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New Sloped Box method to study allelopathic effects of *Acacia dealbata* under competitive interactions

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ABSTRACT

We have developed new 'Sloped Box' technique to investigate the role of allelopathy in spread of *A. dealbata* under competitive interactions. The germination of *Plantago lanceolata* L. and *Trifolium pratense* L. was accelerated in seeds near to *Acacia dealbata* Link. In 3 out of 4-test species, the photochemical efficiency of photosystem II was increased with time, in plants grown around *A. dealbata* and plants in first part of underside of the slope. Interestingly, the above ground biomass of *P. lanceolata* and *Taraxacum officinale* Weber ex F.H.Wigg were lowest in plants closer to *A. dealbata*, followed by the plants grown on the first part of lower side of slope and the highest in plants on the top and bottom of slope. We found that plants around the invasive species were more affected than those on the edges of pots. We concluded that the invasive *A. dealbata* could affect the autochthonous plant species by naturally releasing the organic compounds that act as allelochemicals, although the interaction of other factors cannot be overlooked. Its effect is focussed on plants located near the individual donors. This new method may be adequate to study allelopathy and help in understanding the allelopathic process and competitive mechanisms.

Keywords: *Acacia dealbata,* competitive interactions, invasive species, native species, *Plantago lanceolata,* physiological parameters, Sloped Box technique, *Trifolium pratense*

INTRODUCTION

The exotic species causes the structural changes in ecosystems and loss of biodiversity (7,21,25,33). Not all exotic species are invaders in the environments to which they are introduced (23). These exotic species became invasive when they overcome many barriers (29) and are better competitors than native species, either through resource competition or through allelopathic interference (3).

Allelopathy refers to positive and negative effects effects of one plant on another plants through the release of chemical compounds in the environment, including microorganisms (49). Allelopathy is one of the reasons for the success of invasive *Acacia dealbata*. However, the relative importance of chemical interference in competitive interactions is not known in its invasive process. The negative allelopathic effects caused

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by invasive species are more important than the positive effects (20,23), as the adverse effects are evident on the structure of ecosystems and biodiversity (7,21,28,33). Callaway and Ridenour (6) have suggested that exotics were successful because they released unique allelopathic compounds in the introduced area (*Novel Weapons Hypothesis*) to which native species are not adapted, due to the exotic and native species failing to develop mutual tolerance during the joint evolutionary process (37).

Generally allelopathy is studied in germination bioassays, to know the physiological processes and structural characteristics involved in seed germination, which is important for the establishment of plant species in their natural environment (34). Recently much allelopathy research has been done (6,7,9,12,24,33), but without considering the competitive process. Resource competition is major competitive mechanism that affects the plant success (24). However, allelopathy facilitates the success of invasive plants (12,20,23). The signalling allelopathy should be considered in managing the forest ecosystems threatened by exotic species (4). Generally the possibility that both competition for resources and allelopathy may operate simultaneously (17,20). Recently, new methods to study allelopathy has been developed for specific purposes, such as collecting volatile allelochemicals (44) or searching new target plants for allelopathic compounds (19). However, to evaluate the allelopathic activity in plant invasion process, the activated carbon method is used to assess the allelopathic effects of higher plants, separating the resources competition (22,24,41). Activated carbon absorbs the organic compounds and thus removes some allelochemicals from soil solution (22). Recently, doubts have arisen that activated carbon may also affect the plant growth and the availability of plants nutrients (27,46). However, there is no other method to study the effects of allelopathy, separating the resource competition. Hence, we proposed a new method to overcome the introduction of artefacts in the Experimental Design and is called the ‘Sloped Box’ technique.

Acacia dealbata Link (Australian tree legume), was introduced in Europe as ornamental specie in 19th century (36). Today it has become a serious environmental problem in Northwest Spain, where it has invaded native forests, vacant arable land and watercourses to harm the native understory species (8), establishing monocultures and modifying the ecosystems structure (28,32,33). Its success in NW Spain has been attributed to its allelopathic effects on model test species (8) and on agricultural and native understory species (30,31,33). However, the relative importance of chemical interference and competitive interactions remained unknown in its invasive process. The *A. dealbata*, contains phenolic compounds (ferulic acid, gallic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-vanillin) responsible for many allelopathic interactions (11,38). These and other allelochemicals have been quantified in various *Acacia* species (43). Specifically, González *et al* (18) have identified different phenolic compounds (luteolin, apigenin, vanillin, ellagic acid, egallitannins, 4-hydroxy-3-methoxybenzyl alcohol, ferulic acid, quercetin 3-glycoside and vanillic acid) in bark and leaves of *A. melanoxylon*. Moreover, some phenolic and sesquiterpene compounds found in different extracts of other *Acacias* possess the phytotoxic activity (1,12,13).

This study aimed to understand the relative importance of allelopathy in spread of *A. dealbata* under competitive interactions. Hence, we investigated the allelochemical interference of *A. dealbata* on germination, physiological parameters of stress and biomass of 4-native understory and prairie species (*Plantago lanceolata*, *Trifolium pratense*, *Taraxacum officinale* and *Dactylis glomerata*) by newly developed "Sloped Box" technique, based on sloped area. This method allows growing together the exotic and native species and they interact with each other, separating the allelopathic effects of others and preventing the introduction and possible interference of other compounds (such as activated carbon). We also discussed the potential of this new technique as new bioassay method in allelopathy.

MATERIALS AND METHODS

Plant material

The *Dactylis glomerata* L., *Plantago lanceolata* L., *Taraxacum officinale* Weber ex F.H.Wigg and *Trifolium pratense* L. species were chosen as target species as they are common understory in native forests and agricultural crops in the NW Iberian Peninsula, hence, these are first affected during the invasion processes. The use of such species from different families may facilitates the generalization of results (12). Seeds of donor specie (*A. dealbata*) were obtained from Nindethana Seed Service, Australia and seeds of target species (*D. glomerata*, *P. lanceolata*, *T. officinale* and *T. pratense*) from Herbiseed, U.K.

Field study

The experiment was done in natural greenhouse conditions at University of Vigo, Spain, from June to October 2008. To control stress conditions (drought, nutrients and herbivory), the irrigation and nutrients were applied uniformly and regularly (26). Barriers to prevent entry of small herbivores were made. Seven inclined pots (1 m x 1 m x 40 cm depth) with 60 degrees slope ("Sloped Box") were filled with commercial substrate (Compo Sana®: N 200-450 mg/L; P_2O_5 200-500 mg/L; K_2O 300-550 mg/L; pH ($CaCl_2$) 5.0-6.5). Four plants (20-months-old) plants of *Acacia dealbata* were planted in centre of each Sloped Box, about 5 cm apart. The experimental design ensured the equal distribution of shadow in all directions. The *A. dealbata* plants did not reach the bloom stage. The acacia plants were left for one month to acclimatize and then the Sloped Boxes were fertilized to prevent the nutrients deficiencies (Compo Nitrophoska®: Total N 12 %; P_2O_5 12 %; K_2O 17 %; MgO 2 %; SO_3 15 %; B 0.02 %; Zn 0.01 %; 35 g/m^2). Twenty seeds of each target specie were sown and covered with substrate layer (5 mm) in 4-quadrats (5 cm x 5 cm) placed along the row. In each Plot, 4-rows (spaced 5 cm apart) were prepared from the centre to each side (Fig. 1a). The photosynthetically Active Radiation was measured under *Acacia* plants at target species level at 3-points in each Sloped Box side throughout the experiment using a Hansatech Light Meter (QRT1, United Kingdom). The Sloped Boxes were uniformly watered when necessary (2-3 times weekly). Sprinkler irrigation was given to acacia plants, ensuring uniform water distribution over the plants and Sloped Boxes surface to simulate rainfall and to produce leachate similar to natural leachate. The experiment was done in randomized complete block with seven 'Sloped Box' pots. There was no physical barrier between the plants that prevented the free movement of potential allelochemicals released from any part of the invasive plants.

In this approach, allelochemicals were naturally released by the exotic plants and could affect the physiological processes of target species. This methodology was adapted to simulate solute transport (downward migration of potential allelochemicals released by

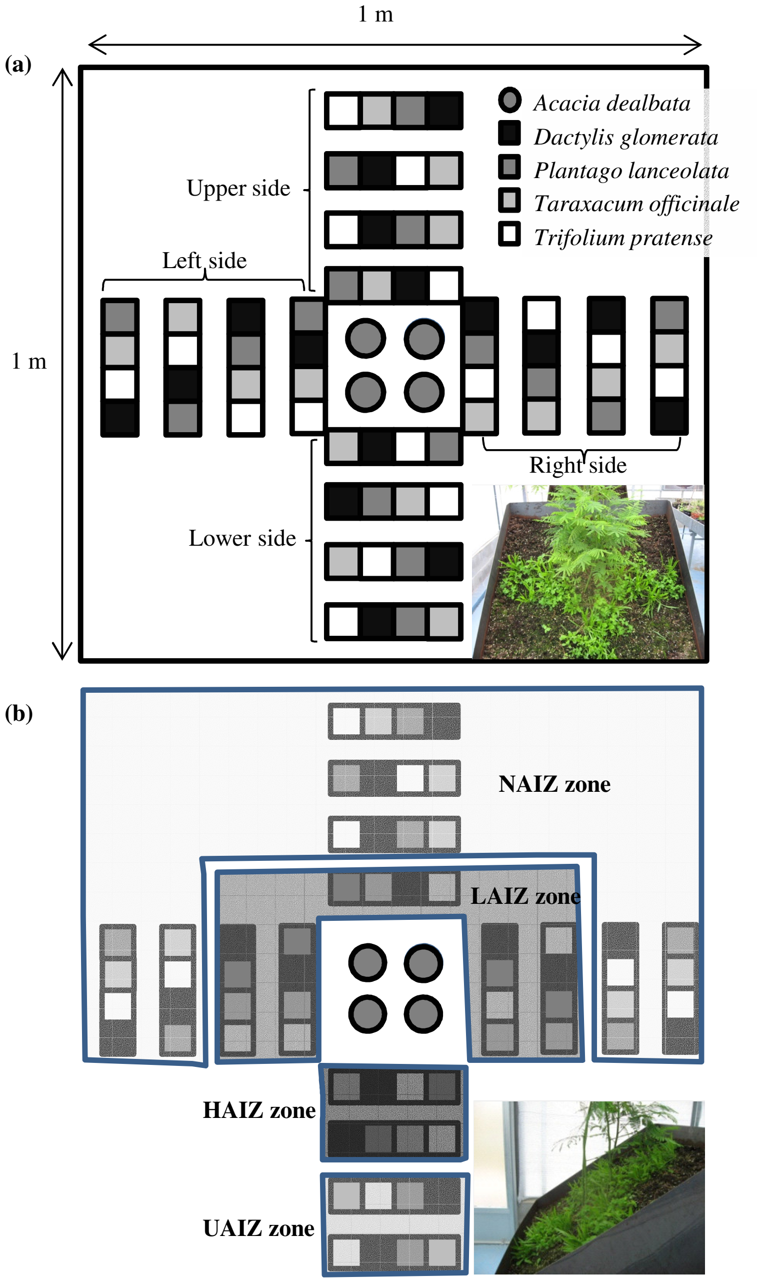

Figure 1. Experimental design scheme of slope box. (a) Native species distribution. (b) Allelochemicals distribution hypothesis, NAIZ: non-allelopathic interaction zone; LAIZ: low allelopathic interaction zone; HAIZ: high allelopathic interaction zone; UAIZ: unpredictable allelopathic interaction zone

A. dealbata). We hypothesised that allelochemicals migrate with soil solution around the donor plants and mainly downward, affecting the target plants (Fig. 1a) as per Carballeira *et al.* (8) and Sanchez-Moreiras *et al.* (42). In our hypothesis, the highest interaction between the allelochemicals and the target plants should be at the downward part, next to the donor plants (the "high allelopathic interaction zone", HAIZ) and the smallest number of target plants affected should be at the top (The "non-allelopathic interaction zone", NAIZ). Lateral plants both right and left, showed the susceptibility of target plants to allelochemicals only when the concentration is sufficient to move sideways (the "low allelopathic interaction zone", LAIZ). Plants at the bottom of slope would be affected in unpredictable ways, depending on the rate of movement and susceptibility of target species (the "unpredictable allelopathic interaction zone", UAIZ). Interspecific competition is supposed to be limited within each zone, so it does not affect the experimental design (Fig. 1b).

Target species measurements

Seeds were considered germinated when the cotyledons elongated to 2 mm above the substrate. The germination of target species was recorded twice a week, until either all of the seeds germinated or no seed germination was observed. Several germination indices [Total germination (G_t), speed of cumulative germination, AS and germination rate coefficient CRG)] were calculated for these species as per Chiapusio *et al.* (10). The plants in each quadrat (5 cm x 5 cm) were thinned to one plant. For each target species, one plant per quadrat x 4-rows per side x 4-sides per Sloped Box x 7-Sloped Boxes. Non-destructive measurements of the efficiency of Photosystem II photochemistry (PSII) (49) and chlorophyll fluorescence (Fv/Fm) (39) were recorded in each of these test plants. The PSII and Fv/Fm were measured, from the youngest expanded leaves with fluorescence monitoring system (Hansatech, United Kingdom) once a month from August to October. At 15-weeks after planting, the target species, the plants in each quadrat were harvested and dry biomass was determined. To calculate the dry weight, the plant material was dried in oven at 60 ºC to constant weight. Due to difficulty in recovering the root biomass of each target plant separately, only aboveground biomass was determined.

Statistical analyses

The photosynthetically active irradiance was determined using the Kruskal-Wallis test. The effects of the allelochemicals released by *A. dealbata* on all of the parameters measured for each target species were determined using one-way ANOVA and the LSD test to determine the main differences in effects between zones. Verification of normality and homogeneity of variance were assessed by the Kolmogorov-Smirnoff test and the Levene test respectively. When homogeneity of variance could not be achieved, the Kruskal-Wallis test was used followed by a *post hoc* Mann-Whitney *U* test whenever there was a treatment effect. We used a two-way ANOVA to test for the effects of zones and sampling date on Fv/Fm and PSII for each species. If the sampling date or its interaction was significant in the two-way ANOVA, we presented and analyzed the data from different samplings separately, using one-way ANOVA with zones as the main effect. If there was no significant effect of the sampling date, we ran a one-way ANOVA on pooled data from August to October. The normality, homogeneity of variance and differences between treatments were checked as before. The level of significance for the differences between the means of the groups was set at $P \leq 0.05$ in all applied tests. The role of the distance from the centre to every side of the sloped box on the effects of released allelochemicals from *A. dealbata* was assessed by correlation analyses between all measured parameters and distance for each target species (Pearson correlation coefficient, significance level $P \leq 0.05$). The effect was evaluated in two transects established from plants of *A. dealbata*, in the centre of the sloped box, toward the topside and underside.

All statistical analyses were performed using SPSS v.17.0 for Windows.

RESULTS AND DISCUSSION

The target plants in Sloped Box received the same solar radiation (Average = 150.01±9.540; $P = 0.055$). The release of allelochemicals from *A. dealbata* did not affect the total germination (G_t) of all target species (Fig. 2a). However, there were significant differences in the rate of accumulated germination (AS) for *P. lanceolata* (Fig. 2b). The AS was significantly lower in the NAIZ and UAIZ than in the HAIZ and LAIZ. The coefficient germination rate (CRG) index showed significant differences in *T. pratense*, in which the LAIZ and UAIZ had the highest values, followed by NAIZ and HAIZ (Fig. 2c). The total germination of all studied test species was not affected by allelochemicals released from *A. dealbata*, but the germination of *P. lanceolata* was accelerated in zones with highest expected concentration of allelochemicals (HAIZ). Surprisingly, the germination of *T. pratense* was accelerated near the *Acacia* plants (LAIZ), delayed in the middle of slope underside (HAIZ) and accelerated again at the bottom of slope underside (UAIZ). Generally, allelochemicals are stimulatory at low concentrations and inhibitory when the concentration is increased (45). In our case, it seemed that *A. dealbata* released little quantity of allelopathic compounds that were not enough to cause inhibitory effects, but were stimulatory (38). These authors found that the germination and growth of target species were stimulated at low concentrations of phenolic compounds. According to the hypothesis on the distribution of allelochemicals (Fig. 1), no interaction was expected at bottom of slope underside, where germination was stimulated. Present findings did not agree with Carballeira and Reigosa (9) and Lorenzo *et al.* (33) in that the germination frequencies of lettuce and maize were depressed by natural washes from *A. dealbata* during its flowering period, which confirmed the different response depending on the target species (14) or the concentration or quality of allelochemicals. These authors previously assayed *A. dealbata* during the flowering period, when its flowers extracts were rich in allelochemicals (9,30,31,33). However, the present test was conducted without flowers of *A. dealbata*. Therefore, the probable low concentration of allelochemicals could be responsible for the observed stimulatory or null effects on germination indexes, although we could not reject the possibility of accumulation of nutrients favoured by the slope. Factors such as the rainfall regime (9,30,31) or plant density of target species (47) could also affect the results. The watering frequency could dilute the concentration of allelopathic compounds present on the surface of seeds and plants. Additionally, allelopathic effects were density-dependent, as high plant densities imply the availability of less potential allelochemical per seed or plant (48).

Aboveground biomass differed depending on the target species (Fig. 3), although

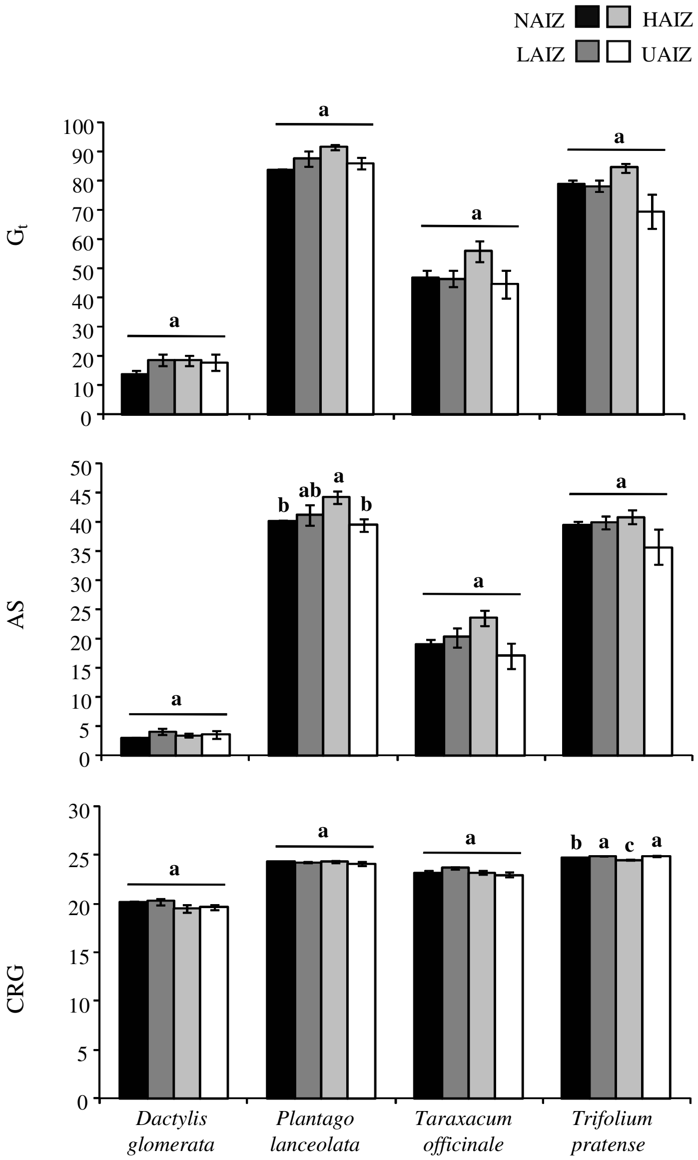

Figure 2. (a) Total germination (Gt), (b) accumulated germination (AS) and (c) coefficient germination rate (CRG) of native species in the presence of *Acacia dealbata.* NAIZ: non-allelopathic interaction zone; LAIZ: low allelopathic interaction zone; HAIZ: high allelopathic interaction zone; UAIZ: unpredictable allelopathic interaction zone. Means ± SE are presented, *n* = 7. LSD test was used when homogeneity of variance was achieved; otherwise the Mann-Whitney *U* test was used. Different letters indicate statistically significant differences ($P \leq 0.05$) in each target species.

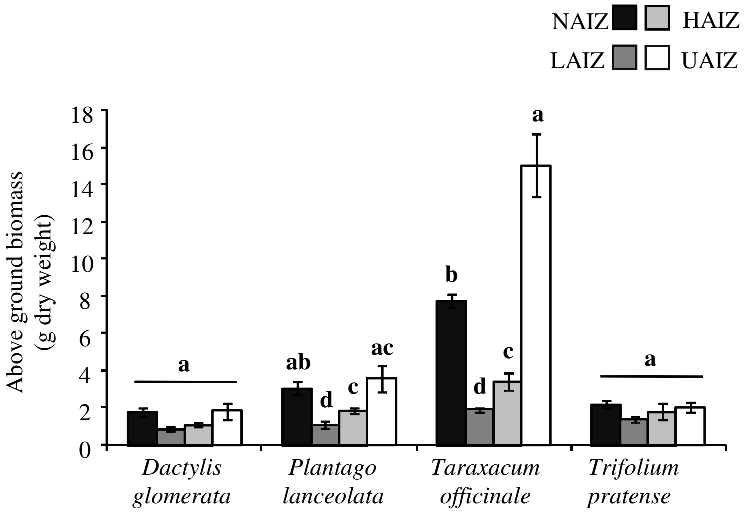

Figure 3. The aboveground biomass of native species in the presence of *Acacia dealbata*. NAIZ: non-allelopathic interaction zone; LAIZ: low allelopathic interaction zone; HAIZ: high allelopathic interaction zone; UAIZ: unpredictable allelopathic interaction zone. Means ± SE are presented, $n = 7$. LSD test was used when homogeneity of variance was achieved; otherwise the Mann-Whitney *U* test was used. Different letters indicate statistically significant differences ($P \leq 0.05$) in each target species.

there was similar pattern between *D. glomerata* and *T. pretense* and between *P. lanceolata* and *T. officinale.* For *D. glomerata* and *T. pretense* there were no significant differences among the treatment zones. However, there were significant differences in *P. lanceolata* and *T. officinale*. Invasive plant species are excellent competitors (3) and their success as an invader is due to winning in competitive process (24). Competition for resource and indirect competition through allelopathy, are components of competitive process (17). The distinction between the direct and indirect competition is very important for invasive plant species (3). Removal of invasive plant could limit the spread of invasion caused by this invader when allelopathy does not interfere with competition for resources. However, at site where resource exploitation was indirect through allelopathy (17,38), removal of invader may not be enough. Ridenour and Callaway (41) proposed a simple formula to determine the relative importance of allelopathy in competitive process between an invasive and target species based on total biomass, but this formula could not be applied to our data due to the different experimental design. Since allelopathy depended on target species, for this experimental design we proposed related equations using aboveground biomass production that showed the relative importance of allelopathy in the competitive process between the donor species and each of the target species. Thus, for *D. glomerata* and *T. pratense* the ratio was as Under:

$$\mathrm{NAIZ}_{\text{aboveground biomass}} = \mathrm{LAIZ}_{\text{aboveground biomass}} = \mathrm{HAIZ}_{\text{aboveground biomass}} = \mathrm{UAIZ}_{\text{aboveground biomass}}.$$

This result suggested that there was no direct or indirect competition between the *A. dealbata* and each test species. The limited effect of allelopathic factor for both *D. glomerata* and *T. pratense* was evident. However, considering that these species were excluded from the understory of *A. dealbata* groves (28) and that in our experiment, we added nutrients, resource competition should not be ignored in invasion process. Therefore, it was possible that other factors could play role in the competitive process in the field. Moreover, there was a gap in our knowledge about the effects of allelochemicals released by *A. dealbata* on root biomass. The ratio for *P. lanceolata* and *T. officinale* were similar as under:

I. Case: $(\text{NAIZ}_{\text{aboveground biomass}} = \text{UAIZ}_{\text{aboveground biomass}}) > \text{HAIZ}_{\text{aboveground biomass}} > \text{LAIZ}_{\text{aboveground biomass}}$

II. Case: $\text{UAIZ}_{\text{aboveground biomass}} > \text{NAIZ}_{\text{aboveground biomass}} > \text{HAIZ}_{\text{aboveground biomass}} > \text{LAIZ}_{\text{aboveground biomass}}$.

In the vicinity of donor plants, the growth of *P. lanceolata* and *T. officinale* was less than at the top side suggesting a predominant allelopathic effect on target species, as there was no limitation of nutrients. Another form of passive interference was the shading effect (15), which reduces the aboveground biomass (17); however, our target plants received the same amount of solar radiation. This means that allelopathic compounds could be concentrated around *Acacia* plants and so allelopathy might play a role compared to resource competition. The fact that allelopathic compounds did not reach the bottom of the slope suggests that these allelochemicals could be absorbed selectively by the target plant, retained by the soil components, or that they were quickly inactivated in soil (5).

The two-way ANOVA found no significant effect of sampling date or its interaction for PSII in *P. lanceolata* and *T. pratense* ($P \geq 0.086$). So, data collected at different times were pooled. The PSII was higher in LAIZ than in NAIZ and intermediate in other two zones for *P. lanceolata*. However, no differences were found among zones in *T. pratense* (Fig. 4). On the contrary, the two-way ANOVA showed a significant effect in *D. glomerata* and *T. officinale* ($P\leq 0.036$). Therefore, data were analyzed for each sampling date. For *D. glomerata*, there were no differences in August, the NAIZ was significantly lower (around 1-folds) in September, and the LAIZ showed the highest value, significantly different from NAIZ, HAIZ and UAIZ zones in October. The PSII of *T. officinale* showed no differences in August, the NAIZ and UAIZ were similar and significantly lower than HAIZ in September, and the UAIZ was 1.0-folds > NAIZ and LAIZ in October (Fig. 5). The sampling date had significant effect on the Fv/Fm parameter in all target species, according to the two-way ANOVA ($P \leq 0.001$). Therefore, the data collected from each sampling date were separately analyzed. For all target species, the Fv/Fm revealed no significant differences among the zones on each sampling date and the value of this parameter was always > 0.85 (Table 1). The emission of fluorescence study indirectly provided us information on photosynthesis (2). In fact, the Fv/Fm parameter was used as sensitive indicator of plant photosynthetic performance, whereby values of around 0.83 indicate the optimal functioning of photosynthetic apparatus and values lower than this indicate that plant is exposed to stress (35). The value of Fv/Fm was always above 0.83, for all target species. This tells us that the target plants were not under strong stress conditions.

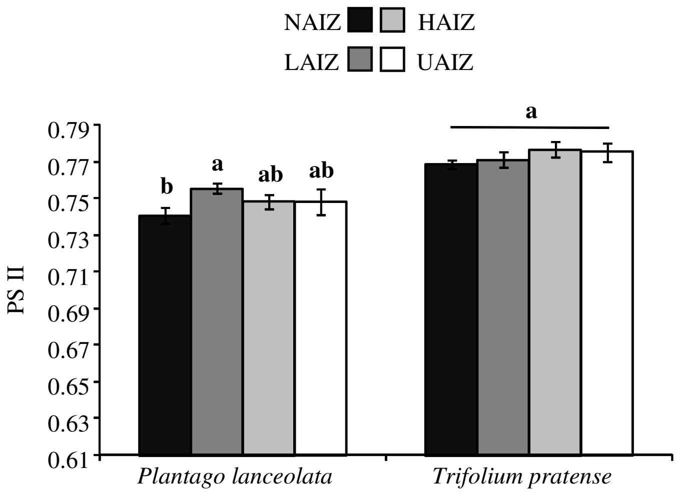

Figure 4. The efficiency of Photosystem II photochemistry (PSII) of native species in the presence of *Acacia dealbata.* NAIZ: non-allelopathic interaction zone; LAIZ: low allelopathic interaction zone; HAIZ: high allelopathic interaction zone; UAIZ: unpredictable allelopathic interaction zone. Means ± SE are presented, $n = 7$. LSD test was used when homogeneity of variance was achieved; otherwise the Mann-Whitney U test was used. Different letters indicate statistically significant differences ($P \leq 0.05$) in each target species.

Table 1. Chlorophyll fluorescence (Fv/Fm) of native species in the presence of *Acacia dealbata.* NAIZ: non-allelopathic interaction zone; LAIZ: low allelopathic interaction zone; HAIZ: high allelopathic interaction zone; UAIZ: unpredictable allelopathic interaction zone. Means ± SE are presented, $n = 7$. LSD test was used when homogeneity of variance was achieved; otherwise the Mann-Whitney U test was used

Sloped box zone	***Dactylis glomerata***			***Plantago lanceolata***		
	August	September	October	August	September	October
NAIZ	0.88±0.004	0.86±0.001	0.86±0.002	0.89±0.004	0.88±0.002	0.87±0.001
LAIZ	0.87±0.005	0.86±0.003	0.86±0.001	0.89±0.004	0.88±0.002	0.88±0.004
HAIZ	0.86±0.004	0.86±0.004	0.86±0.002	0.88±0.004	0.88±0.004	0.88±0.002
UAIZ	0.87±0.004	0.86±0.003	0.86±0.004	0.89±0.004	0.89±0.003	0.88±0.003
	Taraxacum officinale			***Trifolium pratense***		
NAIZ	0.89±0.004	0.87±0.003	0.89±0.004	0.87±0.003	0.89±0.004	0.87±0.003
LAIZ	0.89±0.004	0.88±0.003	0.89±0.004	0.88±0.003	0.89±0.004	0.88±0.003
HAIZ	0.89±0.003	0.88±0.003	0.89±0.003	0.88±0.003	0.89±0.003	0.88±0.003
UAIZ	0.88±0.004	0.88±0.001	0.88±0.004	0.88±0.001	0.88±0.004	0.88±0.001

In general we did not observe any correlation between the germination and distance from *A. dealbata* plants to target plants (Table 2). The Fv/Fm values showed that there was no correlation between the stress values measured in target species and distance.

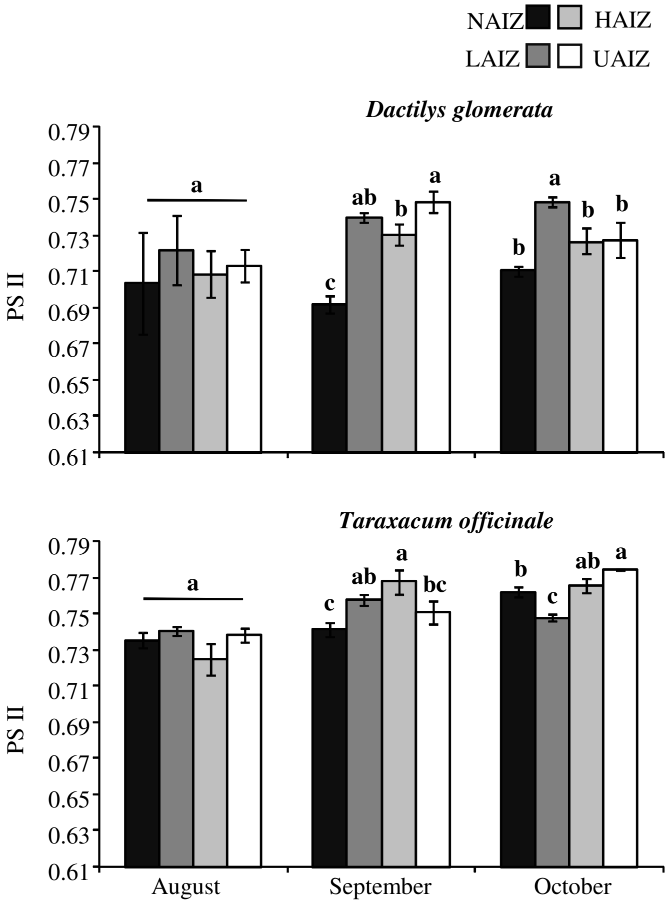

Figure 5. The efficiency of Photosystem II photochemistry (PSII) of native species in the presence of *Acacia dealbata*. NAIZ: non-allelopathic interaction zone; LAIZ: low allelopathic interaction zone; HAIZ: high allelopathic interaction zone; UAIZ: unpredictable allelopathic interaction zone. Means ± SE are presented, $n = 7$. LSD test was used when homogeneity of variance was achieved; otherwise the Mann-Whitney U test was used. Different letters indicate statistically significant differences ($P \leq 0.05$) in each target species.

Correlation in photosynthetic efficiency depends on the time of measurement and the target species (Table 2). However, aboveground biomass was the most affected physiological parameter related to the distance from the *A. dealbata* plants (Table 2). There was always a positive correlation between the biomass of target species and the distance. Our results showed correlations between the distance and PSII (mostly negative) and a strong positive correlation between the distance and aboveground biomass of target plants, with test plants showing stimulated PSII and less biomass with increasing exotic

Table 2. Correlation analyses between each of the measured parameter and distance from the center to target species following the Pearson correlation coefficient

		Dactylis glomerata		*Plantago lanceolata*		*Taraxacum officinale*		*Trifolium pratense*	
		TS	US	TS	US	TS	US	TS	US
G_t	PC	0.27	-0.15	-0.24	-0.30	-0.20	-0.35	-0.22	-0.15
	n	28	27	28	28	28	28	27	25
AS	PC	0.33	-0.14	-0.18	-0.30	-0.36	-0.37	-0.25	-0.03
	n	27	27	27	28	28	28	27	25
CRG	PC	0.06	-0.09	-0.14	0.02	-0.36	-0.11	0.03	0.42(*)
	n	28	27	27	25	28	27	26	27
Fv/Fm August	PC	-0.15	-0.07	0.01	0.14	0.12	-0.20	-0.05	-0.16
	n	11	14	28	28	27	28	27	27
Fv/Fm September	PC	0.06	0.00	0.29	0.34	-0.05	0.05	0.06	0.20
	n	26	23	27	27	27	28	26	28
Fv/Fm October	PC	0.10	0.26	0.09	0.22	0.03	0.07	-0.16	-0.39(*)
	n	26	23	28	27	26	28	26	28
PSII August	PC	0.85	0.40	-0.51(**)	0.23	-0.11	0.24	0.24	0.16
	n	3	6	26	28	28	27	27	27
PSII September	PC	-0.81(**)	0.47(*)	-0.16	0.30	-0.39(*)	-0.39(*)	0.19	-0.11
	n	18	24	27	27	27	28	27	28
PSII October	PC	-0.81(**)	-0.28	-0.17	-0.07	-0.19	0.34	-0.06	-0.27
	n	25	24	27	26	28	28	26	27
Aboveground biomass	PC	0.51(*)	0.07	0.54(**)	0.40(*)	0.63(**)	0.64(**)	0.65(**)	0.37
	n	23	23	27	27	28	24	25	27

TS: transect from *A. dealbata* plants to topside, US: transect from *A. dealbata* plants to underside, PC: Pearson correlation value.
** Correlation is significant at the $P \leq 0.01$ level; * Correlation is significant at the $P \leq 0.05$ level;

species influence. This finding confirmed the previously shown results, the allelopathic effects of allelopathic compounds released by *A. dealbata* on test species was a function of distance from invader plant. This result was in accord with results of Gómez-Aparicio and Canham (16), who found that effects of the invasive tree *Alianthus altisima* were strongly influenced by distance from the tree, in general declining to zero.

In the experimental design we have used different species, to generalize the results (12) and achieving a greater insight with ecological implications. Allelochemicals have different effects on different species due to differences in their physiological processes (14,33,45). Our species were affected differently owing to allelopathy and competitive interactions between them and *A. dealbata*. In an invasion process, native species and their ecological relationships are expected to be affected in one way or another by arrival of exotic species such as *A. dealbata* (6, 40), because exotic and native species have not co-evolved together (37). Moreover, the donor species may have fatal allelopathic effects, weak or non allelopathic effects depending on the target species (45).

CONCLUSIONS

The invasive *Acacia dealbata* showed phytotoxic activity to various physiological processes in native species under competitive interactions. This effect depended on the target species and its distance from the donor, although another type of interactions should not be overlooked. The method presented here provided a realistic procedure, where allelochemicals naturally released by *Acacia dealbata* could be tested by separating resource competition in an ecological approach without adding other potential disorder agent as activated carbon. However, factors such as nutrients and water flows should be measured to improve the resolution of this method. With this improvement, the 'Sloped Box' can be useful tool to address the role of secondary metabolites in other species suspected to be allelopathic. Furthermore, the effect of particular water-soluble phytochemicals can be checked.

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