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Bud dormancy release in elm (*Ulmus* spp.) clones—a case study of photoperiod and temperature responses

LUISA GHELARDINI,[1,2,3] ALBERTO SANTINI,[2] SANNA BLACK-SAMUELSSON,[4] TOR MYKING[5] and MAURO FALUSI[6]

[1] *Department of Plant Biology and Forest Genetics, Swedish University of Agricultural Sciences, Box 7080, S-750 07 Uppsala, Sweden*

[2] *Istituto per la Protezione delle Piante, Consiglio Nazionale delle Ricerche, Via Madonna del Piano 10, 50126 Sesto Fiorentino, Italy*

[3] *Corresponding author (Luisa.Ghelardini@vbsg.slu.se)*

[4] *Swedish Forest Agency, Box 1350, S-751 43 Uppsala, Sweden*

[5] *Norwegian Forest and Landscape Institute, Fanaflaten 4, NO-5244 Fana, Norway*

[6] *Dipartimento di Biologia Vegetale, Università di Firenze, Piazzale delle Cascine 28, 50100 Firenze, Italy*

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Summary Dormancy release as influenced by duration of outdoor winter chilling in Florence (Italy) was studied under different photoperiodic and temperature treatments in collected twigs of two European (*Ulmus glabra* Huds. and *Ulmus minor* Mill.) and four Asian (*Ulmus pumila* L., *Ulmus parvifolia* Jacq., *Ulmus macrocarpa* Hance and *Ulmus villosa* Brandis) elm clones. Photoperiod had no effect on dormancy release, and there was no evidence that photoperiod affected bud burst during quiescence in the studied elm clones. Thermal time (day degrees >0 °C) to bud burst decreased in all the clones with increasing outdoor chilling. Although all the clones exhibited a rather weak dormancy, they significantly differed from each other. Dormancy was released earlier in the Asian than in the European clones, and the clones could be ranked from the *U. pumila* clone (very weak and short dormancy) to the *U. minor* clone (relatively stronger and longer dormancy), the other clones being intermediate. In all the clones except *U. minor*, the observed decrement in thermal time to bud burst was efficiently explained as an inverse exponential function of the number of chill days ≤5 °C received outdoor in autumn and winter. Endodormancy, as measured by the single-node cuttings test, was weak and short in all the clones. The latter result suggests that correlative inhibitions were largely responsible for preventing bud burst during winter in these elm clones.

Keywords: bud burst, *bud rest*, *chilling requirement*, *day length*, *thermal time*.

Introduction

The genus *Ulmus* L. includes trees growing in the temperate and sub-tropical regions of the Northern Hemisphere. Eight species are endemic to North America, three species to Europe, and the major part is native to Asia (Brummitt 1992). Elms were among the most widely used ornamental trees in Europe and North America until Dutch elm disease (DED), perhaps the single most destructive disease to attack forest trees, devastated their populations in the twentieth century (Dunn 2000).

There is considerable variation in resistance to DED among elm species. Generally, European and American species are highly susceptible to DED, whereas Asian species are highly to moderately resistant. Besides, susceptibility to DED displays strong seasonal variation both in susceptible and resistant species. The time of highest susceptibility and the duration of the period during which elms can become infected and express DED symptoms vary greatly among species, provenances and environmental conditions (Banfield 1941, Kais et al. 1962, Smalley 1963, Tchernoff 1965, Smalley and Kais 1966). The reasons for this phenomenon are not well understood, but according to the few studies which correlated DED susceptibility with the host's rhythm of seasonal morphogenesis, the seasonal variation in disease development depends on the pattern of longitudinal and radial growth including the timing of bud burst and reactivation of cambial activity (Pomerleau 1966, Neely 1968, Takai and Kondo 1989, Santini et al. 2005, Solla et al. 2005, Ghelardini et al. unpublished data). A significant relationship between timing of bud burst and DED susceptibility has been reported for the European field elm (*Ulmus minor* Mill.) (Santini et al. 2005), suggesting that variations in the host spring phenology may lead to disease escape through asynchrony between the period of DED susceptibility of the host and the phase of disease transmission of the insect vector. Despite its putative importance for the disease development, very little is known about the environmental control of bud burst and resumption of vegetative growth in elms, and additional information on

the subject would be desirable for DED research and breeding purposes (Ghelardini 2007, Ghelardini and Santini 2009).

Timing of bud burst is triggered by environmental factors, mainly winter chilling and spring temperature. These signals have synergistic effects on bud burst by releasing dormancy and promoting bud development, respectively (Kozlowsky and Pallardy 1997). In some species, light conditions (photoperiod and light spectral composition) may be an additional trigger of bud burst (Heide 1993*b*, Partanen et al. 1998, Linkosalo and Lechowicz 2006). Bud dormancy is a temporary suspension of growth controlled by correlative and environmental factors (Howarth et al. 2003). During the non-growing season, there is a gradual transition from correlative inhibition of bud growth (paradormancy), which is determined by other buds and tissues, to deep dormancy of the bud itself (endodormancy) and then to restriction of bud growth by environmental factors (ecodormancy or quiescence) (Lang et al. 1987). Although endodormancy and paradormancy both inhibit growth, they can be distinguished experimentally by comparing bud burst of whole shoots and isolated nodal cuttings under favourable controlled conditions, the so-called 'single node cuttings test' (Crabbé and Barnola 1996, White et al. 1999, Mazzitelli et al. 2007).

It has long been known that dormancy release depends on sufficient exposure to cold temperatures (chilling requirement). Temperatures between 0 and 15 °C are effective for breaking dormancy, with the optimum generally around 5 °C (Perry 1971). Subsequent bud burst depends on sufficient exposure to warm temperatures over a specific threshold (thermal time requirement) to resume growth (Perry 1971). In many trees, the thermal time required for bud burst decreases with increasing chilling until a level at which the chilling requirement is fully met (Cannell and Smith 1983, Murray et al. 1989). A rapid decrease in thermal time typically occurs during the early stages of dormancy release, whereas a slow decrease characterizes the final stages of the process. Besides chilling, light conditions affect dormancy release in some species. Long photoperiod may release dormancy and promote bud burst in pre-dormancy and post-dormancy phases (Wareing 1953, Falusi and Calamassi 1990, Heide 1993*b*, Linkosalo and Lechowicz 2006). Moreover, in many trees the effect of chilling can be partly replaced by a long photoperiod when the chilling requirement is not satisfied (Downs and Borthwick 1956, Farmer 1968, Falusi and Calamassi 1996, Heide 1993*a*, Myking and Heide 1995, Myking 1997). This compensation manifests itself in a reduction of the thermal time requirement in partially chilled buds subjected to long photoperiods but not in unchilled or fully chilled ones.

Knowledge on dormancy is scarce in the genus *Ulmus*, and direct evidence from controlled conditions experiments is available only for the American elm (*Ulmus americana* L.). Dormancy release in *U. americana* seedlings is mainly due to chilling, with a weak compensating effect by long photoperiod when chilling is short (less than 60 days below 5 °C; Roberts and Main 1965). Some indirect evidence about dormancy in European elms can be drawn from phenological studies in field conditions. On the basis of a modelling analysis of open field phenological data, European elms (*Ulmus glabra* Huds, *Ulmus laevis* Pall. and *U. minor*) have been classified as species with low chilling requirement for dormancy release (Santini et al. 2004, Ghelardini et al. 2006). Other modelling analyses of phenological data series have found chilling to have little or no effect in explaining the observed variation in bud burst date in European (Sparks and Carey 1995, Chuine et al. 1999) and American (Chuine et al. 2000) elm species. However, inferences on dormancy based on modelling analysis of open field data should be confirmed by experiments in controlled environment (Saxe et al. 2001).

Hence, there are two main reasons to study the control of dormancy release and growth resumption in selected elm clones commonly used in breeding against DED: (i) to characterize clones of interest for programming controlled environment studies of the dependence of DED susceptibility on timing of phenological events such as release of endodormancy and onset of ecodormancy in the prospect of a possible exploitation of this disease escape mechanism to breed DED-resistant elm clones; (ii) to define the environmental factors to be considered in order to develop process-based phenological models to predict the bud burst phenology of these clones under variable environmental conditions.

For these purposes, we tested the effect of photoperiod and temperature on dormancy release in clones of two European (*U. minor* and *U. glabra*) and four Asian (*Ulmus pumila* L., *Ulmus parvifolia* Jacq., *Ulmus villosa* Brandis and *Ulmus macrocarpa* Hance) elm species, which are currently used in the research on mechanisms of DED resistance and in the breeding of DED-resistant elms (Dunn 2000, Santini et al. 2008). Excised twigs from field-growing trees were subjected to different photoperiod and temperature treatments after various durations of outdoor chilling during 2 years. Intensity and duration of endodormancy were also investigated by means of single-node cuttings. A phenological model that had previously proved efficient to explain the observed variation in the bud burst date of European elms in field conditions (Santini et al. 2004) was fitted to the data in order to verify its validity under controlled conditions in these clones of interest.

Besides the above-mentioned specific objectives, the study of these clones will contribute new, although circumscribed, knowledge on dormancy in *Ulmus*, a genus that has been very little investigated so far. However, since significant variation among and within population and geographical variation patterns have been shown in bud burst timing both in European and Asian elm species (Santini et al. 2005, Ghelardini et al. 2006, Geng 1989), and the clones included in this study have been sampled from a rather restricted latitudinal range with only one clone representing each species, differences among species could not be tested herein, and the results cannot be generalized.

Table 1. Geographic origin of the elm (*Ulmus* spp.) clones in the experiments.

Species	Clone	Geographic origin	Latitude	Longitude
Ulmus glabra Huds.	CNR055[a]	Trentino Alto Adige, Northern Italy	46°37′ N	11°07′ E
Ulmus macrocarpa Hance	UM001[b]	Horqin Left Wing Rear Banner, Northern China	42°45′ N	122°15′ E
Ulmus minor Mill.	CNR118[b]	Emilia Romagna, Central Italy	44°52′ N	10°58′ E
Ulmus parvifolia Jacq.	'Dinasty' (Santamour 1984) US National Arboretum Accession no: 36 533	Korea	Unknown	Unknown
Ulmus pumila L.	S015[b]	Volgograd Province, Russia	48°40′ N	44°39′ E
Ulmus villosa Brandis	Hilliers Arboretum Accession no: 1989.2869	Sundarnagar Forest Division, Himachal Pradesh, India	31°15′ N	76°11′ E

[a]Italian National Research Council (CNR) code.
[b]International code adopted in the EU-project RESGEN CT96-98 'Coordination for conservation, characterization, collection and utilization of the genetic resources of European elms'.

Materials and methods

Plant material and collection site

Plant material for all the experiments was sampled from four healthy, field-growing trees of the *U. glabra*, *U. macrocarpa*, *U. minor*, *U. parvifolia*, *U. pumila* and *U. villosa* clones listed in Table 1. The trees, obtained by rooted cuttings from adult trees about 20 years earlier, were growing in Florence (Italy, 43°43′ N, 11°22′ E, 170 m a.s.l., climate zone IV_4 according to Walter and Lieth 1960) in the elm clone collection of the Institute for Plant Protection-Italian National Research Council (CNR). Air temperature at the collection site was recorded hourly by means of a normal meteorological station 2 m aboveground. Monthly mean temperatures in the two seasons of study are reported in Figure 1.

Experimental design

Two growth chamber experiments were carried out at the Department of Plant Biology of the University of Florence. In both experiments, 1-year-old twigs, 40–80 cm long and bearing 15–30 buds each, chilled under natural conditions, were collected monthly from October 15 to March 15 and subjected to either two photoperiod (Experiment 1) or two temperature (Experiment 2) treatments. In Experiment 2, single-node cuttings were studied in addition to whole twigs in order to distinguish, by comparing experimental units, dormancy of the bud itself and short-distance influences of the adjacent stem tissues (sensu Crabbé (1994), i.e., communication blocks between the bud and the adjacent stem tissues) from correlative inhibitions (Crabbé and Barnola 1996). Forty-five (Experiment 1) and 30 twigs (either whole twigs or single nodes, Experiment 2) were used per clone and treatment. Twigs and single-node cuttings were cultured on polystyrene boards floating in deionized water. The bases of the twigs were weekly re-cut by a sharp blade in order to prevent vessel occlusion. The upper cut surfaces of single-node cuttings were dipped in paraffin to reduce water loss. Relative humidity inside the growth chambers was maintained at 75–80% to avoid desiccation.

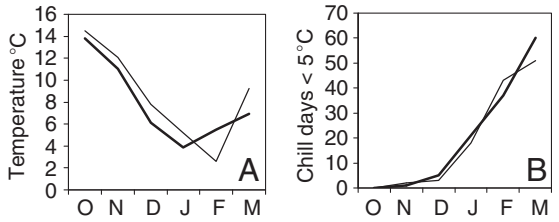

Figure 1. Mean monthly temperature (A) and number of chill days (with mean temperature <5 °C) (B) at the collection site in Florence (Italy, 43°43′ N, 11°22′ E) during autumn and winter 2002–2003 (Experiment 1, thick line) and 2003–2004 (Experiment 2, thin line).

Experiment 1—photoperiod treatments

During the winter season 2002–03 on each intake date, three replicate groups of 15 twigs of the *U. glabra*, *U. macrocarpa*, *U. minor*, *U. parvifolia*, *U. pumila* and *U. villosa* clones listed in Table 1 were randomly assigned to two walk-in growth chambers maintained at constant 21 ± 0.5 °C temperature, under 16 or 8 h photoperiod. A total 1010 buds on average per clone per treatment were observed. Lighting was provided by high-pressure mercury lamps and incandescent lamps (about 215 ± 0.05 μmol m^{-2} s^{-1} photosynthetic active radiance (PAR)). In the 16-h treatment, day length was extended by low-intensity light from only the incandescent lamps (about 3 ± 0.05 μmol m^{-2} s^{-1} PAR).

Experiment 2—temperature treatments

During the winter season 2003–04 on each intake date, three replicate groups of 10 twigs and five replicate groups of 50 single-node cuttings of the *U. minor*, *U. parvifolia*, *U. pumila* and *U. villosa* clones (Table 1) were randomly assigned to two walk-in growth chambers maintained at either 14 ± 0.5 or 26 ± 0.5 °C to promote bud burst. A total 925 buds on average per clone per treatment were observed. Light conditions were as in the 16-h treatment of Experiment 1.

Assessment of bud burst and statistical analyses

In both experiments, all the buds were scored for bud burst every other day until the end of the growth chamber treat-

ments, which were terminated after accumulation of 1050 day degrees (dd) >0 °C, i.e., after 50, 40 and 75 days at 21 (Experiment 1), 26 and 14 °C (Experiment 2), respectively. Bud burst was defined as Stage 3 according to Murray et al. (1989) phenological scale: 1 = slightly swollen; 2 = swollen; 3 = green foliage showing; 4 = elongating. Individual twigs were recorded as flushing on the average bud burst date among all their buds. Percentage bud burst (PB) and thermal time (TT) to bud burst (dd > 0 °C) were calculated for each treatment in both experiments. Twigs that did not flush by the end of the observation period were recorded as flushing after receiving 1050 dd > 0 °C for the purpose of the statistical analyses with TT as dependent variable. PB and TT were subjected to factorial analyses of variance (ANOVA), with intake date (*I*) and photoperiod (*P*) (Experiment 1, Eqs. (1) and (2)) or temperature (*T*) (Experiment 2, Eqs. (3) and (4)) and their interaction as fixed effects, according to the following linear models. Whole twigs data and single-node cuttings data were subjected to separate ANOVAs in Experiment 2. In order to meet ANOVA assumptions for distribution normality and homogeneity of variance, the arcsine transformation of percentage bud burst was performed (Zar 1999).

$$\arcsin\sqrt{\mathrm{PB}_{ij}} = m + P_i + I_j + (P \times I)_{ij} + e_{ij} \quad (1)$$

$$\mathrm{TT}_{ij} = \mu + P_i + I_j + (P \times I)_{ij} + e_{ij} \quad (2)$$

$$\arcsin\sqrt{\mathrm{PB}_{ij}} = m + T_i + I_j + (T \times I)_{ij} + e_{ij} \quad (3)$$

$$\mathrm{TT}_{ij} = \mu + T_i + I_j + (T \times I)_{ij} + e_{ij} \quad (4)$$

In Eqs. (1) and (2), PB_{ij} is the percentage of bud burst, and TT_{ij} is the thermal time to bud burst under photoperiod i of the twigs sampled on intake date j, P_i is fixed effect of photoperiod i (i = 8 or 16 h), I is the fixed effect of intake date j (j = 15 October 2002, 15 November 2002, 15 December 2002, 15 January 2003, 15 February 2003 or 15 March 2003), $P \times I$ is the fixed effect of the interaction between photoperiod and intake date. In Eqs. (3) and (4), PB_{ij} is the percentage of bud burst, and TT_{ij} is the thermal time to bud burst under temperature i of the plant material sampled on intake date j, T_i is fixed effect of temperature i (i = 14 or 26 °C), I is the fixed effect of intake date j (j = 15 October 2003, 15 November 2003, 15 December 2003, 15 January 2004, 15 February 2004 or 15 March 2004), $T \times I$ is the fixed effect of the interaction between temperature and intake date. In all equations, e_{ij} is the random residual.

The inverse exponential model developed by Cannell and Smith (1983) to describe the variation in thermal time as a function of previous chilling (Eq. 5) was fitted to data of Experiment 1.

$$TT = a + be^{(r\mathrm{CD})} \quad (5)$$

In Eq. (5), CD is the number of chill days with mean temperature ≤5 °C received outdoor from 1 October 2002 to the intake date (Figure 1); a, b and r are the model parameters. The parameter r measures the rate at which thermal time decreases when the number of chill days increases: the higher the r absolute value, the faster the decrement. At CD = 0, the expected value of the temperature sum equals $a + b$, whereas it tends to the asymptotic value a as CD tends to infinity when there is no further effect of increased chilling.

The point where the required thermal time to bud burst becomes less than 5% higher than the minimum thermal time (Eq. 6) was used as an arbitrary estimate of chilling requirement for dormancy release in each clone (Hannerz et al. 2003).

$$\mathrm{CD} = |r|^{-1}(lnb - ln0.05a) \quad (6)$$

Statistical analyses and curve fitting were performed with Statistica 6.0 (StatSoft Inc. 1984–2004).

Results

Experiment 1—effect of photoperiod during dormancy release

Photoperiod had no significant effect in any clone at any intake date on PB and TT (Table 2, Figure 2). In all clones, PB increased, and TT decreased with consecutive dates (significant intake date effect). Bud burst was strongly inhibited in mid-October and mid-November. No or sporadic bud burst was observed at the first two intakes except for the *U. pumila* clone (20–30% bud burst), which exhibited a shallower dormancy (Figure 2A–F) than the other clones. Growth inhibition was alleviated earlier in the Asian than in the European clones, although there was variation within each group. PB (Figure 2A–F) increased rapidly in the Asian clones, exceeding 50% from mid-December (*U. pumila*) to mid-January (*U. parvifolia*, *U. macrocarpa*, *U. villosa*) and becoming steady later on (no significant difference among intake dates). In the European clones, PB started to significantly increase only in mid-January (*U. glabra*) to mid-February (*U. minor*), reached 50% in mid-February and continued to increase until the end of the experiment. TT followed a similar pattern over time with the first sharp decline earlier in the Asian (especially *U. pumila*) than in the European (especially *U. minor*) clones (Figure 2G–L).

The observed variation in TT to bud burst was efficiently explained as an inverse exponential function of the number of chill days ≤5 °C received outdoor in autumn and winter (Eq. 5) in all clones (explained variance >80%, Table 3) except *U. minor*, for which the TT did not tend to stabilize within the observed chilling range. Results of the curve fitting are shown in Figure 3. TT decreased especially fast and tended to become steady, i.e., to reach an asymptotic value, after short chilling in the *U. pumila* clone and similarly, although at a slightly lower rate, in the *U. parvifolia* and *U. macrocarpa* clones. TT decrease was slower in the *U. villosa* and slow-

Table 2. *F*-ratio values of the two-way ANOVA for percentage bud burst (PB, data angle transformed) and thermal time (TT, accumulated day degrees >0 °C) in 1-year-old twigs (Experiments 1 and 2) and single-node cuttings (Experiment 2) of elm clones (Table 1) collected on six intake dates (*I*) from 15 October to 15 March and subjected to 21 °C and photoperiods (*P*) of either 8 or 16 h (Experiment 1) or to 16 h and temperatures (*T*) of either 14 or 26 °C (Experiment 2).

Effect	(df1, df2)	*U. pumila*		*U. parvifolia*		*U. minor*		*U. villosa*		*U. glabra*		*U. macrocarpa*	
		PB	TT	PB	TT	PB	TT	PB	TT	PB	TT	PB	TT
Experiment 1 whole twigs													
I	(5, 24)	125.1***	138.8***	894.7***	1023.2***	125.8***	135.9***	225.3***	295.0***	158.5***	1397.3***	97.7***	1023.2***
P	(1, 24)	0.3 ns	0.1 ns	1.1 ns	0.01 ns	0.1 ns	0.01 ns	0.1 ns	0.01 ns	0.2 ns	0.1 ns	0.1 ns	0.01 ns
I × P	(5, 24)	0.5 ns	0.2 ns	0.3 ns	0.2 ns	0.3 ns	0.1 ns	0.6 ns	0.2 ns	0.2 ns	0.3 ns	0.1 ns	0.2 ns
Experiment 2 whole twigs													
I	(5, 24)	83.6***	2034.4***	493.2***	434.61***	119.42***	469.95***	272.86***	280.4***				
T	(1, 24)	31.1***	2033.0***	13.0**	352.38***	19.51***	93.88***	219.22***	508.8***				
I × T	(5, 24)	5.5***	435.3***	26.6***	81.01***	7.59***	9.51***	41.57***	109.6***				
Experiment 2 single-node cuttings													
I	(5, 48)	46.9***	90.9***	64.8***	118.5***	118.3***	397.7***	112.8***	242.0***				
T	(1, 48)	57.1***	106.9***	64.8***	95.5***	45.0***	208.9***	149.8***	409.4***				
I × T	(5, 48)	46.9***	54.0***	64.8***	46.2***	45.3***	79.2***	60.1***	134.6***				

**$P < 0.01$ significance level, ***$P < 0.001$ significance level, and ns = non-significant.

est in the *U. glabra* clone. Chilling requirement, as estimated according to Eq. (6), i.e., as the point where the required TT becomes less than 5% higher than the minimum TT, was lowest for *U. pumila*, followed by *U. parvifolia*, *U. macrocarpa*, *U. villosa* and *U. glabra* (Table 3).

Experiment 2—effect of forcing temperature during dormancy release

Both in single-node cuttings and whole twigs of all clones, PB increased and TT decreased with successive intake dates (significant intake date effect), PB was higher and TT lower under 26 °C compared to 14 °C (significant temperature effect), and differences between forcing temperatures diminished with successive intakes so that towards the end of the experiment bud burst was promoted at 14 °C as well as at 26 °C (significant temperature × intake date interaction) (Table 2, Figure 4).

In whole twigs of all the clones, growth inhibition was strongest in mid-October, although shallower in *U. pumila* than in the other clones, and it was alleviated faster in the Asian than in the European ones (Figure 4) consistently with results of Experiment 1 (Figure 2). PB started to increase, and TT to decrease, by mid-November in *U. pumila* and 1 month (*U. parvifolia*, *U. villosa*) to 2 months (*U. minor*) later in the other clones. In the *U. minor* clone, however, the first strong reduction in growth inhibition occurred 1 month earlier (mid-January) than in Experiment 1 (mid-February, Figure 2). Forcing at 26 °C enhanced bud burst compared to 14 °C, but the effect was observed only at the beginning of the experiment. Later on, the difference became non-significant (significant temperature × intake date interaction), and this happened earlier in the *U. pumila* and *U. parvifolia* than in the *U. villosa* and *U. minor* clones.

In single-node cuttings, PB was higher and TT lower than in whole twigs under both temperature treatments, but the difference between experimental units tended to decrease with successive intakes (Figure 4). This reduction occurred earlier in the *U. pumila* and *U. parvifolia* than in the *U. minor* and *U. villosa* clones (Figure 4). At 26 °C, bud burst was slightly inhibited: PB was always above 80%, complete in the Asian clones and significantly below 100% in *U. minor* on the first intake only. In the *U. pumila* and *U. parvifolia* clones, TT was very low at the first intake (less than 250 dd > 0 °C) and decreased slightly and gradually with time, whereas in the *U. villosa* and especially in the *U. minor* clone, TT was clearly higher at the first intake (about 270 and 400 dd > 0°C, respectively) and decreased steeply from mid-October to mid-November (Figure 4). At 14 °C, bud burst was partially inhibited in mid-October in the *U. pumila* and *U. parvifolia* clones and strongly inhibited in the *U. villosa* and *U. minor* clones. A sharp increment in PB up to 100% occurred in mid-November in the *U. pumila*, *U. parvifolia* and *U. minor* clones, whereas in the *U. villosa* clone, PB started to increase in mid-November and reached 100% in mid-December. TT declined significantly from mid-October to mid-November

in *U. pumila* and *U. parvifolia*, whereas the first significant and sharp decrement in TT was in mid-December for *U. villosa* and *U. minor*. Under both forcing temperatures between mid-February and mid-March, a further steep decrease in TT occurred that was larger in *U. pumila* and *U. parvifolia* than in *U. minor* and *U. villosa*, suggesting that buds had started to accumulate thermal time outdoors before the last intake date in the *U. pumila* and *U. parvifolia* clones.

Discussion

Effect of photoperiod during dormancy release

The literature about photoperiodic effects on growth regulation in the genus *Ulmus* is scarce and not straightforward. Moreover, the majority of reports are on American elm (*U. americana*), which is phylogenetically distant from the European and Asian elm species (Wiegreife et al. 1994) to which the clones studied in this paper belong. The only information on the effect of photoperiod on dormancy release under controlled conditions in elms dates back to 1965, when Roberts and Main reported a partial and weak substituting effect of chilling by long days in *U. americana* seedlings of unknown origin (about 40° N in latitude if a local origin is presumed). Slightly richer is the literature about the effect of photoperiod on growth cessation and dormancy induction in elms. At the stage of seedling and sapling, *U. americana* is sensitive to photoperiod for cessation of growth (shoot apex abscission or set of apical bud) and dormancy induction (Downs and Borthwick 1956, Nitsch 1957, Vaartaja 1959, Millingtown 1963, Laing 1966). Also, in *U. americana*, there is a clear latitudinal trend in responsiveness to photoperiod (variation in both critical night length for growth inhibition and required duration of the inducive treatment), with northern sources responding to shorter treatments of shorter critical night length (Vaartaja 1959). However, even for the northern sources, the effect of photoperiod is moderate in comparison with other trees from the same latitudes (Downs and Borthwick 1956, Vaartaja 1959). Besides, Millington (1963) reported a strong effect of tem-

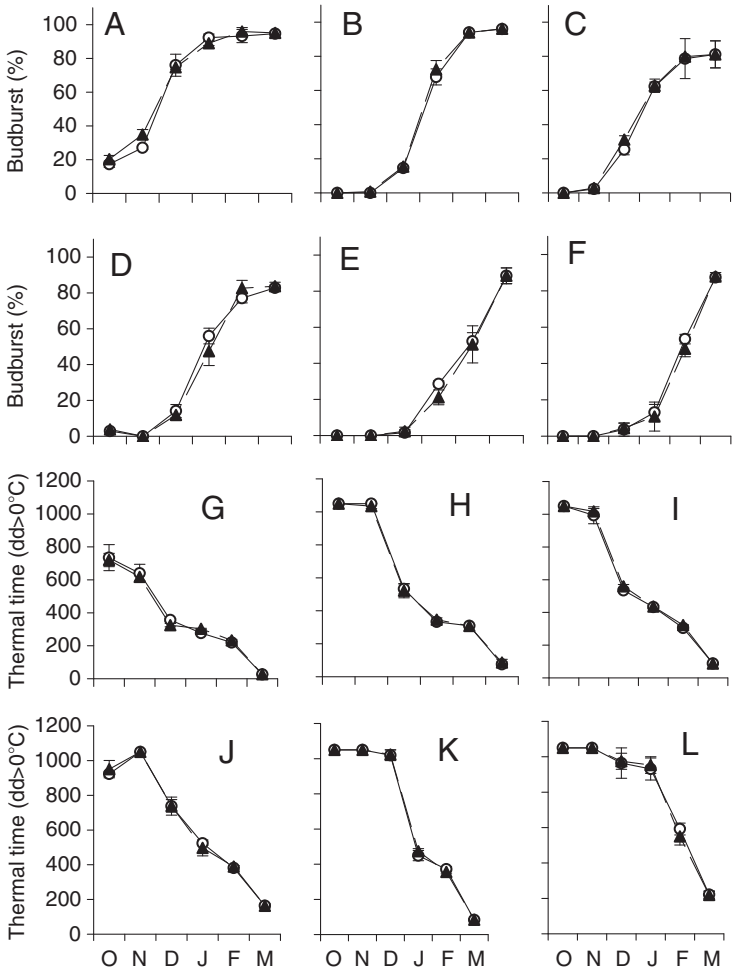

Figure 2. Percentage bud burst (A–F) ± SE and thermal time to bud burst (G–L) ± SE in 1-year-old twigs collected monthly from 15 October 2002 to 15 March 2003 in Florence (Italy, 43°43′ N, 11°22′ E, 170 m a.s.l.) from field-growing *Ulmus pumila* (A, G); *U. parvifolia* (B, H); *U. macrocarpa* (C, I); *U. villosa* (D, J); *U. glabra* (E, K); and *U. minor* (F, L) clones (see Table 1 for details) and subjected to photoperiods of either 16 h (open symbols) or 8 h (closed symbols) under 21 °C constant temperature (Experiment 1).

Table 3. Values of the parameters (a, b, r) in Eq. (5) (see Materials and methods), and estimated number of chill days (CD, days with mean daily temperature <5 °C) required for dormancy release (1.05 times the lowest thermal time of the fitted function, see Eq. (6) in the text) for each clone. e.v.% = percentage explained variance.

Clone	Species	a	b	r	e.v. %	CD, 1.05
CNR055	*U. glabra*	91.0	1014.5	−0.047	95.5	115
UM001	*U. macrocarpa*	238.8	796.4	−0.136	83.8	31
US NA 36 533	*U. parvifolia*	222.4	860.0	−0.174	85.0	25
S015	*U. pumila*	173.4	569.1	−0.243	82.6	17
HA 1989.2869	*U. villosa*	220.9	806.5	−0.059	91.1	72

perature on the timing of shoot apex abscission, and both Millington (1963) and Downs and Borthwick (1956) suggested that temperature could play a major role in growth cessation. This hypothesis is supported by the general observation that local elms in natural conditions often continue to grow during autumn until injured by frost in North America (Downs and Borthwick 1956), Europe (L. Ghelardini, unpublished data) and China (Geng 1989). For European elms, the only report in controlled conditions is that *U. glabra* seedlings originating from the northernmost areas of the natural distribution of the species respond to photoperiod for growth cessation and show a latitudinal cline in the critical day length (Håbjørg 1978). Apart from this report, there is only circumstantial evidence, from the observation of latitudinal trends in common garden experiments, for involvement of photoperiod in growth cessation in *U. glabra* (Myking and Scrøppa 2007), *U. laevis* (Whiteley et al. 2003) and *U. pumila* (Geng 1989). In conclusion, elms do not seem to be especially sensitive to photoperiod for regulation of growth, and their low sensitivity and large latitudinal variation in responsiveness suggest that, at least for southern populations, photoperiod may have little or no effect in field conditions. Other factors than photoperiod could play a major role in dormancy induction in elms, such as decreasing temperatures, which are responsible for dormancy induction in trees insensitive to photoperiod (Heide and Prestud 2005). In regard to dormancy release and growth resumption, this conclusion is indirectly supported by the high efficiency of temperature-based phenological models to explain the variation in the bud burst date of European and American elm species in natural conditions (Sparks and Carey 1995, Chuine et al. 1999, 2000, Santini et al. 2004).

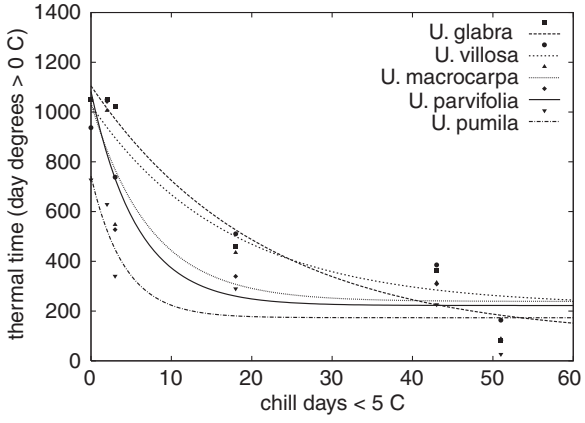

Figure 3. Decrement in thermal time (day degrees > 0 °C) to bud burst following exposure to outdoor chilling (number of chill days with mean temperature ≤ 5 °C since 1 October to the intake date) in the studied *Ulmus* spp. clones as described by curve fitting of Eq. 5 (see Materials and methods) to data of Experiment 1.

The study herein reported provided no evidence that photoperiod influences dormancy release in elms. The partial substituting effect by long photoperiods after short chilling durations shown in *U. americana* seedlings (Roberts and Main 1965) was not confirmed in any of the elm clones in this study, nor was a long day requirement for bud burst found in the late phases of dormancy, which is instead the case for *Fagus sylvatica* L. (Heide 1993*b*). However, the plant material included in this study, i.e., six clones belonging each to a different elm species and covering a rather restricted latitudinal range, is too limited to allow drawing any general conclusion. In fact, timing of bud burst, as a trait with adaptive significance, typically shows geographic variation along climatic gradients, together with large within population and clonal variation (Savolainen et al. 2007), and large intraspecific and clonal variation in this trait has been shown in European elms (Santini et al. 2004, Ghelardini et al. 2006). In addition, as previously mentioned in this section, strong ecotype variation has been shown in photoperiodic response for other phenology traits in some elm species (Vaartaja 1959, Håbjørg 1978). With this reservation in mind, still the fact that independent elm specimens from different species and geographical origins all showed no response to photoperiod in this study could suggest a widespread lack of photoperiodic effect on bud burst in the genus *Ulmus*. To test this hypothesis, experiments on extensive samples of elms from different species and populations, including ecotypes originating from higher latitudes, where photoperiod is the most reliable environmental signal in late spring and early summer, would be needed. In addition, missing effect by photoperiod in this study with respect to Roberts and Main's results could be due to the different age of the plant material, that is shoots of adult trees versus seedlings, respectively. Photoperiodic effects on dormancy release may vary depending on the tree's ontogenetic stage. Partanen et al. (2005) showed that in Norway spruce (*Picea abies* (L.) Karst.) long photoperiod did not affect bud burst in old trees, whereas it partially compensated for lack of chilling in young trees, as it is known to be the

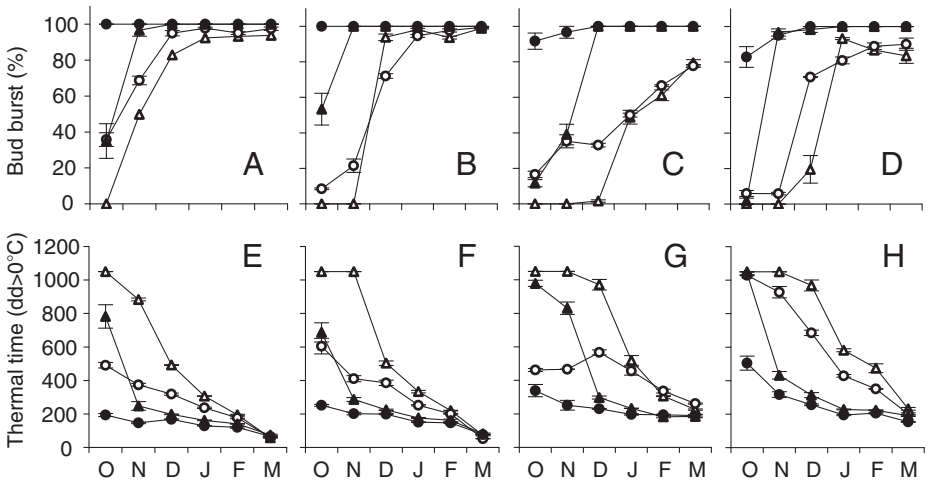

Figure 4. Percentage bud burst (A–D) ± SE and thermal time to bud burst (E–H) ± SE in 1-year-old twigs (open symbols) and single-node cuttings (closed symbols) collected monthly from 15 October 2003 to 15 March 2004 in Florence (Italy, 43°43′N, 11°22′E) from field-growing *Ulmus pumila* (A, E), *U. parvifolia* (B, F), *U. villosa* (C, G) and *U. minor* (D, H) clones and subjected to either 14 °C (triangles) or 26 °C (circles) constant temperature under 16 h photoperiod (Experiment 2).

case also in seedlings of the same species (Nienstaedt 1967). Again, to verify whether the presence of a photoperiodic effect depends on plant age in elms and to draw any more general conclusion, it would be necessary to test plant material from adult trees and seedling of various elm species.

Response to temperature during dormancy release: intensity and duration of dormancy phases

All the clones exhibited maximum dormancy in mid-October (Figures 2 and 4). There was a substantial variability in the strength of this inhibition, the *U. pumila* clone clearly being less dormant than the other clones. According to both variables used as dormancy measures (percentage bud burst and thermal time), dormancy was released earlier in the Asian *U. pumila*, *U. parvifolia*, *U. macrocarpa* and *U. villosa* clones than in the European *U. glabra* and *U. minor* clones. The clones could therefore be ranked from *U. pumila* (very weak and short dormancy) to *U. minor* (relatively stronger and longer dormancy), the other clones being intermediate, with *U. parvifolia* and *U. macrocarpa* less dormant than *U. villosa* and *U. glabra*. For the clones included in both experiments, the ranking in dormancy was consistent across years (Figures 2 and 4).

Like Vegis (1964), we believe that the narrower the temperature range promoting growth, the deeper is dormancy. In twigs, the capacity of bud burst was regained first at high temperature in the following sequence: *U. pumila* (mid-October), *U. parvifolia*, *U. villosa* (mid-November) and *U. minor* (mid-December). Later on, from mid-November to mid-January, a progressive widening towards lower temperatures was observed with the same sequence of bud burst among clones (Figure 4).

Endodormancy (Lang et al. 1987), as measured by the single-node cuttings test (Crabbé and Barnola 1996), was shallow and short in all clones when compared with other tree species studied with the same technique (Bailly and Mauget 1989, Jacques et al. 1989, Falusi and Calamassi 1997, 2003). The result that bud burst in twigs was delayed with respect to single-node cuttings suggests that correlative inhibitions are largely responsible for preventing bud burst during winter in the studied clones. A similar result was reported by Mazzitelli et al. (2007) for *Rubus idaeus* L. Single-node cuttings flushed fully and rapidly at higher temperature, even in the period of maximum inhibition with minor differences among clones (Figure 4). According to Champagnat (1993), a certain percentage of dormant buds may flush at high temperatures (>25 °C), even in the period of maximum inhibition, but not as fast as it was observed in the present study. The *U. pumila* and *U. parvifolia* clones flushed, at the first intake, after less than 10 days, which has been considered the limit defining endodormancy (Balandier et al. 1993), questioning the presence of endodormancy in these clones. Single-node cuttings of the *U. minor* and *U. villosa* clones flushed fast too, but on the first intake they required larger thermal time, which steeply decreased on the second intake.

As in whole twigs, in single-node cuttings the effective temperature range for bud burst widened earlier in *U. pumila* and *U. parvifolia* than in *U. villosa* and *U. minor* clones, which further confirms the ranking in dormancy among clones. Nevertheless, differences among clones were smaller in single-node cuttings than in whole twigs, suggesting that they could be ascribed to a larger extent to correlative inhibition (paradormancy) than to endodormancy.

The capability of the *U. pumila* clone to flush even in the period of strongest growth inhibition (mid-October), although in a narrower temperature range (Figures 2 and 4), suggests that this clone was in a state of conditional dormancy rather than true dormancy. According to Vegis (1964), this is a nor-

mal phenomenon for many species and varieties, whereas for other species the presence or absence of true dormancy seems to depend on the climatic conditions of the year in question. Moreover, inter-annual variations in depth and duration of bud dormancy may depend on autumn temperatures, which, in combination with photoperiod or alone, are responsible for dormancy induction (Heide 2003, Junttila et al. 2003, Heide and Prestud 2005, Søgaard et al. 2008). These effects, which are complex and variable with species and provenances, have not been investigated in the genus *Ulmus*.

Modelling analysis and chilling requirement estimates

On the basis of the thermal time versus chilling curves obtained by fitting a commonly used phenological model (Cannell and Smith 1983, Murray et al. 1989, Hannerz et al. 2003) to open field bud burst data, European elms have been classified as low chilling requirement species (Santini et al. 2004), similar to groups 3 and 4 in Murray et al. (1989). For trees included in these groups (e.g., *Sorbus aucuparia* L., *Corylus avellana* L., *Betula pendula* Roth., *Salix viminalis* L., *Prunus avium* L.), the thermal time to bud burst decreases after short chilling and tends to a stable value when chilling exceeds 100 chill days. Results of the fitting of this model to growth chambers data (Experiment 1, Figure 3 and Table 3) confirm that elms need short chilling for dormancy release. In accordance with their timing of dormancy release (Figure 2), the clones could be ranked on the basis of their estimated chilling requirement from *U. pumila* (<20 CD), *U. macrocarpa* and *U. parvifolia* (<35 CD) to *U. villosa* (72 CD) and *U. glabra* (>100 CD). In *U. minor*, the chilling requirement was highest but could not be estimated within the observed chilling range. Thus, in agreement with previous studies (Ghelardini et al. 2006), the chilling requirement of *U. glabra* and *U. minor* from northern Italy seems not to be fully met in the mild winters of the Mediterranean region. For the *U. minor* clone, this result is further confirmed by the large decrement in thermal time observed in the slightly colder second year of study (Figure 1) in agreement with what is expected when the chilling requirement is not satisfied (Murray et al. 1989).

Conclusions and implications for research on DED resistance

In this study, six clones of different elm species, all involved in studies on DED resistance mechanisms, have been characterized for their responses to photoperiod and temperature during bud dormancy release. In none of the clones were dormancy release and bud burst influenced by photoperiod, but intensity of dormancy and timing of dormancy release greatly differed among them. Knowledge about lack of photoperiodic effect and differences in the time course of dormancy release as a function of temperature in these clones will help to model phenology in these clones and to plan controlled conditions and open field studies on the dependence of susceptibility to DED on timing of phenological events, such as reacquisition of the full competence to grow in favourable environmental conditions, which is followed by the onset of development of a new wood ring (L. Ghelardini et al., unpublished data) and bud burst. We hypothesize that annual variation in DED susceptibility in elms depends on the timing of growth-related processes, which determine how the plant, at the time of the beetle vector's transmission phase in spring, can balance allocation between growth and defence processes. In early spring, the energy reserves of DED-susceptible elms are probably exhausted by the flowering process that occurs in late winter and by the construction of the new porous ring, which has to provide nutrients for the expanding photosynthetic surface. Growing meristems and newly formed leaves behave as strong photosynthetic sinks, and no resources are dedicated to defence, which renders elms especially susceptible to DED. As time passes, growth rate decreases so that photosynthates can be allocated to defence. This could explain why the second generation of vectors in summer is less successful or totally ineffective to cause DED compared to the first generation in spring. In early flushing elms, all these phenological and physiological events, starting with early dormancy release and resumption of competence to grow, take place earlier, so that at the time of DED infection growth rate is reduced and carbohydrates from mature leaves can be allocated to secondary metabolism and defence against DED. This mechanism of DED avoidance by early flushing, possibly combined with other types of resistance, could be exploited for breeding DED-resistant elm clones.

The study of these clones has contributed new, although circumscribed, knowledge on dormancy in the genus *Ulmus*. Some previous findings, such as a general low level of dormancy in European elms (Santini et al. 2004), have been confirmed, and some results, such as a lack of photoperiodic effect on dormancy release, have been reported for the first time and would deserve further investigation in a suitable sample of elm trees.

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