Responses of *Phalaris minor* Rezt. and *Phalaris brachystachys* Link to different levels of soil water availability

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**Abstract**

*Phalaris brachystachys* and *Phalaris minor* are common and troublesome weeds in winter cereals in Mediterranean countries. Different distribution and soil preferences have been found for each species in Andalusia (southern Spain). In irrigated fields *P. minor* is more frequent while *P. brachystachys* has extended its range to semiarid provinces with low rainfall. This different adaptation to irrigation conditions is difficult to explain considering aspects of their biology, herbicide tolerance, or cultivation practices. The objective of this study was to assess the influence of different soil water availabilities over growth and reproductive aspects to explain the differences found in ecology and distribution of *P. brachystachys* and *P. minor*. The experiment was conducted under greenhouse controlled conditions using five levels of water availability: field capacity, light drought, moderate drought, severe drought and extreme drought. Differences between species and among treatments were found in plant height, biomass, tiller number, and reproductive traits. Field capacity and light drought treatments favoured biomass, tiller number, and panicle number in *P. minor*. In contrast, *P. brachystachys* had a positive response only in moderate drought and increased the percentage of mature panicle with increasing drought levels. These results could explain the wider distribution of *P. brachystachys* in fields without supplemental irrigation in semiarid areas, due to its adaptation to moderate drought conditions. It may also clarify the greater frequency of *P. minor* in irrigated fields and in areas with higher rainfall.

**Additional key words**: canary grass; chorology; drought; ecology; field capacity; weeds.

**Resumen**

Respuesta de *Phalaris minor* Rezt. y *Phalaris brachystachys* Link a diferentes niveles de disponibilidad de agua en el suelo

*Phalaris brachystachys* y *Phalaris minor* son malas hierbas frecuentes y problemáticas en los cereales de invierno a lo largo de la cuenca mediterránea. En Andalucía (sur de España) se han encontrado diferencias entre especies en relación a su distribución y preferencias edáficas. En suelos regados *P. minor* es más frecuente mientras que *P. brachystachys* se extiende a las provincias semiáridas con baja precipitación. Esta diferente adaptación a las condiciones de riego no se puede explicar por aspectos relacionados con su biología, tolerancia a herbicidas o prácticas de cultivo. El objetivo de este estudio es evaluar la influencia de diferentes niveles de agua en el suelo sobre el crecimiento y los caracteres reproductivos de *P. brachystachys* y *P. minor*, para explicar las diferencias encontradas en la ecología y distribución de ambas especies. El experimento se realizó en invernadero y se evaluaron cinco niveles de disponibilidad de agua: capacidad de campo, ligera sequía, sequía moderada, sequía severa y sequía extrema. Se encontraron diferencias entre especies en altura de planta, biomasa, número de brotes y en los caracteres reproductivos. Los tratamientos a capacidad de campo y con ligera sequía favorecieron la biomasa, el número de brotes y número de paniculas en *P. minor*. *P. brachystachys* tuvo una respuesta positiva sólo en el tratamiento de sequía moderada, incrementando el porcentaje de paniculas maduras con el incremento de los niveles de sequía. Estos resultados podrían explicar una más amplia distribución de *P. brachystachys* en secano y en zonas semiáridas debido a su adaptación a las condiciones de moderada sequía y una mayor frecuencia de *P. minor* en regadío y en zonas con mayor precipitación.

**Palabras clave adicionales**: alpistes; capacidad de campo; corología; ecología; malas hierbas; sequía.

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Received: 10-12-09; Accepted: 05-10-10.
Introduction

The annual Poaceae species *Phalaris brachystachys* Link (2n = 2x = 12) (short-spiked canary grass), *Phalaris minor* Retz (2n = 4x = 28) (littleseed canary grass), and *Phalaris paradoxa* L. (2n = 2x = 14) (hood canary grass) are common and troublesome weeds in winter cereals in Mediterranean countries (Catizone and Viggiani, 1980; Damnakis, 1983; García-Baudín, 1983), India (Bir and Sidhu, 1979; Chhokar and Sharma, 2008; Vashisht et al., 2008), Pakistan (Waheed et al., 2009) and in California and Arizona (Bell, 1992; Butler et al., 1993). The three species have a similar distribution throughout the world: across the Mediterranean and subtropical regions (Talavera, 1987). In Europe, Tutin (1980) identifies the Mediterranean region and South-western Europe for both *P. brachystachys* and *P. paradoxa*, and the Mediterranean and South-western Europe, northwards to N.W. France for *P. minor*.

In Spain these species infest crops throughout autumn and winter, creating a serious problem for cereal production (Pujadas-Salvá, 1986; Saavedra et al., 1989b; Hidalgo et al., 1990). Due to the high cost and difficulty of control measures (González-Díaz et al., 2009), as well as their high rate of population growth (González-Andújar et al., 2005), farmers consider canary grasses some of the most pernicious weeds of cereal crops. In fact, several studies have indicated that competition between canary grass species and winter cereals is severe (Cudney and Hill, 1979; Dellow and Milne, 1986; Mehra and Gill, 1988; Walker et al., 2001), consequently sustainable weed management practices are being exploited as control strategies (Franke et al., 2007; Jamil et al., 2009).

In Andalucía (southern Spain) *P. brachystachys* is the most frequent and extended of the canary grass species (Saavedra et al., 1989a; González-Andújar and Saavedra, 2003); however, *P. minor* is also widely distributed and produces more severe infestations than *P. brachystachys*. In contrast, *P. paradoxa* produces the most highly-infested areas in terms of field density (Saavedra et al., 1989a), in spite of its reduced distribution. Differences in *P. paradoxa’s* germination capacity, such as greater light dependence (Jiménez-Hidalgo et al., 1997; Taylor et al., 2004) and a more extended period of emergence (Jiménez-Hidalgo, 1993; Mancebo et al., 2007), may explain its reduced and distinct distribution compared to the other species.

To our knowledge, neither differences in biology nor competition between *P. minor* and *P. brachystachys* have been found. In fact, the competitive abilities of *P. minor* and *P. brachystachys* are similar in Andalusia (Jiménez-Hidalgo et al., 1997) and for their biotypes in Greece (Afstoutoli and Eleftherohorinos, 1999). However, specific soil preferences have been found for each species in Andalusia. In irrigated fields, *P. minor* is more frequent in loamy soils, while *P. brachystachys* appears to prefer heavy clay soils (Saavedra, 1987; Saavedra et al., 1990). In dry land fields, however, there were no differences in frequency (Hidalgo, 1988; Hidalgo et al., 1990) between the species. Nevertheless, *P. brachystachys* has extended its range to semiarid provinces with low rainfall (Saavedra et al., 1989a).

This different adaptation to soil and irrigation conditions is difficult to explain with aspects of their biology, herbicide tolerance, or cultivation practices. However, they may indicate a different tolerance to deficient or excessive watering regimens. Other authors (Rodiyati et al., 2005) have related differences in distribution of *Cyperus* species to drought tolerance. In *Phalaris* species, the more extended distribution of *P. brachystachys* could be linked with drought tolerance, but to our knowledge, has not yet been established. Hence, the objective of this study was to assess the influence of different soil water levels over growth and reproductive aspects under greenhouse controlled conditions to explain the differences found in distribution of *P. brachystachys* and *P. minor*.

Material and methods

The experiment was conducted in a greenhouse in Alameda (Córdoba) to evaluate drought tolerance in 1988, from January to May. Temperatures during development, from February to May, ranged from 20 to 30°C at day and from 10 to 18°C at night. Plants were exposed to 14 hours of light and 10 of darkness.

*P. brachystachys* and *P. minor* seeds were previously collected in June in cultivated fields in Córdoba, identified, confirmed (Tutin, 1980), and stored in glass containers in the laboratory during summer and autumn. Ten seeds per species were sown on January 22 in 15 cm diameter pots with 1,700 g of substrate composed in volume of three equal parts of sand, silt, and turf plus 1 g of fertilizer (15-15-15 N-P₂O₅-K₂O) in order to ensure that all plants received sufficient nutrients. The pots were irrigated daily to field capacity until the plants had two leaves. Three plants per pot were selected and the rest removed. Five treatments of irrigation were...
established 24 days after sowing. The treatments differed in irrigation frequency and water volume applied and ranged from field capacity to extreme drought. In order to determine the water volume necessary in each treatment, field capacity was measured in 6 pots only with soil, by soil weight before and after wetting and drainage resulting 0.217 cm$^3$ cm$^{-3}$. Also, in each irrigation event, the water volume applied was adjusted according to the pots weight which was incrementing by the plants biomass and it was calculated as field capacity percentage (Table 1).

The experimental design was a factorial design with 8 repetitions. Factors established were species (two) and irrigation treatment (five) resulting 80 pots with three plants per pot. Additionally three pots per treatment, species, and time of determination (34, 48, 72, 88 DAS: days after sowing) were destructively sampled for biomass, resulting 120 pots. The total number of pots was 200 with three plants per pot.

Periodically, an exhaustive monitoring of the plants growing was performed. The variables evaluated per plant in each time of determination were: height by measuring leaves or panicles, total tiller number, total number of panicles, and distinguishing ripeness stage of panicles (emerged not flowering, beginning flowering, full flowering and beginning ripening and panicle ripe). Aerial biomass at the end of the assay was determined in 8 pots per treatment and species. Grain production was not evaluated due to dehiscence.

The results were analyzed using ANOVA test with the statistical package Statistix 8. Means were separated by application of the Tukey test. It was not necessary to transform the data because variances were homogenous.

### Results

Total mean water consumption per pot (expressed in cm$^3$) was 3,777; 2,803; 1,575; 749 and 378 for *P. minor*, and 4,416; 3,153; 1,600; 674 and 296 for *P. brachystachys* in the treatment T1, T2, T3, T4 and T5 respectively.

### Differences of traits between species and among treatments, and interaction between species and treatments

The ANOVA analysis showed that there were significant differences between the two species for all parameters measured (Table 2). Likewise, treatment within a species were significant ($p < 0.001$), except for maturing panicle percentage ($p = 0.05$). For most of the traits, significant interaction of species versus treatment was detected.

### Growth and aerial biomass

The progression of height and aerial biomass shows that there were differences between species and responses to soil water availability (Fig. 1), both species being affected negatively by drought. Differences in height were very important within 34 days after sowing (DAS) in both species. In *P. minor*, the field capacity and light drought treatments differed greatly from those of severe and extreme drought for both traits. Moderate drought results were intermediate between the two extremes. However, in *P. brachystachys* this trend was different: severe and extreme drought regimens resulted in much lower heights and biomasses than those obtained at other water levels.

At 61 DAS differences in height between the two species were significant for light drought but not for other treatments (Fig. 2), although overall the response of both species in height was similar. However, biomass differences between species were significant for both field capacity and light drought treatments (Fig. 2),

### Table 1. Treatments of irrigation on *Phalaris brachystachys* and *Phalaris minor*

| Treatment | Water level       | Irrigation frequency | Water volume (%FC)$^1$ |
|-----------|-------------------|----------------------|------------------------|
| T1        | Field capacity    | 6 days a week        | 100                    |
| T2        | Light drought     | 2 days a week        | 100                    |
| T3        | Moderate drought  | 2 days a week        | 50                     |
| T4        | Severe drought    | 2 days a week        | 25                     |
| T5        | Extreme drought   | 2 days a week        | 12.5                   |

$^1$ FC: field capacity = 0.217 cm$^3$ cm$^{-3}$. 
Responses of Phalaris sp. to different soil water levels

Tillers and panicles

There were considerable differences between *P. minor* and *P. brachystachys* in terms of tiller and panicle numbers. Soil water availability had a strong influence (Fig. 3a-d) over these two traits. The greatest number of panicles were produced from 46 DAS to 61 DAS in *P. minor* (Fig. 3c) while in *P. brachystachys* this took place one to two weeks later (Fig. 3d).

The total number of tillers and panicles at field capacity were significantly (*p < 0.01*) smaller in *P. brachystachys* compared to *P. minor*. There were no other statistically important differences found for the rest of the treatments (Fig. 4).

Table 2. Summary of ANOVAs for testing species, effects of water availability, and their interactions on the development of *Phalaris minor* and *Phalaris brachystachys*

| Plant traits               | Species (Sp) | Treatment (Tr) | Sp vs. Tr |
|----------------------------|--------------|----------------|-----------|
|                            | d.f. | F     | d.f. | F     | d.f. | F     |
| **Growth**                 |      |       |      |       |      |       |
| Plant height               |      |       |      |       |      |       |
| 34 DAS                     | 1    | 12.09*** | 4    | 57.94*** | 4    | 0.25(ns) |
| 46 DAS                     | 1    | 11.02*** | 4    | 91.17*** | 4    | 1.08(ns) |
| 61 DAS                     | 1    | 0.16(ns) | 4    | 182.61*** | 4    | 3.02*   |
| **Aerial biomass**         |      |       |      |       |      |       |
| Dry weight                 |      |       |      |       |      |       |
| 34 DAS                     | 1    | 19.22*** | 4    | 6.54**  | 4    | 1.99(ns) |
| 48 DAS                     | 1    | 33.21*** | 4    | 33.12*** | 4    | 4.11*   |
| 72 DAS                     | 1    | 14.61**  | 4    | 23.48*** | 4    | 4.52**  |
| 88 DAS                     | 1    | 35.96*** | 4    | 82.04*** | 4    | 12.26***|
| **Tillering**              |      |       |      |       |      |       |
| Tiller number              |      |       |      |       |      |       |
| 34 DAS                     | 1    | 51.08*** | 4    | 39.48*** | 4    | 3.65**  |
| 46 DAS                     | 1    | 89.72*** | 4    | 76.63*** | 4    | 11.89***|
| 61 DAS                     | 1    | 16.85*** | 4    | 40.25*** | 4    | 6.77**  |
| **Reproduction**           |      |       |      |       |      |       |
| Panicle number             |      |       |      |       |      |       |
| 46 DAS                     | 1    | 6.16*   | 4    | 3.43*   | 4    | 2.00(ns) |
| 53 DAS                     | 1    | 25.23*** | 4    | 14.70*** | 4    | 5.16**  |
| 61 DAS                     | 1    | 11.81**  | 4    | 27.77*** | 4    | 6.37**  |
| 67 DAS                     | 1    | 1.64(ns) | 4    | 29.21*** | 4    | 6.20**  |
| 88 DAS                     | 1    | 1.16(ns) | 4    | 25.62*** | 4    | 4.36**  |
| Panicle length             |      |       |      |       |      |       |
| 88 DAS                     | 1    | 218.39*** | 4    | 88.19*** | 4    | 10.37***|
| Total panicle length per plant | 1   | 63.32*** | 4    | 44.12*** | 4    | 10.89***|
| Maturating panicle percentage† | 67 DAS | 1 | 41.03*** | 3 | 3.25* | 3 | 0.45(ns) |

DAS: days after sowing. ns: non-significant (*p > 0.05*). *p < 0.05. **p < 0.01. ***p < 0.001. † Exclude treatment 5.

with *P. minor* reaching greater values than *P. brachystachys*.

**Tillers and panicles**

*P. minor* produced significantly more tillers under field capacity and light to moderate drought levels than under severe and extreme drought conditions (Fig. 3a, 3c and 4). However, the only difference produced in panicle number was between moderate and severe/extreme drought levels (Fig. 4). In *P. brachystachys*, the maximum production of tillers and subsequently the maximum panicle number took place at the moderate drought level (Fig. 4).
Figure 1. Evolution of height (a, b) from 34 to 61 days after sowing (DAS) and aerial biomass (c, d) from 34 to 88 DAS for *Phalaris minor* (a, c) and *Phalaris brachystachys* (b, d) grown under field capacity conditions and different levels of drought: light, moderate, severe, and extreme. Vertical bars represent the standard errors of the means.

Figure 2. Responses of height at 61 days after sowing (DAS) and aerial biomass at 88 DAS of *Phalaris minor* and *Phalaris brachystachys* to field capacity and different levels of drought: light, moderate, severe and extreme. Vertical bars are the standard errors of the means across 8 repetitions. Different letters indicate differences among the soil water conditions within *P. minor* (capital letters) and within *P. brachystachys* (lower-case). *p < 0.05, **p < 0.01 and ***p < 0.001 represent the differences between species.
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Figure 3. Evolution of tiller number (a, b) from 34 to 61 days after sowing (DAS) and panicle number (c, d) from 46 to 88 DAS for Phalaris minor (a, c) and Phalaris brachystachys (b, d) grown under field capacity conditions and different levels of drought: light, moderate, severe, and extreme. Vertical bars represent the standard errors of the means.

Figure 4. Responses of tiller number at 61 days after sowing (DAS) and panicle number at 88 DAS of Phalaris minor and Phalaris brachystachys to field capacity and different levels of drought: light, moderate, severe, and extreme. Vertical bars represent the standard errors of the means across 8 repetitions. Different letters indicate differences among the soil water conditions within P. minor (capital letters) and within P. brachystachys (lower-case). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ represent the differences between species.
Panicle size

Panicle size for *P. brachystachys* was significantly lower than that of *P. minor* \((p < 0.001)\) under field capacity, light, and moderate drought levels and \(p < 0.05\) at severe and moderate drought levels (Fig. 5). Low water availability significantly reduced panicle size in both species. Differences in *P. minor*’s panicle size were detected between the treatments with more water availability (field capacity and light drought) compared to moderate drought, as well as between these levels and those of severe and extreme drought (Fig. 5). The size of *P. brachystachys*’ panicles was significantly different among field capacity, moderate, and extreme drought levels (Fig. 5).

Ripeness

Significant differences were observed between species in the percentage of panicles produced at 67 DAS according to ripeness ranks, although they were not found among treatments (Fig. 6). Between 82 to 91% of *P. minor*’s panicles achieved ripeness levels (or exceeded anthesis stage), in contrast with 47 to 72% of those of *P. brachystachys*. There were no statistical differences among treatments for *P. minor*; however, *P. brachystachys* displayed an accelerated rate in the ripening process when water availability was reduced. This acceleration was manifested with significant statistical differences between field capacity and severe drought treatments (Fig. 6).

Discussion

This study showed that *P. minor* and *P. brachystachys* are significantly influenced in their growing by different levels of soil water availability. Although both species were affected in their development by drought, they exhibited a positive response to moderate levels of water stress. Development was, however, greatly affected by severe and extreme drought conditions. Plant growth was lower under drought stress, although differences between the two species were small. Both species produced seeds and overcame severe or extreme drought periods. This characteristic is typical of weed species in Mediterranean countries and allows them to remain in fields over long time periods. The distribution of both species in Europe (Tutin, 1980), and Southern Spain specifically (Garcia-Baudin, 1983; Saavedra *et al.*, 1989a; Jiménez-Hidalgo *et al.*, 1997) agrees with this response to severe and extreme drought conditions.
Field capacity conditions favoured *P. minor* over *P. brachystachys* that had higher biomass, tiller number, panicle number, and panicle length. These characteristics may allow *P. minor* to produce higher quantity of seeds and therefore more frequent infestations in irrigated fields of Mediterranean countries. This phenomenon has been observed previously by Saavedra *et al.* (1989a) in Southern Spain. This fact may also explain the Paleosubtropical distribution of *P. minor* that Pignatti (1982) observed, as well as the species’ extension northwards into N.W. France (Tutin, 1980).

Furthermore, *P. brachystachys*’ biomass was not increased through exposure to field capacity or light drought levels in comparison with its response to the moderate drought regimen. On the contrary, its number of panicles was remarkably reduced under these conditions. Although at field capacity the panicles were, on average, longer than at other water levels, they also exhibited chlorosis, further indicating adaptation problems to excess of water in this species.

Irrigation in Guadalquivir river Valley produces temporary excess of water, hindering *P. brachystachys*, and therefore, favouring *P. minor*. Moreover, Om *et al.* (2004) showed that *P. minor*’s seeds exhibited tolerance to anoxia during anaerobic respiration in rice. On the contrary, Ohadi *et al.* (2009) in germination studies found differences between irrigated and nonirrigated conditions in the *P. minor* seeds survival at 10 cm deep, with higher seed mortality under irrigated conditions, however, that did not happen at 20 and 40 cm deep.

*P. brachystachys* exhibited a higher tolerance to moderate water stress, producing more panicles without excessively affecting the mean size of panicle. Consequently, this species seems to be more adapted to dry land conditions than *P. minor*, which could explain the wider distribution of *P. brachystachys* across the Spanish provinces with a lower average rainfall (Saavedra *et al.*, 1989a).

Under the experiment conditions, *P. minor* showed a faster growth rate, in accordance with Afentouli and Eleftherohorinos (1999). In addition, the phenological cycle of *P. minor* was shortened compared to *P. brachystachys* and appeared not to be dependent on the level of water availability. However, *P. brachystachys* had the capacity to reduce the time of ripeness for panicles under water stress conditions. This may be an adaptive strategy to dry conditions in the Mediterranean climate, where the rainfall is scarce and irregular.

The results obtained show that the growth and reproductive traits such as biomass, tiller number, or panicle number in both species are influenced by the water availability. This different behaviour of *P. minor* and *P. brachystachys* depending on the soil water levels during the plant development may complicate the assessment of the competitive capabilities if those parameters are used. In our experiment under moderate stress treatments *P. brachystachys* developed a greater number of panicles regarding others treatment. In contrast, *P. minor* produced more panicles when water levels were high (field capacity) which agrees with results obtained by Afentouli and Eleftherohorinos (1999). This is an important consideration because it can limit the use of competition models based solely on panicle numbers, such as that proposed by Jiménez-Hidalgo *et al.* (1997), or biomass. Therefore, the competitive studies based on models which use panicle number or biomass to explain the competitive relationship between *P. minor* or *P. brachystachys* and cereal crops, should take into account the conditions of water availability under which they are performed.

The results of this study, though it was performed once and in controlled conditions, are totally reliable by the great number of repetitions (300 plants of each species) and the exhaustive monitoring of variables evaluated per plant such us height, biomass, tiller number, panicle number and length and different ripeness stage of panicles. This great number of variables could not have been evaluated in field conditions with this detail nor the different treatments of water availability established. However, field trials with lesser number of variables and treatments are mandatory in medium or short-term in order to validate the results obtained in this experiment.

As final conclusion, this study has revealed differences in the responses to different soil water levels for *P. minor* and *P. brachystachys*. Both survived even when were exposed to extreme drought, although, both were negatively influenced in their development by severe and extreme drought levels. Field capacity conditions consistently favoured *P. minor* and harmed *P. brachystachys*. The observed tolerance of *P. brachystachys* to moderate drought (as shown by a greater production of tiller number, panicle number, and an accelerate ripeness), together with the negative effects caused in plants by the field capacity treatment, may explain its wider distribution in rainfall fields and its adaptation to moderate drought conditions. Their distinct response to different soil water levels could explain differences in chorology and ecological preferences between these two species.
Acknowledgements

The authors are greatly indebted to Grant INIA-7636 (MAPA) and PIR 9019 (IFAPA-CICE Junta de Andalucía) for our experiment’s financial support, to Dr. González-Andújar for critical review of this paper, and Andrés Gutiérrez and Trinidad Gutiérrez for technical assistance.

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