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Soil water content and patterns of allocation to below- and above-ground biomass in the sexes of the subdioecious plant *Honckenya peploides*

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• *Background and aims* Dioecious plants often show sex-specific differences in growth and biomass allocation. These differences have been explained as a consequence of the different reproductive functions performed by the sexes. Empirical evidence strongly supports a greater reproductive investment in females. Sex differences in allocation may determine the performance of each sex in different habitats and therefore might explain the spatial segregation of the sexes described in many dimorphic plants. Here, an investigation was made of the sexual dimorphism in seasonal patterns of biomass allocation in the subdioecious perennial herb *Honckenya peploides*, a species that grows in embryo dunes (i.e. the youngest coastal dune formation) and displays spatial segregation of the sexes at the studied site. The water content in the soil of the male- and female-plant habitats at different times throughout the season was also examined.

• *Methods* The seasonal patterns of soil-water availability and biomass allocation were compared in two consecutive years in male and female *H. peploides* plants by collecting soil and plant samples in natural populations. Vertical profiles of below-ground biomass and water content were studied by sampling soil in male- and female-plant habitats at different soil depths.

• *Key Results* The sexes of *H. peploides* differed in their seasonal patterns of biomass allocation to reproduction. Males invested twice as much in reproduction than females early in the season, but sexual differences became reversed as the season progressed. No differences were found in above-ground biomass between the sexes, but the allocation of biomass to below-ground structures varied differently in depth for males and females, with females usually having greater below-ground biomass than males. In addition, male and female plants of *H. peploides* had different water-content profiles in the soil where they were growing and, when differences existed (usually in the upper layers of the soil), the water content of the soil was higher for the female plants had than for the male plants.

• *Conclusions* Sex-differential timing of investment in reproduction and differential availability and use of resources from the soil (particularly water) are factors that probably offset the costs of reproduction in the above-ground growth in males and females of *H. peploides.* The results suggest that the patterns of spatial segregation of the sexes observed in *H. peploides* may contribute to maximize each sex's growth and reproduction.

Key words: Dioecy, biomass allocation, below-ground structures, reproductive effort, spatial segregation, water availability.

INTRODUCTION

Dioecious plant species, in which different individuals perform different sexual functions, often show sex-specific differences in morphological and physiological traits (Dawson and Ehleringer, 1993; Obeso *et al.*, 1998; Dawson and Geber, 1999; Correia and Díaz Barradas, 2000; Retuerto *et al.*, 2000; Verdú, 2004), in ecological characters, such as patterns of defence and herbivory (Krischik and Denno, 1990; Retuerto *et al.*, 2006), survival (Allen and Antos, 1988) or spatial distribution (Iglesias and Bell, 1989), and in patterns of growth and resource allocation (Ågren, 1988; Ramp and Stephenson, 1988; Nicotra, 1999; Leigh *et al.*, 2006). These intersexual differences have commonly been explained as a consequence of the different reproductive functions performed by the sexes. Because females produce seeds and fruits in addition to flowers, many studies have found that females expend proportionally more of their resources on reproduction and less on maintenance and growth compared with males (Lloyd and Webb, 1977; Willson, 1983; Delph, 1999). However, some studies have challenged this generalization, reporting similar or even higher reproductive investment in males due to a greater allocation to floral display (Delph *et al.*, 1993; Leigh *et al.*, 2006) or to pollen in wind-pollinated species such as the herb *Mercurialis annua* (Hesse and Pannell, 2011). Sexual dimorphism in allocation might also arise as a result of selection for traits that may allow each gender to meet the specific resource demands associated with reproduction (Cox, 1981; Cipollini and Stiles, 1991; Nicotra *et al.*, 2003; Harris and Pannell, 2008; Sánchez-Vilas and Pannell, 2011).

Sexual differences in resource allocation may be an important determinant of the performance of each sex in different habitats and therefore could help to explain why some populations of dioecious species show habitat-specific sex-ratio biases (Freeman *et al.*, 1976; Bierzychudek and Eckhart, 1988). Within-population sex-ratio variation, or spatial segregation of the sexes, may occur if the sexes respond differentially to given environmental conditions. Differences in competitive abilities between the sexes (Cox, 1981; Eppley, 2006), morphological or physiological specialization of the sexes to different habitats (Dawson and Ehleringer, 1993; Dawson and Geber, 1999) or intersexual differences in reproductive biology (Lloyd, 1973; Bierzychudek and Eckhart, 1988) have been suggested as possible causes of spatial segregation. If the reproduction is more costly for females, as it has usually been argued, the relative fitness of females will increase as environmental quality improves and, consequently, the sex ratio will become female-biased in high-quality environments and male-biased in stressful or resource-poor habitats. In fact, this is the prevailing pattern emerging in the literature on spatial segregation of the sexes (Freeman *et al.*, 1976; Lloyd and Webb, 1977; Freeman *et al.*, 1980; Bierzychudek and Eckhart, 1988; Geber, 1999; Dawson and Geber, 1999).

Most studies on patterns of biomass allocation in dioecious species have examined sexual differences in biomass distribution at a single point in time (Wallace and Rundel, 1979; Bullock, 1984; Hemborg and Karlsson, 1999). However, because sexes may differ in their timing of development (Lloyd and Webb, 1977), static, or single point estimation of patterns of allocation may not reflect real or time-integrated patterns. The few studies that have examined seasonal patterns of biomass distribution have found significant differences between the sexes in the amount of resources allocated, and in the timing of allocation, to reproductive and vegetative structures (Gross and Soulé, 1981; Ågren, 1988; Hemborg and Karlsson, 1999; Ehlers and Thompson, 2004). More research is required for a thorough understanding and realistic modelling of the quantitative relationships between male and female reproductive effort over the whole growing season. We are not aware of any field study considering below-ground structures in the analysis of the patterns of biomass allocation in dimorphic plants. Most of the field studies on allocation have expressed the reproductive effort as the ratio of the dry mass of reproductive tissues to the total dry mass of the above-ground tissues (Korpelainen, 1992; Leigh *et al.*, 2006; Zunzunegui *et al.*, 2006). Although the extent and vertical distribution of the root system has been found to be critical for water uptake and drought tolerance (Moroke *et al.*, 2005; Yu *et al.*, 2007), we know no studies aimed at finding out if sexes of dimorphic species differ in vertical root distribution.

Honckenya peploides (Caryophyllaceae) is a dimorphic species typically found on the upper beach at the embryo dunes. It presents a breeding system in which male and female flowers are borne on separate plants. Female plants never produce pollen and are constant in their sex expression, whereas 'male' plants, all of which produce pollen, may also produce a small number of seeds. We will refer to this second sexual morph as male since it attains most of its fitness via pollen export. This system, called subdioecy, is close to dioecy in the evolutionary pathway from hermaphroditism to dioecy via gynodioecy (for a review, see Delph and Wolf, 2005). At the location of our study, *H. peploides* displays an extreme spatial segregation of the sexes, with monomorphic patches composed exclusively of individuals of either one sex or the other.

The allocation of biomass among below-ground, above-ground and reproductive structures may be especially crucial for dune plants such as *H. peploides*. Due to the low capacity of sandy soils to buffer against drought, dune plants may experience severe water deficits, especially in spring and summer, when high temperatures lead to high evapotranspiration rates. Under these conditions, sex-specific allocation of biomass to structures specialized in the uptake of water and the scarce nutrients available in the dune systems might result in a differential survival of the sexes in different habitats resulting in spatial segregation of the sexes. Previous research undertaken in individuals of *H. peploides* growing under greenhouse conditions has found greater reproductive effort and smaller size in females than in males, but not sex-specific differences in the below- : above-ground biomass ratio (Sánchez-Vilas and Retuerto, 2011).

Here, we extend our previous study by comparing the seasonal patterns of soil-water availability and biomass allocation in two consecutive years in males and females of *H. peploides* by collecting soil and plant samples in natural populations. Specifically, we aimed to address the following questions: (1) Do males and females of *H. peploides* differ in their seasonal patterns of biomass allocation to below- and above-ground vegetative structures and to reproduction? (2) Do they differ in their vertical distribution of below-ground biomass? (3) Do habitats of male and female plants differ in water availability, as estimated by soil water content?

MATERIALS AND METHODS

Study species

Sea sandwort, *Honckenya peploides* (L.) Ehrh., is a subdioecious perennial plant with a circumpolar distribution (from temperate to arctic zones; anthropochorous in South America). In the Iberian Peninsula, *H. peploides* extends from the Atlantic coast of northern Portugal and northwards and eastwards along the Bay of Biscay (Mar Cantábrico) to France. It is a hemicryptophyte regrowing each spring from long rhizomes that produce compact groups of aerial shoots, forming vegetative patches or mats. These patches are typically found on the upper beach, forming small and unconnected mounds called embryo dunes, which are the youngest dune formations in coastal systems. Flowering takes place from March to June and fruiting occurs from May to the end of August. Flowers are axillary and solitary, and/or in one- to six-flowered terminal cymes, strongly honey-scented. Two types of flower can be found in *H. peploides*, as reported for the subspecies *major* by Tsukui and Sugawara (1992). One type (hereinafter 'female flower') has long styles, short petals and non-functional anthers; the other (hereinafter 'male flower') has short styles, long petals and long stamens that produce pollen grains; this definition of male and female flowers is as per Tsukui and Sugawara (1992), and based on the arguments of Lloyd (1976) and Delph (1990). Male flowers rarely produce seeds, and when they do the number of seeds produced is very small compared with female flowers. Both types of flower have nectaries at the base of the stamens which attract pollinators. Plants reproduce sexually by seed or clonally by adventitious shoots produced by root buds. *Honckenya peploides* is an early colonizer, contributing to stabilization and anchorage of the soil and facilitating the establishment of other species (Houle, 1997; Gagné and Houle, 2001).

Study site

Fieldwork was conducted monthly from April to the end of August of 2006 and 2007 at the Lariño site (42°45′51″N, 9°6′4″W), on the coast of Galicia (north-west Spain). Climatic data (summarized in Fig. 1) were obtained from the nearest meteorological station at Corrubedo (42°33′20″N, 9°01′43″W; 25 km away from Lariño) and were provided by the meteorological service of Galicia (www.meteogalicia.es). We studied all the six segregated patches of *H. peploides* plants existing at that site, three composed exclusively of females and the other of male individuals and all of them facing south-west. Patches were separated by at least 10 m from the nearest patch. Individuals from the six monomorphic patches were sexed on the basis of their floral morphology, and the study was conducted during the flowering and fruiting seasons. We have been studied this population during the last five years and we have not observed inconstancy in sex-expression. Moreover, a previous study using amplified fragment length polymorphism (AFLP) as well as isozyme analysis has found considerably high values of genetic variation, revealing several genets within each of these monomorphic patches (Sánchez-Vilas *et al.*, 2010). In Lariño, *H. peploides* grows forming well-delimited patches, in which it is the dominant species; other species including *Cakile maritima*, *Eryngium maritimum* or *Ammophila arenaria* are sometimes present, but at very low density in the patch.

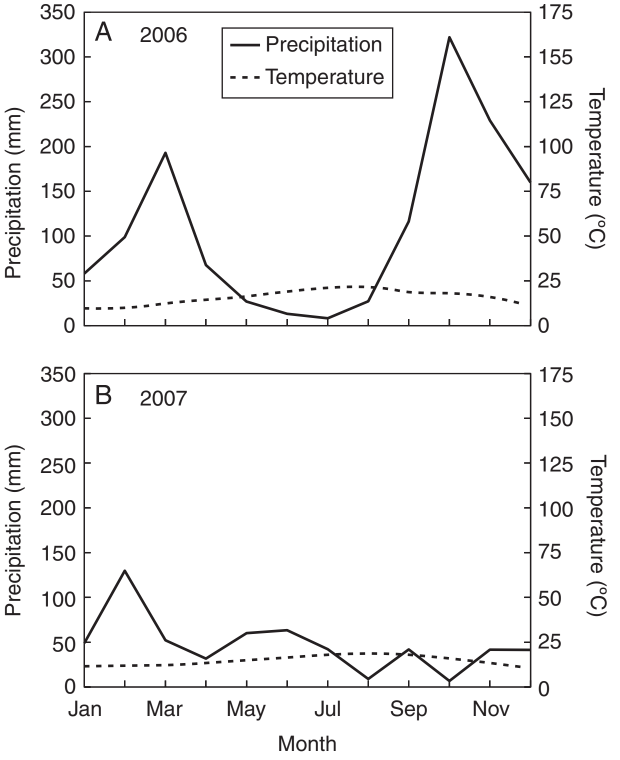

FIG. 1. Gaussen's ombrothermic diagrams for (A) 2006 and (B) 2007 obtained from the meteorological station at Corrubedo (42°33′20″N, 9°01′43″W). Dashed and continuous line represent, respectively, monthly average temperature (°C) and monthly average precipitation (mm) in a 2 : 1 (temperature : precipitation) scale. Drought conditions are considered when the precipitation is lower than twice the temperature values.

Soil and plant samples were collected in the early morning in the patches. In each patch, we established transects perpendicular and parallel to the coast line delimiting an *x*, *y* co-ordinate system. We randomly selected *x*, *y* co-ordinates to collect soil and plant samples within each patch. If *x*, *y* co-ordinates corresponded to bare soil we selected the nearest individual to collect the samples. Similarly, we also avoided those points sampled on previous months. In April and May 2006, we collected two samples in each of the six patches. In June, July and August 2006, the size of four of the patches (two male and two female) had increased so much that we decided to increase the number of samples in these patches to four per patch, maintaining two samples in each of the two smallest patches. In 2007, we also collected samples monthly from April to August following the sampling scheme used in June, July and August 2006. Approximately 2 % of the plant cover in each patch was sampled. We used a cylindrical soil core sampler for collecting soil and plant samples at the same point to determine above- and below-ground plant mass, and soil moisture. The cylindrical sampler (10 cm diameter, 45 cm length) was driven into the soil and a soil core was carefully removed preserving the plant and soil sample as it existed *in situ*. Samples were taken from the 0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm depths, by cutting the soil core at these predetermined depths. The 0–10 cm samples included the above-ground biomass. Each sample was put into a sealed plastic bag and this into a zip-lock plastic bag. Each sample was identified and carried to the laboratory, where samples were weighed at a ±0·01-g precision to obtain their total fresh mass. Then, from each sample, we separated the biomass of *H. peploides* and determined its fresh mass. Fresh biomass was subtracted from the total sample mass to obtain soil sample mass. From the 0–10 cm samples, we separated above- (shoot) and below-ground (rhizomes and roots) mass of *H. peploides*. We split the above-ground mass into vegetative and reproductive (flower and fruits). In these samples, we also counted the number of fruits, and at the peak of the flowering (April), the number of fresh flowers. Below-ground biomass was washed and all plant material was oven-dried at 55 °C for 6 d and weighed at a ±0·0001-g precision (Mettler AJ100, Switzerland) to calculate dry mass of below-ground structures (including rhizomes and roots), shoots, flowers and fruits. Biomass allocation patterns were assessed by calculating below- : above-ground vegetative ratios and reproductive effort, estimated as the ratio of reproductive to vegetative dry mass (i.e. to above- and below-ground vegetative dry mass). Mean flower and fruit dry mass were estimated from dry flower and fruit mass and the number of flowers and fruits, respectively. After separating *H. peploides* biomass, soil samples were placed in an oven at 75 °C for a minimum of 72 h for drying. Soil water content at each predetermined depth was obtained by dividing the difference between wet and dry soil masses by the mass of the dry sample to obtain the ratio of the mass of water to the mass of dry soil (g water kg^{-1} dry soil). Males and females had similar water content in their below-ground structures ($3{\cdot}17 \pm 0{\cdot}147$ $\text{g}{\cdot}\text{g}^{-1}$ and $3{\cdot}12 \pm 0{\cdot}179$ g g^{-1}, respectively; ANOVA test: $F_{1,237} = 1{\cdot}17$, $P = 0{\cdot}280$), calculated as [(below-ground fresh mass – below-ground dry mass)/below-ground dry mass] in a random sample of below-ground structures (roots and rhizomes) of males and females harvested from April to August in 2007 ($n = 118$ and 121, for males and females, respectively).

Statistical analyses

We tested for differences between males and females and among months for total biomass, total above-ground biomass (including vegetative and reproductive biomass), above-ground vegetative biomass, below- : above-ground vegetative biomass ratio and reproductive effort in 2006 and 2007. For each variable, analyses were carried out using linear mixed-effects models using the R 'lme' function from the 'nlme' package (Pinheiro *et al.*, 2009) in which sex and month were fitted as fixed factors and patch was fitted as random effect. In these analyses, tests of significance were carried out using an F-test based on marginal sums of squares using the function 'anova' for each model object (Pinheiro and Bates, 2000). Total biomass, flower biomass and below- : above-ground vegetative biomass ratio were square-root transformed, total below-ground biomass for 2007 was $\log_{10}$-transformed to achieve normality of standardized residuals and homogeneity of variance.

To analyse the below-ground biomass and the water content at different soil depths, we performed linear mixed-effect models using the R 'lmer' function from 'lme4' package (Bates and Maechler, 2010). This function handles models with more complex random structures than 'lme', allowing the nesting of random effects. In these analyses we fitted sex, month and depth as fixed factors, and patch and core (nested within patch) as random effects. Significance of the fixed effects was assessed by means of likelihood ratio tests by calling the function 'anova' to compare models with and without the factor being tested that are fitted using maximum likelihood estimates (Pinheiro and Bates, 2000; Crawley, 2007). Below-ground biomass for 2006 and 2007, and water content for 2007 were $\log_{10}$-transformed.

All statistical analyses were carried out in R version 2·8.1 (R Development Core Team, 2008).

RESULTS

Biomass and reproductive traits

The reproductive effort (proportion of biomass allocated to reproductive structures) of males and females changed differently over time (Fig. 2 and Table 1, sex × month interaction). Males allocated proportionally more to reproductive structures at earlier stages than did females; however, as the season progresses females were allocating more biomass to reproduction than males.

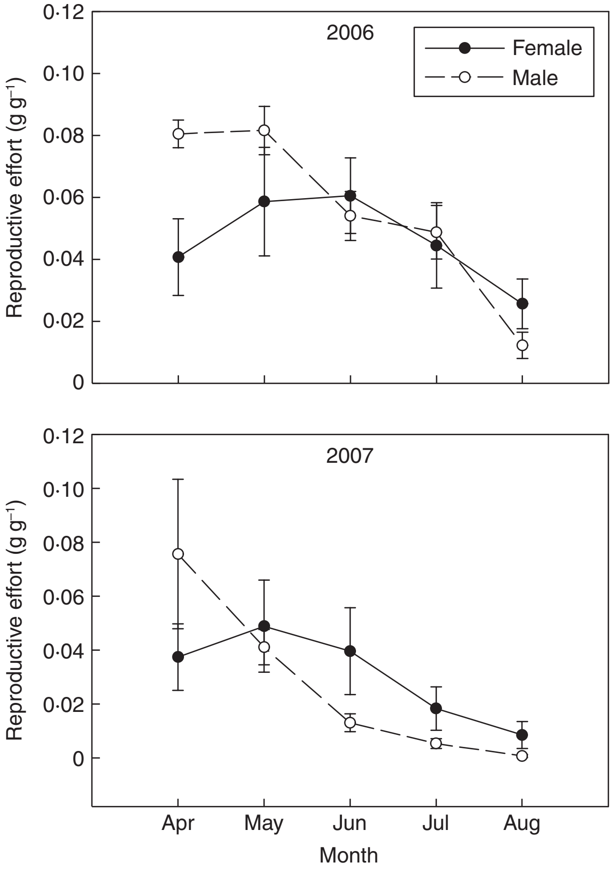

FIG. 2. Seasonal variation in reproductive effort (reproductive/vegetative biomass) of males and females of *Honckenya peploides* during 2006 and 2007. Values represent means ($n = 10$, except $n = 6$ for April and May 2006) ± s.e.

We did not find significant differences in total above-ground biomass, above-ground vegetative biomass, total biomass and below- : above-ground vegetative biomass ratio between males and females (Table 1). However, these variables changed significantly over time (except total biomass in 2007; Table 1). In 2006, total above-ground biomass, above-ground vegetative biomass and total biomass showed an increase from April to May, and then decreased again until reaching similar values to those found in April [Fig. 3A, B for total above-ground and total biomass; similar patterns were followed by above-ground vegetative biomass (not shown in the figure)]. In 2007, total above-ground and above-ground vegetative biomass increased significantly over time (Fig. 3D for total above-ground biomass), whilst no changes were observed in total biomass (Fig. 3E). Below- : above-ground vegetative biomass ratio decreased with time in both years (Fig. 3C, F).

In 2006, we found differences in below-ground biomass of males and females that varied with depth (sex × depth interaction: $\chi^2 = 5{\cdot}48$, $P = 0{\cdot}006$). Both sexes showed similar below-ground biomass on the first layer of soil sampled (0–10 cm), but showed differences in the rest of the layers of soil, with females having greater values than males, especially in the deepest soil layer (30–40 cm) (Fig. 4). Overall, and regardless of the sex and depth, the below-ground biomass declined with time from May to August ($\chi^2 = 40{\cdot}3$, $P < 0{\cdot}0001$; Fig. 4). In 2007, we also found differences in

TABLE 1. *Results of the mixed effect models for the reproductive effort, total above-ground biomass, above-ground vegetative biomass, total biomass and below- : above-ground vegetative biomass ratio*

	2006						2007					
	Sex (1,4)		Month (4,70)		Sex × month (4,70)		Sex (1,4)		Month (4,86)		Sex × month (4,86)	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Reproductive effort	4·42	0·103	2·41	0·058	2·10	0·090	2·54	0·186	2·37	0·058	2·58	**0·043**
Total above-ground biomass	0·133	0·734	5·57	**0·001**	1·28	0·284	0·820	0·416	3·61	**0·009**	1·12	0·351
Above-ground vegetative biomass	0·0387	0·854	4·40	**0·003**	1·13	0·351	1·15	0·343	4·84	**0·001**	1·31	0·273
Total biomass	0·585	0·487	13·3	**< 0·001**	1·19	0·322	0·898	0·397	0·670	0·615	0·810	0·522
Below- : above-ground vegetative biomass	2·47	0·191	6·35	**< 0·001**	1·31	0·276	0·510	0·515	3·79	**0·007**	0·184	0·946

Patch was included in the analysis as random variable (not shown) and sex, month and their interaction were treated as fixed factors. Numbers in parenthesis are the degrees of freedom of numerator and denominator used to test the fixed factors. *P*-values <0·05 are shown in bold type.

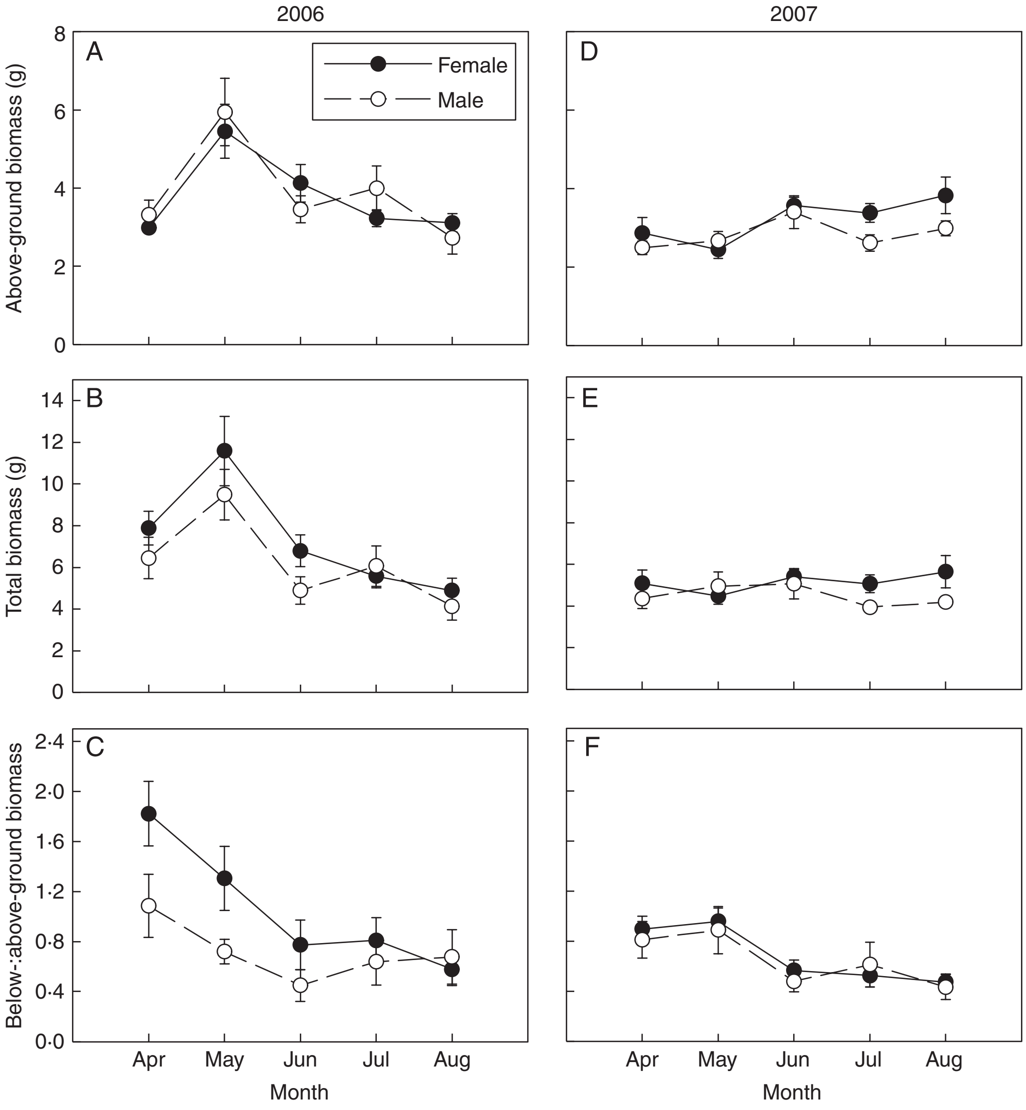

FIG. 3. Mean monthly values (± s.e.) of total above-ground and total biomass, and ratio of below- : above-ground vegetative biomass ratio in males and females of *H. peploides* from April to August in 2006 (A–C) and 2007 (D–F).

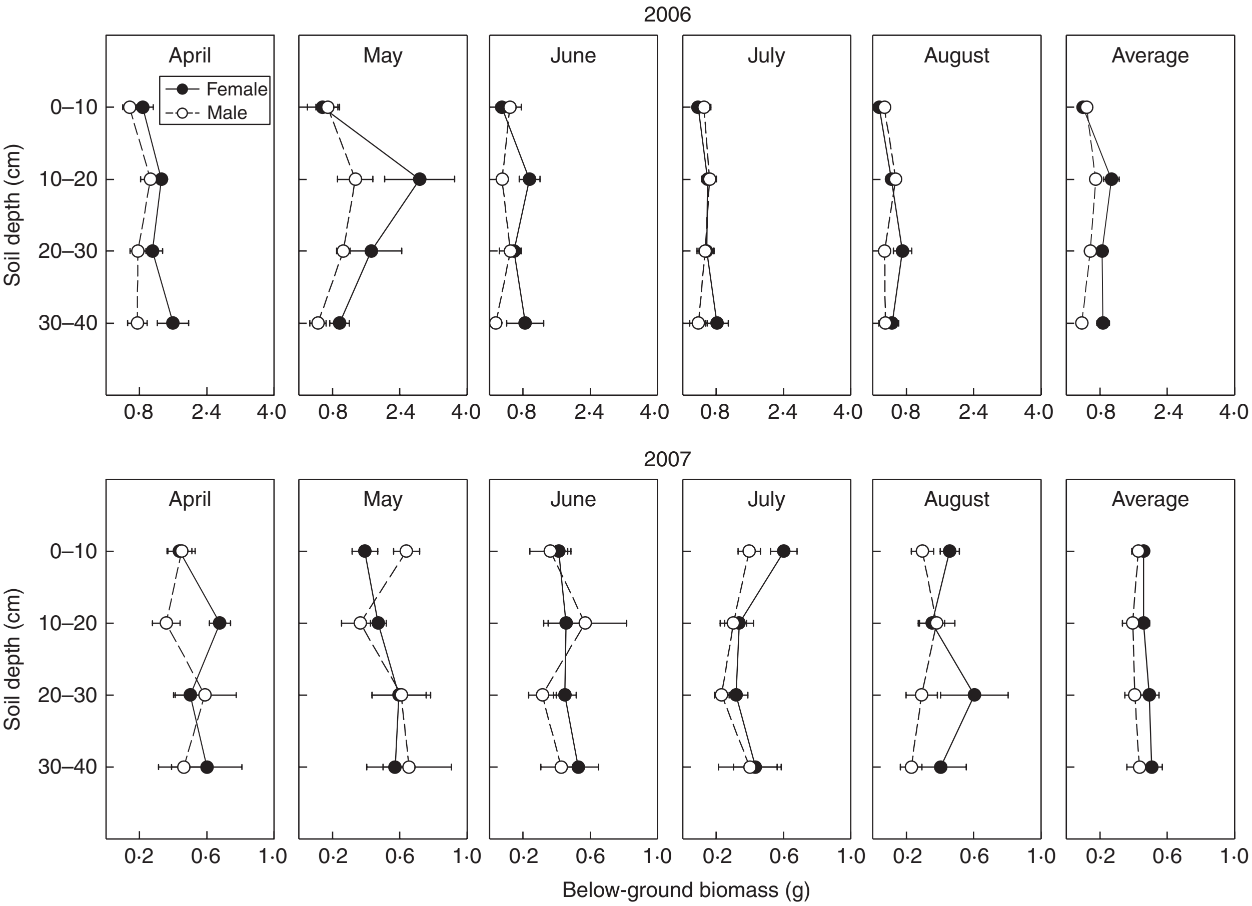

FIG. 4. Vertical distribution of below-ground biomass (g) of males and females of *H. peploides* in the soil profile for April, May, June, July, August and the average for all months in 2006 and 2007. Values represent means ($n = 10$, except $n = 6$ for April and May 2006) ± s.e. Note: range of below-ground biomass (x-axis) in 2006 is four times greater than in 2007.

below-ground biomass of males and females, which varied with depth and time (sex × depth × month interaction: $\chi^2 = 25{\cdot}8$, $P = 0{\cdot}012$). Although this three-way interaction is somewhat complex and differences depended on depth, we can observe that females usually had greater values than males, especially in the months of July and August (Fig. 4).

Soil water content

Differences between soil water content in the habitat of males and females depended on depth in both years (sex × depth interaction: $\chi^2 = 13{\cdot}6$, $P = 0{\cdot}003$ for 2006; $\chi^2 = 27{\cdot}08$, $P < 0{\cdot}001$ for 2007). In 2006, the first layer of the soil (0–10 cm) had a greater soil water content than the second layer (10–20 cm), especially for females, and similar values to the rest of the other layers (Fig. 5). In 2007, water content increases with increasing depth. In both years, 2006 and 2007, we found that females had greater water content near the soil surface than males, especially in the 0–10 cm. However, these differences disappeared in the deepest soil layers (Fig. 5).

In addition, soil water content decreased with time, although this decrease differed among the different soil layers (depth × month interaction: $\chi^2 = 36{\cdot}8$, $P < 0{\cdot}001$ for 2006; $\chi^2 = 28{\cdot}5$, $P < 0{\cdot}001$ for 2007). In particular, the water content at the surface decreased less than in the deepest layers of the soil in 2006, whilst the opposite pattern was true for 2007; Fig. 5). The temporal patterns of soil water content did not vary for males and females (sex × month interaction: $\chi^2 = 3{\cdot}12$, $P = 0{\cdot}538$ for 2006 and $\chi^2 = 5{\cdot}82$, $P = 0{\cdot}589$ for 2007).

DISCUSSION

Sex differences in reproductive allocation

Our study revealed different patterns of biomass allocation to reproduction in males and females of *H. peploides*. Males decreased their relative allocation to reproduction over time due to most of the flowering taking place before May. Females maintained their allocation to reproduction roughly constant from April to June, presumably as a consequence of fruits maturating after flowering has ceased. However, they also showed a reduction in the allocation to reproduction with time, but this was less pronounced than in the case of males, which corresponds to the dispersion of seeds that are lost from the mother plant. The result is that males tended to have greater reproductive effort than females early in the season (April and May) due to a greater investment in

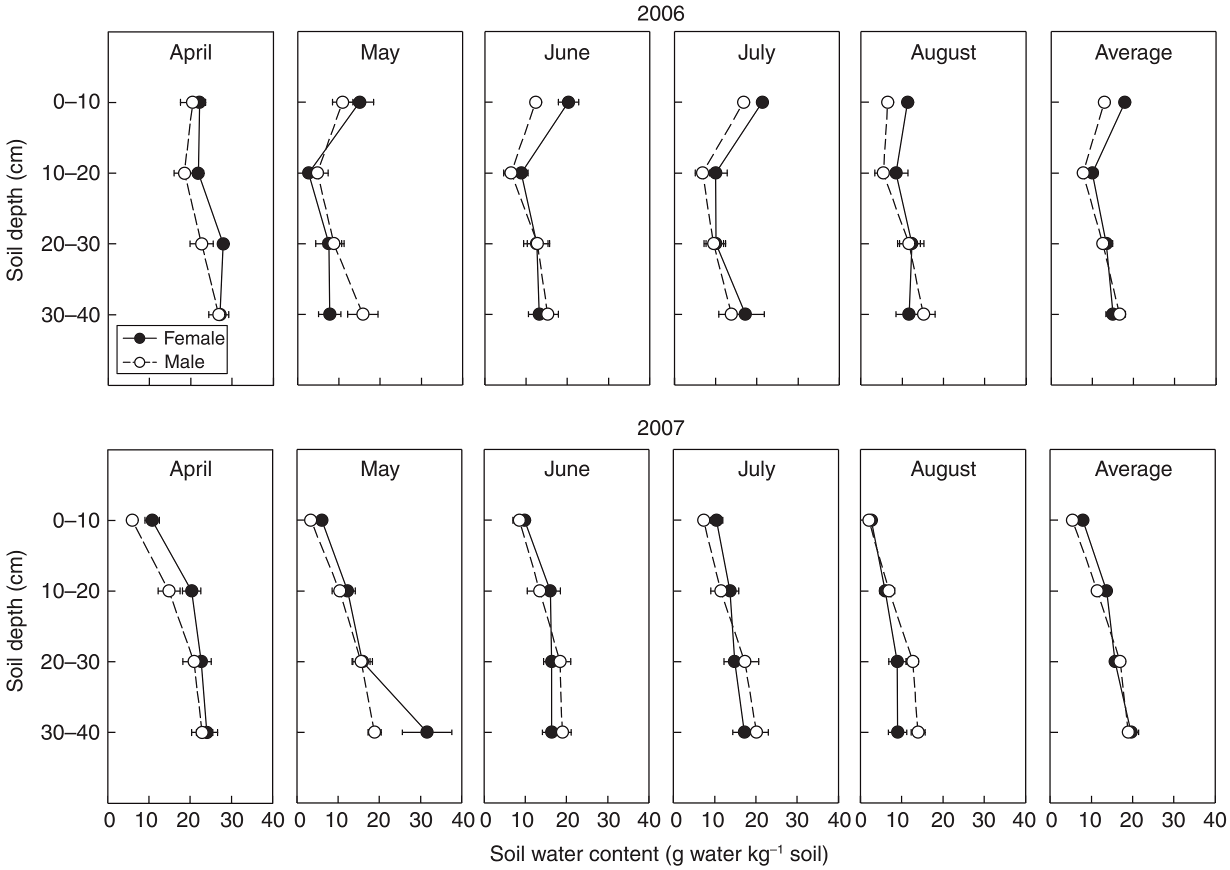

FIG. 5. Soil water content (g water kg^{-1} soil) in male and female habitats of *Honckenya peploides* from April to August 2006 and 2007. Values represent means ($n = 10$, except $n = 6$ for April and May 2006) ± s.e. The average values for all months are also shown.

flowers, but sexual differences became diminished or even reversed as the season progressed. The pattern of allocation to reproduction in females is somewhat surprising, because a much greater increase in May–June due to fruit production could be expected. This low fruit production in females might be attributable to pollen limitation due to the spatial segregation of males and female in different patches. Different patterns in the biomass allocation to reproduction between males and females have been previously identified in dioecious plants, mainly woody species, with males allocating more biomass to reproduction than females at flowering and females more to reproduction than males at fruiting (e.g. Allen and Antos, 1988; Delph *et al.*, 1993; Nicotra, 1999; Obeso, 2002; Leigh and Nicotra, 2003). Since the energetic requirements for producing fruits are generally greater than for flower production, and the maturation of fruits usually extends for a considerable period after flowering, females must continue to expend resources longer than do males, as observed in our study. Consequently, females of dioecious plants typically have been found to have a higher investment in reproduction than males over an entire growing season (Lloyd and Webb, 1977; Gross and Soulé, 1981; Willson, 1986; Popp and Reinartz, 1988; Leigh *et al.*, 2006). For these reasons, many studies have found higher vegetative growth in males of dioecious plants at the end of the growing season, which is consistent with the existence of a trade-off investment between reproductive and vegetative biomass (Herrera, 1988; Vasiliauskas and Aarsen, 1992; Gibson and Menges, 1994; Watson, 1995; Díaz Barradas and Correia, 1999; Hemborg and Karlsson, 1999). A similar trend has been found in *H. peploides* growing under greenhouse conditions, where females had over four times greater reproductive effort than males, which in turn had a greater size than females (Sánchez-Vilas and Retuerto, 2011). However, here we have not found the expected intersexual differences in the above-ground vegetative growth: males and females did not differ in their temporal patterns of growth, and female growth was not reduced relative to males towards the end of the growing season.

The timing of resource investment in reproduction may be as, or more, important than the amount invested in shaping sexual differences in growth (Gross and Soulé, 1981; Eckhart and Seger, 1999; Case and Ashman, 2005; Sánchez-Vilas and Pannell, 2011). The greater allocation of resources to reproduction in males early in the season may divert those resources away from investment in photosynthetic machinery that might otherwise contribute to an increased growth rate (Obeso, 2002). This may partly explain why comparatively greater reproductive effort in females towards the end of the season does not negatively impact on their growth in comparison to males. In addition, the lack of sex-specific differences in growth may be due to a similar cost of reproduction in both sexes, as a consequence of the low fruit production of females in the two years of the study.

Sex differences in below-ground allocation

Despite of the lack of differences in above-ground vegetative biomass between males and females of *H. peploides*, we did observe sexual differences in the patterns of allocation to below-ground structures. On the one hand, females had greater below-ground biomass, but only at certain soil depths. The greater below-ground biomass in females than in males found in our study could be due either to an increased amount of fine roots or/and to an increased rhizome growth; the particular contribution of each of these two components to the below-ground biomass is unknown in our study. A greater amount of fine roots could be expected if females had a greater demand of water and nutrients due to fruit production and maturation. However, since males and females showed similar reproductive effort, especially in 2006, other factors may be shaping this difference. Due to the perennial habit of this species, the sex-differential investment in below-ground biomass may also be related, as pointed out above, to sex-specific differences in the patterns of allocation to storage (i.e. rhizomes). Sex-specific differences on biomass allocation to storage organs have been found previously in other dioecious species, e.g. in *Borderea pyrenaica* (Garcia and Antor, 1995) and *Corema album* (Alvarez-Cansino *et al.*, 2010). On the other hand, regardless of the sex, we observed temporal variability in below-ground biomass: the below-ground biomass in the different layers of soil changed differently throughout the season. Optimal allocation theory predicts that plants will maximize growth under a given set of resource conditions by allocating biomass to the organ responsible for acquiring the most limiting resource (Bloom *et al.*, 1985; Bazzaz, 1997). Plants should thus increase allocation to shoots in conditions of carbon stress (e.g. resulting from shading) and to roots in conditions of nutrients or water stress (Bloom *et al.*, 1985). We might also expect an increase in allocation to the storage organs (rhizomes) as the season progressed. However, we found a decrease in the below- : above-ground vegetative biomass ratio as the season progressed and water stress beccame greater (see below). Different precipitation patterns in 2006 and 2007 indicate that water stress may be not so strong in the summer of 2007 (see Fig. 1). In 2007, the spring was quite dry in comparison to the previous year, but in summer the amount of precipitation was higher than in the same period for 2006. The result is that above-ground biomass increased with time in 2007, which may have shifted the below- : above-ground vegetative biomass ratio towards lower values as the season progressed in this year. Nevertheless, the decrease in below- : above-ground vegetative biomass ratio is somewhat surprising, especially for 2006, where summer drought is intense, considering that a larger proportion of assimilates allocated to root production may be particularly significant in maintaining an adequate water balance (Aronson *et al.*, 1992; Retuerto and Woodward, 1993; Fitter and Hay, 2002). Although we know no field studies examining seasonal changes in below-ground allocation in dioecious plants, some authors have reported results consistent with our findings. In *Silene latifolia* the proportion of biomass allocated to roots decreased in time with allocation to reproduction (Gehring, 1993). Escarré *et al.* (1990) reported reduced below- : above-ground vegetative biomass ratios in females of *Rumex acetosella* during flowering and fruiting. Similarly, Cibils *et al.* (2005) found that females of *Atriplex canescens* produced fewer roots than males when soil moisture was deficient. These authors suggested that females were constrained by their relatively inefficient use of water and were unable to produce sufficient photosynthates to sustain a greater allocation to the root system. Our results highlight the possibility that the ability to alter timing and placement of root proliferation, as indicated by temporal variability and differences with depth in below-ground biomass, may be more important for plant success than changes in the partitioning of biomass between below- and above-ground structures (Reynolds and D'Antonio, 1996).

Sex differences in habitat

Sexes of many dioecious plant species are spatially segregated along a gradient of habitat quality, with females being more common under less-stressful conditions, i.e. in sites that are moister, less exposed, less saline, and/or with higher nutrient concentrations (Bierzychudek and Eckhart, 1988). In line with this, in our study the first layers of the soil where females were growing showed higher water content than those of males. However, the differences were less clear at other depths, which makes it difficult to relate solely this factor to the presence/absence of the sexes as previously have been done for other species (Dawson and Bliss, 1989; Dawson and Ehleringer, 1993).

Soil water content decreased towards the end of the season, in both male and female habitats. Climatic, characterized by increasing temperatures and radiation and lower precipitation as summer progresses, may explain this decrease in the content of water in the soil. However, and contrary to what we might expect, this decrease was similar in female and in male habitats (or even tended to be more pronounced in female than in male habitats). Towards the end of the season, females are making greater investment in reproduction than males, so reduced water availability may critically affect their growth. However, no differences between males and females were found in above-ground growth. A plausible explanation for this unexpected result is a differential use of this limiting resource by males and females. Of course, our study did not measure directly the plant water status, and therefore we lack empirical evidence for such an explanation. Nonetheless, in support of this hypothesis, we found greater below-ground biomass in females, which presumably contributes to increase the uptake of water more in females than in males when this becomes scarce. This greater use of water by females would also explain why the water content of the habitats of females and males was similar in the deepest layers of the soil, despite the water content being greater in the upper layers of the females' habitat. Greater below-ground biomass, as pointed above, may be also related to greater allocation to rhizomes, which are mainly storage organs. A high capacity for water storage by rhizomes has been found in species of arid environments, e.g. *Leymus chinensis*, playing an important role in regulating plant growth (Wang *et al.*, 2008). In this sense, a greater water storage capacity of females would be in accordance with previous evidence that the different sexes of *H. peploides* have different strategies to cope with water stress, with females displaying mechanisms to maintain a more favourable water content in their tissues than males (Sánchez-Vilas and Retuerto, 2009). These results would indicate some degree of sexual specialization in resource acquisition and utilization, suggesting that males and females may benefit if they occupy different niches within the environment (i.e. niche partitioning; Freeman *et al.*, 1976; Onyekwelu and Harper, 1979; Cox, 1981; Vitale *et al.*, 1987; Bierzychudek and Eckhart, 1988; Freeman *et al.*, 1997; Dawson and Geber, 1999; Sánchez-Vilas and Pannell, 2010).

Finally, our seasonal analysis of soil water content suggests that timing of investment in reproduction in males occurs when moisture in the soil is high as pointed out above. Males of *H. peploides* tend to allocate proportionally more biomass to reproduction than females early in the season. Since water is less limiting at this time than later in the season, males would not need to invest as many resources as females in root production, resulting in greater below-ground biomass in females than in males.

Conclusions

Males and females of *H. peploides* had different profiles of water content in the soil where they were growing. Overall, when differences existed (usually in the upper layers of the soil), females had higher soil water content than males. Similarly, the allocation of biomass to below-ground structures also varied in depth differently for males and females, with females usually having greater below-ground biomass than males. Although males and females differed in patterns of reproductive effort, no differences were found in above-ground growth. Sex differential timing of investment in reproduction – males invest more biomass to reproduction early in the season, when water content is high – and differential availability and use of resources from the soil (particularly water) are all factors that probably offset the costs of reproduction in above-ground growth in males and females of *H. peploides*. Therefore, our results found evidence to support the theory that the patterns of spatial segregation of the sexes observed in *H. peploides* may contribute to maximize each sex's growth and reproduction, although further studies are needed to fully understand the mechanisms that create such patterns.

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