

Received May 30th, 2003

Geographic distribution and seed propagation of *Urtica dioica* L. from Portugal

ORLANDA PÓVOA ⁽¹⁾

ANA MONTEIRO ⁽²⁾

TERESA VASCONCELOS ⁽²⁾

ABSTRACT

The main objective of this project was to verify whether it is possible to mass-produce *Urtica dioica* L. for medicinal purposes. It therefore sought to study *Urtica dioica* L. propagation by seed, given different seed treatments and germination conditions.

The species' geographic distribution was determined on the basis of information from bibliographic research and several Herbariums located around mainland Portugal. Seeds collected from the Tapada da Ajuda Botanical Garden were submitted to a pre-treatment of 24 hours immersion in a KNO₃ 10-3 M solution at room temperature (20°C); a control without pre-treatment was also carried out. Germination tests were performed in light and temperature-controlled incubations at 15 °C, 20 °C, 25 °C and 30°C, as well as under alternate 15/25°C and 20/30°C regimes in total darkness and with 12-hr daily photoperiods. The seed treatment method did not significantly influence seed germination. Significantly large differences were obtained for the different modalities of temperature and photoperiod regime, as well as for the interaction between temperature and photoperiod. The best germination result (29 %) was obtained with seeds that had not been subjected to seed treatment, at 20 °C and with a 12-hour daily photoperiod. Under the remaining light modalities seed germination was below 10 %. In total darkness germination was null, except for the seeds at 15 °C, which returned a 1% germination rate. Seed germination began after 6 days. The plants that were obtained in the germination essays were transplanted to 12x25 cm black plastic bags. The dry biomass was obtained after 3 and 6

⁽¹⁾ Escola Superior Agrária de Elvas, Rua de Alcamim, nº 19, 7350-903 Elvas, Portugal. opovoa@esaelvas.pt.

⁽²⁾ Departamento de Protecção das Plantas e de Fitoecologia, Secção de Fitoecologia e Herbologia, Instituto Superior de Agronomia, Tapada da Ajuda, P 1349-017 Lisbon, Portugal. anamonteiro@isa.utl.pt.

months, using an average of 30 plants. In the final 3 months the plants increased their dry biomass tenfold; the aerial part increased more than the rest. After 6 months the species presented an average of 4.73 g/plant.

Keywords: Distribution; Seed; Germination; Survival essay; Medicinal plant; *Urtica dioica*.

RESUMO

Estudou-se a possibilidade de produção massiva de *Urtica dioica* L. para fins medicinais, tendo sido realizados ensaios para averiguar as melhores condições de propagação seminal. Com base na informação existente em herbários portugueses efectuou-se também o levantamento da sua distribuição geográfica em Portugal Continental. Os estudos foram realizados com sementes colhidas no Parque Botânico da Tapada da Ajuda, submetidas ou não a pré-tratamentos de quebra de dormência durante 24-hr em imersão numa solução de KNO_3 a 10^{-3} M à temperatura ambiente (20 °C). A germinação decorreu em condições controladas de temperatura constante de 15 °C, 20 °C, 25 °C e 30 °C, bem como às temperaturas alternadas de 15/25 °C e 20/30 °C e em total escuridão ou com um fotoperíodo de 12-hr de luz.

O pré-tratamento das sementes não afectou a germinação. Percentagens de germinação significativamente diferentes foram todavia observados nos vários regimes de temperatura e fotoperíodo, observando-se uma interacção entre estes dois factores. A melhor taxa de germinação (29%) foi obtida com sementes sem pré-tratamento a 20 °C com 12-hr luz. Nas restantes modalidades a germinação foi inferior a 10 %. As escuras a germinação foi nula, excepto a 15 °C, com 1%. A germinação iniciou-se 6 dias após o início do ensaio. As plântulas obtidas nas emergências foram transplantadas para vasos de polietileno com 12 x 25 cm. A produção de biomassa seca em estufa, média de 30 plantas, foi determinada 3 e 6 meses após a transplantação. A biomassa total apresentou um acréscimo de 10 vezes, 3 meses após a transferência para os vasos, com a parte aérea a crescer mais que a parte radicular. A biomassa média era de 4,73 g/planta, 6 meses após a transplantação das plântulas.

Palavras-chave: Semente; Germinação; Sobrevivência; Planta medicinal.

1. Introduction

Urtica dioica (stinging nettle) is a plant from the *Urticaceae* family that is widely distributed in temperate regions, mainly on nitrogen-rich cultivated land and in sunny or semi-shaded places (Bown, 1995).

On the Portuguese mainland it is a common species, albeit rare in the CW and S. It occurs along roadsides or in cultivated, moist or shaded places (Franco, 1971).

It is commonly considered to be a weed, but both humans and animals can also use it as food or for medicinal purposes.

Amongst other things, young leaves, rhizomes and roots possess astringent, diuretic and homeostatic properties that justify its medical use, and the Swiss

Pharmacopoeia recognises the use of the aerial parts of the plant (Conseil Fédéral Suisse, 1995). Young leaves may also be used for food (Delaveau *et al.*, 1983; Bown, 1995).

Urtica dioica is a frost-resistant plant that propagates by seed or rhizome fragments in the spring; flowering plants for medicinal purposes are collected at the beginning of the summer (Bown, 1995).

The best yield results have been obtained with early planting (September) and when plants are harvested at later developmental stages (Stepniak & Szewczuk, 2001).

In the field essays undertaken by Bomme & Unterholzner (1996), leaf production was greater in the second and third years of cultivation; root biomass (8.8 t year⁻¹) was greater when plants were installed as rhizomes; the cultivation methods tested did not affect chemical composition.

Laboratory studies were conducted to evaluate the effects of temperature and gibberelic acid (at 0.005%) on germination. Temperature, which was altered every 24 hrs (4 °C/20-22 °C), exhibited a significant effect on germination. The initial wetting of seeds with a 0.005% gibberelic acid (G.A.) solution at the various temperatures also had a stimulating effect on germination. The best result (94 %) was obtained with the combination of the variable temperature regime and the wetting with the G.A. solution (Szewczuk, 2001).

Urtica dioica seeds germinated irregularly in the first year after harvest, depending on the climatic conditions during seed set and seed ripening. Only those seeds from years with a high rainfall (about 300 mm from June-July) displayed a high germination capacity immediately after harvest (>70%). In dry years, seeds matured after harvest. Seeds stored in an unheated room germinated well over a period of five years (>90%), and even after more than 10 years (with exposure to light). Seeds germinated in the light, on Petri dishes containing blotting paper and at temperatures of 20/30°C (Kozłowski & Szczyglewska, 1995).

2. Material and methods

2.1. Geographic distribution

The species' geographic distribution was determined on the basis of information from bibliographic research and several Herbariums located around mainland Portugal. The following Herbariums were consulted: ISA (LISI), Lisbon University Faculty of Science (LISU), the National Agronomy Station (LISE) in Oeiras, the Plant Improvement Station (ENMP) in Elvas, Coimbra University (COI), the Gonçalo Sampaio Botanical Institute at Oporto University (IBGS-PO) and UTAD in Vila Real (HVR).

The location of each specimen in the herbariums was then attributed to a 10x10 km square UTM. This task was difficult because information was missing from some of the herbarium files, particularly the older ones. The exact location of the sites described in the herbarium files was achieved using 1/100,000 and 1/25,000 scale Army Cartographic Maps and a dictionary of place names and locations (Frazão, 1981). Some of the most difficult herbarium files were treated with personal assistance from Professor João Amaral Franco.

The final step in the production of the distribution maps was to input this information into the interactive software programme for mainland Portugal developed by the Phytoecology and Herbology Section of the Higher Agronomy Institute's (ISA) Plant Protection and Phytoecology Department.

2.2. Seed material

The *Urtica dioica* seeds that were used in the germination essay were collected from the Tapada da Ajuda Botanic Garden in July 1997. They were stored indoors in paper bags at room temperature (± 20 °C) until the germination trials, which began in November of 1997.

2.3. Germination tests

The seeds were submitted to a pre-treatment of 24-hrs immersion in a KNO_3 10^{-3} M solution at room temperature (20°C); a control without pre-treatment was also carried out.

Germination tests were performed in light and temperature-controlled incubations at 15 °C, 20 °C, 25 °C and 30°C, as well as under the alternate 15/25°C and 20/30°C regimes in total darkness and at 12-hr daily photoperiods.

Four replications (100 seeds each) were placed in controlled environmental chambers for each temperature trial. Seeds for each replicate were placed on filter paper in 9-cm diameter Petri dishes to which 8 ml of distilled water was added.

Final germination percentages were determined after 30 days of daily observations. The germination data were subjected to an analysis of variance (STATISTICA Program); the variance was stabilised by angular transformation of the germination percentage. Mean separations were conducted by Newman-Keuler test at the 5% and 1% levels.

2.4. Survival tests and biomass evaluation

The plants obtained in the germination essays were transferred to other Petri dishes and then transplanted to 12x25 cm black plastic bags. The dry biomass was obtained after dissection (80°C for 24 hours, followed by 60 °C for 48 hours), using an average of 30 plants.

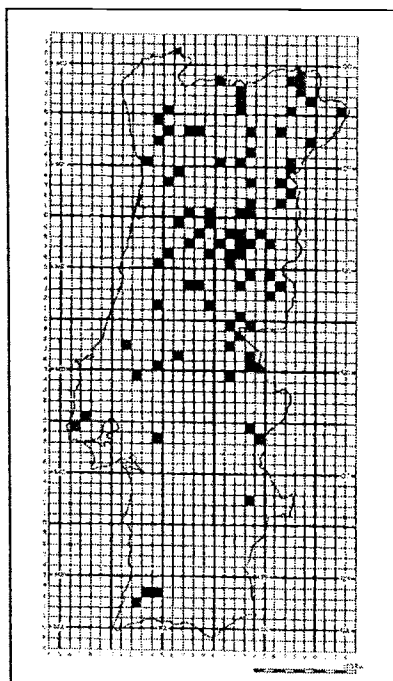
3. Results

3.1. Geographical distribution

Figure 1 shows the geographic distribution of *Urtica dioica* in mainland Portugal. The species appears in most of the study area, but is rarely found south of the River Tagus, except along the Monchique mountain ridge, where it appears frequently.

Figure 1

Geographic distribution of Urtica dioica in mainland Portugal
(Adapted from Póvoa, 1999)



3.2. Germination tests

The results of seed germination using different seed treatment methods did not reveal significant differences ($p < 0.05$). Significantly large differences ($p < 0.01$) were obtained for the different modalities of temperature and photoperiod regime, as well as for the interaction between temperature and photoperiod (Table 1).

The best germination result (29 %) was obtained with the seeds that had not been treated, at 20 °C and with a 12-hour daily photoperiod (Figures 2 and 3). Seed germination began after 6 days; germination time was 11 days for the modality with the best germination result. The germination curves display an exponential form until, on approximately the 5th day following germination, they begin to evolve more gradually.

Table 1

*Analysis of variance output for the effect of seed treatment, temperature and light regimes on the seed germination of *Urtica dioica**

Factor	Germination ⁽¹⁾ (%)
Seed treatment (PRET)	
None	1.0
KNO ₃	0.7
Statistical difference	n.s.
Temperature (TEMP)	
15 °C	2.4
20 °C	6.2
25 °C	0.6
30 °C	0.3
15/25 °C	0.1
20/30 °C	0
Statistical difference	**
Photoperiod (FOT)	
Total darkness	0
12 hours of light	3.4
Statistical difference	**
Interactions	
PRET X TEMP	n.s.
PRET X FOT	n.s.
TEMP X FOT	**
PRET X TEMP X FOT	n.s.

⁽¹⁾ Average without transformation. ns, **, Non-significant, or significant at the 1% level, respectively. Mean separations within columns were performed by Newman-Keuler test at the 5% and 1% levels. Brackets indicate that the factor is involved in significant interactions and that therefore no mean separation was performed for the main effect.

The species barely germinated at 15 °C in total darkness, and germination was null for the rest of the dark modalities (Table 2). With the exception of the modality that produced the best result, germination in the 12-hour daily photoperiod modalities was below 10 %.

Figure 2

*Accumulated germination (%) curves for *Urtica dioica* at the different temperature regimes with a 12-hr daily photoperiod, without seed treatment*

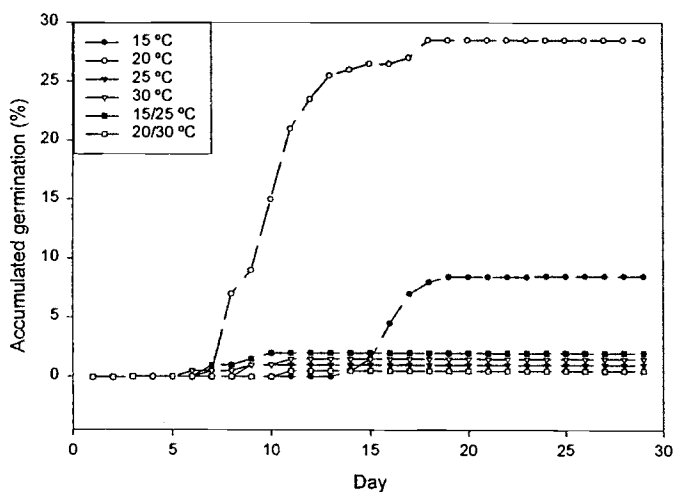
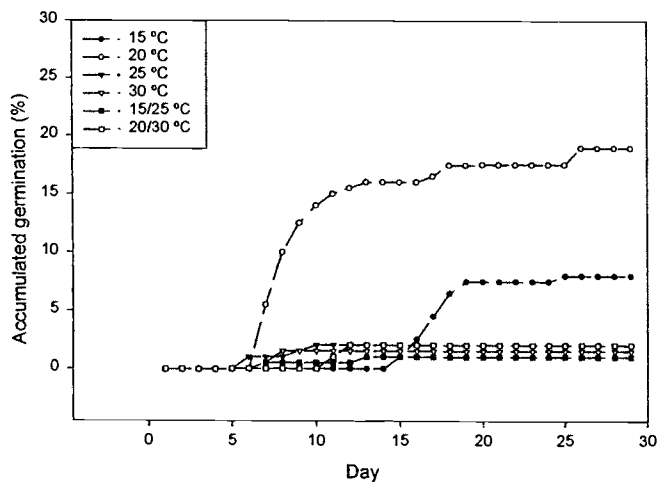


Figure 3

*Accumulated germination (%) curves for *Urtica dioica* at the different temperature regimes with a 12-hr daily photoperiod, with a KNO_3 10^{-3} M solution seed treatment*



The germination rates of the 4 replicates present a high square deviation, with a maximum of 3.3 (Table 2). In previous work by Kozłowski & Szczyglewska (1995) and Szewczuk (2001) the germination rate was over 90%. In both cases this result was obtained with seed that had been stored for at least one year. According to Kozłowski & Szczyglewska (1995), in dry years seeds matured after harvest and the climatic conditions during seed set and seed ripening influenced seed germination.

Table 2

*Germination (%) of *Urtica dioica* seeds, untreated and with a 24-hr wetting in a KNO_3 10^{-3} M solution seed treatment; seeds collected in 1997 from the Tapada da Ajuda Botanic Garden (average of four replicates \pm standard error)*

Temperature regime	Untreated control		24-hr wetting in KNO_3 10^{-3} M	
	12-hr light	24-hr dark	12-hr light	24-hr dark
15 °C	8.5 \pm 3.2	0.50 \pm 0.5	8.0 \pm 2.9	1.0 \pm 0.6
20 °C	28.5 \pm 3.3	0	19.0 \pm 4.4	0
25 °C	1.0 \pm 0.6	0	2.0 \pm 0.8	0
30 °C	1.5 \pm 0.5	0	1.5 \pm 1.5	0
15/25 °C	1.5 \pm 1.0	0	1.0 \pm 0.6	0
20/30 °C	1.0 \pm 1.0	0	1.0 \pm 0.6	0

The germination results obtained in this study display an irregular, low germination rate, which is contrary to the high rate of success (weed) that the species achieves in the wild.

The data from the Tapada da Ajuda Meteorology Station for the period between 1951 and 1980 (ISA, 2003) give a very low average precipitation rate (< 30 mm) for the months between June and August, and 1997 was no exception. The low precipitation rate during this period could justify the low and irregular germination rate in the first year following collection and confirms the results obtained by Kozłowski & Szczyglewska (1995). However, the possibility that these results could be due to the particular seeds that were collected cannot be excluded.

3.3. Survival tests and biomass evaluation

Urtica dioica seedlings obtained in the germination essays were transplanted to plastic bags at the end of December of 1997. The initial establishment of the plants

was successful. The plant's dry biomass (roots and aerial parts) was determined after 3 and 6 months (Table 3).

In the final 3 months the plant increased its dry biomass tenfold; the aerial part increased more than the rest. After 6 months the species presented an average of 4.73 g/plant⁻¹. The square deviation was high, due to high variability between plants. These figures should be considered indicative because the plants were confined in small plastic bags with light and soil competition between plants. At the end of the essay the plants were at the flowering stage.

Table 3

Urtica dioica dry biomass production

	Dry biomass (g plant ⁻¹)	
	3 months	6 months
Root part	0.14	1.36
Aerial part	0.30	2.93
Total	0.44	4.29
Square deviation	0.11	1.02
Confidence interval*]0.25-0.63[]2.57-6.02[

* - P<0.01

In previous field essays by Bomme & Unterholzner (1996) and Stepniak & Szewczuk (2001), the best yield results were obtained with early planting and plants harvested at later developmental stages; leaf production was greater in the 2nd and 3rd years of cultivation and root biomass (8.8 t year⁻¹) was greater when rhizomes were installed.

The yields obtained in this study are not comparable with those in previous published works, because the plants were confined in small plastic bags with high competition between plants.

4. Conclusions

The results indicate that it is possible to successfully propagate *Urtica dioica* L. using seeds that originate in Portugal. The best germination rate (29 %) at 20 °C was obtained with a 12-hr daily photoperiod and without seed treatment to break seed dormancy. The plants survived transplantation into plastic bags very well and grew until the flowering stage.

Studies on this species need to continue and to include both tests of germination rates following different storage times and field trials to evaluate yield. It is also

important to study a wide range of seeds from different geographic locations in mainland Portugal, in order to determine the best origins for the species' cultivation in different soil and climate conditions.

REFERENCES

- BOMME, U.; UNTERHOLZNER, S. (1966) — Most favorable methods for cultivation of stinging nettle. *Gemüse Munchen*, 32 : 233-234.
- BOWN, D. (1995) — *The Royal Horticultural Society Encyclopaedia of Herbs & their Uses*. Dorling Kinderley, London.
- CONSEIL FÉDÉRAL SUISSE (1995) — *Pharmacopeia Helvetica*. 7^e Ed. Département Fédéral de l' Interieur, Berne.
- DELAVEAU, P.; LORRAIN, M.; MORTIER, F.; RIVOLIER, C.; RIVOLIER, J.; SCHWEITZER, R. (1983) — *Segredos e Virtudes das Plantas Medicinais*. Selecções do Reader's Digest, Lisboa.
- ISA (2003) — Summary online treatment of data from the Lisbon/Tapada da Ajuda Meteorology Station for 1951-1980. ISA Agricultural Section. At <http://agricultura.isa.utl.pt/agricultura/agribase/estacoes.asp>, accessed 21st May 2003.
- FRANCO, J.A. (1971) — *Nova Flora de Portugal (Continente e Açores)*, vol.I, *Lycopodiaceae-Umbelliferae*. [s.n.]. Lisbon.
- FRAZÃO, A.C.A. (1981) — *Novo Dicionário Corográfico de Portugal (Continente e Ilhas)*, Reviewed, expanded and updated by A.A. Dinis Cabral, Editorial Domingos Barreira, Oporto.
- KOZŁOWSKI, J.; Szczyglewska, D. (1995) — Biology of germination of medicinal plant seeds. XVII. Seeds of *Urticaceae* family. *Herba Polonica*, 41(4): 178-184.
- PÓVOA, O. L. V. (1999) — *Distribuição e Propagação de Espécies com Interesse Medicinal*. Diss. Curso Mestrado Gestão de Recursos Naturais, Inst. Sup. Agronomia, Univ. Téc. Lisboa.
- STEPNIAK, M.; Szewczuk, C. (2001) — Yielding of stinging nettle (*Urtica dioica* L.) depending upon time of rhizome planting and development stage of harvested plants. *Annales Universitatis Mariae Curie Skłodowska. Sectio EEE, Horticultura*. 2001, 9: Supplementum, 121-128.
- SZEWCZUK, C. (2001) — Effect of variable temperature and solution of gibberellic acid on the germination of stinging nettle seeds (*Urtica dioica* L.). *Annales Universitatis Mariae Curie Skłodowska. Sectio EEE, Horticultura*, 9: Supplementum, 333-337.
- URBANCZYK, J.; E. Hanczakowska; M. Swiatkiewicz (2002) — Herb mixture as an antibiotic substitute in pig feeding. *Medycyna-Weterynaryjna*, 58(11): 887-889.