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The physiology of drought tolerance in Tedera (*Bituminaria bituminosa*)

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Abstract. Tedera [*Bituminaria bituminosa* (L.) C.H. Stirton; Fabaceae] has been used as a traditional forage crop due to its high nutritional value and biomass production with a smaller water demand with respect to alfalfa. In water-limited environments such as southern Spain, different strategies allow the survival of several varieties of tedera. The aim of this work was to determine how four Spanish populations, from differing climatic conditions, respond when faced with drought. A summer field assay was carried out in La Alberca (Murcia, Southern Spain), comparing a watered with a non-watered treatment. Tolerance was expressed as inhibition of shoot growth relative to watered plants. Among the plant physiological parameters that were determined, we observed significant differences between the populations with respect to leaf water potential, osmotic potential, pressure potential, relative water content, specific leaf area, starch, P and K. Under drought, population Albomarginata (Lanzarote) maintained better its growth and its water relations. Population Beal (Murcia) was the most drought-sensitive in terms of growth, perhaps due to its inability to maintain leaf water potential. The behavior of the other two varieties was intermediate.

Keywords. *Bituminaria bituminosa* – Drought – Water relations – Physiological responses.

La physiologie de la tolérance à la sécheresse chez Tedera (*Bituminaria bituminosa*)

Résumé. *Tedera [*Bituminaria bituminosa *(L.) C.H. Stirton; Fabaceae] a été utilisée comme culture fourragère traditionnelle en raison de sa haute valeur alimentaire et de sa production de biomasse avec une demande plus faible en eau par rapport à la luzerne. Dans des environnements où les ressources en eau sont limitées comme le Sud de l'Espagne, des stratégies différentes permettent la survie de plusieurs variétés de Tedera. Le but de ce travail était de déterminer comment quatre populations espagnoles, provenant de différentes conditions climatiques, répondent face à la sécheresse. Un essai estival aux champs a été effectué à La Alberca (Murcia, Sud de l'Espagne), en comparant un traitement irrigué avec un autre non irrigué. La tolérance a été exprimée sous forme d'une inhibition de la croissance des pousses par rapport aux plantes non irriguées. Parmi les paramètres physiologiques de la plante qui ont été déterminés, nous avons observé des différences significatives entre les populations concernant le potentiel d'eau de la feuille, le potentiel osmotique, le potentiel de turgescence, la teneur relative en eau, la surface foliaire spécifique, l'amidon, P et K. Sous sécheresse, la population Albomarginata (Lanzarote) a mieux maintenu sa croissance et ses paramètres hydriques. La population Beal (Murcia) était sensible à la sécheresse en termes de croissance, peut-être dû à son incapacité de maintenir le potentiel en eau de la feuille. Le comportement des deux autres variétés était intermédiaire.*

Mots-clés. Bituminaria bituminosa *– Sécheresse – Paramètres hydriques – Réponses physiologiques.*

I – Introduction

Bituminaria bituminosa (L.) C.H. Stirton (Fabaceae), a perennial species widely distributed in the Mediterranean Basin and Macaronesia, is used as a forage crop. Its nutritional value for livestock has been analyzed (Ventura *et al.*, 2004), its net energy content being 4.6-5.3 MJ kg^{-1} dry matter, similar to *Medicago sativa*. The production of biomass of tedera in relation to the supply of water (up to 40 Tm FW ha^{-1} $year^{-1}$) and to the nutritional quality of this forage could justify the cultivation of tedera for hay (Méndez *et al.*, 2000). Additionally, it contains furanocoumarins like psoralen, object of numerous investigations related to diseases of the skin, and traditionally used in the so-called PUVA therapies (psoralen+UVA) to fight melanomas and other diseases of the skin like psoriasis, vitiligo and dermatitis (Mariano *et al.*, 2002). Each variety or population of *B. bituminosa* develops the pertinent adaptations that allow it to survive in its place of origin. Our purpose is to select high-biomass varieties with tolerance to drought and cold, and preferably with low contents in furanocoumarins, to provide forage.

II – Materials and methods

We compared four populations of *B. bituminosa* originating from sites of differing climate (Table 1).

Table 1. The studied varieties and their origins

Variety	Location (N, W)	Origin	Rainfall (mm)	Altitude (m asl)	Mean temp. (^{o}C) coldest/hottest months
Albomarginata	29^{o}07', 13^{o}31'	Lanzarote	150	600	11.1/22.8
Beal	37^{o}36', 00^{o}47'	Llano del Beal, Murcia	250	300	9.7/26.1
Crassiuscula	28^{o}14', 16^{o}34'	Mt. Teide, Tenerife	600	2200	4.0/17.4
Perdiz	37^{o}52', 01^{o}35'	Sierra Espuña, Murcia	350	850	5.9/25.8

Forty days after sowing, plants were transplanted from pots of soil to blocks in the experimental field [sandy-loam; water-holding capacity (%) =34.9; pH = 7.46 and EC (dS m^{-1}) = 1.72, both saturated paste]. All were watered every 2-3 days. Sixty days after transplanting, rainfall supposed the last contribution of water for the blocks that would be exposed to water stress. Only the control blocks continued to receive irrigation. After 30 days ± drought (30ºC mean temperature, PAR= 2000 µE m^{-2} s^{-1}), we determined leaf relative water content (RWC) and specific leaf area (SLA). Leaf water potential (Ψ_w) was measured using a Scholander chamber. The same leaves of the determination of the Ψ_w were used in the estimation of the osmotic potential (Ψ_π). Osmotic adjustment (OA) and pressure potential (Ψ_p) were calculated using the equations OA= $[\Psi_\pi*(RWC/100)]_{drought}$ - $[\Psi_\pi*(RWC/100)]_{control}$ and $\Psi_p = \Psi_w - \Psi_\pi$ respectively. Proline and amino acids were determined according to Bates *et al.* (1973). For the determination of soluble sugars and starch we followed the protocol described by Buysse and Merckx (1993). The concentrations of K and P were determined by inductively coupled plasma-optical emission spectroscopy. Differences among the means were tested using the least significant difference (LSD) test at the 0.05 probability level after an ANOVA analysis. Normality was achieved by arc-sin transformation for the percentages.

III – Results and discussion

After 30 days, in non-watered plots, the water content was decreased significantly (0.02 g H_2O g $soil^{-1}$) compared with control soil (0.06 g H_2O g $soil^{-1}$). This produced consequences in the RWC of all varieties except Albomarginata (Table 2). Fresh biomass production (shoot g FW $plant^{-1}$) was reduced significantly by drought for each variety; although DM production was reduced by 35-53% (Table 2), the effects were not significant (P = 0.13). In absolute terms, of relevance for forage purposes, DM yield under drought was in the order: Albomarginata > Beal, Crassiuscula > Perdiz. Dry mass under drought as a percentage of control plants declined in the order: Albomarginata (65%) > Crassiuscula (60%) > Beal (52%) > Perdiz (47%). Fresh mass under drought as a percentage of control plants declined in the order: Albomarginata (50%) >

Crassiuscula (48%) > Perdiz (42%) > Beal (38%). While it has been suggested that increased thickness (lower SLA) can decrease net photosynthesis per g leaf DM (Meziane and Shipley, 2001), thicker leaves are reported to have higher photosynthetic capacity (Mott and Michaelson, 1998).

Table 2. Mean shoot parameters in the four varieties of *B. bituminosa*

Variety	Treatment	Shoot g FW plant^{-1}	Shoot g DW plant^{-1}	RWC% (arc-sin)	SLA m^2 kg^{-1}DW
Albomarginata	Control	86.5	17.60	81.4 (1.12)	15.5
	Drought	42.8	11.42	77.3 (1.07)	10.6
Beal	Control	98.4	17.91	89.8 (1.25)	14.3
	Drought	37.1	9.34	71.2 (1.00)	9.8
Crassiuscula	Control	83.3	14.65	80.3 (1.11)	12.4
	Drought	40.0	8.81	73.0 (1.03)	9.5
Perdiz	Control	66.6	10.59	85.8 (1.19)	10.5
	Drought	27.8	5.00	78.3 (1.10)	8.7
ANOVA	F	4.19	1.94	7.96	14.1
	Sig	0.01	0.13	<0.001	<0.001
	LSD	38.28	9.33	0.07	1.6

Drought did not modify significantly the content of sugar expressed in DM (29-46 g kg^{-1}), but significant increases appear for concentrations in the tissue water (mmol L^{-1}) which are relevant for osmotic adjustment (Table 3). The order according to the increase in the concentration of sugar was: Beal (78%) > Crassiuscula (55%) > Albomarginata (44%) > Perdiz (41%). The greater decrease in RWC for Beal was an important factor in this. Albomarginata had the greatest values under both control and drought conditions, contributing to its OA. The starch concentration increased greatly for all populations except Perdiz. The proline concentration increased in the four varieties under water stress, but the values reached are relatively near to the controls if these are compared with the values that were reached in the same varieties in a previous pot experiment (surpassing 60 µmol g^{-1}DM^{-1}). This could be due to the fact that RWC did not fall below 71%, or because of the interaction of drought with heat stress, where proline could be toxic to the cells (Nanjo *et al.*, 2003). Drought provoked significant reductions in the shoot concentrations of K and P for all the populations. Under drought, particularly for populations Albomarginata and Beal, there could have been K deficiency (Table 3).

Table 3. Effect of drought on the shoot DM concentrations of soluble sugars, starch, proline, K and P

Variety	Treatment	Sugar mmol l^{-1}H20	Starch g kg^{-1}	Proline µmol g^{-1}	K mmol kg^{-1}	P mmol kg^{-1}
Albomarginata	Control	74.6	86.4	2.21	305	50.4
	Drought	132.4	112.7	8.09	220	27.6
Beal	Control	56.1	73.1	2.68	389	59.8
	Drought	99.7	111.6	7.74	215	29.8
Crassiuscula	Control	47.6	97	2.75	409	54.6
	Drought	73.9	154	3.31	262	33.8
Perdiz	Control	40.4	86.2	2.24	437	61.5
	Drought	56.8	91.3	5.23	328	35.1
ANOVA	F	14.03	6.04	1.94	14.47	16.07
	Sig	<0.001	<0.001	0.13	<0.001	<0.001
	LSD	24.39	30.5	5.25	67	10

The Canarian populations (Albomarginata and Crassiuscula) were more drought-tolerant in terms of growth, relative to control plants, and showed the smallest declines in Ψ_w (Table 4). Albomarginata and Crassiuscula higher Ψ_p under drought. It has been stated that growth declines only if bulk tissue Ψ_p falls below a threshold, although loss of the Ψ_w gradient between fully-grown and expanding tissues may be more important (Nonami, 1998). The lack of decrease in Ψ_p seen here and the OA values indicate that *B. bituminosa* is able to perform sufficient OA to avoid such growth inhibition – thus, it possesses constitutive *resistance* to drought but certain populations possess greater *tolerance*.

Table 4. Water relations

Variety	Treatment	Ψ_w (MPa)	Ψ_π (MPa)	Ψ_p (MPa)	Osmotic adjustment
Albomarginata	Control	-0.53	-1.25	0.72	-0.654
	Drought	-1.03	-2.16	1.16	
Beal	Control	-0.75	-1.27	0.52	-0.277
	Drought	-1.72	-2.23	0.51	
Crassiuscula	Control	-0.73	-1.19	0.45	-0.361
	Drought	-0.80	-1.97	1.17	
Perdiz	Control	-0.86	-1.21	0.35	-0.564
	Drought	-1.71	-2.03	0.32	
ANOVA	F	5.33	20.22	3.67	
	Sig	<0.001	<0.001	<0.001	
	LSD	0.61	0.24	0.61	

IV – Conclusions

Although *B. bituminosa escapes* summer drought by shedding its leaves, tolerant populations will be better able to withstand short drought periods in spring and autumn and will be useful for breeding of high-biomass lines for forage and high-furanocoumarin lines for pharmaceutical applications. Our results suggest that Perdiz and Beal, from Murcia (mainland Spain), were the most drought-sensitive and that Albomarginata, from a low-rainfall area in Lanzarote, achieved both the greatest biomass production under water stress, and the lowest growth inhibition, relative to watered conditions, maybe due to its OA, allowing it to *avoid* internal stress.

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