

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

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12

1 **Abstract**

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

19

20 **Keywords:** Herbivory, plant-herbivore interactions, forest pests, pest management,  
21 genetic resistance, maritime pine, pine weevil

22

## 1 **Introduction**

2 Pine weevil (*Hylobius abietis* L., Coleoptera: Curculionidae) is one of the most harmful  
3 forest pests in Europe, causing important economic losses in young plantations mainly  
4 of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* Karst) but also of  
5 other conifer species and even broadleaved species (Langstrom and Day 2004). Pine  
6 weevil adults are attracted in mass by volatile compounds emitted from fresh wood after  
7 clear-cuttings. They lay their eggs on the stumps, and new emerged adults feed on the  
8 outer bark and phloem of young seedlings during the following years, causing important  
9 growth losses, stem deformities and mortality (Leather et al. 1999). Several silvicultural  
10 methods are used to minimize the pine weevil damage, including delayed planting, soil  
11 scarification, shelterwood, feeding barriers, and insecticide applications (e.g. Petersson  
12 and Orlander 2003; Sydow 1997). None of these practices alone is completely effective,  
13 and in northern Europe seedling mortality may be as high as 70% if no pesticide is  
14 applied (Orlander and Nordlander 2003; Pitkänen et al. 2005). Within the framework of  
15 insecticide reduction policies in Europe, biological control using entomopathogenic  
16 nematodes is also a promising alternative against this pest (Brixey et al. 2006; Dillon et  
17 al. 2006), while the possibility of breeding for tree resistance has also been suggested  
18 (Zas et al. 2005).

19 In northern Spain, *H. abietis* also hampers natural and artificial regeneration of *Pinus*  
20 *pinaster* Ait. (Zas et al. 2005; 2006a) and *Pinus radiata* D. Don (Cobos and Ruíz 1990)  
21 in felled conifer sites. Recently, Zas et al. (2006a) warned about undesired effects of  
22 establishment fertilization on pine weevil damage, which has been shown to strongly  
23 increase damage in second rotation *P. pinaster* plantations. Debarked area of fertilized  
24 trees was up to 2.9-fold greater than in unfertilized trees, inducing a 3.7-fold greater  
25 leader loss in the former. The response was notably higher when calcium-phosphate was

1 included in the fertilization treatment. If this effect is confirmed in *P. radiata*,  
2 fertilization recommendations for this species should be revised. *P. radiata* suffers  
3 important nutritional disorders throughout its range in Northern Spain (Romanyà and  
4 Vallejo 1996; Zas 2003; Zas and Serrada 2003), with productivity depending strongly  
5 on nutritional status (Romanyà and Vallejo 2004; Sánchez-Rodríguez et al. 2002).  
6 Phosphorus and magnesium are the usual limiting nutrients whereas nitrogen seems to  
7 be well supplied or even in excess. Fertilization is thus a common practice in *P. radiata*  
8 management, especially in second-rotation plantations, where the large nutrient losses  
9 through harvesting may aggravate the nutritional status of the new plantation (Merino et  
10 al. 2005; Ouro et al. 2001).

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

22 Within the Galician (NW Spain) *P. radiata* breeding program, several progeny ×  
23 fertilization trials were established to test how soil fertility may affect the performance  
24 of the genetic material (Zas et al. 2006b). In one of these trials, located next to the *P.*  
25 *pinaster* trial analyzed by Zas et al. (2005, 2006a), *H. abietis* caused important damage

1 during the first year after planting. The aim of this paper is to confirm if the effects of  
2 pine genotype and fertilization on weevil damage are also important in *P. radiata*.  
3 Specifically, the objectives were i) to analyze the effect of fertilization on the intensity  
4 of *H. abietis* attack on *P. radiata* seedlings, ii) to measure the genetic variation in  
5 susceptibility to pine weevil damage in a subset of the current *P. radiata* breeding  
6 population in Galicia, iii) to examine the viability of including different damage- and  
7 symptom traits in the goal for operational breeding, and iv) to explore the genetic and  
8 phenotypic correlations between different growth- and damage traits.

9

## 10 **Material and Methods**

### 11 *The site*

12 The study was conducted in a progeny × fertilization trial located near the Atlantic coast  
13 of Galicia (Rianxo, NW Spain, 42.60° N, 8.77° W, altitude 90 m). The trial was  
14 established in March 2003, adjacent to a similar *P. pinaster* trial in which the pine  
15 weevil damage was extensively studied (Zas et al. 2005, 2006a). About four months  
16 after planting the trial suffered an intensive pine weevil attack, which was ideal for  
17 analyzing the variable incidence of the pest.

18 The site was previously occupied by a mature *P. pinaster* stand which was clear-cut one  
19 year before trial establishment. The climate is maritime, temperate humid with high  
20 annual precipitation (2000 mm; mean annual temperature 14 °C), low summer drought,  
21 and low annual temperature fluctuation. The soil, derived from granite, has a sandy  
22 texture, acidic pH (pH in H<sub>2</sub>O = 4.5), high organic matter content (170 g ash-free dry  
23 weight kg<sup>-1</sup>), high total Kjeldahl nitrogen (8.3 g N kg<sup>-1</sup>) and low concentrations of other  
24 nutrients, especially of available phosphorus (Olsen P = 5.2 mg kg<sup>-1</sup>).

25

1 *Plant material*

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

10

11 *Experimental design and fertilization treatments*

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

19 The nine fertilizer treatments (eight combinations of four commercial fertilizers, plus an  
20 unfertilized control, Table 1) were applied at establishment. The fertilizers were  
21 combined to allow the effect of combinations of single fertilizers (phosphorus (P),  
22 potassium (K) and magnesium (Mg)) with nitrogen (N) to be tested (see Zas et al.  
23 2006b for details). The fertilizers were spread by hand over a 20-cm radius around each  
24 seedling.

25

## 1 *Assessments*

2 Height ( $H$ ) and ground-surface diameter ( $D$ ) were measured in all living plants, one and  
3 two years after planting. During the first year, the pine weevil damage led to stem  
4 girdling and leader loss in many plants. In this first year, “actual height” was defined  
5 as the height of the live stem, i.e. up to the girdling, whereas “potential height” was  
6 defined as the total height in plants with living leaders (plants with dead leader were  
7 dropped from the analyses) (Zas et al. 2006a). No stem girdling was observed in the  
8 second year.

9 Pine weevil damage was measured after the first growing season (February of 2004) by  
10 evaluating the wounds in the stem caused by the insect. To diminish subjectivity, the  
11 stem height was divided in ten parts using an elastic ruler, and wounds were evaluated  
12 by estimating the relative debarked area in each resulting tenth using a 4-levels scale (0  
13 = undamaged, 1 = some wounds, 2 = many wounds, and 3 = death due to girdling). The  
14 sum of these ten values by plant was the damage trait ‘wounds’ ( $WND$ , 0-30 score).  
15 Additionally, the leader loss ( $LL$ ) due to stem girdling by the pine weevil was also  
16 recorded as a binary variable (leader alive:  $LL = 0$ , leader dead:  $LL = 1$ ).

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

22

## 23 *Spatial analysis*

24 The incidence of pests and diseases in forest stands commonly follow heterogeneous  
25 spatial structures that hinder proper statistical analysis (Díaz et al. 2007; Zas et al.

1 2007). When spatial heterogeneity is present, the violation of the requisite of data  
2 independence of standard statistical analyses may led to erroneous conclusions, and  
3 sophisticate spatial-analysis procedures should be used (Zas et al. 2007). We use  
4 geostatistics to test whether the weevil damage was homogeneously distributed in the  
5 study area or whether there was a spatial pattern not absorbed by the block design (Zas  
6 et al. 2006a). The spatial structure of the *WND* trait and that of the residuals of the  
7 model defined in equation [2] for this trait was analyzed using a semivariogram, which  
8 plots the semivariance between trees as a function of the distance separating them. The  
9 semivariance  $\gamma(h)$  was calculated as:

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

13 where  $n$  is the number of observation pairs separated by distance  $h$ ,  $z(s_i)$  is the value of  
14 the variable of interest at location  $s_i$ , and  $z(s_{i+h})$  is the value for a tree located at a  
15 distance  $h$  from  $s_i$ . For randomly distributed data, little change in the semivariance will  
16 be obtained when  $h$  increases, and the semivariogram will be essentially flat. If spatial  
17 dependence is present, semivariance will be lower at short distance, it will increase for  
18 intermediate distances and it will typically reach an asymptote for long distance. The  
19 distance at which the asymptote begins, if present, indicates the range or patch size of  
20 heterogeneity below which data are stochastically interdependent (Cressie 1993). The  
21 two variables were previously standardized to a  $N(0, 1)$  in order to attain an equivalent  
22 scale. The semivariogram was constructed using the VARIOGRAM procedure of the  
23 SAS System (SAS-Institute 1999).



1 *Statistical analysis*

2 Analyses of variance (ANOVA) were performed on all traits using the MIXED  
3 procedure of the SAS System (SAS-Institute 1999) and the following mixed model:

4

$$5 \quad Y_{ijk} = \mu + F_i + G_j + B_k + FG_{ij} + FB_{ik} + GB_{jk} + \varepsilon_{ijk} \quad [2]$$

6

7 where  $\mu$  is the general mean,  $F_i$ ,  $G_j$  and  $B_k$  are the main effects of fertilizer treatment  $i$  ( $i$   
8 = 1 to 9), family  $j$  ( $j = 1$  to 31) and block  $k$  ( $k = 1$  to 10),  $FG_{ij}$ ,  $GB_{jk}$  and  $FB_{ik}$  are the  
9 corresponding interactions, and  $\varepsilon_{ijk}$  is the experimental error. To analyze the whole-plot  
10 factor (i.e. fertilization) with the appropriate error term, the F×B interaction was  
11 considered a random effect (Littell et al. 1996).

12 Binary variables ( $LL$  and survival) were analyzed, in order to test for fertilizer effects,  
13 on a whole-plot mean basis. Whole-plot means were transformed (arcsin(square root))  
14 and then analyzed assuming a randomized complete block design, and using the  
15 following model:

16

$$17 \quad Y_{ik} = \mu + F_i + B_k + \varepsilon_{ik} \quad [3]$$

18

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

20 Statistical comparison of treatment means was conducted using the LSMEAN statement  
21 of the MIXED procedure (Littell et al. 1996). Data are shown as mean  $\pm$  S.E.

22

23 *Genetic parameters*

1 To estimate variance components, model [2] was applied again considering the family  
 2 effect ( $G_k$ ) and all the interactions involving this factor ( $FG_{ij}$  and  $GB_{jk}$ ) as random  
 3 factors. The analysis was restricted to the 27 families of the Galician breeding program.  
 4 Individual ( $h^2_i$ ) and family ( $h^2_f$ ) heritabilities were estimated as:

$$h^2_i = \frac{\sigma_A^2}{\sigma_g^2 + \sigma_{gf}^2 + \sigma_{gb}^2 + \sigma_e^2} \quad [4]$$

$$h^2_f = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gf}^2/F + \sigma_{gb}^2/B + \sigma_e^2/FB} \quad [5]$$

11 where  $\sigma_A^2$  is the additive variance which was assumed to be  $\sigma_A^2 = 4 \cdot \sigma_g^2$ ,  $\sigma_g^2$  is the  
 12 family variance,  $\sigma_{gf}^2$  and  $\sigma_{gb}^2$  are the variance of the family  $\times$  fertilization and family  $\times$   
 13 block interactions, respectively,  $F$  and  $B$  are the number of fertilization treatments and  
 14 blocks, respectively, and  $\sigma_e^2$  is the residual variance. Approximate standard errors of  
 15 individual-tree and family-mean heritabilities were estimated according to Wright  
 16 (1976).

17 Heritabilities of binary variables (survival and leader loss) were adjusted by the method  
 18 of Dempster and Lerner (1950). This method assumes an underlying continuous normal  
 19 variable, which at a given threshold point changes the outward observable variable into  
 20 a yes or no response. The following equation relates the heritability of the outward scale  
 21 ( $h^2_{01}$ ) to the heritability of the continuous underlying scale ( $h^2$ ):

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

1 where  $z$  is the height of the ordinate of the normal distribution at the threshold point  
2 which correspond to the observed incidence of the trait ( $\Phi$ ).

3

4 Genetic correlation between traits  $x$  and  $y$  was estimated as:

5

6

$$r_G = \frac{COV_g(xy)}{\sqrt{\sigma_{fx}^2 \cdot \sigma_{fy}^2}} \quad [7]$$

7

8 where  $COV_g(xy)$  is the genetic covariance between the two traits, and  $\sigma_{fx}^2$  and  $\sigma_{fy}^2$  are  
9 the family variance of traits  $x$  and  $y$ , respectively. The covariance between the two traits  
10 was calculated as:  $COV_g(xy) = 1/2 (\sigma_{f(x+y)}^2 - \sigma_{fx}^2 - \sigma_{fy}^2)$  where  $\sigma_{f(x+y)}^2$  is the family variance  
11 of the sum trait  $x + y$ . Data were standardized (mean = 0, standard deviation = 1) for  
12 each trait to remove scale effects prior to calculating genetic correlations. Standard  
13 errors of genetic correlations were estimated as in Falconer (1989).

14 Phenotypic correlations between family means ( $n = 27$ ) of different traits were also  
15 calculated using the CORR procedure of SAS (SAS-Institute 1999).

16

## 17 **Results**

### 18 *Pine growth*

19 Fertilization had an important effect on pine growth one and two years after planting  
20 (Table 2). Both diameter and potential height (i.e., height of those plants with leaders  
21 alive) were significantly higher when any fertilizer was added. On the contrary,  
22 fertilization did not affect actual height (height up to the girdling) in the first growing  
23 season, suggesting larger height losses due to more severe pine weevil damage in  
24 fertilized plants (Figure 1, see later). During the second growing season, the pine  
25 weevil damage did not cause stem girdling, and fertilization significantly increased both

1 height and diameter (Table 2). Among the different fertilization treatments, the  
2 inclusion of P and Mg without including N (treatments F2 and F7, Table 1) produced  
3 the greatest growth responses. On the other hand, the absence of calcium phosphate  
4 (treatments F3 and F6) led to the lowest growth responses (Figure 1).

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

8

### 9 *Pine weevil damage*

10 The pine weevil attack affected more than 94% of all seedlings in the trial, causing  
11 leader loss and death of 43.5% and 27.5% of all plants, respectively. Height of those  
12 plants with leaders alive (called potential height) was  $52.9 \pm 0.4$  cm after the first  
13 growth period, more than double the height of the plants that lost their leaders ( $23.2 \pm$   
14  $0.6$  cm).

15 The flat semivariogram of the residuals of the mixed model for the *WND* trait (Figure 2)  
16 indicated that the pine weevil attack was uniformly distributed around all the  
17 experimental area, or, at least, that the block structure accounted well for the eventual  
18 spatial variation. Actually, the semivariogram for *WND* fitted very well to a spherical  
19 model, denoting that this trait showed a small-scale spatial heterogeneity up to distances  
20 of around 30 m. This range, or patch size, corresponded well with the average size of  
21 the whole-plots. Considering the highly significant effect of fertilizer on pine weevil  
22 damage (see later), the spatial structure of the *WND* trait can be attributed to the  
23 variation among whole-plots fertilized with different treatments.

24 Pine weevil damage was significantly influenced by fertilization (Table 3), with all  
25 fertilization treatments leading to significantly more wounds and leader losses, although

1 the absence of calcium-phosphate led to significantly lower weevil damage (Figure 3a,  
2 b). Survival was also highly influenced by fertilization (Table 3), unfertilized plants and  
3 plants fertilized without calcium-phosphate (F3 and F6) showing significantly higher  
4 survival (Figure 3c), although treatment F7 which includes calcium-phosphate did not  
5 led to higher mortality. The debarked area score, the percentage of plants that lost their  
6 leaders, and mortality were around twice as high in fertilized plants than in unfertilized  
7 control.

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

18 Family  $\times$  fertilization interaction was not significant for the damage trait *WND*, but was  
19 significant for the symptoms traits *COL* and *DEF* (Table 3). This interaction led to  
20 relatively lower family and individual heritability estimates for these symptom traits  
21 (Table 4).

22

### 23 *Correlation among traits*

24 All, damage and symptom traits were significantly correlated among each other with  
25 high genetic correlation estimates (Table 5). Judging from the strong relationship

1 between the wounds in the stem and the family mean colour (Figure 4a), screening for  
2 pine weevil resistance could be carried out by simply assessing the average colour of the  
3 seedlings. Survival was also significantly related with pine weevil damage (Figure 4b),  
4 with genetic correlation close to unity, suggesting pine weevil to be the main cause of  
5 mortality. Pine growth and weevil damage were not significantly related (Figure 4c).

6

## 7 **Discussion**

8 Fertilization and pine family had a strong influence on both pine growth and weevil  
9 damage. Growth response to fertilization agreed well with the common nutritional  
10 disorders found in *P. radiata* stands in northern Spain. Phosphorus and magnesium  
11 deficiencies are the most widespread, especially on acid soils over granites such as that  
12 of the studied site, whereas nitrogen is commonly well supplied (Romanyà and Vallejo  
13 1996; Zas 2003; Zas and Serrada 2003). According to these nutritional disorders,  
14 productivity of northern Spanish *P. radiata* plantations has been shown to be positively  
15 correlated with available soil P and foliar P, and negatively with total soil- and foliar N  
16 (Romanyà and Vallejo 2004; Sánchez-Rodríguez et al. 2002). The strong response to P  
17 and Mg fertilization, and the lack of response to N addition was expected. Indeed, the  
18 responses to the fertilization treatments were almost the same as those found in other  
19 similar unattacked *P. radiata* trials in Galicia (Zas et al. 2006b). It can be concluded  
20 that growth in the studied plantation was P- and Mg-limited.

21 An increase of insect herbivory through fertilization has been reported before by several  
22 authors and for many insect-plant systems. Generally, fertilized plants may be more  
23 susceptible to insects due to reduced chemical and/or physical defences (Blodgett et al.  
24 2005; Kytö et al. 1999; Mutikainen et al. 2000; Turtola et al. 2002; vanAkker et al.  
25 2004; Zas et al. 2006a), better nutritive value (Ayres et al. 2000; Giertych et al. 2005),

1 or stronger insect attraction through higher or differential volatile emission (e.g.  
2 Gouinguené and Turlings 2002). From an evolutionary point of view, plants need to  
3 modulate how to allocate the available resources to growth, defence and reproduction.  
4 Resource constraints result in a trade-off between the high growth rate needed to  
5 outgrow competing plants, and the allocation processes that led to increased defences  
6 against herbivores, pathogens and other potential damage agents (Holopainen et al.  
7 1995). In resource-poor environments, the importance of herbivory relative to  
8 competition increases and more resources are allocated to defence. Conversely, in  
9 resource-rich soils, competition becomes relatively more important and plants invest  
10 less in defence (Agrawal 2006). These arguments agree with several ecological  
11 hypotheses, such as the carbon-nutrient balance (CNB) hypothesis which predicts that  
12 fertilization increases growth, resulting in less carbon available for the production of  
13 carbon-based secondary metabolites (Bryant et al. 1983).

14 Additionally, plant size could also play a relevant role explaining the pine weevil  
15 preferences for fertilized plants. Incidence of *Pissodes strobi* (Peck) on interior spruce  
16 (*Picea glauca* (Moench) Voss, *Picea engelmannii* Parry, and their hybrids) increased  
17 with fertilization intensity, and VanAkker et al. (2004) explained this trend in terms of  
18 increased resources available for insect feeding as a result of increased leader size and  
19 bark thickness. Seedling size is also known to affect *H. abietis* incidence. For example,  
20 *H. abietis* usually prefers Scots pine over Norway spruce, because Norway spruce is  
21 traditionally planted as bare-root seedlings which are commonly thicker than Scots pine  
22 and hence are less palatable (Toivonen and Viiri 2006). Several authors have also found  
23 a significant positive correlation between seedling diameter and survival (Orlander and  
24 Nilsson 1999; Thorsen et al. 2001). According to this relationship, a threshold of 10-12  
25 mm for root collar diameter has been suggested to avoid serious pine weevil damage in

1 Norway spruce and Scots pine plantations (Thorsen et al. 2001; Wallertz et al. 2005).  
2 However, the relatively large root collar diameter of the planting stock, and the lack of a  
3 significant correlation between growth and damage in the present study (Table 5), made  
4 us think that seedling size alone cannot explain the positive fertilizer effect on weevil  
5 preference. Further research is needed to better understand the causes behind the  
6 positive effect of fertilizer on weevil damage. The nutritional value, the chemical and  
7 physical defences, and the influence of fertilizer on the emission of volatiles, should be  
8 analyzed in new fertilizer experiments to give some light about this.

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

16 Irrespective of the proximate causes explaining the effect of fertilization on weevil  
17 damage, the practical consequence of this result is that, as indicated before (Zas et al.  
18 2006a), fertilization of second-rotation plantations on clear-felled coniferous areas at  
19 establishment is not a recommendable management practice. During the first growing  
20 season, pine weevil damage caused the loss of the growth benefits of fertilization.  
21 However, contrary to what occurred with *P. pinaster* in similar conditions (Zas et al.  
22 2006a), the effect of fertilization on height and diameter after the second growing  
23 season became significant due to overcompensatory regrowth in fertilized plants,  
24 suggesting that growth losses due to weevil damage were not as great as the growth  
25 gains due to fertilization. The higher nutrient demand of *P. radiata* may explain these



1 differences. VanAkker (2004), studying the effect of fertilizer on *P. strobi* damage,  
2 found similar results, and concluded that fertilization is a feasible option for increasing  
3 productivity of interior spruce plantations. However, taking into account the effects on  
4 survival and on the stem deformities derived from the leader losses, fertilization should  
5 be avoided when the risk of *H. abietis* attack is high. The recommendation of no  
6 fertilization or delayed fertilization for *P. pinaster* (Zas et al. 2006a) should therefore be  
7 extended to *P. radiata* plantations, and considered as another prophylactic measure to  
8 be combined with the traditional methods employed against this pest (e.g. Petersson and  
9 Orlander 2003; Sydow 1997).

10 The results of the present study also indicated substantial genetic variation in pine  
11 weevil susceptibility. Family-mean heritability was high whereas individual-tree  
12 heritability was only moderate. Both estimates were of the same order of those found  
13 for susceptibility to this insect in *P. pinaster* (Zas et al. 2005). This genetic variation  
14 could be exploited by breeding, selecting families and/or individuals to be used in high-  
15 risk plantations such as in recently clearfelled areas. The high family-mean heritability  
16 suggests high genetic gains through family selection, which may be considered when  
17 roguing clonal seed orchards. Additionally, highly resistant and susceptible genotypes  
18 could be developed through recurrent breeding, from which the mechanisms of  
19 resistance could be studied. Results presented here are based on a single test site, and  
20 may be subject to the incidence of genotype by environment interactions, which may  
21 hamper the broad applicability of the results. However, the lack of a significant family  $\times$   
22 fertilization interactions suggests that tree resistance is relatively stable, at least,  
23 regarding the pine nutritional status.

24 The high correlation between the symptom- and damage traits (Table 5) indicates that  
25 screening for resistance can be carried out by simply assessing the average colour of the

1 seedlings. However, the significant fertilizer  $\times$  family interaction found in the *P. radiata*  
2 trial for the symptom traits (Table 3) indicates that the symptoms of the weevil damage  
3 could vary with pine nutritional status. Thus, special attention should be paid to the  
4 nutritional conditions in future genetic trials if screening for resistance will be based on  
5 symptom traits.

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

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

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

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

14

## 15 **Conclusions**

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

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

23 Growth and pine weevil susceptibility were not significantly correlated, suggesting that  
24 growth and weevil resistance can be easily improved together.

25

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Table 1. Codes and chemical composition of the fertilization treatments. The + and – signs indicate presence and absence of a given compound.

Nutrient	Fertilizer	Dose	Treatment Code									
			control	F1	F2	F3	F4	F5	F6	F7	F8	
N	Ammonium nitrate	5 g N plant <sup>-1</sup>	-	+	-	+	+	+	+	-	-	-
P-Ca	Calcium phosphate	10 g P plant <sup>-1</sup>	-	+	+	-	+	+	+	-	+	+
K	Potassium sulphate	15 g K plant <sup>-1</sup>	-	+	+	+	-	+	+	+	-	+
Mg	Magnesium sulphate	5 g Mg plant <sup>-1</sup>	-	+	+	+	+	+	-	+	+	-

Table 2. Results of the mixed model for growth traits of *P. radiata* seedlings during the first two years after planting. F values and probability levels are showed.

Effect	DF	Error term	First growth period			Second growth period	
			Diameter	Actual height <sup>1</sup>	Potential height <sup>2</sup>	Diameter	Height <sup>3</sup>
Fertilization (F)	8	FxB	11.47 ***	1.68	13.11 ***	5.22 ***	6.31 ***
Block (B)	9	FxB	8.40 ***	3.39 **	2.18 *	2.84 **	4.02 ***
Family	30	error	3.33 ***	2.27 ***	3.43 ***	2.14 ***	2.36 ***
Fert x Fam	240	error	0.87	0.90	0.89	0.93	0.94
Fam x B	270	error	0.99	0.93	0.95	0.91	1.02

Significance levels \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

<sup>1</sup> Height of the live part of the stem, all plants considered.

<sup>2</sup> Plants with leader loss were dropped from the analysis.

<sup>3</sup> No stem girdling was observed in the 2<sup>nd</sup> year so only one height was evaluated.

Table 3. Results of the mixed model for damage- and symptom traits caused by the pine weevil *Hylobius abietis* on *P. radiata* seedlings. F-values and probability levels<sup>1</sup> are shown.

Effect	DF	Error term	Damage			Symptoms	
			Wounds	Leader loss <sup>2</sup>	Survival <sup>2</sup>	Color	Defoliation
Fertilization (F)	8	FxB	12.22 ***	7.29 ***	3.13 **	5.48 ***	4.70 ***
Block (B)	9	FxB	2.67 **	2.52 *	1.41	1.83	3.19 **
Family	30	error	4.63 ***			2.53 ***	1.98 **
Fert x Fam	240	error	1.08			1.17 *	1.19 *
Fam x Block	270	error	0.96			0.89	0.99

<sup>1</sup>Significant levels \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

<sup>2</sup>Leader loss and survival were assessed as binary variables. Analysis were performed on transformed (arcsin(sqrt)) whole-plot mean values.

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 the pine weevil *Hylobius abietis*.

	$h_i^2$	$h_f^2$	$CV_A$ (%)
Growth (1 <sup>st</sup> 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 <sup>1</sup>	0.16 ± 0.04	0.70 ± 0.09	34.3
Survival <sup>1</sup>	0.04 ± 0.02	0.30 ± 0.04	8.8
Symptoms			
Color	0.08 ± 0.03	0.60 ± 0.08	18.0
Defoliation	0.04 ± 0.02	0.45 ± 0.06	13.3

<sup>1</sup> Heritability estimates of binary variables were adjusted by the method of Dempster and Lerner (1950).

Table 5. Phenotypic correlation of family means ( $n = 27$ , below diagonal) and genetic correlation  $\pm$  standard errors (above diagonal) between different traits. Significant phenotypic Pearson correlation coefficients ( $P < 0.05$ ) are in boldface.

	Height	Diameter	Wounds	Leader loss	Color	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	<b>0.61</b>		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	<b>0.93</b>		-0.94 $\pm$ 0.05	-0.88 $\pm$ 0.06	-1.01 $\pm$ 0.01
Color	0.29	-0.10	<b>-0.87</b>	<b>-0.91</b>		0.85 $\pm$ 0.11	0.92 $\pm$ 0.08
Defoliation	0.24	-0.03	<b>-0.84</b>	<b>-0.89</b>	<b>0.87</b>		0.88 $\pm$ 0.08
Survival	0.14	-0.08	<b>-0.82</b>	<b>-0.93</b>	<b>0.86</b>	<b>0.89</b>	

### Figure captions

Figure 1. Effect of the fertilization treatments on diameter (a) and actual height (b) of *P. radiata* seedlings during the first and second growing seasons after planting. Data are treatment least square means  $\pm$  standard errors. Different letters indicate significant differences ( $P < 0.05$ ) among treatments. n.s. = no significant differences.

Figure 2. Semivariogram for the wounds caused by the pine weevil (WND, black squares) and for the residuals of the mixed model (white squares).

Figure 3. Effect of the fertilizer treatments on the wounds caused by the pine weevil (a), the frequency of plants that lost their leaders (b), and survival of *P. radiata* seedlings (c). Data are treatment least-squares means  $\pm$  standard errors. Different letters indicate significant differences ( $P < 0.05$ ) among treatments.

Figure 4. Relationship between the wounds in the stem caused by the pine weevil and the foliage colour (a), survival (b) and stem diameter (c) of the *P. radiata* seedlings after the first growing season. Black dots denote family means and hollow circles denote the controls. TC denotes the unimproved control.



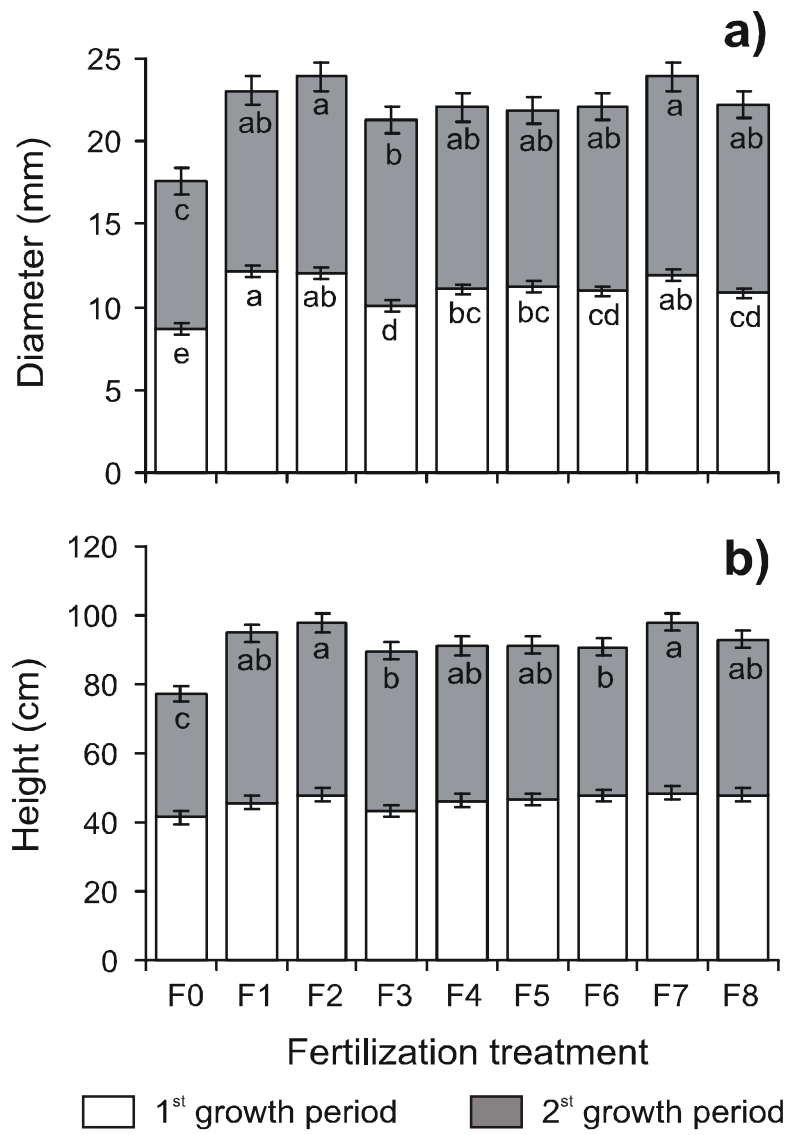


Figure 1.

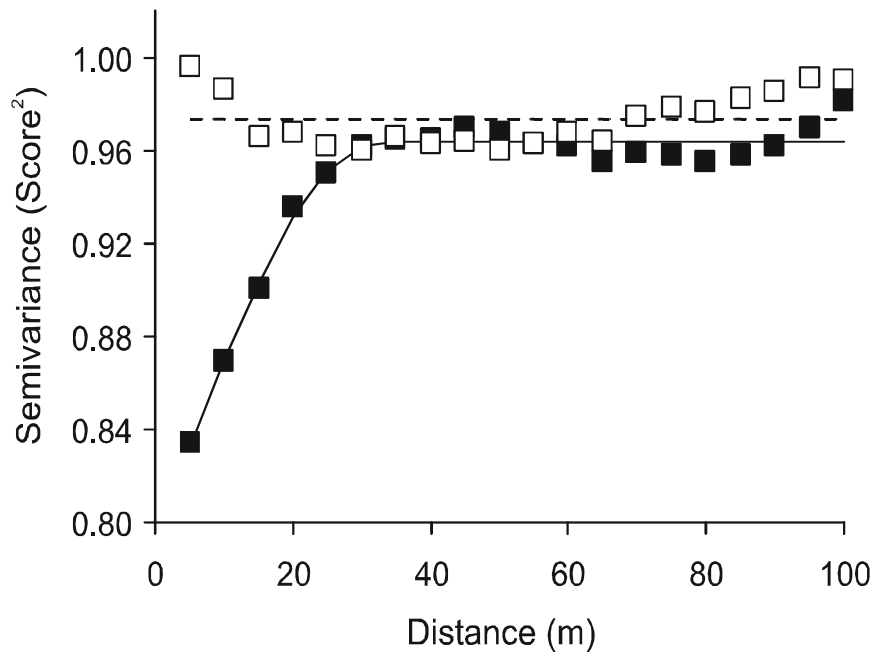


Figure 2.

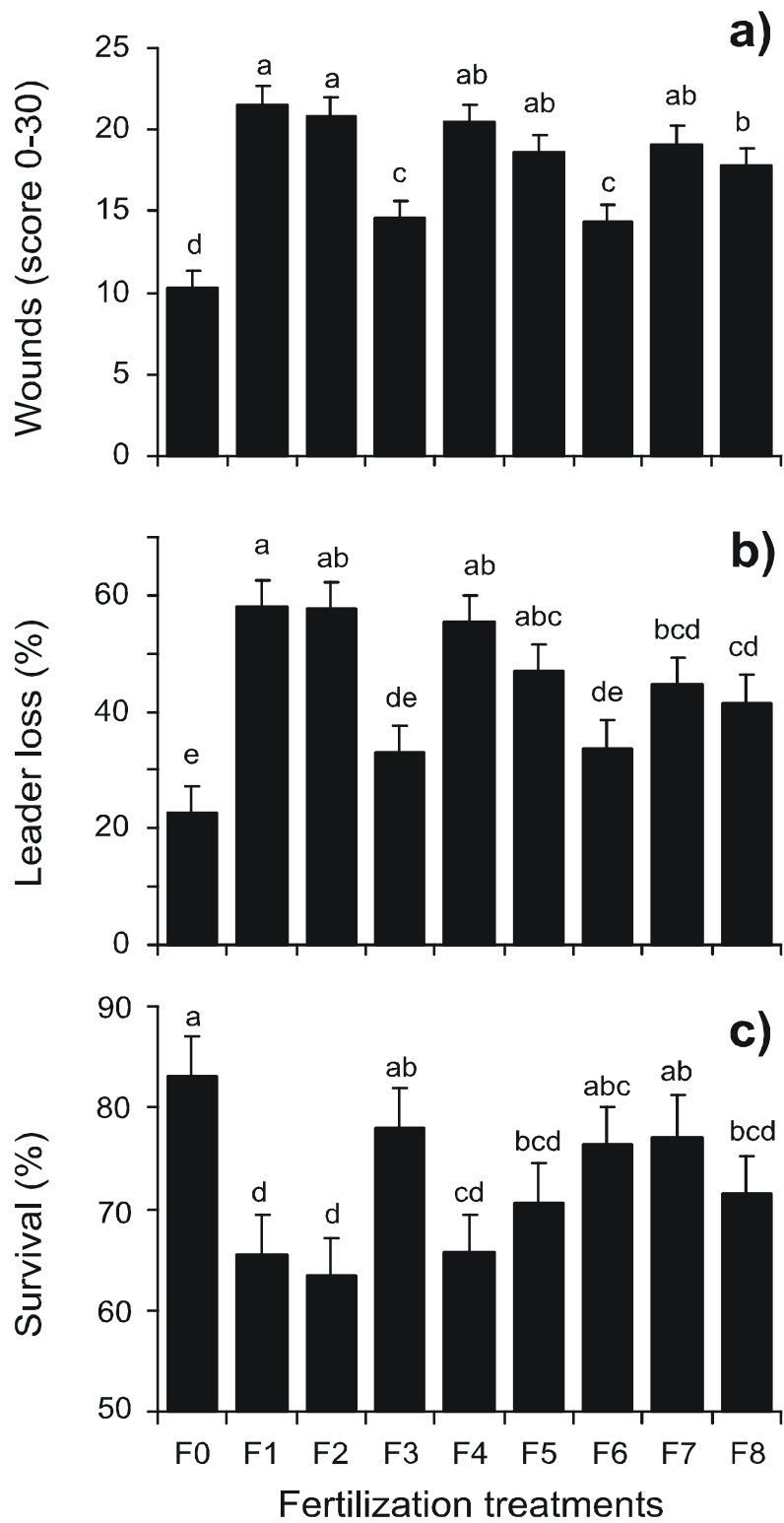


Figure 3.

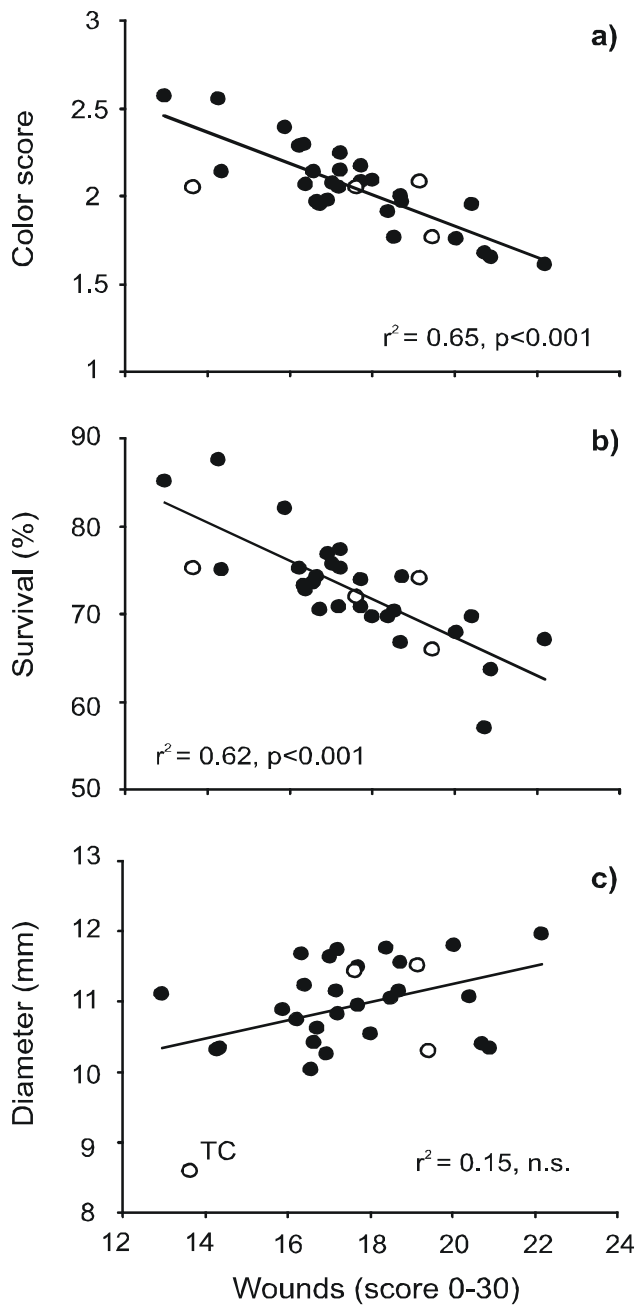


Figure 4.