991). Additionally, the willingness of people to collaborate in written questionnaires is often low and may be biased (White et al. 2003). To avoid these issues, we developed a novel and visual questionnaire that can cope with those issues presented above and can be used in various conservation contexts. As people often value organisms through subjective criteria such as their aesthetics and usefulness, a more visual approach could be useful to study biodiversity perceptions (Bayne et al. 2012, Shwartz et al. 2013a, Lindemann-Matthies 2016). Hence, our objectives in this study are threefold: first, quantify any differences in the social perception of the parakeet amongst pre-defined parts of the general public; second, to test the usefulness and reliability of a simple novel visual tool in assessing public perceptions on IAS and finally, understand to what extent perceptions change when people are informed or reminded that the parakeet is non-native. This question is important in establishing to what extent efforts to increase awareness to IAS can result in a social norm with respect to non-native species that can influence attitudes and behaviour regarding IAS.

Material and methods

Novel visual tool and questionnaire design

We conducted structured interviews, in which different members of the public who use parks and green spaces in Seville filled-up a close-ended questionnaire, to test the

variation in the perception of the parakeet amongst different stakeholders. We specifically targeted predefined groups which *a priori* we believed might have different perceptions and attitudes towards the parakeet. For the first part of the survey, we developed a plate with 20 images of twenty species of birds present in the parks and gardens of the metropolitan area of Seville, including the rose-ringed parakeet. We then asked each respondent to choose ten of these twenty birds which he or she would like to have present in the environment in which the survey was done. We expect that if the parakeet is perceived more positively, it will be included in this set of 10 species with a greater probability. By not asking anything specific about the parakeet, we avoid the risk of people providing biased responses towards the parakeets or other species.

The selection of species, size, quality and position of the bird images can influence the choices of participants. We therefore carefully selected both colourful species and species with single or dark colours and ensured that our selection represented different functional groups of bird species. To avoid biases with respect to visibility or conspicuousness, we decided to depict species with different sizes to more or less the same size on the plate. We also developed three different plates using three different images for each of the same 20 species: one in which all of them appeared with muted and rather unimpressive colours, one intermediate and another with bright colours. When we did not find three suitable images for some species, we adjusted the contrast and brightness of an image to obtain the desired effect (see Suppl. material 1: Figure SM1). With these three types of images we made plates with duller, intermediate or brighter images. Next, the location of a species in each plate was selected randomly, so that in each plate they appeared in a different position (also in Suppl. material 1: Figure SM1). With this approach, we tried to avoid effects in the selection of the parakeet due to its position in the plate or due to the appearance of the parakeet in any particular image, thereby aiming to obtain more general results.

We included four non-native species, including the rose-ringed parakeet, in each plate and to test whether there is a social norm that acts against non-native species, we made two versions of each of the three different plates presented above, where one version indicated the non-native species by placing the text non-native next to it (see Suppl. material 1: Figure SM2). We expected that people would be less inclined to select a non-native species in their set of 10 preferred species if they think that non-natives have negative effects, whereas they may be more inclined to select them if they think non-natives have positive effects, like contributing novelty.

In order to confirm the validity of this novel visual tool, we asked respondents about their perception of the parakeet using a more traditional question-based survey. In the second part of the survey, we explored attitudes towards the rose-ringed parakeet using a modified version of the companion animal scale (Poresky et al. 1988) (see Suppl. material 1: Figure SM3). This scale was developed for measuring attitudes towards pets, with several items assessing attitude dimensions that would not be expected in human-wildlife relationship, for example loving to not loving, trusting to fearful (Perry-Hill and Prokopy 2014). We therefore reduced the scale's original 18 measurement items down to six, which were suitable for the rose-ringed parakeet and added

five additional adductive pairs: harmless to dangerous, useful to plague, silent to noisy, abundant to rare and muted colour to colourful (following Perry-Hill and Prokopy 2014). Altogether, respondents were asked to indicate the number that best described their opinion for each attitude item, for example, unpleasant to pleasant, clean to dirty, along a 7-point scale, ranging between the positive and negative adjective. To avoid automated answers without paying too much attention, we inverted the order of the 11 adjectives, such that negative adjectives could be either on the left or the right side of the scale that were re-aligned later for data analysis. We expect that if people have a positive opinion about the parakeet, they would score towards the positive adjectives. This was then used to validate the visual approach, by exploring whether people who gave high scores to the parakeets also tend to select the parakeet in the set of ten species in the visual survey. Finally, with this set of characteristics, we can also test which ones contribute most to variation in social perception.

Study design and data collection

The survey was conducted with five pre-defined groups of people between 26 May and 19 June 2014 during two daily periods (8:00–13:00 h and 18:00–20:30 h) in order to test whether social perception of the parakeet depends on prior experience and potential effects of parakeet presence. These groups were: (1) people who live near the roost sites of the parakeets; (2) visitors of parks with parakeets; (3) visitors of parks without parakeets; (4) farmers/agriculturists/gardeners with crops near the city; and (5) people who work in parks with parakeets (gardeners, waiters, street vendors etc.). We carried out between 50–60 surveys per group. In the case of visitors of parks, we selected these people in an unbiased way by inviting every third person encountered to carry out the survey. When the third person was less than 16 years old or someone who was not living in the area, such as foreign tourists or visitors from other cities, we again took the third person encountered. In the case of park workers and farmers, due to the limited numbers of people available in these groups, the surveys were taken with all suitable subjects encountered. To interview visitors of parks without parakeets, we selected parks without parakeets in Seville, as well as parks in towns nearby the Seville metropolitan area in which the parakeets at the time of survey did not breed and can be observed only occasionally. The surveys with visitors of parks with and without parakeets and park workers were each realised in six different parks in order to avoid specific location effects on the results. The surveys with the group of people living close to the roost were realised only in the neighbourhood “Tablada”, since this is where the main parakeet roost was located. Finally, we conducted the surveys with the group of farmers/agriculturists in different urban community vegetable gardens and crop fields around the city.

After the visual part of the survey, we asked respondents three questions while pointing to the image of the parakeet to evaluate the level of knowledge and personal experience of rose-ringed parakeet: (1) Do you know this bird? (2) Could you indicate its name? and (3) Have you seen this bird here? We then used the modified version of

the companion animal scale (Poresky et al. 1988) to explore the attitudes towards the rose-ringed parakeet. In the last section of the questionnaire, we collected socio-demographic information, which might also influence social perception and, moreover, which might differ between our pre-defined groups. We recorded information about gender, year of birth, the childhood environment (town, small city or large city), the current environment (same categories) and the last educational degree that has been achieved (up to secondary school graduate, Spanish *Baccalaureate*, intermediate level professional training, superior level professional training, university degree). These data were recorded and stored without the identity or any contact details of the respondent and hence participation was completely anonymous. Participants were informed verbally about the broad aims of the research and chose whether they wanted to answer the questionnaire. Our research activities fall within the scope of categories exempt from IRB approval. Finally, before conducting the survey, we piloted the questionnaire with 35 participants in order to explore the wording and internal validity of the questions used.

Data preparation and analysis

All analyses were done in the R environment (R Core Team 2008). We first confirmed the reliability and consistency of the scores given by the respondents for the 11 items by measuring the attitudes towards the rose-ringed parakeet. Since the responses people gave may be due to one or several underlying dimensions or latent factors that reflect their opinions, we performed a factor analysis of the responses, using the *psy* package (Falissard 2012). We produced a scree plot of the eigenvalues of the 11 possible factors, with an overlaid distribution of random factors based on 1,000 simulations assuming no correlation structure in the data. We only retained factors with eigenvalues greater than the random expectation. The responses to this questionnaire reflected a single latent factor. Only the first factor had an eigenvalue (5.8) clearly higher than the randomly generated first factor (range: 1.2–1.5); the rest of the factors had eigenvalues equal or lower than randomly generated factors. All its factor loadings were positive and mostly large (Table 1). After verifying internal consistency (i.e. whether respondents answer similar questions in similar ways: Cronbach's $\alpha = 0.9$; values larger than 0.7 are typically taken to indicate internal survey consistency, Rattray and Jones 2007), we summed the scores given by the respondents for each of the 11 items to create a new variable called '*attitude*'. Since all questions were first ordered in the same direction from negative to positive, high attitude score represents positive attitudes towards the parakeet.

To explore which variables determined whether the rose-ringed parakeet was included into the set of 10 preferred bird species, we performed Generalised Linear Models using the binomial error distribution and a logistic link function (MuMIn package, Barton 2017). The main effects (explanatory variables) of interest were the five different groups of people and the availability of information on non-natives. We also tested

Table 1. Exploration of why parakeets are selected in the visual survey. Loadings of each variable on the first and only latent factor of our second, question-based survey (ordered from high to low; 47.9% of the variance captured).

Variable	Loading
Bad/good	0.88
Harmful/harmless	0.83
Worthless/valuable	0.83
Plague/useful	0.81
Unpleasant/pleasant	0.79
Dirty/clean	0.71
Friendly/not friendly	0.67
Noisy/silent	0.56
Abundant/rare	0.54
Ugly/beautiful	0.43
Muted colour/colourful	0.34

for the additional and potentially confounding effects of socio-economic factors, prior knowledge and experience with the species and the type of plate. We therefore fitted a full model using as fixed effects: group (5 categories), information on non-natives (yes or no), gender (male or female), decade of birth (6 categories), childhood environment (3 categories), current environment (3 categories), last educational degree (5 categories), familiar with species (yes or no), experience with species (i.e. whether respondent had previous interaction with the species; yes or no) and type of plate (3 categories). Similarly, we built additional linear models (with Gaussian error structure) to determine the relationships between the above mentioned independent variables and the attitudes toward the rose-ringed parakeet, using 'attitude' as the dependent variable. We also fitted a simple generalised linear model with binomial error structure to explore the relationship between the two different types of attitudes variables measured, the visual and the written questionnaire, with the selection of the parakeet (visual) as the dependent variable and the 'attitude' score as the only independent variable.

We used the R package MuMIn (Barton 2017) to determine the effects and importance of each explanatory variable. Applying this package, we constructed and ran all possible models using subsets of variables, in this case, 512 models, ranked these by Akaike's information criterion corrected for small sample size (AICc) and calculated the Akaike model weights (w_i) (Burnham and Anderson 2002). We then obtained for each variable their model-weighted parameter estimates (Burnham and Anderson 2002). We also obtained the relative importance of each variable by summing the Akaike weights (w_i) of the models in which each variable was included. As a rule of thumb, an importance >0.5 corresponds to roughly a p-value <0.05 (Shwartz et al. 2013b). We think this model-averaged and information theoretic approach gives a much more integrative overview of the variable's effects than the results of a single model, especially for models including many explanatory variables such as ours.

Results

Altogether, 276 people participated in our survey and were distributed across the experience groups as follows: 54 workers in parks, 60 visitors in parks with and 60 in parks without parakeets, 50 participants living near the roost and 52 farmers.

People generally recognise the parakeet but personal experience varies

Across the survey, 80.1% of the respondents indicated that they knew the rose-ringed parakeet. The vast majority of workers in parks (90.7%), people who live near the roost (88.0%) and farmers (84.6%) said they knew the parakeet. Although the percentages of all groups were high, always exceeding 50%, only 78% of the visitors of parks without parakeets and 62% of the visitors of parks with parakeets, indicated that they knew the species. When asked for the name of the bird, none of the respondents gave an answer that indicated they misidentified it as a parakeet: all people mentioned names that are associated with the family Psittacidae (Parrots). Almost half (52.9%) said parrot, 18.5% said parakeet -without saying the complete name- and 11.2% said names of other parrots such as macaw or lovebird. Only one person named the species correctly and a very small fraction (4.7%) chose the option 'Do not know/ Do not answer'. A total of 56.9% of the respondents replied that they had seen the species before. Workers and people living close to the roost obtained higher percentages (87.0% and 80.0%, respectively); visitors of parks without parakeets obtained the lowest percentage (17%), as expected.

Preference for parakeets is generally low and differs between groups of people

In the visual survey, 34.8% (out of N=276) of participants chose the parakeet as one of the 10 preferred birds. Since random choice would yield on average 50%, this lower percentage indicates an overall aversion towards the parakeet. There were notable differences amongst the groups of people in this respect. The group that chose the parakeet most was the visitors of parks with parakeets (53.3%, out of N=60); this was the only group in which the parakeet was selected by more than half of the respondents. However, after controlling for social and demographic differences between groups, no group chose the parakeet more than half of the times (Figure 1). The groups which selected the parakeets least were farmers (17.3%, out of N=52) and neighbours living close to the roost (22.0%, out of N=50). The group of workers in parks selected it 37.0% of the times (out of N=54). These group differences are statistically important, even when controlling for potentially confounding social and demographic variables (Table 2). With respect to these, gender was important: men selected parakeets less frequently than women. Finally, people who had seen the parakeet before had a reduced probability of choosing the parakeet.

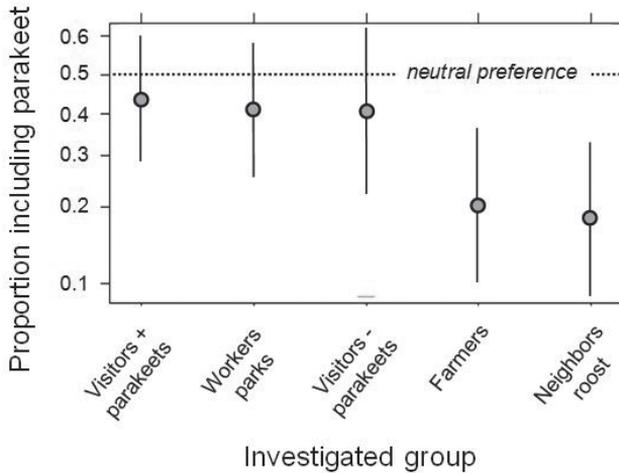


Figure 1. Differences amongst people in their response towards parakeets. Proportion of individuals per pre-defined survey group that included the parakeet into their list of 10 preferred birds, ordered from high to low (and corrected for all variables included in the statistical analysis, see Table 1). Since people could choose 10 out of 20 birds on offer, a neutral preference would result in a 50% probability of inclusion (dotted line).

A mild but generalised disliking of non-natives

Providing information that the parakeet was non-native did not importantly change its probability for selection, although it did decline (Table 2). In addition, for the other three non-native species, we found similar and often much larger declines in their probability to be chosen when it was indicated that they were non-native: selection rates of the red avadavat (*Amandava amandava*), rock dove (*Columba livia*) and common waxbill (*Estrilda astrild*) were reduced by 23.8%, 6.82 % and 13.0%, respectively. In accordance, in separate models for each of these species, information on being non-native was always an important contributor (importance estimates for each species: 1.00 ($\approx p < 0.001$), 0.59 ($\approx p < 0.05$) and 0.81 ($\approx p < 0.05$), respectively).

The two different survey methods give very similar and meaningful results

There was no effect of which plate people used in the visual survey, suggesting the results reflect true preference. In accordance with this, the GLM results to explain the results of the visual survey were very similar to the results for the variable 'attitude', both qualitatively, ranking and sign and quantitatively, relative importance (Table 2). In addition, a model explaining the results of the visual survey with the attitude values had an AIC value that was 167.7 points lower than a model that only included the intercept, i.e. the attitude values predicted the probability to choose the parakeet.

Table 2. Pattern of covariance amongst the 11 attitude items. Effects of different characteristics of respondents on their probability to include the rose-ringed parakeet in the set of 10 preferred birds in the first, visual survey (“*Parakeet chosen*”), ranked by their relative importance in the models. Estimates (and their standard errors) given are the untransformed and model-weighted coefficients from all possible binomial regression models; relative importance of each variable is the sum of the Akaike weights (w) of the models in which each variable was included. As a rule of thumb, an importance >0.5 corresponded to roughly a p-value <0.05 (*) and importance >0.95 to $p <0.01$ (**) (Shwartz et al. 2013b). In the two right-most columns the same is provided, but then for the summed attitude score towards the parakeet from the second, question-based survey (“*Attitude*”). Note the similarity in ranking, relative importance and sign of the estimates between the two different survey methodologies. (Reference levels for the variables were as follows: gender=female, experience with parakeet=no, group=visitors to parks without parakeets, current environment=large city, information available on non-native=no, familiar with=no, childhood environment=large city, plate used=bright, last diploma=Spanish baccalaureate, year of birth=1930–1949).

Variable	Parakeet chosen		Attitude	
	Estimate (SE)	Importance	Estimate (SE)	Importance
Intercept	0.80 (0.65)		60.5 (3.06)	
Gender (male)	-0.81 (0.34)	0.96 **	-5.72 (1.44)	1.00**
Experience with parakeet? (yes)	-0.90 (0.41)	0.94*	-9.35 (1.81)	1.00**
Group (farmers)	-0.63 (0.62)	0.85*	-4.37 (2.47)	1.00**
“ (roost)	-0.68 (0.73)		-8.39 (2.83)	
“ (visitors parks with parakeets)	0.29 (0.61)		0.75 (2.62)	
“ (workers)	0.20 (0.63)		-3.66 (2.77)	
Current environment (small city)	-0.95 (1.03)	0.58*	-1.47 (3.17)	0.29
“ (town)	-0.43 (0.50)		-0.77 (1.62)	
Information available on non-native (yes)	-0.06 (0.17)	0.30	-0.02 (0.65)	0.25
Familiarity with parakeet? (yes)	-0.07 (0.26)	0.30	-1.28 (1.93)	0.47
Childhood environment (small city)	-0.15 (0.41)	0.25	-0.70 (1.81)	0.23
“ (town)	-0.11 (0.25)		0.08 (0.77)	
Plate used (dark)	-0.03 (0.14)	0.13	0.09 (0.61)	0.13
“ (intermediate)	0.004 (0.12)		0.14 (0.67)	
Last diploma (university degree)	-0.04 (0.18)	0.07	-0.24 (1.00)	0.08
“ (professional training superior level)	-0.001 (0.13)		-0.17 (0.91)	
“ (professional training interm. level)	0.0005 (0.09)		-0.18 (0.77)	
“ (primary/secondary school graduate)	-0.04 (0.19)		-0.26 (1.05)	
Year of birth (1950–59)	0.0004 (0.07)	0.01	0.20 (1.47)	0.17
“ (1960–69)	-0.003 (0.07)		-0.41 (1.62)	
“ (1970–79)	-0.002 (0.07)		-0.004 (1.30)	
“ (1980–89)	0.0006 (0.07)		0.32 (1.54)	
“ (1990–99)	-0.004 (0.09)		0.64 (2.23)	

Discussion

Recently, Courchamp et al. (2017) summarised the key problems and possible solutions in the field of invasion biology. They argued that “one major impediment to the support of studies and action in biological invasions is the huge sympathy capital for many IAS” and especially the ones that are beautiful and cute. Results from a few reports and other anecdotal evidence support this claim for alien parakeets. For instance, in Europe, three surveys conducted in France and Germany have identified that the majority of respondents find the rose-ringed parakeet attractive and like to see them in gardens and parks (Scalliet 1999, Wegener 2004, Wolff and Touratier 2010). These

attitudes and perceptions may cause public objections towards any attempt to manage problems posed by charismatic IAS (as is currently the case for the parakeet in Seville, where the political decision to eliminate the parakeet was subsequently halted by animal protection groups). In the search of a solution, Courchamp et al. (2017) suggest to change the science communication paradigm in invasion biology from the deficit model, which postulates that knowledge transfer to the public will convince the latter about the importance and reliability of the issue, to the dialogue model, which calls for open interchange with the public. Our study embraces and facilitates this approach by developing a simple and novel visual tool for surveying people's attitudes towards charismatic alien species. Using this novel methodology, we find that the parakeet is not overly preferred compared to other local species, but that attitudes may vary strongly between different stakeholders. Importantly, preference appears related to the type and degree of exposure to parakeets.

Social perception of an invasive parakeet: variation amongst people

A few recent studies have highlighted a lack of ecological knowledge in identifying the names of common species in urban areas (reviewed by Pett et al. 2016). Our results demonstrate that this was not the case for the rose-ringed parakeet, which the vast majority of participants were able to identify correctly. Nonetheless, the majority of participants (65.2%) did not choose the parakeet. This suggests that the species is less popular than the average bird species depicted on the plates we used (see Suppl. material 1: Figure SM1) and that there is general aversion towards the parakeet. Similar results were obtained in Paris (France), where the rose-ringed parakeet was only placed in 8% of the gardens people designed, ranking 29th out of 32 species proposed (Shwartz et al. 2013a).

The popularity of the parakeet differed systematically amongst participants. In both the visual survey and the question-based survey, men were less likely to include the parakeet. Other studies have identified some mixed results regarding the effect of gender. For instance, men provided more accurate estimations of the richness of birds, flowers and insects in urban gardens (Shwartz et al. 2014), but included fewer animal and flower species in ideal gardens they designed (Shwartz et al. 2013a). While we do not have a clear explanation for this, it is known that there are fundamental differences in the ethical perceptions, values and attitudes of men and women on issues such as wildlife control and ecological problems (Lauber et al. 2001, Dougherty et al. 2003, Bremner and Park 2007). This might have played a role, together with other differences in perceptions and preferences between men and women (e.g. women place greater importance on aesthetics, see Lindemann-Matthies 2016). Therefore, in any kind of survey, gender of the respondent is a factor that needs to be taken into account, both in design or analysis and interpretation.

Another effect in both surveys was that people, who had seen the parakeet before, had a more negative opinion. These results somewhat contradict recent studies that demonstrate how interaction with charismatic species, notably mammals and even dangerous ones, can yield positive attitudes towards their conservation (Bjurlin and Cypher 2005, Bruskotter

et al. 2017, Shwartz et al. 2013a). A possible explanation for our result may be that prior information about the parakeet and its impacts (e.g. coverage in the media) increase both a negative attitude towards the parakeet and the probability that it is detected/recognised. This may be especially true for stakeholders potentially exposed to negative impacts by the parakeets, like farmers, people who live close to the roost and park managers, which were not covered by the studies mentioned above which focused on the general public.

In fact, one of the aims of our survey was to test if there are differences in the social perception of the parakeet amongst pre-defined parts of the general public. Indeed we found, in both types of surveys, that the perception of the parakeet was different amongst our groups, even after statistically controlling for socio-demographic variables. This may be due to the type of interactions that these groups have with the parakeet, as such a result has also been found in other studies that included groups with diverse types of interaction with the subject of study (García-Llorente et al. 2008). Visitors to parks with and without parakeets had a more favourable attitude towards the parakeet. These people only go to the parks infrequently and do not necessarily have negative or any interaction with the parakeets. Some visitors may like to have parakeets in the city and had an informed favourable opinion about them. In contrast, people involved in agriculture and those living close to the roost had a more negative attitude towards the parakeet. These people probably have had more interactions with the parakeets and may have been exposed to or know about negative impacts, such as damage to agricultural crops and pollution with noise and faeces (Bremner and Park 2007, Canavelli et al. 2013). Overall then, the perception of the parakeet appears to depend on the level of interaction with and knowledge about the species; people who suffer the risk of the noise, dirt or damage from the parakeets have a worse opinion about the parakeets and in majority do not want parakeets in their city or town when given a choice. As the species becomes more common and is affecting more people working in agriculture and living close to one of the roosts, it is likely that the average attitude will only become more negative in the future. This could provide a major argument in the open discussion with the public, when considering early action against establishing non-natives.

Such information about heterogeneity in public opinion is crucial when employing the dialogue model to management (Crowley et al. 2016, Courchamp et al. 2017). First, it is important that all relevant stakeholders are engaged in the discussion. In addition, however, it is important that different stakeholders sections are informed about each other's opinion and the reasons behind them. This may reduce "human-human" conflict which results from conflicting values and interests of different sections of the society (White and Ward 2010) and thereby promote consensus across sections, i.e. a beneficial interaction between the dialogue and the deficit model.

The results of the Factor Analysis indicated that there was only a single underlying latent factor representing the responses to the question-based survey. However, the variables reflecting the parakeet's aesthetic characteristics were not very correlated with this latent factor as they had the lowest factor loadings. Hence, the general opinion is dominated by characteristics that we could call more pragmatic characteristics, for example bad/good, harmful/harmless, worthless/valuable, plague/useful etc. Indeed,

when talking to respondents after the survey was concluded, they often commented on the use or role of parakeets in nature and in urban and rural communities, instead of on their aesthetic characteristics.

Suitability of the visual survey approach to assess social perception

Several studies have highlighted the usefulness of adopting a visual approach when studying people's preference for nature or biodiversity (e.g. Bayne et al. 2012, Shwartz et al. 2013a, Lišková and Frynta 2013), but this has only rarely been done for invasive species (Lindemann-Matthies 2016). Here we used a rather novel approach to investigate people's perception of a focal non-native species, by presenting an image of it mixed with those of other species and then asking people to select a fixed-number subset of species they prefer. This design has several advantages. First, we did not ask people to somehow qualify the images according to some of our pre-established criteria, but to just use their own. Second, it remains unknown that one (or a few) of the species is a focal species of the study, avoiding biased answers. And third, a generalised positive (or negative) response bias towards all species is impossible, since only a limited set of species can be positively selected and the rest has to be selected against.

Due to its ease, visual attraction and 'game-like' nature, in our experience respondents were very keen to participate in the survey. Similar advantages were also recorded in a study that used a visual approach and gamification, a user-friendly 3-dimensional computer programme that allows people to design their ideal garden and to explore the biodiversity people want in urban green spaces (e.g. Shwartz et al. 2013a). We feel that such user-friendly visual approaches can increase the probability of involving various social groups in the research, for example, elderly people or children, those with a lower formal education or people without a special interest in nature, as is often not the case in other studies (White et al. 2003, Lindemann-Matthies 2016). This approach can thus be useful for reducing the self-selection bias for people with positive attitudes to nature and response bias (i.e. respondents' tendency to provide answers that are socially acceptable; Paulhus 1991), as no information on the special interest for the focal species was given in the visual part of the survey.

Our results confirmed that people recognised that the focal species depicted in the plate was a parrot of some sort, indicating that the information we obtained is relevant for the species of interest. Next, the usage of a specific image or its location on a plate could influence the probability that the image is selected, but we did not find any effect of using different plates which varied in quality and location of the images. Nonetheless, in visual surveys a random subset of images and locations could be used to generate variation amongst plates which are then randomly presented to a respondent, in order to avoid any image and location biases. In view of the above, we conclude that, in our surveys, the decision to include the parakeet in the subset of preferred species was made consciously. In addition, the visual survey and the classical question-based survey yielded very similar results and one could predict the results of the other. Hence, the similarity in

results between the two types of survey, novel versus classical, confirms that the respondents took part in the visual survey while taking their opinions and feelings specifically about the parakeet into account, even when they did not know this was our focal species. This implies that the novel visual survey is a valid method to assay social perception.

Is there a generalised disliking of non-native species?

There is a controversial aspect as to whether the origin of certain species influences attitudes in conservation (Van der Wal et al. 2015). In our results, we saw a mild and statistically non-important reduction in the probability to include the parakeet in the preferred set when presented as a non-native. In the case of the other three non-native species included in the survey (*Estrilda astrild*, *Amandava amandava* and *Columba livia*), there was also a consistent, sometimes large and always statistically important reduction when indicating they were non-native. Similar patterns were also found in Paris (France), when participants were asked to design their ideal gardens: most non-local species were excluded from the gardens (Shwartz et al. 2013a). Altogether these results may indicate that, overall, people do care whether species are native or non-native and take this information into account when selecting species. If so, it means that all sorts of information and campaigns on the potential negative effects of non-native species have managed to influence people's opinions about non-natives and have formed a social norm against some IAS. In that case, it also suggests that support for active management against non-native species, or prevention of future introductions, can be increased by information campaigns (Courchamp et al. 2017, Novoa et al. 2017).

Conclusion

The novel visual approach we present here suggests that the obtained information is reliable. Importantly, the visual survey is easy to take, has a very high participation rate and the data are fast and easy to analyse. Moreover, it gives an indication of perception and attitude relative to other species. Such a ranked perception could facilitate decision-making, since management is often about setting priorities in the face of limited resources. We therefore think our visual approach might be a good tool for conservation biologists who need to collect information on social perception on any kind of topic (as long as it can be captured in images), including biological invasions. In our case, it allowed us to establish that different sections of the public have different perceptions about the invasive rose-ringed parakeet, that perceptions are worse in those sections that are exposed more to negative impacts of parakeets and that there appears to be a social norm against invasive species. These are insights that should be helpful when deciding over actions against invasive species (Crowley et al. 2017). For example, for the parakeet in Seville, it might be helpful for managers to realise that public experience with the species decreases its desirability, suggesting a generally

worse perception as the species continues to increase. In addition, it might be helpful to communicate that, while some sections of the public are rather indifferent, others are decidedly negative in view of concrete interactions (people living close to roosting sites) – recognition of this may promote support for actions by the entire public. In general, information on social perception can be essential for effective policy, management and communication with the public.

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Supplementary material I

Figures SM1–SM3

Authors: Álvaro Luna, Pim Edelaar, Assaf Shwartz

Data type: multimedia

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Low litter cover, high light availability and rock cover favour the establishment of *Ailanthus altissima* in forests in southern Switzerland

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Abstract

Future forest composition is uncertain in many areas due to climate change. The spread of non-native species adds to these uncertainties, particularly in forests recently colonised by novel tree species. To anticipate future forest composition, and thus the provision of ecosystem services, a thorough understanding of the factors influencing the establishment of non-native tree species is essential. We studied the presence and abundance of regeneration of *Ailanthus altissima* (Mill.) Swingle in 89 plots on a regular grid in three sites in southern Switzerland to determine the main drivers of its establishment. All sites are located in abandoned, i.e. currently unmanaged stands of *Castanea sativa* Mill. Propagule pressure is caused by single mature *A. altissima* that colonised the area ca 40 years ago. We found high rock cover, low litter cover and high light availability to be the most important predictors for the presence of *A. altissima* regeneration, whereas its abundance was positively influenced by high light availability, low litter cover and high browsing on regeneration of competing species. However, the presence models performed much better than the abundance models. Interestingly, the most important factors favouring the establishment of *A. altissima* in recently undisturbed sites were found to be similar in a nearby site after a severe forest fire, which suggests a similar establishment strategy after a disturbance as in recently undisturbed forests. Based on our results we expect a further expansion of the species in lowland forests currently dominated by *C. sativa*, likely controlled primarily by light availability.

Keywords

Ailanthus altissima (tree of heaven), browsing, *Castanea sativa* (sweet chestnut), Gap Light Index (GLI), invasive tree, light availability, litter cover, non-native tree, propagule pressure

Introduction

Many forest ecosystems are expected to face compositional changes as a consequence of climate change, the introduction and spread of novel tree species, or interactions thereof (Lindner et al. 2010; Hickler et al. 2012). As shifts in tree species composition are first detectable in the regeneration layer (Grund et al. 2005; Moser et al. 2010; Fisichelli et al. 2013; Rigling et al. 2013), a thorough understanding of the factors underlying the successful establishment of novel tree species is crucial to anticipate future community development. Knowledge on the possible future forest composition is a prerequisite for evidence-based, anticipatory management decisions (Lindner et al. 2014), which may enable a continuous provisioning of forest ecosystem services (Gret-Regamey et al. 2013; Seidl et al. 2016). This is particularly important in forests that have only recently started to be colonised by non-native trees, i.e. where the future development is often highly uncertain.

The establishment of non-native tree species has mainly been studied in disturbed forest ecosystems (e.g., Maringer et al. 2012). On the one hand, because colonisation rates are generally slow in closed-canopy forests due to extended periods without gap formation and thus colonisations are difficult to detect and quantify at early stages (Martin et al. 2009; Essl et al. 2011; Wangen and Webster 2006). On the other hand, the majority of non-native tree species that are able to establish and spread outside of their native range are shade intolerant (Martin et al. 2009) and thus depend on large scale disturbances for their establishment. However, also disturbance dependent non-native trees have been able to persist in patches of undisturbed forests. *Prunus serotina* Ehrh., for example, can maintain a short-lived seedling bank, which enables the species to reach the canopy after gap formation (Closset-Kopp et al. 2007; Vanhellefont et al. 2009). Furthermore, *Acacia dealbata* Link, generally considered to be light demanding, was found to be able to grow below the canopy of native trees in south-central Chile (Fuentes-Ramírez et al. 2011).

In this study, we focus on the establishment of the early-successional tree species *Ailanthus altissima* (Mill.) Swingle. Its spread in forest ecosystems has often been associated with disturbances induced by forest management (Call and Nilsen 2003; Radtke et al. 2013; Berg et al. 2017; Rebbeck et al. 2017) or natural processes (Xi 2008; Maringer et al. 2012; Kasson et al. 2013). Despite the positive association of *A. altissima* with disturbance, the species also proved to be capable of establishing in forests lacking a large scale disturbance by virtue of its fast juvenile height growth (Knapp and Canham 2000; Martin et al. 2010; Knüsel et al. 2016). However, the environmental factors underlying the successful establishment of *A. altissima* in recently undisturbed forests remained largely unexplored. To shed light on these factors, we studied its generative regeneration in recently unmanaged lowland forests of southern Switzerland that started to be colonised by *A. altissima* following the general abandonment of the forests dominated by *Castanea sativa* Mill. in the 1950s. Specifically, we focused on the current distribution frontier where only few canopy *A. altissima* are present, which enabled us to study generative regeneration rather than vegetative sprouts that typically grow faster and can persist in shadier conditions (Kowarik 1995; Knüsel et al. 2016). We addressed the following research questions:

- i) What are the most important environmental factors that drive the presence and abundance of regeneration of *A. altissima* in recently undisturbed forests?

Once identified, we compared these factors with the environmental drivers of the post-fire establishment of *A. altissima* in the same study region (Maringer et al. 2012), leading to the second research question:

- ii) What are the key differences in the environmental drivers of the establishment of *A. altissima* in disturbed versus recently undisturbed forests?

Knowledge on key differences in the establishment requirements of *A. altissima* in forests after a disturbance versus recently undisturbed forests may facilitate predictions on the spread of the species.

Materials and methods

Study species

Ailanthus altissima is a dioecious early successional tree species originating from China that was brought to Europe around the 1740s as an ornamental tree (Kowarik and Säumel 2007). The spread of *A. altissima* has often been associated with natural disturbances (Xi 2008; Maringer et al. 2012; Kasson et al. 2013) or disturbance caused by forest management (Call and Nilsen 2003; Radtke et al. 2013; Berg et al. 2017; Rebbeck et al. 2017). Radtke et al. (2013) found frequent clear-cuts in coppice forests to favour the establishment and spread of *A. altissima* in northern Italy, while the time since the last timber harvest was the best predictor of *A. altissima* presence and density in state forests in Ohio, USA (Rebbeck et al. 2017). Moreover, natural disturbances such as fire (Maringer et al. 2012), windthrow (Xi 2008) or insect defoliation (Kasson et al. 2013) were found to lead to an increase in the abundance and/or the area colonised by *A. altissima*. In particular, light availability (Knapp and Canham 2000; Maringer et al. 2012; Martin et al. 2010; Berg et al. 2017; Rebbeck et al. 2017) and nutrient availability (Soják and Löffler 1988; Berg et al. 2017) were found to increase the growth and abundance of *A. altissima* regeneration.

Study area

The study was conducted in the sweet chestnut (*C. sativa*) forests of southern Switzerland. The climate in the study area is characterized by a mild average annual temperature of ca 12.7 °C and a mean annual precipitation of 1862 mm (climate normal 1981–2010, MeteoSwiss station Locarno-Monti, 383 m a.s.l.). A large amount of precipitation falls in short and heavy spells during the growing season (1023 mm

from May to September) followed by a relatively dry period in winter. The soils are classified as haplic podzol (cryptopodzol) on crystalline bedrock (Blaser et al. 2005).

The studied forests used to be dominated by *C. sativa*, which was introduced by the Ancient Romans more than 2000 years ago (Tinner et al. 1999) and intensively cultivated in monocultures as coppice forests or orchards (Conedera et al. 2004). The cultivation of *C. sativa* dropped in the 1950s as a consequence of a general abandonment of agriculture and the simultaneous spread of the chestnut blight (*Cryphonectria parasitica* (Murr.) Barr.). In the following decades, chestnut forests were increasingly colonised by native (*Fraxinus* spp., *Tilia* spp., *Prunus* spp.) and non-native tree species (*Robinia pseudoacacia* L., *A. altissima*, *Paulownia tomentosa* (Thunb.) Steud.) facilitated by the generally low competitiveness of *C. sativa* (Conedera et al. 2001; Pividori et al. 2005), which was further weakened by repeated outbreaks of pathogens such as the ink disease (*Phytophthora* spp.; Prospero 2017), the chestnut blight (Rigling and Prospero 2018), and since 2009 the arrival of the chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu; Gehring et al. 2017).

Study sites

We selected three study sites in southern Switzerland based on an occurrence map of *A. altissima* (Gurtner et al. 2015). We looked for sites at the current distribution frontier of the species with no vegetative regeneration. All sites (named Locarno, Sementina, San Vittore) are situated in the chestnut belt at elevations ranging from 400 to 550 m a.s.l. Their slopes are facing SW to SE with an average inclination of 70° ($\pm 18.0^\circ$ standard deviation, Fig. 1). In all three sites, *C. sativa* comprises the largest fraction of basal area (77–98%), followed by deciduous *Quercus* spp. (0–15%). All other species have a basal area <5% (*Betula pendula* Roth, *Robinia pseudoacacia* L., *Tilia* spp., *Fraxinus* spp., *Prunus avium* (L.) L., *Sorbus aria* (L.) Crantz, *Paulownia tomentosa* Steud., *Populus tremula* L., *Populus alba* L., *A. altissima*). All studied forests used to be managed as coppices except for a small part (ca 1 ha) of the site in San Vittore, which was managed as a chestnut orchard. In all three sites, no management has taken place since the 1950s apart from occasional cuttings of single trees or small forest patches (<0.2 ha).

To get an overview of the colonization history as well as the existing propagule pressure of *A. altissima* in the study sites, we searched for and mapped all seed-bearing female *A. altissima* within the boundary of the study sites and in a buffer of approximately 300 m around the studied forests. Of all accessible female trees, an increment core was extracted at a height of ca 1.3 m to determine their age. In the site Locarno, only a single female tree was found with an age of ca 18 years. In Sementina, the oldest of the four female trees was ca 27 years old, while the other three were only ca 15 years old. In the site San Vittore, several adult and at

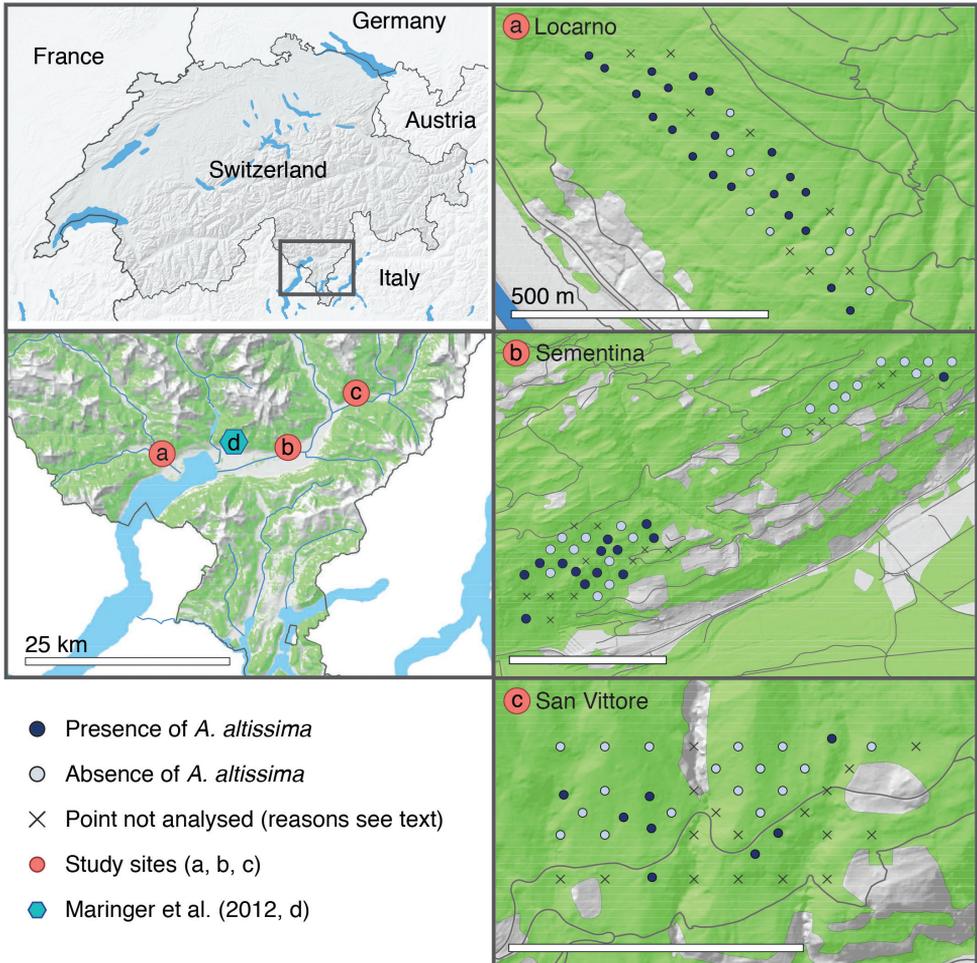


Figure 1. Study area and study sites. Study area in southern Switzerland (left column) and selected study sites: Locarno (a; $46^{\circ}10'36''\text{N}$, $8^{\circ}46'12''\text{E}$), Sementina (b; $46^{\circ}10'53''\text{N}$, $8^{\circ}58'13''\text{E}$) and San Vittore (c; $46^{\circ}14'19''\text{N}$, $9^{\circ}04'46''\text{E}$; right column with scale bars representing 500 m). (d) Represents the study area analysed by Maringer et al. (2012) on the establishment of non-native species after a forest fire, which was used for comparison in this study.

least 40-years-old *A. altissima* trees that grew along the road crossing the study site (Fig. 1) were girdled in 2010 and felled in winter 2014 (Luca Plozza, pers. comm.). About 10 adult trees remained in the forest, of which the oldest female tree was ca 40 years old. Although we searched for seed-bearing female *A. altissima* during the sampling (summer 2015) and in the following winter after leaf shedding, it cannot be ruled out entirely that further and yet undiscovered seed-bearing trees exist in inaccessible parts around the study sites.

Sampling design

We applied a systematic sampling design that was centred on *A. altissima*. An adaptation was necessary to obtain a sufficient number of sampling points with regeneration of *A. altissima*, as the studied forests are located at the distribution frontier where the species occurs at low densities only. To this end, we placed a regular grid with a mesh size of 50 m over all three study sites and defined potential sampling points at the nodes of the grid. At each of these points we searched for *A. altissima* saplings (>40 cm tall and <12 cm DBH) in a circular area of 800 m² (ca 16 m radius) around the sampling points. Plants smaller than 40 cm were not considered to avoid individuals that had germinated in the year of the field survey (Knüsel et al. 2016). This resulted in 44 sampling points with saplings of *A. altissima* in the vicinity (later referred to as *presence points*) and 45 without (later referred to as *absence points*), i.e. a total of 89 sampling points (Fig. 1). These numbers already exclude several sampling points that were inaccessible ($n = 15$), experienced heavy management ($n = 8$, i.e. below a power line and close to a building inside the forest), or were close (<10 m) to a forest road ($n = 9$). In addition, six sampling points were excluded because of mature *A. altissima* (DBH >12 cm) in the 800 m² circle, which may support conspecific regeneration via root grafts (O’Neal and Davis 2015).

At the absence points, two nested, slope-corrected circular plots with sizes of 100 and 200 m² were temporarily installed at the nodes of the grid. At presence points, in contrast, the nested plots were not installed at the nodes of the grid, but were moved to have the nearest *A. altissima* sapling in the centre.

A range of environmental variables were then measured in each plot. First, the light environment was recorded at the plot centre with a hemispherical photograph (Canon EOS 50D camera with a Sigma EX DC 4.5 mm fisheye lens). The photograph was taken horizontally at a height of 1.7 m to avoid shading by small regeneration. If the *A. altissima* sapling representing the plot centre (later referred to as *central A. altissima*) at presence points was taller than 1.7 m it was bent to the forest floor to avoid self-shading. In addition, the age of the central *A. altissima* was assessed by counting growth units (Heuret et al. 2003) for individuals <6 years and by taking an increment core or a stem disc at 40 cm height for older individuals. Furthermore, we excavated the central *A. altissima* to verify its seed origin in case a mature *A. altissima* occurred within 20 m. All excavated central *A. altissima* originated from seeds.

In the 100 m² plot, we surveyed ground cover as well as seedlings (10–40 cm tall) and saplings (>40 cm tall) of all tree species. Percentage cover of bare soil (later referred to as *mineral soil cover*), litter, herbs and shrubs (later referred to as *vegetation cover*), coarse woody debris and vegetation-free rock (later referred to as *rock cover*) were assessed visually in 10% classes. Ground cover values were assessed so that they added up to 100%. For tree seedlings, the species and the presence of recent (i.e., last three years) browsing damages were noted. For saplings, we additionally recorded height, DBH and damage by fraying (only for saplings >130 cm tall).

In the 200 m² plot, we assessed stand structure and signs of management (i.e., presence of tree stumps). For all trees and coppice shoots or stools (DBH >12 cm), DBH, height and the vitality class following Tinner et al. (2010) were recorded.

Establishment models

Model types

We used two types of multiple regression models to analyse *A. altissima* establishment. First, we used logistic models (LOG) to analyse the presence or absence of *A. altissima* saplings. Second, we used negative binomial models (NB) to analyse the abundance (number) of *A. altissima* saplings (see Methods S1 for details).

Explanatory variables

The light environment was characterized by the Gap Light Index (GLI), which integrates diffuse and direct irradiation over a growing season. GLI was calculated from the hemispherical photographs with the program *Hemisfer* (www.schleppi.ch/hemisfer; Schleppi et al. 2007). As an additional measure, we quantified canopy closure, i.e. the proportion of the sky covered by vegetation when viewed from a single point (Jennings et al. 1999; Gonsamo et al. 2013). Canopy closure was further used as a single explanatory variable in a separate simple LOG model, since it represents a more intuitive and easily obtainable measure of the light environment that can be estimated visually by practitioners. Based on this simple model, we calculated a threshold canopy closure value that best differentiated the presence or absence of *A. altissima* saplings by optimizing for the sum of sensitivity and specificity based on the ROC curve with the *R* function *coords* in the *R* package *pROC* (Robin et al. 2011).

Three of the five assessed ground cover variables, mineral soil, litter, and rock cover were used as explanatory variables (Table 1). Vegetation cover was excluded due to a variance inflation factor (VIF) >5, calculated with the *vif* function in the *R* package *car* (Fox and Weisberg 2011). Coarse woody debris was excluded due to the small variance in the data (Table 1) and a lacking ecological justification, since no regeneration growing on woody debris was found (personal observation). Furthermore, we approximated propagule pressure by the minimal distance of each sampling point to the nearest seed-bearing tree (later referred to as *seed source*). The distance to the next seed source was calculated using ArcGIS. In addition, we characterised stand density by the total sum of living basal area ($\text{m}^2 \text{ha}^{-1}$) of all trees on the plot above 12 cm DBH. Due to the high browsing pressure on competing tree species (but not on *A. altissima*), we calculated an average proportion of browsed co-occurring species (excluding *A. altissima*) for each sampling point. Finally, we analysed interactions of saplings of *A. altissima* with saplings of other tree species. To this end, we selected the number of seedlings and the number of saplings of the four most abundant species as additional explanatory variables. Moreover, we selected the sum of living basal area of the four tree species with the highest total basal area to analyse interactions with canopy tree species. The number of other tree species' seedlings and saplings, as well as the living basal area were not used in the process of model selection, but were only added to the most parsimonious models after model selection.

All continuous variables were standardized before running the models by subtracting the mean and dividing the result by the standard deviation.

Table 1. Environmental parameters recorded for the establishment models.

Continuous predictors	Mean (SD)	Range
Light environment:		
Gap light index (GLI)	21 (14.6)	4–73
Ground cover:		
Mineral soil cover (%)	15 (12.4)	0–52
Litter cover (%)	32 (20.8)	0–85
Rock cover (%)	18 (13.9)	0–70
Coarse woody debris cover (%)	5 (6.1)	0–32
Vegetation cover (%)	29 (24.7)	0–92
Propagule pressure:		
Distance to seed source (m)	127 (65.1)	15–303
Stand characteristics:		
Basal area per plot (m² ha⁻¹)	36 (21.3)	5–121
Browsing:		
Browsed competitors (%)	40 (18.0)	0–100
Categorical predictors	Levels	<i>n</i>
Site	Locarno	29
	San Vittore	25
	Sementina	35

Bold labels indicate predictors that were used for model selection.

Model selection

Starting with the eight selected variables (Table 1), we generated a full submodel set with the *dredge* function in the *R* package *MuMIn* (Bartoń 2018). Three main models were chosen for reporting: (1) null model (later referred to as NB_{null}, LOG_{null}), (2) most parsimonious model of those models that differed by less than 2 AIC_C points compared to the model with the lowest AIC_C score (later referred to as NB_{pars}, LOG_{pars}), (3) average of all models within 2 AIC_C points (later referred to as NB_{avg}, LOG_{avg}). All models within 4 AIC_C points as well as the average thereof can be found in Tables S1–S4.

For the averaging of all models within 2 AIC_C points, we used the zero method to compute parameter estimates (Burnham and Anderson 2002; Nakagawa and Freckleton 2010), where a parameter estimate of zero is added for a parameter not occurring in a model selected for averaging. In addition, a relative importance value for each explanatory variable occurring in at least one of the averaged models was calculated. It represents the fraction of models used for averaging in which a certain variable occurred.

Model performance

Goodness-of-fit of the models was assessed by comparing the most parsimonious models against the null model with a Chi-squared test. For the LOG models we further calculated AUC values with the *R* package *pROC* (Robin et al. 2011) to quantify the predictive power of the models. As no similar measure exists for NB models, we computed the Pearson correlation of the observed number of *A. altissima* saplings versus the predicted number using NB_{pars}.

Comparison with post-fire establishment

To discriminate factors driving the establishment of *A. altissima* after a forest fire (Maringer et al. 2012) versus in recently undisturbed stands (this study, no forest management or other large-scale disturbances for the last ca 60 years), we re-analysed the data of Maringer et al. (2012). The authors studied the establishment of non-native species in plots affected by a forest fire four years before and in adjacent control plots (Maringer et al. 2012). The study was conducted in the same study region (Fig. 1).

In particular, we aimed at quantifying differences in ground cover and light availability in presence and absence points in this study and in fire affected plots in Maringer et al. (2012). Due to deviations in the sampling protocols we did not directly compare values between the studies, but used two-sided Wilcoxon tests instead to quantify differences within but not across the studies. Ground cover values have been assessed in a circle of 200 m² size in Maringer et al. (2012) as opposed to a 100 m² circle in our study. In addition, Maringer et al. (2012) assessed herb, fern and shrub cover separately. Hence, vegetation cover in this study (sum of herb and shrub cover) was compared to herb cover only of Maringer et al. (2012). Further, canopy closure in Maringer et al. (2012) was measured from eight photographs taken in subplots of the 200 m² plot, whereas in our study it was calculated from a single hemispherical photograph taken at each sampling point.

Results

Presence and abundance of regeneration

The presence points were on average slightly closer to seed sources (118 m, ± 61.3 m standard deviation) than the absence points (136 m, ± 68.1 m), however not significantly ($P > 0.2$). In the 44 presence points, a total of 243 saplings of *A. altissima* were recorded. They had a mean height of 224 cm (± 179.9 cm), a mean DBH of 1.0 cm, and a mean age of 4 years (± 1.8 yr), with a maximum of 11 years. When present, *A. altissima* saplings often dominated the regeneration at the sampling point ($57\% \pm 31.3\%$ of all saplings, Table 2). The four most abundant saplings of other species were *C. sativa* (total = 181), *R. pseudoacacia* (80), *Fraxinus* spp. (50) and *S. aria* (31).

In addition, we recorded a total of 188 seedlings of *A. altissima*, accounting on average for 16% ($\pm 18.1\%$) of all seedlings at the presence points (Table 2). The four most abundant seedlings of other species were *Fraxinus* spp. (total = 1560), *Quercus* spp. (1241), *C. sativa* (1186) and *R. pseudoacacia* (202).

Only *A. altissima* showed an increasing relative frequency of individuals with increasing height (Fig. 2). In contrast, the most frequent co-occurring species at the earliest stages, such as *Quercus* spp. and *Fraxinus* spp., featured a strong reduction in stem numbers with increasing height.

Table 2. Presence and abundance of regeneration. Shown are seedlings (10–40 cm tall) and saplings (>40 cm tall and <12 cm DBH) of *A. altissima* and the four most abundant co-occurring seedlings or saplings. Values are averages per sampling point, with standard deviations in parentheses.

Class	Species	Sampling points			
		With <i>A. altissima</i> saplings		Without <i>A. altissima</i> saplings	
		n*ha ⁻¹	proportion per sampling point (%)	n*ha ⁻¹	proportion per sampling point (%)
Seedlings	<i>A. altissima</i>	494 (513)	16 (18.1)	163 (177)	4 (6.5)
	<i>Fraxinus</i> spp.	4023 (11312)	35 (26.1)	1082 (1812)	20 (18.1)
	<i>Quercus</i> spp.	1161 (1375)	28 (22.4)	2146 (3329)	37 (31.0)
	<i>C. sativa</i>	1068 (1377)	20 (18.6)	1912 (3174)	42 (27.5)
	<i>R. pseudoacacia</i>	563 (392)	15 (10.5)	500 (831)	10 (8.7)
Saplings	<i>A. altissima</i>	560 (603)	57 (31.3)	–	–
	<i>C. sativa</i>	391 (429)	30 (23.3)	465 (470)	72 (32.0)
	<i>R. pseudoacacia</i>	492 (776)	26 (20.3)	300 (490)	18 (12.4)
	<i>Fraxinus</i> spp.	257 (234)	16 (9.6)	275 (236)	45 (46.1)
	<i>S. aria</i>	500 (693)	23 (31.6)	229 (138)	46 (29.1)

Proportions per sampling point are an average over all sampling points in which the respective species occur.

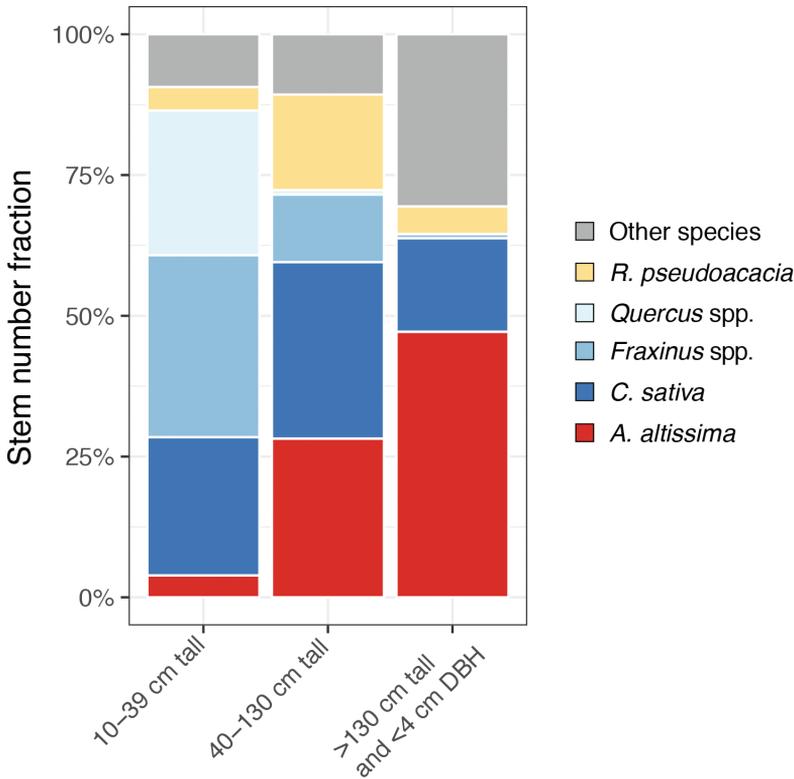


Figure 2. Fractions of stem numbers per species for three different height classes. Data were pooled from all three sites.

Establishment models

Drivers of *A. altissima* occurrence

The occurrence of *A. altissima* saplings was positively ($P < 0.05$) affected by rock cover and negatively ($P < 0.001$) by litter cover in LOG_{pars} (Table 3). LOG_{pars} fitted the data significantly better ($P < 0.001$) than LOG_{null} as indicated by a Chi-squared test. LOG_{avg} identified rock cover as the only significant variable ($P < 0.05$), which also had the highest relative importance. Additionally, LOG_{avg} identified the GLI, distance to seed source, mineral soil cover and site as explanatory variables, which were not included in LOG_{pars} . However, only GLI and distance to seed source were included in a larger number of averaged models (>60%). Both LOG_{pars} and LOG_{avg} had similar AUC values (0.88 and 0.91, respectively).

Table 3. Results of the logistic models of the occurrence of *A. altissima* saplings. The null model (LOG_{null}), the most parsimonious model (LOG_{pars}) as well as an average of all models ($n = 8$) within 2 AIC_C points (LOG_{avg}) are shown. Significant predictors are written in bold, values in parentheses represent standard errors.

	LOG_{null}	LOG_{pars}	LOG_{avg}
(Intercept)	-0.05 (0.21)	-0.19 (0.29)	0.21 (0.65)
Rock cover (%)		0.74* (0.36)	0.82* (0.41)
Litter cover (%)		-1.86*** (0.42)	-1.11 (0.71)
Gap Light Index (-)			0.92 (0.68)
Distance to seed source (m)			-0.44 (0.43)
Mineral soil cover (%)			0.12 (0.25)
Site - San Vittore			-0.19 (0.51)
Site - Sementina			-0.52 (1.00)
AIC_C	123.99	82.41	-
AUC	0.50	0.88	0.91
Relative importance			
Rock cover			1.00
Litter cover			0.87
Gap Light Index			0.83
Distance to seed source			0.69
Mineral soil cover			0.27
Site			0.26

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

The three most important variables according to LOG_{avg} , i.e. rock cover, litter cover and GLI, showed significant differences ($P < 0.01$) between presence- and absence points (Fig. 3a, b, e, red boxes). In addition, vegetation cover, which was excluded from the models due to multicollinearity, was significantly higher ($P < 0.01$) at presence points (Fig. 3d). As a more intuitive measure, the light environment was additionally characterized by canopy closure (Fig. 3f). At a threshold value of 86% canopy closure, the highest correct classification rate could be achieved based on canopy closure alone (Fig. 3f, red dashed line).

Drivers of *A. altissima* abundance

According to NB_{pars} , the abundance of *A. altissima* saplings was significantly positively affected by GLI and the percentage of browsed competitors, whereas litter cover had a significantly negative effect (Table 4). As for the logistic model, NB_{pars} fitted the data significantly better ($P < 0.001$) than NB_{null} , but displayed a poor predictive power with a low correlation ($r = 0.17$) between observed and predicted numbers of *A. altissima* saplings (Fig. S1).

NB_{avg} identified the percentage of browsed competitors, GLI and litter cover as the most important variables as well, supplemented by mineral soil cover, total basal area and rock cover. However, the influence of total basal area and rock cover were marginal only (Table 4).

Browsing and fraying

Overall, *A. altissima* regeneration (10–300 cm tall) experienced less ($P < 0.01$) browsing damage compared to the four most frequent other species (*Fraxinus* spp., *C. sativa*, *Quercus* spp. and *R. pseudoacacia*) and an average of all other species (Fig. 4).

Compared to the two species with most abundant saplings taller than 130 cm (*C. sativa* and *R. pseudoacacia*) as well as an average over all other species, *A. altissima* was frayed significantly less ($56\% \pm 33\%$ standard deviation, $P < 0.05$) than *C. sativa* ($81\% \pm 27\%$) but did not differ significantly ($P > 0.4$) from *R. pseudoacacia* ($75\% \pm 35\%$) or an average of all other species ($52\% \pm 31\%$, Fig. S2).

Comparison with post-fire establishment

Two of the three most important variables for the occurrence of *A. altissima* saplings identified in this study, litter cover and light availability, also differed significantly ($P < 0.01$) between presence- and absence points in a study conducted after a forest fire (Maringer et al. 2012, Fig. 3b, f). Mineral soil cover, in contrast, was significantly higher ($P < 0.01$) in presence points after fire (Maringer et al. 2012), but not in this

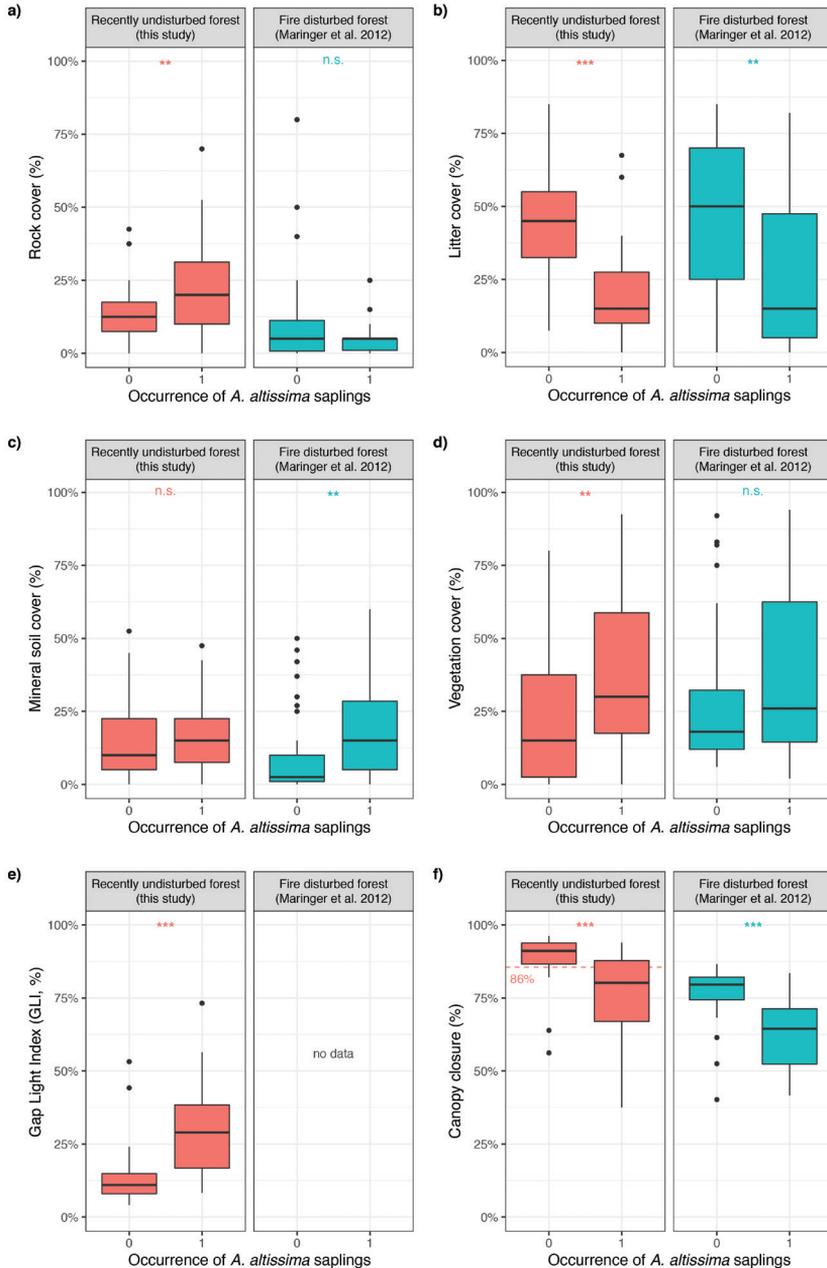


Figure 3. Comparison of ground cover (a–d) and light environment (e, f) between sampling points with or without saplings (>40 cm tall and <12 cm DBH) of *A. altissima*. Values originate from recently undisturbed forests (this study), and from a forest analysed four years after a fire (Maringer et al. 2012). The dashed line in (e) shows a threshold value that is optimized for sensitivity and specificity to distinguish sampling points with or without saplings of *A. altissima* in recently undisturbed forests. Asterisks indicate significant differences between sampling points with and without saplings of *A. altissima* (** $P < 0.01$, *** $P < 0.001$). See text for differences in the sampling procedure between this study and Maringer et al. (2012).

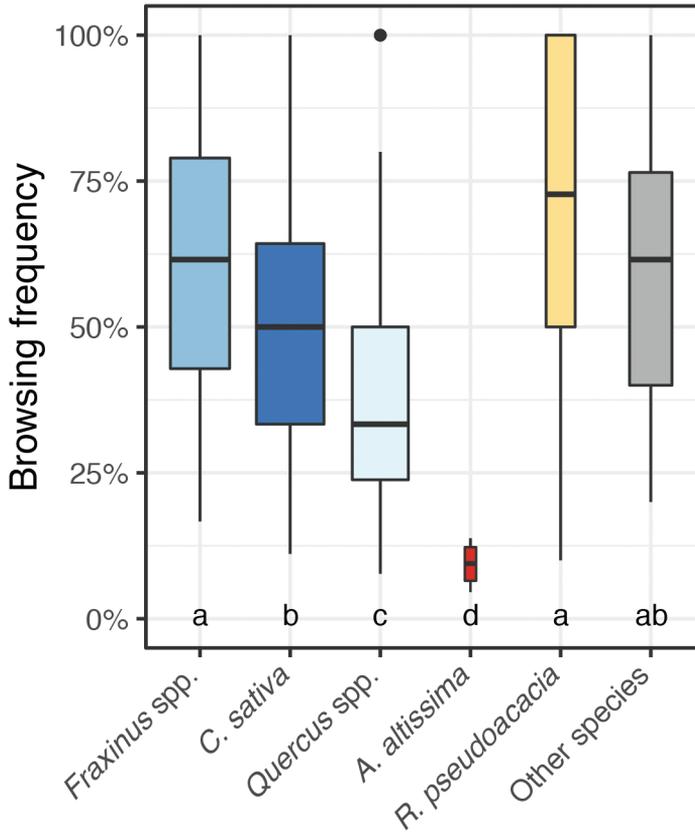


Figure 4. Proportion of browsed regeneration. Browsing damages were recorded on saplings 10–300 cm tall for the three most recent years. Widths of the bars are proportional to the number of individuals recorded ($n > 360$). Different lowercase letters indicate significant ($P < 0.05$) differences between proportions.

study (Fig. 3c). In addition, rock cover was generally lower in the area studied by Maringer et al. (2012) and did not differ significantly between presence- and absence points, while the difference was significant ($P < 0.01$) in this study (Fig. 3a). Moreover, vegetation cover was higher in presence points in both studies (Fig. 3d), but only differed significantly ($P > 0.01$) in this study.

Interactions with other species

The number of *A. altissima* saplings was significantly negatively ($P < 0.05$) correlated with the number of *S. aria* saplings only (Table S6). However, *S. aria* was merely present in 10 sampling points, with a maximum of 13 saplings at one sampling point (cf. Table 2). The three species with most abundant saplings (*C. sativa*, *R. pseudoacacia* and *Fraxinus* spp.), in contrast, were not significantly related ($P > 0.2$) to the number of *A. altissima* saplings.

Table 4. Results of the negative binomial model of the number of *A. altissima* saplings. The null model (NB_{null}), the most parsimonious model (NB_{pars}) as well as a model average of all models ($n = 6$) within 2 AIC_C points (NB_{avg}) are shown. Significant predictors are written in bold, values in parentheses represent standard errors.

	NB _{null}	NB _{pars}	NB _{avg}
(Intercept)	1.01*** (0.21)	0.53** (0.19)	0.51** (0.19)
Gap Light Index (-)		0.70** (0.24)	0.75** (0.26)
Browsed competitors (%)		0.45* (0.18)	0.45* (0.18)
Litter cover (%)		-0.64* (0.28)	-0.53 (0.33)
Mineral soil cover (%)			0.23 (0.23)
Total basal area (m ² ha ⁻¹)			0.05 (0.13)
Rock cover (%)			0.01 (0.07)
AIC _C	349.95	330.73	-
Relative importance			
Gap Light Index			1.00
Browsed competitors			1.00
Litter cover			0.89
Mineral soil cover			0.61
Total basal area			0.24
Rock cover			0.11

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

The number of *A. altissima* saplings was positively ($P < 0.001$) correlated with the number of seedlings of *R. pseudoacacia* and *Fraxinus* spp., while there was no significant relationship ($P > 0.5$) with the other two most frequent seedling species, *C. sativa* and *Quercus* spp. (Table S5).

The amount of basal area of live trees of the four most prominent tree species (*C. sativa*, *Quercus* spp., *B. pendula* and *R. pseudoacacia*) at presence points did not show a significant relationship ($P > 0.05$) with *A. altissima* sapling density (Table S7).

Discussion

Regeneration models

Drivers of *A. altissima* occurrence

The occurrence of *A. altissima* saplings was mainly favoured by high rock cover, low litter cover and high light availability (Table 3). A beneficial effect of a high rock cover on the presence of *A. altissima* has not been found in other studies that analysed the

establishment of the species in forests (Maringer et al. 2012; Radtke et al. 2013). However, *A. altissima* is known to thrive in ruderal urban sites (Celesti-Grapow and Blasi 2004; Kowarik and Säumel 2007) that partly resemble rocky forest patches. Rock cover has been suggested to reduce competition and serve as a refuge for early successional species (Frelich and Reich 2002), even more if they are drought tolerant (Plieninger et al. 2010). Both effects may partially explain the association of *A. altissima* with rocky forest patches.

The negative effect of litter cover for the occurrence and the abundance of regeneration of *A. altissima* may have been caused by a delay in germination and an increased invertebrate herbivory and seedling mortality, as found in an early successional site (Facelli and Pickett 1991; Facelli 1994). Similarly, Kostel-Hughes et al. (2005) found reduced robustness of seedlings that emerged from deep litter (ca 5 cm deep) in an experimental study, although there was no difference in the probability of emergence between different litter treatments (no litter, 1–2 cm and ca 5 cm deep litter).

As expected, light availability played a major role for the occurrence of the early successional *A. altissima*. Although the species is able to persist in low light levels for several years (Knapp and Canham 2000; Knüsel et al. 2016), most of the presence points experienced light levels above 10% GLI (Fig. 3e). A study of Martin et al. (2010) conducted in forests in north-western Connecticut (USA) found that radial growth of *A. altissima* exceeded native regeneration at levels >10% of full sunlight (GLI), but not at light levels below 3%. However, we did not measure such low light levels in this study (Fig. 3e). Moreover, Huebner et al. (2018) found canopy closure of ca 85% and above to reduce the establishment probability for *A. altissima*. The threshold suggested by Huebner et al. (2018) also matches the 86% canopy closure level found in this study (Fig. 3f, red dashed line), at which the probability of false positive and false negative classifications of *A. altissima* occurrence was minimal.

Drivers of *A. altissima* abundance

The models for the abundance of *A. altissima* saplings had a much lower predictive power than the occurrence models, which implies that the following interpretations represent tendencies only. While high light availability and low litter cover were also important for the density of *A. altissima* regeneration, rock cover did not influence sapling abundance. This might be related to the lower availability of suitable sites for establishment in rocky forest patches (Frelich and Reich 2002). In contrast, sapling density of *A. altissima* was positively influenced by an increased cover of mineral soil (Table 4, Fig. 3c), which is known to be an important factor for the establishment of many light-demanding early successional tree species (Shields et al. 2007; Zouhar et al. 2008), including *A. altissima* (Call and Nilson 2003; Martin and Canham 2010). Furthermore, the observed high browsing pressure on competitors may have indirectly favoured the largely unbrowsed regeneration of *A. altissima* through reduced competition.

However, the distribution of tree species is determined not only by the availability of suitable microsites for their establishment, but also by seed production and dispersal

(Nathan and Muller-Landau 2000; Turnbull et al. 2000). Limitations in propagule pressure are often particularly important for expanding non-native tree species (Pyšek et al. 2009; Terwei et al. 2013; Conedera et al. 2017; Sullivan and Franco 2017; Dyderski and Jagodziński 2018). Accordingly, the distance to the next seed source played a role for the occurrence of *A. altissima* (Table 3), which is in line with previous studies (Radtke et al. 2013; Höfle et al. 2014). Thus, a further increase in seed-bearing female *A. altissima* is likely to lead to an increase in the distribution range of the species. Yet, the distance to the next seed source was not important for the density of *A. altissima* (Table 4). This may suggest that the density of *A. altissima* is mainly driven by the amount of suitable establishment sites in case seeds are available. Nevertheless, seed addition experiments would be required to quantify the relative importance of seed limitation for the occurrence and density of *A. altissima* in the studied forests (Turnbull et al. 2000).

Vegetation cover, which was excluded from the models due to multicollinearity with light availability and litter cover, was significantly higher at presence points (Fig. 3d). This may contradict the oftentimes reported negative effect of herbs and shrubs on the establishment of tree regeneration (Maguire and Forman 1983; Picon-Cochard et al. 2006). However, as high light availability tends to simultaneously enhance both the amount of vegetation (Vockenhuber et al. 2011) and the probability of occurrence and abundance of *A. altissima* (Tables 3, 4), high vegetation cover may rather be the consequence of suitable germination conditions. Nevertheless, it is possible that very high vegetation cover ($\geq 75\%$) hindered the establishment of *A. altissima* at two sampling points with ample light availability (Fig. 3e, outlier points on the left). Yet, *A. altissima* regeneration was still present at three out of five sampling points with vegetation cover $\geq 75\%$, probably owed to its fast juvenile height growth (Knapp and Canham 2000; Martin et al. 2010). Hence, we surmise that the role of vegetation cover for the establishment of *A. altissima* may be different depending on the timing of its development and its coincidence with seed rain.

We did not consider soil characteristics as *A. altissima* is known to tolerate a broad range of soil conditions (Kowarik and Säumel 2007). In other studies, *A. altissima* occurred slightly more often on moister soils (Radtke et al. 2013) and was associated with sites with high indicator values for nutrients (Berg et al. 2017). However, soil characteristics were not identified as the main factors influencing the establishment of *A. altissima*.

Browsing and fraying

Ailanthus altissima suffered almost no damage from ungulate browsing compared to co-occurring regeneration (Fig. 4), which agrees with findings of Maringer et al. (2012). We hypothesize that browsing may enhance the establishment success of *A. altissima* through reduced competition by co-occurring regeneration, as suggested by the abundance models. Reduced herbivory by ungulates or insects is common for many novel non-native species and may influence successional pathways of colonised forest stands (Cappuccino and Carpenter 2005; Knapp et al. 2008). However, differences in herbivore damage are thought to decrease with time since introduction (Brändle et al. 2008; Schilthuizen et al.

2016). Furthermore, results from Hungary (Mátrai et al. 2004) and the USA (Hunter 1995; Carter and Fredericksen 2007) demonstrate that *A. altissima* is sometimes browsed as frequently as co-occurring trees. In addition, fraying damages on larger regeneration, which were to our knowledge considered for the first time in this study, were as frequent on *A. altissima* as on other species (Fig. S2). However, fraying did not seem to cause mortality, as several saplings with sealing callus growth and wound closure were found. This is in line with findings of Gurtner (2015) in the study region.

Comparison with post-fire establishment

Forest fires temporarily increase the availability of light and nutrients, and reduce competition (Tyler and D'Antonio 1995; Keeley et al. 2003), which tends to promote the establishment of *A. altissima* (Maringer et al. 2012; Crandall and Knight 2018). These same factors, i.e. high light availability and reduced competition via high rock cover or browsing on competing regeneration were also important for the establishment of *A. altissima* in recently undisturbed forests analysed here. Furthermore, low litter cover seemed to be similarly important for the establishment of *A. altissima* after a fire and in recently undisturbed forests. Moreover, *A. altissima* seems to benefit from the higher availability of mineral soil after a fire for its establishment. Based on the similar patterns in the establishment drivers of *A. altissima* in fire-disturbed and recently undisturbed forests (Fig. 3), we conclude that *A. altissima* follows the same establishment strategy in both cases. This is in line with findings of Martin et al. (2010), who suggest that *A. altissima* is restricted by a trade-off between low light survivorship and high light growth also when growing in closed-canopy forests, unlike other non-native tree species such as *Acer platanoides* L. (Martin and Marks 2006; Martin et al. 2010; Shouman et al. 2017). While early generative regeneration of *A. altissima* may only persist for an average of three years at low light levels (< 5% GLI; Martin et al. 2010; Knüsel et al. 2016), *A. platanoides* showed almost no mortality at such light levels after five years (Martin et al. 2010), but was still able to grow at similar rates once released from shade (Martin and Marks 2006; Martin et al. 2010; Shouman et al. 2017). However, the strong vegetative regeneration potential of *A. altissima*, which was not considered in this study, may enable the species to increase its persistence in unfavourable conditions after it successfully established in a forest stand (Kowarik 1995; Knüsel et al. 2016).

Interactions with other species

Due to the early successional character of both *A. altissima* and *R. pseudoacacia* (Radtko et al. 2013; Berg et al. 2017), we expected a positive interaction between these species, as observed by Call and Nilsen (2003). However, only seedlings but not saplings of *R. pseudoacacia* were positively associated with *A. altissima* saplings (Tables S5, S6). This may be related to the high browsing pressure on *R. pseudoacacia* (Fig. 4) that may have strongly reduced the number of saplings of *R. pseudoacacia* (Fig. 2).

Future development of analysed forest stands

The increasing relative abundance of *A. altissima* with increasing development stage (Fig. 2) suggests that the fraction of basal area of *A. altissima* will increase in the analysed forests. Several factors may further favour the future establishment and spread of *A. altissima*. First, an increase in the number of seed-bearing trees may augment both the occurrence and abundance of *A. altissima*, as the dispersal of non-native species is oftentimes limited by propagule pressure (Křivánek et al. 2006; Edward et al. 2009). Second, ungulate browsing may continue to act stronger on co-occurring species, thereby reducing understorey competition for *A. altissima*. Third, natural disturbances such as fire, windthrow or uprooting are expected to increase with climate change (Vogt et al. 2006; Pezzatti et al. 2016), thus creating suitable establishment conditions for *A. altissima* (Martin et al. 2010). Fourth, the high drought tolerance of *A. altissima* already at the sapling stage (Trifilò et al. 2004) may make this species less vulnerable to anticipated prolonged droughts (CH2011 2011; Lindner et al. 2010). Yet, this study only focused at the first generation of *A. altissima*. The transition from the first established canopy trees to the next generation can be particularly important for future forest succession, as seen for declining early-successional *R. pseudoacacia* in northern Italy (Motta et al. 2009).

Conclusions

High light availability, low litter cover and to a lesser degree high rock cover resulted as the most important factors determining the occurrence and abundance of *A. altissima* regeneration in forests lacking of recent large scale disturbances like fire or coppice management (research question i). Interestingly, very similar factors were also important for the establishment of *A. altissima* after a disturbance (research question ii), suggesting a consistent establishment strategy across sites with different disturbance intensities. Thus, light availability remains a key factor that controls the establishment and spread of the species, particularly for generative regeneration analysed in this study.

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Supplementary material I

Justification for models used on the abundance of *A. altissima*

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Data type: (models)

Explanation note: We used models with a negative binomial distribution to characterize the abundance of *A. altissima* saplings.

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