Jber. naturwiss. Ver. Wuppertal 48 154 - 175 Wuppertal, 15. 6. 1995

New acquirements in the taxonomy of allogamous species of the *Epipactis helleborine* (L.) Crantz group in western Europe

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With 5 figures and 7 tables

Summary

New acquirements in the taxonomy of allogamous species of the *Epipactis helleborine* (L.) Crantz group in western Europe. Multivariate analyses performed on western European allogamous populations of the *E. helleborine* group showed that *E. distans* Arvet-Touvet, *E. neerlandica* (Vermeulen) J. & P. Devillers-Terschuren, *E. tremolsii* Pau and *E. lusitanica* Tyteca are four welldefined, clearly separable entities. The paper centers mainly on southeastern French populations previously identified as representatives of *E. tremolsii*. Although several individuals in these populations show close quantitative morphological proximity with either *E. lusitanica* or *E. neerlandica*, the initial identification is maintained, mainly based on qualitative, ecological as well as phenological arguments. In order to cope with situations such as the one encountered in southeastern France, the definition of the species *E. tremolsii* has to be somewhat enlarged with respect to the previous statements.

Zusammenfassung

Neue Erkenntnisse über die Taxonomie der allogamen Arten von *Epipactis helleborine* (L.) Crantz - Gruppe in Westeuropa. Bei westeuropäischen allogamen Populationen der *E. helleborine* Gruppe durchgeführte Multivariablen-Analysen ergaben, daß *E. distans* Arvet-Touvet, *E. neerlandica* (Vermeulen) J. & P. Devillers-Terschuren, *E. tremolsii* Pau und *E. lusitanica* Tyteca vier gut abgegrenzte klar unterscheidbare Sippen sind. Diese Arbeit konzentriert sich hauptsächlich auf südostfranzösische Populationen, die bisher *E. tremolsii* zugeordnet worden sind. Obwohl mehrere Exemplare in diesen Populationen in quantitativen morphologischen Merkmalen eine große Annäherung entweder an *E. lusitanica* oder an *E. neerlandica* zeigen, wird die ursprüngliche Bestimmung hauptsächlich aus qualitativen, ökologischen und auch phänologischen Gründen beibehalten. Um Fällen wie in Südost-Frankreich zu begegnen, muß die Artdefinition von *E. tremolsii* mit Rücksicht auf vorherige Vorstellungen erweitert werden.

Résumé

Nouvelles données sur la taxonomie des espèces allogames du groupe d'*Epipactis helleborine* (L.) Crantz en Europe occidentale. Des analyses multivariables réalisées sur des populations allogames du groupe d'*E. helleborine* en Europe occidentale ont montré que *E. distans* Arvet-Touvet, *E. neerlandica* (Vermeulen) J. & P. Devillers-Terschuren, *E. tremolsii* Pau et *E. lusitanica* Tyteca sont quatre entités bien définies et clairement séparables. L'article est centré principalement sur des populations du sud-est de la France, identifiées précédemment comme représentantes d'*E. tremolsii*. Malgré que plusieurs individus de ces populations montrent des caractéristiques morphologiques quantitatives proches de celles d'*E. lusitanica* ou d'*E. neerlandica*, l'identification initiale est maintenue, principalement sur base d'arguments qualitatifs, écologiques et phénologiques. Pour pouvoir prendre en compte des situations telles que celles rencontrées dans le sud-est de la France, la définition de l'espèce *E. tremolsii* doit être quelque peu élargie par rapport aux points de vue antérieurs.

1. Introduction

The complexity of the genus *Epipactis* is widely recognised. Most problems are concentrated in the *E. helleborine* (L.) Crantz group. Several subgroups have recently received particular attention, as briefly reported in a companion paper (TYTECA & DUFRÊNE 1994). In that study, our attention was centered on allogamous taxa from the *E. helleborine* group with the most occidental distribution, namely, *E. helleborine* s. str., *E. lusitanica* Tyteca, *E. tremolsii* Pau, *E. distans* Arvet-Touvet and *E. neerlandica* (Vermeulen) J. & P. Devillers-Terschuren. Multivariate analyses, based on morphological characteristics, helped us to confirm the validity of the latter four as autonomous taxonomic units, distinct from *E. helleborine*. Generally speaking, there are several significant vegetative as well as floral differences. The distinction is more critical between *E. tremolsii* and *E. lusitanica*, for which the differences are chiefly vegetative. The five taxa also possess distinctive ecological characteristics and, though transitional forms sometimes occur, can be regarded as independent species, in the context of the present trend to split taxonomic units into species.

This study concentrates mainly on one of the problems that until now remained largely unsolved, namely, the characterisation and distribution of *E. tremolsii* in south-eastern France. Indeed, in the previous research (TYTECA & DUFRÊNE 1994), only *E. tremolsii* samples coming from Spain and Portugal were taken into account. It is useful to replace the *E. tremolsii* problem in a more global and historical perspective. Long after the original description by PAU (1914), the taxon received large acceptance and recognition only after the studies by NIESCHALK (1971) and

KLEIN (1979). Following that period, BAUMANN & KÜNKELE (1982) published a distribution map covering a part of North-Africa and the southeastern part of the Iberic peninsula (from southern Portugal to Catalunya). Immediately after, we identified two populations from France (in departments Drôme and Var) as *E. tremolsii*, and this was the first time that the species was reported from France (DELFORGE & TYTECA 1982). By that time, we did not make use of any peculiar technique, statistical or other, and simply acted by visual comparison with the plants from Catalunya. After that time, the species was reported from a few other French departments (JACQUET 1988).

In the recent literature, there still remains a great deal of controversy about the existence of *E. tremolsii* as an independent taxon, not only in France but also in the Iberic peninsula, as commented by TYTECA (1994). Lately, *E. tremolsii* was also reported from other places in Europe, the most noteworthy of these being Sardinia (DAISS et al. 1990). However, in that island, the taxon also gave rise to serious problems of delimitation with respect to *E. helleborine* s.str., which apparently grows in neighbouring areas, accompanied by various intermediate plants (GIOTTA & PICCITTO 1993).

One of the main purposes of the present study is to reconsider in depth the comparison between the typical forms of *E. helleborine* from northern or median Europe and plants recently assigned to *E. tremolsii* in southern Europe. The samples of the latter come from Portugal and Catalunya, as in the previous research (TYTECA & DUFRÊNE 1994), but also from five southeastern French localities, including the two „original" ones indicated above.

2.Materials and methods

2.1. Samples

The biometric data exploited here come from 28 localities visited between 1988 and 1993, the characteristics of which are given in Table 1. In addition to the five taxa of the *E. helleborine* group, data coming from four populations of two species of the *E. atrorubens* group (*E. atrorubens* and *E. parviflora*) were also collected. As the latter are well-known, widely recognized species, these data are used as reference points to which the four critical taxa of the *E. helleborine* group can be compared, mainly as regards the statistical differences between taxa. Twenty-one of the 28 populations studied are easily distributed within the seven aforementioned taxa, as indicated in Table 1. The identification of two of the other populations (Mogadouro and Reguengo do Fetal) as either *E. tremolsii* or *E. lusitanica* is not definitely settled, as discussed

by TYTECA & DUFRÊNE (1994). The remaining five populations, from southeastern France, are (at least provisionally) attributed to *E. tremolsii*, as indicated in the previous section.

2.2. Characters

For each individual plant studied, 28 quantitative characters, listed in Table 2 (n. 1 to 28), are measured from the plant parts. Additional characters are defined as ratios between the absolute characters and enter the definition of Gölz and Reinhard's taxonomic distance (GÖLZ & REINHARD 1973; TYTECA & DUFRÊNE 1993). These 24 secondary or relative characters are listed and defined in Table 2. The means and standard deviations of the populations, regrouped under seven entities, are given in TYTECA (1994).

In addition to the quantitative characteristics specified in Table 2, a few qualitative characters were also studied. These characters, listed in Table 3, are those for which a satisfactory scoring procedure (indicated in Table 3) could be set out and/or those with highest taxonomic significance for the taxa under study. Some of these characters have been suggested by DEVILLERS-TERSCHUREN & DEVILLERS (1989).

2.3. Analyses

The statistical analyses used here have been described in previous papers (DUFRÊNE et al. 1991; TYTECA & DUFRÊNE 1993). In this paper we use principal coordinates analyses based on Mahalanobis distances as well as discriminant functions. Other types of graphical representations were also used, such as „scale" representations (see e.g. GÖLZ & REINHARD 1979). All analyses were performed with the R package for multivariate analysis and spatial analysis (LEGENDRE & VAUDOR 1991) and with custom coded routines.

TABLE 1. List and characteristics of the *Epipactis* populations analysed[1].

#	Taxon	Locality (dept. or prov.)	Date	Biotop	Alt (m)	n	Spl.	Liter ref
1	*E. parviflora*	Serra de Prades (Tarragona, E)	7/6/92 8/6/92	Pinewoods, road sides	600 400	6	T	-
2		Arboli (Tarragona, E)	9/7/92	Pinewood on northern slope	700	5	T	-
3	*E. atrorubens*	Bure (Namur, B)	14/7/90	Chalk grassland with pines	270 290	8	T	-

TABLE 1. continued.

#	Taxon	Locality (dept. or prov.)	Date	Biotop	Alt (m)	n	Spl.	Liter ref
4	*E. atrorubens*	Wéris (Luxem., B)	11/7/90	Grassland on calcarous schists	245	9	G	-
5	*E. helleborine*	Rochefort (Namur, B)	3/8/91	Beechwoods on limestone	290 220	8	T	-
6		Belvaux (Namur, B)	28/7/90	Pine plantation on chalk grassland	195	6	T	-
7		Louvain-la-Neuve (Brabant, B)	20/7/91 28/7/91	Gardens, *Cotoneaster*, clear wood	140 90	16	T	-
8	*E. distans*	La Bâtie des Fonds (Drôme, F)	12/7/91 15/7/91	Xeric pinewood on limestone, SW	1180	12	T	CT
9		Les Costes (Htes-Alpes, F)	14/7/91	Xeric pinewood on limestone, SSE	1180	8	T	CT
10	*E. tremolsii*	Beaufort-sur-Gervanne (Drôme, F)	14/6/93	Garrigue, old vineyard, on limestone	400	6	T	DT
11		Combe Curnier (Vaucluse, F)	12/6/93	Calcarous scree	475	8	T	-
12		Pernes (Vaucluse, F)	13/6/93	Scrubby garrigue, on limestone	360	8	T	-
13		Château Grime (Var, F)	10/6/93	Open cork-oak wood	305	10	T	DT
14		Col de Gratteloup (Var, F)	9/6/93	Scrubby cork-oak wood	200	6	T	-
15		Serra de Prades (Tarragona, E)	4/6/92	Garrigues, pinewoods, road sides	600 900	7	T	-
16		La Febro (Tarragona, E)	4/6/92 8/6/92	Hazelnut groves, road sides	800	8	T	-
17		Marco (Algarve, P)	13/4/92	*Cistus* scrub, cultureland on limestone	190	8	T	-

TABLE 1. continued.

#	Taxon	Locality (dept. or prov.)	Date	Biotop	Alt (m)	n	Spl.	Liter ref
18	*E. tremolsii*	Benafim (Algarve, P)	14/4/92	Garrigues, oak scrub, road sides	290	6	T	-
19	*Epipactis* sp.	Mogadouro (Trás-os-Montes, P)	1/6/90 2/6/90	Pinewood, chestnut grove with *Cistus*	700 650	5	TG	-
20		Reguengo do Fetal (Estremadura, P)	27/5/90	Garrigues, oak scrub on limestone	180	8	TG	-
21	*E. lusitanica*	Amoreiras (Alentejo, P)	24/5/90	Cork trees with *Cistus, Arbutus*	280	4	TG	-
22		Aracena (Huelva, E)	21/5/90	Cork trees with *Cistus*	750	12	TG	-
23		Cachopo (Algarve, P)	22/5/90	*Cistus* scrub on schists	350	6	TG	-
24		Monchique (Algarve, P)	16/5/88 17/5/88	Cork, chestnut trees *Eucalyptus, Arbutus*	240 550	12	T	T
25		Marmelete (Algarve, P)	24/5/90	Pinewood on cristalline soil	350	8	TG	-
26		Barão de São João (Algarve, P)	6/4/90	Parasol pines, *Eucalyptus, Cistus*	170	10	T	-
27	*E. neerlandica*	De Panne (W-Vlaanderen, B)	17/8/91 25/7/92	Sand dunes with *Salix repens* scrub	10	10	T	C
28		Oostduinkerke (W-Vlaanderen, B)	29/7/92	Sand dunes with *Salix repens* scrub	15	6	T	C

[1] # = number of sample;
countries: B = Belgium, E = Spain, F = France, P = Portugal;
Alt. = altitude;
n = number of individuals in the sample;
Spl. = sampler: G = J.-L. Gathoye, T = D. Tyteca, TG = both;
Liter(ature) ref(erence) : C = COULON (1989), CT = CHAS & TYTECA (1992), DT = DELFORGE & TYTECA (1982), T = TYTECA (1988).

3. Results

TABLE 2. List of quantitative characters used in the biostatistical study of *Epipactis*.

a) Vegetative and general aspects

1. - Plant height (cm)
2. - Number of cauline leaves
3. - Number of the longest cauline leave
4. - Length of second leave, from the base (cm)
5. - Width of second leave (cm)
6. - Length of the longest leave, from the base (cm)
7. - Width of the longest leave (cm)
8. - Uppermost leave length (cm)
9. - Insertion level of first cauline leave, from soil level (cm)
10. - Uppermost internodium length (cm)
11. - Stem diameter under inflorescence (mm)
12. - Stem diameter under lowermost leave (mm)
13. - Number of flowers
14. - Inflorescence length (cm)
15. - Length of inflorescence axis between the insertion points of first and fifth flowers (cm)

b) Floral aspects (measures in mm taken on fourth flower from inflorescence base)

16. - Bract length
17. - Bract width
18. - Ovary length
19. - Lateral sepals length
20. -Lateral sepals width
21. - Petals length
22. - Petals width
23. -Hypochile length
24. -Hypochile width
25. -Hypochile depth
26. -Width of constriction between epichile and hypochile
27. -Epichile length
28. -Epichile width

c) Relative characters (ratios between absolute characters - used in the Gölz and Reinhard distance)

29. - # 1/# 6	35.- # 6/# 7	41. - # 19/# 20	47. - # 23/# 27
30. - # 1/# 10	36.- # 8/# 10	42. - # 19/# 21	48. - # 24/# 25
31. - # 1/# 11	37.- # 13/# 14	43. - # 20/# 22	49. - # 24/# 26
32. - # 1/# 14	38.- # 16/# 17	44. - # 21 /# 22	50. - # 24/# 28
33. - # 2/# 3	39.- # 16/# 18	45. - # 22/# 28	51. - # 26/# 28
34. - # 4/# 5	40.- # 16/# 28	46. - # 23/# 24	52. - # 27/# 28

TABLE 3. - List of qualitative characters analysed and values of the corresponding coefficient.

Leaves arrangement:	on two opposite ranks	1
	± on two opposite ranks	2
	in all directions	3
Leaf margins:	non-undulate	1
	slightly undulate	2
	undulate	3
	stongly undulate	4
Ovary pubescence:	none (glabrous)	1
	light	2
	medium	3
	strong	4
Colour of ovary pedicel:	green	1
	pinkish green	2
	purplish	3
Flowering opening:	slight	1
	medium	2
	wide	3
Epichile orientation:	forward	1
	downward	2
	backward	3

3.1. Populations

Figs. 1 and 2 show principal coordinates analyses of the 28 populations, based on Mahalanobis distances. Several subsets can be observed: (1) a subset composed of samples from the *E. atrorubens* group, clearly separated on axes 1 and 3 (Fig. 2), (2) a subset including the *E. helleborine* samples; (3) a subset composed of the *E. distans* samples (axes 1 and 2 : Fig. 1); (4) a subset in which all *E. lusitanica* samples take part (including the Reguengo sample, n° 20 - Fig. 2); (5) a last subset surrounding the latter (locally as a half-circle : Fig. 1) and composed of all remaining samples, i.e., those refered to as *E. tremolsii* and *E. neerlandica*. The *E. neerlandica* samples (n° 27 and 28) cannot be separated from the French *E. tremolsii* samples (n° 10 to 14), which, on the other hand, are somewhat segregated from the other *E. tremolsii* samples (n° 15 to 18, on axis 2 - see Fig. I).

3.2. Groups of populations

Hereafter we concentrate on critical groups of populations, according to the analysis of § 3.1; namely, we consider the following entities: (1) *E. helleborine*, (2) *E. tremolsii*

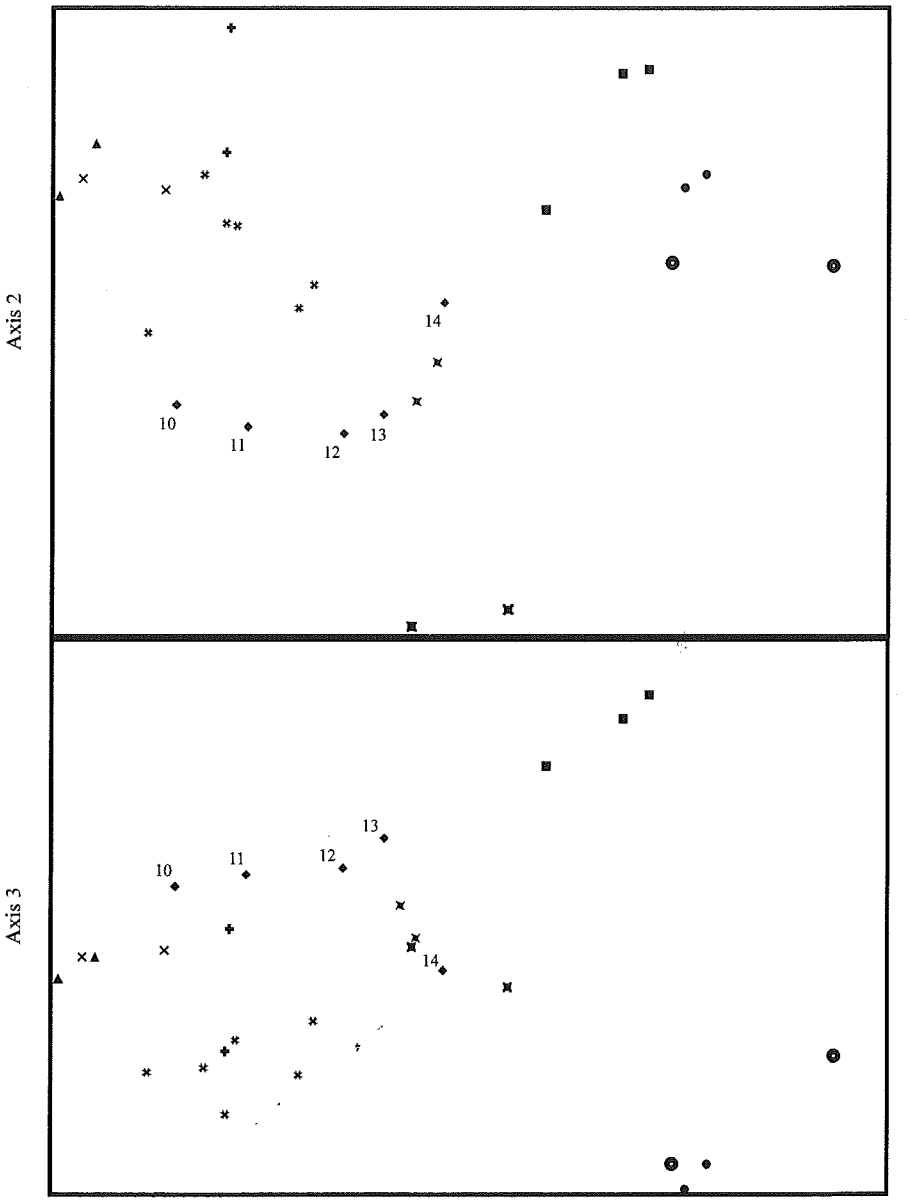
- France, (3) *E. tremolsii* - Spain, (4) *E. tremolsii* - Portugal, (5) *E. lusitanica* (excluding the Mogadouro and Reguengo populations), and (6) *E. neerlandica*. At this stage, we no longer consider *E. atrorubens*, *E. parviflora* nor *E. distans*, all three of which were found to be clearly separated from the rest of samples (see also TYTECA 1994). Nevertheless we maintain *E. helleborine* as a reference point, since all remaining taxa were formerly considered as making part of that species.

Table 4 gives the GÖLZ & REINHARD distances between the six aforementioned groups, taken two by two. The figures of that table enable us to build a three-dimension representation of the relationships between the six groups of samples (Fig. 3). In that figure, transitive relationships are of course respected; that is, the distance (not indicated) between two groups separated by a third one on a (almost) straight line, approximately equals the sums of the distances between that third group and each of the other two. For example, the distance between *E. neerlandica* and *E. tremolsii* - Spain, which is 63 (Table 4), is about equal to the distance between *E. neerlandica* and *E. tremolsii* - France (31) plus the distance between the latter and *E. tremolsii* - Spain (29).

In Fig. 3 one can roughly retrieve the same relationships as previously. That is, *E. helleborine* is neatly distinct from the other five groups, the distance being superior or equal to 30. We can represent *E. helleborine* as exterior to a plane including all remaining five groups (Fig. 3). The relations between the latter are more subtle; we can sketch them as a quadrilateral with *E. neerlandica*, *E. lusitanica*, *E. tremolsii* - Portugal and *E. tremolsii* - Spain as vertices. *E. tremolsii* - France appears at the middle of the largest side, which gives it a central position in the plane (Fig. 3).

TABLE 4. - GÖLZ and REINHARD distances among six sets of samples in *the E. helleborine* group.

	E. hell	E. tre-F	E. tre-P	E. tre-E	E. lus	E. nee
E. hell	—	30	47	39	45	46
E. tre-F	30	—	24	29	22	31
E. tre-P	47	24	—	19	29	45
E. tre-E	39	29	19	—	47	63
E. lus	45	22	29	47	—	22
E. nee	46	31	45	63	22	—


Axis 2
14
10
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Axis 3
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14
Axis 1

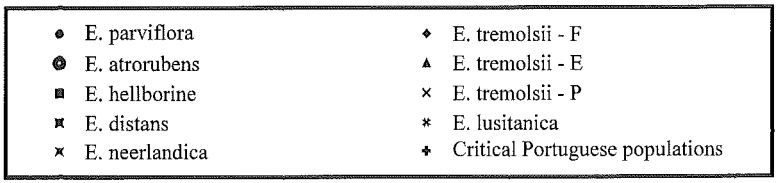

Fig. 1.-2. - Principal coordinates analysis (axes 1 and 2; axes 1 and 3) of the 28 considered populations, based on Mahalanobis distances. The numbered samples are those from southeastern France; see Table 1.

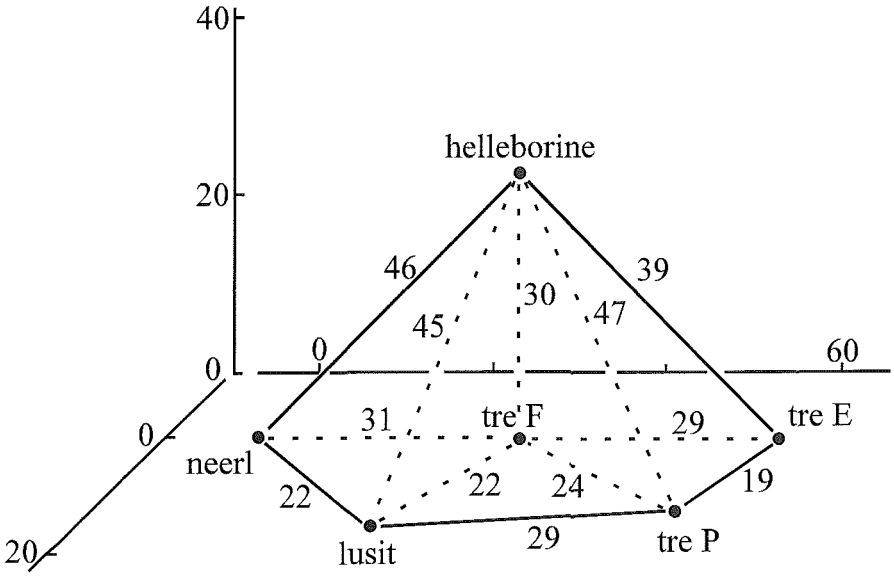

Fig. 3. - Three-dimensional representation of the relationships between six sets of samples; based on GÖLZ and REINHARD distances.

In view of the above facts, we could consider „*E. tremolsii* - France" as having an intermediate position between *E. neerlandica* and *E. tremolsii* - Spain, or between *E. neerlandica* and *E. tremolsii* - Portugal, or between *E. lusitanica* and *E. tremolsii* - Spain. All three comparisons have been tested on a scale representation (TYTECA 1994). The most elegant representations, that is, those with the fewest excentric points, are the first and third ones (Figs. 4 and 5). This could be interpreted as the fact that the French *E. tremolsii* populations should be regarded as transitional from the Spanish („true") *E. tremolsii* populations to the very extreme *E. lusitanica* and/or *E. neerlandica*. More will be said on that subject in Section 4.2.

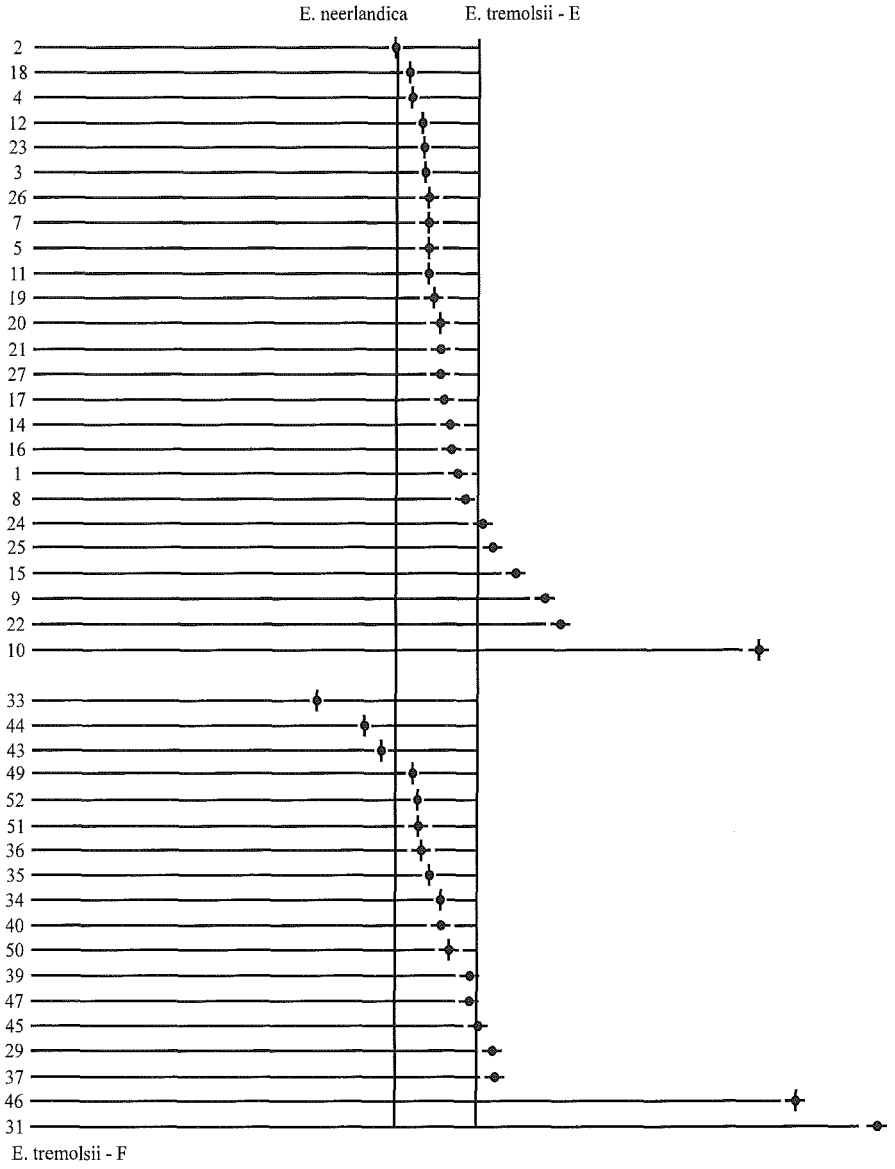

Fig. 4. - Scale representation of the characters of the French *E. tremolsii* populations with respect to those of the *E. neerlandica* and the Spanish („E") *E. tremolsii* populations. Only characters significantly distinct (at the 99 % level) from those of one or both of these two species are represented; the vertical and horizontal bars crossing the circles indicate significant distinction with respect to the Spanish *E. tremolsii* and *E. neerdandica*, respectively. The character numbers refer to Table 2.

3.3. Identification of French „*E. tremolsii*" populations using quantitative data

For the five species of the *E. helleborine* group, Table 5 gives variation intervals of characters that appear to offer a good compromise between, on the one hand, a sufficiently general discriminating power (accounting for the results obtained in TYTECA & DUFRÊNE 1994) and, on the other hand, easy use for field identification and research. Two options are provided for *E. tremolsii*, one based on the mean of Spanish and Portuguese populations, the other based on the whole set of populations, including French ones. The logic of that procedure will appear more clearly hereafter.

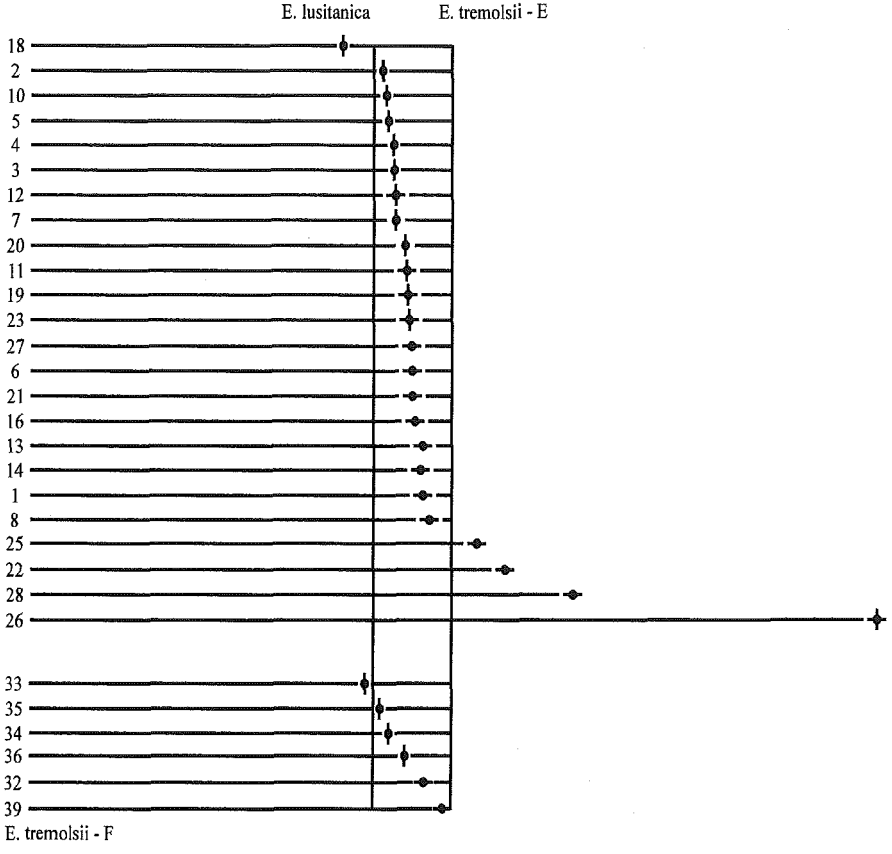

Fig. 5. - Scale representation of the characters of the French *E. tremolsii* populations with respect to those of the *E. lusitanica* and the Spanish („E") *E. tremolsii* populations. Only characters significantly distinct (at the 99 % level) from those of one or both of these two species are represented; the vertical and horizontal bars crossing the circles indicate significant distinction with respect to the Spanish *E. tremolsii* and *E. lusitanica*, respectively. The character numbers refer to Table 2.

TABLE 5. - Intervals of values (mean ± 1 standard deviation) of 16 morphological characters. Values for *E. tremolsii* based on Spanish and Portuguese samples [„E.tre(E-P)"], and on Spanish, Portuguese and French samples [„E.tre(E-P-F)"].

	E.hel	E.dis	E.nee	E.tre (E-P)	E.tre (E-P-F)	E.lus
Number of leaves	5-9	3-6	5-9	7-11	6-10	5-8
2nd leaf length/width	1.3-2.2	1.1-1.7	1.4-1.9	0.9-1.3	0.9-1.5	1.1-1.7
Longest leaf length (cm)	8.8-12	4.8-6.6	4.3-6.9	5.9-8.4	5.4-8.0	4.5-6.6
Heigth lst leaf - soil (cm)	6.2-13.0	3.0-9.0	0.6-3.6	0.4-5.3	1.2-6.1	1.7-4.8
Plant heigth/stem diameter	180-274	107-148	103-134	100-140	100-167	114-172
Number of flowers	16-45	10-48	12-44	24-46	20-44	10-26
Plant heigth/upp. internod.	5.9-12.7	4.8-8.6	4.6-12.1	7.0-16.6	6.3-13.9	4.3-10.9
Number flowers/cm inflor.	1.0-2.1	1.2-2.6	1.6-2.9	1.6-2.6	1.4-2.4	0.8-2.0
Bract length (mm)	15-37	21-38	11-20	21-34	17-33	14-24
Bract length/width	3.8-5.4	4.2-5.8	3.1-4.3	3.1-4.5	3.2-4.5	2.9-4.2
Sepal length/width	1.8-2.2	1.8-2.0	1.8-2.2	1.7-2.0	1.7-2.0	1.6-2.0
Hypochilium width (mm)	4.5-5.3	4.0-5.2	3.8-4.6	4.5-5.5	4.4-5.7	4.1-5.4
Hypochilium depth (mm)	3.1-3.9	2.8-3.7	2.7-3.2	3.0-3.8	3.0-3.9	2.7-3.6
Lip constriction (mm)	.54-.94	.41-.67	.64-1.0	1.2-2.0	.89-1.8	1.2-2.0
Epichile length (mm)	4.1-5.2	4.0-4.5	3.8-4.5	4.5-5.9	4.3-5.7	3.7-4.9
Epichile width (mm)	4.8-5.8	3.8-4.6	5.2-6.1	5.2-6.8	5.3-6.8	4.7-6.1

Table 6 gives a summary of the classification of the French populations identified so far as *E. tremolsii* into one of the five taxa, in each of the following three contexts : (1) using the linear discriminant functions computed in TYTECA & DUFRÊNE (1994) (recall that the French *E. tremolsii* populations did not make part of that study), (2) using the characters of Table 5 with *E. tremolsii* averages based on Spanish and Portuguese samples, and (3) using the characters of Table 5 with *E. tremolsii*

averages based on all samples, including French ones. In the latter two contexts, the results are simply obtained by counting the number of times each character of each individual plant of the sample matches the interval of Table 5, and by summing over all indicated characters; the taxon that obtains the largest sum in elected for that plant. This is of course a strong simplification with respect to the discriminant functions but, as said above, the emphasis is on easy field identification and on an understanding of the behaviour of the French „E . tremolsii" samples.
The results obtained in Table 6 indicate that the situation is far from being simple for the latter category of samples. While the other samples (not shown in Table 6) are „correctly" classified on the whole, even with restricted sets of characters (TYTECA & DUFRÊNE 1994), the identification is rather erratic for the French „tremolsii" in the first two of the three aforementioned contexts (first two columns of Table 6). Only when the third context is considered, is there more stability, but that could be expected as an a posteriori result (recall the definition of the third context), and the number of individuals classified as *E. tremolsii* in those samples then only ranges from 50 to 67 %, which is far from the 100 % sometimes observed in other samples. These results strongly plead in favour of the consideration of additional characteristics, e.g. qualitative, ecological and/or phenological. This will be further discussed in section 4.2.

TABLE 6. - Identification of the French „*E. tremolsii*" individuals in three different contexts (see text). For each population, the table gives the species most often elected and the corresponding %.

		Discrim. function	16 char. trem E-P	16 char. trem E-P-F
Beaufort		trem (50)	lus (56)	trem (58)
Curnier		neer (63)	lus/trem (38)	trem (50)
Pernes		neer (50)	trem (50)	trem (67)
Grime		neer (80)	hell (35)	trem (65)
Gratteloup		neer (83)	trem (50)	trem (50)
Total (%)	hel	5.3	15.4	8.8
	nee	**63.2**	14.0	12.7
	tre	15.8	**37.7**	**58.8**
	lus	13.2	**32.0**	18.9
	dis	2.6	0.9	0.9

3.4. Qualitative characters

Table 7 shows the results obtained for a few qualitative characters observed in all studied samples, according to the scoring procedure depicted in Table 3. Fig. 6 gives the result of a principal component analysis performed with the data of Table 7. On the whole, it appears that the French *E. tremolsii* populations show better qualitative resemblance with the other *E. tremolsii* populations than with the other sets of samples.

4. Discussion

4.1. *Epipactis distans*, *E. lusitanica* and *E.neerlandica*

These three taxa are relatively well distinct, not only on morphological ground, but also on ecological and phenological bases. From a multivariate viewpoint, each of them readily separates from the other taxa in the *E. helleborine* group. This was detailed in TYTECA & DUFRÊNE (1994) and also follows from the results of sections 3.1 and 3.4, though in this research the inclusion of the French *E. tremolsii* samples somewhat complicated the picture as regards *E. neerlandica*. However, for the latter taxon, there are definite ecological and phenological arguments to support the distinction.

TABLE 7. Averages of coefficients reflecting qualitative characteristics, defined in Table 3, for seven groups of samples.

	Leaves arrang.	Leaf margins	Ovary pubesc.	Pedicel color	Flower opening	Epichil. orient.
E. helleborine	2.67	1.71	1.89	2.40	2.23	1.93
E. distans	2.10	3.10	2.00	1.05	1.70	2.00
E. tremolsii - France	2.26	2.87	2.00	2.18	2.29	2.21
E. tremolsii - Spain	2.80	3.87	2.00	1.67	2.47	2.20
E. tremolsii - Portugal	2.79	3.57	1.86	2.00	2.17	1.92
E. lusitanica	2.02	2.87	2.12	1.87	1.45	1.88
E. neerlandica	2.63	2.44	1.94	2.31	2.00	1.75

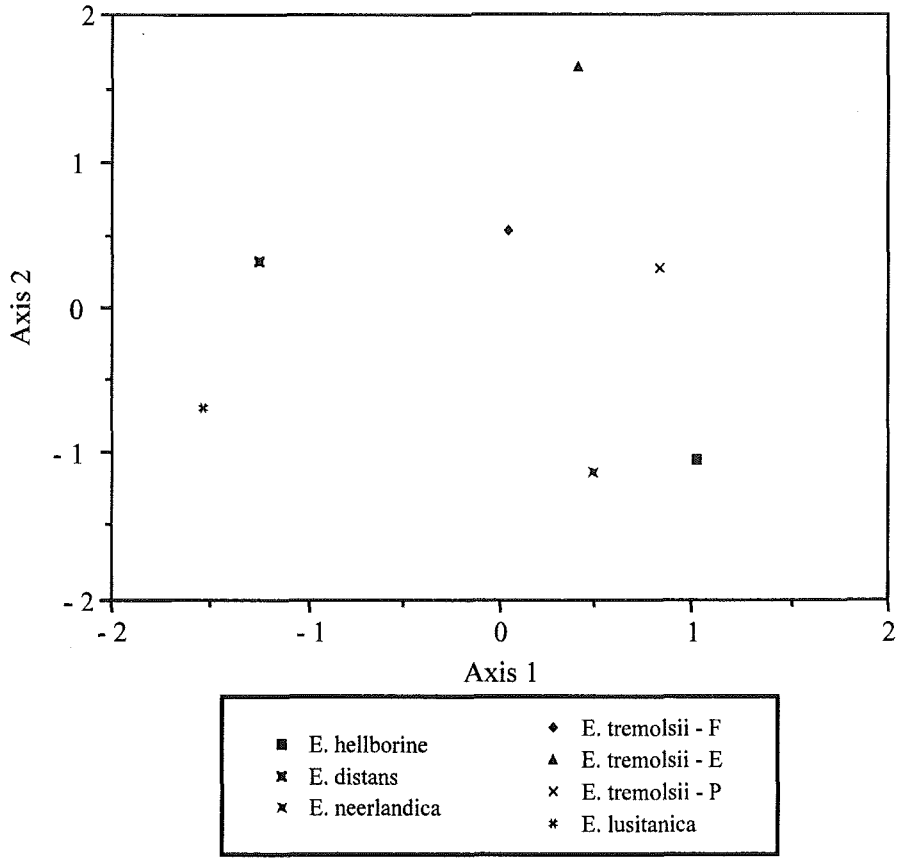

Fig. 6. - Principal component analysis (axes 1 and 2) based on qualitative morphological characteristics of seven sets of samples, performed from the figures of Table 7.

4.2. Identification of the xerophilous, southeastern French populations and further implications

In view of the results reported in section 3.3 (Table 6), one could hesitate about the identity of the French populations so far assigned to *E. tremolsii*. Hereafter a few arguments are developed towards maintaining that identification. First of all, it looks surely unreasonable to classify these populations as *E. helleborine*. Many of the results of section 3 preclude from doing so. Figs. 1 and 2 and Table 6 are especially explicit in this regard : even when considering individual plants, with a simplified identification scheme (Tables 5 and 6), only 5 to 8 plants over 38 can be assigned to that species. Qualitative results suggest the same diagnostic (Table 7 and Fig. 6).

Finally, from ecological as well as phenological standpoints, the plants also significantly differ from the Belgian populations of *E. helleborine*.

When considering character comparisons (section 3.3 and Figs. 4-5), the French populations seem to provide a link between *E. neerlandica*, *E. lusitanica* and the Spanish and Portuguese *E. tremolsii*. This is also apparent in Fig. 3 and is reflected by the results in Table 6. Therefore, one way to view the situation would be to suppress the distinction between *E. tremolsii* and the closely allied *E. neerlandica* and *E. lusitanica*. In that case, the latter two names, more recent, would simply fall in the synonymy of *E. tremolsii*, or these taxa could be considered as subspecies or varieties of *E. tremolsii*. However, this would ignore the phenological and ecological pecularities of *E. neerlandica* and *E. lusitanica*, which on the other hand show marked differences when we consider them with respect to Spanish or even Portuguese populations of *E. tremolsii*. It is not because the differences from the French „*E. tremolsii*" are weaker that we must assimilate all three taxa into only one.

One could be tempted to compare the French populations with the Italian lately described *E. latina* (Rossi & E. Klein) B. & H. Baumann (ROSSI & KLEIN 1987; BAUMANN 1988). While it is clear that there are similarities between both, at least at the ecological level, there is one morphological feature of *E. latina* that would prevent from such an assimilation, namely, the sudden contrast between the large basal leaves and the very small uppermost leaves and bracts, accompanied by the long uppermost internodium (between the last leave and the inflorescence). This attitude might be reconsidered as soon as we have sufficient statistical data from Italy at our disposal.

It would certainly be unreasonable to blindly proceed as Table 6 would suggest, by identifying the French populations individual by individual. This would lead to aberrant situations where three, four or even five species of the *E. helleborine* group would be present in the same biotope, whereas there are obviously no clear discontinuities allowing us to do so. It is much wiser to consider that all plants in each of these populations belong to the same taxon.

Moreover, the results illustrated in Figs. 1 and 2 (and others reported in TYTECA 1994) suggest that in fact all five populations are representatives of one unique species. In this regard, there is one point that is worthy of closer examination, namely, an obvious gradient from sample n° 10 to n° 14. This gradient can easily be paralleled with a geographical gradient, since populations 10 to 14 are arranged by decreasing latitudes. And it is astonishing to observe that the further north we proceed, the closer the plants are to *E. tremolsii* (though the opposite would be expected) while in the south, they resemble more closely to *E. neerlandica*. This appears in both

Figs. 1 - 2 and in Table 6. In a sense, this gives an additional argument to consider all five populations as *E. tremolsii*. Furthermore, qualitatively speaking, the French populations show more affinities with the Spanish and Portuguese *E. tremolsii* than with *E. neerlandica* (Table 7 and Fig. 6).

From a phenological point of view, there is one important aspect that was not yet dealt with, namely, that as well in southeastern France as in Catalunya or in southern Portugal, the flowering period of *E. tremolsii* is contemporary with that of many other orchids (e.g., *Ophrys scolopax, O. apifera, Platanthera bifolia, Anacamptis pyramidalis, Himantoglossum hircinum, Cephalanthera rubra, Serapias* div. sp., ...), whereas *E. neerlandica* is found in flower as one of the latest orchids. The phenological difference is thus quite significant. The biotopes of *E. neerlandica* are also quite peculiar (sand dunes with *Salix repens*), though both that species and *E. tremolsii* can be considered as well adapted to xeric and sunny situations, contrasting in that respect with *E. helleborine*. To close that point, let us mention that *E. lusitanica*, while flowering at about the same period as *E. tremolsii* in southern Spain and Portugal, has ecological preferences that are quite distinct in these regions. However, as we proceed towards southern France or northern Portugal, *E. tremolsii* appears occasionally to show an ecological behaviour that recalls that of *E. lusitanica*, while remaining morphologically closer to the southern Portuguese or Catalonian *E. tremolsii*. One could therefore infer that the morphological differenciation between *E. tremolsii* and *E. lusitanica* can only be considered achieved in southern Spain and Portugal (where it parallels the ecological differenciation), and that *E. lusitanica* probably does not yet (?) exist elsewhere.

From the above discussion, we can conclude that the analysed southern French, xerophilous populations can reasonably be determined as *E. tremolsii*. The latter can be considered as a „big" species, with ample morphological as well as ecological variations, while *E. lusitanica* and *E. neerlandica* can be viewed as more specialised entities, with a narrower morphological and ecological amplitude, and can be called „small", local species. The taxonomic rank of species is preferable to that of subspecies, since there is partial sympatry (between *E. lusitanica* and *E. tremolsii*), while considering *E. neerlandica* as a subspecies of *E. tremolsii* would be somewhat premature, at least as we do not yet know where the distribution of the latter extends to. One other point implied by the above discussion is that we have to enlarge somewhat the definition of *E. tremolsii* (as suggested in the third context of Table 6), in order to cope with the difficulties observed with the French populations. An identification framework, accounting for all characteristics used so far, that is, morphological (quantitative and qualitative), ecological, phenological and even geographical whenever possible, is provided as a table in TYTECA (1994).

5. Concluding remarks

The final word has certainly not been said about the identification and classification of allogamous populations in *the E. helleborine* group. In this research, we have once again stressed the necessity of a populational and multivariate approach, that is, an approach in which the local population, and not the individual plant, is the operational unit, and an approach that accounts for a sufficient number of characteristics before a decision can be reached on the diagnostic. We have also recognised the need to incorporate other considerations, ecological and phenological, into the characteristics to be taken into account for identifying a species. Now there are obviously other factors to be taken into account, such as caryological and molecular factors, but these could hardly participate in field investigations, and would only contribute to a better knowledge of the species as well as provide a means of checking field determinations. Beside the research on molecular and caryological aspects, there are other important questions that still need to be addressed :

- The distribution of *E. tremolsii* remains largely unknown. While it can reasonably be admitted that the distribution covers whole Portugal, where does it stop in central Spain and in southern France ? Are the localities in Vercors(dept. Drôme) the northernmost of that taxon, as they are for many other mediterranean species ? An hypothesis that is here tentatively considered, and should be checked in the next years, is that *E. tremolsii* could have (at least in France) a distribution area similar to those of typical mediterranean orchids such as *Barlia robertiana*, *Ophrys arachnitiformis* or *O. bertolonii* sensu latissimo, as results of cartographic investigations would tend to indicate (JACQUET 1988).

- The same type of remarks is certainly true for the other three taxa (*E. distans*, *E. lusitanica* and *E. neerlandica*), while connexions with other allied, neighbouring taxa should be investigated, such as, in priority, *E. latina*, which shows obvious (at least ecological) similarities with *E. tremolsii*.

- Closer attention should be directed towards situations such as described in Sardinia (GIOTTA & PICCITTO 1993), where *E. helleborine* seems to coexist with *E. tremolsii* and various intermediate forms. Clearly, such situations should be studied, not only from a multivariate standpoint, such as performed in the present research, but also with more sophisticated tools such as molecular analysis.

- In connexion with the latter point, there is certainly one aspect worthy of investigation, namely, rigorously establishing the correlations between ecological characteristics, morphological aspects, and with the taxon identity. In this regard, one point remains to investigate, that is, now that we have

given *E. helleborine* a somewhat more restricted definition (see TYTECA 1994), will all populations living in the most xerophilous situations be identified as one of the other taxa (in France, *E. tremolsii* or *E. neerlandica*)? It is probably significant to observe that in northern France and in Belgium, really xerophilous forms of *E. helleborine* s.l. are rather rare, with the exception of *E. neerlandica* populations, whereas in southern France and elsewhere in the Mediterranean region, where conditions are much more stringent, xerophilous populations are more frequent (and are probably to assign all to *E. tremolsii*). Moreover, during years with dry spring conditions, as was the case in 1993, *E. helleborine* populations in the north, even under shady conditions, were deeply affected, while the xerophilous populations (*E. tremolsii* and *E. neerlandica*) were almost untouched. The latter observation indeed pleads in favour of the splitting into distinct taxa.

Acknowledgements

This research greatly benefitted from discussions with, among others, Jean-Louis GATHOYE (Univ. of Liège), Marc DUFRÊNE and Philippe GOFFART (Univ. of Louvain-la-Neuve). The former also actively participated in the data collection program (see Table 1). For field investigations I am indebted to, among others, Edouard CHAS (Gap), Pierre DELFORGE (Rhode-Saint-Genèse), Jean and Pierre DEVILLERS-TERSCHUREN (Inst. Royal des Sc. Natur. de Belgique, Brussels), Roland MARTIN (Avignon) and Eduardo and Manuela SAMPAIO FRANCO (Lisboa).

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