

Effect of fertilization and genetic variation on susceptibility of *Pinus radiata* seedlings to *Hylobius abietis* damage

Rafael Zas, Luis Sampedro, Xoaquín Moreira, and Patricia Martínez

Abstract: The effects of establishment fertilization and pine genotype on pine weevil (*Hylobius abietis* L.) damage was studied in a radiata pine (*Pinus radiata* D. Don) naturally infected family × fertilization genetic trial in Galicia (north-western Spain). Fertilization strongly increased both growth and *H. abietis* damage, especially when calcium phosphate was included in the fertilization treatment. Fertilized plants showed higher degree of debarking, greater leader loss, and higher mortality than unfertilized controls. Because of the greater leader loss, fertilization did not significantly increase the actual height (height of live stem) 1 year after planting. In contrast, after the second growing season, fertilized plants overcompensated for the weevil damage and reached greater height than the unfertilized controls. However, considering the effects on survival and the stem deformities resulting from the leader losses, fertilization should be avoided (or delayed) in *P. radiata* plantations on clear-cut coniferous areas if the risk of *H. abietis* is high. Our results also indicate a strong genetic variation in *H. abietis* susceptibility within the Galician *P. radiata* breeding population. The high family-mean and the moderate individual-tree heritability estimates suggest that improving resistance to this pest by conventional breeding techniques is possible. The use of planting stock with improved resistance should be considered as another prophylactic measure to supplement the traditional methods employed against this pest.

Résumé : Les effets d'une fertilisation lors de la plantation et ceux du génotype du pin sur les dommages causés par le charançon (*Hylobius abietis* L.) ont été étudiés dans un test génétique (familles × fertilisation) établi en Galice, au nord-ouest de l'Espagne, où les plants de pin de Monterey (*Pinus radiata* D. Don) étaient naturellement infestés. La fertilisation a fortement augmenté la croissance ainsi que les dommages causés par *H. abietis*, particulièrement lorsque le phosphate de calcium était inclus dans le traitement de fertilisation. Les plants fertilisés subissaient un degré plus élevé de perte d'écorce, une perte plus élevée de la pousse terminale et une mortalité plus élevée que les plants témoins non fertilisés. À cause de la perte plus fréquente de la pousse terminale, la fertilisation n'a pas significativement augmenté la hauteur réelle (hauteur des tiges vivantes) 1 an après la plantation. Par contre, après la deuxième saison de croissance, les plants fertilisés ont surcompensé les dommages causés par le charançon et ont atteint une hauteur plus élevée que les plants témoins non fertilisés. Cependant, considérant ses effets sur la survie et les déformations de la tige dues à la perte de la pousse terminale, la fertilisation devrait être évitée (ou retardée) dans les plantations de *P. radiata* dans les zones de conifères coupées à blanc si *H. abietis* présente un risque élevé. Nos résultats indiquent aussi qu'il y a une très forte variation génétique dans la susceptibilité de *H. abietis* dans la population de sélection de *P. radiata* en Galice. Les estimations de l'héritabilité, élevées pour les moyennes des familles et modérées pour les arbres individuels, indiquent qu'il est possible d'améliorer la résistance à ce ravageur avec les méthodes conventionnelles d'amélioration. On devrait considérer l'utilisation de plants plus résistants comme autre méthode prophylactique à titre de complément aux méthodes traditionnelles utilisées pour lutter contre ce ravageur.

[Traduit par la Rédaction]

Introduction

Pine weevil (*Hylobius abietis* L.; Coleoptera: Curculionidae) is one of the most harmful forest pests in Europe, causing important economic losses in young plantations mainly of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) but also of other conifer species and even broadleaved species (Langstrom and Day 2004). Pine weevil adults are attracted in mass by volatile compounds emitted

from fresh wood after clear-cuttings. They lay their eggs on the stumps, and newly emerged adults feed on the outer bark and phloem of young seedlings during the following years, causing important growth losses, stem deformities, and mortality (Leather et al. 1999). Several silvicultural methods are used to minimize the *H. abietis* damage, including delayed planting, soil scarification, shelterwood, feeding barriers, and insecticide applications (e.g., Petersson and Örlander 2003; Sydow 1997). None of these practices alone is completely effective, and seedling mortality in northern Europe may be as high as 70% if no pesticide is applied (Örlander and Nordlander 2003; Pitkänen et al. 2005). Within the framework of insecticide reduction policies in Europe, biological control using entomopathogenic nematodes is also a promising alternative against this pest (Brixey et al. 2006; Dillon et al. 2006); the possibility of breeding for tree resistance has also been suggested (Zas et al. 2005).

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In northern Spain, *H. abietis* also hampers natural and artificial regeneration of maritime pine (*Pinus pinaster* Ait.; Zas et al. 2005, 2006a) and radiata pine (*Pinus radiata* D. Don; Cobos and Ruíz 1990) in felled conifer sites. Recently, Zas et al. (2006a) warned about undesired effects of establishment fertilization on *H. abietis* damage, which has been shown to strongly increase damage in second-rotation *P. pinaster* plantations. Debarked area of fertilized trees was up to 2.9-fold greater than in unfertilized trees, inducing a 3.7-fold greater leader loss in the former. The response was notably higher when calcium phosphate was included in the fertilization treatment. If this effect is confirmed in *P. radiata*, fertilization recommendations for this species should be revised. *P. radiata* suffers important nutritional disorders throughout its range in northern Spain (Romanyà and Vallejo 1996; Zas 2003; Zas and Serrada 2003), with productivity depending strongly on nutritional status (Romanyà and Vallejo 2004; Sánchez-Rodríguez et al. 2002). Phosphorus (P) and magnesium (Mg) are the usual limiting nutrients, whereas nitrogen (N) seems to be well supplied or even in excess. Thus, fertilization is a common practice in *P. radiata* management, especially in second-rotation plantations, where the large nutrient losses through harvesting may aggravate the nutritional status of the new plantation (Merino et al. 2005; Ouro et al. 2001).

Pinus radiata is an important timber species in northern Spain, where it occupies more than 300 000 ha. Several breeding programs are developing for this species in Spain aiming to improve growth, stem form, branching habit, and (or) disease resistance (Espinell and Aragones 1997; Zas et al. 2006b). Knowledge of genetic variation in susceptibility to *H. abietis* available within the breeding populations of these programs would be highly desirable not only because of the possibility of improving resistance through breeding, but also because it would provide plants with different susceptibilities in which the morphological and physiological mechanisms involved in resistance could be studied. In *P. pinaster*, high genetic variation in susceptibility to this pest has been reported, with high family-mean and moderate individual-tree heritability estimates (Zas et al. 2005).

Within the Galician (northwestern Spain) *P. radiata* breeding program, several progeny × fertilization trials were established to test how soil fertility may affect the performance of the genetic material (Zas et al. 2006b). In one of these trials, located next to the *P. pinaster* trial analyzed by Zas et al. (2005, 2006a), *H. abietis* caused important damage during the first year after planting. The aim of this paper is to confirm whether the effects of pine genotype and fertilization on weevil damage are also important in *P. radiata*. Specifically, the objectives were (i) to analyze the effect of fertilization on the intensity of *H. abietis* attack on *P. radiata* seedlings, (ii) to measure the genetic variation in susceptibility to *H. abietis* damage in a subset of the current *P. radiata* breeding population in Galicia, (iii) to examine the viability of including different damage and symptom traits in the goal for operational breeding, and (iv) to explore the genetic and phenotypic correlations between different growth and damage traits.

Materials and methods

The site

The study was conducted in a progeny × fertilization trial

located near the Atlantic coast of Galicia (Rianxo, northwestern Spain, 42.60°N, 8.77°W, altitude 90 m). The trial was established in March 2003, adjacent to a similar *P. pinaster* trial in which the *H. abietis* damage was extensively studied (Zas et al. 2005, 2006a). About 4 months after planting, the trial suffered an intensive *H. abietis* attack, which was ideal for analyzing the variable incidence of the pest.

The site was previously occupied by a mature *P. pinaster* stand that was clear-cut 1 year before trial establishment. The climate is maritime, temperate humid with high annual precipitation (2000 mm, mean annual temperature 14 °C), low summer drought, and low annual temperature fluctuation. The soil, derived from granite, has a sandy texture, acidic pH (pH in H₂O = 4.5), high organic matter content (170 g·kg⁻¹ soil ash-free dry mass), high total Kjeldahl N (8.3 g N·kg⁻¹) and low concentrations of other nutrients, especially of available P (Olsen P = 5.2 mg·kg⁻¹).

Plant material

The study material consisted of open-pollinated families of 30 maternal plus trees selected for superior growth and form in mature plantations of *P. radiata* in Galicia (27 plus trees) and in the Basque Country (northeastern Spain; three plus trees). The Galician plus trees represent a random subset of the first-generation breeding population of *P. radiata* in Galicia, whereas the three open-pollinated families from the Basque Country were randomly selected from the actual breeding program there and were considered as controls. One unimproved seed source that was commonly used for reforestation in the coastal area of Galicia (TC) was also included as a control.

Experimental design and fertilization treatments

The experimental design was a split plot replicated in 10 blocks, with 9 fertilizer treatments acting as whole plots and 31 pine families as split plots. The fertilizer treatments were randomly assigned to the whole plots within each block replicate, and one tree of each family was randomly distributed within each whole plot. Most of the whole plots were arranged as rectangles of 4 × 8 seedlings. To complete the resulting 32 plants, one unimproved *P. pinaster* seedling was included in each whole plot but was not considered in the analyses. Spacing was 3 m × 2 m.

The nine fertilizer treatments (eight combinations of four commercial fertilizers, plus an unfertilized control) (Table 1) were applied at establishment. The fertilizers were combined to allow the effect of combinations of single fertilizers (P, Mg, and potassium (K)) with N to be tested (see Zas et al. 2006b for details). The fertilizers were spread by hand over a 20 cm radius around each seedling.

Assessments

Height (*H*) and groundline diameter (*D*) were measured in all living plants, 1 and 2 years after planting. During the first year, the *H. abietis* damage led to stem girdling and leader loss in many plants. In this first year, “actual height” was defined as the height of the live stem, i.e., up to the girdling, whereas “potential height” was defined as the total height in plants with living leaders (plants with dead leader

Table 1. Codes and chemical composition of the fertilization treatments.

Nutrient	Fertilizer	Dose (g·plant ⁻¹)	Treatment code									
			Control	F1	F2	F3	F4	F5	F6	F7	F8	
N	Ammonium nitrate	5	–	+	–	+	+	+	–	–	–	
P and Ca	Calcium phosphate	10 (P)	–	+	+	–	+	+	–	+	+	
K	Potassium sulphate	15	–	+	+	+	–	+	+	–	+	
Mg	Magnesium sulphate	5	–	+	+	+	+	–	+	+	–	

Note: The plus and minus signs indicate presence and absence of a given compound, respectively.

were dropped from the analyses) (Zas et al. 2006a). No stem girdling was observed in the second year.

Hylobius abietis damage was measured after the first growing season (February 2004) by evaluating the wounds in the stem caused by the insect. To diminish subjectivity, the stem height was divided in 10 sections using an elastic ruler, and wounds were evaluated by estimating the relative debarked area in each resulting section using a four-level scale (0, undamaged; 1, some wounds; 2, many wounds; and 3, death due to girdling). The sum of these 10 values by plant was the damage trait “wounds” (WND, 0–30 score). Additionally, the leader loss (LL) due to stem girdling by the pine weevil was also recorded as a binary variable (LL = 0, leader alive; LL = 1, leader dead).

The WND trait was a reliable measure of *H. abietis* damage, but it was laborious to assess. To explore other practical traits for operational breeding, seedlings were visually scored for foliage colour (COL) and foliage density (DEN). These traits were subjectively assessed on a scale from 1 (yellow foliage and low foliage density) to 4 (deep green foliage and no needle loss). All assessments were made by the same person.

Spatial analysis

The incidence of pests and diseases in forest stands commonly follow heterogeneous spatial structures that hinder proper statistical analysis (Díaz et al. 2007; Zas et al. 2007). When spatial heterogeneity is present, the violation of the requisite of data independence of standard statistical analyses may lead to erroneous conclusions, and sophisticated spatial-analysis procedures should be used (Zas et al. 2007). We use geostatistics to test whether *H. abietis* damage was homogeneously distributed in the study area or whether there was a spatial pattern not absorbed by the block design (Zas et al. 2006a). The spatial structure of the WND trait and that of the residuals of the model defined in eq. 2 for this trait was analyzed using a semivariogram, which plots the semivariance between trees as a function of the distance separating them. The semivariance $\gamma(h)$ was calculated as

$$[1] \quad \gamma(h) = \frac{1}{2n} \sum_{i=1}^n [z(s_i) - z(s_{i+h})]^2$$

where n is the number of observation pairs separated by distance h , $z(s_i)$ is the value of the variable of interest at location s_i , and $z(s_{i+h})$ is the value for a tree located at a distance h from s_i . For randomly distributed data, little change in the semivariance will be obtained as h increases, and the semivariogram will be essentially flat. If spatial dependence is present, semivariance will be lower at a short distance, it will increase for intermediate distances, and it will typically reach an asymptote for long distance. The distance at which

the asymptote begins, if present, indicates the range or patch size of heterogeneity below which data are stochastically interdependent (Cressie 1993). The distributions of the two variables were previously standardized (mean = 0, SD = 1) to attain an equivalent scale. The semivariogram was constructed using the VARIOGRAM procedure of SAS (SAS Institute Inc. 1999).

Statistical analysis

Analyses of variance (ANOVA) were performed on all traits using the MIXED procedure of SAS (SAS Institute Inc. 1999) and the following mixed model:

$$[2] \quad Y_{ijk} = \mu + F_i + G_j + B_k + FG_{ij} + FB_{ik} + GB_{jk} + \epsilon_{ijk}$$

where μ is the general mean, F_i , G_j , and B_k are the main effects of fertilizer treatment i ($i = 1-9$), family j ($j = 1-31$), and block k ($k = 1-10$), respectively, FG_{ij} , GB_{jk} , and FB_{ik} are the corresponding interactions, and ϵ_{ijk} is the experimental error. To analyze the whole-plot factor (i.e., fertilization) with the appropriate error term, the fertilization \times block interaction was considered a random effect (Littell et al. 1996).

To test for fertilizer effects, binary variables (LL and survival) were analyzed on a whole-plot mean basis. Whole-plot means were arcsine square root transformed and then analyzed assuming a randomized complete block design and using the following model:

$$[3] \quad Y_{ik} = \mu + F_i + B_k + \epsilon_{ik}$$

where Y_{ik} is the transformed whole-plot mean.

Statistical comparison of treatment means was conducted using the LSMEAN statement of the MIXED procedure in SAS (Littell et al. 1996). Data are presented as means \pm SEs.

Genetic parameters

To estimate variance components, model 2 was applied again considering the family effect (G_k) and all the interactions involving this factor (FG_{ij} and GB_{jk}) as random factors. The analysis was restricted to the 27 families of the Galician breeding program.

Individual (h_i^2) and family (h_f^2) heritabilities were estimated as

$$[4] \quad h_i^2 = \frac{\sigma_A^2}{\sigma_G^2 + \sigma_{GF}^2 + \sigma_{GB}^2 + \sigma_e^2}$$

$$[5] \quad h_f^2 = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_{GF}^2/F) + (\sigma_{GB}^2/B) + (\sigma_e^2/FB)}$$

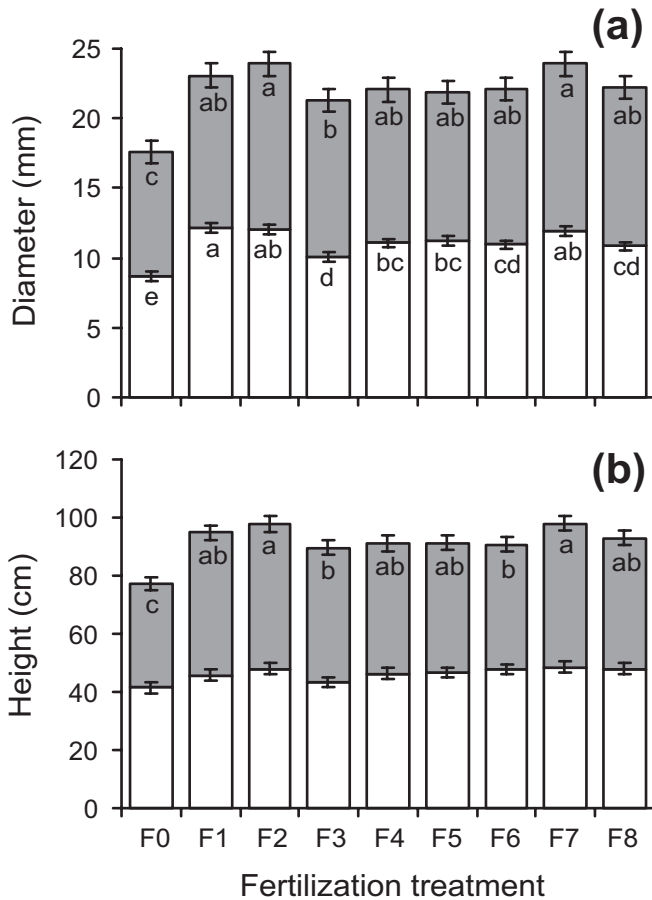
where σ_A^2 is the additive variance, which was assumed to be

Table 2. Results of the mixed-model ANOVA for growth traits of *Pinus radiata* seedlings during the first 2 years after planting.

Effect	df	Error term	First growth period			Second growth period	
			Diameter	Actual height ^a	Potential height ^b	Diameter	Height ^c
Fertilization (F)	8	F × B	11.47*** ^d	1.68	13.11***	5.22***	6.31***
Block (B)	9	F × B	8.40***	3.39**	2.18*	2.84**	4.02***
Family (G)	30	Error	3.33***	2.27***	3.43***	2.14***	2.36***
F × G	240	Error	0.87	0.90	0.89	0.93	0.94
G × B	270	Error	0.99	0.93	0.95	0.91	1.02

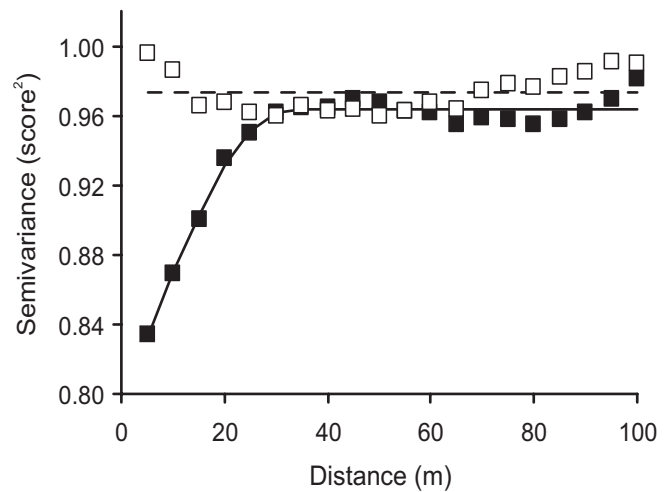
^aHeight of the live part of the stem, all plants considered.
^bPlants with leader loss were dropped from the analysis.
^cNo stem girdling was observed in the second year so only one height was evaluated.
^dANOVA *F* values are given. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

Fig. 1. Effect of the fertilization treatments on (a) diameter and (b) actual height of *Pinus radiata* seedlings during the first growing season (open bars) and second growing season (shaded bars) after planting. Bars are treatment least square means, and error bars are SEs. Bars with different letters are significantly different (*P* < 0.05). See Table 1 for fertilization treatment codes.



$\sigma_A^2 = 4\sigma_G^2$; σ_G^2 is the family variance; σ_{GF}^2 and σ_{GB}^2 are the variance of the family × fertilization and family × block interactions, respectively; *F* and *B* are the number of fertilization treatments and blocks, respectively; and σ_c^2 is the residual variance. Approximate SEs of individual-tree and family-mean heritabilities were estimated according to Wright (1976). Heritabilities of binary variables (survival and leader loss) were adjusted by the method of Dempster and Lerner (1950). This method assumes an underlying continuous normal var-

Fig. 2. Semivariogram for the wounds caused by *Hylobius abietis* (solid squares) and for the residuals of the mixed-model ANOVA (open squares).



iable, which at a given threshold point changes the outward observable variable into a yes or no response. The following equation relates the heritability of the outward scale (h_{01}^2) to the heritability of the continuous underlying scale (h^2):

$$[6] \quad h_{01}^2 = \frac{h^2 \times z^2}{\Phi(1 - \Phi)}$$

where *z* is the height of the ordinate of the normal distribution at the threshold point that corresponds to the observed incidence of the trait (Φ).

Genetic correlation between traits *x* and *y* was estimated as

$$[7] \quad r_g = \frac{COV_g(xy)}{\sqrt{\sigma_{F_x}^2 \times \sigma_{F_y}^2}}$$

where $COV_g(xy)$ is the genetic covariance between the two traits and $\sigma_{F_x}^2$ and $\sigma_{F_y}^2$ are the family variance of traits *x* and *y*, respectively. The covariance between the two traits was calculated as $COV_g(xy) = (\sigma_{F_{x+y}}^2 - \sigma_{F_x}^2 - \sigma_{F_y}^2)/2$, where $\sigma_{F_{x+y}}^2$ is the family variance of the sum trait *x* + *y*. Data were standardized (mean = 0, SD = 1) for each trait to remove scale effects prior to calculating genetic correlations. Standard errors of genetic correlations were estimated as in Falconer (1989).

Phenotypic correlations between family means (*n* = 27) of

Table 3. Results of the mixed-model ANOVA for damage- and symptom traits caused by *Hylobius abietis* on *Pinus radiata* seedlings.

Effect	df	Error term	Damage			Symptoms	
			Wounds	Leader loss ^a	Survival ^a	Colour	Defoliation
Fertilization (F)	8	F × B	12.22*** ^b	7.29***	3.13**	5.48***	4.70***
Block (B)	9	F × B	2.67**	2.52*	1.41	1.83	3.19**
Family (G)	30	Error	4.63***			2.53***	1.98**
F × G	240	Error	1.08			1.17*	1.19*
G × B	270	Error	0.96			0.89	0.99

^aLeader loss and survival were assessed as binary variables. Analyses were performed on arcsin square root transformed whole-plot mean values.

^bANOVA *F* values are given. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

different traits were also calculated using the CORR procedure of SAS (SAS Institute Inc. 1999).

Results

Pine growth

Fertilization had an important effect on pine growth 1 and 2 years after planting (Table 2). Both diameter and potential height (i.e., height of those plants with leaders alive) were significantly greater when any fertilizer was added. On the contrary, fertilization did not affect actual height (height up to the girdling) in the first growing season, suggesting larger height losses due to more severe *H. abietis* damage in fertilized plants (Fig. 1) (see below). During the second growing season, the *H. abietis* damage did not cause stem girdling, and fertilization significantly increased both height and diameter (Table 2). Among the different fertilization treatments, the inclusion of P and Mg without including N (treatments F2 and F7) (Table 1) produced the greatest growth responses. On the other hand, the absence of calcium phosphate (treatments F3 and F6) led to the lowest growth responses (Fig. 1).

Differences among families were highly significant for both diameter and height in the 2 years (Table 2). No statistically significant fertilizer × family interaction was evident, indicating that all families responded similarly to the nutrient additions.

Hylobius abietis damage

The *H. abietis* attack affected more than 94% of all seedlings in the trial, causing leader loss and death of 43.5% and 27.5% of all plants, respectively. Height of those plants with leaders alive (called potential height) was 52.9 ± 0.4 cm after the first growth period, more than double the height of the plants that lost their leaders (23.2 ± 0.6 cm).

The flat semivariogram of the residuals of the mixed model for the WND trait (Fig. 2) indicated that the *H. abietis* attack was uniformly distributed around all the experimental area or, at least, that the block structure accounted well for the eventual spatial variation. Actually, the semivariogram for WND fitted very well to a spherical model, denoting that this trait showed a small-scale spatial heterogeneity up to distances of approximately 30 m. This range, or patch size, corresponded well to the mean size of the whole plots. Considering the highly significant effect of fertilizer on *H. abietis* damage (see below), the spatial structure of the WND trait can be attributed to the variation among whole plots fertilized with different treatments.

Hylobius abietis damage was significantly influenced by fertilization (Table 3), with all fertilization treatments leading to significantly more wounds and leader losses, although the absence of calcium phosphate led to significantly lower weevil damage (Figs. 3a and 3b). Survival was also highly influenced by fertilization (Table 3); unfertilized plants and plants fertilized without calcium phosphate (F3 and F6) showed significantly higher survival (Fig. 3c), although treatment F7 (which includes calcium phosphate) did not lead to higher mortality. The debarked area score, the percentage of plants that lost their leaders, and mortality were approximately twice as high in fertilized plants than in unfertilized control.

Highly significant differences were also observed among the pine families for all the damage and symptom traits (Table 3). The additive genetic coefficient of variation varied between 13.3% and 34.3% for different damage and symptom traits (Table 4). The WND in the stem of the worst attacked family was 1.7-fold greater than in the least attacked one, whereas family survival varied between 52% and 82%. The family mean heritability estimates were high for the damage traits (Table 4) suggesting important genetic gains in *H. abietis* resistance through family selection. Individual heritability for WND and LL was only moderate. The unimproved coastal seed source (TC) was one of the less attacked, whereas the families from the Basque Country breeding program showed intermediate damage levels (Fig. 4).

Family × fertilization interaction was not significant for the damage trait WND but was significant for the symptoms traits COL and DEF (Table 3). This interaction led to relatively lower family and individual heritability estimates for these symptom traits (Table 4).

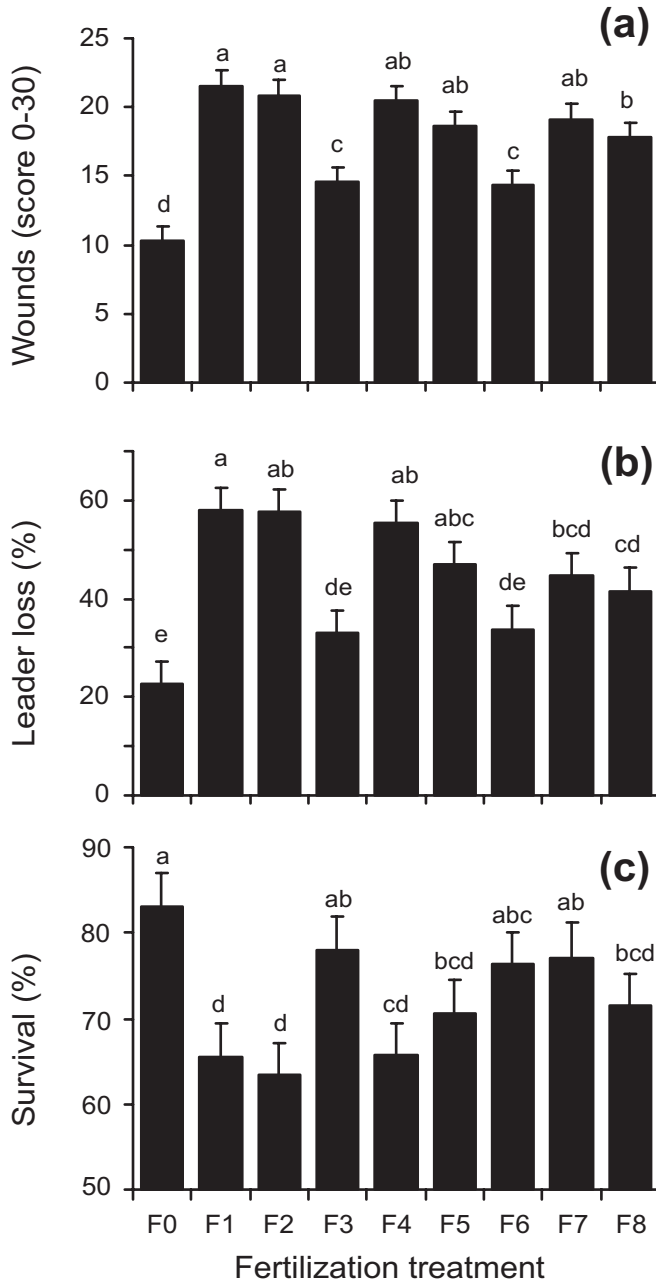
Correlation among traits

All damage and symptom traits were significantly correlated among each other with high genetic correlation estimates (Table 5). Judging from the strong relationship between the wounds in the stem and the family mean colour (Fig. 4a), screening for *H. abietis* resistance could be carried out by simply assessing the mean colour of the seedlings. Survival was also significantly related with *H. abietis* damage (Fig. 4b), with genetic correlation close to unity, suggesting *H. abietis* was the main cause of mortality. Pine growth and weevil damage were not significantly related (Fig. 4c).

Discussion

Fertilization and pine family had a strong influence on

Fig. 3. Effect of the fertilizer treatments on (a) the wounds caused by *Hylobius abietis*, (b) the percentages of plants that lost their leaders, and (c) survival of *Pinus radiata* seedlings. Bars are treatment least-squares means, and error bars are SEs. Bars with different letters are significantly different ($P < 0.05$). See Table 1 for fertilizer treatment codes.



both pine growth and weevil damage. Growth response to fertilization agreed well with the common nutritional disorders found in *P. radiata* stands in northern Spain. Phosphorus and Mg deficiencies are the most widespread, especially on acid soils over granites such as that of the studied site, whereas N is commonly well supplied (Romanyà and Vallejo 1996; Zas 2003; Zas and Serrada 2003). According to these nutritional disorders, productivity of northern Spanish *P. radiata* plantations has been shown to be positively correlated with available soil P and foliar P, and negatively with total soil and foliar N (Romanyà and Vallejo 2004; Sánchez-

Table 4. Individual-tree (h_i^2) and family-mean (h_f^2) heritability estimates and estimated additive genetic coefficients of variation (CV_A) for growth of *Pinus radiata* seedlings and damage by *Hylobius abietis*.

	h_i^2	h_f^2	CV_A (%)
Growth (first growing season)			
Diameter	0.08±0.03	0.64±0.08	7.3
Height	0.09±0.04	0.67±0.09	11.3
Damage			
Wounds	0.16±0.05	0.78±0.13	20.7
Leader loss ^a	0.16±0.04	0.70±0.09	34.3
Survival ^a	0.04±0.02	0.30±0.04	8.8
Symptoms			
Colour	0.08±0.03	0.60±0.08	18.0
Defoliation	0.04±0.02	0.45±0.06	13.3

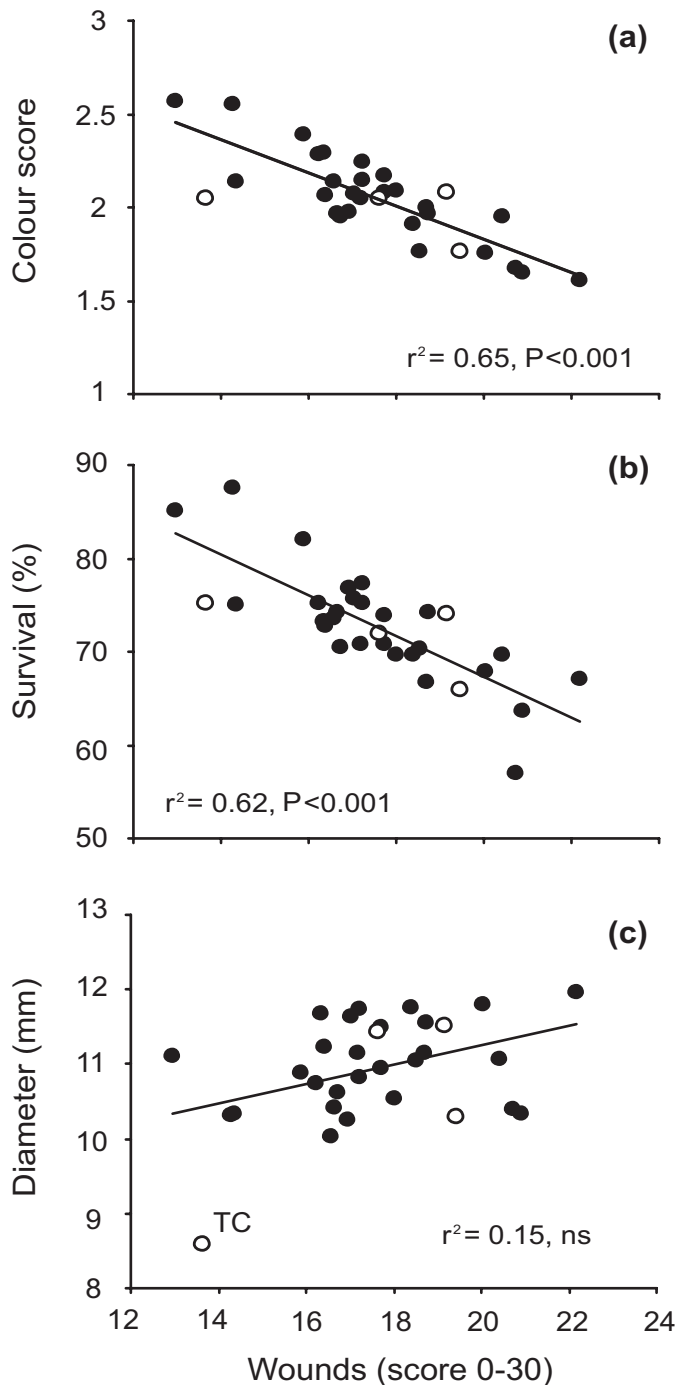
^aHeritability estimates of binary variables were adjusted by the method of Dempster and Lerner (1950).

Rodríguez et al. 2002). The strong response to P and Mg fertilization, and the lack of response to N addition was expected. Indeed, the responses to the fertilization treatments were almost the same as those found in other similar unattacked *P. radiata* trials in Galicia (Zas et al. 2006b). It can be concluded that growth in the studied plantation was P and Mg limited.

An increase of insect herbivory following fertilization has been reported before by several authors and for many insect-plant systems. Generally, fertilized plants may be more susceptible to insects because of reduced chemical and (or) physical defences (Blodgett et al. 2005; Kytö et al. 1999; Mutikainen et al. 2000; Turtola et al. 2002; vanAkker et al. 2004; Zas et al. 2006a), better nutritive value (Ayres et al. 2000; Giertych et al. 2005), or stronger insect attraction through higher or differential volatile emission (e.g., Gouinguéné and Turlings 2002). From an evolutionary point of view, plants need to modulate how to allocate the available resources to growth, defence, and reproduction. Resource constraints result in a trade-off between the high growth rate needed to outgrow competing plants and the allocation processes that led to increased defences against herbivores, pathogens, and other potential damage agents (Holopainen et al. 1995). In resource-poor environments, the importance of herbivory relative to competition increases, and more resources are allocated to defence. Conversely, in resource-rich soils, competition becomes relatively more important, and plants invest less in defence (Agrawal 2006). These arguments agree with several ecological hypotheses, such as the carbon-nutrient balance hypothesis, that predict that fertilization increases growth, resulting in less carbon available for the production of carbon-based secondary metabolites (Bryant et al. 1983).

Additionally, plant size could also play a relevant role explaining *H. abietis* preferences for fertilized plants. Incidence of *Pissodes strobi* (Peck) on interior spruce (*Picea glauca* (Moench) Voss, *Picea engelmannii* Parry, and their hybrids) increased with fertilization intensity, and vanAkker et al. (2004) explained this trend in terms of increased resources available for insect feeding as a result of increased leader size and bark thickness. Seedling size is also known

Fig. 4. Relationship between the wounds in the stem caused by *Hyllobius abietis* and (a) the foliage colour, (b) survival, and (c) stem diameter of *Pinus radiata* seedlings after the first growing season: ●, family means; ○, controls; TC, unimproved control; ns, no significant relationship.



to affect *H. abietis* incidence. For example, *H. abietis* usually prefers *P. sylvestris* over *Picea abies*, because *Picea abies* is traditionally planted as bareroot seedlings which are commonly thicker than *P. sylvestris* and, hence, are less palatable (Toivonen and Viiri 2006). Several authors have also found a significant positive correlation between seedling diameter and survival (Örlander and Nilsson 1999; Thorsen et al. 2001). According to this relationship, a threshold of 10–

12 mm for root collar diameter has been suggested to avoid serious *H. abietis* damage in *Picea abies* and *P. sylvestris* plantations (Thorsen et al. 2001; Wallertz et al. 2005). However, the relatively large root collar diameter of the planting stock, and the lack of a significant correlation between growth and damage in the present study (Table 5), made us think that seedling size alone cannot explain the positive fertilizer effect on weevil preference. Further research is needed to better understand the causes behind the positive effect of fertilizer on weevil damage. The nutritional value, the chemical and physical defences, and the influence of fertilizer on the emission of volatiles should be analyzed in new fertilizer experiments to attempt to explain this effect.

Increased susceptibility to fungal pathogens through fertilization has been also reported in conifers and explained in the same terms as for herbivores, i.e., lower plant defences or improved growth environment for the fungus (Blodgett et al. 2005; Entry et al. 1991). In fact, many plant defence mechanisms, especially the constitutive defences, are effective against both herbivores and pathogens (Loehle 1996). Thus, the knowledge of nonhost resistance mechanisms may be exploited to improve resistance in a broad sense.

Irrespective of the proximate causes explaining the effect of fertilization on weevil damage, the practical consequence of this result is that, as indicated before (Zas et al. 2006a), fertilization of second-rotation plantations on clear-felled coniferous areas at establishment is not a recommendable management practice. During the first growing season, *H. abietis* damage caused the loss of the growth benefits of fertilization. However, contrary to what occurred with *P. pinaster* in similar conditions (Zas et al. 2006a), the effect of fertilization on height and diameter after the second growing season became significant because of overcompensatory regrowth in fertilized plants, suggesting that growth losses due to weevil damage were not as great as the growth gains due to fertilization. The higher nutrient demand of *P. radiata* may explain these differences. In a study of the effect of fertilizer on *Pissodes strobi* damage, vanAkker et al. (2004) found similar results and concluded that fertilization is a feasible option for increasing productivity of interior spruce plantations. However, taking into account the effects on survival and on the stem deformities derived from the leader losses, fertilization should be avoided when the risk of *H. abietis* attack is high. Therefore, the recommendation of no fertilization or delayed fertilization for *P. pinaster* (Zas et al. 2006a) should be extended to *P. radiata* plantations and considered as another prophylactic measure to be combined with the traditional methods employed against this pest (e.g., Petersson and Örlander 2003; Sydow 1997).

The results of the present study also indicated substantial genetic variation in *H. abietis* susceptibility. Family-mean heritability was high whereas individual-tree heritability was only moderate. Both estimates were similar to those found for susceptibility to this insect in *P. pinaster* (Zas et al. 2005). This genetic variation could be exploited by breeding and selecting families and (or) individuals to be used in high-risk plantations such as in recently clear-felled areas. The high family-mean heritability suggests high genetic gains through family selection, which may be considered when roguing clonal seed orchards. Additionally,

Table 5. Phenotypic correlation of family means ($n = 27$, below diagonal) and genetic correlation \pm SEs (above diagonal) between different traits.

	Height	Diameter	Wounds	Leader loss	Colour	Defoliation	Survival
Height		0.68 \pm 0.16	-0.33 \pm 0.23	-0.57 \pm 0.19	0.25 \pm 0.39	0.00 \pm 0.30	-0.25 \pm 0.32
Diameter	0.61		0.34 \pm 0.24	-0.24 \pm 0.27	-0.32 \pm 0.37	-0.28 \pm 0.28	-0.29 \pm 0.32
Wounds	-0.29	0.28		1.00 \pm 0.00	-0.93 \pm 0.05	-0.94 \pm 0.03	-1.09 \pm 0.06
Leader loss	-0.35	0.07	0.93		-0.94 \pm 0.05	-0.88 \pm 0.06	-1.01 \pm 0.01
Colour	0.29	-0.10	-0.87	-0.91		0.85 \pm 0.11	0.92 \pm 0.08
Defoliation	0.24	-0.03	-0.84	-0.89	0.87		0.88 \pm 0.08
Survival	0.14	-0.08	-0.82	-0.93	0.86	0.89	

Note: Significant phenotypic Pearson's correlation coefficients ($P < 0.05$) are given in boldface.

highly resistant and susceptible genotypes could be developed through recurrent breeding, from which the mechanisms of resistance could be studied. Results presented here are based on a single test site and may be subject to the incidence of genotype \times environment interactions, which may hamper the broad applicability of the results. However, the lack of a significant family \times fertilization interactions suggests that tree resistance is relatively stable, at least regarding the pine nutritional status.

The high correlation between the symptom and damage traits (Table 5) indicates that screening for resistance can be carried out by simply assessing the mean colour of the seedlings. However, the significant fertilizer \times family interaction found in the *P. radiata* trial for the symptom traits (Table 3) indicates that the symptoms of the weevil damage could vary with pine nutritional status. Thus, special attention should be paid to the nutritional conditions in future genetic trials, if screening for resistance will be based on symptom traits.

Breeding for resistance to pests is relatively common in agriculture, although it is known that it cannot give complete protection. Plant resistance may be only effective against specific biotypes of the pest, and pest adaptation to improved resistant varieties is also possible (Gatehouse 2002). However, breeding for resistance is considered an effective mechanism to combat pests within integrated pest management programs, which seek to minimize insect damage through a combination of different management tools. This idea may be particularly relevant in the case of *H. abietis*, against which no single method is completely effective (Petersson and Örlander 2003; Sydow 1997). Breeding for insect resistance has been used to control pests in several tree species, e.g., in *P. pinaster* against the moth *Dyorictria sylvestrella* Ratz. (Kleinhentz et al. 1998) or in interior spruce against the weevil *Pissodes strobi* (e.g., Alfaro et al. 2004).

Despite insect pests being a common problem of *P. radiata* stands in various countries where it is grown commercially, little information about the genetic variation of *P. radiata* in susceptibility to pests is available. However, researchers are concerned about the potential risks of many pests and, on the basis of the apparent lack of specific insect-resistance factors in this species, have sought to develop insect resistance *P. radiata* through biolistic transformation (Grace et al. 2005). On the other hand, genetic variation of *P. radiata* in susceptibility to fungal diseases has been widely studied and exploited in several breeding programs by recurrent breeding to develop more resistance genetic material (e.g., Bradshaw 2004; Gordon 2006). For example,

in New Zealand, resistance to *Dothistroma pini* Hulbary (red-band needle blight) is one of the selection traits routinely assessed in several breeding programs, and important genetic gains in resistance to this disease have been achieved (Jayawickrama and Carson 2000). The results of the present paper denote a genetic variation in pest susceptibility in *P. radiata* that shows promise of improving pest resistance by breeding.

No clearly adverse genetic correlations were evident, unlike what was found in *P. pinaster* where growth and weevil susceptibility were genetically positively correlated (Zas et al. 2005). This apparent lack of correlation indicates that breeding for resistance and growth can be carried out at the same time without problems, facilitating the incorporation of resistance in the breeding program. However, we again observed higher susceptibility in the Galician breeding material than in the unimproved commercial seedlot. Caution should then be used when using improved material in high-risk areas. Resistance of the Basque Country breeding material was intermediate.

Conclusions

Fertilization significantly increased *H. abietis* damage on *P. radiata*, especially when calcium phosphate was added. These results support the previous recommendation of no fertilization or delayed fertilization in second-rotation plantations on felled coniferous areas.

Strong genetic variation was also observed in damage caused by this insect, with high family and moderate individual heritability estimates. Judging from these results, breeding for resistance against this pest is possible.

Growth and *H. abietis* susceptibility were not significantly correlated, suggesting that growth and weevil resistance can be easily improved together.

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