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Allelopathic potential in the laurel forest of Tenerife, Canary Islands

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Abstract. Laboratory and field experiments were conducted in order to assess the allelopathic potential of a laurel forest of Tenerife, Canary Islands. Decomposing litter and leaching from leaves or other plant parts can have inhibitory effects on seed germination and seedling growth. Two native species to the laurel forest were selected to test for allelopathic effects, *Laurus azorica* and *Persea indica*, since results from former studies have shown toxicity against other organisms. The target species were two annual bioassay species, *Lepidium sativum* L. (cress) and *Lactuca sativa* L. (lettuce). Both leaves from *L. azorica* and fruits of *P. indica* were shown to negatively affect germination and seedling development in Petri dishes as well as in sowing experiments. Samples from the forest floor were also influencing seedling growth of the bioassay species, although somewhat contradictory, in the laboratory experiments. From the field experiments another factor possibly influencing seedling survival was detected, the slug *Plutonia lamarcki*. These two factors, allelopathy and slug predation, could together have great influence in shaping structure and composition of this forest type.

Sammanfattning. Laboratorie- och fältexperiments utfördes i syfte att studera allelopatisk potential i en lagerskog på Tenerife, Kanarieöarna. Nedbrytning av förna och utsöndring från bladverk, blad och/eller andra växtdelar kan ha hämmande inverkan på grodd och unga plantors tillväxt. Två inhemska arter från lagerskogen valdes ut för att testas för allelopatiska effekter, *Laurus azorica* (atlantlager) och *Persea indica* (madeiramahogny), eftersom resultat från tidigare studier visat toxicitet mot andra organismer. Två annuella försöksarter användes i experimenten, *Lepidium sativum* L. (kryddkrassing) och *Lactuca sativa* L. (sallat. Blad från *L. azorica* och frukter från *P. indica* visade sig ha negativa effekter på groning och utveckling av groddplantor både i Petriskålar och i såddexperiment. Jordprover från lagerskogen påverkade också tillväxt av groddplantor, även om resultaten var något tvetydiga, i laboratorieexperimenten. Från fältförsöken uppdagades ytterligare en faktor som kan ha påverkan på groddplantors överlevnad, snigeln *Plutonia lamarcki*. Dessa två faktorer, allelopati och snigelpredation, kan tillsammans ha stor inverkan på struktur och sammansättning i den här typen av skog.

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1 Introduction

1.1Background

The laurel forest (or laurisilva) is an ancient ecosystem that, based on fossil findings, is considered to have its origin in the Mediterranean basin. It is believed to have colonised the geographic region of Macaronesia during Miocene (Bramwell, 1976). It is now restricted to this area, which comprises the Atlantic islands of the Azores, Cap Verde, Madeira and the Canary Islands. The constitutional species of the laurel forest can be considered as paleo-endemics (Bramwell, 1976). The laurisilva is an evergreen, subtropical mountain moist forest growing on altitudes between 600 and 1200 meters above sea level, almost exclusively on the northern, windward slopes of the islands with higher mountains (Fernández-Palacios 1992). These altitudinal limits also correspond with the zone of the so called cloud sea formed by the thermic inversion of the predominating NE trade winds (Fernández-Palacois & de Nicolás, 1995).

One of the characteristics of this forest is a high rate of asexual reproduction, more than 75 % of the tree species regenerate with basal sprouts (Fernández-Palacios & Arévalo, 1998). The resulting dominance by a few species is one of the characteristics of remnant communities (Eriksson, 1996). The tree species can be divided into different ecological groups based on degree of shade tolerance, reproductive strategies and correspondence between canopy and seed bank (Arévalo et al., 2000, Arévalo et al., 2003).Larger gaps do not occur frequently in this system, and the forest dynamics is rather affected by small-scale disturbances (Arévalo & Fernández-Palacios, 1998).

Studies of the chemical ecology of some tree species in the laurel forest of the Canary Islands have revealed both plant-mammal, and plant-insect interactions (Delgado García 2000, González-Coloma et al., 1990 and 1992). The black rat (*Rattus rattus)* has been observed being intoxicated when feeding on plant tissue from the tree species *Persea indica* L. Spreng (Lauraceae), natural to the laurel forest (González-Coloma et al., 1990). Both field and laboratory studies show a positive feeding response of the rodents, suggesting a balance between the beneficial, nutritional components and the toxic secondary metabolites (González-Coloma et al., 1990). Furthermore, the feeding activity of the rats on the *P. indica* is highest during the months of April and May, indicating temporal differences in the chemical composition of the plant (González-Coloma et al., 1990). Extracts of the terminal branches of *P. indica* were also shown to have strong insecticidal activity on the two species *Macaronesia fortunata* (Lepidoptera: Lymantriidae) and *Heliothis armigera* (Lepidoptera: Noctuidae), both endemics causing outbreaks with serious damage in the Canarian pine forest (González-Coloma et al., 1992). Laboratory assays have furthermore shown strong toxicity of both leaves and fruits of *Laurus azorica* (Seub.) Franco (Lauraceae) against seeds of annual species by inhibiting their germination and radical elongation (not published).

The interference between plant species and micro-organisms, where substances are released to the surrounding in beneficial purpose is called allelopathy (Rice, 1979). It is differed from competition where resources are removed from the surrounding. Observations in the laurel forest have been made, indicating that allelopathic interactions exist. Very few exotic species have established inside the forest, although no studies have been made on this subject. An important question to answer is therefore if there is some kind of intrinsic resistance against invasions of new species in this ecosystem. Since former studies on the chemical ecology of the forest revealed toxic substances to mammals and insects, these compounds are suspected to have negative influence on plant species as well. Thus, the focal question for this project has been: Could allelopathic interactions have an impact on the laurel forest dynamics?

The aim of this study was to analyse if possibly toxic substances from different parts of the two tree species *Laurus azorica* and *Persea indica* (both Lauraceae) affect the germination of seeds of species not natural to the laurel forest. Seeds from exotic species were selected to test the hypothesis that new species are prevented from settling inside the forest. From the beginning, seeds from native species as well as exotics were intended to be used in the assays. Unfortunately, difficulties with germination of the native species i.e. mould attacks and delay in germination time, prevented this comparison. The approach has been to perform experiments in the laboratory as well as in field sites.

2 Material and methods

The nomenclature is according to Hansen and Sunding (1985).

2.1 Study site

Tenerife, with an area of 2068 km², is the largest of the Canary Islands and of the Macaronesian region as a whole. All the islands are of volcanic origin and the volcano Teide (3718 m) of Tenerife is the highest in the region. On Tenerife, the size of the laurel forest has decreased with approximately 90 % due to human exploitation, as logging activities, charcoal production and clearing to produce new farmland, since the arrival of the Europeans in the late 15th century (Arévalo et al, 1999). The remaining fragments, 4000 ha or 10 % of its potential, were formally protected in 1988 but the forest has experienced minimal human disturbances since the 1950's according to aerial photographs showing a similar forest to the present (Arévalo et al, 1999).

The study sites were located in the Anaga Natural Park (NE Tenerife; 28°19'N,16°34'W), which, with its 130 km^2 , comprises all of the extant laurel forest of the island except for small fragments in the southern part and another in the north-west (Arévalo & Fernández-Palacios, 1998). The park occupies the hills of a basaltic massif, one of the oldest parts of the island, with the age of around 7 million years and with soils classified as Entisols (Fernández-Palacios, 1992). Such soils are found in areas with a high slope, and maintain a high grade of humidity due to the content of hydrate aluminium silicates (Arévalo & Fernández-Palacios, 2000). They have a high organic content, 10%, and a pH around 5.5 (Fernández-Palacios & Arévalo, 1998). The mineral composition and the nutrient availability do not differ much within the park due to its homologous, volcanic origin (Fernández-Palacios, 1992). Mean annual temperature varies between 13-15° C, with no frost events during winter and no summer drought, and mean annual precipitation is >700 mm (Fernández-Palacios, 1992) a quantity that can be doubled considering the fog dripping (Fernández-Palacios & Arévalo,1998). The laurel forest contains 19 tree species, and the canopy height varies between 10 and 20 meters with the maximum heights found on the basin floor decreasing progressively towards the basin border (Arévalo et al, 1999). The dominating species include *Erica arborea* L., *Ilex canariensis* Webb&Berth, *Laurus azorica* (Seub) Franco, *Prunus lusitanica* (Willd.) Franco, *Viburnum tinus* Vent. and *Myrica faya* Aiton, forming a matrix into which other species are inserted depending on different environmental conditions (Fernández-Palacios & Arévalo, 1998).

Two sites on the windward slope, ‘El Moquinal’ (700 m a.s.l.) and ‘Barranco del Nieto’ (800 m a.s.l.), and one on the leeward slope, ‘Monte Aguirre’ (900 m a.s.l.), were selected for the field experiments. These sites are considered to be among the best conserved areas of the laurel forest of the Anaga Natural Park (Arévalo & Fernández-Palacios, 2000).

2.2 Laboratory experiments

Several different experiments were conducted in order to estimate the potential toxicity of the two species *Laurus azorica* and *Persea indica* (both *Lauraceae*). Different vegetative parts of the two species as well as samples of the forest floor were used in the bioassays. In all experiments, seeds from the two bioassay species lettuce (*Lactuca sativa* L.) and cress (*Lepidium sativum* L.) were used to assess potential inhibition of germination and radical- and/or stem elongation by the different treatments. These species were selected because of their rapid germination and use in former bioassay experiments (Nilsen et al, 1999, Rice, 1979).

Soil extracts. In November 2004, leachates from the organic layer of the soil from the three study sites were retreived to test for possible differences, in terms of inhibitory effects on germination and growth, between them.

The leachates were obtained through adding 3 ml of distilled water per 1 g of soil from the first two centimetres of the organic layer, followed by filtration. Two filter papers were moistened with 3 ml of the extracts, and placed in Petri dishes. 50 seeds of either cress or lettuce were spread out randomly on the dishes and they were thereafter sealed with Parafilm and left in room temperature for four days. Controls were made in the same manner with 3 ml of distilled water added. Three Petri dishes of each treatment were included, which means a total of 150 seeds. On the fourth day, the germination percentage was noted and the root length was measured.

Soil layer samples. In February 2005, samples from three different layers in the forest soil were collected to test for possible difference in toxicity. In Entisoils, horizons are normally not clearly distinguished, but differences in colour and texture between the decomposition layer, the organic and the soil mineral layer, were the basis of the soil sampling. The litter layer was removed, before collecting the upper 2 cm of each soil layer.

20 g of each of the three soil layers (litter, organic and mineral) were placed in Petri dishes and 10 ml of distilled water was added. 50 seeds of either cress or lettuce were placed upon a double layer of filter paper in each of the dishes. Controls were prepared as above. Two Petri dishes were made of each treatment, a total of 100 seeds, and they were left for four days before measurements of radical elongation.

Fruits from Persea indica. In November 2004, experiments were performed to assess the toxicity of fruits of the tree species *Persea indica.* The pulp from the fruit was removed and 2.5 g of it were placed in Petri dishes with double layer of filter paper. 3 ml of distilled water were added, 50 seeds of cress or lettuce were randomly spread out and then the dishes were sealed with Parafilm and left in room temperature for four days.

The seed of the fruit was cut in pieces and 2.5 g were placed in Petri dishes and thereafter the same treatment as with the pulp was applied. Controls were made by moistening filter paper with distilled water and pseudo replicates as above, 150 seeds per treatment. Measurement of root length was made after four days.

Extracts of leaves from Laurus azorica. In December 2004, fresh leaves from mature individuals of *Laurus azorica* were collected directly from the trees. The leaves were thoroughly moulded before adding 3 ml of distilled water per g leaves and finally filtered to achieve a solvent. By diluting the extract three different concentrations were obtained; the original 1g/3 ml, 1 g/6 ml and 1 g/12 ml.

To study possible effects, as absorption or adsorption, of soil on the toxicity, extract was added to planting pots filled with commercial pot soil. For each of the dilutions, 8 ml was added to five pots with 10 seeds of lettuce or cress, a total of 50 seeds per treatment. Control pots were the same treatment but with distilled water. The seeds were left in the pots for 7 days before measurements of both shoot, and radical length.

3 ml of the undiluted extract, 1g/ 3 ml, were added to Petri dishes with double filter paper and 50 seeds of cress or lettuce were randomly placed in the dishes before sealing them with Parafilm. Two Petri dishes, a total of 100 seeds, were made for the solution and for the controls with distilled water. The dishes were left in room temperature for four days and then the radical length was measured.

In February 2005, the experiment was amplified by collecting both fresh leaves directly from the trees and fallen, brown leaves from the upper litter layer. The extracts and dilutions were prepared in the same manner as above.

From the fresh leaves, the dilutions of 1 g/6 ml and 1 g/12 ml were tested for in Petri dishes with 50 seeds of either cress or lettuce in each. Two dishes were made for each dilution, and controls as above, a total of 100 seeds per treatment. They were left for four days in laboratory before measurements of radical elongation.

From the brown leaves, three different concentrations were applied; 1 g/3 ml, 1 g/6 ml and 1 g/12 ml and experiments were performed in the same way as for the fresh leaves, i.e. 100 seeds per species and treatment plus controls.

Sowing experiments. In November 2004, sowing experiments were conducted to try to simulate natural conditions in the laurel forest. Three soil types were used; commercial pot soil, litter from the forest floor and the upper 2 cm of the organic soil layer in the laurel forest. The two latter were selected since the seeds from the laurel forest species germinate and develop in either of the two layers. To determine whether or not light and nutrients were important factors limiting seedling growth, the two forest soil types were exposed to four different treatments; 1) light without nutrients added, 2) light with nutrients added, 3) shade without nutrients added and 4) shade with nutrients added. Fertiliser (concentration N P K 14-13-13 %) was added as pearls, three per pot, approximately 0.1 g. No fertiliser was added to the commercial pot soil. Half of the pot trays were placed in a dark room to simulate shadow, and the other half in a room with light. This means 10 different treatments in total. Of each treatment, 8-9 pots were filled and seeded with 4 seed of either cress or lettuce, 32-36 seeds per treatment in total. The pots were irrigated daily with common tap water and the root and shoot length of seedlings were measured after seven days.

2.3 Field experiments

Pot trays were prepared in exactly the same way as in the laboratory sowing experiments (described in 2.2 Laboratory experiments) and then placed on the forest floor in the three different sites described in 2.1 Study sites. The field experiments were conducted in November and December 2004. At each site trays were placed in either a small gap/clearing or under a closed canopy. The experiment was made twice, and both times the germination percentage and the stems of the seedlings were measured after seven days.

3 Results

SPSS 12.0 for Windows was used to analyse the experimental data. A large proportion of the data sets were not normally distributed and therefore, all sets were analysed with non-parametric tests. In some cases parametric tests were applied, but these results must be interpreted with some precaution.

3.1 Laboratory experiments

Soil extracts. No difference was found between the three sites of the laurel forest, regarding the germination and radical elongation of both cress and lettuce. For the lettuce seeds, a difference between the controls and the three sites was detected regarding radical elongation (p=0.000 df=3, Kruskal-Wallis' test). A one-way ANOVA of the lettuce data set showed significant differences between each of the three sites and the control (p=0.000; df=3; F=18.19), the seeds growing better in the extracts of all three sites as compared to the control (Fig 1). For the cress, no difference was detected between the mean root length of the seeds germinating in the controls as compared to the three sites (p=0.093; df=3; F=2.15; one-way ANOVA, Fig 2.). Since no differences were detected between the soil extracts of the three selected sites, all soil samples in the following experiments were collected at the same site, "Barranco del Nieto".

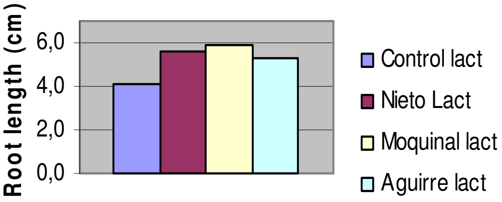

Fig 1

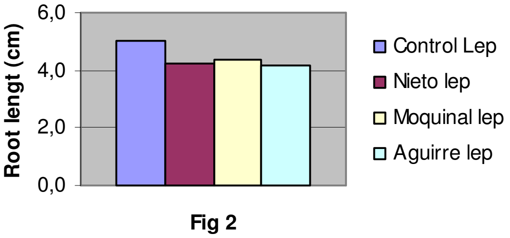

Fig 2

Figure 1. Mean root length of seedlings of *Lactuca sativa* after four days.
Figure 2. Mean root length of seedlings of *Lepidium sativum* after four days.

Soil layer samples. The germination percentage was high in all groups for the lettuce (control 97%, decomposition layer 98%, organic layer 92% and the soil mineral layer 92%) and there was a significant difference for the mean root length between the different treatments (p=0.000, df=3 Kruskal-Wallis test). A one-way ANOVA showed significant differences between the treatments (df=3, F=44.94, p=0.000) except for the comparison between the decomposition and the organic soil layers (p=0.247, Tukey HSD). The mean difference was most evident between the control and the decomposition layer (mean difference=2.05 *, SE=0.19, p=0.000 Tukey HSD) but also between the control and the soil mineral layer the mean difference (1.09, SE=0.19) was significant (p=0.000 Tukey HSD). (Fig 3.)

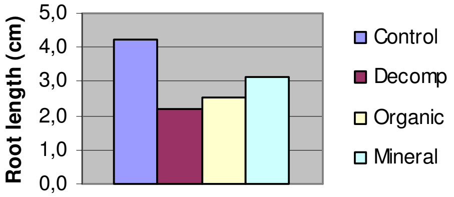

Fig 3

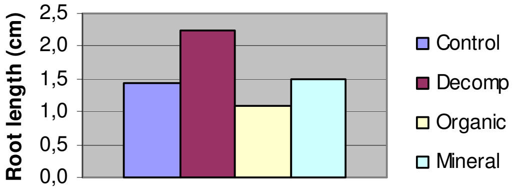

Fig 4

Figure 3. Mean root length of seedlings of *Lactuca sativa* after four days.
Figure 4. Mean root length of seedlings of *Lepidium sativum* after four days.

For the cress seeds, the germination percentage differed between the treatments (control 94%, decomposition 89%, organic layer 75% and the soil mineral layer 82%) and there was also here a significant difference between the treatments regarding the radical elongation (p=0,000, df=3 Kruskal-Wallis test). The one-way ANOVA revealed a significant difference between means (p=0.000, df=3, F=7.56) and the Tukey test showed that the biggest difference in means was between the decomposition layer and the organic layer (mean difference 1.16 cm, p=0.000). In this experiment, the cress seeds grew better in the decomposition layer (mean=2.24 cm) than in the controls (mean=1.45 cm). (Fig 4.)

Fruits from Persea indica. The germination percentage for the lettuce differed significantly between the control (97%), the pulp (0%) and the seeds (87%), as did the mean root length of the lettuce seedlings (p=0.000, df=2 Kruskal-Wallis test), (Fig 5.).
There was a significant difference in the germination percentage of the cress seeds between the control (99 %), the pulp (27%) and the seeds from *P. indica* (80%) The difference between the mean root length of the cress seeds was also significant between the three treatments (p=0.000, df=2 Kruskal-Wallis test), (Fig 6.).

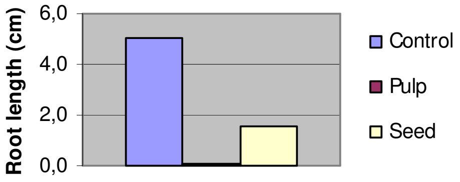

Fig 5

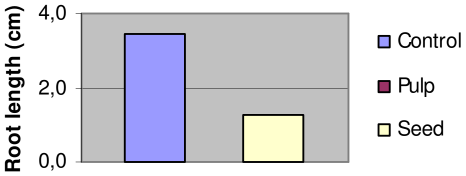

Fig 6

Figure 5. Mean root length of seedlings of *Lepidium sativum* after four days.
Figure 6. Mean root length of seedlings of *Lactuca sativa* after four days.

Extracts of leaves from Laurus azorica. From the experiments with the extract of laurel in pot soil (Nov. -04), the mean length of both root and shoot were calculated, and both differed significantly between the different concentrations and the controls (p=0.000, df=3 Kruskal-Wallis test, both lettuce and cress considering both stem and radical elongation), (Fig 7-10).

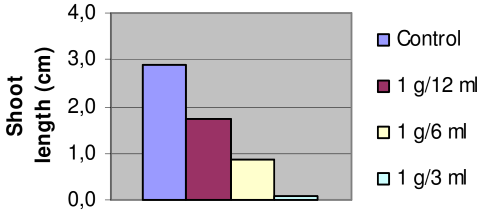

Fig 7

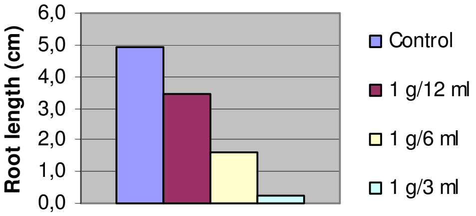

Fig 8

Figure 7. Mean stem length of seedlings of *Lepidium sativum* in extract from laurel in pot soil after 7 days.
Figure 8. Mean root length of seedlings of *L. sativum* in extract from laurel in pot soil after 7 days.

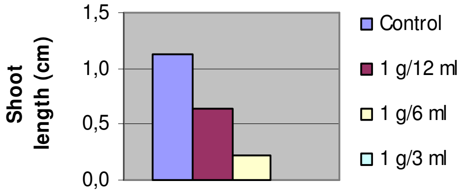

Fig 9

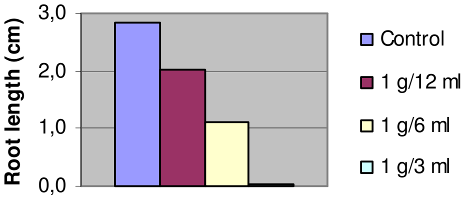

Fig 10

Figure 9. Mean stem length of seedlings of *Lactuca sativa* in extract from *L. azorica* in pot soil after 7 days.
Figure 10. Mean root length of seedlings of *Lactuca sativa* in extract from *L. azorica* in pot soil after 7 days.

In November 2004, the experiment only included the concentration of 1 g fresh leaves per 3 ml distilled water. The germination percentage for the cress was 0%, as compared to the control which had 100 %, why measurements only are displayed from the controls (Fig 11). The germination percentage for the lettuce was also strongly affected by the strong extract from the laurel leaves, only 1 % germinated compared to the control which had 69 % germination, and the root length mean also differed significantly (p= 0.000; df=1, Kruskal-Wallis test), (Fig 12).

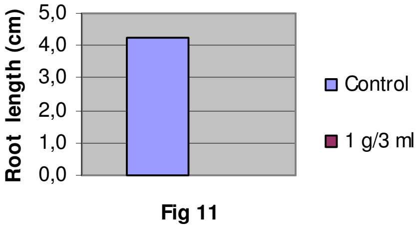

Fig 11

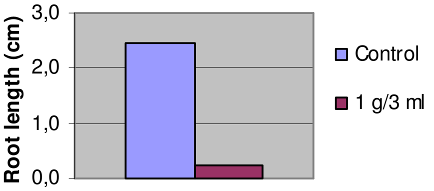

Fig 12

Figure 11. Mean root length of seedlings of *Lepidium sativum* in Petri dishes, after four days in extract from *Laurus azorica*.
Figure 12. Mean root length of seedlings of *Lactuca sativa* in Petri dishes, after four days in extract from *Laurus azorica*.

In February 2005 the experiments with leaves from *Laurus azorica* in Petri dishes were continued. The two concentrations, 1 g/6 ml and 1 g/12 ml, of the extracts of the fresh leaves inhibited the radical elongation of both cress and lettuce seeds as compared to the controls (for both cress and lettuce: df=2, p=0.000 Kruskal-Wallis test), (Fig 13 and 14). The germination percentage was high in all groups for both the cress (1 g/6 ml 75%, 1 g/12 ml 90%, and control 94%) and the lettuce (97% all treatments).

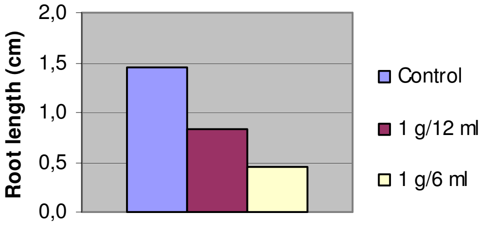

Fig 13

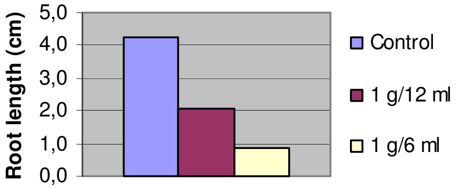

Fig 14

Figure 13. Mean root length of seedlings of *Lepidium sativum* in Petri dishes, after four days in extract from *Laurus azorica.*
Figure 14. Mean root length of seedlings of *Lactuca sativa* in Petri dishes, after four days in extract from *Laurus azorica.*

The results from the assays with the brown leaves in February 2005 showed clear toxicity of the fallen leaves on both cress and lettuce seeds, the two seed types growing worse in the different concentrations of the extract as compared to the controls (df=3, p=0.000 Kruskal-Wallis test for both cress and lettuce seeds), (Fig 15 and 16). A one-way ANOVA for the lettuce experiment also showed a significant difference between the treatments (p=0.000, df=3, F=156.71), and the following Tukey HSD showed significant difference between the mean values of all groups. For the cress seeds, the one-way ANOVA showed significant differences between the treatments (p=0.000, df=3, F=35.78), but the Tukey HSD showed that there was no significant difference in the mean values for the two strongest concentrations, 1 g/3 ml and 1 g/ 6 ml. The germination percentage for the cress seeds differed between the control and the three dilutions of the extract (control: 94%, 1 g/12 ml: 82%, 1 g/6 ml: 75%, 1 g/3 ml: 40%) but was high in all treatments for the lettuce seeds (control: 97%, 1 g/12 ml: 94%, 1 g/6 ml: 96%, 1 g/3 ml: 95%).

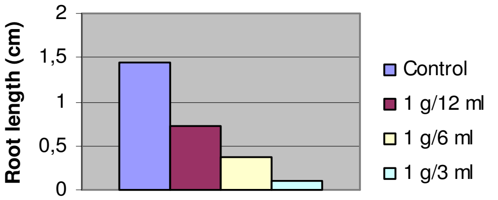

Fig 15

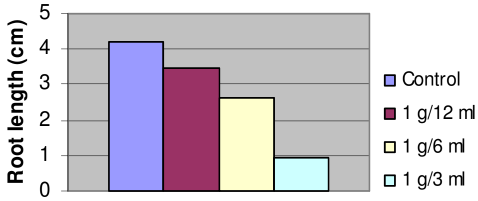

Fig 16

Figure 15. Mean root length of seedlings of *Lepidium sativum* in Petri dishes, after four days in extract from brown leaves of *Laurus azorica.*
Figure 14. Mean root length of seedlings of *Lactuca sativa* in Petri dishes, after four days in extract from brown leaves of *Laurus azorica.*

Sowing experiments. In the data sets from November 2004 all 10 categories were analysed together (five in light and five in shade, see Laboratory experiments 2.2). The results were interpreted in three groups to assess if difference in growth, both shoot and root, could be explained by either light or nutrient availability or soil type. Both the Kruskal-Wallis test and the one-way ANOVA showed significant differences between means, regarding both seed types and the two measurements (Kruskal-Wallis: Lactuca/Lepidium shoot/root, all combinations: p=0.000, df=9, ANOVA: Lactuca shoot df=9, F=19.57 p=0.000, Lactuca root df=9, F=5.28, p=0.000, Lepidium shoot df=9, F=5.50, p=0.000, Lepidium root df=9, F=9.91, p=0.000). Results from the Post hoc Tukey HSD for the three groups, light, fertiliser and soil effect, are shown in Table 1-3.

Table 1. Light effect on radical and shoot elongation of *Lactuca sativa* and *Lepidium sativum*. Differences between treatment in shade versus light are given in cm. *=significant at the 0.05 level, also indicated in bold.

Light effect	**Lactuca, root**	**Lactuca, shoot**	**Lepidium, root**	**Lepidium, shoot**
Litter, non-fertilised: Shade vs Light	**-1.06 (*)**	0.59 (n.s.)	0.63 (n.s.)	0.64 (n.s)
Litter, fertilised: Shade vs Light	-0.72 (n.s.)	**1.83 (*)**	-0.35 (n.s.)	**1.24 (*)**
Organic soil, non-fertilised: Shade vs Light	-0.16 (n.s.)	**2.04(*)**	0.55 (n.s.)	0.87 (n.s.)
Organic soil, fertilised: Shade vs Light	0.05 (n.s.)	**1.54 (*)**	0.09 (n.s.)	0.90 (n.s.)
Commercial soil: Shade vs Light	**-0.91 (*)**	**2.02 (*)**	-0.91 (n.s.)	1.03 (n.s.)

Light effect. The light had its clearest effect on the shoot length of *Lactuca sativa,* shoots growing higher in shade compared to light in all treatments but in the non-fertilised litter. The roots of the same species grew significantly better in light in pots with non-fertilised litter and in those with commercial soil. No effect of light conditions was detected regarding root length of *Lepidium sativum.* The shoot length of the same species only differed significantly in the pots with fertilised litter, resulting in shoots growing longer in shade (Table 1).

Table 2. Fertiliser effect on radical and shoot elongation of *Lactuca sativa* and *Lepidium sativum*. Differences between treatment without versus with fertiliser are given in cm. *=significant at the 0.05 level, also indicated in bold

Fertiliser effect	**Lactuca, root**	**Lactuca, shoot**	**Lepidium, root**	**Lepidium, shoot**
Litter, shade: non-fertilised vs fertilised	-0.36 (n.s.)	**-1.36 (*)**	0.52 (n.s.)	-0.47 (n.s.)
Litter, light: non-fertilised vs fertilised	-0.03 (n.s.)	-0.12 (n.s.)	-0.46 (n.s.)	0.14 (n.s.)
Organic, shade: non-fertilised vs fertilised	0.30 (n.s.)	0.82 (n.s.)	0.51 (n.s.)	0.76 (n.s.)
Organic, light: non-fertilised vs fertilised	0.46 (n.s.)	0.33 (n.s.)	0.05 (n.s.)	0.78 (n.s.)

Fertiliser effect. Commercial soil was excluded in the comparison for fertiliser effect, since no fertiliser was added in those pots. The only significant difference in mean value was for *Lactuca sativa* shoot length in the pots with litter placed in shade, resulting in longer shoots with fertiliser (Table 2).

Soil effect. The root length of the seedlings of *Lacutca sativa* was significantly shorter in organic soil as compared to litter when plants grew in light conditions and fertiliser was added, and also as compared to the commercial soil under the same conditions (Table 3). In no other treatments there were significant differences.

Regarding the shoot length of *Lactuca*, it was longer in litter as compared to organic soil, pots placed in shade and both with or without fertiliser added (Table 3). Under no other conditions, the mean values differed significantly. The shoot length of the *Lepidium sativum* seedlings was not significantly affected by the different soil types under any conditions (Table 3). The roots of the seedlings showed the strongest response to the soil effect, and they always grew better in the litter as compared to the organic soil. Under light conditions roots were longer in commercial soil as compared to the organic (Table 3).

Table 3. Soil effect on radical and shoot elongation of *Lactuca sativa* and *Lepidium sativum*. Differences between treatments with different soil types are given in cm. *=significant at the 0.05 level.

Soil effect	**Lactuca, root**	**Lactuca, shoot**	**Lepidium, root**	**Lepidium, shoot**
Shade, non-fertilised; litter vs organic	-0.53 (n.s.)	**-1.82 (*)**	**1.34 (*)**	-0.33 (n.s.)
Shade, fertilised; litter vs organic	0.14 (n.s.)	0.36 (n.s.)	**1.32 (*)**	0.90 (n.s.)
Shade; non-fertilised litter vs commercial soil	-0.46 (n.s.)	**-1.41 (*)**	0.96 (n.s.)	-0.07 (n.s.)
Shade; fertilised litter vs commercial soil	-0.10 (n.s.)	-0.04 (n.s.)	0.44 (n.s.)	0.46 (n.s.)
Shade; non-fertilised organic vs commercial soil	0.07 (n.s.)	0.42 (n.s.)	-0.38 (n.s.)	0.32 (n.s.)
Shade; fertilised organic vs commercial soil	-0.23 (n.s.)	-0.41 (n.s.)	-0.88 (n.s.)	-0.44 (n.s.)
Light, non-fertilised; litter vs organic	0.37 (n.s.)	-0.38 (n.s.)	**1.26 (*)**	-0.09 (n.s.)
Light, fertilised; litter vs organic	**0.87 (*)**	0.07 (n.s.)	**1.77 (*)**	0.55 (n.s.)
Light; litter non-fertilised vs commercial soil	-0.31 (n.s.)	0.02 (n.s.)	-0.58 (n.s.)	0.39 (n.s.)
Light; fertilised litter vs commercial soil	-0.28 (n.s.)	0.14 (n.s.)	-0.12 (n.s.)	0.25 (n.s.)
Light; organic non-fertilised vs commercial soil	-.,68 (n.s.)	0.40 (n.s.)	**-1.84 (*)**	0.48 (n.s.)
Light; fertilised organic vs commercial soil	**-1.14 (*)**	0.07 (n.s.)	**-1.89 (*)**	-0.30 (n.s.)

3.2 Field experiments

During the first set out of trays in November 2004, there were heavy rains for almost the whole week. Partly because of this heavy rain, very few seeds or seedlings were left in the pots at the Barranco del Nieto and the El Moquinal sites and the data obtained were too few to allow a powerful statistical analysis. Nevertheless, trends could be detected. Both the lettuce and the cress seeds grew better in the pots with litter as compared to the other soil types. The litter is very coarse and drains the rain water well in contrast to the compact organic soil and the commercial pot soil, where the pots probably were filled with water during some days. Individuals of the slug species *Plutonia lamarcki* (*Insulivitrina reticulata*) (Férussac) were observed to have a great impact on the growth of the seedlings, since many of their shoots were grazed and mucus from the molluscs covered the pots. One week after setting out trays for the second time, basically no seedlings or seeds were left and even more slugs were present. This can probably be linked to the fact that during that week it rained moderately and conditions seemed to be optimal for the slugs. No data from the second trial were therefore obtained. In the Monte Aguirre site, hardly anything grew neither the first nor the second time of placing out the trays, precluding statistical analyses.

4 Discussion

4.1 Laboratory experiments

In the laboratory experiments allelopathic potential in the laurel forest was detected. Although contradictory, the experiments testing for toxicity in the forest floor showed that the inhibitory effect differed with depth in the soil. Strong evidence for toxic substances that can negatively affect both germination of seeds and early development of seedlings of the bioassay species used was found for both the leaves from *Laurus azorica* and the fruits of *Persea indica*.

Soil extracts. The extracts of the organic soil samples of the three different study sites had no negative effect on the germination and growth of the cress seeds, and the lettuce seeds grew even better in the leachates as compared to the controls with distilled water. A similar result, where the test species grew better in leachates from the organic layer of the forest floor, was found in a study of *Rhododendron maximum* L. (Ericaceae) in the Appalachian Mountains (Nilsen et al., 1999). *R. maximum* is a subcanopy shrub forming near ground thickets, and inhibits seedling establishment and survival of canopy species (Nilsen et al., 1999). In their study, Nilsen et al (1999) tested for allelopathic interference of *Rhododendron maximum* on germination and early development of native tree species and the two bioassay species, and on the synthesis of ectomycorrhizal fungi. Leachates and substrate from forest with presence of *R. maixmum* did not affect germination of native species, and the fungi species were to a greater extent inhibited by leachates from the canopy species than from *R. maixmum* (Nilsen et al., 1999) Only leachates of the litter layer had inhibitory effect on radical elongation of the bioassay species. The authors suggest that the suppressing characteristics of *R. maximum* could not directly be explained by allelochemicals, but rather indirectly by their possibly negative effects on bacterial and invertebrate action resulting in reduced nutrient availability in the soil (Nilsen et al., 1999).

Soil layer samples. The results from this experiment were contrasting to the results from the soil extract experiments. The lettuce seeds grew significantly worse in the decomposition layer, the organic and the soil mineral layers, as compared to the control with distilled water. The cress seeds did not show that strong response, but still significant differences in growth was found in the following declining order; decomposition, control, soil mineral and organic soil. Cress seeds and seedlings grew and germinated worse in February 2005 as compared to in November 2004, why the data sets might have been more difficult to analyse statistically. The different soil layers seem to have a potential to negatively affect the seedling development. The difference in results between the two experiments regarding soil effect could have the following explanations: 1.) When preparing the soil leachates, the mixture of soil and distilled water was only left for 2-3 hours before filtering. If the active substances are strongly bound to the soil particles, it would be more appropriate to leave it soaking for several days, or alternatively heat the mixture to accelerate the laeching process. 2.) The climatic conditions differed between the two sampling and experimental events. In November 2004, temperature was higher and precipitation was lower as compared to February 2005. Because of the greater amount of precipitation in February, the leaching process might have been higher and the soil could have had a higher concentration of free toxic substances. 3.) The contrary: Because of the growth of the forest on relatively steep slopes, much of the direct precipitation is washed away immediately and this could explain why the soil does not contain concentrations of the secondary metabolites sufficiently toxic to affect the germination of the bioassay species. The flow-through rate may be too high for these substances to accumulate in the forest floor.

Persea indica fruits. Evident inhibitory effect of the fruit parts of *Persea indica* on the two bioassay species was shown in the experiments. Other causes than toxins released from the fruits, such as mould or bacterial attacks on the germinating seeds, can not be excluded when interpreting the results. Since fruits from this species are shed all year round (Fernández-Palacios J. M., personal communication), their inhibitory potential on other plant species could affect seedling establishment. Future studies of the toxicity of this species should include other plant parts, such as leaves and shoots. Besides testing on native species of the laurisilva, it would also be appropriate to test for auto-toxicity.

Extracts Laurus azorica. Both the extracts of fresh leaves and of shed leaves from the litter layer affected seedling growth negatively on lettuce as well as on cress. Regarding the fresh leaves, the probability that these compounds ever reach inhibitory concentration in the soil is very low, why the results from the brown leaves are more interesting. Even in the most diluted concentration both cress and lettuce seeds grew significantly worse compared to the controls.

Both the experiments with the *Persea indica* fruits and the *Laurus azorica* leaves coincide with results from former studies on the species' toxicity on both insects and mammals (Delgado García 2000, González-Coloma et al, 1990 and 1992), amplifying its effects to include plant-plant interactions.

Sowing experiments. The two bioassay species responded differently to the treatments in this experiment. The lettuce seedling shoot length was the character with the most evident response to the light/shade treatment, growing longer in shade. Regarding the possible nutrient deficiency in the forest floor, no effect of adding fertiliser was shown. It could be more appropriate to use liquid fertiliser, rather than pearls to achieve a rapid effect.

The cress seedlings were significantly affected in half of the different soil treatments, regarding the radical elongation. The roots grew worst in the organic layer, both as compared to the commercial soil and the litter layer, in both light and shade. Shoot length showed no such differences between treatments. The lettuce seedling root length responded in the same way, but only under light conditions. Of the two soil layers used in this experiment, it seems like the organic soil has the greatest potential to negatively affect development of seedlings. In the laurel forest, *Laurus azorica* show significant aggregation patterns as adult trees, juveniles and saplings, occur within short distances (Arévalo & Fernández-Palacios, 2003). To asses whether this distributional pattern could be explained by allelopathy or by resource competition advantage, further experiments need to be conducted and several methods can be used to differ between the two interference types.

In a study of an aggressive invasive plant, *Lantana camara* L. (Verbenaceae), in Australian forest communities three different treatments were used to determine whether the plant species' ability to spread and suppress native rainforest species could be explained by allelopathy or by resource competition (Gentle & Duggin, 1997). In field plots the invasive plant was 1.) completely removed, 2.) cut and left in place or 3.) burnt. In the sites where the *L. camara* had been removed, the seedlings of the two indigenous species showed a negative pattern of seedling mortality with increased density, compatible with density-dependent resource competition. In contrast, a positive response on seedling density was observed in the treatments where *L. camara* was either cut and left in place or burnt. The authors suggested that the results could be interpreted as allelopathic interactions rather than resource competition, with the following line of argument: a dilution of phytotoxins will take place through uptake by neighbouring plants, with the degree of dilution increasing with density. In this way, survival and growth should therefor be positively correlated with density, contrasting the results of resource competition in the absence of allelopathy (Gentle & Duggin, 1997).

Activated carbon was shown to have adsorptive capacity on allelochemicals, and reduced the inhibitory effect of the Swedish boreal species *Empetrum hermaphroditum* L. (Empetraceae) on Scots pine (*Pinus sylvestris*), both in green house experiments and when added to field plots (Nilsson, 1994). Resource competition was controlled by growing the seedlings in root exclusion tubes, and thus the reduction of inhibition caused by activated carbon was attributed to allelopathic effects (Nilsson, 1994).

A similar approach was used in a study of another shrub species, *Kalmia angustifolia* L. (Ericaceae), and its effects on black spruce (*Picea mariana* (Mill.) BSP) seedlings (Wallstedt et al., 2002). In this study, the charcoal treatment had negative effect on the seedling biomass under low levels of *Kalmia* cover, and belowground competition was concluded to be an important factor to suppression of black spruce seedlings. At high levels of *Kalmia* cover, the authors suggested that the inhibition of black spruce seedling growth was due to *Kalmia* influencing their nutrient acquisition by immobilising N in the organic layer and by influencing the composition of ectomychorrizal fungi on the seedlings (Wallstedt et al., 2002).

Wardle et al (1998) suggested that the allelopathic potential of one species, the invading thistle (*Carduus nutans*), could be regulated by another species in a reciprocal interference with the native grass species (*Lolium perenne*) in New Zealand pastures. In the laurel forest, the two tree species (and possibly more species) *Laurus azorica* and *Persea indica* both indigenous to this ancient ecosystem, could be an example of co-evolution of reciprocal allelopathy, either by regulating the toxic potential of the other species or by having developed immunity to the other species' phytotoxins.

4.2 Field experiments

In this study, difficulties in detecting the effects of the secondary metabolites in the field, revealed another factor probably affecting the survival and growth of the seedlings of the two bioassay species. It seems plausible that the slug *Plutonia lamarcki* plays an important role, concerning the settlement of new and existent species, in the laurel forest. The slugs are abundant (personal observation) but no studies have been made to assess their impact on different levels of the ecosystem.

In a coniferous forest in northern Sweden, field experiments were conducted to study predation levels of the slug *Arion subfuscus* (Drap.) on young Scots pine (*Pinus sylvestris*) seedlings (Nystrand & Granström, 1997). To assess the effect of forest floor moisture content on the slug predation activity, young Scots pine seedlings were transplanted into field plots with three different treatments: 1.) screened plots with low moisture content, 2.) unmanipulated plots, and 3.) watered plots with high floor moisture content . The predation levels were significantly higher in the watered plots as compared to the screened plots. In the unmanipulated plots, predation levels were highly variable and related to the forest floor water content. The authors conclude that slug predation can be an important mortality factor on Scots pine seedlings, and the highest rate of predator activity coincide with conditions favourable for seedling establishment, i.e., when forest floor is moist (Nystrand & Granström, 1997).

Transferring the results of Nystrand and Granström (1997) to the laurel forest, the slug *Plutonia lamarcki* could have a great potential to affect the stand composition and spatial patterns of trees. With an annual precipitation mean around 700 mm and no summer drought (Fernández-Palacios, 1992), conditions should be optimal not only for year round seedling establishment but also for slug predation activity.

4.3 Conclusions

As emphasised by authors of former allelopathic studies (Nilsen et al., 1999, Wallstedt et al., 2002 and Wardle et al., 1998), it seems more appropriate to study this type of interference in a higher level perspective than as a plant-plant interaction. In the great majority of studies made on this interference type, focus has been set on exotic species invading natural communities. I suggest the opposite situation, where invading plants are suppressed by native species with allelopathic potential, being equally possible.

In this sclerophyll moist forest, the majority of the constitutional species has leaves with rigid structure and at least two of them (*Laurus azorica* and *Persea indica*) produce secondary metabolites with strong toxic potential to other plant species. This could have negative effects on the litter quality and thereby on the soil microflora and -fauna, on fungal symbiosis and on the soil invertebrates. That in turn could deteriorate soil structure, decrease decomposition rate and thereby the rate of nutrient mineralisation and affect plant nutrient acquisition ability. As an effect, the phytotoxins could indirectly have a negative effect on plant growth and thereby plant community structure. This could influence water relationships in the soil, and thereby slug activity and seedling predation. These relationships remain to be studied.

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