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Variation in timing of bud-burst of *Ulmus minor* clones from different geographical origins

Luisa Ghelardini, Mauro Falusi, and Alberto Santini

Abstract: The vegetative bud-burst phenology of field elm (*Ulmus minor* Mill.) clones originating from a large geographical range within the species natural area was studied at three European sites over a 5-year period in ex situ collections constituted for the conservation of elm genetic resources. At all the sites and in all the years of study, the date of bud burst and the required thermal time to bud burst were directly related to latitude and altitude of origin of the clones. Nevertheless, the differences among origins were reduced with increasing the chilling duration, suggesting that the clones from more northern latitudes and higher altitudes required longer chilling for dormancy release. Analyses of variance performed on selected groups of clones from different latitudinal origins showed that the order of bud burst was stable among years and that the thermal time requirements decreased in all the groups with increasing chilling, with significant differences among groups; southern clones satisfied their chilling requirements after a shorter chilling duration.

Résumé : La phénologie de l'éclosion des bourgeons végétatifs de clones d'orme champêtre (*Ulmus minor* Mill.) provenant d'une vaste étendue géographique dans l'aire de répartition naturelle de l'espèce a été étudiée dans trois sites européens. L'étude s'est poursuivie pendant cinq ans dans des collections ex situ constituées pour la conservation des ressources génétiques de l'orme. Dans tous les sites et durant toutes les années de l'étude, la date du débourrement et le temps thermique nécessaire au débourrement étaient directement reliés à la latitude et à l'altitude du point d'origine des clones. Néanmoins, les différences entre les provenances diminuaient avec l'augmentation de la durée de la période de froid, ce qui indique que les clones qui proviennent des latitudes plus boréales ou d'endroits plus élevés en altitude exigent une période de froid plus longue pour la levée de la dormance. Les analyses de variance réalisées sur des groupes choisis de clones provenant de différentes latitudes ont montré que l'ordre du débourrement était stable d'une année à l'autre et que le temps thermique requis diminuait dans tous les groupes avec l'augmentation de la période de froid. Il y avait des différences significatives entre les groupes dont les clones provenant des régions situées les plus au sud qui satisfaisaient leur besoin en froid après une plus courte période de froid.

[Traduit par la Rédaction]

Introduction

Dutch elm disease (DED), perhaps the single most infamous and destructive disease to attack forest trees (Gibbs 1978), is still a threat to European elm genetic diversity. As a result of two consecutive epidemics (in the 1930s and 1970s) of enhanced virulence caused by *Ophiostoma ulmi* (Buisman) Nannfeldt and *Ophiostoma novo-ulmi* (Brasier 2000), the great majority of mature field elm (*Ulmus minor* Mill.) trees in Europe has been wiped out.

The conservation of native European elm genetic resources is currently realized by means of ex situ clone collections (EU Res-Gen project CT96-78). Clones maintained in collections are studied for different adaptive characters in

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the perspective of their reintroduction into the environment. Timing of bud burst has been widely used as a reference for adaptedness and is especially important in evaluating the risk of damage from spring frost. Moreover, recent results suggested that, in field elm, the precocity of bud burst may possibly relate with avoidance of DED (Santini et al. 2005).

Timing of bud burst is thought to be an adaptive character under strong genetic control (Worrall and Mergen 1967; Worrall 1983; Von Wuehlisch et al. 1995), and it has been shown to vary along geographical gradients within the distribution area of many species (Campbell and Sugano 1979; Von Wuehlisch et al. 1995; Falusi and Calamassi 1997). Other studies showed an intraspecific variation in the control of dormancy release, because of differences in chilling requirements among ecotypes from different both latitudinal (Kriebel and Wang 1962; Nienstaedt 1967; Farmer 1968; Myking and Heide 1995) and altitudinal origins (Myking 1997). Although little is known about control of dormancy release and bud burst in elms, exposure to low temperatures during winter is thought to be the main factor for the breaking of dormancy in most tree species, including *Ulmus americana* L. (Perry 1971; Roberts and Main 1965). Whenever the effects of temperature have been quantitatively evaluated, strong correlations emerged between timing of bud burst and accumulated thermal time above a threshold tem-

perature (Campbell 1974; Owens et al. 1977; Eriksson et al. 1978; Campbell and Sugano 1979). As previously reported for several other tree species that require chilling to break dormancy (Cannell and Smith 1983; Murray et al. 1989), it has been recently shown that for European *Ulmus* species, the thermal time to bud burst decreases exponentially with increasing winter chilling, down to a minimum constant and species-specific value (Santini et al. 2004). For *U. minor*, Santini et al. (2004) showed that the thermal time to bud burst tends to stabilize when chilling exceeds 90 days, so that this species could be included among the species with short chilling requirement and short dormancy, as defined by Murray et al. (1989). However, these authors emphasized that, in the case of *U. minor* with its broad distibution, it is likely a geographic variation in the relationship between chilling and thermal time to bud burst. To date, reports about the geographical variability in timing of bud burst within the European elm species are also scarce. Whiteley et al. (2003) found the sequence of bud flush of five *Ulmus laevis* Pall. populations from across Europe to follow the latitude of origin, with high-latitude populations flushing first. Differences in timing of bud burst for different field elm varieties are mentioned by Richens (1983). The aim of this paper was to investigate the variability in timing of bud burst of *U. minor* as a function of the geographical origin, by using the existing clone collections established for the conservation and characterization of the genetic resources of European elms.

Materials and methods

Elm material

The original material for most of the clones came from young individuals of natural populations across Europe, and a few clones came from adult individuals that had survived DED epidemic. The experimental material was agamically propagated via cuttings. One- or two-year-old rooted cuttings, about 1.0–1.5 m tall, were planted at 1 m × 4 m spacing in level and homogeneous sites, according to a fully randomized design, with four to eight replicates per clone. The experimental fields were completely cleared, cultivated prior to planting, and weeded and cultivated at least once a year after transplanting.

Phenological notations

The vegetative bud-burst phenology was studied in the field in three clone collections, two in France and one in Italy, over a 5-year period. Data were recorded at (*i*) Nogent-sur-Vernisson (France, 47°51′N, 2°45′E, 130 m asl, climate zone V(IV)2 according to Walter and Lieth 1960) in springs 2000, 2001, and 2002 from a collection of up to 118 clones from France, Italy, Spain, southern Sweden, England, Belgium, Germany, and Greece (latitude range from 37°11′N to 57°22′N; altitude range from the sea level to 900 m) that was established in 1999–2000 (Fig. 1*a*); (*ii*) Guémené-Penfao (France, 47°38′N, 1°50′W, 15 m asl, climate zone V(IV)$_2$ according to Walter and Lieth 1960) in spring 2000 and 2001 from four clones from northern France (N group) and four clones from southern France (S group) planted in 1999–2000 (Fig. 1*a*); and (*iii*) Antella (Italy, 43°43′N, 11°22′E, 170 m asl, climate zone IV$_4$ according to Walter and Lieth

1960) in spring 2000, 2001, 2003, and 2004. Here, two clone banks were set up in 1995 and 1999 for a total of 272 different clones. In the years 2000 and 2001, 65 *U. minor* clones from Italy and France (latitude range from 38°34′N to 47°59′N; altitude range from the sea level to 1100 m) were studied. In 2003 and 2004, 272 clones from Italy, France, and Spain (latitude range from 36°54′N to 49°50′N; altitude range from the sea level to 1200 m) were studied (Fig. 1*b*).

Phenological notations were taken for each tree according to a common protocol from February to May at least once a week using a three-step scale (1, dormant buds; 2, buds swollen, but scales closed; 3, bud scales open, and the extremities of the first leaf visible at the apex of the buds). For each individual tree, the bud-burst date (BBD) was defined as the day since January 1 (Julian day), when one-half of the lateral buds had reached stage 3. For each clone, the mean BBD and the required thermal time to bud burst (TT, accumulated day degrees >5 °C since February 1 to bud burst) were calculated at each location for each year. The duration of chilling (CD, number of days with mean daily temperature ≤5 °C from November 1 of the previous year to bud burst) was calculated at each site for each year. Method and threshold temperature for chilling and thermal time estimation were chosen in agreement with the results of previous comparison analyses (Santini et al. 2004). November 1 was chosen as the starting date for chilling accumulation, according to Murray et al. (1989), because few chill days occur before this date in central and southern Europe and also because buds may not be dormant in October. This is especially true in the case of elms, which can continue growing into late autumn. February 1 was chosen as an arbitrary date for the TT accumulation, which has been used in the literature (Cannell and Smith 1983), because choosing different starting dates for heat-sum accumulation from January to March do not yield significant differences in the assessment of the relationship between BBD and TT (Castonguay at al. 1984; Nizinski and Saugier 1988). Maximum and minimum daily air temperatures were recorded in the study areas or in their immediate vicinity by normal meteorological stations 2 m aboveground.

Statistical analyses

At Nogent-sur-Vernisson, the effect of the latitude of origin on BBD and TT was studied in 2002 by means of a simple linear regression analysis. The effect of the altitude of origin on BBD and TT was evaluated by means of a simple linear regression analysis in a sample of 11 clones originating from a wide altitudinal range (from 0 to 920 m asl) within a narrow latitudinal range (43°22′N – 44°35′N), because clones from high altitudes were not represented at high latitudes.

At Antella, clones from different latitudes and altitudes were evenly represented; therefore, the effects of the latitude and altitude of origin on BBD and TT were studied by means of multiple regression analyses.

To study the variability of the BBD and the effect of chilling on TT among the years and sites for clones from different origins, four factorial ANOVAs were performed on two selections of clones. At Nogent-sur-Vernisson and at Guémené-Penfao, four clones from northern France (N group) and four clones from southern France (S group) were

Fig. 1. Geographical origin of the clones of *Ulmus minor* studied at (*a*) Nogent-sur-Vernisson, France (47°51′N, 2°45′E, 130 m asl, open arrow) and Guémené-Penfao, France (47°38′N, 1°50′W, 15 m asl, solid arrow) and (*b*) Antella, Italy (43°43′N, 11°22′E, 170 m, shaded arrow).
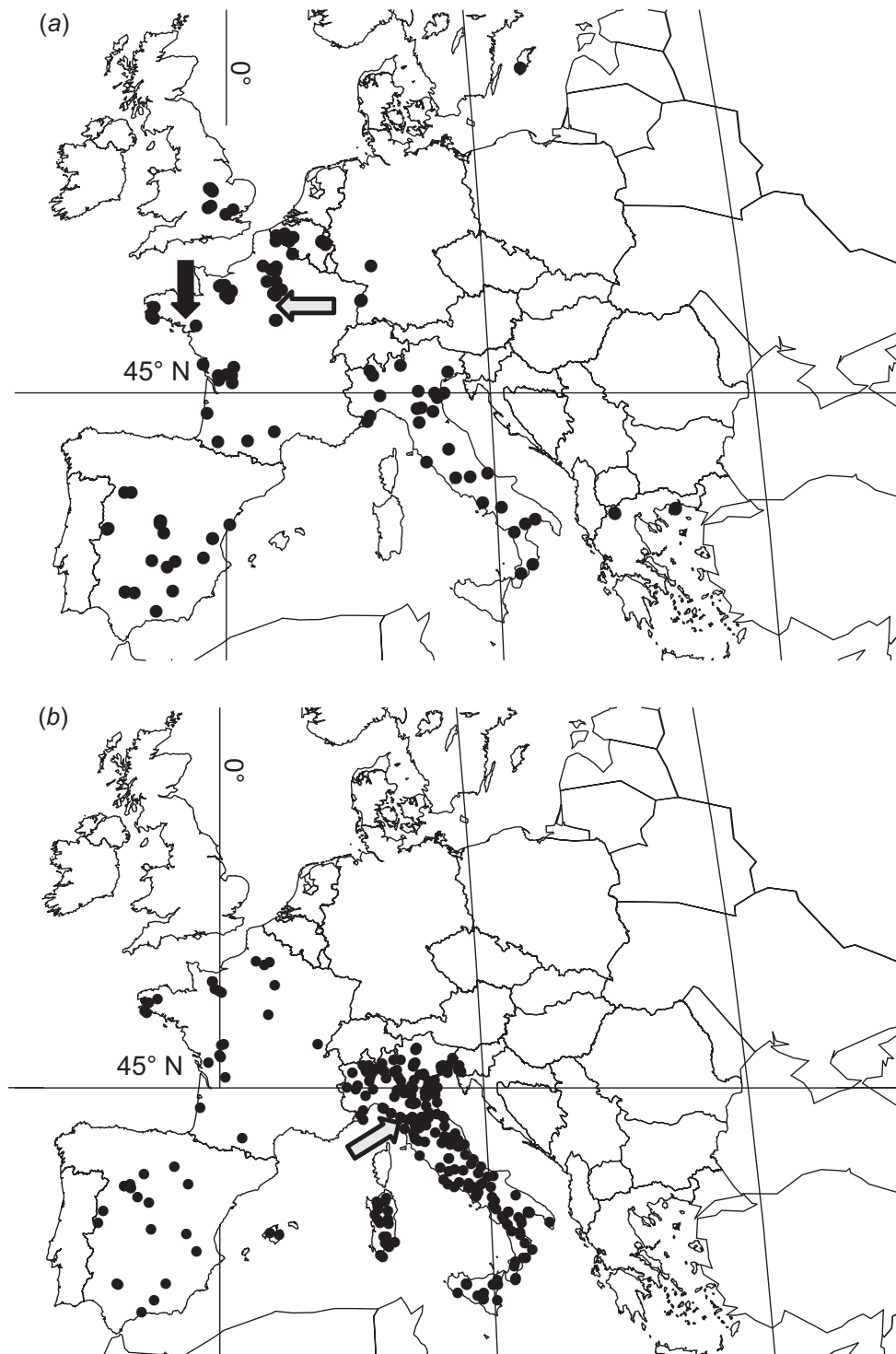
studied. At Antella, eight clones were studied: two from northern France (NF group), two from southern France (SF group), two from northern Italy (NI group), and two from southern Italy (SI group). Two of these clones were the same as those grown on the French sites. All of the clones came from altitudes below 350 m asl (Table 1).

Data collected at Nogent-sur-Vernisson and at Guémené-

Penfao were pooled and subjected to two factorial ANOVAs according to the following linear models:

$$[1] \quad TT_{ij} = \mu + G_i + CD_j + (G \times CD)_{ij} + e_{ij}$$

$$[2] \quad BBD_{ijk} = \mu + G_i + S_j + Y_k + (G \times S)_{ij}$$

$$+ (G \times Y)_{ik} + (S \times Y)_{jk} + e_{ijk}$$

Table 1. Geographical origin of the latitudinal groups of clones used in ANOVA analyses.

Group	Clone	Longitude	Latitude	Altitude (m asl)
Northern group	CEM017	0°29′29″W	49°04′39″N	116
	CEM020	0°29′29″W	49°04′39″N	116
	CEM023	0°33′04″W	49°06′11″N	75
	CEM063	0°20′31″W	48°48′46″N	244
Southern group	CEM361	1°06′17″E	43°22′21″N	270
	CEM362	1°06′17″E	43°22′21″N	270
	CEM370	1°10′00″W	44°23′46″N	8
	CEM400	1°12′06″E	43°15′30″N	305
Northern France group	CEM085	0°09′48″W	48°49′07″	—
	CEM392	4°25′22″W	47°59′12″N	72
Southern France group	CEM370	1°10′00″W	44°23′46″N	8
	CEM400	1°12′06″E	43°15′30″N	305
Northern Italy group	CNR009	8°38′00″E	45°03′00″N	90
	CNR025	9°14′00″E	44°21′00″N	50
Southern Italy group	CNR203	17°06′00″E	40°31′00″N	5
	CNR227	16°05′00″E	38°34′00″N	350

Note: The northern and southern groups were grown and studied at Nogent-sur-Vernisson and at Guémené-Penfao, respectively. The northern France, southern France, northern Italy, and southern Italy groups were grown and studied at Antella. The codes used for identifying clones refer to a European code adopted in the frame of the EU Res-Gen 96–98 Project.

In eq. 1, TT_{ij} is the required thermal time to bud burst in latitudinal group i after number of chill days j, G_i is the fixed effect of latitudinal group i (i = N or S), CD_j is the fixed effect of number of chill days j (j = 28, 33, 41, or 62), $G \times CD$ is the effect of interaction between latitudinal group and number of chill days, and e_{ij} is the random residual. Each chilling duration (j) in eq. 1 refers to a different year at Nogent-sur-Vernisson or Guémené-Penfao (see Table 2). In eq. 2, BBD_{ijk} is the number of Julian days to bud burst in latitudinal group i in site j in year k, G_i is as defined for eq. 1, S_j is the fixed effect of site j (j = Guémené-Penfao or Nogent-sur-Vernisson), Y_k is the fixed effect of year k (k = 2000 or 2001), $G \times S$ is the effect of the interaction between latitudinal group and site, $G \times Y$ is the effect of the interaction between latitudinal group and year, $S \times Y$ is the effect of the interaction between site and year, and e_{ijk} is the random residual.

Data collected at Antella were likewise subjected to two factorial ANOVAs according to the following linear models:

$$[3] \quad TT_{ij} = \mu + G_i + CD_j + (G \times CD)_{ij} + e_{ij}$$

$$[4] \quad BBD_{ij} = \mu + G_i + Y_j + (G \times Y)_{ij} + e_{ij}$$

In eq. 3, G_i is the fixed effect of latitudinal group i (i = NF, SF, NI, or SI), CD_j is the fixed effect of number of chill days j (j = 15, 48, or 63) on variable thermal time. Each chilling duration (j) in eq. 3 refers to a different year at Antella (see Table 2). In eq. 4, G_i is as defined in eq. 3, Y_j is the fixed effect of year j (j = 2000, 2001, or 2004) on variable BBD. In both equations, e_{ij} is the random residual.

Duncan's test was applied for multiple comparisons in all the analyses.

Results

The duration of chilling in the years of study at the different sites is reported in Table 2.

**Table 2.** Duration of chilling at each site over the course of the study.

Site	Year	Chill days
Nogent-sur-Vernisson	2000	62
	2001	41
	2002	67
Guémené-Penfao	2000	33
	2001	28
Antella	2000	48
	2001	15
	2003	52
	2004	63

Note: Chill days is the number of days with mean temperature ≤5 °C since 1 November of the previous year.

At Nogent-sur-Vernisson in 2002, BBD and TT were positively correlated with the latitude and altitude of origin (Fig. 2). Clones from more northern latitudes and higher altitudes tended to flush later and to require greater TT than clones from more southerly latitudes and lower altitudes. Regressions of BBD on latitude and on altitude were highly significant (R^2 = 0.35, $p < 0.001$, and R^2 = 0.63, $p < 0.01$, respectively). Regressions of TT on latitude and on altitude were also significant ($p < 0.001$ and $p < 0.05$, respectively) and explained similar percentages of the observed variance (R^2 = 0.39 and R^2 = 0.53, respectively) (Fig. 2).

The statistical analyses performed on the data collected at Antella confirmed the results obtained at Nogent-sur-Vernisson. The partial correlations of BBD and TT on latitude and on altitude were positive and significant ($p \leq 0.05$) in all the years, although the correlations with latitude were stronger than the correlations with altitude (data not shown). The multiple regressions of BBD and TT on latitude and altitude were all significant ($p \leq 0.05$) in 2000 (R^2 = 0.37 and R^2 = 0.35, for BBD and TT, respectively), 2001 (R^2 = 0.32 and R^2 = 0.34 for BBD and TT, respectively), 2003 (R^2 =

Fig. 2. Bud-burst date (BBD) and thermal time to bud burst (TT) of the *Ulmus minor* clones observed at Nogent-sur-Vernisson in 2002 as a function of latitude (*a* and *c*) and altitude (*b* and *d*) of origin.
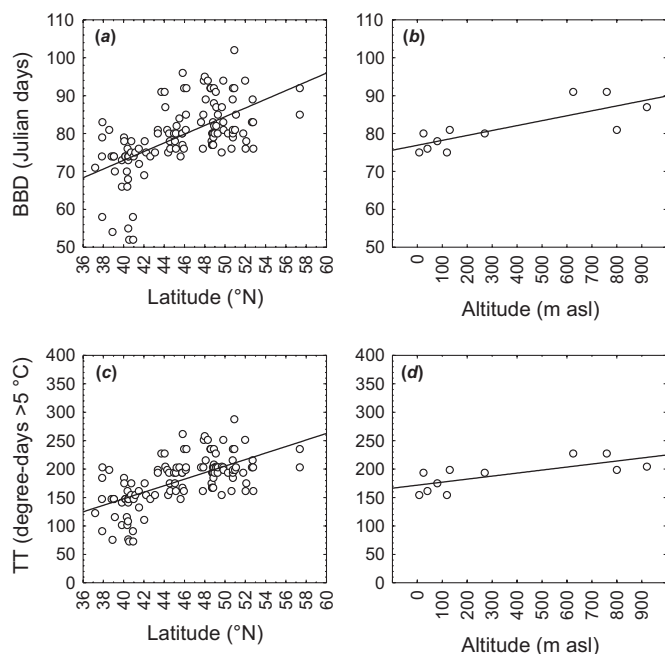
0.30 and R^2 = 0.30, for BBD and TT, respectively), and 2004 (R^2 = 0.27 and R^2 = 0.25, for BBD and TT, respectively) (Figs. 3 and 4).

Differences in the relationship between chilling and TT among the selected latitudinal groups of clones

At Guémené-Penfao and Nogent-sur-Vernisson, TT decreased with increasing number of chill days for both latitudinal groups in all the observed chilling range (Fig. 5*a*). The analysis of variance (Table 3) showed significant main effects of CD and *G* but revealed no differences in the effect of chilling among latitudinal groups (no CD × G interaction, *p* = 0.45). Actually, the N group required significantly greater (*p* < 0.001) TT to bud burst than the S group in all years irrespective of chilling duration. The ratio, R^2, of the model sum of squares to the total sum of squares was 0.90.

The ANOVA of the Antella data set confirmed significant main effects of CD and *G* and further revealed a significant effect of CD × G interaction (Table 3). Groups from different latitudinal origins responded differently to increasing CD (Fig. 5*b*). The TT requirement of SI group significantly decreased from 15 chill days to 48 chill days (*p* < 0.001) but stabilized thereafter with no significant differences (*p* = 0.33) between 48 chill days and 63 chill days. In contrast, the TT requirements of NF, SF, and NI groups also decreased from 15 chill days to 48 chill days (*p* < 0.001) but continued decreasing from 48 chill days to 63 chill days (*p* < 0.001). In all years, the NF group required larger TT than the other groups. However, the gap in TT between NF group and both SF and NI group did not change with increasing the CD, whereas it progressively decreased between the NF and SI groups. The SF and NI groups, which came from a similar latitude range, did not differ at any CD, and their TT

requirements continued to decrease over the observed chilling range (*p* < 0.001). With increasing CD, the difference in TT requirement of both the SF and NI groups in comparison with the SI group progressively decreased until it was no longer significant after 63 chill days (*p* = 1.00 and *p* = 0.99, respectively). The R^2 of the model was 0.92.

Differences in the variation of the BBD among sites and years among the selected latitudinal groups of clones

At Nogent-sur-Vernisson and at Guémené-Penfao, the ANOVA showed significant main effect of *G*, *S*, and *Y* on BBD, whereas none of the interactions was significant (Table 3, Fig. 5*c*). The R^2 of the model was 0.63. On the average, the N group flushed about 10 days later (*p* < 0.01) than the S group. The gap between the BBDs of the N and S groups remained stable at both sites and in all years (no significant *G* × *S* × *Y* interaction). Bud burst occurred 5.7 days later at Guémené-Penfao than at Nogent-sur-Vernisson (*p* < 0.001). On average, bud burst occurred 6.1 days earlier (*p* < 0.001) in 2001 than in 2000. The delay of BBD between the N and S groups was similar in both years (10.15 days and 9.89 days in 2000 and 2001, respectively) and at both sites (10.58 days and 11.71days in 2000 and 2001, respectively). In 2000, both groups flushed later at Guémené-Penfao than at Nogent-sur-Vernisson. In 2001, both groups flushed later at Guémené-Penfao than at Nogent-sur-Vernisson.

The ANOVA for Antella confirmed significant main effects of *G* and of *Y* on BBD and, in addition, revealed a significant *G* × *Y* interaction (Table 3, Fig. 5*d*). The model ratio R^2 was 0.84. The NF group flushed the latest in all the years at Antella. In all years, the SF and NI groups flushed together (no significant differences between their BBDs in 2000, *p* = 0.32; 2001, *p* = 0.85; and 2004, *p* = 0.26) at dates between the other groups. The SI group flushed first in 2000 and 2001; however, in 2004, its BBD was not significantly different from those of the NI and SF groups (*p* = 0.29 and *p* = 0.90, respectively). The earliest BBD was in 2001 for all the groups. Relative changes in the BBD among the other years were different among groups. The latest flushing date of the NF group was in 2000. Thus, the NF group showed a significant advance (4.1 days, *p* < 0.05) in the BBD from 2000 to 2004. Similarly, the SF and NI groups flushed earlier (5.5 days, *p* < 0.01, and 5.7 days, *p* < 0.01, respectively) in 2004 than in 2000, whereas they flushed at the same date in 2001 and 2004 (*p* = 0.18 and *p* = 0.75, respectively). The SI group was substantially different from all the others, since it flushed significantly later (5 days, *p* < 0.01) in 2004 than in 2000.

Discussion

In this study, a significant and stable relationship between BBD and geographical origin of the clones, i.e., the southern and lowland clones always flushed earlier, was observed in two *U. minor* clone collections, one from France and the other from Italy. In the literature, conflicting results have been reported concerning the pattern of bud-burst variation among latitudinal and altitudinal provenances in field testing (Wang and Perry 1958; Worrall and Mergen 1967; Sharik and Barnes 1976; Worrall 1975, 1983). In the genus *Ulmus*,

Fig. 3. Bud-burst date (BBD) of the *Ulmus minor* clones in (*a*) 2000, (*b*) 2001, (*c*) 2003, and (*d*) 2004 in the Antella, Italy, clone collection (43°43′N, 11°22′E, 170 m asl) as a function of latitude (*x*) and altitude (*y*) of origin of the clones. Jd, Julian days (days from the beginning of the year); dd, degree-days.
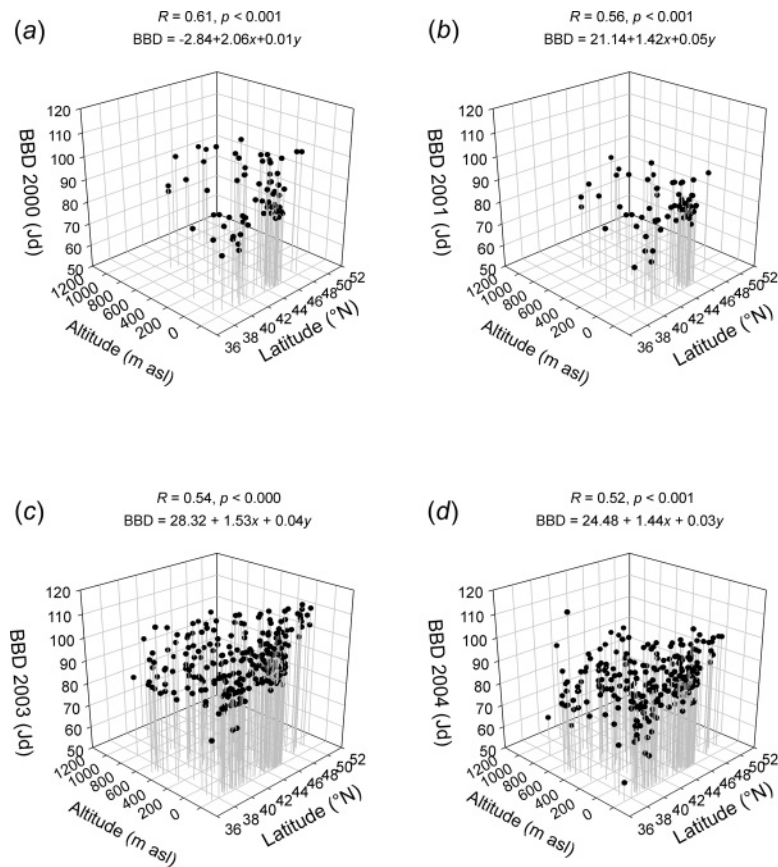
latitudinal patterns in timing of flushing were observed by Whiteley et al. (2003) for *U. laevis* in Sweden and by Geng (1989) for *Ulmus pumila* L. in China. Different or even opposite geographical trends in timing of bud burst have been explained at colder or warmer sites than at the site of origin as a consequence of the interplay between chilling and thermal time requirements for bud burst (Kriebel and Wang 1962; Nienstaedt 1974; Cannell and Smith 1984; Murray et al. 1989). Our results were obtained in climatic conditions where chilling (15–67 chill days) was presumably always inadequate to fully satisfy the chilling requirements of *U. minor*, which have been shown to be about 90 chill days (Santini et al. 2004). Thus, the latitudinal and altitudinal trends herein reported can be explained as the consequence of a south–north trend of variation in the chilling requirement of the observed *U. minor* clones, with extensive requirements in high-latitude and high-altitude origins. Greater chilling requirements for northern than for southern provenances have been reported for *Acer saccharum* Marsh. (Kriebel and Wang 1962), *Acer rubrum* L. (Perry and Wang 1960), different *Picea* species (Nienstaedt 1967), *Liquidambar styraciflua* (Farmer 1968), *Pseudotsuga menziesii* (Mirb.) Franco (Campbell and Sugano 1979). The north–south sequence in the flushing date of six *U. laevis* populations reported by Whiteley et al. (2003) under Swedish climatic conditions, where chilling requirement is likely to be fully met, could be instead attributed to differences

among populations in the thermal time requirement to reach bud break or in the base temperature for growth, as reported by Myking and Heide (1995) for *Betula pendula* Roth and *Betula pubescens* Ehrh. ecotypes.

The altitudinal trends that we observed, although consistent with the latitudinal ones, were always less strong. This result was expected considering that, in mountainous regions. climatic factors to which plants are adapted may vary peculiarly and in hardly predictable ways, i.e., the local topography can produce microclimatic variation incongruous with the general trend of variation (Sharik and Barnes 1976).

The ANOVAs showed a great stability in the order of bud burst among latitudinal origins, suggesting that the timing of bud burst in *U. minor* is a character under strong genetic control, as also reported for the congener *U. laevis* (Whiteley et al. 2003) and for other species (Eriksson et al. 1978; Bradshaw and Stettler 1995). The observed interannual variation in the BBD, whatever the latitudinal origin, also indicated an effect of environmental factors, especially interannual temperature variation, in addition to genetic effects.

The hypothesis that northern clones have longer chilling requirements than southern ones is strengthened by the observation that the regression parameters for the effect of latitude decreased when chilling was longer (Figs. 2 and 4), i.e., the difference among origins were reduced with increasing CDs. The expected increment in TT for 1° of latitude at

Fig. 4. Thermal time to bud burst (TT) of the *Ulmus minor* clones in (*a*) 2000, (*b*) 2001, (*c*) 2003, and (*d*) 2004 in Antella, Italy, clone collection (43°43′N, 11°22′E, 170 m asl) as a function of latitude (*x*) and altitude (*y*) of origin of the clones.
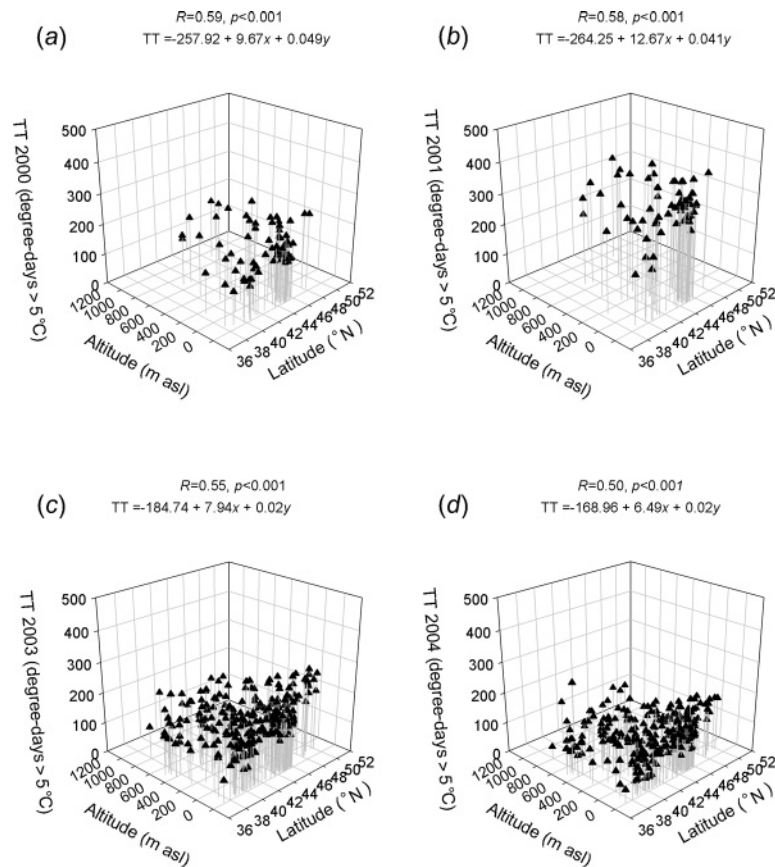

Table 3. Factorial ANOVA summary for thermal time to bud burst (TT) and bud burst date (BBD).

|	Guémené-Penfao and Nogent-sur-Vernisson				Antella			
	df	SS	F	p	df	SS	F	p
TT (degree-days >5 °C)								
Latitudinal group (G)	1	54 184	108.42	<0.001	3	217 901	130.10	<0.001
Duration of chilling (CD)	3	158 153	105.49	<0.001	2	707 289	633.44	<0.001
G × CD	3	1 337	0.89	0.448	6	19 265	5.75	<0.001
Error	102	50 975			145	19 265		
BBD (Julian days)								
Latitudinal group (G)	1	3 189	107.16	<0.001	3	5 893	167.74	<0.001
Site (S)	1	1 448	48.67	<0.001				
Year (Y)	1	983	33.05	<0.001	2	1 551	66.21	<0.001
G × S	1	6	0.21	0.647				
G × Y	1	2	0.07	0.789	6	493	7.02	<0.001
S × Y	1	6	0.20	0.652				
G × S × Y	1	50	1.66	0.200				
Error	102	3 035			145	1 698		

Note: Julian days are the number of days since 1 January.

Antella was the highest (12.67 degree-days) in 2001, when chilling was only 15 chill days, but it gradually fell in 2000 (9.26 degree-days), 2003 (7.94 degree-days), and 2004 (6.49 degree-days), when chilling rose to 48 chill days, 52 chill days, and 63 chill days, respectively. At Nogent-sur-Vernisson in 2002, when chilling reached 67 chill days, the

expected difference in TT was lower (5.74 degree-days) than in all the other years at Antella.

In this hypothesis, northern clones were penalized when chilling was short, but their TTs decreased with increasing chilling more than those of southern clones, so that the differences among latitudinal origins were gradually reduced.

Fig. 5. Least squares means ± SEs for the variable thermal time to bud burst (TT) of the selected group of clones as a function of chilling duration at (*a*) the French sites and (*b*) the Italian site. Each chilling durations corresponds to a different year (see Table 2 for details). Least squares means ± SEs for the variable bud-burst date (BBD) at Nogent-sur-Vernisson (Nog) and Guémené-Penfao (Gue) (*c*) and at Antella (*d*) in each year of study. N, northern group; S, southern group; NF, northern France group; SF, southern France group; NI, northern Italy group; SI, southern Italy group (see Table 1 for details).
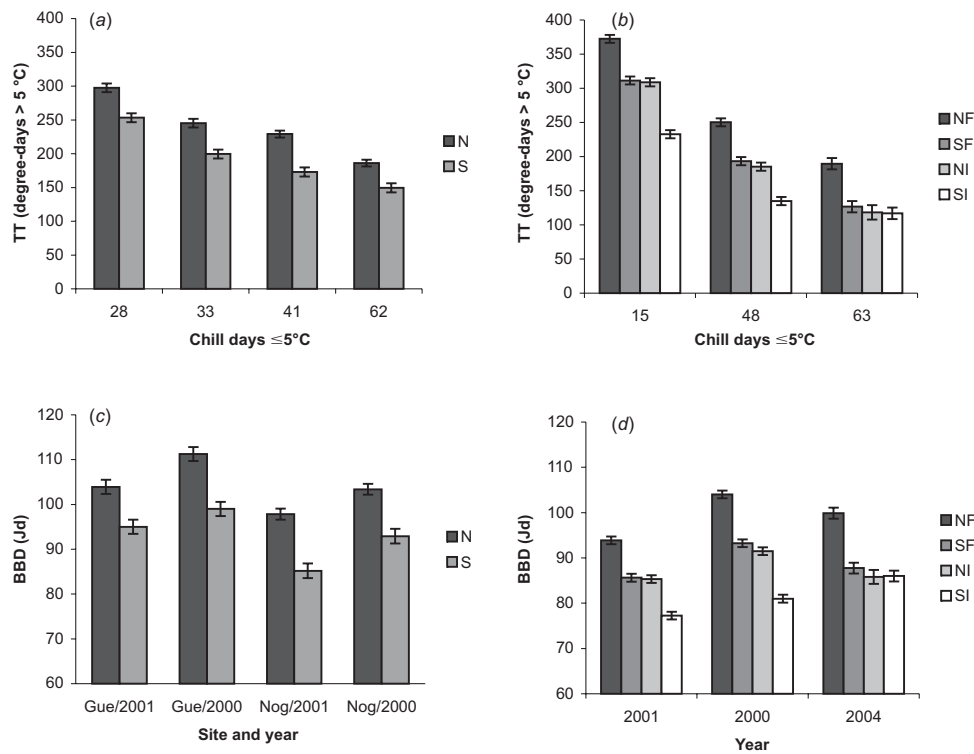
ANOVA results for TT also supported the existence of a latitudinal trend in chilling requirements. At Antella, the chilling requirements of the NF, SF, and NI groups were not satisfied by CDs shorter than 63 chill days, whereas the chilling requirement of SI group was fulfilled by 48 chill days (Fig. 5*b*). The comparison between the TT requirements at the French sites and those at Antella further indicated that the relationship between chilling requirement and latitude is strong and stable. Groups from similar latitudes required almost the same TT irrespective of year and site, provided that chilling had similar duration (Fig. 5).

In the literature, different phenological trends among provenances have been explained as a result of adaptation to differences in the degree of climate uncertainty, in the frequency of late frosts, and in the impact of summer droughts (Campbell and Sugano 1979; Myking and Heide 1995; Von Wuehlisch et al. 1995; Hamann et al. 2001). In our study, the main climatic difference between the northern and southern parts of the sampled area is in the rainfall distribution: the growing season is strongly restricted by drought in the Mediterranean climate. Thus, we suggest that, following Campbell and Sugano (1979), the precocity in flushing of southern U. minor clones observed in our trials results from an adaptation to the Mediterranean climate, where mild and rainy periods are present starting in late winter, wheras drought occurs in late spring and lasts all summer. Such an effect of drought as selective pressure for timing of bud burst has been also reported for *U. pumila* by Geng (1989).

Although the duration of winter chilling allowed us to ef-

fectively explain the observed variation in TT among sites, year and geographical origins, the course of temperature during the spring (forcing temperature) has to be taken into account to explain the variation in the BBD in field conditions (Hunter and Lechowicz 1992).

In our study, a delay in the BBD among sites or years in association with a decrease in chilling duration was observed in the absence of significant differences in forcing temperature, as was the case in both years between the two French sites. The chilling requirement of both groups were not satisfied at all of the French sites, so that at Guémené-Penfao bud burst was later than at Nogent-sur-Vernisson, because chilling was shorter and forcing conditions were very similar between sites in both 2000 and 2001 (Fig. 6*a*). Instead, an advancement in the BBD was observed, despite a reduction in chilling, when higher forcing temperatures and faster heat accumulation occurred in spring. In 2001, bud burst was earlier both at Guémené-Penfao and at Nogent-sur-Vernisson (Fig. 5*c*), despite a reduction of the chilling duration and an increase in TT at both sites (Fig. 5*a*), because heat accumulation from February to April was very much faster in 2001 than in 2000 (Fig. 6*a*). Similarly, at Antella the especially warm temperatures from February to April in 2001 (Fig. 6*b*) induced earlier bud burst in all latitudinal groups (Fig 5*d*), notwithstanding the very mild winter conditions, the lowest chilling duration, and the largest TT requirement (Fig. 5*b*). The phenological trends reported in the literature for the genus *Ulmus* are consistent with our results: in *Ulmus procera* Salisb., Sparks and Carey (1995) showed that, in mild winter

Fig. 6. Accumulated degree-days >5 °C per week during the weeks shortly preceding bud burst and during the bud-burst period at Guémené-Penfao (Gue), Nogent-sur-Vernisson (Nog) and Antella (Ant) in 2000, 2001, and 2004.
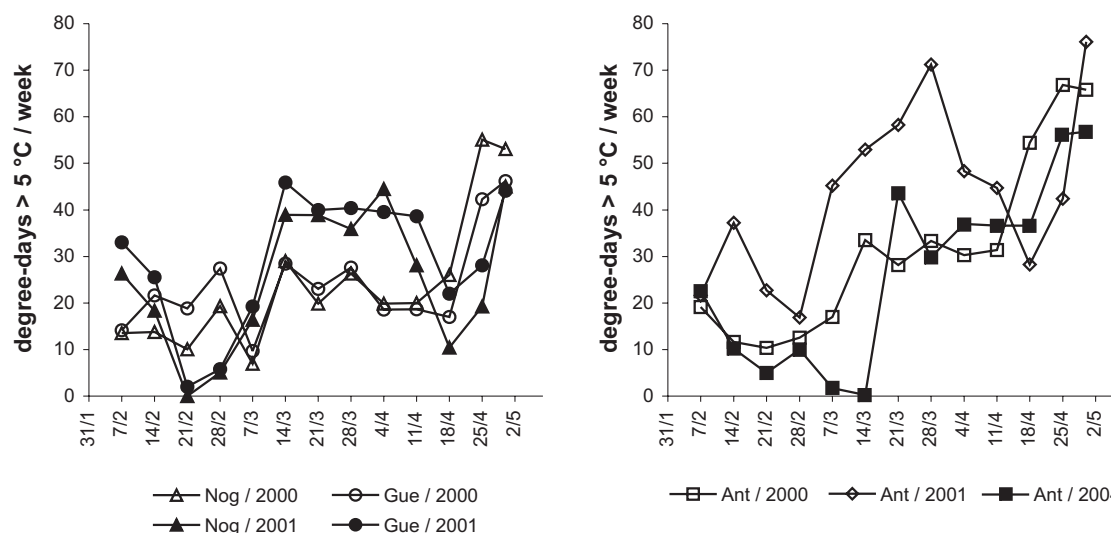
conditions of England where chilling is limiting for elm, an increase in winter temperature enlarged the required thermal sum to bud burst, thus causing either a delay or an advancement of bud burst, depending on whether the temperature in spring remained constant or increased, respectively. Chuine et al. (2000) showed that, in Ohio, where chilling requirements were widely exceeded, *U. americana* flowered significantly earlier over the second half of the 20th century in concurrence with climatic warming, as a consequence of the increased forcing temperature.

Santini et al. (2005) found a statistically significant relation between susceptibility to DED and latitude in *U. minor*: southerly clones showed less disease following artificial inoculations than northerly ones. They also found a significant relationship between disease severity and timing of bud burst: early flushing clones were less affected by DED than late flushing ones. The results obtained here showed that the clones from southern Europe maintain their precocity in flushing under different environmental conditions. In addition, the order of bud burst was shown to be stable over the years and sites, and this was due to the fact that southern clones have short chilling requirements and that precocity is mainly dependent on chilling satisfaction. This stability gives hope for a successful use of early flushing clones in new plantings in northern sites as material more resistant to DED, always providing that their precocity does not render them unsuitable for other reasons, e.g., because of susceptibility to late frosts. Precocity in flushing is an easy observable character, and these results may open the possibility of selecting "locally" resistant clones among native elms that, so far, are considered to be unsuitable because of their susceptibility.

Acknowledgements

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