

# Tolerance and induced resistance in a native and an exotic pine species: relevant traits for invasion ecology

Rafael Zas<sup>1</sup>, Xoaquín Moreira<sup>2</sup> and Luis Sampedro<sup>2\*</sup>

<sup>1</sup>Misión Biológica de Galicia, Consejo Superior de Investigaciones Científicas Apdo. 28, E-36080 Pontevedra, Spain; and <sup>2</sup>Centro de Investigación Forestal de Lourizán. Unidad Asociada MBG-CSIC. Apdo. 127, E-36080 Pontevedra, Spain

## Summary

1. Current hypotheses predict contrasting roles for natural enemies in determining the success or failure of plant invasions. Differences in plant-induced resistance and tolerance to native herbivores between native and exotic species might contribute to resolve this controversy.

2. We examined the differences between the native *Pinus pinaster* and the exotic *P. radiata* in constitutive resistance, inducibility of chemical defences, realized resistance and tolerance to the large pine weevil *Hylobius abietis* in NW Spain. In this region, both pine species closely coexist and are threatened by the weevil, a harmful phloem feeder that causes extensive mortality and growth reduction in young pine stands.

3. We performed two *in vitro* cafeteria bioassays, two induction experiments with direct exposure to the weevil and spraying methyl jasmonate and an exhaustive field study of the genetic variation in tolerance and resistance in forestry genetic trials.

4. The weevil significantly preferred the native to the exotic pine when twigs were offered as cut material in Petri dishes. However, the pattern in the field was the opposite, with greater damage on the exotic. Inducibility of stem oleoresin did not differ between species when elicited by the application of methyl jasmonate. However, after a 72-h experimental exposure to the weevil, stem resin content in the native pine was double that in the exotic pine, suggesting a lower capability of the exotic pine to respond to the insect damage. In the field, family relationships between early damage and several pine fitness correlates revealed a significantly greater tolerance of the native pine to the insect damage. Furthermore, only the native pine showed genetic variation in tolerance to the damage.

5. *Synthesis.* The preference of the herbivore for the native species was counterbalanced by a lower capability for expressing induced resistance to the weevil and reduced tolerance in the exotic species, resulting in no apparent fitness advantage of the exotic *P. radiata* over the native *P. pinaster*. Differences in inducibility by and tolerance to native enemies between exotic and native host congeners emerge as key traits for understanding how native enemies might contribute to preventing an introduced species from becoming invasive.

**Key-words:** biological invasions, biotic resistance hypothesis, enemy release hypothesis, exotic plants, herbivory, *Hylobius abietis*, invasion ecology, *Pinus pinaster*, *Pinus radiata*, plant–herbivore interactions

## Introduction

There are currently several much-debated hypotheses about the differences that should be expected in the impact of specialist and generalist herbivores on native and exotic plant species and how these differences may explain the invasive behaviour of some exotics when planted out of their natural ranges (Cat-

ford, Jansson & Nilsson 2009 and references therein). In particular, the enemy release hypothesis (ERH) predicts a lower impact of herbivores on introduced plants because specialist enemies of the introduced plants are absent in the new range, and generalist enemies would be better adapted to consume native than novel exotic species (Maron & Vilá 2001; Keane & Crawley 2002). Alternatively, the biotic resistance hypothesis (BRH) predicts that native herbivores can limit invasions as introduced species may be poorly adapted for defending

\*Correspondence author. E-mail: lsampe@uvigo.es

against them (Elton 1958; Maron & Vilá 2001). Lacking a common evolutionary history with the resident herbivores, exotic plant species may be particularly vulnerable to the new enemies in the introduced range, as they have been not selected to evolve defences against them (Parker, Burkepile & Hay 2006).

These hypotheses have been widely tested in different plant–animal systems, and results are controversial (Maron & Vilá 2001; Keane & Crawley 2002; Colautti *et al.* 2004; Levine, Adler & Yelenik 2004; Parker & Hay 2005; Liu & Stiling 2006; Parker, Burkepile & Hay 2006; Chun, van Kleunen & Dawson 2010). There is increasing evidence that richness of herbivorous insects, particularly of specialists, is greater in the native than in the introduced ranges. This apparent release from enemies, however, is only partially translated into a lower incidence of herbivory on the exotics than on the native congeners (Colautti *et al.* 2004; Liu & Stiling 2006), whereas differences in damage may not necessarily result in greater performance of the introduced species (Chun, van Kleunen & Dawson 2010). Results are especially variable and inconclusive when the incidence of herbivory in exotic and native congeners occurring in the same community is compared (community studies *sensu* Colautti *et al.* 2004). Some studies found exotics to be more damaged or more impacted in terms of fitness by herbivores than were native species (e.g. Parker & Hay 2005; Morrison & Hay 2011), whereas others found the opposite (e.g. Siemann & Rogers 2003; Hill & Kotanen 2009). These apparently contradictory results have prompted considerable research efforts during recent decades. Surprisingly little attention, however, has been paid to exploring the role played by different plant defensive strategies in explaining the observed patterns.

Plant defences against herbivores include two different and complementary mechanisms: those aiming for reducing the damage (resistance) and those aiming for reducing the impact of damage on plant fitness (tolerance). Resistance can be further divided into constitutive resistance, which is permanently expressed irrespective of the incidence of herbivores, and induced resistance, which includes traits that are activated in response to herbivore attack. All these mechanisms are considered adaptive and have evolved in response to the selection pressure imposed by the herbivores with which they have shared an evolutionary history (Núñez-Farfán, Fornoni & Valverde 2007). Although the simultaneous evolution and expression of different mechanisms can be constrained (Koricheva, Nykanen & Gianoli 2004; Leimu & Koricheva 2006), the combination of different defensive strategies appears to be quite common and advantageous, especially in trees (Leimu & Koricheva 2006). Their large size and long lifespan make trees very apparent to a wide range of herbivores, and commonly their resistance mechanisms do not completely exclude damage (Haukioja & Koricheva 2000).

Interpretation of the results from studies on invasion ecology may substantially differ if different plant defensive strategies are considered (Ashton & Lerdau 2008; Chun, van Kleunen & Dawson 2010). For instance, Lombardero, Vázquez-Majuto & Ayres (2008) found that the attack of a bark beetle was significantly greater in a native pine than in a

co-occurring exotic pine, but the impact of the insect on pine growth was notably greater in the exotic. Thus, their results support the predictions of the ERH when damage (i.e. resistance) is considered, but they agree with the predictions of the BRH when the impact of herbivores on plant fitness (i.e. tolerance) is taken into account. Native and introduced species can therefore differ in how they tolerate the damage inflicted by native enemies. However, to date, little attention has been paid to exploring how these differences may influence the likelihood of a given introduced species of becoming invasive (Ashton & Lerdau 2008; Chun, van Kleunen & Dawson 2010).

On the other hand, induced responses to herbivory caused by native insects may be elicited more strongly in native than in alien plants (Stout & Bostock 1999). There is evidence that plants have evolved sophisticated mechanisms to detect herbivore-derived molecules that act as signals of herbivore damage and mediate the specificity in the response in plant defence responses (Heil 2009; Hilker & Meiners 2010). Induced responses to the damage of native herbivores may therefore be more efficiently elicited in native plant species, which have co-evolved with the herbivore, than in exotics, which may lack mechanisms to recognize the native insect as a harmful enemy. This would imply that native herbivores could limit invasions, as suggested by the BRH, but as far as we know, no previous studies have specifically tested this appealing idea.

The aim of this paper was to explore the role of different defensive strategies in explaining the diverse impact of a harmful native insect herbivore on the native *P. pinaster* and the exotic *P. radiata*. Specifically, we analysed the differences between the two pine species in (i) constitutive resistance to this insect, by means of *in vitro* bioassays, (ii) inducibility of chemical defences elicited by chemical elicitors and insect feeding, (iii) realized resistance (constitutive plus induced) and (iv) short- and medium-term tolerance to the damage, and their genetic variation, under field conditions. Although invasive in many countries of the Southern Hemisphere, *P. radiata* is not invasive in Europe (Carrillo-Gavilán & Vilá 2010). It has been suggested that the populations of phytophages and pathogens from closely related host species in the introduced area might contribute to the inability of this species to invade in Europe, but this hypothesis remains untested. We aimed to gain insight into the implications of constitutive and induced resistance and of tolerance and resistance strategies in explaining how native herbivores might contribute to prevent an introduced species from becoming invasive.

## Materials and methods

### STUDY SYSTEM

Our study was carried out in the coastal area of Galicia (NW Spain), where two main pine species currently dominate the forested area: the native *Pinus pinaster* and the introduced *P. radiata*. The former, Maritime pine, occurs naturally in the western part of the Mediterranean basin, where it is an important forest species with high ecological and economic value. The natural distribution of *radiata* pine is restricted

to a very small area in California (USA). Nowadays, however, it is among the most widely planted tree species in the world, especially in the Southern Hemisphere (Lavery & Mead 1998), where no pine species occurs naturally, and where this pine species is considered a threatening invasive plant causing important economic and ecological problems (Richardson & Higgins 1998). In Spain, *P. radiata* was introduced in the nineteenth century, although it was not widely planted until the mid-twentieth century. Here, however, it rarely spreads from planting sites (Carrillo-Gavilán & Vilá 2010).

In Spain, both pine species coexist closely, are planted in similar sites and exhibit similar ecological habits. They are light pioneer species with high growth rates and similar dispersal strategies (Barberó *et al.* 1998; Lavery & Mead 1998). As in other sun-loving colonizer species, early growth is related to plant fitness, because only the fast growers can take their crowns to the vital sunlight and thus survive and reproduce. Because they are obligate seeders living in fire-prone regions, early reproduction is also crucial for fitness of the species (Barberó *et al.* 1998; Richardson & Higgins 1998).

In Spain, both pines are attacked by diverse insect herbivores that cause important growth reduction and mortality, especially at the seedling stage. The native large pine weevil, *Hylobius abietis*, in particular, stands out as an herbivore that directly affects pine fitness by causing extensive tree mortality at young ages (Lieuter *et al.* 2004). This pine weevil is a polyphagous herbivore that feeds on the bark and phloem of conifer seedlings and small branches of adult trees, mainly *Pinus* sp. and *Picea* sp. If the damage is intense, the wounds on the stems of young seedlings may cause stem girdling and, consequently, the loss of the leader and even the death of the seedling (Lieuter *et al.* 2004). This weevil occurs naturally in Europe and northern Asia where it is one of the most harmful pests affecting the regeneration of managed coniferous forests. Clear-felling of conifers provides ideal conditions for weevil breeding, leading to outbreaks that can severely damage the natural or artificial regeneration in the cleared area (Nordlander 1991). After 1 or 2 years, adults fly away to find new breeding sites. Although weevils can also feed on small branches of adult stands and in young stands established on areas not previously occupied by conifers, the damage inflicted on those stands, and its impact on plant fitness, is minimal (Orlander *et al.* 2000). Indeed, this insect herbivore is considered a 'silvicultural pest' that causes serious damage only after the clear-felling of large areas of conifers (Lieuter *et al.* 2004). Genetic variation in resistance to this insect has been found in both pine species (Zas *et al.* 2006, 2008), although the weevil has only coexisted with the native *P. pinaster*, not with the exotic *P. radiata* (Lieuter *et al.* 2004).

To address our research questions, we used three different and independent experimental approaches. First, we evaluated the realized resistance and tolerance to *H. abietis* damage in an extensive field genetic trial with both pine species naturally infected by the weevil, where damage, subsequent growth and other fitness correlates were assessed over 5 years. Additionally, we studied pine growth in a neighbouring identical twin trial not attacked by the weevil. Second, the preference of adult weevils for cut twigs of the two pine species was evaluated in *in vitro* feeding bioassays. Third, differences in inducibility between the two pines were evaluated by assessing the increase in concentration of chemical defences (stem resin) after exposing the pine seedlings to either chemical induction with methyl jasmonate (MeJa) or biotic induction by direct *H. abietis* feeding.

#### FIELD TRIALS

A family  $\times$  fertilization trial of *P. pinaster* and *P. radiata*, located in Rianxo (Galicia, NW Spain; 42.36° N; 8.46° W; altitude

90 m a.s.l.) and planted in March 2003, was naturally infected by the large pine weevil *H. abietis* soon after planting. The site had previously been occupied by a mature stand of *P. pinaster*, clearcut in 2002. Weevils were attracted to the cut area by the volatiles emanating from the fresh conifer stumps, where females lay their eggs (Nordlander 1991). Both the newly arrived adults and the new generation emerging from the stumps caused an intensive *H. abietis* attack on the established seedlings that lasted until the end of the second season. All the seedlings of the two trials had the appropriate size for being fed on by the weevil, with diameter distributions at the time of damage (99% confidence intervals of 4.8–16.6 and 4.4–15 mm for *P. radiata* and *P. pinaster*, respectively) within the range of twig diameters that the weevil prefers (Orlander *et al.* 2000). The spatial analysis of the damage severity across all the seedlings in the trial revealed that the infestation was spatially uniform in the experimental area (see variograms in the study by Zas *et al.* 2006, 2008).

Two adjacent independent trials were established, one where the main species was *P. radiata* and *P. pinaster* was used as a control, and the other with *P. pinaster* as the main species and *P. radiata* as the control. The experimental layout, exactly the same for both species, consisted of a split-plot design superimposed across a randomized block design with 10 blocks, with 9 fertilization treatments randomly assigned to the 9 whole plots in which each block was divided and 32 genetic entries randomly distributed within each whole plot. Fertilization (see details in the study by Zas *et al.* 2006, 2008) was not a central focus of the present study but was considered in the statistical analyses to account for the corresponding variation.

The plant material for each species consisted of open-pollinated families of mother trees from natural stands or mature plantations in the coastal area of Galicia. In the *P. pinaster* section, the 32 genetic entries included 28 half-sib families and 3 commercial seedlots of *P. pinaster* without family structure. One mixed seedlot of *P. radiata* was interspersed within the *P. pinaster* seedlings to complete the 90 main rectangular experimental units (whole plots) of 31 + 1 plants (8 columns  $\times$  4 rows). Conversely, in the *P. radiata* section, there were 30 half-sibs and 1 commercial seedlot of *P. radiata*, with a mixed seedlot of the material included in the attached *P. pinaster* experiment. In total, within each trial, 2790 seedlings of the main pine species were interspersed with 90 seedlings of the opposite species, providing a unique experimental design for studying the feeding behaviour of *H. abietis* on these species.

Plant growth (total height and basal stem diameter) was measured yearly from December 2003 to December 2007 (1–5 growing seasons after planting) in all plants of the two trials. Reproductive effort of each tree was also evaluated at age five and considered as another early fitness correlate. Reproductive effort was assessed according to Climent *et al.* (2008) as the total count of cones per tree, including the two female strobili cohorts that grow together in the crown.

*Hylobius abietis* damage was measured after the first growing season (February 2004) by evaluating the wounds in the stem caused by the insect. The stem height of each tree was divided into 10 sections; the damage was measured in each section according to a four-level wounding scale (0 = undamaged, 1 = some wounds, 2 = many wounds and 3 = full of wounds or death because of stem girdling below the given section). The weevil damage on each plant was the sum of the 10 stem sections (0–30 score, Zas *et al.* 2006). We assumed that the lower the site of the stem girdling, the smaller the amount of living tissue remaining alive, and thus the greater the damage caused to the plant. We accounted for this by assigning the maximum score to all stem sections recorded above the site of girdling.

## CAFETERIA EXPERIMENTS

Two independent *in vitro* feeding bioassays, a two-choice and a non-choice test, were performed to test for differences in the preference of the weevil for the two species. Ingestion rate (debarked area) was considered a proxy of constitutive resistance against the pine weevil between the two pine species.

Plant material for both experiments was randomly selected from the open-pollinated families included in the field trials. Seeds were sown in May 2006, and seedlings were cultivated in 2-L pots filled with 1 : 1 peat : perlite (v : v) in a greenhouse, following conventional nursery watering and fertilizing protocols. One year after they were sown, seedlings of each pine species were grouped in pairs of similar basal diameter and harvested; needles were gently removed and stems placed on ice in coolers.

For the two-choice experiment, three 2.5-cm-long twigs (upper, middle and lower thirds of the stem) from each seedling were sampled and paired with twigs belonging to the other pine species according to their relative position within the plant. Each pair of fresh cuttings was placed in an 8-cm Petri dish with a moistened filter paper and offered to one preweighed pine weevil for 48 h at room temperature (c. 22 °C). The total number of plates was 60, corresponding to 20 pairs of seedlings of similar diameter, with 3 stem twigs per seedling. Sampling several twigs from each seedling allowed us to account for the possible effects of heterogeneous levels of defences along the stems of pine seedlings.

For the nonchoice feeding test, we used 20 seedlings of each species which were grouped according to their diameter into 5 groups, with four plants in each group. We sampled 5 twigs of 2.5 cm from each seedling. Twigs of the four seedlings of each group were grouped according to their position along the stem and offered together in Petri dishes to one preweighed weevil as described earlier. This allowed us to analyse the sum of the debarked areas in the four twigs of each plate, without losing information regarding the possible effects of the seedling size and the part of the stem from which the twigs were sampled. The total number of plates was 50, corresponding to 2 species, 5 diameter groups and 5 twigs per seedling.

Pine weevils were collected at a neighbouring clear-felled *P. pinaster* forest (see the study by Moreira *et al.* 2008a) 2 weeks before the experiments and maintained at room temperature in the laboratory in plastic buckets with moistened sawdust and bark and twigs of both pine species. Sex was not considered in the bioassays because previous studies have revealed little difference in feeding behaviour between sexes (e.g. Borg-Karolson *et al.* 2006). The weevils were starved for 24 h before the test period.

After 48 h, weevils were removed and the debarked area in each twig was estimated with the aid of a transparent millimetric grid.

## INDUCTION EXPERIMENTS

To assess weevil-driven-induced responses, we covered 40 one-year-old seedlings of each pine species with a plastic net cage (plant material and culture as described earlier). We confined 1 preweighed adult weevil within the net in half of the seedlings following a completely factorial design with 20 replicates. After a 72-h feeding period, weevils were removed, debarked area was evaluated according to the same scale as in the field trials, and seedlings were harvested to analyse the concentration of resin acids in the stem. The whole stem was cut into 5-mm sections, extracted twice with hexane in an ultrasonic bath (15 min at 20 °C) and then for 24 h at room temperature, and the nonvolatile resin residue was determined gravimetrically and referred to the dry mass of each stem (Sampedro, Moreira & Zas 2011). Resin

concentration in pine stems has been shown to be inversely related to wounding by the weevils (Moreira, Sampedro & Zas 2009) and thus represents an effective resistance trait against this insect.

To check for differences between species in their inducibility potential, we evaluated the pine response in chemical defences to the exogenous application of MeJa. Methyl jasmonate is a well-known plant phytohormone involved in the signalling and triggering of induced responses after insect damage. Experimental MeJa application to conifer seedlings has been reported to induce chemical defensive responses similar to those caused by insects (Moreira, Sampedro & Zas 2009; Sampedro, Moreira & Zas 2011), and it has been shown to be very useful as a reference induction value for comparing induction capabilities to different agents (Hudgins, Christiansen & Franceschi 2004).

Twelve seedlings from each pine species were cultivated as described earlier, and during the second growth period (August 2008), half of the seedlings were sprayed with a suspension of 80 mM MeJa (cat. #39270-7; Sigma-Aldrich, St. Louis, MO, USA) in deionized water with 2.5% (v/v) ethanol, and the remaining half was treated only with the carrier solution. Both treatments were applied with a hand sprayer to runoff at an average dose of about 3 mL of solution per plant. The MeJa was applied in a different space of the greenhouse, and the seedlings remained in that isolated room for 24 h to allow the volatilization of excess MeJa. Then, all seedlings were distributed in the greenhouse according to a factorial randomized block design with six blocks. Fifteen days after the application of the induction treatments, i.e. when MeJa effects are assumed to be maximized (Martin *et al.* 2002), the plants were harvested and the nonvolatile resin content in the stem was determined as described earlier.

## STATISTICAL ANALYSES

All analyses were carried out in SAS System, using the GLIMMIX and MIXED procedures for analyses of generalized and general linear mixed models, respectively (Littell *et al.* 2006).

Damage data from the field experiments were analysed with a generalized mixed model appropriate for split-plot designs, in which the two main factors (fertilization and genetic entry) are applied to nested experimental units of different size. The dependent variable, which ranged from 0 to 30, was assumed to be similar to a count-data variable and was thus modelled with a Poisson distribution. Fertilization, genetic entry and fertilization by genetic-entry interaction were considered fixed effects. Blocks and the block  $\times$  fertilization interaction (i.e. the whole plots) were considered random factors to analyse each factor with the appropriate error term (Littell *et al.* 2006). To avoid any possible confounding effects associated with size differences between the two pine species, we also included the diameter at the time of the attack as a covariate in the model. Within each site and species, differences in consumption between the pine species by the pine weevil were analysed with specific contrasts on linear combinations of the family means, using the CONTRAST statement of the GLIMMIX procedure in SAS. Family least-squares means were derived from the generalized mixed models and used to estimate the relationship between the damage by the insect and pine survival at the family level. Survival being a clear component of pine fitness, the slope of this relationship reflects the tolerance to the insect attack (Simms 2000). How pines were able to recover growth after insect damage was also considered as a measure of tolerance to the insect. Thus, for each pine species, we estimated the relationship at the family level between damage by the insect and consecutive pine growth 1 and 5 years after the attack. Consecutive pine growth was measured as the relative growth rate in height or diameter after the attack (e.g.  $(H_5 - H_0)/H_0$ , where

$H_5$  and  $H_0$  are the pine heights in year 5 and in the year of the attack). This measure was considered to represent the relative investment of plant resources in recovering after attack. Differences in tolerance between the two pine species were estimated by testing for differences in the slope of these regressions with an analysis of covariance (ANCOVA). To double check that the differences in the slopes between the two species are not because of different ranges of weevil damage, we further confirmed the results of this analysis using only the common range of weevil damage (i.e. restricting the analyses to scores from 12 to 20).

Intraspecific genetic variation for tolerance to *H. abietis* damage was evaluated by analysing different pine fitness correlates with a general mixed model equivalent to that described before for the analysis of weevil damage, but including the damage and damage  $\times$  family interaction as covariates (Pillson 2000). A significant family  $\times$  damage interaction indicates that the effect of damage on pine fitness, that is tolerance, varies among families.

The two-choice test bioassay was analysed with a doubly repeated-measures analysis where the two dimensions of repeated measures were represented by the pair of pine seedlings (with three parts of the same subjects) and the Petri dish in which they shared the same pine weevil. Analysis was performed with a repeated-measures mixed model including the species as a between-subject factor, and the stem part and their interaction as within-subject factors. The Petri dish and the pine weevil weight were also included in the model as a random effect and a fixed covariate, respectively. The covariance structure of the repeated measures was modelled with the Huynh–Feldt structure, as it gave the lowest Akaike information criteria and was consistent with the sample schedule (Littell *et al.* 2006). The Kenward–Roger correction was used for the estimation of the degrees of freedom, as recommended by Littell *et al.* (2006, p. 188).

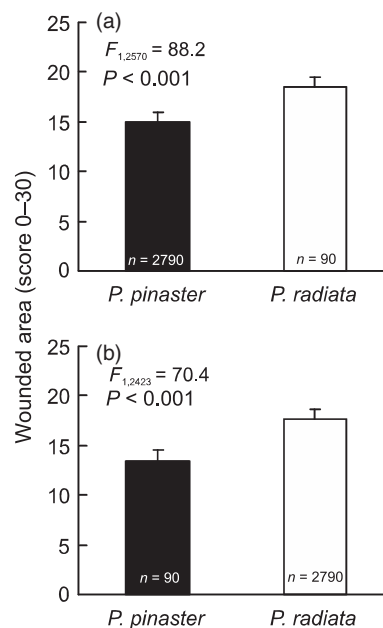
The nonchoice experiment was analysed with a repeated-measure mixed model, where the five stem parts are repeated measures of the same subjects. The mixed model was fitted for the sum values of the four wounding-area values per plate, and the pine weevil weight was included as a covariate. A first-order autoregressive covariance structure was used to model the covariance of the repeated measures. Degrees of freedom were again estimated by the Kenward–Roger method.

The induction of chemical defences by either weevil feeding or MeJa application was analysed with a factorial ANCOVA using the debarked area as covariate and assuming all effects (species, induction treatment and their interaction) as fixed factors.

## Results

### WEEVIL DAMAGE ON THE TWO PINE SPECIES

Results from the field trials indicate that *H. abietis* fed significantly more on *P. radiata* than on *P. pinaster* seedlings. The *P. radiata* seedlings growing interspersed within the *P. pinaster* trial were attacked the most (Fig. 1a; see also Table S1 in Supporting Information), and they were significantly more consumed than 24 of the 31 *P. pinaster* genetic entries in the trial (data not shown). Consistently, the *P. pinaster* seedlings growing interspersed within the *P. radiata* trial were significantly less attacked (Fig. 1b, Table S1 in Supporting Information), and 26 of the 31 *P. radiata* genetic entries were significantly more damaged than the control *P. pinaster* seedlings (data not shown).

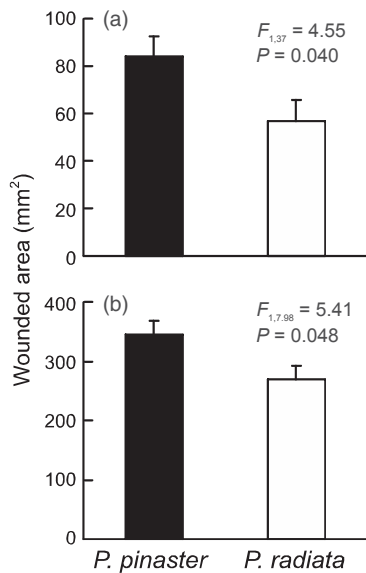


**Fig. 1.** Realized resistance of the native *Pinus pinaster* (black bars) and the exotic *P. radiata* (white bars) to the pine weevil *Hylobius abietis* in the field conditions. The pine weevil consumed more on the exotic *P. radiata* than on the native *P. pinaster*, both when *P. radiata* was planted surrounded by *P. pinaster* (a), and when *P. pinaster* was planted surrounded by *P. radiata* (b). The  $F$  ratio and associated  $P$ -values are presented for the specific contrast between species within the generalized mixed model (see also Table S1 in Supporting Information); sample size is indicated within each bar. Diameter did not differ between species.

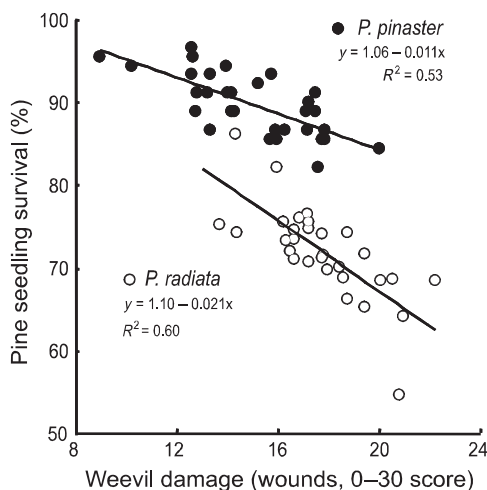
However, pine weevils preferred *P. pinaster* (the native) more than *P. radiata* (the exotic) twigs when both species were offered as cut material in Petri dishes (Fig. 2, Table S2 in Supporting Information). Weevil feeding was significantly more intense in *P. pinaster* than in *P. radiata*, both in the two-choice (Fig. 2a, Table S2A in Supporting Information) and in the nonchoice (Fig. 2b, Table S2B in Supporting Information) experiments.

### SHORT-TERM TOLERANCE TO PINE WEEVIL DAMAGE

The relationship at the family level between actual damage and subsequent survival in the field was negative and very strong in both species (Fig. 3). Damage by the pine weevil explained more than 50% of the family variation in survival. The slopes significantly differed between the two pine species (ANCOVA  $F_{1, 58} = 9.34$ ,  $P = 0.003$ ), with a steeper relation in the case of *P. radiata*, indicating that the native *P. pinaster* was more tolerant of the damage than the exotic *P. radiata* (Fig. 3). Results remained the same when the analysis was restricted to the common range of damage scores for the two species (scores 12–20; ANCOVA  $F_{1, 51} = 4.53$ ,  $P = 0.038$ ). The relationships with survival at age 5 followed the same trends, confirming the relevance of the early attack as a determinant of medium-term mortality.



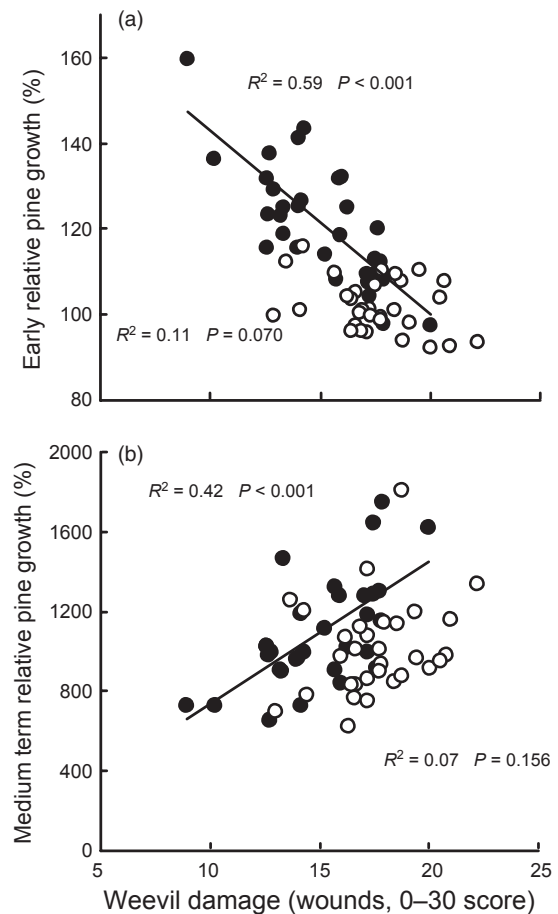
**Fig. 2.** Preference of the native large pine weevil *Hylobius abietis* for the native *Pinus pinaster* (black bars) and the exotic *P. radiata* (white bars) evaluated in two independent *in vitro* cafeteria experiments: a two-choice (a) and a nonchoice (b) feeding tests (see complete results of the corresponding mixed models in Table S2 in Supporting Information).  $N = 60$  and  $N = 25$ , respectively. Bars represent the debarked area consumed by adult individuals in 2.5-cm-long twigs of both tree species. Twig diameter was used as covariate. Least-squares means  $\pm$  SE.



**Fig. 3.** Short-term tolerance to *Hylobius abietis* damage as revealed by the family relationship between *H. abietis* damage and pine survival 1 year after the insect attack for *Pinus pinaster* (filled dots) and *P. radiata* (open dots). Seedlings were planted in the same field trial, naturally and homogeneously infected by the pine weevil. Both relationships were significant at  $P < 0.001$ . Each dot is an open-pollinated family least squares mean ( $N = 90$ ).

#### MEDIUM-TERM TOLERANCE TO THE PINE WEEVIL DAMAGE

The family relationship between insect damage and the immediate consecutive pine growth in *P. pinaster* was significant



**Fig. 4.** Family relationship between *Hylobius abietis* damage and subsequent pine growth in *Pinus pinaster* (black dots) and *P. radiata* (open dots). (a) Early relative pine growth is the relative diameter growth rate 1 year after the insect attack  $((D_1 - D_0)/D_0)$ , where  $D_0$  and  $D_1$  are the stem pine diameter in the year of the attack and 1 year after weevil damage. (b) Medium-term compensatory growth, measured as the relative growth rate 5 years after the attack  $((H_5 - H_0)/H_0)$ , where  $H_5$  and  $H_0$  are pine height at age 5 and at the year of insect attack, respectively. Seedlings were planted in the same field trial naturally and homogeneously infected by the pine weevil. Each dot is an open-pollinated family least-squares mean ( $N = 90$ ).

and negative (Fig. 4a). Pine growth after insect damage was less in those families that were more damaged and greater in those where the damage was less, indicating that the insect attack disrupted regular growth. This relationship, which could be interpreted as early growth costs of tolerance or induced resistance, was not significant in the exotic *P. radiata* (Fig. 4a), where differences in consecutive family growth appeared to be unaffected by the damage level. Figure 4b summarizes the medium-term vegetative tolerance, that is, the potential of each family for height recovery 5 years after the insect attack, measured as 5- to 1-year relative growth regressed against the early damage. Relative compensatory effort in the 5 years following attack was greater in the more damaged families of *P. pinaster*, whereas we found no significant response in *P. radiata*.

Comparing the growth patterns in the studied stands with those in two neighbouring twin trials of the two pine species

that remained free of weevil damage also suggested that the native pine was better able to recover from weevil damage (see Appendix S1 in Supporting Information). The negative effect of the weevil damage on pine growth disappeared at age five in *P. pinaster*, but still remained significant in *P. radiata*, suggesting a compensatory growth in the native but not in the exotic.

#### GENETIC VARIATION IN TOLERANCE

Growth and survival during the period studied were strongly determined by the damage produced by the pine weevil at age 1, as evidenced by the significant covariation of the initial damage on the studied variables in the ANCOVA analyses (Table 1). We found evidence of genetic variation for tolerance to *H. abietis* in *P. pinaster*, but not in *P. radiata*. In the case of *P. pinaster*, the damage  $\times$  family interaction was marginally significant for pine survival after the insect damage, representing the proximal effects of the attack, and significant for pine height at age 5, suggestive of vegetative compensatory effort and opportunity fitness. However, the covariation of weevil damage on the early reproductive effort did not vary among the *P. pinaster* families (Table 1). We did not detect genetic variation in tolerance in *P. radiata* in any case, nor was there covariation of weevil damage with early reproductive effort in this species. Radiata pine families survived and grew similarly irrespective of the level of damage suffered, whereas early cone production was not affected by insect damage.

#### INDUCED RESPONSE OF PINE TO WEEVIL DAMAGE

We did not detect significant differences between species in the constitutive resin content in control plants (Fig. 5a). After exposure to insect feeding for 72 h, both species reacted to the weevil damage by increasing the resin concentration in the whole stem. However, the quantitative response in resin differed between the two species (significant species  $\times$  induction interaction). The response of the native *P. pinaster* was 2-fold that of the exotic pine, even though no significant differences were observed in the wounding intensity between the species ( $F_{1,27} = 1.2$ ,  $P = 0.274$ ).

The application of MeJa also significantly increased the resin content in the two pine species. The response to MeJa application was, however, similar in the two pines (no significant species  $\times$  MeJa interaction, Fig. 5b). Thus, the resin content in the stem of both constitutive and MeJa-induced plants did not differ between species, suggesting similar physiological capabilities to express MeJa elicited induction.

## Discussion

#### INDUCED RESISTANCE AND BIOLOGICAL INVASIONS

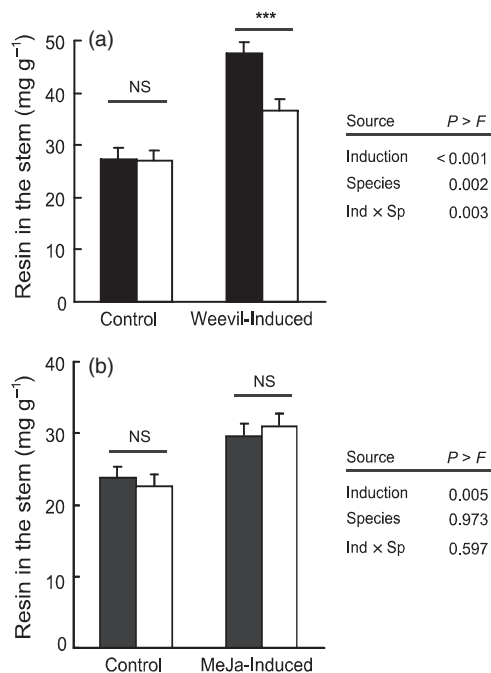
In contrast to the expectations of the BRH, the large pine weevil consumed more on the native *P. pinaster* than on the exotic *P. radiata* when both species were offered as cut material in *in vitro* bioassays. As cut stem twigs are unable to respond to the damage caused by the weevils, results of the *in vitro* bioassays likely reflect the variation in preference because of constitutive resistance between the two species, which appeared to be higher in the exotic than in the indigenous pine species. However, under field conditions, damage by the pine weevil was clearly greater on the exotic than on the native species, suggesting lower effective resistance of the former. Both species were planted simultaneously, and the pine weevil attack started early after planting and lasted several months until weevil damage was assessed. During this time, weevils were continuously feeding on the pine seedlings, which should have had enough time to respond by activating induced resistance mechanisms. As an evidence of this, the resin canal density in the xylem of the *P. pinaster* seedlings in the attacked stand was nearly twice as high as that found in the nearby unattacked stand; the differences were attributed to the defensive response induced by the weevil damage (Moreira *et al.* 2008b).

The results of our induction experiment revealed large differences between the species in the induction of resin in the stem after wounding. Although the damage inflicted by the weevil during the induction experiment did not differ between pine species, the increment in the resin content in the indigenous species just 72 h after insect damage was more than 2-fold than in the nonindigenous species. Moreover, differences between species in this main resistant trait were only significant

**Table 1.** Tolerance and genetic variation in tolerance to *Hylobius abietis* damage in *Pinus pinaster* and *P. radiata*. The table shows the  $F$ -values and corresponding  $P$  values of the mixed model for the covariation of the initial weevil damage (wounds) and damage  $\times$  family interaction with several components of pine fitness. A significant damage  $\times$  family interaction indicates that the effect of damage on the subsequent fitness, that is, tolerance, varied between families

Fitness component	<i>Pinus pinaster</i>				<i>Pinus radiata</i>			
	Wounds at age 1		Wounds $\times$ family		Wounds at age 1		Wounds $\times$ family	
	$F_{1,1876}^*$	$P > F$	$F_{30,1876}^*$	$P > F$	$F_{1,1244}^*$	$P > F$	$F_{30,1244}^*$	$P > F$
Survival after attack	340.5	< 0.001	1.5	0.051	583.5	< 0.001	0.8	0.737
Height at year 5	152.3	< 0.001	1.5	<b>0.031</b>	17.5	< 0.001	1.3	0.133
Cone production year 5	16.5	< 0.001	0.8	0.801	0.1	0.819	0.9	0.587

\*Denominator degrees of freedom in the case of survival were 2394 for *P. pinaster* and 2262 for *P. radiata*. Significant  $P$  values ( $P < 0.05$ ) are in bold.



**Fig. 5.** Inducibility of pine chemical resistance elicited by the pine weevil (a) and by the exogenous application of methyl jasmonate (MeJa) (b) in *Pinus pinaster* (black bars) and *P. radiata* (white bars) seedlings. *P* values in the associated tables resume the results from the mixed models. Both species responded significantly to both chemical and biotic induction. Although response to MeJa was similar, response to weevil feeding markedly differed between the two species. Within each induction treatment (Control, Induced) differences between species are indicated by the associated significance levels (n.s. = no significant; \*\*\**P* < 0.001). Least-squares means  $\pm$  SE of two independent experiments are shown: *n* = 20 in (a) and *n* = 6 in (b).

after exposure to the insect. Conversely, the response to the application of MeJa did not differ between the species, suggesting similar MeJa-inducible physiological capabilities (i.e. inducibility potential) between the two species. Based on the differences between the response to the pine weevil and to the MeJa application, we infer, as predicted by the BRH, that the native pine species is better adapted to this local insect, being able to elicit a stronger induced resistance mechanism that likely will deter further damage. These results suggest that because of the greater induced resistance in the native species, the initial preference of the insect for the native pine could switch with time towards greater damage on the exotic, as observed in the field. The lack of preference between the two pine species shown by the weevil in the induction experiment does not conflict with this idea, as the period of time that the weevils were feeding on the seedlings (just 72 h) was probably insufficient for the induced defences to become effective against the weevil.

Induced resistance is a key strategy for defending *Pinus* sp. against bark beetles and phloem feeders (Lieuter *et al.* 2004; Franceschi *et al.* 2005) and varies greatly among species of the Pinaceae (Hudgins, Christiansen & Franceschi 2004). Plants are known to be able to recognize specific cues of their enemies and elicit the appropriate defensive mechanisms (Heil 2009;

Hilker & Meiners 2010). Thus, plant species can respond specifically to different enemies (Lieuter 2002), with larger responses of the trees to the enemies that normally attack them (Raffa 1991). Results presented here are in concordance with this cue specificity of the induced responses, which were more intensely elicited in the pine species with a common evolutionary history with the enemy.

Empirical evidences of the ability of plants to recognize different herbivorous species and elicit differential responses against them are, however, equivocal (Heil 2009). For example, the specificity of the tree response to the bark beetle–fungus association may be more related to protein–protein interactions with the fungus species than to plant recognition of the insect species (Karban & Kuc 1999). *Hylobius abietis* could also vector different native pathogenic fungus species to which *P. radiata* may be not adapted, but information on the transmission of diseases by weevils is very limited (Lieuter *et al.* 2004).

Although other factors (e.g. the environmental and ontogenetic differences between the field trials and the bioassays with greenhouse potted plants) may have differentially affected the results, our findings indicate that the intensity and/or rapidity of the induced responses to a native insect can differ between native and non-native host species. If induced defences are more specific mechanisms than constitutive resistance (Franceschi *et al.* 2005), the differences in inducibility between native and exotic species may be relevant for understanding the role of local enemies in invasion ecology. The lower inducibility of the exotic pine species supports the predictions of the BRH (Elton 1958), as it could imply greater damage in the field, which could contribute towards preventing the exotic pine from becoming invasive in the area. As pointed out by Orians & Ward (2010), however, little attention has been paid to the implications of induced resistance in invasion ecology. To date, only two studies have examined induced defences in exotic plants, comparing patterns of inducible defences in native and introduced ranges (Cipollini *et al.* 2005; Eigenbrode *et al.* 2008). These studies explore how induced defences have evolved after the relaxation of herbivore pressure in the exotic range, using either chemical elicitors or mechanical wounding to assess inducibility. However, responses to specific herbivore species can potentially differ from those to artificial induction treatments (as observed in the present study), and these differences could be highly relevant in the context of invasion ecology. To our knowledge, results presented here are the first reporting how failure to elicit effective induced responses against local herbivores might be a factor in impeding an introduced species from becoming invasive.

#### TOLERANCE IN THE CONTEXT OF BIOLOGICAL INVASIONS

Differences in tolerance to herbivore damage can also be important in interpreting the invasion ecology hypotheses (Ashton & Lerda 2008; Chun, van Kleunen & Dawson 2010). Besides the release from specialist enemies, the ERH also assumes a lower impact of generalist enemies on the introduced



species, because native consumers will be better adapted to consume native, rather than exotic hosts (Keane & Crawley 2002). According to the BRH, however, the exotic hosts may be either more heavily damaged by the resident herbivores than native plants or just poorly adapted to tolerate the damage caused by novel enemies with which they lack an evolutionary history (Parker & Hay 2005). This idea is well accepted among foresters, who commonly consider that trees can be more susceptible to pests and pathogens when they are planted off-site, i.e. outside the soil, climate system and biotic interactions to which they are adapted (Lombardero, Vázquez-Majuto & Ayres 2008).

In support of the BRH, we found that the native pine was more tolerant of the coevolved herbivore than the exotic pine, suggesting no fitness advantage of the exotic pine species in its introduced area. For the same level of insect damage, survival of *P. pinaster* families was higher than that of *P. radiata*, indicating a lower impact of the herbivore on the former. Fitness evaluation is especially complicated in forest trees with long lifespans and reproduction. However, early survival is a clear component of pine fitness, and thus the relationship between survival and damage can be utilized to assess tolerance to the attack. Early growth in light-demanding species such as the pines studied is another component of plant fitness, which evaluates the opportunity of a tree to arrive to the dominant stratus, and thus reproduce and contribute to the next generation. Slow-growing pines are expected to be suppressed by dominant trees and die. Considering early growth, the native *P. pinaster* appeared to be, again, more tolerant than the exotic *P. radiata*. Although the pine weevil reduced the expected regular size of *P. pinaster* during the immediate following years after the attack, the positive relationship between initial weevil damage and relative medium-term growth reveals that this pine species was able to likely compensate after just 5 years. In contrast, in *P. radiata*, neither immediate consecutive growth nor medium-term relative growth was related to initial damage, suggesting no compensatory growth capability in the exotic pine. The differences in the growth trends in the attacked and unattacked stands were in concordance with this finding, although the results from this comparison should be interpreted with care as the two sites may not have been equally suitable for the two pine species (see Appendix S1 in Supporting Information).

The detection of intraspecific genetic variation in tolerance of *P. pinaster* to the insect supports the possibility of evolution of tolerance traits as an adaptation to this (and other) local insects. The cost–benefits balance, together with the temporal and spatial heterogeneity in the selective pressure imposed by these (and other) insects, would explain why these apparent adaptive traits are not fixed within the native population and genetic variation for tolerance is maintained.

The lower tolerance of the exotic pine species suggest poor adaptation to its new range, which is in consonance with the predictions of the BRH. In a recent study, Lombardero, Vázquez-Majuto & Ayres (2008), studying the impact of the local bark beetle *Tomicus piniperda* on the same pine species in the same region, also found the native *P. pinaster* to be more toler-

ant than the exotic *P. radiata*. Although the insect damage was more than twice as great in the native *P. pinaster* as in the exotic *P. radiata*, the productivity of the exotic was more adversely affected, suggesting higher tolerance of the native species. The results were consistent across a wide range of tree ages and beetle incidence levels. Additionally, productivity of the non-native *P. radiata* was further reduced by the pathogen *Sphaeropsis sapinea*, a fungus that seems to be associated with *T. piniperda* and was only present in the exotic pine.

Few studies have considered the importance of tolerance in invasion ecology (Chun, van Kleunen & Dawson 2010). After the formulation of the evolution of increased competitive ability (EICA) hypothesis (Blossey & Notzold 1995), however, it has been postulated that the invasiveness of exotic species might be related to increased tolerance of the introduced populations as a result of a relaxation in herbivore pressure in the new range (Stastny, Schaffner & Elle 2005; Zou, Rogers & Siemann 2008). However, *P. radiata* was introduced in Spain very few generations ago, so few opportunities to evolve tolerance are expected in this species. Besides, the lack of genetic variation in tolerance found in the present study would further constrain the possibility to evolve towards higher tolerance. As stated by Lombardero, Vázquez-Majuto & Ayres (2008), the most obvious implications of EICA in forestry would be for tree breeding programmes, because natural selection is constrained in trees by their long generation times.

In summary, we found that *P. pinaster* and *P. radiata* coexisting in NW Spain differed markedly in their responses to a native insect herbivore. Although the insect intrinsically preferred the native pine species with which it shared an evolutionary history, the native *P. pinaster* responded more strongly to the attack and was more tolerant of it than the exotic *P. radiata*. Thus, in this area, the exotic *P. radiata* shows no advantages compared with the native pine against this enemy. These findings are in agreement with the fact that *P. radiata* is not invasive in this area (Carrillo-Gavilán & Vilá 2010). Other authors have already suggested that the failure of invasions by alien conifers in Central Europe might be, among other reasons, because of the presence of pathogens and phytophages in the introduced range because of the presence of native conifer congeners (Adamowski 2004; Carrillo-Gavilán & Vilá 2010). Indeed, exotic conifers in Europe are known to be colonized by as much herbivore insect species as native conifers (Bertheau *et al.* 2009), and the majority of native insects species that colonize alien trees are generalist herbivores (Bertheau *et al.* 2010). Additionally, there is some evidence that herbivory by generalist vertebrates might limit the invasiveness of *P. radiata* in some areas of the Southern Hemisphere (Becerra & Bustamante 2008). However, that *P. radiata* might show signs of invasiveness in Europe in the absence of herbivory and that this pine has become invasive in other parts of the world because of escape from herbivory remain untested. Based on the results presented here, the weak ability to elicit induced defences and the low tolerance of the exotic species against native herbivores appeared as key concepts for understanding how native enemies may contribute to limit the invasiveness potential of exotic plants, as stated by the BRH. Further stud-

ies are needed to confirm this by testing whether *P. radiata* may show signs of invasiveness in the absence of herbivory in Europe.

## Acknowledgements

Research was supported by the grants RTA2007-100 and AGL2010-18724. L.S. and X.M. received financial support from DOC-INIA and PREDOC-INIA grant programs, respectively. We thank field technicians from the Lourizán Center (Xunta de Galicia) for their help in field trial establishment and maintenance. We thank Rodrigo Costas and Ana Soliño for assistance with the bioassays, and Patricia Martins, Diana Blanco, Beatriz Rodríguez-Morales, Sara Varela and Benito Santos for their valuable help in field assessments. Comments and suggestions by Dr. María Josefa Lombardero, Prof. Matthew Ayres and two anonymous referees helped to improve the manuscript. We are also very grateful for the exhaustive language edition by Prof. Alison Moldenke and David Brown.

## References

- Adamowski, W. (2004) Why don't alien conifers invade the Białowieża Forest? *Weed Technology*, **18**, 1453–1456.
- Ashton, I.-W. & Lerdau, T. (2008) Tolerance to herbivory, and not resistance, may explain differential success of invasive, naturalized, and native North America temperate vines. *Diversity and Distributions*, **14**, 169–178.
- Barberó, M., Loisel, R., Quézel, P., Richardson, D.M. & Romane, F. (1998) Pines of the Mediterranean Basin. *Ecology and Biogeography of Pinus* (ed. D.M. Richardson), pp. 153–170. Cambridge University Press, Cambridge, UK.
- Becerra, P.I. & Bustamante, R.O. (2008) The effect of herbivory on seedling survival of the invasive exotic species *Pinus radiata* and *Eucalyptus globulus* in a Mediterranean ecosystem of Central Chile. *Forest Ecology and Management*, **256**, 1573–1578.
- Bertheau, C., Salle, A., Rossi, J.P., Branhead, S., Pineau, X., Roux-morabito, G. & Lieutier, F. (2009) Colonisation of native and exotic conifers by indigenous bark beetles (Coleoptera: Scolytinae) in France. *Forest Ecology and Management*, **258**, 1619–1628.
- Bertheau, C., Brockerhoff, E.G., Roux-Morabito, G., Lieutier, F. & Jactel, H. (2010) Novel insect-tree associations resulting from accidental and intentional biological 'invasions': a meta-analysis of effects on insect fitness. *Ecology Letters*, **13**, 506–515.
- Blossey, B. & Notzold, R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants – a hypothesis. *Journal of Ecology*, **83**, 887–889.
- Borg-Karlson, A.-K., Nordlander, G., Mudalige, A., Nordenhem, H. & Unelius, C.R. (2006) Antifeedants in the feces of the Pine Weevil *Hylobius abietis*: identification and Biological Activity. *Journal of Chemical Ecology*, **32**, 943–957.
- Carrillo-Gavilán, M.A. & Vilá, M. (2010) Little evidence of invasion by alien conifers in Europe. *Diversity and Distributions*, **16**, 203–213.
- Catford, J.A., Jansson, R. & Nilsson, C. (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions*, **15**, 22–40.
- Chun, Y.J., van Kleunen, M. & Dawson, W. (2010) The role of enemy release, tolerance and resistance in plant invasions: linking damage to performance. *Ecology Letters*, **13**, 937–946.
- Cipollini, D., Mbagwu, J., Barto, K., Hillstrom, C. & Enright, S. (2005) Expression of constitutive and inducible defenses in native and invasive populations of *Alliaria petiolata*. *Chemical Ecology*, **31**, 1255–1267.
- Climent, J., Aránzazu-Prada, M., Calama, R., Chambel, R., Sánchez-de-Ron, D. & Alía, R. (2008) To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis*, Pinaceae). *American Journal of Botany*, **95**, 833–842.
- Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, **7**, 721–733.
- Eigenbrode, S.D., Andreas, J.E., Cripps, M.G., Ding, H., Biggam, R.C. & Schwarzländer, M. (2008) Induced chemical defenses in invasive plants: a case study with *Cynoglossum officinale* L. *Biological Invasions*, **10**, 1373–1379.
- Elton, C.S. (1958) *The Ecology of Invasions by Animals and Plants*. University of Chicago Press, Chicago.
- Franceschi, V.R., Krokene, P., Christiansen, E. & Krekling, T. (2005) Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist*, **167**, 353–375.
- Haukioja, E. & Koricheva, J. (2000) Tolerance to herbivory in woody vs. herbaceous plants. *Evolutionary Ecology*, **14**, 551–562.
- Heil, M. (2009) Damage-self recognition in plant herbivore defence. *Trends in Plant Science*, **14**, 356–363.
- Hilker, M. & Meiners, T. (2010) How do plants "notice" attack by herbivorous arthropods. *Biological Reviews*, **85**, 267–280.
- Hill, S.B. & Kotanen, P.M. (2009) Evidence that phylogenetically novel non-indigenous plants experience less herbivory. *Oecologia*, **161**, 581–590.
- Hudgins, J.W., Christiansen, E. & Franceschi, V.R. (2004) Induction of anatomically based defense responses in stems of diverse conifers by methyl jasmonate: a phylogenetic perspective. *Tree Physiology*, **24**, 251–264.
- Karban, R. & Kuc, J. (1999) Induced resistance against pathogens and herbivores: an overview. *Induced Plant Defenses Against Pathogens and Herbivores* (eds A.A. Agrawal, S. Tuzun & E. Benti), pp. 1–16. The American Phytopathological Society, St. Paul, Minnesota, USA.
- Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, **17**, 164–170.
- Koricheva, J., Nykanen, H. & Gianoli, E. (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? *The American Naturalist*, **163**, E64–E75.
- Lavery, P.B. & Mead, D.J. (1998) *Pinus radiata*: a narrow endemic from North America takes the world. *Ecology and Biogeography of Pinus* (ed D.M. Richardson), pp. 432–449. Cambridge University Press, Cambridge, UK.
- Leimu, R. & Koricheva, J. (2006) A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos*, **112**, 1–9.
- Levine, J.M., Adler, P.B. & Yelenik, S.G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, **7**, 975–989.
- Lieutier, F., Day, K.R., Battisti, A. & Gregoire, J.C. (2004) *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer Academic Publishers, The Netherlands.
- Lieutier, F. (2002) Mechanisms of resistance in conifers and bark beetle attack strategies. *Mechanisms and Deployment of Resistance in Trees to Insects* (eds M.R. Wagner, K.M. Clancy, F. Lieutier & T.D. Paine), pp. 31–77. Kluwer Academic Press, Dordrecht, The Netherlands.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. & Schabenberger, O. (2006) *SAS System for Mixed Models*, 2nd edn. SAS Institute, Cary, North Carolina, USA.
- Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, **8**, 1535–1545.
- Lombardero, M.J., Vázquez-Majuto, P. & Ayres, M.P. (2008) Role of plant enemies in the forestry of indigenous versus nonindigenous pines. *Ecological Applications*, **18**, 1171–1181.
- Maron, J.L. & Vilá, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypothesis. *Oikos*, **95**, 361–373.
- Martin, D., Tholl, D., Gershenzon, J. & Bohlmann, J. (2002) Methyl jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. *Plant Physiology*, **129**, 1003–1018.
- Moreira, X., Sampedro, L. & Zas, R. (2009) Defensive responses of *Pinus pinaster* seedlings to exogenous application of methyl-jasmonate: concentration effect and systemic response. *Environmental and Experimental Botany*, **67**, 94–100.
- Moreira, X., Costas, R., Sampedro, L. & Zas, R. (2008a) A simple method for trapping *Hylobius abietis* (L.) alive in northern Spain. *Investigación Agraria. Sistemas y Recursos Forestales*, **17**, 188–192.
- Moreira, X., Sampedro, L., Solla, A. & Zas, R. (2008b) Alterations of the resin canal system of *Pinus pinaster* seedlings after fertilization of a healthy and of a *Hylobius abietis* attacked stand. *Trees Structure and Function*, **22**, 771–777.
- Morrison, W.E. & Hay, M.E. (2011) Herbivore preference for native vs. exotic plants: generalist herbivore from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS ONE*, **6**, e17227.
- Nordlander, G. (1991) Host finding in the pine weevil *Hylobius abietis*: effects of conifer volatiles and added limonene. *Entomologia Experimentalis et Applicata*, **59**, 229–237.
- Núñez-Farfán, J., Fornoni, J. & Valverde, P.L. (2007) The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 541.
- Orians, C.M. & Ward, D. (2010) Evolution of plant defenses in nonindigenous environments. *Annual Review of Entomology*, **55**, 439–459.

- Orlander, G., Nordlander, G., Wallertz, K. & Nordenhem, H. (2000) Feeding in the crowns of Scots pine trees by the pine weevil *Hyllobius abietis*. *Scandinavian Journal of Forest Research*, **15**, 194–201.
- Parker, I.M., Burkepile, D.E. & Hay, M.E. (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science*, **311**, 1459–1461.
- Parker, J.D. & Hay, M.E. (2005) Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters*, **8**, 959–967.
- Pilson, D. (2000) The evolution of plant response to herbivory: simultaneous consideration of resistance and tolerance in *Brassica rapa*. *Evolutionary Ecology*, **14**, 457–489.
- Raffa, K.F. (1991) Induced defensive reactions in conifer-bark beetle systems. *Phytochemical Induction by Herbivores* (eds D.W. Tallamy & M.J. Raupp), pp. 245–276. John Wiley, New York, USA.
- Richardson, D.M. & Higgins, S.I. (1998) Pines as invaders in the southern hemisphere. *Ecology and Biogeography of Pinus* (ed. D.M. Richardson), pp. 450–474. Cambridge University Press, Cambridge, UK.
- Sampedro, L., Moreira, X. & Zas, R. (2011) Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *Journal of Ecology*, **99**, 818–827.
- Siemann, E. & Rogers, W.E. (2003) Herbivory, disease, recruitment limitation, and success of alien and native tree species. *Ecology*, **84**, 1489–1505.
- Simms, E.L. (2000) Defining tolerance as a norm of reaction. *Evolutionary Ecology*, **14**, 563–570.
- Stastny, M., Schaffner, U. & Elle, E. (2005) Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness?. *Journal of Ecology*, **93**, 27–37.
- Stout, M.J. & Bostock, R.M. (1999) Specificity of induced responses to arthropods and pathogens. *Induced Plant Defenses Against Pathogens and Herbivores* (eds A.A. Agrawal, S. Tuzun & E. Bent), pp. 183–210. APS Press, St. Paul, Minnesota, USA.
- Zas, R., Sampedro, L., Prada, E., Lombardero, M.J. & Fernández-López, J. (2006) Fertilization increases *Hyllobius abietis* L. damage in *Pinus pinaster* Ait. seedlings. *Forest Ecology and Management*, **222**, 137–144.
- Zas, R., Sampedro, L., Moreira, X. & Martins, P. (2008) Effect of fertilization and genetic variation on susceptibility of *Pinus radiata* seedlings to *Hyllobius abietis* damage. *Canadian Journal of Forest Research*, **38**, 63–72.
- Zou, J., Rogers, W.E. & Siemann, E. (2008) Increased competitive ability and herbivory tolerance in the invasive plant *Sapium sebiferum*. *Biological Invasions*, **10**, 291–302.

Received 15 March 2011; accepted 28 June 2011

Handling Editor: Ray Callaway

## Supporting Information

Additional supporting information may be found in the online version of this article:

**Appendix S1.** Medium-term growth trends of both pine species in the attacked and the healthy stands.

**Table S1.** Results of the generalized linear mixed model for the analysis of the damage by *H. abietis* in the *P. pinaster* and *P. radiata* field trials.

**Table S2.** Results of the mixed model for the analysis of the debarked area in (A) the two-choice feeding experiment and (B) the nonchoice feeding experiment.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

**Appendix S3.** Medium-term growth trends of both pine species in the attacked and the healthy stands are consistent with the differences in tolerance observed when analyzing only the attacked stand.

When analyzing growth of attacked pines in the Rianxo trials, we observed a significant positive family relationship between damage by the weevil and subsequent growth rates for *P. pinaster* but not for *P. radiata*, suggesting a compensatory growth in the former but not in the later (see Fig. 4 in main text). In this Appendix we compare the growth of both pine species in the attacked stands with that in a twin neighbouring trial of each pine species that was not attacked by the weevil.

The two twin family  $\times$  fertilization trials were located in Rebordelo (42.46° N; 8.48° W), just 30 km away from the Rianxo trials. As for the attacked stands, there were two neighbouring independent trials, one where the main species was *P. radiata* and *P. pinaster* was used as a control, and the other with *P. pinaster* as the main species and *P. radiata* as the control. The four trials (two in Rianxo and two in Rebordelo) were planted at the same time, with exactly the same plant material and the same experimental design (see Material and Methods within the main text). Climatic and edaphic conditions were almost the same in the two locations (see details in Moreira et al. 2008 and Sampedro et al 2009). Before trial establishment, the site at Rebordelo was occupied by shrubs, mainly *Ulex europaeus*, whereas the trials in Rianxo were established on a clear-felled area within a mature *P. pinaster* stand. In Rianxo there were fresh conifer stumps that greatly attract *H. abietis* for breeding. The two trials at Rebordelo, on the contrary, remained completely free of these harmful insects. We measured height growth in all plants of the four trials during the first five years after planting. For each pine species, height growth in the attacked and the healthy stands was compared with the aid of a t-test analysis (Fig. 1).

Growth differences between the two *P. pinaster* stands were only significant in the years immediately following the attack, and they disappeared at age 5 (Fig S1.a). On the contrary, growth differences between the attacked and the healthy *P. radiata* stands were significant throughout the period studied, with height differences tending to increase with time (Fig S1.b). These results were consistent with our findings when analyzing the growth trends in both pine species only in the attacked stand, and support that the native *P. pinaster* was more tolerant to the pine weevil than the exotic *P. radiata*.

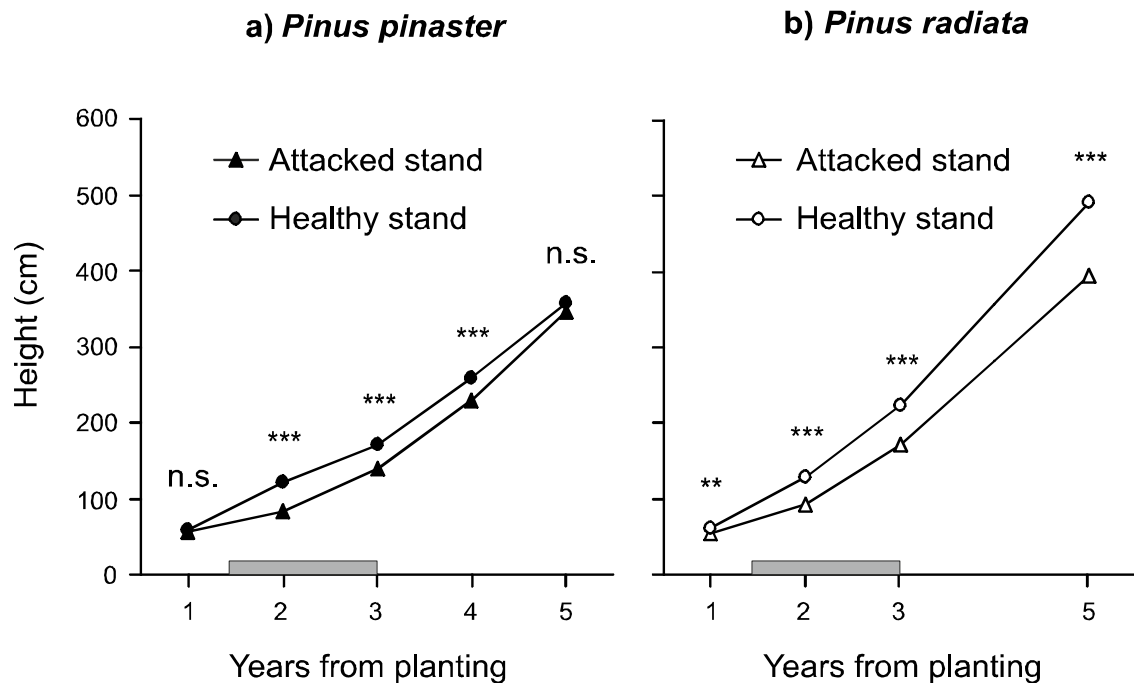


Figure S1. Height growth during five years after planting of (a) *P. pinaster* and (b) *P. radiata* in two twin neighboring trials, one attacked by the pine weevil *H. abietis* (circles) and one unattacked (triangles). The comparison between sites is consistent with the observed differential medium-term tolerance to *H. abietis* between the two species. Gray bars along the x-axes denote the period during which weevils were feeding on the plants. Differences between the attacked and the healthy sites within each year are indicated by asterisks. Significance levels: \*\*\* =  $p < 0.001$ , \*\* =  $p < 0.01$ , n.s. = no significant.  $N = 2790$  trees for each species in each site.

However, due the limitations of this ‘natural experiment’ that compares only two stands (attacked vs non-attacked), results from the comparison in this Appendix are simply confirmatory, not conclusive, because they rely on the assumption that, for each species, the

two sites would have produced equivalent growth in the absence of insect attacks (Sampedro et al., 2009). Alternatively, the attacked stand could have a higher site index for *P. pinaster*, whereas the healthy stands may be located in a better site for *P. radiata*. The comparison of stem heights at year one could, indeed, suggest this, as mean initial height significantly differ between the attacked and the healthy stands of *P. radiata* (Figure S1). However, the comparison of heights at year one should be interpreted with care as height measurements in the attacked stands were strongly altered by the leader loss of many pine seedlings due to stem girdling by weevil feeding. Based on our experience, and taking into account the climatic and edaphic data of the two sites, as well as the site index comparisons of neighbour pine stands (see details in Moreira et al. 2008 and Sampedro et al 2009) we believe that the insect attack was the main cause differing growth patterns between the healthy and the attacked stands.

#### **Literature cited**

- Moreira, X., Sampedro, L., Zas, R. & Solla, A. (2008) Alterations of the resin canal system of *Pinus pinaster* seedlings after fertilization of a healthy and of a *Hylobius abietis* attacked stand *Trees*, **22**, 771-777.
- Sampedro, L., Moreira, X., Martíns, P. & Zas, R. (2009) Growth and nutritional response of *Pinus pinaster* after a large pine weevil (*Hylobius abietis*) attack. *Trees*, **23**, 1189-1197.

**Appendix S1** Results of the generalized linear mixed model for the analysis of the damage by *H. abietis* in the *P. pinaster* and *P. radiata* field trials.

Table S1. Results of the generalized linear mixed model for the analysis of the damage by *H. abietis* in the *P. pinaster* and *P. radiata* trials showing the F-ratio, the associated probability levels and the degrees of freedom in the numerator (DFn) and denominator (DFd) for the main fixed effects, as well as the specific contrast for the comparison of the wounds on the main and the complementary pine species within each trial.

Effect	<i>Pinus pinaster</i> trial				<i>Pinus radiata</i> trial			
	DFn	DFd*	F	P > F	DFn	DFd*	F	P > F
Fertilization	8	70.7	27.28	<.0001	8	69.8	14.84	<.0001
Genotype	31	2570	35.81	<.0001	31	2570	35.81	<.0001
Fert x Genotype	248	2570	6.77	<.0001	248	2423	5.81	<.0001
Diameter	1	2570	168.09	<.0001	1	2423	5.87	0.0155
Specific Contrast <i>P pinaster</i> vs <i>P radiata</i>	1	2570	88.2	<.0001	1	2423	70.39	<.0001

\* Degrees of freedom were estimated with the Kenward-Roger approximation.

**Appendix S2** Results of the general mixed models for the analysis of the debarked area in the two-choice and non-choice feeding experiments.

**Table S2A** Results of the mixed model for the analysis of the debarked area in the two-choice feeding experiment, showing the between subjects effect (differences between the two species) and the within subjects effects (differences between stem parts and the interaction), as well as the covariation with the weevil weight.

<b>Effect</b>	<b>NumDF</b>	<b>DenDF*</b>	<b>FValue</b>	<b>ProbF</b>
Species	1	37.0	4.55	0.0397
Stem part	2	75.5	0.67	0.5153
Species × Stem part	2	74.2	0.17	0.8457
Weevil weight	1	73.6	4.22	0.0435

\* Degrees of freedom were estimated with the Kenward-Roger approximation.

**Table S2B** Results of the mixed model for the analysis of the debarked area in the non-choice feeding experiment, showing the between subjects effect (differences between the two species) and the within subjects effects (differences between stem parts and the interaction), as well as the covariation with the weevil weight.

<b>Effect</b>	<b>NumDF</b>	<b>DenDF*</b>	<b>FValue</b>	<b>ProbF</b>
Species	1	8.0	5.41	0.0485
Stem part	4	23.6	0.7	0.6015
Species × Stem part	4	23.6	0.71	0.5963
Weevil weight	1	38.8	21.65	<.0001

\* Degrees of freedom were estimated with the Kenward-Roger approximation.