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Nutlet production and germination in female and hermaphrodite plants of *Thymus albicans* Hoffmanns. & Link (*Lamiaceae*)

Abstract

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Some reproductive aspects are studied in *Thymus albicans* Hoffmanns. & Link, endemic to SW Iberian Peninsula. Percentage of hermaphrodite plants fruit set, nutlet production, seed set, reproductive efficiency and percentage of germination of seeds produced by female and hermaphrodite plants are studied. Percentage of female and hermaphrodite plants is very variable between populations. Nutlet production and reproductive efficiency are higher in female than in hermaphrodite plants, and this, together with a slightly advanced germination of seed produced by females, may increase female fitness in natural populations.

Introduction

Gynodioecy is the occurrence of female and hermaphrodite individuals in the same natural populations of a species. It is frequently the result of the regular occurrence of male sterile genes in otherwise hermaphrodite populations (Richards 1986). Female plants can be considered as hermaphrodites that have lost their male function, a process in which both nuclear and cytoplasmic genes may be involved (Belhassen & al. 1991).

Gynodioecy can be considered a well-stablished breeding system in the *Lamiaceae*; within a population, frequencies of hermaphrodite and female plants show little variation with time (Richards 1986). Models of gynodioecy involving the effects of selfing postulate that female are maintained in the population because their offspring are obligatory outcrossed and hence more fit than the progeny of hermaphrodites often resulting from self-fertilization (Cox 1988).

Gynodioecy occurs in most species of *Thymus* (Morales 1986) and has been studied in detail in *T. vulgaris* L. by Dommêe & al. (1978) and Assouad & al. (1978). As in many other gynodioecious Angiosperms (Delph 1996), the corolla in female flowers is smaller than in hermaphrodite flowers, and while in the latter there is a well marked protandry, with the stigma becoming receptive four days after anthesis, in female flowers the stigma is receptive inmediately after flower opening (Morales 1986).

Thymus albicans Hoffmanns. & Link is one of the two Iberian Peninsula species of sect.

Mastichina (Miller) Bentham, characterized by their flat leaves, which are never ciliate at the base, and capituliform flower-heads with small white flowers with ciliate calyx-teeth. *T. albicans* differs from the other species in the section, *T. mastichina* (L.) L., by the size of the calyx, higher in *T. albicans*, and size of calyx teeth, as long as or shorter than tube in *T. mastichina*, but quite longer than the tube in *T. albicans*. It is a gynodioecious endangered species endemic of SW Iberian Peninsula where it lives in coastal sandy soils of a reduced area in S Portugal around Faro (Algarve) and in Cádiz province (Spain), where its distribution area is now in regresion. Its presence in Huelva and Sevilla provinces (Morales 1986) has not been recently confirmed.

The aims of this paper is to quantify the sexual reproductive capacity of this species. Sex distribution, nutlet production, reproductive efficiency and seed germination capacity have been studied in several populations. Possible differences between female and hermaphrodite plants for these parameters have been checked.

Material and Methods

Five populations were chosen near Chiclana (Cádiz province) (Fig. 1), all living on sandy soils with similar climatic conditions. Populations 1, 2 and 3 grow as part of the

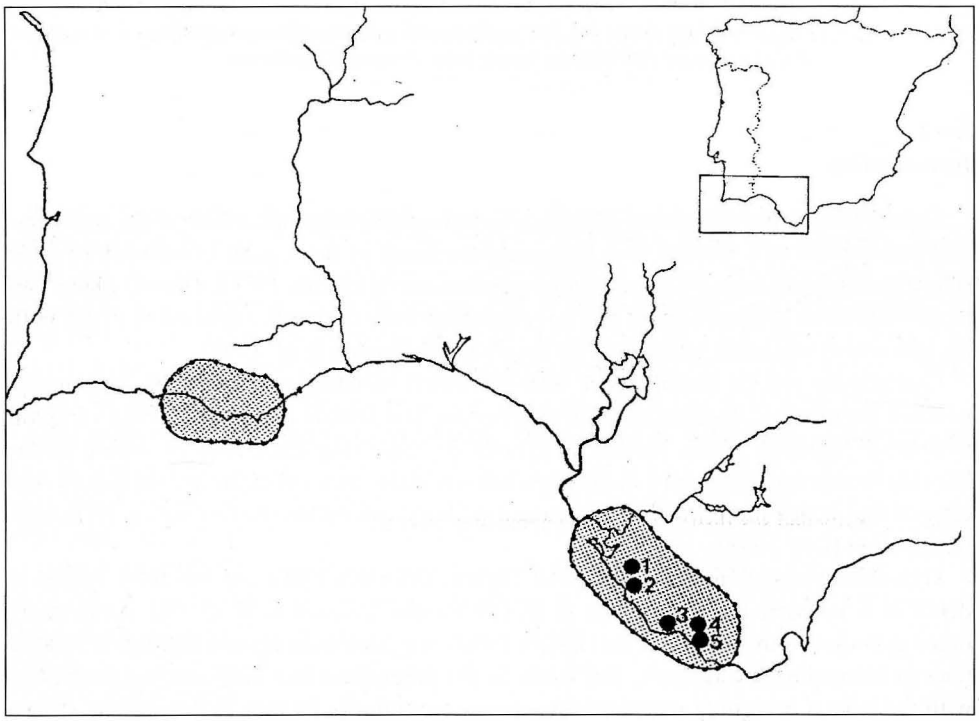

Fig. 1. General distribution of *Thymus albicans* Hoffmanns. & Link. and origin of the populations studied.

open underscrub of *Pinus pinea* forest, and populations 4 and 5 in open and sunny areas around the forest.

Percentage of female and hermaprodite plants was estimated by counting all specimens in four populations.

Fruit set (% fr/fl), nutlet production, seed set (% S/O) and percentage of abortion was measured in several female and hermaprodite plants in the four populations in 1995 and 1996. Plants of similar size, separate for an equivalent distance and distributed at random in each population were chosen. Five replicates of 50 calyces per plant, all separate at random, were used to evaluate these parameters. Fruit set was estimated as percentage of flowers which produced fruiting calyces, this is, with at least one nutlet. Nutlet production per calyx was estimated as number of nutlets produced by each fruiting calyx. Seed set was estimated as percentage of ovules which produced nutlets. Percentage of abortion refers to all fruiting calyces studied; aborted fertilized ovules were recognizable as seedless nutlets, which were always smaller than ripe ones. Reproductive efficiency was measured according to Wiens & al. (1987) as % S/O x fr/fl.

Percentage and dynamic of germination of seeds produced by female and hermaphrodite plants of four populations were studied in laboratory conditions. Four replicates of each sample, of 25 nutlets each, were sown in October 1995 and December 1996, in sterile Petri dishes with wet filter paper. Seeds were grown in natural day light with temperatures ranging from 18 to 28 °C. Seed germination was followed daily during 40 days. The number of days elapsed from sowing to germination of the first seed (t0) and from sowing to 50% germination (t50) have been used as indicators of germinaton dynamic.

Results

Percentages of gynodioecy.- Size of population, number of plants, density and sex distribution in the five populations studied are indicated in table 1. Percentage of hermaphrodite and female plants is very variable from one population to another, ranging from 39.13% to 63.15% of hermaphrodites. In these perennial plants, no differences in sex percentages have been observed in two consecutive years (1995 and 1996) except for population 1, which was partially destroyed by building activities in the spring of 1996.

Fructification.- Fruit set, nutlet production, seed set, reproductive efficiency (PERS) and percentage of abortion in female and hermaphrodite plants of the four populations

Table 1. Density and sex distribution in the population studied.

Population	Surface (m^2)	N° plants	N° plants/m^2	% Hermaphrodite
1	360	61	0.16	46.15
2	675	83	0.12	39.13
3	372	95	0.25	40.47
4	42	19	0.45	63.15
5	4234	2032	0.47	62.22

Table 2. Fructification in 1995 and 1996 in female (F) and hermaphrodite (H) plants of the populations 1, 3, 4 and 5. N, number of plants studied.

A) 1995

Population	N	% fr/fl (mean ± s.d.)		nutlets/calyx	% S/O	PERS	% abortion
3-F	3	10.39	4.20	0.38	9.71	1.01	57.63
3-H	4	12.41	7.29	0.60	15.25	1.89	40.46
4-F	4	57.19	16.59	0.65	16.29	9.31	40.68
4-H	1	11.36	4.10	0.41	10.40	1.18	58.32
5-F	5	29.34	13.42	0.68	17.24	5.06	38.70
5-H	4	13.61	8.13	0.29	7.08	0.96	70.68

B) 1996

Population	N	% fr/fl (mean ± s.d.)		nutlets/calyx	%S/O	PERS	% abortion
1 - F	4	17.62	5.55	0.48	11.98	2.11	52.06
1 - H	4	10.15	6.86	0.42	10.58	1.07	41.03
3 - F	9	36.68	10.67	0.64	15.91	5.84	34.60
3 - H	9	20.24	9.58	0.37	9.28	1.88	54.90
5 - F	4	14.28	1.42	0.39	9.75	1.39	60.99
5 - H	4	20.78	10.34	0.39	9.82	2.04	54.47

studied in 1995 and 1996 are indicated in table 2. Percentages of fruiting calyces with 0, 1 and 2 ripe nutlets in female and hermaphrodite plants are shown in Figs. 2a and 2b. No statistical tests have been carried out owed to the heterogeneity in the number of plants studied in each population.

In populations 1 and 4 nutlet production and reproductive efficiency are higher in female than in hermaphrodite plants. In populations 3 and 5, where these values were estimated for two consecutive years, results are rather contradictory. In population 3 both nutlet production per fruiting calyx and fruit set were lower in female than in hermaphrodite plants in 1995, but considerably higher in 1996. In population 5, on the contrary, both parameters were quite higher in female than in hermaphrodite plants in 1995, while they were very similar in 1996.

As shown in Figs. 2a and 2b, the number of ripe nutlets per calyx is rather low, with a reduced percentage of calyces with two ripe nutlets. No calyx was found with three or four

ripe nutlets. Number of nutlets produced by ripe calyx tends to be higher in female than in hermaprodite plants.

Seed germination.- Percentage and dynamics of germination of seeds for four populations studied and level of statistic significant differences for Tukey HSD test are given in table 3. There are no statistically significant differences between populations 3 and 4 as studied in 1995. In 1996 only population 1 showed a percentage of seed germination sig-

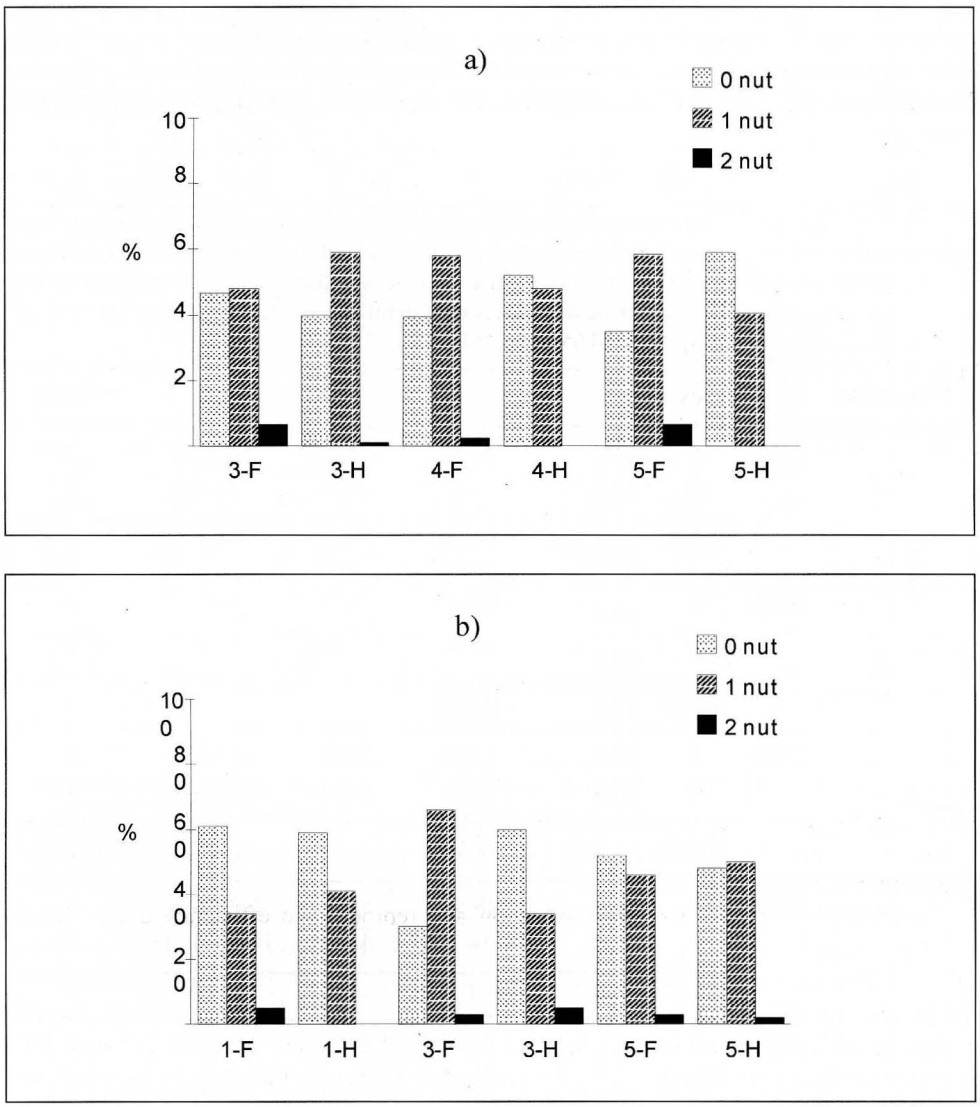

Fig. 2. Percentage of fruiting calyces with 0, 1 and 2 nutlets in female (F) and hermaphrodite (H) plants: a, data for populations 3, 4 and 5 (1995); b, data for populations 1, 3 and 5 (1996).

nificantly lower than in populations 3 and 5, which did not differ one from the other. No differences have been found either between 1995 and 1996 in population 2.

Data for female and hermaphrodite plants of these populations are shown separately in table 3. The statistic comparison between pairs of means in female versus hermaphrodite plants (Mann-Whitney-U non parametric test) has shown only significantly differences at the 0.05 level in population 3 for 1995, but at the 0.1 level in population 5 for 1996, with a lower percentage in female versus hermaphrodite plants.

Data on dynamics of germination of seeds are also given in table 3, where the parameters t0 and t50 have been indicated. Germination usually begins earlier in nutlets produced by female than by hermaphrodite plants, but in populations 3 no difference was found in 1996. The parameter t50 is ussually reached 1-3 days earlier in nutlets produced by females than in those produced by hermaphrodites, but once more population 3 differs at this respect.

Table 3. Percentage (%) and dynamics (t0 and t50, in days) of germination of seeds in female (F) and hermaphrodite (H) plants from populations 1, 3, 4 and 5. Mean and standard deviation (in brackets) for % germination, and mean and range of variation (in brackets) for t0 and t50, are indicated. The same letter upper means implies statistic differences with significance at level as follows: a= $p<0.01$, b= $p<0.001$ [Tukey HSD test]; x= $p< 0.05$, y= $p<0.1$ [Mann-Whitney-U].

Population	year	sex	%		t0	t50	n° replic.
1	1996	F	49.0	(8.9)	3 (2-5)	7 (4-8)	4
		H	44.0	(-)	4 (4)	5 (5)	1
		Total	48.0 a,b	(0.8)			5
3	1995	F	59.0 x	(22.2)	3 (3)	4 (4)	4
		H	84.0 x	(3.3)	3.5 (3-4)	7 (7)	4
		Total	71.5	(20.0)			8
	1996	F	81.0	(7.6)	3 (3)	4.5 (4-6)	4
		H	85.0	(8.2)	3 (3)	4.25 (3-5)	4
		Total	82.8 a	(7.6)			8
4	1995	F	83.0	(8.9)	3.5 (3-4)	6.2 (4-7)	4
		H	68.6	(14.1)	4 (4)	10.5 (7-14)	2
		Total	78.8	(11.6)			6
5	1996	F	78.0 y	(4.0)	2.5 (2-3)	3 (3)	4
		H	86.0 y	(6.9)	2 (2)	4 (3-5)	4
		Total	82.0 b	(16.4)			8

Discussion

There is a wide variability in the fructification parameters analyzed, which together with the differences in number of individuals studied from each population complicates statistical comparisons. However, data suggest that percentage of fructification is very low in all populations. Percentage of autogamy in hermaphrodite plants is equally very low, as it has been observed a percentage of fructification lower than 5%, with a PERS of c. 0.1 (unpublished data). This indicates the low survival potenciality of this species.

Seed abortion, sometimes as high as 70%, seems to greatly affect fruit set, and there is a negative correlation between number of nutlets per calyx and degree of seed abortion, as deduced from table 2. Besides, a high, but not evaluated, style predation by small larvae has been observed in most female and hermaphrodite plants. Both seed abortion and style predation could explain the low fruit set observed, and also the differences found in a particular population in consecutive years.

On the other hand the results show a general tendency to a higher nutlet production and reproductive efficiency in female than in hermaphrodite plants, which agree with the general tendency found in gynodioecious populations of other species of *Thymus* (Dommêe & al. 1978, Assouad & al. 1978), and with results found by several authors in other groups (Maki 1993, Eckhart 1992).

Seed germination percentage is generally high, with a total mean of c. 74%. This indicates that there are not special requirements for seed germination and that this does not make any problem for the survival of this species. Population 1 is the only one which significantly differs from the others by its low germination percentage, but no logical explanation has been found to this difference, as population 1 is not the smallest and occupies a similar habitat that the other three. At the other side, as deduced from table 3, there is no relationship between percentage of germination and sexuality. Only significant differences are found in population 3 in 1995, but not in the same population in 1996.

As indicated above, nutlets produced by female plants seem to germinate earlier than those produce by hermaphrodites. As germination occurs after Autumn rainfall, this difference in germination may favour female offspring. However, further observations could confirm this tendency.

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