Lagascalia 19(1-2): 857-864 (1997)

COMPARATIVE OBSERVATIONS ON THYMUS STRIATUS VAHL AND T. STRIATUS VAR. OPHIOLITICUS LACAITA IN CENTRAL ITALY

LAURA BINI MALECI, PIER LUIGI CIONI, GUIDO FLAMINI,
GUIDO SPINELLI, ORIETTA SERVETTAZ

Abstract

The study of micromorphological characters as well as chemical compounds distribution and heavy metals content show that these characters can be used to strengthen morphological differences betweey *Thymus striatus* subsp. *striatus* and *Th. striatus* subsp. *ophioliticus*, and confirms the separation of the serpentinicolorus subsp. *ophioliticus* from the typical subspecies.

Introduction

Thymus striatus Vahl (*Labiatae*) is a very polymorphous species, whose areal is reported by JALAS (1972) to extend from the Balkan peninsula to South and Central Italy. Nevertheless, as far as the Italian flora is concerned, some taxonomical problems are still open.

In 1911 Lacaita (LACAITA, 1911) substituted the name of *T. striatus* Vahl with *T. acicularis* W.K., and within this taxon described a new variety, growing on serpentine in Tuscany, as *T. acicularis* W.K. var. *ophioliticus* Lac. Afterwards, the same author (LACAITA, 1919) resumed the name *T. striatus* Vahl, and finally (LACAITA, 1925) reorganized the *T. striatus* group into 11 subspecies, one of which was *T. striatus* Vahl subsp. *ophioliticus* Lacaita, the taxon growing on serpentine in Tuscany being promoted to a subspecific range.

In the same year, on the contrary, FIORI (1925) again quotes the same taxon as a variety (*acicularis* W.K.) of *T. striatus* Vahl, recording it for Central and Southern Italy.

Flora Europaea (JALAS, 1972) mentions *T. striatus* only without any subdivision, emphasizing however its great variability; *T. acicularis* is here confined in synonimy. PIGNATTI (1982) reports *T. striatus* Vahl for Southern Italy, mentioning for Central Italy the same *T. acicularis* W.K. present in the Balkan peninsula, and quoting the variety *ophioliticus* possibly worthy of the specific range. ARRIGONI & al. (1983) returns to the old literature (LACAITA, 1911), placing in synonimy the taxa both of LACAITA (1925) and FIORI (1925). Finally, *Med Check List* (GREUTER & al., 1986) considers *T. striatus* Vahl as in *Flora Europaea*.

The present research aims at finding more differing characters in order to better define the status of the serpentine-restrained taxon.

Therefore, macro- and micro-morphological as well as phytochemical characters of the serpentine taxon, growing at Impruneta (FI), were evaluated and compared with those of a population of *T. striatus*, growing at Gola del Furlo (PS) on calcareous soil. Moreover, owing to the significant substrate differences, it seemed us useful to compare the heavy metals content in specimens growing in the two habitats.

In this paper we follow the nomenclature of LACAITA (1925).

Plant material

Specimens of *T. striatus* have been collected at Gola del Furlo (PS) on calcareous soil. Specimens of *T. striatus* subsp. *ophioliticus* have been collected at Impruneta near Florence on serpentine soil. Vaucher specimens have been deposited in the herbarium of the Dipartimento di Biologia Vegetale, University of Florence.

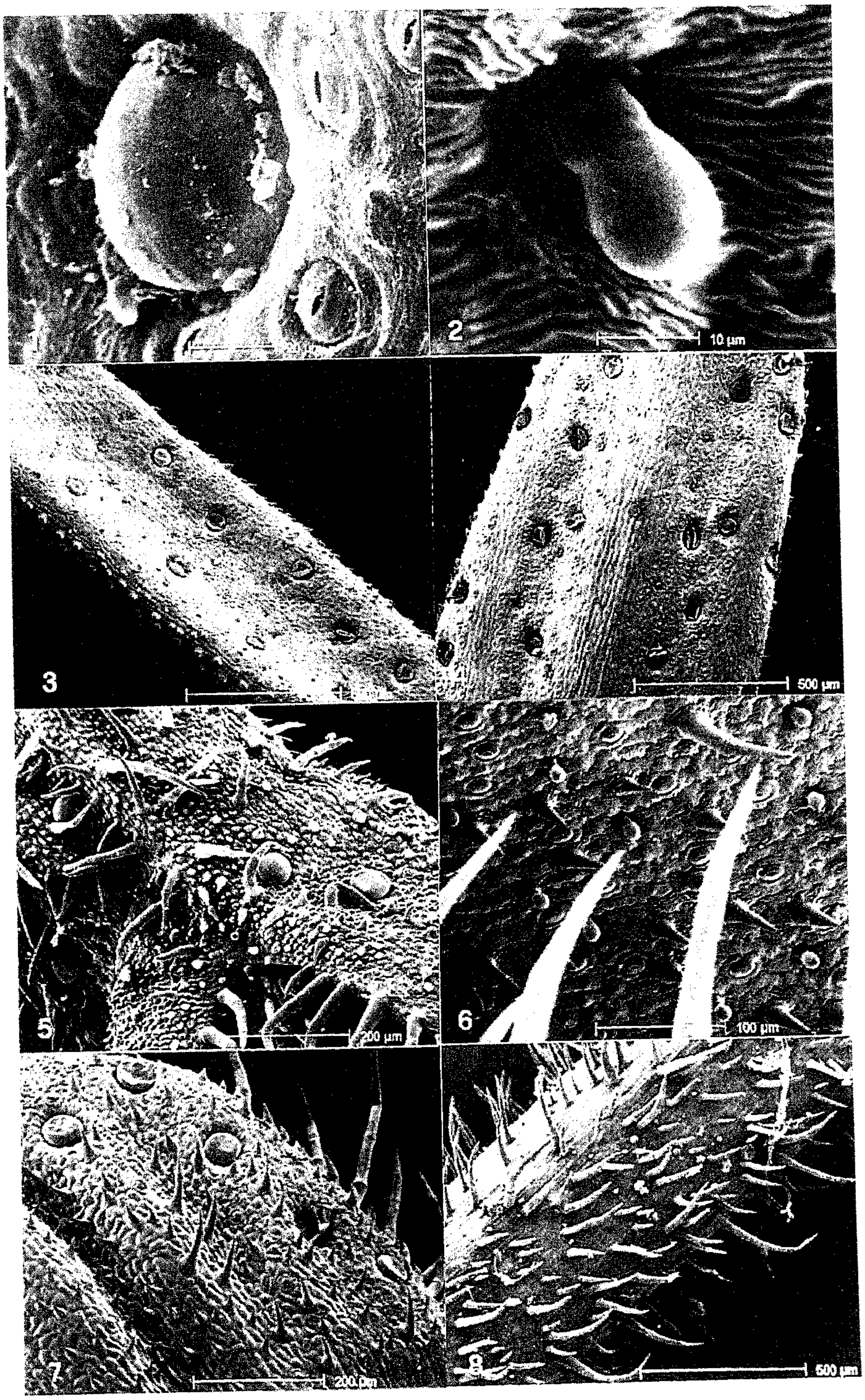
Methods

The epidermal surfaces as well as stem sections were preliminarly direct observed by light microscopy utilizing hand cut sections. In order to display lignification, phloroglucinol test has been performed (JENSEN, 1962). For SEM observations small pieces of material were fixed by 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 and postfixed in 2% OsO_4. The material has been dehydrated with acetone, followed by critical point drying and carbon gold coated. An electron microscope Philips 515 was utilized.

Volatile compounds have been obtained from fresh plants by steam distillation. Analyses were carried out by gas chromatography on an HP 5890 selective II equipped with MS detector HP 5972, and with capillary column HP-5MS (30 m x 0.25 mm, 5% Ph Me silicone), temperature program: 60° C/10'; 5° C/min up to 220° C; 10° C/min up to 270° C. Injector temp. 250° C, carrier gas He at 21 KPa (0.6 ml/min), split ratio 48.0.

For heavy metals analysis, samples were air dried, finely ground and then dissolved in HNO_3 suprapure according to ANGELONE & al. (1993). Afterwards, AAS analyses were carried out for Fe, Mn, Cu, Pb, Zn, Ni, Cr, Cd by Perkin Elmer 303 and Perkin Elmer 372 H6A 2200 Hitachi 055 recorder spectrometers.

Fig. 1. Micromorphological characters of Thymus striatus. 1, Peltate trichome on *T. striatus* subsp. *ophioliticus*. 2, Clubbed trichome on *T. striatus* subsp. *striatus*. 3, Leaf adaxial side of *T. striatus* subsp. *ophioliticus*. Note peltate and clubbed trichomes. 4, Leaf abaxial face of *T. striatus* subsp. *striatus*. The same trichomes as on the adaxial face are present. 5, Calix external surface of *T. striatus* subsp. *ophioliticus*. Peltate, clubbed and non grandular trichomes are present. 6, Calix internal surface of *T. striatus* subsp. *ophioliticus* with only clubbed and non glandular trichomes. 7, Bract abaxial side of *T. striatus* subsp. *ophioliticus* with peltate and non glandular trichomes. 8, Stem of *T. striatus* subsp. *striatus*. Numerous non glandular trichomes and few glandular trichomes (peltate and clubbed) are present.


2
10 µm
3
500 µm
5
200 µm
6
100 µm
7
200 µm
8
500 µm

Results and conclusions

At a macromorphological level *T. striatus* subsp. *ophioliticus* differs from the nominate race in size and colour: stems are shorter because of closer internodes and are intensely red coloured by anthocyanins, flowers are likewise deeper coloured. Bracts and calyces also contain the red pigment and are about 20% smaller than in subsp. *striatus*. This size difference is highly evident in the leaves, subsp. *ophioliticus* showing here a brightness diminution of up to 40% in comparison with subsp. *striatus* (1 mm bright against 1.8 mm). Furthermore, the stem pith is much more lignified in subsp. *ophioliticus*.

At a micromorphological level we can see peltate trichomes (Fig. 1.1) and little crubbed trichomes (Fig. 1.2) as described by BRUNI & MODENESI (1983) and by MODENESI & al. (1984) in *Thymus vulgaris*; furthermore, many non glandular trichomes are present (Figs. 1.5-1.8). Distribution of different kinds of trichomes in different plant organs of the two taxa is illustrated in Figs. 3-8 and in Table 1. The hereby recorded data demonstrate that both taxa are very similar as far as trichomes are concerned. As a matter of facts, previous researches by HRUSKA (1981) on epidermal cell size allowed to slightly differentiate the two taxa on a variety level.

Heavy metals analysis in specimens growing on the two different substrates showed that in serpentine-adapted plants Fe, Pb and Zn are less abundant than in the limestone plants; Mn, Cu and Cr, on the contrary, resulted slightly more abundant. Ni is the only one element present in the serpentine plants up to 10 times in comparison with the nominate race (from 0.36 ppm in the Furlo specimens to 3.8 ppm in the Impruneta plants). This datum is in agreement with what is known on serpentine vegetation (VERGNANO GAMBI, 1992; ANGELONE & al., 1993).

In Table 2 the volatile compounds composition of different plant parts of the two taxa has been reported. Because of the smallness of the plants, we could separately analyse only stem plus leaves and inflorescences. The latter ones include bracts, calyces and corollas altogether, organs sometimes known to differ considerably in kind and distribution of trichomes (BINI MALECI & SERVETTAZ, 1991; SERVETTAZ & al., 1992).

Thymus striatus (aerial parts of specimens from Central Yougoslavia) has been previously studied by KARUZA-STOJAKOVIC & al. (1989). According to these authors, terpinen-4-ol was the major component of the essential oil, followed by linalol and terpinyl acetate. In our specimens, collected at Passo del Furlo, the major components (Table 2) are ß-cubebene (up to 26% and 18% in leaves and inflorescences resp.), ß-caryophyllene (up to 18% and 15%), and g-terpinene (up to 15% in inflorescences). In *T. striatus* subsp. *ophioliticus* ß-myrcene is largely prevailing (up to 21 and 13% in leaves and inflorescences), followed by ß-cubebene and eucaliptol. Moreover, this taxon showed the exclusive presence of nerolidol (up to 16% in leaves and 6% in inflorescences), as well as of one unknown compound, eluted immediately after nerolidol (up to 9% in inflorescences). On the contrary, the subsp. *ophioliticus* lacks borneol, present in more than 4% ratio in *T. striatus*. This datum on borneol is in agreement with PÉREZ-ALONSO & VELASCO-NEGUERUELA, (1984), who observed that the essential oil of *Thymus villosus* subsp. *lusitanicus* contained borneol when grown on basic soils, but, on the contrary, linalol when grown on siliceous soils.

Kind of trichomes	*T. striatus* subsp. *striatus*							*T. striatus* subsp. *ophioliticus*						
	Stem	Leaf		Bract		Calyx		Stem	Leaf		Bract		Calyx	
		adax.	abax.	adax.	abax.	inner	outer		adax.	abax.	adax.	abax.	inner	outer
Peltate	±	++	++	-	+	+	-	±	++	+	-	+	+	-
Clubbed	-	+	++	±	±	±	++	+	++	+	±	+	+	++
Non glandular	++	-	-	-	-	++	++	++	-	-	+	+	++	++

Table 1 . Trichomes distribution in *Thymus striatus* subsp. *striatus* and *T. striatus* subsp. *ophioliticus*. Symbols indicate progressive increase of trichome numbers, from no trichomes (-), very few trichomes (±) to a progressive increase (+) and (++).

Components	*T. striatus* subsp. *striatus*		*T. striatus* subsp. *ophioliticus*	
	stems/leaves	inflorescences	stems/leaves	inflorescences
α-thujene	—	0.75	0.18	—
α-pinene	1.48	2.03	1.75	2.51
camphene	2.62	3.16	2.22	2.42
sabinene	—	0.38	0.70	1.06
β-pinene	—	0.45	1.40	1.44
1-octen-3-ol	—	0.19	0.55	0.73
β-myrcene	10.85	6.25	21.19	12.96
α-phellandrene	—	0.09	—	—
α-terpinene	0.71	2.54	0.15	0.21
p-cymene	2.31	1.41	0.44	—
limonene	1.05	1.81	1.96	3.09
eucaliptol	1.09	1.38	10.39	10.61
β-ocimene	2.99	9.31	3.76	7.30
γ-terpinene	4.72	15.61	0.60	0.72
2-menthen-1-ol	—	—	1.04	—
terpinolene	—	0.10	0.08	—
linalol	—	2.35	1.72	3.79
camphor	1.90	2.26	0.68	0.72
borneol	4.85	4.62	—	0.49
terpinen-4-ol	—	0.50	0.50	0.41
α-terpineol	—	0.18	1.03	—
decanal	—	—	0.09	—
isobornyl acetate	—	0.12	—	—
thymol	—	0.81	0.07	0.75
4(15)-bourbonene	0.90	0.17	1.42	—
β-caryophyllene	18.79	14.90	6.97	9.36
germacrene D	—	0.11	0.22	—
α-caryophyllene	—	0.82	0.28	0.39
1(10)-aristolene	—	0.56	—	—
β-cubebene	26.28	18.14	11.98	18.98
germacrene B	0.59	0.42	0.85	—
α-farnesene	—	1.63	0.23	0.55
δ-cadinene	—	—	0.27	0.30
calamenene	—	0.22	—	—
β-elemol	—	0.58	—	—
nerolidol	—	—	16.24	6.80
unknown	—	—	4.35	7.17
caryophyllene oxide	3.17	—	1.94	—
TOTAL	84.30	93.85	90.90	92.76

Table 2. Volatile components in different plant parts of *T. striatus* subsp. *striatus* and *T. striatus* subsp. *ophioliticus* (percentage of the total steam distillate)

The meaning of the above reported differences is very difficult to evaluate, since it is well known that the volatile compounds composition highly depends from environment, season behaviour, collecting time, weather in the collection day. Althought all efforts have been made to obtain comparable analytical data, further researches are needed in order to better clarify the phytochemical differences. At the moment, we do not think it reasonably safe to separate the two taxa on the basis of the chemical composition alone.

According to the above described characters, we conclude that the checked differences are chiefly due to the substrate, and that the taxon growing on serpentine may represent an ecotype, which may be considered a subspecies of *T. striatus*. Indeed, our macromorphological, micromorphological and phytochemical observations allow to confirm LACAITA's proposal (1925) of ascribing the taxonomical rank of subspecies to the taxon growing on serpentine.

Acknowledgements

We thank Ministero dell'Università e Ricerca Scientifica (Rome) for financial aid.

References

ANGELONE, M., O. VASELLI, C. BINI & N. CORADOSSI (1993). Pedogeochemical evolution on trace elements availability to plants in ophiolitic soils. *Sc. Total Envir.* **129**: 291-309.

ARRIGONI, P. V., C. RICCERI & A. MAZZANTI (1983). *La vegetazione serpenticola del Monte Ferrato di Prato in Toscana.* Arti Grafiche Prioreschi, Catena di Quarrata (Pistoia).

BINI MALECI, B. & O. SERVETTAZ (1991). Morphology and distribution of trichomes in Italian species of Teucrium sect. Chamaedrys (Labiatae) - a taxonomical evaluation. *Pl. Syst. Evol.* **174**: 89-91.

BRUNI, A. & P. MODENESI (1983). Development, oil storage and dehiscence of peltate trichomes in Thymus vulgaris (Lamiaceae). *Nord. J. Bot.* **3**: 245-251.

FIORI, A. (1925). *Nuova flora analitica d'Italia* (ristampa 1969) **2**: 451-455. Edagricole, Bologna.

GREUTER, W., H. M. BURDET & G. LONG (1986). *Med-Checklist* **3**: 392-393. Conservatoire et Jardin Botaniques, Genève.

HRUSKA, K. (1981). Su alcune caratteristiche dell'epidermide fogliare di *Thymus striatus* Vahl. *Inform. Bot. Ital.* **13**: 140-141.

JALAS, J. (1972). Thymus in: G. TUTIN & al. (eds.), *Flora Europaea* **3**: 172-182. Cambridge.

JENSEN, W. A. (1962). *Botanical Histochemistry.* Freeman & Co., S.Francisco and London.

KARUZA-STOJAKOVIC, L., S. PAVLOVIC, P. ZIVANOVIC & B. TODOROVIC (1989). Kolicina i sastav etarskog ulja razlicitih vrsta roda Thymus L. *Arh. farm.* **39**: 105-111.

LACAITA, C. (1911). Piante italiane critiche o rare, III. *Bull. Soc. Bot. Ital.* **18**: 116.

— (1919). Piante italiane critiche o rare, LV. *Nuovo Giorn. Bot. Ital.* **25**: 221.

— (1925). Le sottospecie del Thymus striatus Vahl. *Bull. Soc. Bot. Ital.* **111**: 108.

MODENESI, P., G. SERRATO VALENTI & A. BRUNI (1984). Development and secretion of clubbed trichomes in *Thymus vulgaris* L. *Flora* **175**: 211-219.

PÉREZ-ALONSO, M. J. & A. VELASCO-NEGUERUELA (1984). Essential oil analysis of Thymus villosus subsp. lusitanicus. *Phytochemistry* **23**: 581-582.

PIGNATTI, S. (1982). *Flora d'Italia* **2**: 489. Edagricole, Bologna.

SERVETTAZ, O., L. BINI MALECI & A. PINETTI (1992). Micromorphological and phytochemical characters of Teucrium marum and T. subspinosum (Labiatae) from Sardinia and Balearic Islands. *Pl. Syst. Evol.* **179**: 129-139.

VERGNANO GAMBI, O. (1992). The distribution and ecology of the vegetation of ultramafic soils in Italy, in: B. A. ROBERTS & J. PROCTOR (eds.), *The ecology of areas with serpentinized rocks. A world view*: 217-247. Kluwer Academic Publ., Netherlands.

Addresses of the authors:

Dr. L. Bini Maleci, Dipartimento di Biologia Vegetale, Universitá di Finenze, via la Pira 4, 5012 Firenze, Italy; Dr. P. L. Cioni & Dr. G. Glamini, Dipartimento di Chimica Bioorganica dell'Universitá, via Bonanno, 56100 Pisa, Italy; Dr. G. Spinelli, USL 6 Servizio Multizonale Prevenzione, via Marradi 116, 57100 Livorno, Italy; Dr. O. Servettaz, Dipartimento di Biologia dell'Università, vía Celonia 26, 20133 Milano, Italy.