ARE GLAUCOUS THURIFEROUS JUNIPERS MORE PHOTOPROTECTED AGAINST ENVIRONMENTAL STRESS THAN GREEN ONES?

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Resumen La sabina (*Juniperus thurifera* L.) presenta una importante variabilidad fenotípica en el contenido de ceras epicuticulares en las hojas, lo que genera la coexistencia de individuos más o menos glaucos con otros de follaje verde. En el presente trabajo pretendemos verificar la hipótesis de que las sabinas glaucas se encuentran más fotoprotegidas frente a los factores ambientales de estrés al reducirse la cantidad de luz interceptada, o si, por el contrario, esta menor captación de luz reduce la eficiencia fotosintética cuando las condiciones ambientales están próximas al óptimo. La glaucescencia sería por tanto una característica fenotípica ventajosa en unas condiciones y negativa en otras, lo que justificaría la coexistencia de ambos fenotipos en poblaciones naturales. Hemos verificado que estas ceras contribuyen a reflejar en torno a un 5% de la energía luminosa, sin embargo las hojas blancas mostraron mayores síntomas de estrés que las hojas verdes, sugiriendo que estos compuestos se acumulan en respuesta al estrés ambiental más que representar una característica fenotípica. Hemos utilizado como parámetro de evaluación la relación entre los pigmentos fotosintéticos y las ceras además de la relación entre los registros de crecimiento anual y las condiciones climáticas. El estudio del comportamiento de estos fenotipos puede resultar de especial relevancia en un contexto de cambio climático, que implica un progresivo endurecimiento de las condiciones de estrés ambiental en la región Mediterránea.
Palabras clave: *Juniperus thurifera, fotoprotección, reflectancia, ceras*

ARE GLAUCOUS THURIFEROUS JUNIPERS MORE PHOTOPROTECTED AGAINST ENVIRONMENTAL STRESS THAN GREEN ONES?

Abstract Thuriferous juniper (*Juniperus thurifera* L.) presents a large phenotypic variability in the content of epicuticular waxes in leaves that generate the coexistence of glaucous individuals with others characterised by greenish leaves. In the present work we have tried to verify the hypothesis that glaucous plants are more protected against environmental stress factors as light intensity intercepted by photosynthetic tissues is attenuated. On the other hand, the lesser light absorption can lead to a reduced efficiency in photosynthetic energy conversion when environmental conditions are close to the optimum. Glaucous leaves would then increase plant fitness under some conditions but would be negative in others, justifying the co-existence of both phenotypes in natural populations. We have assessed that these waxes can reflect approximately 5% of light energy. However glaucous leaves showed higher symptoms of light stress than green ones suggesting that these compounds are produced as a response to stress conditions more than a mere phenotypic characteristic. As stress indicators, we have used pigments and waxes, as well as annual growth and climatic conditions for individuals with different epicuticular wax contents. The study of these phenotypes could be of special relevance in the context of climatic change that implies a progressive hardening of the environmental conditions in the Mediterranean Region.
Key words: *Juniperus thurifera, photoprotection, reflectance, waxes*

SONT-ILS LES GENÉVRIERS THURIFÈRES GLAUQUES PLUS PROTÉGÉS FACE AU STRESS DÛ A L'ENVIRONNEMENT(O STRESS ENVIRONNEMENTAL) QUE LES GENÉVRIERS THURIFÈRES VERTS?

Résumé Il existe une importante variabilité phénotypique dans les feuilles du genévrier thurifère (*Juniperus thurifera* L.), en ce qui concerne le contenu de cires épicuticulaires. Ceci origine la coexistence d'exemplaires plus ou moins glauques avec d'autres à feuillage vert. Dans cette étude on essaie de vérifier l'hypothèse de si les genévriers glauques sont plus protégés face aux facteurs de l'environnement quand la quantité de lumière interceptée par les tissus photosynthétiques diminue., ou si, au contraire, cette mineure capture de lumière les rend moins efficaces dans la photosynthèse quand les conditions de l'environnement sont près de l'optimum . Les feuilles glauques représenteraient donc un trait phénotypique avantageux dans certaines conditions et négatif dans d'autres, ce qui justifierait la coexistence des deux phénotypes dans des peuplements naturels. Dans cette communication on étudie le phénomène à partir de deux perspectives, les différences des feuilles si on compare les différences de contenu

des pigments en fonction de la quantité de cires épiticulaires. Nous avons établi que ces cires peuvent refléter environ 5% de l'énergie lumineuse. Cependant les feuilles pourvues d'une pellicule de cire ont montré des symptômes de stress plus grands que les feuilles vertes, ce qui laisse supposer que ces composants sont davantage produits en réaction aux conditions de stress que par le fait de pures caractéristiques phénotypiques. Pour cela, on a vérifié l'hypothèse de si les exemplaires d'une couleur plus verte ont plus de sensibilité aux conditions climatiques que ceux d'une couleur plus glauque, utilisant comme paramètre d'évaluation la relation entre la croissance annuelle et les conditions climatiques. L'étude du comportement de ces phénotypes peut être spécialement relevante dans un contexte de changement climatique, qui implique un progressif durcissement des conditions de stress de l'environnement dans la région méditerranéenne.
Mots-clés: *Juniperus thurifera,* photoprotection, réfléchissement, cires.

INTRODUCTION

Juniperus species are highly stress-tolerant (MARTINEZ-FERRI *et al.* 2000; Mueller *et al.* 2005). In photosynthetic tissues environmental stress may lead to the generation of photooxidative damage. To avoid this, plants possess several photoprotection mechanisms that operate coordinately. These mechanisms are specially relevant in relation to the double stress period that characterises the Mediterranean climate (summer and winter stresses) (LARCHER, 2000). There are basically two main photoprotective strategies. The first one is based on morphological modifications that include changes in leaf orientation or accumulation of reflective structures or molecules such as hairs, anthocyanins and epicuticular waxes, while the second operates at cellular level and is based on the process of energy dissipation and the antioxidative metabolism (For a review see NIYOGI 2000). As the content of these compounds is strongly modulated by environmental conditions, their content is strongly influenced by stress, and in fact, can be used as indicators of the physiological state of plants (TAUSZ *et al.* 2003). This is also the case of epicuticular waxes that accumulate during unfavourable conditions (BONDADA *et al.* 1996). It has been proposed that waxes would decrease leaf temperature and consequently losses in transpiration processes (BLUM, 1975), but the exact role of this accumulation is not completely established. Alternatively waxes increase leaf reflectance, and could be a mechanism to reduce the excess of light intercepted by chlorophylls as light intensity intercepted by photosynthetic tissues is attenuated. Glaucous leaves would then increase plant fitness under some conditions but would be negative in others, justifying the co-existence of both phenotypes in natural populations. The aim of the present work was to test this hypothesis in natural populations of thuriferous juniper (*Juniperus thurifera* L.) which shows the coexistence of green and glaucous phenotypes. The study of these phenotypes could be of special relevance in the context of climatic change that implies a progressive hardening of the environmental conditions in the Mediterranean Region.

MATERIALS AND METHODS

Plant material for the study was collected in January 2006 in Cabrejas (Soria, Spain). The day was sunny with temperatures ranging between –6 and 0 °C. Twenty-four healthy juniper plants (12 glaucous and 12 green) were selected for the study. Measurements were performed in the field at midday or after 12 h of dark incubation at room temperature to allow the recovery from winter photoinhibition and provide fully comparable conditions (GARCÍA-PLAZAOLA *et al.* 2000). Leaf samples were immediately frozen in liquid nitrogen and stored at –80 °C until biochemical analyses.

Lipophilic antioxidants (carotenoids and tocopherols) and photosynthetic pigments were extracted and measured by reverse-phase HPLC following the method of GARCÍA PLAZAOLA & BECERRIL (1999). Chlorophyll *a* fluorescence was measured in the

laboratory using a portable modulated fluorimeter (OS 5-FL, Optisciences, Tyngsboro, USA). Initial (Fo) and maximal fluorescence (Fm) were measured in dark adapted leaves with a saturating pulse of 0.8 s. The maximal photochemical efficiency of PSII was estimated by the ratio Fv/Fm = (Fm-Fo)/Fm. Leaf reflectance was measured with a spectroradiometer (UNISPEC, PP Systems, Amesbury, MA), before and after removing epicuticular waxes by dipping the leaves in chloroform as described in HOLMES & KEILLER (2002). Chronic and dynamic photoinhibition were calculated according to WERNER *et al.* (2002). Statistical significance of linear regressions were analysed with SPSS 11.0 package.

RESULTS AND DISCUSSION

The reflectance spectra of glaucous and green leaves are shown in figure 1. Total reflectance was higher in glaucous leaves, and this effect was reduced after chloroform treatment, confirming that epicuticular waxes are responsible for the increase in reflectance. On average, waxes increased reflectance by 4.35 % (Table 1), thereby reducing the same proportion of light intercepted by chlorophyll. This value is lower than others reported for *Eucalyptus* (15 %) (HOLLMES & KEILLER 2002) or barley (20%) (FEBRERO *et al*. 1998), suggesting a limited role for waxes as reflectors in *J. thurifera*.

Assuming a photoprotective role for waxes, it could be expected a negative relationship between reflectance and photoprotection mechanisms. However, none of the photoprotection parameters analysed (xanthophyll cycle pigments –VAZ-, deepoxidation state –AZ/VAZ-, tocopherol and Fv/Fm) correlated negatively with reflectance (Figure 2). Furthermore, the total VAZ pool, which is a highly sensitive indicator of the stress level (NIINEMETS *et al.* 2003) increased significantly with reflectance. These results suggest that waxes accumulate as a response to stress rather than representing a phenotypic characteristic. This does not exclude the initial hypothesis that waxes act as photoprotective structures, but furthermore reinforces it. The accumulation of epicuticular waxes in response to stress has been described under drought conditions in cotton (BONDADA *et al.* 1996) and with elevation and latitude in boreal conifers (RICHARDSON *et al.* 2003). It remains to be elucidated whether the ability to produce waxes under stress conditions is an individual characteristic or not.

An alternative approach to test the initial hypothesis that greenish plants are responsive to environmental conditions is to study their responses to inter-annual changes in climatic conditions by the analyses of the pattern of growth of the annual rings. In a preliminary approach we aim to build a master chronology for this species for the last one hundred years. This temporal limit is established in order to avoid the effects of herbivorism that were suffered by trees until the end of the 19th century. This master chronology is based on the common response of all individuals to high frequency factors (mainly inter-annual climatic variability). For each tree a sensitiveness factor can be obtained, that would indicate the intensity of the responses of this individual to the annual variations of the master chronology. Following our hypothesis, sensitivity of glaucous trees should be lower than that of greenish trees.

Environmental conditions during the study period were highly photoinhibitory, with sunny weather and temperatures always below 0°C. However, photochemical efficiency and the operation of the xanthophylls cycle showed a dynamic character (Figure 3), contrasting with other conifers that remain strongly and irreversibly photoinhibited during winter (ADAMS *et al*. 2002). In fact chronic (non reversible) photoinhibition only accounted for a small proportion (16 % in green and 20% in glaucous plants) of total photoinhibition (data not shown). It implies that this species is able to maintain its photosynthetic activity during winter

when environmental conditions are favourable and its strategy during winter is not to behave as a stress avoider species.

CONCLUSIONS

Epicuticular waxes are responsible for the increase in reflectance in glaucous leaves. These waxes accumulate as a response to stress rather than representing a phenotypic characteristic. It remains to be elucidated whether the ability to produce waxes under stress conditions is an individual characteristic or not.

An alternative approach is to study the responses of plants´ inter-annual changes in climatic conditions by the analyses of the pattern of growth of the annual rings For each tree a sensitiveness factor can be obtained, that would indicate the intensity of the responses of this individual to the annual variations of the master chronology. Following our hypothesis, sensitivity of glaucous trees should be lower than that of greenish trees.

Last but not least conclusion is that this species is able to maintain its photosynthetic activity during winter when environmental conditions are favourable and its strategy during winter is not to behave as a stress avoider species.

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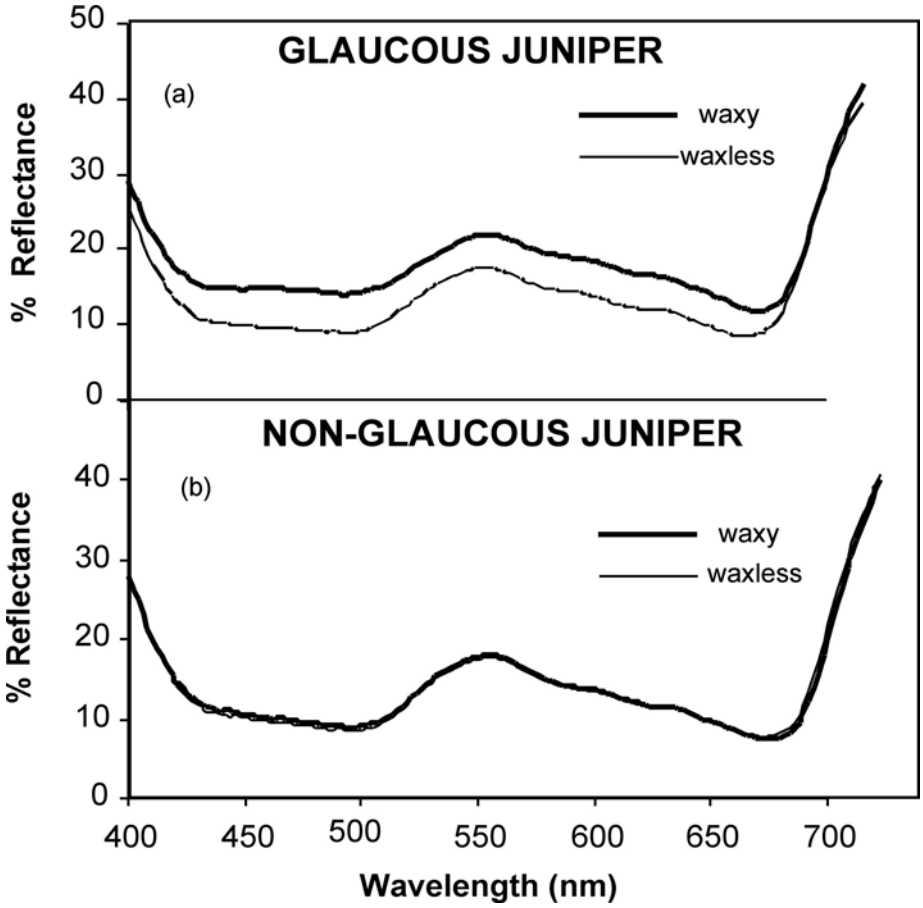

Figure 1**.** Spectral reflectance over the range 400-730 nm for a glaucous *(a)* and non-glaucous *Juniperus thurifer(b).* Each line is the average of two different scans on twelve different leaves. Bold lines represent reflectance before wax removal; dashed lines are reflectance after wax removal.

1. Total reflectance over the range 400-730 nm for a glaucous and non-glaucous *Juniperus thurifer* before and after wax removal.

REFLECTANCE	Wax	Waxless	Due to waxes
Glaucous	18,26±0,44	13,91±0,52	**4,35±0,09**
Non-glaucous	**14,09±0,50**	**14,02±0,52**	**0,06±0,04**

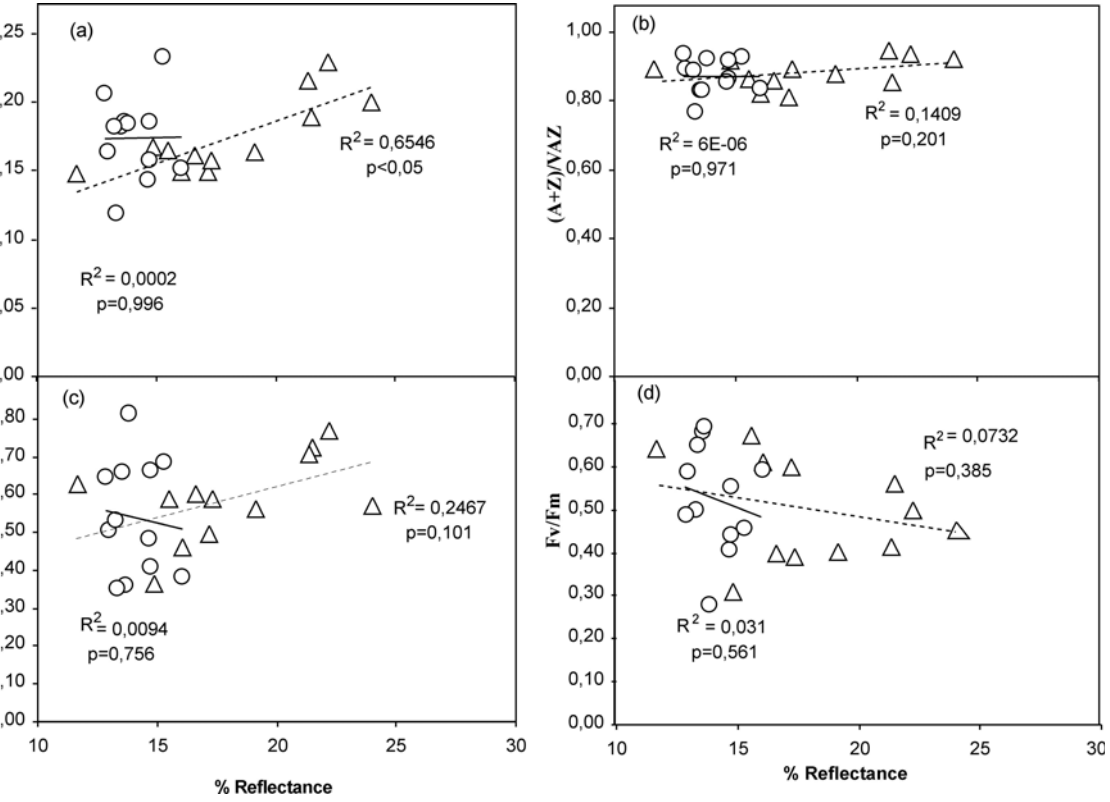

Figure 2. Relationships between the percentage of reflectance and the xantophyll cycle pigmets –VAZ- (a), deepoxidation state –AZ/VAZ- (b), tocopherol pools (c) and Fv/Fm (d). Triangles and dashed lines represent glaucous trees; circles and dashed lines indicates non-glaucous trees.

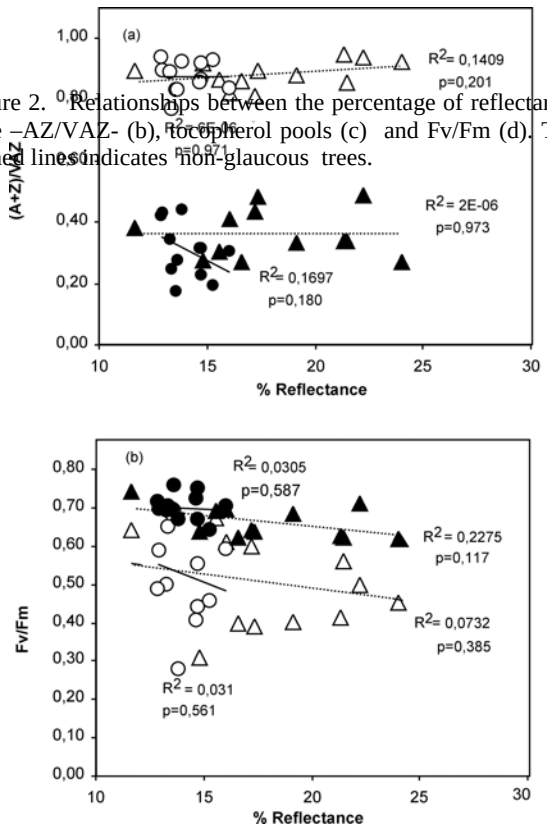

Figure 3. Relationships between the percentage of reflectance and the deepoxidation state –AZ/VAZ- (a) and Fv/Fm in noon (white figures) and predawn (dark figures) (b). Triangles and dashed lines represent glaucous trees; circles and dashed lines indicates non-glaucous trees.