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ORIGINAL ARTICLE

Population decrease of *Thymelaea hirsuta* (L.) Endl. in Liguria: conservation problems for the North Tyrrhenian sea

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Abstract

This study is focused on the decrease of *T. hirsuta* populations along the North Tyrrhenian Coast, on the northern edge of the species areal, and on the necessity to preserve this species within this area. The drastic drop in population density is prejudicing the survival of this species along the North Tyrrhenian Coast, which features high mortality rates among adult plants and seedlings. In the Ligurian station of Bergeggi, a drop in population was noticed after 3 years of observation, with an average of 49.18%. At the end of the study period all specimens appeared to have a smaller number of branches while the total volume of plants dropped by 71.91%. Fruit production varied seasonally, though in general low rates of ripe fruit and fruit germinability (0.28%) were recorded. *Ex situ* seeds showed low rates of germination too. Reproduction by cuttings yielded very poor results and *in vitro* micropropagation was unsuccessful. In defining possible conservation strategies for the future of the plant in this area, sexual breeding was found to be the only way to regenerate the natural population, while vegetative reproduction seemed to be difficult to implement.

Key words: *Conservation activities, population decrease,* Thymelaea hirsuta.

Introduction

Thymelaea hirsuta (L.) Endl. is a perennial evergreen woody shrub with a circum-Mediterranean distribution. Phenological observations in different geographical zones of its distribution area highlighted different classes of sex with a clearly consistent tendency to become a dioecious species (Dommée et al., 1990, 1995; Shaltout & El-Keblawy, 1992; Ramadan et al., 1994; El-Keblawy et al., 1995, 1996a; El-Keblawy & Freeman, 1999).

This plant is very abundant in the central and southern parts of its areal. Elsewhere, in contrast, it appears to be particularly sensitive to environmental modifications, resulting in a reduction in populations and specimens.

T. hirsuta clearly exhibits a population mortality everywhere along the Mediterranean coast and is significantly affected by habitat and size-class. Moreover, mortality rates among flowering individuals vary from place to place depending on plant interaction and relationship with the natural vegetation (El-Keblawy et al., 1997).

Previous studies highlighted an extremely low presence of this species along the Ligurian Coast (Minuto et al., 1995; Minuto & Casazza, 2001). Recent herbaria and in-the-field investigations, enlarged to the North Tyrrhenian sea (600 km of coastline), reported a similar demographic behaviour. The 11 populations of the species [Cape d'Antibes, Antibes and Nice (Department des Alpes Maritimes – France); Capo Noli, Bergeggi, Portofino (Liguria, Italy); Donoratico, Piombino, Bocca di Coria, Castiglione della Pescaia, Talamone (Tuscany – Italy)], recorded at the beginning of the 20th Century, were reduced to three sites: Antibes, Bergeggi and Talamone. Such sites also featured a low number of specimens and a very low number of young plants and plantlets (Minuto & Casazza, unpublished data).

A similar population decrease of *T. hirsuta* is to be found all over its areal, both for adults and seedlings, which is highly dependent upon habitats. In general, increasing aridity is associated with greater mortality rates among plants of all sizes (El-Keblawy et al., 1997).

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Moreover, in experimental conditions, seedling survival rates seem to be much higher than in nature (El-Keblawy et al., 1997). Although *ex situ* conditions have been indicated as better than in nature for the growth of new seedlings, seed germination rates are still quite low (Dommée et al., 1995; El-Keblawy et al., 1996b).

To date, *ex situ* propagation with cuttings has mostly proved unsuccessful. Many attempts were undertaken in Greece and Italy, however no results were obtained (Papafotiou et al., 1991; Grassotti, 1999).

Starting from our experience on micropropagation of threatened Ligurian endemic species (Casazza et
4 al., 2002; Savona et al., in press), we decided to implement the a.m. conservation techniques on *T. hirsuta*. The use of micropropagation techniques in the conservation of endangered species has proved useful for a wide range of species (Martinez & Rubluo, 1989; Fay & May, 1990; Iriondo & Pérez, 1990; Sharma et al., 1991; Martin & Pérez, 1992, 1995; Lledó et al., 1993), producing a large stock of plants from a minimum of original plant material, with a low impact on the endangered native population.

Our study pin-pointed the population survey of the relict station in Bergeggi, which is near the extreme northern border of the area. In choosing this marginal position, we had several different aims: (a) study and investigate the demographic development of the population; (b) investigate population regeneration and its ability to produce new generations; (c) attempt *ex situ* multiplication by sowing seeds, growing cuttings and *in vitro* micropropagation; and (d) define general conservation policies for the species.

Material and methods

Study species

The twigs of *T. hirsuta* bear many inflorescences in the form of small capitula, each having 3–15 yellow flowers with a variable diameter. In the early pilose primordium stage all the flowers are bisexual, with a superior gynoecium and eight stamens attached to a perianth formed by four sepals. The future of these primordia then varies depending on the sexual development of the individual. In female flowers, the perianth is small (2–2.5 mm dia) and greenish-yellow while the gynoecium measures 1.8 × 1.5 mm and consists of a single carpel containing a single ovule. Male flowers (3.5–4 mm dia) have a larger yellow perianth, with two cycles of four stamens each joined to the perianth. Hermaphrodite flowers have a perianth and an androecium similar to that of male flowers, but a smaller gynoecium (1 × 0.8 mm). Both female and perfect flowers can set an indehiscent fruit (3.8 × 2.5 mm) containing only one seed (Dommée et al., 1990).

The flowering season lasts from June to April, and is characterized by two high production periods: one in September/October, the other in February/April
(Minuto et al., in revision). 3

Sex expression in *T. hirsuta* is complex and labile (Ramadan et al., 1994; El-Keblawy et al., 1995). In one of our previous studies, we monitored gender phenotypes held by the species in the study area over
a period of 2 years (Minuto et al., in revision). Two 3
main categories were defined: plants that were completely stable in their sex expression (totally female or male), and plants that were sexually labile to different degrees (protandrous, protogynous, others). These results were similar to those of other studies carried out on Egyptian and French populations of *T. hirsuta* (Dommée et al., 1990; El-Keblawy et al., 1995).

At least three different gender phenotypes are consistently able to produce seeds: female, protandrous and protogynous (El-Keblawy et al., 1995;
Minuto et al., in revision). 3

Study area and natural populations

The studied localities were Bergeggi (Savona – Liguria), Antibes (Nice – Dept des Alpes Maritimes) and Talamone (Grosseto – Tuscany) (Figure 1), in order to understand demographic behaviour in the northernmost population along the Tyrrhenian coast.

The population of Bergeggi was distributed over quite a small area (1500 m^2) and was formed by 61 (now 31) specimens; the other two populations in Talamone and Antibes both had a smaller area of 600 m^2, with 38 (now 25) and 63 (now 40) specimens respectively.

Geographical, geological and climatic data of the three localities are reported in Table I.

The sites have a Mediterranean climate and the vegetation recorded was always typically Mediterranean evergreen maquis.

The three sites are subject to a moderate degree of disturbance, mainly due to seagull reproduction and human activities (harbours, fishing and tourism).

The pH values of all soil types involved were calculated. Percentage of $CaCO_3$ and the presence of other chemical, physical and organic elements were not calculated, according to their independence from *T. hirsuta* activity, as suggested by some authors (Ramadan et al., 1994; El Keblawy et al., 1997).

Demography

From August 2000 to August 2003, three permanent plots were established on three sites. Transects were

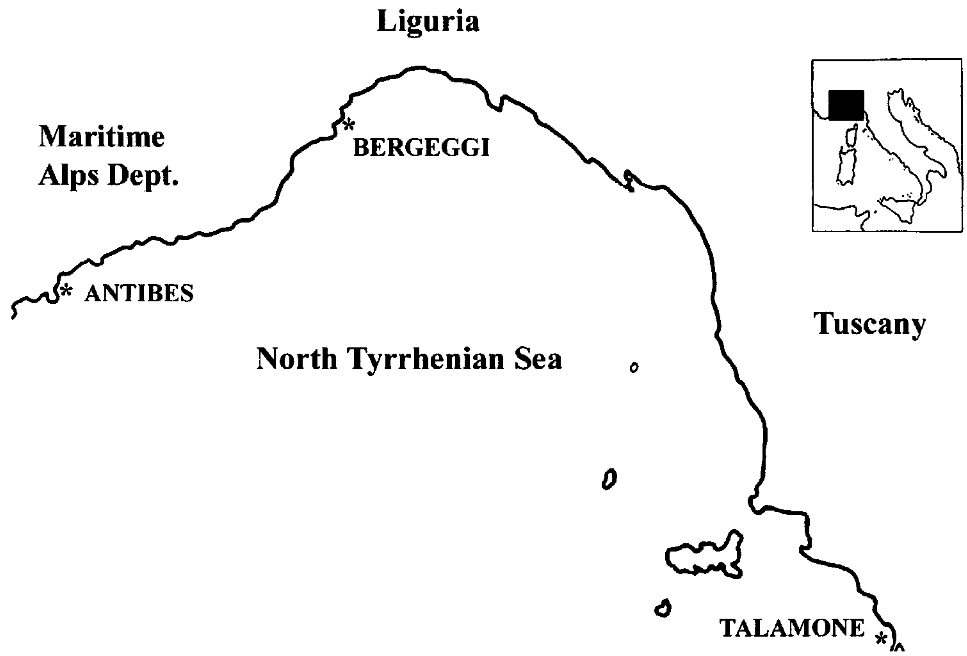

Figure 1. Geographical distribution of *T. hirsuta* populations in the North Tyrrhenian Sea.

Table I. Geographical and ecological elements of the three study sites: Bergeggi (Liguria – Italy), Antibes (Dep. Des Alpes Maritimes) and Talamone (Toscana – Italy).

	GPS	Geol.substrate	Ann.mean Min. (°C)	Ann.mean Max. (°C)	Mean annual rainfall (mm)	Vegetation
Bergeggi	44°15′N 8°27′E	dolomite	7.1	23.9	1068	maquis
Antibes	43°34′N 7°08′E	calcareous	11.5	18.6	994	maquis
Talamone	42°33′N 11°08′E	calcareous	7.1	23.1	667	maquis

large enough to cover the entire area population, with a plot size ranging from 200 to 500 m^2. Maps were compiled showing the spatial distribution of *T. hirsuta* individuals in each plot and individual plants were permanently marked with small flags. Periodical visits enabled us to record the number of dead/living plants, the number of fruits borne and the presence of new seedlings.

The demography of the plotted populations was calculated on a monthly basis in Bergeggi, in order to individuate mortality periods, and once a year on the two other sites. The results of the demographic observation from Liguria were compared with their logarithmic tendency.

Further investigations on fruit production, canopy diameter and volume were only attained on Bergeggi population.

Fruit production was calculated on site every month, through careful examination of the whole plant and precise counts of ripe fruits.

Each shrub's canopy diameter and height were measured and its crown volume (interpreted as a cylinder) was calculated at the beginning and at the end of the study. The size frequency of individuals was classified according to six crown-diameter classes (I-VI) and three canopy volume classes (I-III) as suggested by Ramadan et al. (1994). Such data had been useful when calculating percental variation in crown diameter, the variation of crown and volume classes and population percental vegetative growth rate (Ramadan et al., 1994; El-Keblawy et al., 1997).

Ex situ multiplication

Even though *ex situ* seed germination and *in vivo* propagation had already been tested in the past by a number of authors (Papafotiou et al., 1991; Dommée et al., 1995; El Keblawy et al., 1996; Grassotti, 1999), we decided to attempt the same techniques for further testing purposes and to add micropropagation owing to the high number of successes scored by this particular methodology.

Seed germination rates (850 seeds in total, from the three sites) were tested under experimental conditions, according to literature (Shaltout & El-

Shourbagy, 1989): a 10 min immersion treatment in 95% H_2SO_4 (acid scarification) was applied. After removal from the acid, the seeds were washed thoroughly in water, sown in Petri dishes with 500 mg l^{-1} of gibberellic acid and kept in the dark at 15°C for 24 h. The seeds were then transferred to Petri dishes with absorbent paper filled with water and kept at 15°C in the dark. Seedling emergence was recorded every 3 days for 3 months and germination percentages calculated. Germinated seeds were transplanted and cultured *in vitro* on a semi-solid Murashige and Skoog (1962) medium. Mortality was recorded weekly, and survival was calculated as the number of seedlings present at the end of the month as a percentage of the total number of seedlings that had germinated.

Plant material for cuttings was harvested in April, at the beginning of the period of greatest vegetative activity of the species, as indicated in the literature (Dommée et al., 1990). Several stems were cut from mother plants. Segments10 – 15 cm long (with several apical buds) were planted in three different pot sets with different degrees of moisture retention. The first set was placed in plastic pots, filled with 25% gravelly clay and 75% basic soil (60% beech leaf soil, 30% heather soil, 10% cut conifer leaves). The second set was placed inside identical plastic pots filled with 100% heather soil. The third set was placed into crocks, filled with the same types of soils described above. All pots were kept in a growth chamber at a temperature of 24 ± 2°C with 12/12 h light/darkness cycles. To maintain a high degree of humidity of the cultures, the pots were covered with clear plastic bags for 3 weeks. The plastic covers were then gradually removed to reduce the humidity of the pots and to adapt the cuttings to greenhouse conditions. After being kept for a further 2 weeks in greenhouse conditions, the pots were moved outside to enable complete adaptation to the natural climate. Survival rates were then calculated.

The plant material for *in vitro* cultivation was harvested in October, January, April and July and several stems were cut from mother plants. Segments 20 – 30 mm long (with apical buds) were excised and sterilized with the following procedure: 3 min in 95% alcohol; 15 min in soap and water; a first rinse in sterile distilled water; 15 min in soap and water again; a second rinse in water; 10 min in commercial 5% sodium hypoclorite solution at 1:1 concentrations. After a final 10 min rinse, the explants were placed in the culture medium.

The sterilized explants were cultured *in vitro* on a semi-solid Murashige and Skoog (1962) (MS) medium to which different concentrations of various hormones were added: 6-benzylaminopurine (BA) (0.02; 0.05; 2; 4 mg l^{-1}), kinetine (K) (4 mg l^{-1}), 2,4 dichlorophenoxyacetic acid (2,4D) (2 mg l^{-1}), kinetin, naftalenacetic acid, 2,4 dichlorophenoxyacetic acid (KND) (2:2:2 mg l^{-1}) and 6-bemzylaminopurine, 2,4 dichlorophenoxyacetic acid (BAD) (2:0.1 mg l^{-1}) at pH 5.5; K (4 mg l^{-1}), gibberellic acid (Ga_3) (4 mg l^{-1}), 2,4D (4 mg l^{-1}) at pH 6.8. One set of explants was cultured *in vitro* on a Hoagland's (Hoagland & Arnon, 1938) medium (pH 6.8) hormone free. All media were solidified with 0.8% agar. Cultures were kept in a growth room at 25 ± 1°C and exposed to 2000 lux of light by means of daylight fluorescent tubes with 12/12 h light/darkness cycles.

Results

Demography

As reported in Table II, a population decrement was recorded throughout the study area with a mean percentage value of 40%. In particular, an increasing mortality value recorded according to the latitude of the sites: mortality decreased from the extreme northern margin towards the southern one.

In Bergeggi, a drop in density was noticed over 3 years of observation: from 61 specimens in August 2000 to 31 specimens in August 2003 (Figure 2). The population experienced a high mortality rate, with an average rate of 49.18%. The periods of death were at the end of summertime, probably following distress caused by the hottest and driest period of the year. As indicated in Table III the highest mortality was recorded among individuals belonging to the diameter classes I, II and VI, i.e. individuals with either a small or a very large crown.

According to canopy volume, classes I and III showed an important decrease in specimens. The high mortality value scored by size III class was due to the death of 1 protandrous and 2 female plants – the highest producers of the population (69,067 flowers – 61% of the whole population production).

The frequency distribution of individuals of each of the five sex forms is given in Table IV. In 2000, female (4 samples from II and III size classes) and protandrous (3 samples from II and III size classes) shrubs attained greater overall canopy volumes than

Table II. Population survey of flowering individuals from the three study populations over 3 years of observations (2000–2003). The global numeric (D n°) and percentage (D %) decrement of individual in each site is reported.

	Aug 2000	Aug 2001	Aug 2002	Aug 2003	D n°	D %
Bergeggi	61	50	40	31	27	49.18%
Antibes	63	54	47	40	23	36.51%
Talamone	38	31	28	25	13	34.21%

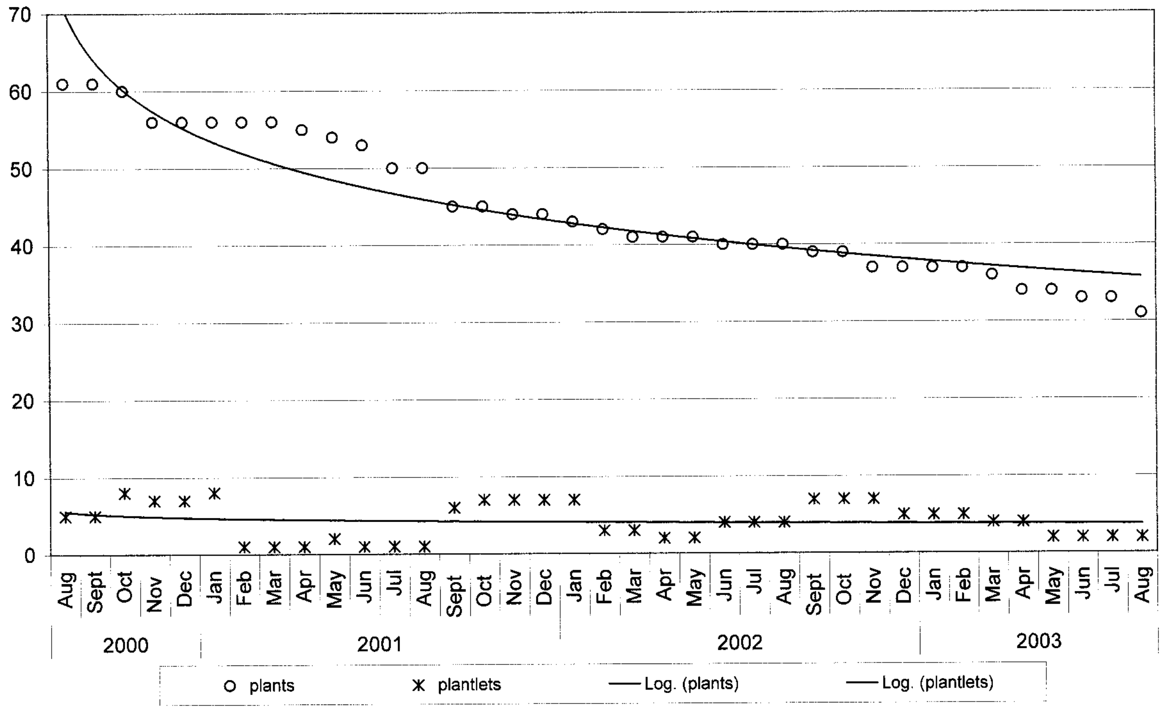

Figure 2. Demographic decrement recorded at Bergeggi (SV). Adult plants (○) and seedlings (*) life by August 2000 to April 2003.

Table III. Crown diameter (I–VI) and canopy volume (I–III) classes (Ramadan *et al.*, 1994) in 2000 and 2003 for the population of Bergeggi. The number of specimens for each classes and the relative mortality percentage (Δ Mort%) are reported.

Dia	Aug 2000	Aug 2003	Δ Mort.%
I	1	0	100.0
II	11	4	63.6
III	29	15	48.3
IV	12	7	41.7
V	6	4	33.3
VI	2	0	100.0
Volume	**Aug 2000**	**Aug 2003**	**Δ Mort.%**
I	53	27	44.4
II	4	3	25.0
III	4	1	75.0

males and other dichogamous plants. By considering the drop in plant population according to sex phenotypes, results more or less complied with population mean mortality value. Young plants, not yet sexually developed, recorded a higher mortality value.

The total volume of population showed a heavy drop: from 19.11 m^3 of live branches in August 2000 to 5.37 m^3 in August 2003 (a 71.91% decrease).

Variations in crown volume (Table V) were measured on the plants which survived the study period: respectively 29 belonged to I, 1 belonged to II and 1 belonged to III size classes. Attention should be drawn to the fact that no plant's crown increased enough to be moved to a higher size class. On the contrary two members of the II class were de-classed to I class. Sexual phenotypes maintained their relative proportions. Moreover only females and males showed an little increment in crown volume, whereas protandrous plants crown volume decreased. Other sexual forms had a variable behaviour.

Fruit production varied seasonally, however, generally speaking, a 5.7% rate was assessed on female flowers and fruits (Table VI). Mature fruits fell in January and July, 3 months after the two flower production peaks. The final number of mature fruits available for regenerating was calculated at 6386 units (5.67% of borne flowers). According to the number of seedlings observed (18), the *in situ* germination rate was estimated at an average of 0.28% of the mature fruits of the whole population.

Very few seedlings (ranging from 1 to 9) were observed during the study throughout the populations (Figure 2), and seedling establishment was recorded mainly in September and October, 6 months after the preceding flowering period. Most of the new autumnal seedlings died suddenly during the Winter. There were very few new recruitments and the life-span of almost all the seedlings did not exceed 1 year.

Table IV. Size-frequency distribution of *T. hirsuta* individuals in Bergeggi in relation to sex phenotype before and over the study.

		Sex phenotype					
	Size class	F	M	FM	MF	O	nf
2000	I	8	10	10	7	10	8
	II	1	–	1	2	–	–
	III	3	–	–	1	–	–
2003	I	5	4	6	4	6	2
	II	–	–	1	2	–	–
	III	1	–	–	–	–	–

F = female; M = male; FM = protogynous; MF = protandrous; O = other labile sex phenotypes; nf = no flowered specimens.

Table V. Variations in canopy volume of survived individual measured in Bergeggi. Volume variation percentage (Δ vol.%), canopy volume classes (in 2000 and 2003), sex phenotype of each individual are reported.

2000	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Δ vol.%	8,33	– 23,20	58,20	81,48	82,29	– 20,00	81,48	200,00	44,62	– 87,50	– 28,57	– 75,00	0,00	– 12,50	25,00
2003	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
	F	F	F	F	F	M	M	M	M	FM	FM	FM	FM	FM	FM

2000	I	I	I	I	I	I	I	I	I	I	I	II	II	II	III
Δ vol.%	– 65,82	77,78	42,38	0,00	0,00	– 96,88	102,80	76,94	– 23,44	0,00	162,50	– 44,00	– 69,38	– 78,40	– 22,15
2003	I	I	I	I	I	I	I	I	I	I	I	I	II	I	III
	MF	MF	MF	MF	O	O	O	O	O	NF	NF	MF	FM	MF	F

Table VI. Ripe fruit production in the 19 main plants in Bergeggi. Female flowers (♀), fruits and fruit/♀ ratio are reported for each over the three production seasons (2001–2003) covered by the study.

	2001	2002	2003	Total
♀	29622	47637	35739	112720
Fruits	1638	2774	2001	6386
Fruit/♀	5.53%	5.82%	5.60%	5.67%

Ex situ multiplication

Laboratory tests on seeds of *T. hirsuta* showed rates of germination varying from 0 to 2.6% (with an average of 1.78%). Transplanted seedlings had a short survival period of about 20–40 days. After 1 month the survival rate was 20% ca., and by the end of the second month only one seedling was still alive.

Reproduction by cuttings yielded very poor results. No rooting or shooting was observed and all the stem segments died within a month from cultivation activities. The different behaviour patterns were recorded on the different sets of pots. Survival averages were initially higher among crock-cultivated cuttings and heather-soil-cultivated cuttings, however all the cuttings died within a month.

In vitro micropropagation of *T. hirsuta* was unsuccessful. No growth of new buds or new plantlets occurred as a result of experimental testing. Hormones (simple and combined) at different concentrations added to the medium, normalized at pH 5.5, never induced shoot- or root formation, results scored also on non-growth-regulators-enriched medium; at highest hormone concentration of BA (4 mg l^{-1}) there was a higher survival rate up to 2 months. A further experimental medium was used (Hougland's); however, no positive results were recorded – though a higher survival rate was noticed during initial growth stages. The best survival rates were obtained with media normalized to the same pH found in nature (i.e. 6.8), even though the addition of growth regulators at different concentrations did not lead to the results we were hoping for.

Discussion

Demography

The population survey of *T. hirsuta* in the North Tyrrhenian Sea highlighted a worrying condition of the species (Minuto et al., 1995; Minuto & Casazza, 2001, and unpublished data): 3 of the 11 populations reported in literature are still present on the area. It is also interesting to add that all the recognized populations lived in coastal rocky habitats, whereas many others (sandy places, beaches, etc.) were described.

The drastic drop in population is actually prejudicing the survival of this species along the North Tyrrhenian coasts, which features high mortality rates among both adult plants and seedlings, though *T. hirsuta* appears to be in distress throughout the Mediterranean. Data from the Egyptian desert (El-Keblawy et al., 1997) reported an annual mortality rate ranging between 5 and 20%. By comparing all data available for *T. hirsuta*, it might be supposed that the species shows an increasing mortality rate from low latitudes towards the northern border of its distribution areal. This behaviour might be related to the fact that the plant's population mortality is significantly affected by habitat, size-class (El-Keblawy et al., 1997) and age.

One reason for this progressive populations and individuals decrement might be given by a gradient of increasing aridity; also in the inland of Egypt, where the aridity rate is higher, this environmental element is associated with a pattern of lower population growth rates and greater mortality of all size classes of *T. hirsuta* plants (El-Keblawy et al., 1997).

However, this climatic feature does not account entirely for a similar behaviour at higher latitudes where rainfalls are usually more abundant than along southern Mediterranean coasts and north Africa inland. Moreover, in the north, human pressure should be added to this stressing situation, where the frequent presence of mankind deeply affects the stability. This fact is evident in Bergeggi where direct damages to plants (breakage and crushing) made by fishermen and bathers should be added to other environmental pressures such as a nearby motorway, harbour and power station. In this situation human pressure enhances the acuteness of natural disease: in many occasion fungal rusts, for example, were observed on the twigs and inside capitula and flowers (Caporali, verbal communication).

The analysis of size-sex relationship confirmed the particular behaviour of *T. hirsuta,* as already seen in Egypt. On the Egyptian coastal dunes and non-saline depressions of the inland, female and functional female plants (protandrous) were significantly larger than males and functional males (Ramadan et al., 1994). This element clashes with the observations in France where plant size and sex appeared to be independent (Dommée et al., 1990).

However, in our study mortality rates in the populations seemed to be related to the crown size rather than sex, since high rates of mortality were recorded among small plants (both in diameter and volume). However, a small size might be insufficient to react to environmental and human stress since a important decrement was evident among large size individuals, too. In this case, sex seemed to be important: the dead plants were all female or functional female and particularly high producers within the population.

An explanation for this behaviour might be the reproductive effort expressed by the previous plants: high producers express such a high effort that they are more exposed to habitat diseases (Ramadan et al., 1994). In this way it is possible to link the high production of flowers recorded during the study with the heavy decrement in plant number. Old and large specimens yielded a special production before their death. This final result is due to the fact that, in older individuals of all sex forms of *T. hirsuta*, a high proportion of energy is spent in self-maintenance (El-Keblawy et al., 1987). 1

As reported for the populations from Egypt, the rate of increment in crown diameter was significantly higher in younger shrubs than in older ones (Ramadan et al., 1994).

Regeneration of the population

Ripe fruit production was limited in proportion to the number of flowers borne by the population, and it was mainly restricted to two sexual types: female and protandrous plants. Similar results had already been reported for the French and Egyptians populations of *T. hirsuta* (Dommée et al., 1990; El-Keblawy et al., 1996a).

Recent anatomical studies on the reproductive organs of *T. hirsuta* have highlighted difficulties in embryonal development. Ripening fruits show sudden embryo alterations (Caporali, verbal communication). This fact might support data recorded in Bergeggi: low percental rates of flowers/ripe fruits and the seedling emergencies *in situ*.

Some studies have suggested that environmental conditions prevailing during seed maturation might have important effects (Miao et al., 1991) on the quality of the seed and its capability in germination, and finally contribute to offspring phenotype (Roach & Wulff, 1987) and some adult plant traits (Alexander & Wulff, 1985; Parrish & Bazzaz, 1985).

In *T. hirsuta* the expression of maternal gender effects on some early traits of progeny depends upon maternal habitat (El Keblawy et al., 1996).

The frequency and abundance of rainfall seem to be extremely important for the regeneration of *T. hirsuta* populations from seed (El-Keblawy et al., 1997). The habitat present in Liguria (climate and human pressure) might be one of the reasons for the very low number of mature fruits and seedlings recorded in the population of *T. hirsuta*.

Ex situ multiplication

In our study we recorded lower germination rates than in other previous works due to the fact that most of the seeds originated from a distressed population. No problems of seed dormancy are recorded in literature (Dommée et al., 1995; El-Keblawy et al., 1996b), but we believe that the low rate of seed germination could be due to plant breeding problems. The frequent incomplete maturity of fruit could to be linked to an incomplete development of the embryo.

The results of the cutting propagation experiments were as unsuccessful as those of other studies reported in literature (Papafotiou et al., 1991; Grassotti, 1999).

Rooting activity from secondary meristematic cells seemed to be very low in this species, as per results of *in vitro* micropropagation. The production of new plants by this technique seems yet to be a distant goal. None of the hormones or chemicals added to the medium were of any use in inducing regeneration activities. Our study, however, did reveal that an unusually high pH (6.8), similar to that recorded in nature, gave rise to higher survival rates among the explants.

This peculiarity of pH value, similar in all study sites, might be linked to a particular plant rhizosphere, and the presence of fungi around *T. hirsuta* roots had already been described by other authors
2 (Abu El-souod et al., 1989). In fact, current histological studies on *T. hirsuta* (Zotti, verbal communication) suggest that there may well be an endo-micorrhizal partner. Further investigation will be necessary to confirm if such a partner may interact with the metabolism and the vegetation activity of the plant under examination.

Conclusions

On the basis of our results, we can confirm the reduction of *T. hirsuta* along the North Tyrrhenian coast and everywhere in the Mediterranean. Also, as already suggested by other authors, most populations of *T. hirsuta* may be drifting towards extinction (El-Keblawy et al., 1997).

We assumed that *T. hirsuta* is affected by external factors (e.g. climate and human pressures) inducing drastic modifications in vegetation growth and breeding system, and that several biological changes have taken place: e.g. flowering periods have lengthened and tend to last throughout the year. Therefore, on the one hand the abundant availability of pistillate flowers should lead to greater production of fruits or to a higher probability of fertilization, while on the other, fruit production induces a higher reproductive effort, and therefore greater general weakness against stressful habitat conditions.

The definition of effective conservation strategies for the future of the plant is now both necessary and urgent, and researchers should bare in mind that sexual breeding is now the only way to regenerate the natural population, while vegetative reproduction seems to be impossible to implement.

A solution to this problem is now urgent since the already decimated populations still living in the North Tyrrhenian Sea are constantly exposed to drastic genetic lost and impoverishment.

A draft conservation policy for *T. hirsuta* might be based on the following elements: (a) conservation of natural habitats by reducing human presence; (b) *ex situ* multiplication by sowing seeds from the natural population, in order to obtain a larger number of new specimens in protected condition; (c) reinforcements of the natural population by introducing new individuals in the mother populations; and (d) further studies on the biology of the reproduction and on the physiology of this species in order to gain better knowledge of both its meristematic activity and its possible symbiosis with endophytes.

References

Abu El-Souod SM, El-Shourbagy MN, El-Refai EM. 1988. Effect 2
of different growth stages on the rhizosphere and rhizoplane fungi of *Thymelaea hirsuta* at different localities. Ann Microbiol 38(29):15–27.

Alexander HM, Wulff R. 1985. Experimental ecological genetics in *Plantago*. X. The effects of maternal temperature on seed and seedling characters in *P. lanceolata*. J Ecol 73:271–82.

Casazza G, Savona M, Carli S, Minuto L, Profumo P. 2002. Micropropagation of *Limonium cordatum (L.) Mill.* for conservation purposes. J Hortic Sci Biotech 77:541–5.

Dommée B, Biascamano A, Denelle N, Bompar JL, Thompson JD. 1995. Sexual tetramorphysm in *Thymelaea hirsuta (Thymelaeaceae)*: morph ratios in open-pollinated progeny. Am J Bot 82(6):734–40.

Dommée B, Bompar JL, Denelle N. 1990. Sexual tetramorphism in *Thymelaea hirsuta (Thymelaeaceae)*: evidence of the pathway from heterodichogamy to dioecy at the infraspecific level. Am J Bot 77(11):1449–62.

El-Keblawy A, Freeman DC. 1999. Spatial segregation by gender of the subdioecious shrub *Thymelaea hirsuta* in the Egyptian desert. Int J Plant Sci 160(2):341–50.

El-Keblawy A, Lovett-Doust J, Lovett-Doust L, Shaltout KH. 1995. Labile sex expression and dynamics of gender in *Thymelaea hirsuta*. Ecoscience 2:55–66.

El-Keblawy A, Lovett-Doust J, Lovett-Doust L. 1996a. Gender variation and the evolution of dioecy in *Thymelaea hirsuta (Thymelaceae)*. Can J Bot 74:1596–1601.

El-Keblawy A, Shaltout KH, Lovett-Doust J, Lovett-Doust L. 1996b. Maternal effects on progeny in *Thymelaea hirsuta*. New Phytol 132:77–85.

El-Keblawy A, Shaltout KH, Lovett-Doust J, Ramadan A. 1997. Population dynamics of an Egyptian desert shrub, *Thymelaea hirsuta*. Can J Bot 75:2027–37.

Fay MF, May NWM. 1990. The in vitro propagation of *Nescodom mauritanicus*. Botanic Gardens Micropropagations News, 1:6–8.

Grassotti A. 1999. Caratterizzazione e valorizzazione di specie da fiore reciso e da fronde ornamentali adatte a zone agricole svantaggiose. http//biodiversita.ba.cnr.it/gras99.htm

Hoagland DR, Arnon DI. 1938. The water-culture method for growing plants without soil. Berkley, CA: University of California Collection Agriculture Experimental Station. pp 347 – 53.

Iriondo JM, Pérez C. 1990. Micropropagation of an endangered species: *Coronopus navasii (Brassicaceae)*. Plant Cell Rep 8:745 – 8.

Lledó M, Crespo M, Amo-Marco J. 1993. Preliminary remarks on micropropagation of threatened *Limonium* species *(Plumbaginaceae)*. Botanica Gardens Micropropagation News 1(6):72 – 4 (Spain).

Martin C, Pérez C. 1992. Multiplication in vitro of *Limonium estevei* Fdez. Casas. Ann Bot 70:165 – 7.

Martin C, Pérez C. 1995. Micropropagation of five endemic species of *Limonium* from the Iberian Peninsula. J Hortic Sci Biotech 70:97 – 103.

Martinez V, Rubluo A. 1989. In vitro pagation of the near extinct *Mamillaria sanangelensis* Sànchez Mejorada. J Hortic Sci Biotech 4:99 – 105.

Miao SL, Bazzaz F, Primack R. 1991. Resistance of maternal nutrients effects in *Plantago major:* the third generation. Ecology 72:1634 – 42.

Minuto L, Casazza G. 2001. *Thymelaea hirsuta* (L.) Endl.: monitoraggio della popolazione ligure. Atti 96° Congresso della Società Botanica Italiana 1:90.

3 Minuto L, Casazza G, Profumo P. ????. Sexual polymorphism and spatial segregation of *Thymelaea hirsuta* in Liguria (NW Italy). in revision

Minuto L, Paola G, Barberis G. 1995. *Thymelaea hirsuta* (L.) Endl. *(Thymelaceae)* specie minacciata in Liguria. Giorn Bot Ital 129(2):103.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol 15:473 – 97.

Papafotiou M, Triandaphyllou N, Chronopoulos J. 1991. Studies on propagation of species of the xerophytic vegetation of Greece with potential floricultural use. IV International Symposium on New Floricultural Crops. ISHS Acta Horticulturae 541:269 – 72.

Parrish JAD, Bazzaz F. 1985. Nutrient contents of *Abutilon theophrasti* seeds and competitive ability of the resulting plants. Oecologia 65:247 – 51.

Ramadan AA, El-Keblawy A, Shaltout KH, Lovett-Doust J. 1994. Sexual polymorphism, growth and reproductive effort in Egyptian *Thymelaea hirsuta (Thymelaeaceae)*. Am J Bot 81(7):847 – 57.

Roach DA, Wulff R. 1987. Maternal effects in plants. Annu Rev Ecol Syst 18:209 – 35.

Savona M, Ruffoni B, Minuto L, Carli S, Profumo P. in press. 4
Leucojum nicaeense Ard.: application of the in vitro culture to preserve the biodiversity. Italus Hortus.

Shaltout KH, El-Keblawy A. 1992. Sex expression in Egyptian *Thymelaea hirsuta* populations. Plant Syst Evol 181:133 – 41.

Shaltout KH, El-Shourbagy MN. 1989. Germination requirements and seedling growth of *Thymelaea hirsuta* (L.) Endl. Flora 183:429 – 36.

Sharma N, Chandel KPS, Srivastava VK. 1991. In vitro propagation of *Coleus forskohlii* Briq., a threatened medicinal plant. Plant Cell Rep 2:67 – 70.

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