PRESENTAZIONE

Il fascicolo 2000/2 di "Comunicazioni di ricerca" edito dall'Istituto Sperimentale per l'Assestamento Forestale e per l'Alpicoltura di Trento, contiene 4 pubblicazioni riguardanti la maggiorana (*Origanum majorana* L.).

Le prove sperimentali descritte nei successivi articoli sono state eseguite nell'ambito del progetto CE FAIR 3 CT 96 1914 intitolato: *Origanum sp. and Salvia sp.: Integrated breeding research to improve homogeneity and quality of multifunctional secondary products.*

Gli obbiettivi generali del progetto, iniziato nel febbraio del 1997 e che terminerà a maggio del 2000, sono i seguenti: a) migliorare e rendere più omogenea la qualità della maggiorana (*Origanum majorana* L.), dell'origano (*Origanum sp*.) e di due specie di salvie (*Salvia officinalis* L. e *Salvia fruticosa* Miller); b) saggiare le potenzialità produttive in seme della maggiorana in ambienti non tradizionali; c) valutare la variabilità genetica di maggiorana, origano e salvia relativamente alle loro proprietà antimicrobiche ed antiossidanti.

A questo progetto hanno partecipato istituti di ricerca e ditte sementiere e di trasformazione di sei paesi europei: Institut für Angewante Botanik der Veterinärmedizinischen Universität di Vienna per l'Austria (Coordinatore); ditta DARBONNE-DAREGAL di Milly la Forêt per la Francia; il Bundesanstalt fuer Zuechtungsforschung an Kulturpflanzen di Quedlinburg e le ditte Chrestensen di Erfurt e MAWEA di Aschersleben per la Germania; lo Scottish Agricultural College di Auchincruive per la Gran Bretagna; il Mediterranean Agronomic Institute di Chania per la Grecia ed il Dipartimento di Agronomia della Facoltà di Agraria di Bari e l'ISAFA di Villazzano-Trento per l'Italia.

Il primo lavoro pubblicato nel presente numero descrive la maggiorana dal punto di vista botanico e fornisce informazioni sulle caratteristiche qualitative e sugli impieghi dei prodotti che da questa pianta si possono ricavare (droga ed olio), nonché sull'importanza economica della specie.

Il secondo ed il terzo lavoro illustrano due prove sperimentali eseguite nel Trentino e finalizzate alla valutazione delle potenzialità produttive della maggiorana in questo ambiente, relativamente alla produzione di droga (foglie + infiorescenze), di olio e di seme.

Il quarto lavoro, scritto dal prof. Thanos della Facoltà di Biologia di Atene che in collaborazione con il MAICH di Creta ha condotto le ricerche sulla fisiologia del seme, si riferisce a prove eseguite su campioni di seme di maggiorana prodotti in Trentino.

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(Responsabile del gruppo di
ricerca sulle piante officinali)

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*Costas A. Thanos**

PHYSIOLOGY OF SEED GERMINATION IN MARJORAM (*Origanum majorana* L.)

Fisiologia della germinazione del seme di maggiorana (Origanum majorana *L.*)

Parole chiave: *Origanum majorana*, fisiologia del seme, germinazione
Key words: Origanum majorana, *seed physiology, germination capacity*

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Introduction

Marjoram (*Origanum majorana* L.), an important aromatic and medicinal plant, is relatively largely cultivated (particularly in the central European region) but it faces a serious availability problem of multiplication material ('seeds', i.e. nutlets). The origin of the species is placed in Western Asia and marjoram seems to be adapted to relatively warm and dry conditions; this fact may account for the inability of the species to complete its reproductive cycle (and produce sound seeds) under the cool autumn temperatures of the central European zone. To tackle this problem, and within the context of a wider project financed by the European Union (FAIR3 CT96 1914), we investigate the germinability of seeds from various marjoram cultivars grown under various cultivation regimes in the European south (N. and S. Italy).

Although marjoram is a plant well-known in the Mediterranean rim (*amarakos* or *amarakon* in Ancient Greek; THEOPHRASTUS, *HP* 1.9.4., 6.1.1., 6.7.4., 6.8.3., 9.7.3.) only very little has been done on its germination (ELLIS *et al.* 1986). THEOPHRASTUS (*HP* 6.7.4.) notes that *'marjoram grows in either way, from pieces torn off or from seed; it produces a quantity of seed, which is fragrant with a delicate scent; it can also be transplanted'*. A few, relatively marginal works refer to seed germination of marjoram itself (CSERESNYES & BALEANU 1978, MENGHINI & VENANZI 1978) while several others have dealt with related species of the genus *Origanum* (e.g. HEIT 1948, KADIS 1995, KADIS & GEORGHIOU 1993, KRETSCHMER 1989, MACCHIA *et al.* 1983, PONS 1991a & 1991b, PUTIEVSKY 1983, THANOS & DOUSSI 1995, THANOS *et al.* 1995).

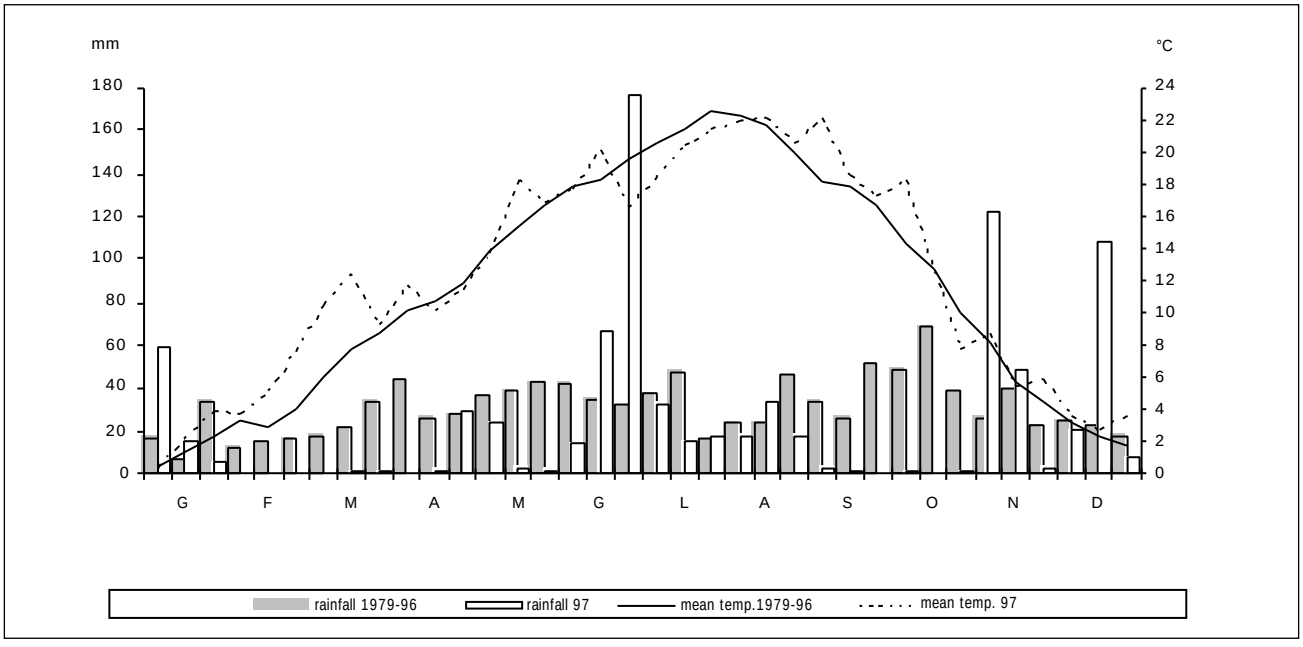
Materials and Methods

Tests were performed with 16 seedlots of marjoram, *Origanum majorana* L., furnished by the ISAFA-Trento, Italy in the spring 1998, after a period of 6 (reproduced seed) or 18-months (original seed) long storage at room conditions. The 16 accessions belong to 4 'cultivars': 'Aegyptischer' (=Egizia), 'Erfo', 'Marcelka' and 'SAIS'. The original seed (A0, E0, M0 and S0, respectively) were sown in Villazzano in spring 1997 and their progeny were harvested at three dates, Sep. 8, Oct. 8 and Nov. 18, 1997. The nutlets thus collected are hereafter coded as A1, A2, A3, E1, E2 etc (where 1, 2 and 3 correspond to each of the harvesting dates).

Average precipitation at Villazzano during the cultivation season (April to October) seems to be distributed quite evenly (as shown by the historical data in the Figure 1). The monthly average is around 100 mm throughout April to September while October is considerable rainier (*ca.* 150 mm). Nevertheless, the actual precipitation during the cultivation period April – October 1997 was markedly different from the average pattern: June 1997 was considerably (almost 3-fold) rainier than average while all the other months were more or less drier (September and October almost totally dry). On the other hand, air temperature during 1997 followed very closely the average trend of 1979-96.

After an initial cleaning involving removal of chaff, marjoram nutlets were placed in light- and water-proof plastic tins (film boxes) and stored at room temperature (20 ± 5 °C). Measurements of average nutlet weights were carried out with an electronic balance

Figure 1 - Ten days data of the rainfall and of the mean temperature recorded in the period 1979/96 and in 1997 (Villazzano, Trento)



(with 4 decimal digits of accuracy, 0.1 mg) on 5 samples of 100 nutlets each (due to the tiny size of the nutlets) from each of the 16 accessions.

Germination tests were performed in glass Petri dishes (7 cm in diameter) lined with two filter paper discs and moistened with 3 mL of distilled water. The criterion of germination was visible radicle protrusion and, after each count, germinated nutlets (hereafter called seeds for simplicity) were discarded. The tests were terminated when no additional seeds germinated. All manipulations of imbibed seeds were carried out under a dim, green safelight. Unless otherwise indicated, each value is the mean of five replicates of 50 seeds ± standard error (SE); in preliminary tests. T_{50} is the time needed for 50% of the final germination value and is calculated by linear interpolation from the two germination values closest to median germination.

For germination experiments in darkness, Petri dishes were incubated within light-proof, metal containers in controlled temperature cabinets (Model BK 5060 EL, W.C. Heraeus GmbH, Germany) where temperature was kept constant within ± 0.5 °C of the value set. Seed germination experiments under various light regimes were performed in temperature and light-programmable growth benches (model GB48, Conviron, Controlled Environments, Winnipeg, Canada) equipped with a lamp canopy of 48 incandescent bulbs (Sylvania 50A19, 50 W, 277 V) and 10 fluorescent tubes (Sylvania Cool White FR96T12/CW/VHO-235/1).

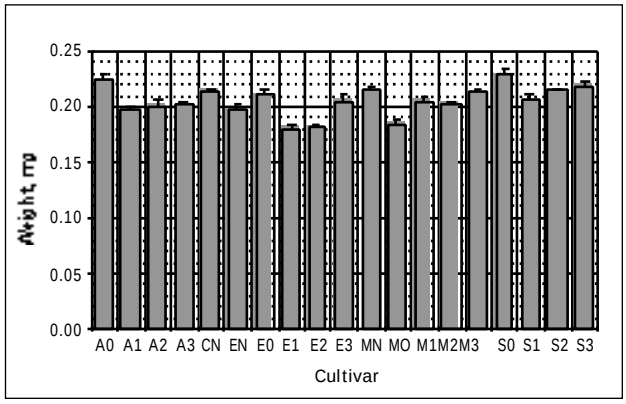
In order to simulate the natural conditions encountered during springtime (May in particular), temperature and light programs were automatically changed according to the average climatic data (period 1955-1987) of a representative meteorological station (Ellinikon Airport, Athens). Thus, temperature and light programs were changed every week while temperature was programmed to change every 3 h and lights were turned on 30 min before official sunrise and off 30 min after official sunset, while both the quality and quantity of light were automatically adjusted several times during the 'day'. Average photoperiod was 13 h while the daily temperature fluctuation was 14-21 °C.

Photon flux densities were measured with a spectroradiometer (Licor 1800, LICOR, USA); calculations of flux densities and values of ζ and ϕ were made with a previously described software application. White light (W) had a value of ζ (660/730 nm photon ratio) equal to 1.119, while the phytochrome photostationary state ratio (ϕ =[Pfr]/[Ptot]) was 0.641, and the total photon flux density at the visible range (400-800 nm) was 103.0 ζmol m^{-2} s^{-1}. When this light was filtered through three sheets of Plexiglas filters (two blue, 527, and one red, 601, 3-mm thick each; Röhm GmbH, Darmstadt, Germany) a 'canopy simulating', far-red-rich light (FR) was obtained. In this case, the values of ζ, ϕ and total flux density (ζmol m^{-2} s^{-1}) were 0.001, 0.062 and 13.6, respectively.

Results and Discussion

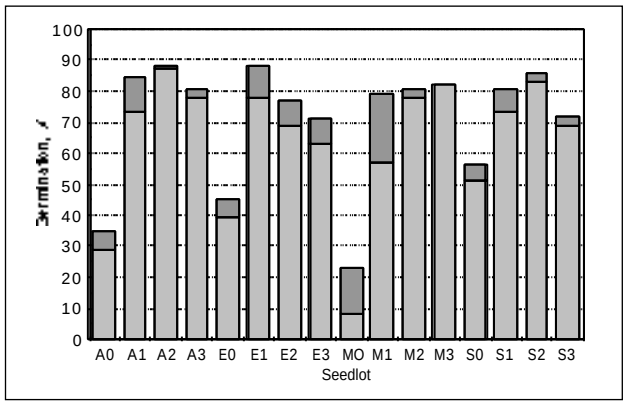
Nutlet weights (Figure 2) did differ among various 'cultivars' and harvest dates but the overall variability might be considered as non-significant. The actual average values ranged between 0.18 and 0.23 mg with a grand mean equal to 0.206 mg and an overall variation *ca.* 10% ± of this mean.

Figure 2. Average air-dry weights of marjoram nutlets. Each bar represents 5 samples of 100 nutlets from each of the 16 seedlots. A, E, M and S correspond to cultivars "Aegyptischer", "ERFO", "Marcelka" and "SAIS", respectively, while 0 refers to initial seeds (1996 harvest) and 1, 2 and 3 those produced in 1997 and harvested on Sep. 8, Oct. 8 and Nov. 18, 1997, respectively. Vertical lines over bars represent standard errors (SE).



Preliminary germination tests with 100 nutlets from each of the 16 accessions clearly showed satisfactory final levels only for the 12 seedlots produced in 1997 (Figure 3). The initial seedlots failed to germinate significantly and judging from the external morphology of the seeds they were probably deteriorated and/or non-viable. Final germinability after 7 days at

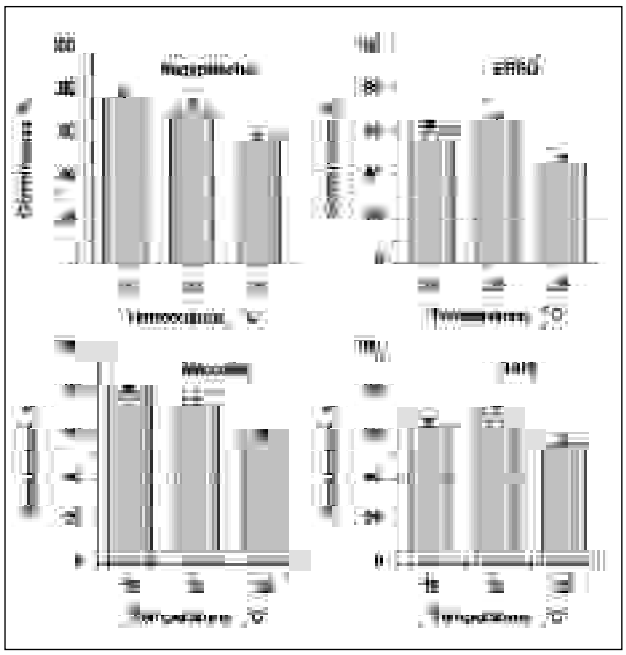
Figure 3. Preliminary germination for each of the 16 marjoram seedlots (codes as in Fig. 1). 100 nutlets per seedlot were imbibed in a single Petri dish for 7 days at 20 °C in the dark (lower part of each bar in light gray) and afterwards transferred for an additional period of 9 days at laboratory conditions, under alternating white light and darkness and 20 ± 5 °C (upper part of each bar in dark gray).



20 °C in the dark varied between a minimum of 57% and a maximum of 87% while an additional 9-day-long period at laboratory conditions (fluctuating temperatures and light during the day) slightly improved the final levels (min 71%, max 88%). The overall germination mean of all 12 seedlots was 74% after 7 days in the dark at 20 °C while it was slightly increased to 81% after the additional period at laboratory conditions.

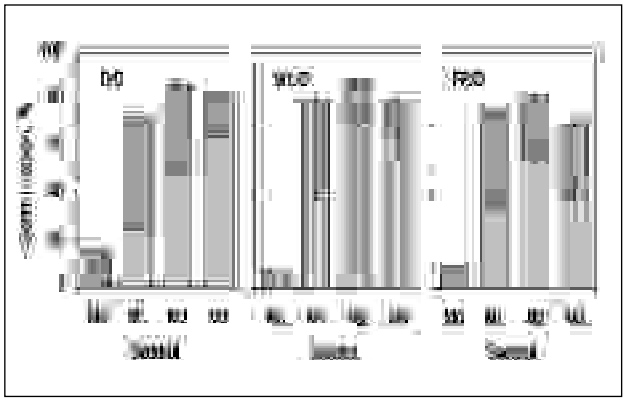
An assessment of the germination levels from the previous preliminary experiment did not reveal any significant differences among the cultivars or among the 3 harvest dates. Nevertheless, as far as final levels and germination rates were concerned, the 2nd harvest seedlots showed a higher consistency as a group than the other 2 ones. Therefore, it was decided to use these seedlots for further experimentation. Figure 4 shows dark germinability as a function of the constant temperatures 15, 20 and 25 °C. It is clearly shown that the former 2 were almost equally optimal for germination (overall means 71.0 and 70.3%, respectively) while at 25 °C a decrease was obtained (55.2%).

Figure 4. Final germination in the dark (after 7 days) as a function of constant temperatures for each of the 4 marjoram seedlots of the 2nd harvest (Oct. 8, 1997). Vertical lines over bars represent standard errors (SE).



The last experiment aimed to investigate the possible intervention of light to germination, as it has been previously found in other *Origanum* species (*Origanum vulgare* PONS 1991; *O. vulgare* ssp. *hirtum* THANOS *et al.* 1995; *O. cordifolium* KADIS 1995; *O. dictamnus* THANOS & DOUSSI 1995). Figure 5 presents germination data after 2 and 7 days at springtime-simulated conditions and under 3 contrasting regimes for Marcelka, the unique cv tested: darkness throughout (D/D), white light during the day (WL/D) and far-red light (simulating canopy-filtered radiation) during the day (FR/D). Apart from the poorly germinating old seeds (M0), no clear effect of light on Marcelka marjoram germination can be deduced from Figure 5. However a very slight decrease on the final germination level was obtained under FR/D (76.1% from all 3 seedlots as compared with 80.8 and 82.7% for D/D and WL/D, respectively). Moreover, the rate (speed) of germination was significantly increased under WL/D as shown by the 2-day germination levels (58.5% compared with 45.9 and 43.7% under D/D and FR/D, respectively). Therefore, it is quite straightforward that under optimal germination conditions, the effect of light is very marginal (if any) but, at suboptimal temperatures, the possibility of a White Light promotion of germination rate and a Far-Red suppression of germinability cannot be excluded. Thus further experimentation on the effect of light has to include a diverse array of temperature regimes and clearly more 'cultivars' and seedlots.

Figure 5. Nutlet germination of the Marcelka marjoram seedlots (codes as in Fig. 1) as a function of the light regime. Germination took place under springtime-simulating conditions (alternating white light and darkness, 13/11 h; daily temperature fluctuation 14-21 °C) and it was assessed at 2 (lower part of each bar in light gray) and 7 days (upper part of each bar in dark gray) after the onset of imbibition. Vertical lines over bars represent standard errors (SE).



Finally, two additional conclusions can be drawn from the data presented in Figure 5. The germination rate of marjoram nutlets seems to be surprisingly fast (at least in comparison to other *Origanum* species; e.g. THANOS *et al.* 1995, THANOS & DOUSSI 1995). The T_{50} value (time needed for 50% of the final germination level) is less than 2 days. In regard to the variability among the seedlots harvested at different dates, a slightly higher germination level was shown by the seeds of the 2nd harvest (85.7% from all 3 light regimes as compared with 75.9 and 78.0% from the 1st and 3rd harvests, respectively). Moreover, the germination rate of the seeds from the 1st harvest was significantly lower (34.5% at 2 days as compared with the corresponding 58.7 and 54.9% of the 2nd and 3rd harvests).

Conclusions

1. The average seed (nutlet) weight was *ca* 0.20 mg and did not differ significantly among the 16 seedlots studied (0.18 – 0.23 mg).

2. The germinability of all the 12 seedlots produced in 1997 at Villazzano (4 'cultivar-types' by 3 consecutive harvests) was sufficiently good (70-90%).

3. On the contrary, germinability (viability) of the 4 initial seedlots was very poor (due perhaps to unknown but obviously detrimental storage conditions).

4. Only slight germination differences were observed among seeds of the three harvest dates. On the basis of these (slight) differences as well as nutlet maturity and cultivation convenience, the 2nd harvest seems preferable.

5. The study of the temperature dependence of germination (in the range 15-25 °C) showed an optimum at both 15 and 20 °C while at 25 °C a certain (but not dramatic) decrease occurred.

6. The rate of germination is particularly fast and 50% of the seeds can germinate (at optimal conditions) within 2 days while germination is complete within a week.

7. The role of light seems rather minor (if any); germination was carried out in continuous darkness or under white or far-red light.

Acknowledgements

This work is part of a European Union funded project (FAIR3 CT96 1914) titled "*Origanum* sp. and *Salvia* sp.: Integrated breeding research to improve homogeneity and quality of multifunctional secondary plant products" coordinated by Prof. Dr. Chlodwig Franz, University of Veterinary Medicine, Wien, Austria. The author is also thankful for the collaboration of Ms Melpo Skoula-Johnson (MAICh, Chania, Greece) and Dr Carla Vender (ISAFA, Villazzano, Italy).

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SUMMARY

The tests were performed with 16 seedlots of marjoram, *Origanum majorana* L., belonging to 4 cultivars (Aegyptischer, Erfo, Marcelka and SAIS), stored at room conditions after a period of 6 (reproduced seed) or 18-months (original seed) long storage. The original seed were sown in Villazzano (Trento) in spring 1997 and their progeny were harvested at three dates, September, October and November 1997.

The average seed (nutlet) weight was *ca* 0.20 mg and did not differ significantly among the 16 seedlots studied (0.18 – 0.23 mg).

The germinability of all the 12 seedlots produced in 1997 at Villazzano was sufficiently good (70-90%). On the contrary, germinability (viability) of the 4 initial seedlots was very poor (due perhaps to unknown but obviously detrimental storage conditions).

Only slight germination differences were observed among seeds of the three harvest dates, but the 2nd harvest (October) seems preferable.

The study of the temperature dependence of germination (in the range 15-25 °C) showed an optimum at both 15 and 20 °C while at 25 °C a certain (but not dramatic) decrease occurred.

The role of light seems rather minor.

RIASSUNTO

Le prove sono state eseguite su 16 lotti di seme di 4 cultivar di maggiorana, *Origanum majorana* L. (Aegyptischer, Erfo, Marcelka e SAIS), conservati a temperatura ambiente rispettivamente per 6 (seme riprodotto) e 18 mesi (seme originale). Il seme era stato seminato a Villazzano (Trento) nella primavera del 1997 e la sua progenie era stata raccolta in tre date successive (settembre, ottobre e novembre).

Il peso medio di un seme è oscillato da 0,18 a 0,23 mg (media 0,2 mg) e non sono emerse differenze significative fra i lotti.

La germinabilità dei 12 lotti riprodotti nel '97 è stata abbastanza buona (70-90 %); al contrario quella del seme originale è stata molto bassa, probabilmente a causa delle non idonee condizioni di conservazione.

Fra le epoche di raccolta, sono emerse soltanto piccole differenze e la raccolta di ottobre sembra preferibile.

Le temperature adottate (da 15 a 25 °C) hanno dimostrato che l'optimum si verifica fra 15 e 20 °C mentre temperature superiori (25 °C) sono negative.

Il ruolo della luce è apparso secondario.