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Leaf morphology and bud-burst variation in *Ulmus minor* from Italy and France

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Abstract

More than 40 *Ulmus minor* clones were characterised on the basis of 10 foliar morphological traits and budburst phenology in two successive years. Ramets, 4- and 5-years-old, were obtained by means of self-rooted cuttings from elms located in northern, central and southern Italy and in France. Measurements were taken in an open field in the vicinity of Florence (Italy). The morphological traits were found not to be appropriate for describing the variability between clones of different origins, even if some of these traits indicated a xeric adaptation in clones originating from southern Italy. On the contrary, the phenological traits were valid describers of the origin of the clones. Indeed, the southern-Italian clones flushed earlier than the others, while the French clones were more delated. A comparison of the results of the two years showed that the chilling requirements on the trial site were not satisfied. The actual state of knowledge regarding dormancy in the *Ulmus* genus does not enable to speculate further on the results of this research. Morphological characters seem to show a greater phenotypic plasticity with respect to phenological traits.

Key words: field elm clones, phenology.

Resumen

Variaciones en la morfología de las hojas y brotes de yema de *Ulmus minor* en Italia y Francia

Más de 40 clones de *Ulmus minor* han sido caracterizados basándose en 10 características morfológicas foliares y en la fenología de la apertura de yemas foliares en dos años sucesivos. Los ramets, de 4 y 5 años de edad, se obtuvieron por medio de estaquillas procedentes de olmos localizados en Italia septentrional, central y meridional, y en Francia. Las mediciones se realizaron al aire libre en las cercanías de Florencia (Italia). Se encontró que las características morfológicas no fueron adecuadas para describir la variabilidad entre clones de distintos orígenes, incluso cuando estas características indicaban, en clones del sur de Italia, una adaptación a condiciones xéricas. Por el contrario, las características fenológicas sirvieron como descriptores válidos del origen de los clones. De hecho, los clones de Italia meridional brotaron antes que los otros, mientras que los clones franceses fueron más tardíos. La comparación de los resultados de ambos años mostró que los requerimientos de frío no fueron satisfechos en la parcela de ensayo. El actual estado de conocimiento en relación con la quiescencia en el género *Ulmus* no permite realizar conjeturas con los resultados de este estudio. Las características morfológicas parecen mostrar una mayor plasticidad fenotípica que las características fenológicas.

Palabras clave: olmo campestre, fenología.

Introduction

During the 20th century, elm-trees suffered heavy losses, due to epidemic waves of Dutch Elm Disease (DED), caused by two closely-related species of fungi: *Ophiostoma ulmi* (Buism.) Nannf. and *O. novo-ulmi* Brasier, the latter species being more aggressive.

In the frame of the RES GEN CT 96-78 EU project (Coordination for conservation, characterisation, collection and utilisation of genetic resources of European elms), some Institutions have set up clonal collections for germplasm conservation and the plant material has been evaluated for the purpose of identifying possible desirable characteristics. In the light of a possible reintroduction of these elms into the environment, it is of interest to characterise them and to assess their adaptability and phenotypic plasticity.

Leaf morphology is usually closely related to the growing environment. Richens (1983) suggested that leaves provide the most useful characteristics to distinguish between different elms. In addition, leaves also provi-

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de separate and precisely measurable characteristics that are independent of one another, but that are relatively constant for a given elm (Richens, 1983). The timing of vegetative budburst is considered to be of primary concern in evaluating the risk of damage from spring frost. Moreover, this knowledge is important in assessing consequences of a climatic change: rapid climatic warming is likely to affect clone adaptation to the local climatological situation (Kramer, 1997).

The various phenological models utilised (Kramer, 1994) have often shown an appreciable degree of accuracy in predicting the date of budburst, at least on a local scale. However, wide-scale geographic predictions require a more detailed knowledge of the complex relations between the various environmental factors that control the growth rhythm in the different species (Hunter and Lechowicz, 1992). In particular, dormancy release and budburst depend greatly on winter chilling and on the thermal time (Vegis, 1964; Campbell, 1978; Campbell and Sugano, 1979; Lavender, 1980; Thomson and Moncrieff, 1982; Heide, 1993a). However, in some species, this response is modified by the photoperiod (Wareing, 1953; Campbell and Sugano, 1979; Heide, 1993b). Unfortunately, the literature does not contain any indications in regard to *Ulmus minor*.

The aim of this paper is to study leaf morphology and the timing of budburst in several *U. minor* clones from Italy and France.

Material and Methods

In 1998, 42 *Ulmus minor sensu latissimo* clones were planted in the field. 19 originated from northern Italy, 13 from central Italy, 6 from southern Italy, and 4 from France (Normandie, Bretagne, Aquitaine, Midi-Pyrenees). A completely randomised design (4 hardwood cuttings for each clone) was used, in a homogeneous flat land belonging to the «Monna Giovannella» experimental farm at Antella, near Florence (43°43'N 11°22'E; 170 m a.s.l.). Cuttings were planted spacing 1 m × 4 m. *U. glabra* seedlings were planted as a border, in order to avoid side-effects.

Leaf morphology

In June 2001, for each clone 4 short shoots, one from each ramet, were harvested, dried and scanned on both sides. According to Richens (1983) and Jeffers (1999) leaves from short shoots can be used for typical comparitive studies among elms. In particular, all the characteristics of the second and third leaves from the shoot apex were usually very similar (Jeffers, 1999). Thus, whenever possible, the second leaf from the apex was chosen for comparative measurements. For this pourpose, the leaf length, breadth, base asymmetry —defined as the distance between the lowest points on each side of the leaf blade divided by the leaf lenght, Richens, 1983—, and the petiole length were measured; similarly the breadth, length and depth of the primary teeth, on the leaves at a random point in their sub-apical region. Additional measurements, leaf area and two ratios were also considered: leaf thinness index, (ratio between length and breadth of the leaf), and tooth depth index (ratio between the depth of the tooth and half the breadth of the leaf). In order to evaluate the degree of variability of the morphological traits a nested analysis of variance was calculated (provenances, clones within provenances, ramets within clones), according to the following model:

$$Y_{ijk} = \mu + O_i + C_{ij} + R_{ijk}$$

where Y is the morphological trait of the *k*th ramet of the *j*th clone of the *i*th provenance, O is the effect of the provenances of the clones, C is the effect of the clones within the provenances and R for the ramets within the clones.

Phenological notations

From mid-March to early May during 2000 and 2001, budburst phenology was observed weekly in all ramets. Observation were carried out until plants had unfolded their leaves, according to the following scale: 1 buds dormant; 2 buds swollen, but scales still closed; 3 budburst: scales open, and extremities of the first leaves visible at the apex of the buds; 4 extremities of all leaves out; 5 two leaves or more completely spread out. A plant was considered to reach a certain stage (from 1 to 5) as soon as more than 50% of the lateral buds showed that stage. In order to establish the lateness/earliness of each clone, three criteria were considered: the mean phenological stage reached by each clone at the date of highest overall variance for each year of observation (criterion 1); the mean number of days from January 1 to reach stage 3 (bud burst) for each clone in each year (criterion 2); and mean thermal time of each clone in each year (accumulated day degrees > 5°C received by the plants from January 1 to the date of bud-

burst) (criterion 3). In order to evaluate the degree of variability in timing of bud burst a nested analysis of variance was calculated (provenances, clones within provenances, ramets within clones), with a model similar to that used for morphological traits.

Results

Leaf morphology

Degrees of variability among clones were revealed by analysis of variance (Table 1). Independently of their origin, some clones showed significant differences in leaf measurements and derived indexes. The main significant differences were found among base asymmetry (in plants originated in northern Italy were more asymmetric than the southern ones), length of the petiole (in plants originated in France, petioles were shorter than those of northern and central Italy), the thinness index (the leaves of the plants originated in south Italy were thinner than the others) and the tooth depth index (the teeth of the plants originated in south Italy were deeper than those of the northerners, and those of France were less deep) (Table 2).

Phenological notations

As shown by ANOVA test, significant differences were noted between the clones and between their ori-

Table 1. Nested analysis of variance considering each leaf morphological trait among clones as dependent variable and provenances, *Ulmus minor* clones within provenances and ramets within clones sources of variation

Trait	Source	df	SS	MS	F-ratio
Teeth breadth	Clones	38	32.52	0.86	3.076**
	Provenance	4	1370.56	342.64	1231.570**
	Error	126	35.05	0.28	
Teeth length	Clones	38	34.41	0.9055	2.823**
	Provenance	4	1407.734	351.9335	1097.238**
	Error	126	40.414	0.3207	
Teeth depth	Clones	38	14.976	0.394	2.442**
	Provenance	4	372.051	93.013	576.302**
	Error	126	20.336	0.161	
Length	Clones	38	6343.4	166.93	4.372**
	Provenance	4	298764.6	74691.15	1956.086**
	Error	126	4811.2	38.18	
Breadth	Clones	38	2607.3	68.61	5.595**
	Provenance	4	122715.6	30678.89	2501.533**
	Error	126	1545.3	12.26	
Leaf area	Clones	38	4717357	124141	5.652**
	Provenance	4	57694305	14423576	656.730**
	Error	126	2767303	21963	
Base asymmetry	Clones	38	98.6958	2.5973	3.517**
	Provenance	4	626.7937	156.6984	212.194**
	Error	126	93.0468	0.7385	
Petiole length	Clones	38	445.722	11.730	6.396**
	Provenance	4	5598.181	1399.545	763.142**
	Error	126	231.074	1.834	
Leaf thinness index	Clones	38	3.4956	0.0920	4.414**
	Provenance	4	415.3151	103.8288	4982.095**
	Error	126	2.6259	0.0208	
Tooth depth index	Clones	38	0.096185	0.002531	2.980**
	Provenance	4	2.119900	0.529975	623.909**
	Error	126	0.107030	0.000849	

SS: sum of squares. MS: mean square. df: degree of freedom. ** Significant for $p < 0.001$.

Table 2. Mean dimensions calculated for each morphological characteristic in each of the provenances. Means sharing the same letters do not differ for Tukey's test

Trait	France	Italy		
		North	Central	South
Teeth breadth	2.88ab	2.72a	3.04b	2.87ab
Teeth length	2.90	2.84	2.96	2.92
Teeth depth	1.42	1.43	1.52	1.62
Length	42.52	42.19	42.00	42.23
Breadth	28.24	27.28	27.10	25.15
Leaf area	612.99	592.60	584.41	549.00
Base asymmetry	1.61b	2.21b	1.71b	1.59a
Petiole length	4.19a	6.44c	5.57b	4.78ab
Leaf thinness index	1.53a	1.55a	1.55a	1.69b
Tooth depth index	0.10a	0.11a	0.11ab	0.13b

gins, independently of the criterion used (Tables 3 and 4). *A posteriori* comparison of the four origins of the clones, calculated by means of the Tukey-Kramer test, always led to the same result for both years: flushing was significantly earlier in elms from southern Italy and later in those from France, while plants from northern and central Italy did not differ among themselves, but were significantly different from those from southern Italy and France. The same conclusions were reached while examining the phenological phase reached on the day of maximum variance. In 2001, this day was reached fifteen days earlier than in 2000. The thermal time needed to reach phase 3 in both 2000 and 2001 was significantly shorter for the southern Italian clones and longer for the French clones. Plants from northern and central Italy did not differ among themselves, but were significantly different from those of southern Italy and France.

Discussion

The morphological traits and ratios utilised proved to be functional for elm species identification and for the characterisation of local populations (Richens, 1983; Richens and Jeffers, 1975; Jeffers, 1999). However, they were found to have little utility for comparison and for describing variation within *U. minor* clones of different origin growing in the same site. This situation could reflect inherent uniformity of these traits between the clones, or, conversely, a high plasticity in the leaf morphology. The fact that significant differences existed between clone origins with regard to the two indexes (leaf thinness and tooth dept) was probably the most interesting result. These two inde-

Table 3. Nested analysis of variance of the different phenological criteria used, considering provenances, *Ulmus minor* clones within provenances, ramets within clones as sources of variation

Criterion	Source	df	Sum of squares	Mean squares	F
13 April 2000	Clones	38	75.124	1.9769	4.700**
	Provenance	4	2709.876	677.4690	1610.58**
	Error	126	53.000	0.4206	
29 March 2001	Clones	38	89.315	2.3504	21.937**
	Provenance	4	2135.185	533.7962	4982.098**
	Error	126	13.500	0.1071	
Days to stage 3-2000	Clones	38	9739	256.3	381.0**
	Provenance	4	1341002	335250.5	498425.6**
	Error	126	85	0.7	
Days to stage 3-2001	Clones	38	3184	83.8	125.7**
	Provenance	4	1234125	308531.4	462797.1**
	Error	126	84	0.7	
Thermal time 2000	Clones	38	215104	5661	3856.6**
	Provenance	4	4935540	1233885	840648.3**
	Error	126	185	1	
Thermal time 2001	Clones	38	226183	5952	2038**
	Provenance	4	14553288	3638322	1245730**
	Error	126	368	3	

df = degree of freedom. ** Ssignificant for $p < 0.001$

Table 4. Mean phenological criterion in each provenance. Means sharing the same letters do not differ for Tukey's test

Criterion	France	Italy		
		North	Central	South
13 April 2000	3.12a	3.97b	4.04b	4.62c
29 March 2001	2.62a	3.58b	3.70b	4.66c
Days to stage 3-2000	99.75d	89.76c	88.46b	82.51a
Days to stage 3-2001	91.19c	85.72b	85.56b	82.19a
Thermal time 2000	222.70d	171.67c	164.99b	142.47a
Thermal time 2001	340.45d	303.28c	284.31b	250.42a

xes clearly differentiated from the others the clones of southern Italian origin, which were also characterised by a smaller foliar area. The tendency towards a decrease in foliar dimensions with a decrease in latitude, could be related with the effects that they produced on the boundary layer (Slatyer, 1967), indicating an adaptation to a warmer and/or more xeric environment (Hinckley *et al*., 1981; Pallardy, 1981). Given the modest extent of the differences observed, further research on the subject will be necessary.

Concerning the phenological results, the observed sequence of budburst (earlier in southern Italy clones) can be considered reliable, because of different winter thermal regimes (data not reported) during the two years of observations. The different climatic trend enabled further evaluations. In 2001, a year characterised by a particularly mild winter, the thermal time was found to be 60-75% higher than in 2000. Despite this increment, the flushing date remained unchanged or happened a little earlier. After an increase in the temperatures, this result suggests that, on the trial site, the chilling requirement was not completely satisfied for any of the provenances (Murray *et al*., 1989). The high values of the thermal time to budburst, above all during the second year of observations, confirm this evaluation. The differences between the 2000 and 2001 in the phenological stage values obtained from different origins during the day of maximum variance —which occurred, as it is well to remember, 15 days early in 2001, compared to 2000— do not seem to contradict the substantial stability of the budburst mentioned earlier. For the French clones, the stage reached in 2001 was lower, compared to 2000 (2.6 vs. 3.1). For southern Italy, instead, it was identical (4.6 vs. 4.6), just as for the days to budburst (82.1 vs. 82.5). This can probably be explained by the differing influence of the temperature during different phenological stages: shoot elongation for southern Italian clones (stage 4.6-4.7) and bud opening for the French clones (stage 2.6-3.1). Furthermore, the same transition times between the various phenological stages gave very different results in the two provenances examined (data not reported). When this parameter (stage transition) was examined, as for example in *Fagus sylvatica* L. (Falusi and Calamassi, 1990; 1996), differences of this type, linked to provenance and to the thermal requirement for the budburst, were brought out.

The most noticeable result of this research, i.e. the early flushing of the southern Italy clones, is not univocally confirmed by references relative to other tree species (Champagnat, 1993; Farmer, 1983). In previous research, the results obtained are often not in agreement (Wuehlisch *et al.*, 1995; Falusi and Calamassi, 1996; Borghetti *et al.*, 1993; Heide, 1993b). The growth of trees with different latitudinal and/or altitudinal origins on a certain site that is colder or warmer than the original, could lead to different effects on flushing, depending upon where —on the curve of thermal time-chill days— the plant will lie in the new site. In investigations of the prediction capabilities of phenological models depending on a rise in the mean temperature, the variations in the budburst date of the different species under test were systematically different, at times even opposite (Chuine *et al.*, 2000; Sparks and Carey, 1995; Menzel and Fabian, 1999). Murray *et al*. (1989) calculated that, in different localities, an increase in the mean temperature of 1-3°C could give opposite results for a given species.

The present lack of knowledge regarding the characteristics of dormancy and its evolution in gen. *Ulmus* limits a more in-depth interpretation of these results. Several indirect indications may be inferred from the research on phenological models (Hunter and Lechowicz, 1992; Sparks and Carey, 1995), which nevertheless should be considered with caution regarding the weak relationship existing between the prediction accuracy of a model and the biological bases of the phenological responses of the plants. Direct indications are few and far between, and are limited to *U. americana*. Downs and Borthwick (1956) consider this species to be among the least sensitive species to short days as inductive agents of the growth cessation. Roberts and Main (1965) consider winter chilling to be the primary agent in the release of dormancy, and

have identified a weak surrogate effect of long days in the case of low levels of chilling. The results of these authors, which again concern *U. americana*, are similar to —even if less stressed than— those of Garber (1983) on *Pinus taeda* and of Falusi and Calamassi (1996, 1997) on *Fagus sylvatica*. Future research should focus on these issues, in order to assess the ability of this species to adapt to a changing climate.

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