Influence of plant maturity, shoot reproduction and sex on vegetative growth in the dioecious plant *Urtica dioica*

Marta Oñate and Sergi Munne-Bosch*

Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 645, E-08028 Barcelona, Spain

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**Key Results** Vegetative growth rates in mature plants were drastically reduced compared with juvenile ones (48% and 78% for number of leaves and leaf biomass produced per day, respectively), which was associated with a loss of photosynthetic pigments (up to 24% and 48% for chlorophylls and carotenoids, respectively) and increases of α-tocopherol (up to 2.7-fold), while endogenous levels of phytohormones did not differ between mature and juvenile plants. Reductions in vegetative growth were particularly evident in reproductive shoots of mature plants, and occurred similarly in both males and females.

**Conclusions** It is concluded that (a) plant maturity reduces vegetative growth in *U. dioica*, (b) effects of plant maturity are evident both in reproductive and non-reproductive shoots, but particularly in the former, and (c) these changes occur similarly in both male and female plants.

**Key words:** Dioecious plant, growth rates, herbaceous perennial, maturity, phytohormones, reproduction, *Urtica dioica*.

**INTRODUCTION**

Living organisms undergo sequential phases from birth to death that are characterized by their chronological age, but also by their reproductive capacity, among other distinctive traits that are sometimes used to mark different phases of development or ontogeny. Thus, pre-reproductive plants are considered juveniles and the transition phase is marked by the onset of flowering and seed production, hence fully reproductive plants are considered mature (Bond, 2000). Perennials maintain the capacity to develop new leaves and continue to grow throughout their life thanks to the fact that at least some vegetative meristems remain indeterminate for more than one growth season (Rohde and Bhalerao, 2007). Meristems are formed by meristematic stem cells, which are relatively undifferentiated cells defined by their abilities for self-renewal and for generating differentiated cells. The interaction between meristem determinacy and the sequential or progressive senescence of lateral organs determines the longevity of the axis (Thomas, 2002). In perennials, whole-plant senescence can occur only when all shoot meristems differentiate to give flowers or inflorescences and/or lose their capacity to divide and form new vegetative tissues and organs during the next season (Munne-Bosch, 2008). At the whole-plant level, it has been proposed that meristems behave very much like individual organisms (Trewavas, 1983). Even, a plant can be considered a colony of semi-autonomous meristems, simultaneously competing with one another, but serving the whole plant over time by subtly altering their behaviour (Greenwood, 1995).

Unisexual plants often display sexually dimorphic reproductive and somatic characters. These may result from different resource constraints on sexual functions (Darwin, 1877) and ecological differentiation of male and female plants (Meagher, 1984). In dioecious plants, it has been generally accepted that females often show higher proportional investments in reproduction than males (Hancock and Bringhurst, 1980; Barrett and Helenurm, 1981; Gross and Soule, 1981; Korpelainen, 1992; Cipollini and Whigham, 1994). However, the theory that females grow less than males as a consequence of higher investment in reproduction appears to be true for woody perennials, but it is not so clear or it is simply not applicable in herbaceous perennials (Obeso, 2002). Instead, in herbaceous perennials, the female advantage of larger size leads to higher fecundity and, at the same time, smaller size in males might be associated with the advantage of earlier...
maturation with related demographic advantages of precocious reproduction (Andersson, 1994).

Possible candidates for the regulation of changes in vegetative growth during plant maturity are auxins, cytokinins and abscisic acid. Besides fulfilling an important role in regulating cell growth (mainly in cell expansion), auxins have multiple functions in patterning and organogenic processes. During post-embryonic growth of plants, differential auxin activities form developmental hallmarks of de novo organogenesis for leaves, flowers and floral organs. First, auxin accumulates at the location of organ initiation and, next, an auxin gradient is established along the growth axis of the developing primordium with the maximum at its tip (Benková et al., 2003). Moreover, differential auxin distribution appears to be important within developing leaves, where increased auxin activity corresponds with the formation of vascular tissue during leaf venation (Mattsson et al., 2003; Scarpella et al., 2006). Aside from their role in cell cycle progression, cytokinins control phloem unloading and sink organ strength (Zaffari et al., 1998; Haberer and Kieber, 2002). Therefore, it has been proposed that high cytokinin levels are needed for leaf initiation, flower induction and flower development (Bernier et al., 1993; Chang et al., 1999). Abscisic acid has also been shown to be involved in regulating the transition from the vegetative to reproductive phase in several woody perennials (Finkelstein et al., 2002). The occurrence of changes in abscisic acid in relation to differences in the rate of vegetative growth is consistent with the increase of this growth inhibitor observed in leaves of mature plants, in which the vegetative growth rate decreases (Haffner et al., 1991; Valdés et al., 2004; Munne-Bosch and Lalauze, 2007; Oñate and Munne-Bosch, 2008). Furthermore, cytokinins, in conjunction with light, induce the production of chlorophyll, chloroplast differentiation and the maintenance of the photosynthetic apparatus in leaves (Haberer and Kieber, 2002), while abscisic acid can favour photo-oxidative stress in chloroplasts by inducing stomatal closure and consequently decreasing the activity of the Calvin cycle as a sink for ATP and reducing equivalents produced in photosynthetic electron transport (Sminoff, 1993). Thus, it is of interest to evaluate how plant maturity influences vegetative growth and the hormonal balance of leaves, and whether or not the possible changes in growth and phytohormones occur in parallel with changes in the contents of photosynthetic pigments and chloroplastic antioxidants in mature plants, an aspect not investigated thus far. In addition, the possible influence of plant maturity and dioecy on the contents of other regulators, such as jasmonic acid and salicylic acid, which are known to play a key role in determining pollen development and fertility, and plant maturation in Arabidopsis thaliana, respectively, is largely unknown (Li et al., 2004; Martínez et al., 2004).

The genus Urtica, which belongs to the family Urticaceae, is composed of annual and perennial herbs and also a few shrubs and small trees, distinguished, among other features, by their stinging hairs. The most common Urtica species are the perennial U. dioica and the annual U. urens. They show a wide range of distribution extended from Europe, North Africa to Asia, up to North and South America and South Africa. These species have been known for a long time as medicinal plants (Kavalali, 2003). Urtica dioica is a herbaceous, dioecious perennial plant that can reproduce sexually through seeds and asexually through rhizomes (Srůtek and Teckelmann, 1998). Although some studies have focused on understanding better the inheritance pattern of the sex ratios in this species (De Jong et al., 2005; Glawe and De Jong, 2007), it is still unknown how reproduction at the whole-plant and shoot-levels, as well as dioecy, affect vegetative growth in U. dioica.

The aim of the present study was to evaluate the effects of plant maturity, shoot reproduction and sex on the vegetative growth of this herbaceous, dioecious perennial species and examine whether or not the possible effects on growth occur in parallel with changes in the contents of phytohormones, photosynthetic pigments and chloroplastic antioxidants. Growth rates of apical shoots, together with foliar levels of phytohormones (auxins, cytokinins and abscisic acid, together with salicylic acid and jasmonic acid) and other indicators of the leaf physiology [water contents, chlorophylls and carotenoids, including the xanthophyll cycle, \( \alpha \)-tocopherol and the maximum efficiency of photosystem II (PSII) photochemistry – the \( F_v/F_m \) ratio] were measured in juvenile and mature plants, with a distinction between reproductive and non-reproductive shoots in both male and female plants. Vegetative growth was not only evaluated in apical shoots of field-grown plants, but also in cuttings obtained from these plants.

MATERIALS AND METHODS

Plant material, growth conditions and sampling

This study was conducted using Urtica dioica L. It is a herbaceous, sub-dioecious perennial native to Europe, Asia, Africa and America, and it is the best known member of the genus Urtica. Urtica dioica grows 1–2 m tall, it has very distictively yellow, widely spreading roots, and soft green leaves, which are 3–15 cm long and with a strongly serrated margin. Generally, U. dioica plants have a strong association with human habitation. Indeed, human and animal waste may be responsible for elevated levels of phosphate and nitrogen in the soil, providing an ideal environment for this species (Bolós and Vigo 1984; Castroviejo et al., 1996).

Plants used for the experiments were obtained as follows. Urtica dioica seeds from Sand Mountain Herbs (Fyffe, AL, USA) were sown in 0.1-L pots containing a mixture of soil : peat : perlite (1 : 1 : 1, v/v/v) during 4 October 2006. After 20 d of growth, plants were transplanted to 0.5-L pots and were maintained in a greenhouse with a controlled temperature (24/18 °C, day/night) and watered twice a week with Hoagland’s solution. After 3-5 months, plants were transplanted to the experimental fields of the University of Barcelona (Barcelona, Spain). Before the plants were transferred, the soil (Calcic Luvisol; FAO) was ploughed and treated with N : P : K (1 : 1 : 1) fertilizer at the rate of 100 kg ha\(^{-1}\) to avoid mineral deficiency in plants. Fifteen plants were homogeneously distributed in a square plot of 6.25 m\(^2\), so that all plants had the same orientation to the sun. During June 2007, seeds obtained from these plants were germinated and grown under the same conditions as mentioned before. During mid-September, these juvenile plants
were transplanted to the experimental fields in a square plot of the same dimensions situated just next to the mature plants and were grown under identical conditions before and during the experiment. Juvenile and mature plants were therefore grown under Mediterranean field conditions and received water exclusively from rainfall during the growth and study period.

Experiments were conducted during autumn 2007, so that juvenile and mature plants were 4 months and 1 year old, respectively, at the beginning of the experiment. All measurements were performed during an active vegetative growth phase in autumn, a few months after mature plants reproduced during spring and summer. Mature and juvenile plants differentiated each other by morphological features intrinsic to plant maturity, including increased size, rhizome storage capacity or lateral rooting. However, all leaves taken for measurement were of a similar age (fully expanded young leaves that appeared after later summer rainfalls) and fully exposed to the sun. Since not all shoots and not all vegetative meristems of a shoot differentiate to give reproductive structures in *U. dioica*, leaves of reproductive and non-reproductive shoots of mature plants could be compared during vegetative growth in autumn. Leaves were collected at midday during 29 October (1500 μmol quanta m$^{-2}$ s$^{-1}$, air temperature 21.0 °C and relative humidity 54 %) and 26 November (1200 μmol quanta m$^{-2}$ s$^{-1}$, air temperature 17.3 °C and relative humidity 43 %) to measure growth parameters [leaf biomass and area, and leaf mass per area (LMA) ratio], levels of phytohormones (cytokinins, auxins and abscisic acid, together with jasmonic acid and salicylic acid) and other indicators of leaf physiological status (water content, chlorophyll and carotenoid, including the xanthophyll cycle, α-tocopherol, and the $F_v/F_m$ ratio, indicative of damage to PSII). For phytohormone, photosynthetic pigments and tocopherol analyses, leaves were collected, immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Furthermore, growth rates were estimated in field-grown plants and in cuttings obtained from these plants, as described below.

**Estimation of leaf growth**

Leaves collected in the field were immediately weighed to estimate the fresh matter and leaf area was measured using a flatbed scanner (model GT-5000; Epson, Nagano, Japan) and an image-processing program. Samples were dried at 65 °C to constant weight to estimate the dry matter, and growth parameters were therefore calculated, including the leaf biomass, leaf area and LMA ratio.

**Phytohormone analyses**

Levels of the cytokinins, zeatin and zeatin riboside, indole-3-acetic acid, abscisic acid, jasmonic acid and salicylic acid were simultaneously analysed by HPLC-MS/MS as described by Abreu and Munné-Bosch (2009).

**Leaf water content and chlorophyll fluorescence**

The relative leaf water content (RWC) was determined as 100 × (FW – DW)/(TW – DW), where FW is the fresh matter, TW is the turgid matter after re-hydrating the leaves for 24 h at 4 °C in darkness, and DW is the dry matter after oven-drying the leaves to constant weight at 65 °C. The maximum efficiency of PSII photochemistry ($F_v/F_m$) was calculated from chlorophyll fluorescence data obtained with a portable fluorimeter mini-PAM (Walz, Effeltrich, Germany) from leaves maintained at least 1 h in darkness, by using the equations described by Van Kooten and Snel (1990).

**Photosynthetic pigments and α-tocopherol**

The extraction and HPLC analyses of chlorophylls, carotenoids and α-tocopherol were carried out as described by Munné-Bosch and Alegre (2000), except that an HPLC system HP1100 Series with a diode array detector (Agilent Technologies, Santa Clara, CA, USA) was used for analyses.

**Growth rates in field-grown plants and cuttings**

Apical shoots were marked just below the two latest leaves to emerge during 18 October 2007 to estimate the number of leaves and leaf biomass produced per apical meristem. The period of growth analysed was from 18 October to 9 November. After this period, leaves were collected, the number of leaves counted and leaf biomass were estimated as described before (see ‘Estimation of leaf growth’ section).

Cuttings were obtained by excising the apical shoots of field-grown plants, keeping the axillary meristems of three nodes besides the shoot apical meristem. The same shoots used before to estimate growth rates in field-grown plants were used here to obtain the cuttings, so that cuttings were of the same size and devoid of leaves. After excision, cuttings were immediately put inside 100-mL pots containing a mixture of soil : peat : perlite (1:1:1, v/v/v), kept in a constant-environment chamber (16-h photoperiod; 90–110 μmol quanta m$^{-2}$ s$^{-1}$; air temperature between 21 and 23 °C), covered with a plastic film (with small holes) during the whole period and watered with Hoagland’s solution. Twenty days later, leaves were collected, the number of leaves counted and leaf biomass estimated.

**Statistical analyses**

Statistical differences between groups were analysed by a two-way factorial analysis of variance (ANOVA) using fixed factors or Student’s *t*-tests with the SPSS package (Chicago, IL, USA). Differences were considered significant at a probability level of $P \leq 0.05$.

**RESULTS**

Plant maturity and shoot reproduction reduce leaf growth irrespective of sex

Leaf growth parameters were measured in juvenile and mature plants (Fig. 1), with a distinction in the latter between reproductive and non-reproductive shoots in both males and females (Fig. 2). Plant maturity reduced leaf biomass and leaf area by up to 72 % (two-way ANOVA; leaf biomass: $F = 54.38$, $P < 0.01$; leaf area: $F = 57.49$, $P < 0.01$; Fig. 1),
while LMA and RWC did not differ significantly between plant groups (two-way ANOVA; $P > 0.05$; Fig. 1). A comparison between reproductive and non-reproductive shoots in mature plants revealed a specific effect of reproduction at the shoot level. Leaf biomass was much smaller in reproductive shoots than in non-reproductive ones, with reductions ranging from 45 to 69% (two-way ANOVA; $F = 14.45$, $P < 0.01$; Fig. 2). Decreases in leaf area were even more evident, which led to increases in the LMA in reproductive shoots of mature plants, with values around 45% higher than in non-reproductive shoots (two-way ANOVA; leaf area: $P < 0.01$; Fig. 2).
F = 47.73, P < 0.01; LMA: F = 7.59, P = 0.01; Fig. 2). RWC values remained around 80% in all plant and leaf groups examined. No differences between sexes were observed in the growth parameters analysed (two-way ANOVA; P > 0.05; Fig. 2).

**Differences in leaf physiology between juvenile and mature plants**

Endogenous levels of the cytokinins, zeatin and zeatin riboside, indole-3-acetic acid, abscisic acid, jasmonic acid and salicylic acid were not significantly different between juvenile and mature plants (two-way ANOVA; P > 0.05; Fig. 3). However, some differences in phytohormone levels could be observed between reproductive and non-reproductive shoots of mature plants (Fig. 4). While levels of cytokinins, auxins, jasmonic acid and salicylic acid did not differ between reproductive and non-reproductive shoots of mature plants (two-way ANOVA; P > 0.05; Fig. 4), endogenous contents of abscisic acid were smaller in the former (two-way ANOVA; F = 12.26, P < 0.01; Fig. 4). No differences between sexes were observed in the endogenous concentrations of phytohormones (two-way ANOVA; P > 0.05; Fig. 4).

While phytohormone levels were not affected by plant maturity, significant reductions in the levels of photosynthetic pigments were observed. Chlorophylls, lutein, β-carotene and the xanthophyll cycle pool decreased by up to 24%, 48%, 41% and 47%, respectively, in mature plants compared with juvenile ones (two-way ANOVA; chlorophyll a + b: F = 25.29, P < 0.01; lutein: F = 17.56, P < 0.01; β-carotene: F = 11.67, P < 0.01; xanthophyll cycle pool: F = 12.67, P < 0.01; Fig. 5). The Fv/Fm ratio also decreased slightly, but significantly, in mature plants compared with juvenile ones (two-way ANOVA; F = 5.37, P = 0.02; Fig. 5). Furthermore, increases in α-tocopherol levels (up to 2.7-fold) and the chlorophyll a/b ratio (up to 84%) were observed in mature plants compared with juvenile ones (two-way ANOVA; α-tocopherol: F = 6.73, P = 0.01; chlorophyll a/b: F = 10.29, P < 0.01; Fig. 5).
Chlorophyll levels and the Fv/Fm ratio were significantly smaller in reproductive shoots compared with non-reproductive ones, with a chlorophyll loss of approx. 40% and Fv/Fm ratios below 0.80 in leaves of reproductive shoots (two-way ANOVA; chlorophyll a+b: F = 35.61, P < 0.01; Fv/Fm ratio: F = 14.45, P < 0.01; Fig. 6). The chlorophyll a/b ratio and α-tocopherol levels were also higher in reproductive shoots compared with non-reproductive ones (two-way ANOVA; chlorophyll a/b: F = 10.70, P < 0.01; α-tocopherol: F = 7.22, P = 0.01; Fig. 6). Although carotenoid levels (lutein, β-carotene and xanthophyll cycle pools) tended to be smaller in reproductive shoots compared with non-reproductive ones, no significant differences were observed (two-way ANOVA; P > 0.05; Fig. 6). In addition, no differences between sexes were observed in the levels of photosynthetic pigments (chlorophylls or carotenoids), the Fv/Fm ratio or α-tocopherol (two-way ANOVA; P > 0.05; Fig. 6).

Growth rates in apical shoots of field-grown plants and cuttings

Growth rates of apical shoot meristems were measured in juvenile and mature plants, with a distinction between reproductive and non-reproductive shoots in both males and females, both in field-grown plants and in cuttings obtained from these plants (Figs 7 and 8). Growth rates of apical shoots, estimated by the number of leaves and leaf biomass produced per day revealed that plant maturity reduces the vigour of vegetative meristems. The number of leaves and leaf biomass produced per day decreased by 48% and 78%, respectively, in mature compared with juvenile plants (Student’s t-test; P ≤ 0.05; Fig. 7). To examine whether or not this reduction in vegetative growth was under local control, we measured the growth rates of cuttings, and a similar pattern was observed, with decreased growth rates in mature compared with juvenile plants (Student’s t-test;
As occurred in field-grown plants, growth rates of cuttings were not significantly different between sexes (two-way ANOVA; \( P < 0.05 \); Fig. 8). Reproductive shoots showed reduced vigour compared with non-reproductive shoots both in field-grown plants and in cuttings obtained from these plants, although differences were only statistically significant in field-grown plants. In these plants, the number of leaves and leaf biomass produced per day decreased up to 56% and 91%, respectively, in reproductive shoots compared with non-reproductive ones (two-way ANOVA; number of leaves: \( F = 18.53, P < 0.01 \); leaf biomass: \( F = 24.72, P < 0.01 \); Fig. 8).
DISCUSSION

Development of plants usually progresses from a strictly vegetative juvenile phase to the sexually competent mature phase. The process of maturation can be achieved within a few weeks, as it occurs in some annuals, or over several years, as it occurs in long-lived perennials, such as some trees. When lacking reproductive structures, juvenile and mature phases may still be distinguishable by leaf phyllotaxy, shape, size...
and colour; stem branching and tropism; thorniness; and other vegetative characteristics. Tissue culturists and plant propagators regard as most significant the diminished competence for organogenesis and plant regeneration in explants or cuttings obtained from mature plants. Adventitious root and shoot formation is substantially reduced, often absent, in explants or cuttings from mature plants, especially at advanced developmental stages, and it is generally accepted that plant maturity reduces vegetative growth rates, both in herbaceous and woody perennials (Huang et al., 1992; Obeso, 2002). Indeed, increased mortality rates were observed in cuttings of mature plants compared with those of juvenile plants (18% vs. 0%, respectively, with all deaths observed in cuttings of reproductive shoots), thus confirming that plant maturity and, more specifically, reproduction reduces the rooting success of plants, probably due to changes in hormone sensitivity associated with plant maturity (Day et al., 2002). Increased vegetative growth rates, both in herbaceous and woody perennials (Huang et al., 1992; Obeso, 2002). Indeed, increased mortality rates were observed in cuttings of mature plants compared with those of juvenile plants (18% vs. 0%, respectively, with all deaths observed in cuttings of reproductive shoots), thus confirming that plant maturity and, more specifically, reproduction reduces the rooting success of plants, probably due to changes in hormone sensitivity associated with plant maturity (Day et al., 2002). It is shown in the present study that plant maturity strongly reduces vegetative growth in U. dioica, the effects being evident both in reproductive and non-reproductive shoots, but particularly in the former, and occurring similarly in both male and female plants. Reductions in vegetative growth in mature plants were associated with large decreases in photosynthetic pigments, but not with variations in the endogenous levels of phytohormones. However, vigour of vegetative meristems was drastically affected by plant maturity, effects being most evident in reproductive shoots.

An altered hormonal balance associated with the transition phase of plants reaching maturity has been reported in woody perennials. Among other phytohormones, cytokinins, auxins and abscisic acid appear to play a central role in the regulation of reduced growth rates as shrubs and trees reach the mature stage (Haffner et al., 1991; Finkelstein et al., 2002; Valdés et al., 2002, 2004; Munné-Bosch, 2007; Munné-Bosch and Laluzea, 2007). As far as is known, the present study is, however, the first report examining the effects of plant maturity on the endogenous levels of phytohormones in leaves of herbaceous perennials. Although plant maturity reduced the vigour of vegetative meristems, it did not significantly alter the endogenous contents of any of the phytohormones tested in leaves, including cytokinins, auxins and abscisic acid. How is it therefore possible that vigour of vegetative meristems is drastically reduced in mature U. dioica plants, while endogenous levels of phytohormones in leaves were not affected by plant maturity? Although endogenous levels of the phytohormones measured were not significantly different in leaves of mature plants compared with those of juvenile ones, it is likely that meristems contain different amounts of phytohormones or other growth regulators. In support of this hypothesis, it was shown in the present study that cuttings of mature plants grew less than those of juvenile ones, thus indicating that vegetative growth is under local control at the meristem level. In addition, the endogenous content of abscisic acid in mature plants was

![Field conditions](image_url)

![Cuttings](image_url)

**Fig. 7.** Growth rates, estimated as number of leaves and leaf biomass produced per day, of apical shoot meristems of juvenile and mature field-grown U. dioica plants and of cuttings obtained from these plants. For field experiments, apical shoots were marked during 18 October and measurements were made in situ on newly appeared leaves after 22 d, when leaves reached full expansion. Cuttings were obtained during 13 November, rooted in pots under controlled environmental conditions and growth parameters were analysed after 20 d of growth, when new leaves reached full expansion. Data represent the mean ± s.e. of n = 12. Significant differences between groups are indicated inside panels (Student’s t-test, P ≤ 0.05).
lower in leaves of reproductive shoots compared with those of non-reproductive ones, thus indicating an asymmetric distribution of abscisic acid levels within the canopy of mature plants.

Factors inherent to plant maturity, such as shoot reproduction, but also increases in plant size, rhizome storage capacity or the number of lateral shoots and roots that ultimately determine resource allocation between different meristems can indeed exert an effect on the reductions in meristem vigour. While other studies have shown that plant size is an important factor influencing leaf growth rates of mature plants, not only in woody perennials (Mencuccini et al., 2005; Vanderklein et al., 2007; Oñate and Munné-Bosch, 2008), but also in herbaceous perennials, such as some epiphytic bromeliads (Zotz et al., 2001, 2002), the present study shows that reproduction at the whole-plant level in *U. dioica* severely affects the vigour of vegetative meristems, particularly of the meristems of shoots that have reproduced. Indeed, reductions in vegetative growth in mature plants were more evident in reproductive than in non-reproductive shoots, both in field-grown plants, and to a lesser extent also in cuttings obtained from these plants. As shown in Figs S1 and S2 in the Supplementary Data (available online), this conclusion is supported by the fact that leaves were smaller (both in biomass and area) in shoots of mature plants compared with juvenile ones, the effects being most evident in reproductive shoots. Moreover, when foliar phytohormone levels of shoots of mature plants were compared with those of juvenile ones (Table S1 in Supplementary Data), it turned out that abscisic acid and jasmonic acid levels were significantly different in reproductive shoots of mature plants compared with juvenile ones, while no significant differences in any phytohormone were observed between non-reproductive shoots of mature plants and juvenile plants, thus confirming that changes induced by plant maturity were most evident in reproductive shoots. This is in agreement with studies performed on annual plants, in which it has been shown that vegetative growth can be stimulated by removing reproductive structures (Leopold et al., 1959; Lockhart and Gottschall, 1961). However, it is worthy to note that vegetative growth was also reduced in non-reproductive shoots of mature plants compared with juvenile ones, despite the fact that phytohormone levels did not differ between them. Taken together these results suggest that the signal responsible of reducing growth operates at the local level (in meristems) and it seems to be preferentially transferred within the same reproductive shoots, but also between different shoots (reproductive and non-reproductive) of *U. dioica* plants.

Interestingly, physiological changes associated with plant maturity occurred similarly in both male and female *U. dioica* plants, thus indicating that both sexes were similarly affected by reproduction at the whole-plant and shoot levels. Although the present study is based on some physiological
parameters only and therefore not completely conclusive in this regard, results do not support the hypothesis of differential reproductive costs between sexes. Indeed, this hypothesis has proven to be applicable in several (although not all) woody perennials, but not in the herbaceous perennials tested thus far (Obeso, 2002; Ortiz et al., 2002).

A further understanding of the physiological processes described here is possible when considering the particularities of the plant species studied from an evolutionary point of view. *Urtica dioica* is a perennial plant with congeners that are annuals, such as *U. urens*. From annuality to perenniality the importance of the determinacy of meristems has been pointed out (Thomas et al., 2000; Rohde and Blahtero, 2007; Munné-Bosch, 2008) and, according to Raunkiaer (1934), the formation of resting structures and the progressive programmed senescence and death of organs also play a critical role, so that plants survive for as long as young proliferating tissues can keep ahead of the wave of senescence and tissue death behind them. In *U. dioica*, shoots that reproduce are committed to death. Although reproductive shoots of mature plants keep some of the vegetative meristems in an indeterminate state in the same shoot, the leaves they produce are smaller and show symptoms of physiological deterioration, such as reduced $F_v/F_m$ ratios and leaf yellowing (as indicated by loss of photosynthetic pigments). Indeed, it was observed in the present study that leaves entered a senescing process a few weeks after sampling and all reproductive shoots died back later, while non-reproductive shoots kept alive. It seems therefore that shoots of *U. dioica* behave in a similar way to several monocarpic plants, in which reproduction is associated with programmed senescence and death. Perenniity in *U. dioica* is therefore based on keeping at least one shoot in the vegetative stage.

It is concluded that (a) plant maturity reduces vegetative growth in *U. dioica*, (b) effects of plant maturity are evident both in reproductive and non-reproductive shoots, but particularly in the former, and (c) these changes occur similarly in both male and female plants. Further research is needed, however, to understand better the mechanisms involved in the regulation of maturity, shoot reproduction and dioecy in *U. dioica*, as well as to unravel the physiological basis and mechanisms involved in determining perenniality in *U. dioica*.

**SUPPLEMENTARY DATA**

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following files. Figure S1: Details of shoots of juvenile and mature plants growing in the experimental fields. Figure S2: Details of cuttings obtained from juvenile and mature plants growing in the experimental fields. Table S1: Statistical differences in the physiological parameters measured between leaves of reproductive (R) and non-reproductive (NR) shoots of mature plants compared to leaves of juvenile plants.

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**LITERATURE CITED**


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