Bud Burst Phenology, Dormancy Release and Susceptibility to Dutch Elm Disease in Elms (*Ulmus* spp.)

Luisa Ghelardini
Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology and Forest Genetics
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2007

Acta Universitatis Agriculturae Sueciae
2007: 134

Cover illustration courtesy of Ignazio Graziosi. Twig of a natural hybrid between *Ulmus minor* Mill. and *Ulmus pumila* L.

ISSN 1652-6880
ISBN 978-91-85913-33-6

Tryck: SLU Service/Repro, Uppsala 2007

Abstract

Ghelardini, L. 2007. Bud Burst Phenology, Dormancy Release and Susceptibility to Dutch Elm Disease in Elms (*Ulmus* spp.). Doctor's dissertation.
ISSN 1652-6880, ISBN 978-91-85913-33-6

European elms (*Ulmus glabra, U. laevis* and *U. minor*) have been damaged and are still threatened by an alien hypervirulent pathogen, *Ophiostoma ulmi s.l.*, the agent of Dutch elm disease (DED). Therefore, several *ex situ* clone collections were established throughout Europe for breeding and conservation purposes. This thesis was carried out within the RESGEN CT96-78 project, which launched the EU-coordinated evaluation of these collections. The aim of this thesis was to analyse the variation in bud burst date and to acquire basic knowledge on the environmental control of this adaptive trait with regard to DED susceptibility.

Bud burst date variation observed among collections and years in European elms was explained by a phenological model based on an inverse exponential relationship between thermal time and chilling to bud burst. According to the fitted curves, European elms have low dormancy and short chilling requirement for dormancy release.

Bud burst date in *U. minor* was directly related to latitude and elevation. The order of bud burst was stable among years. The observed geographic trends were largely determined by difference in chilling requirement for dormancy release which increased with latitude and elevation.

The effects of photoperiod and temperature on dormancy release in clones of European and Asian species were studied in partially controlled conditions in Italy. Dormancy was generally low and short in all clones. There was no evidence that photoperiod influenced dormancy release in these elms.

Susceptibility to DED was assessed in the Italian clone collection. Susceptibility varied greatly among taxonomic groups and within the most represented species, *U. minor.* In this species, DED susceptibility was directly correlated with geographic origin and date of bud burst, southern and early flushing clones showing the least symptoms. The results suggest that earliness of bud burst represents a mechanism of disease avoidance owing to an asynchrony between the susceptible period in the host and the time of natural infection by *Scolytus* insects, the main vectors of DED.

Key words: chilling, disease avoidance, *Ophiostoma ulmi*, photoperiod, rest, thermal time, *Ulmus glabra*, *Ulmus laevis, Ulmus minor*

Author's address: Luisa Ghelardini, Istituto per la Protezione delle Piante – Consiglio Nazionale delle Ricerche C.N.R., Via Madonna del Piano 10, 50019 Sesto fiorentino (FI), Italy. Current address: Department of Plant Biology and Forest Genetics, SLU, Box 7080, SE-750 07 Uppsala, Sweden. *E-mail*: Luisa.Ghelardini@vbsg.slu.se

Riassunto

Ghelardini, L. 2007. Bud Burst Phenology, Dormancy Release and Susceptibility to Dutch Elm Disease in Elms (*Ulmus* spp.). Tesi di Dottorato.
ISSN 1652-6880, ISBN 978-91-85913-33-6

Gli olmi europei (*Ulmus glabra*, *U. laevis* and *U. minor*) sono stati severamente danneggiati e sono tuttora minacciati dal fungo *Ophiostoma ulmi* s.l., agente della grafiosi, una malattia letale dell'olmo. Varie collezioni di cloni *ex situ* sono state pertanto costituite in Europa per la selezione e la conservazione del germoplasma. Questa tesi è stata svolta nell'ambito del progetto RESGEN CT96-78, che ha avviato lo studio coordinato di queste collezioni a livello europeo. Scopo della tesi è stato quello di analizzare la variazione nella data di germogliamento e di acquisire conoscenze di base sul controllo ambientale del germogliamento in relazione alla suscettibilità alla grafiosi.

La variazione nella data di germogliamento degli olmi europei osservata tra le collezioni nei diversi anni è stata spiegata con un modello fenologico basato su una relazione esponenziale inversa tra il thermal time e il chilling necessari per il germogliamento. Sulla base delle regressioni cosí ottenute, si è dimostrato che gli olmi europei sono caratterizzati bassa dormienza e basse richieste di chilling.

La data di germogliamento in *U. minor* si è rivelata direttamente correlata a latitudine e altitudine di origine delle piante. L'ordine di germogliamento si è dimostrato stabile negli anni. I trend geografici osservati sono largamente determinati da differenze nelle esigenze di chilling per l'uscita dalla dormienza, direttamente proporzionali a latitudine e quota.

Inoltre sono stati studiati gli effetti del fotoperiodo e della temperatura sull'uscita dalla dormienza in cloni di olmi europei ed asiatici in condizioni parzialmente controllate in Italia. La dormienza era complessivamente bassa e il fotoperiodo non aveva alcuna influenza sull'uscita della dormienza nei cloni studiati.

La suscettibilità alla grafiosi nella collezione clonale saggiata variava largamente tra gruppi tassonomici e all'interno della specie più rappresentata, *U. minor*. In questa specie la suscettibilità era direttamente correlata all'origine geografica e alla data di germogliamento: i cloni meridionali, più precoci, mostravano una sintomatologia attenuata. Questi risultati suggeriscono che i cloni a germogliamento più precoce possano sfuggire alla malattia grazie all'asincronia che si instaura tra il periodo di suscettibilità dell'ospite e quello del volo degli insetti del gen. *Scolytus*, principali vettori della grafiosi.

Parole chiave: chilling, disease avoidance, *Ophiostoma ulmi*, photoperiod, rest, thermal time, *Ulmus glabra*, *Ulmus laevis, Ulmus minor*

Indirizzo dell'autore: Luisa Ghelardini, Istituto per la Protezione delle Piante – Consiglio Nazionale delle Ricerche C.N.R., Via Madonna del Piano 10, 50019 Sesto fiorentino (FI), Italy. Recapito attuale: Department of Plant Biology and Forest Genetics, SLU, Box 7080, SE-750 07 Uppsala, Svezia. *E-mail*: Luisa.Ghelardini@vbsg.slu.se

A Francesco (dopo la pioggia il sole)

Quando, circondata
dal suo intimo diurno buio,
la neve crocchia, costipata
resiste e ti parla attraverso
le tue scarpe pesanti,
stride, asciutta e dura,
rimastica e inghiotte il freddo,
non penseresti mai
che se ne possa andare o
qualcos'altro cambiare.

Contents

Appendix

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Santini, A., Ghelardini, L., Falusi, M., Bohnens, J., Buron, M., Collin, E., Solla, A. & Van den Broeck, A. 2004. Vegetative bud-burst variability of European elms. In: New approaches to elm conservation. Proceedings of the 2nd International Elm Conference, 20-23 May 2003, Valsaìn, Spain. *Investigatión Agraria: Sistemas y Recursos Forestales* 13 (1): 37-45.

II. Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L. & Mittempergher, L. 2005. Variation among Italian and French elm clones in their response to *Ophiostoma novo-ulmi* inoculation. *Forest Pathology* 35: 183-193.

III. Ghelardini, L., Falusi, M. & Santini A. 2006. Variation in timing of bud-burst of *Ulmus minor* clones from different geographical origins. *Canadian Journal of Forest Research* 36: 1982-1991.

IV. Ghelardini, L., Santini, A., Black-Samuelsson, S., Myking, T. & Falusi, M. Bud dormancy release in elm (*Ulmus* spp.) clones – photoperiod and temperature effects. (Manuscript)

Additional publications

Santini, A., La Porta, N., Ghelardini, L. & Mittempergher, L. 2007. Breeding against Dutch elm disease adapted to the Mediterranean climate. *Euphytica* DOI 10.1007/s10681-007-9573-5

Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L. & Mittempergher, L. 2007. 'Fiorente'and 'Arno' elm trees. *HortScience* 42(3): 712-714.

Glossary

Bud burst	The opening of bud scales in spring followed by elongation of new shoots
Chilling	The cold during winter typically referring to temperatures below about 10 °C that act to release buds from dormancy
Clone	A plant that is genetically identical to its parent having developed by vegetative reproduction, e.g. cutting or graft
Day degrees	Number of temperature degrees accumulated daily above a threshold temperature to provide a measure of thermal time
DED	Dutch elm disease
Disease avoidance	A mechanism of disease escape which occurs whenever susceptible plants do not become infected because the factors necessary for disease (susceptible host, virulent pathogen, and favourable environment) do not coincide and interact at the proper time or for sufficient duration
Dormancy	A temporary suspension of visible growth of any plant structure containing a meristem
Ecodormancy	A phase of dormancy due to an environmental inhibition of growth, also referred to as ‘quiescence’
Endodormancy	A phase of dormancy due to an endogenous inhibition within the affected organ, also referred to as ‘true dormancy’ or ‘rest’
Growth cessation	Termination of growth, often referring the time at which shoot elongation ends
Paradormancy	A phase of dormancy due to endogenous inhibition within the organism but outside the affected organ, also referred to as ‘correlative inhibition’
Phenology	The study of the timing of recurrent events in the life cycles of organisms, as influenced by environmental factors, mainly seasonal variations in temperature and precipitation
Photoperiod	The length of time during which an organism is exposed to sunlight each day, generally equivalent to daylength
Resistance	A hereditary capability to limit pathogen growth
Thermal time	Growth-promoting heat units accumulated over a period of time

Introduction

Background

Many tree species native to the temperate mixed broadleaved forests in Europe are currently endangered because of various human induced threats. Human activities reduce natural habitats and introduce alien organisms, such as pathogens, pests or competitors, potentially dangerous for native species. European elms are an excellent example of this as they suffer from both of these threats. The habitat of *Ulmus laevis* Pall., which typically grows in the riverain deciduous forests of Central and Eastern Europe, has been largely reduced as a consequence of human activities. All three European elm species (*U. laevis*, *Ulmus minor* Mill. and *Ulmus glabra* Huds.) have been severely damaged during the last century and are still threatened by an alien hypervirulent pathogen, *Ophiostoma novo ulmi*, the agent of Dutch elm disease (DED).

As a consequence, European elms are undergoing extraordinary *ex situ* conservation measures (Eriksson 2001; Collin *et al.* 2004). Several national initiatives for collecting germplasm have been undertaken in Europe and different *ex situ* clone collections of European elms have been established. In order to provide these diverse initiatives a unitary design and concept within a European scope, a common conservation strategy was defined for the European elm species within the European Forest Genetic Resources (EUFORGEN) cooperative program (Collin *et al.* 2002) and the "Conservation of Elm Genetic Resources" EU project (RESGEN CT96-78) was set up in the late 1990s.

Ex situ collections are effective for dynamic conservation as far as they contain high genetic variability in adaptive traits, which is the indispensable requisite for adaptation and evolution as well as for genetic improvement by breeding. Genetic variability within the established *ex situ* collections of European elms has been investigated within the RESGEN project with DNA markers and in quantitative traits with adaptive value, i.e. resistance to DED and phenology. Special attention was given to the timing of bud burst which, apart from its general ecological importance, potentially affects DED susceptibility (reviewed in Paper II).

In the work presented here, we analysed the variability in susceptibility to DED within the Italian elm collection in relation to the timing of bud burst under field conditions (paper II). We also studied the variability in timing of budburst of the European elm species across collections and years as a function of environmental factors and geographic origin (paper I and III). Under controlled conditions we studied photoperiod and temperature effects during dormancy release in clones of four Asian and two European elm species (paper IV).

The study genus – *Ulmus*

Elms belong to the genus *Ulmus* L. (Ulmaceae Mirb.) which includes deciduous and semi-deciduous woody plants native to temperate and subtropical regions of the Northern Hemisphere (Buchel 2000) (Figure 1).

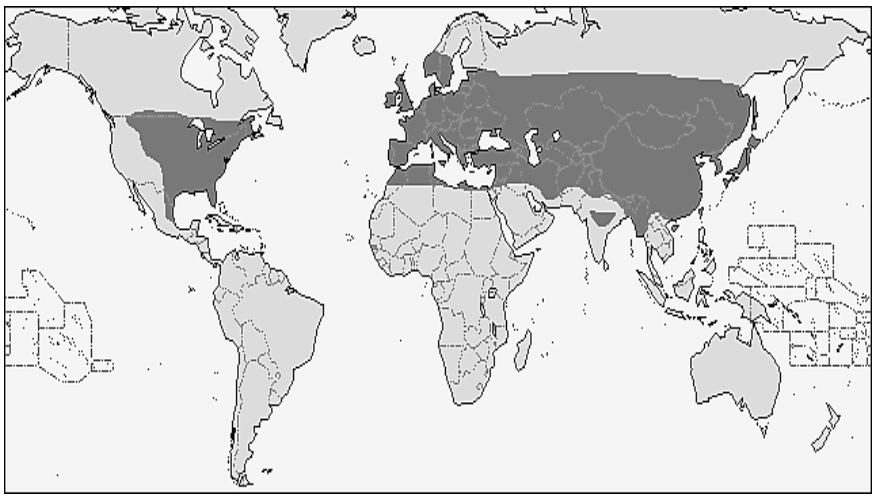

Figure 1. Natural distribution range of the genus *Ulmus* Mirb. From Stipes & Campana (1981). Redrawn by Ignazio Graziosi.

The taxonomy of *Ulmus* is controversial owing to high morphological variability, ease of hybridisation, which produces a large variety of fertile intermediate forms of difficult attribution, and long history of utilization by humans. Elms have since ancient times been cultivated and introduced outside their natural range, allowing hybridisation between taxa otherwise naturally separated (Richens 1983). On the basis of morphological characters, mainly of leaves and fruits, and more recently on the basis of molecular markers, 30 to 45 species of *Ulmus* have been classified and placed in five to nine taxonomic sections (Richens 1983; Wiegrefe *et al.* 1994; Buchel 2000).

Elms are allogamous and hermaphroditic, having small perfect flowers which, being wind-pollinated, are apetalous. Flowers generally appear in late winter or early spring before foliation but a small number of species flower in late summer or fall. The fruit is a roundish wind-dispersed samara containing one non dormant seed which is able to germinate soon after dissemination (Cronquist 1981; Richens 1983). Elms have ring-porous wood, with 1 to 3 rows of earlywood vessels. Vessels in latewood are grouped in tangential to oblique bands together with vascular tracheids and parenchyma. Conduction is restricted to the outermost growth layers (Greenidge 1955; Ellmore & Ewers 1985). The ground-tissue is thick-walled. Paratracheal parenchyma is abundant in earlywood and among vessel groups in latewood (IAWA Committee 1989).

Elms are generally able to adapt to a wide range of soils and pH levels. They have a deep root system which makes them tolerant to wind and drought. Elms grow fast and regenerate rapidly by seed, are resistant to pruning and root damage, and adapt exceptionally well to unfavourable site and environmental conditions. They have nutrient leaves, especially precious as fodder for cattle, and hard wood for construction. Because of all these qualities, elms have been utilized for a long time in agriculture as well as in urban forestry (Heybroek 2000). Elms were among the most widely used ornamental trees in cities and the countryside of both Europe and North America until DED led to the loss of hundred of millions of elms in the 20th century (Dunn 2000).

The study species

This thesis focuses mainly on clones of European elms. We also studied a small number of clones belonging to four Asian species, which have been introduced in Europe and North America as ornamental trees and for landscape or breeding use since they generally show higher resistance to DED. The *Ulmus* species are briefly presented in the following section.

The European elms

The European elms are majestic, deciduous trees growing scattered in temperate mixed broadleaved forests. They are used also in agriculture and as urban trees. Their timber is of high quality and consequently they are sought after for various purposes although their role in traditional forestry is minor.

Ulmus glabra Huds. is native to most of Europe, ranging from Scandinavia, where it reaches the polar circle along the Norwegian coast (Lid & Lid 1998 in Myking & Skrøppa 2007), to the Mediterranean and from Spain to the Ural Mountains (Richens 1983). It grows at elevations of up to 1200 m in southern Europe. It is moderately light-demanding, requires deep and rich soils, and does not tolerate flooding or prolonged drought (CAB International 2005).

Ulmus laevis Pall. has a central-eastern European distribution from France to the Ural Mountains and from Southern Finland to the Balkans (Richens 1983). It typically grows in alluvial and riparian forests along rivers, as it tolerates wet soils and periodic flooding, but it can also be found as a scattered species in mixed forests of moderately dry environments. It grows at elevations of up to 300 m. In the western part of its range, where its habitat is fragmented, *U. laevis* occurs in small and scattered populations, whereas in the central part of its range it is more abundant (Collin 2003 a, b; CAB International 2005).

Ulmus minor Mill. has a more southern distribution. Its range extends from Spain and Great Britain to the Volga river and the Caucasus; from Southern Sweden to the Mediterranean, reaching Northern Africa and Asia Minor (Richens 1983). On its southernmost sites it can reach 1000 m of elevation. *U. minor* has been cultivated since Roman times and probably since prehistoric times in the Mediterranean region, so its indigenousness is dubious in the marginal parts of its range (Richens 1983). Even though *U. minor* is light-demanding, it can tolerate a moderate shade on humid sites. It prefers fresh, deep and fertile alkaline soils but

easily adapts to changing humidity as well as to clay soils (CAB International 2005).

The Asian elms

Ulmus macrocarpa Hance is a deciduous tree up to 20 m tall, whose natural range extends from far Eastern Russia to China. It is resistant to extreme cold and drought and generally grows in harsh conditions in the mountains of Northern China and in the sandy lands of North-Eastern China. Further south it is also found in mixed temperate forests at elevations from 700 to 1800 m (Fu & Yquin 2000; Fu *et al.* 2002). As an ornamental species it has been introduced into Northern America and Europe. It requires well-drained soils and does not tolerate prolonged soil wetness or inundation (Ware 1995). Even though *U. macrocarpa* is moderately resistant to DED, it is considered valuable for breeding since it is resistant to other pests and diseases such as elm yellows and elm leaf beetle that generally damage European elms (Ware 1995).

Ulmus parvifolia Jacq. is a deciduous or semi-deciduous tree up to 25 m tall, native to temperate and tropical regions of Eastern Asia (Fu *et al.* 2002). Despite its geographical distribution in warm temperate zones, it can be surprisingly resistant to cold, although considerable variation has been observed among provenances (Ware 1995). It adapts easily to a large variety of soils and climatic conditions, tolerating harsh conditions including drought and exposure to salt aerosol along windy sea coasts. *U. parvifolia* is not susceptible to DED and it is today the most ubiquitous elm species, being successfully introduced in Europe, Northern America, Southern Africa and New Zealand.

Ulmus pumila L. is an important fast-growing deciduous tree up to 25 m tall, with a Central-Eastern Asian distribution from Siberia to Mongolia, Northern China and Korea. It occurs naturally in temperate, warm temperate and subtropical zones. It is a light-demanding species with a deep root system, but it is intolerant of wet soils. *U. pumila* is extremely cold resistant, very tolerant to drought, to saline-alkali soils and to air pollution (CAB International 2005). It is widely used in agroforestry, erosion control, reforestation of sandy and degraded land, and as a windbreak, as a road-side tree and as an ornamental within and outside its natural range (Geng 1989; Santamour & Bentz 1995). Many European countries have introduced *U. pumila* as a gene source for breeding resistance to DED (Smalley & Guries 2000). It has predominantly sexual reproduction and in Europe it naturally hybridises with *U. minor* (Cogolludo-Agustin *et al.* 2000; Goodall-Copestake *et al.* 2005).

Ulmus villosa Brandis is a medium to large deciduous tree native to the north western sub-Himalayas at elevations from 1200 to 2500 m (Melville & Heybroek 1971). It adapts to several soil conditions but requires well drained soils and has moderate cold resistance (Santamour 1979). The species is highly valued for timber, fuel and fodder. It is considered one of the most important agro-forestry trees in the Kashmir region. It also has a great potential outside its natural range for use on degraded land (Singh 1982; Bhardwaj & Mishra 2005). It is moderately susceptible to DED (Santamour 1979) and has been introduced in Europe and North America as an ornamental tree and for breeding purposes.

Dutch elm disease (DED)

Dutch elm disease is one of the most studied forest tree diseases (Dunn 2000). In this introduction I will briefly describe the history of DED epidemics, the causal pathogen, the disease cycle, and the main evidences on host resistance.

The causal fungus

DED is a severe wilt disease caused by two *Ophiostoma* species probably introduced in Europe and Northern America from Asia (Brasier 2000). *Ophiostoma ulmi* (Buisman) Nannf. was responsible for the first DED pandemic which started in Europe in the 1910s (Schwarz 1922) and rapidly devastated elm populations in Europe and thereafter in North America. The epidemic was initially very intense but in Europe it started to decline in the 1940s, remaining at a relatively low level during the following decades, whereas in North America no decline was observed (Brasier 2000). The current pandemic of DED, which started in Europe in the 1970s, is mainly caused by *Ophiostoma novo ulmi* Brasier subsp. *novo-ulmi* and subsp. *americana* (Brasier 1991; Brasier & Kirk 2001). *O. ulmi* and *O. novo-ulmi* differ for several morphological and biological traits, but the most important difference is that whereas *O. ulmi* is a relatively weak pathogen, *O. novo-ulmi* is very aggressive (Brasier 1991). Because of its higher fitness (Brasier 2001), *O. novo ulmi* replaced *O. ulmi* and is now the more prevalent DED pathogen both in Europe and North America.

Disease symptoms and cycle

External initial symptoms of DED include discolouration and wilting of the leaves. The progression of symptoms depends on the route of infection. Trees infected in the upper crown by feeding of insects carrying the fungus show rapid wilting, yellowing and drying of the leaves on small branches high in the tree. Symptoms progress on other branches and eventually affect the whole crown. Symptom development is more rapid and severe when DED infection occurs in spring than in summer. If the infection occurs through root grafts with infected trees, wilt symptoms are massive and severe throughout the crown and the tree dies rapidly. A typical internal symptom of the disease is the formation of brown streaks in infected sapwood.

During pathogenesis *Ophiostoma novo-ulmi* develops in the xylem vessels of elms. The process involves production by the fungus of cell wall degrading enzymes and toxins (Richards 1993; Binz & Carnevascini 1996; Temple & Horgel 2000), and is accompanied by the formation of vessel plugs such as tyloses and gels (Stipes & Campana 1981; Rioux *et al.* 1998; Ouellette *et al.* 2004 a, b; Et-Touil *et al.* 2005), resulting in vessel blocking and cavitation (Newbanks *et al.* 1983), and ultimately in a wilt syndrome.

DED has a complex infection cycle involving three organisms: the pathogenic fungus, the host tree and the vector insect. The disease is transmitted by bark beetles of the genus *Scolytus* Geoffroy (Coleoptera Scolitidae) (Webber & Brasier 1984). In Europe, the principal vectors are *S. scolytus* and *S. multistriatus*,

(Webber 2004). Overwintering young beetles emerge in spring from their hibernating quarters in the bark of dying elms when the threshold temperature necessary for flight (about 20°C) is reached for some days previously (Rudinsky 1962; Webber & Brasier 1984). They fly to healthy elms for feeding at the crotches of young twigs in the crown. This transient phase is very important for disease transmission as it is the only way for the fungus to reach and infect isolated, healthy trees. Infected beetles contaminate elms by carrying the pathogen's spores into feeding injuries, in direct contact with the host's vascular tissues. Spores germinate into a growing mycelium and reach the xylem where the pathogen spreads into the vessels through a yeast multiplication phase (Webber & Brasier 1984). The effectiveness of this process depends on the environmental conditions at the inoculation site, on the amount of inoculum carried by the insect and on the host's susceptibility and physiological state (Sutherland *et al.* 1997; Webber 2000). Later on, the beetles move to dying elms for breeding into maternal galleries dug in the inner bark of trunks or branches, which provide an ideal environment both for larvae to develop (Rudinsky 1962) and for the fungus to produce fruiting bodies (Webber & Brasier 1984). The new infected beetle generation which emerges from the bark completes the cycle. Depending on the climatic conditions, *Scolytus* species may produce one or more generations per year (Rudinsky 1962), but, as elms display a seasonal variation in DED susceptibility with the most susceptible period soon after bud burst, the beetle generation most effective in transmitting DED is the first one in spring.

Scolytus vectors of DED are elm-specific (Dixon 1964; Pajares 2004) secondary insects, whose reproduction in healthy and vigorous trees is generally impeded by the strong reaction of the plant. For reproduction they depend on the availability of old or weak elms with cortical tissues thick enough for hosting breeding and larvae development, but with low reaction to insect colonization (Rudinsky 1962). The introduction of the DED pathogens therefore provided the vector beetles suitable plant material (diseased elms) for breeding. Thus the population dynamics of the insects undergo phases of outbreaks and reduction following the dying-out of adult elms and the development of new elm populations (Pajares 2004).

Host resistance

There is considerable variation in host resistance to DED between elm species. The most resistant elm species are the Asian species *U. parvifolia*, *U. pumila*, and *U. wallichiana* (Smalley & Kais 1966; Ware 1995; Smalley & Guries 2000), whereas the Asian *U. macrocarpa* and *U. villosa* are moderately resistant (Santamour 1979; Ware 1995). The North American elms are generally highly susceptible and the European species are moderately to very susceptible to DED (Gibbs 1978; Dunn 2000). *U. glabra* is very susceptible to DED (Solla *et al.* 2005 a) although it may remain unaffected at the northernmost latitudes and highest elevations where no efficient vector is available (Eriksson 2001; Collin 2003a). *U. laevis* is susceptible to DED but its populations and individual plants have in many cases escaped the disease, probably because this species is less attractive as a food plant to the *Scolytus* vectors than the other two European elms (Sacchetti *et al.* 1990; Webber 2000). Considerable within-species variability in resistance is found in Asian

(Smalley & Guries 1993, 2000) and in European elms, especially *U. minor* (Smalley & Kais 1966; Solla *et al.* 2005 a; Pinon *et al.* 2005; Paper II). It should be noticed that resistant elms are not immune to the disease: they become infected but are able to efficiently circumscribe and overcome the infection (Rioux & Ouellette 1991 a, b).

The physiological and molecular bases of host resistance to DED are not well understood. Several defense reactions have been addressed in the literature to be involved in DED resistance, but no persuasive evidence for a major role of any of them has been obtained so far, and conflicting results have been reported (Duchesne 1988, 1993; Dunn 2000; Ouellette *et al.* 2004 a, b). A number of minor factors of resistance, either pre-existing as anatomical features, or induced as the formation of chemical or histological barriers, may act in concert. Several genes are supposed to be involved in DED resistance (Lester & Smalley 1972; Hubbes 2004), but so far no genomic data is reported.

Resistance to DED is strictly associated with the host's capacity to quickly localize the infection, preventing the pathogen from both spreading in the vascular system (Sinclair *at al.* 1975) and reaching the cambium (Shigo & Tippet 1981; Bonsen *et al.* 1985). A systemic infection drastically reduces the hydraulic conductivity in the functional xylem, which in elms is limited to the current year ring (Ellmore & Ewers 1985), mainly because of embolism development and progression (Zimmerman & McDonough 1978; Newbanks *et al.* 1983). The process results in a severe wilt syndrome which may cause the tree to die rapidly (MacHardy & Beckman 1973). Ellmore & Ewers (1985) reported that the large vessels formed in spring at the beginning of the current growing ring are responsible for 96% of the flow rate in *U. americana*. When the cambium is damaged, the formation of modified tissue for localizing the infection and the formation of new vessels for replacing the damaged ones are prevented (Bonsen *et al.* 1985).

A convincing conceptual framework dealing with elm resistance to DED was proposed by Shigo & Tippet (1981). Their idea is that the localization of the pathogen follows the CODIT model (Compartmentalization Of Decay In Trees; Shigo 1984). Trees capable of quickly compartmentalize the pathogen by means of barrier zone formation will survive the infection, whereas trees with delayed compartmentalization are severely damaged. This model was initially proposed for *U. americana* (Shigo & Tippet 1981), but was found to apply also to other elm species (Bonsen *et al.* 1985) and non-host species (Rioux & Ouellette 1989, 1991a, b). The formation of barrier zones may involve a variety of induced defense reactions and is facilitated by pre-existing defense structures (Rioux 1996). Thus, the CODIT model brings together different evidence about factors related to DED resistance. Anatomical characters, such as low vessel diameter and length (Elgersma 1970; Sinclair *et al.* 1975; Solla & Gil 2002) or small size of vessel groups (Pope 1943; McNabb *et al.* 1970), represent a pre-existing defense strategy. Reactions taking place as a consequence of the infection may be both anatomical and chemical, and they represent a second inducible defense strategy. These reactions include blockage of vessels by tyloses, embolisms, accumulation of

pectic and hemicellulosic substances (Elgersma 1982; Shigo 1982; Ouellette & Rioux 1992; Rioux *et al.* 1998), formation of chemicals, such as phytoalexin-like sesquiterpenes (Jeng *et al.* 1983; Duchesne *et al.* 1985; Sticklen *et al.* 1991), and histological barriers typically containing phenolic compounds and suberin (Riuox & Ouellette 1991a, b; Ouellette *et al.* 2004 a, b; Et-Touil *et al.* 2005).

Seasonal variation in host resistance and relation with growth rhythm

It has long been known that susceptibility to DED displays strong seasonal variation both in resistant and susceptible species. The time of highest susceptibility, and the duration of susceptibility, i.e. of the period during which elms can become infected and express DED symptoms, varies greatly among elm species and even between provenances and experimental conditions (Banfield 1941, 1968; Smalley 1963; Smalley & Kais 1966; Pomerleau 1965, 1968; Tchernoff 1965; Neely 1968, 1970). However, according to the few studies which correlate DED susceptibility with the seasonal development of the host (Pomerlau 1966; Neely 1968, 1970; Takay & Kondo 1979; Solla *et al.* 2005 b; Santini, Ghelardini & Falusi unpublished data) the time of maximum susceptibility coincides with the time of maximum growth rate, the initiation of leaf expansion and the formation of large-size spring vessels. The decrease in susceptibility is correlated to the seasonal changes in wood anatomy from spring to late wood (Pope 1943; Solla *et al.* 2005 b). Hence, disease development is highly dependent on the host's rhythm of seasonal morphogenesis, including the time of bud burst and reactivation of cambial activity as well as the pattern of longitudinal and radial growth. Since the compartmentalization efficiency depends on the plant's energetic status, and a slow compartmentalisation obliges the plant to keep building new barriers which in turn limit the accessibility of its own reserves in the stem (Bonsen *et al.* 1985), the CODIT model well addresses also the classic seasonal variation of susceptibility. Susceptibility is highest when the tree's energy reserves are at their minimum, growth rate is highest, photosynthetic apparatus is not fully efficient and vessels are large. Variation in susceptibility as related to growth rhythm, either seasonal variation or variation observed among fast and slow growing plants (Sutherland *et al.* 1997), fit well in the Growth-Differentiation Balance Hypothesis (Herms & Mattson 1992). Elms experiencing high resource availability, as is generally the case in spring, allocate a larger proportion of assimilates to growth than to differentiation, which include the production of secondary metabolites which are essential in defense against pathogens and herbivores in plants (Dethier 1954; Whittaker & Feeny 1971). Hence, elms experience a classical trade-off between growth and defence which renders them especially susceptible to DED during spring, exactly in the period of the beetle vector’s infective phase.

As susceptibility undergoes seasonality, and as elms are inoculated by vector insects in a transitory and short phase of their cycle (twig-crotch feeding phase), the disease develops only if the insect's disease-transmission phase and the host susceptibility period coincide. As a consequence, a tree whose maximum susceptibility period would be anticipated and short relative to the presence of insects in their phase of disease transmission has potentially low risk of being infected naturally. An asynchrony between host phenology and insect cycle may

allow host trees to avoid the disease, thus representing a kind of resistance (disease escape). This resistance could be exploited in a breeding program, provided that knowledge is acquired on the host's pattern of seasonal growth and the environmental factors (particularly temperature) regulating it. Within such a breeding program, it would be necessary to study how budburst date and growth rhythm vary in different climate conditions but also how environmental factors control the cycle of the *Scolytus* vectors.

Genetic improvement programs for DED resistance

A large part of the research work carried out to fight DED has concentrated on the exploitation of natural host resistance for breeding resistant elms (Mittempergher & Santini 2004). Since the first appearance of DED in Europe and North America, several traditional breeding programs have been carried out to develop cultivars of elms combining desirable characters for urban and landscape use with DED resistance. These programs are based on extensive screening for resistance by means of artificial inoculations followed by breeding and recurrent selection (Tchernoff 1965). The technique is time-consuming because of the long generation time typical of woody species and because elm seedlings less than three years old cannot be inoculated since they display transitory DED resistance (Heybroek 1957). Attempts have been carried out to find methods alternative to inoculation, based either on anatomical or chemical markers of resistance, for screening elms at least in the early stages of the selection process (Martin *et al.* 2005; Solla *et al.* 2005 b).

Most breeding programs in Europe and North America have introduced resistance from Asian species into native elms. Few programs have focused on identifying and cloning native elm individuals that have moderate resistance. (Heybroek 1993; Smalley & Guries 1993; Smalley *et al.* 1993; Townsend 2000; Mittempergher & Santini 2004). As a result of decades of elm breeding, several clones with good resistance to Dutch elm disease are now available (Rohring 1996; Guries 2001; Santini *et al.* 2002, 2007 a).

Dormancy in vegetative tree buds

The climate of boreal and temperate regions is characterised by a seasonal change in environmental conditions which follows a predictable course over the year. Perennial plants are exposed to frost and dehydration stress during winter. Their ability to survive depends on an evolved mechanism by which plants enter a state of inhibited growth called dormancy and they also develop cold hardiness (Arora *et al.* 2003; Howarth *et al.* 2003). These processes are synchronised with the annual course of temperature at the tree's natural site (Perry 1971; Hänninen & Kramer 2007). Cessation and initiation of growth are key processes in this adaptation as they mark the shift between frost resistant and vulnerable phases.

According to the classical definitions, dormancy in a general sense is a state 'in which visible growth is temporary suspended' (Samish 1954) or 'in which a tissue predisposed to elongate does not do so' (Doorenbos 1953). More recently, Lang (1987) defined dormancy as 'a temporary suspension of visible growth of any structure containing a meristem'.

Dormancy phases

The nature of dormancy varies during the non-growing season. Dormancy is a progressive transition among different physiological conditions rather than a uniform state (Saure 1985; Arora *et al.* 2003). Although terminology is confusing (see Kozlowsky & Pallardy 1997), dormancy phases in tree buds are conceptually well recognised. A gradual transition occurs from 1) an inhibition of bud growth within the plant but outside the bud owing to influences from other organs and tissues to 2) an inhibition within the bud itself and 3) to a restriction of bud growth by external conditions, mainly temperature and photoperiod (Dennis 1994; Arora *et al.* 2003). The presence and relative duration of these phases may differ among species and environments (Vegis 1964; Champagnat 1989). Lang (1987) proposed the terms 'paradormancy', 'endodormancy' and 'ecodormancy' to describe these types of dormancy, respectively. A similar classification was proposed earlier by Chouard (1956) using the terms 'correlative inhibitions', 'dormancy' and 'quiescence'. The phase of endogenous inhibition has also been called 'true dormancy' (Vegis 1964) and 'rest' (Saure 1985).

According to Champagnat (1983 a, b, 1989) bud dormancy can be regarded as a continuous process of plant morphogenesis in which the development of buds into new shoots is restricted by apical dominance during spring, by correlative inhibitions from leaves, other buds and stem tissues during summer, and by inhibitions from within the bud itself in late summer or autumn. The seasonal succession of these diverse inhibitions in tree buds can be shown as in Figure 2.

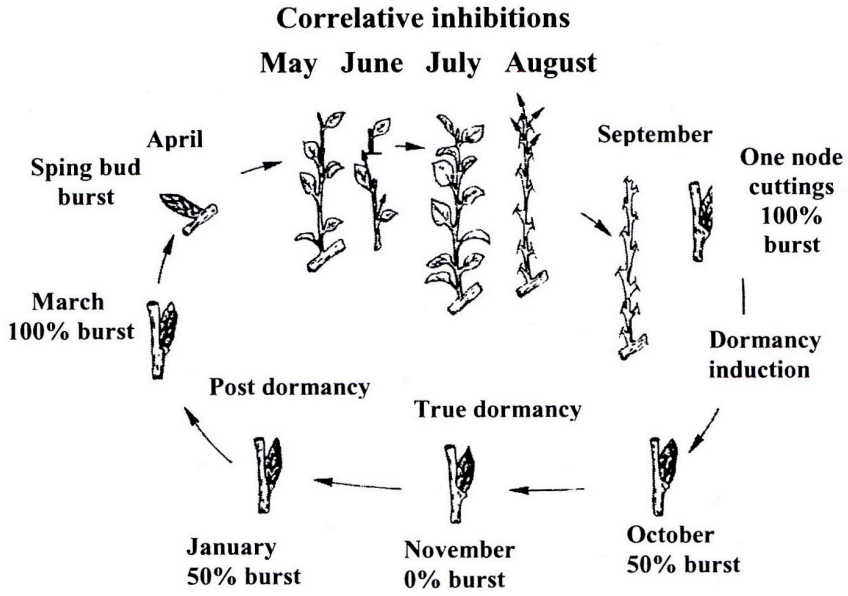

Figure 2. Developmental cycle of lateral buds on a tree shoot in a temperate climate. Apical dominance after bud burst in spring is revealed by apex removal, which initiates growth of lateral buds. Later on, apex removal must be accompanied by complete defoliation to initiate growth of lateral buds. From July-August onward, even defoliation becomes ineffective. True dormancy has not started yet, since at this stage isolated lateral buds (single-node cutting) burst after a short delay under favourable temperature. Buds are under the influence of a correlative inhibition originating from the bearing axis. Between mid-September and mid-October, even single-node cuttings do not burst under favourable conditions. At this stage, dormancy has been induced. Modified from Champagnat 1989.

Bud burst delay of single-node cuttings has long been used as a measure of dormancy intensity and variation during winter or following experimental chilling in woody plants, fruit and forest trees (Crabbé 1968; Barnola 1976; Jourdan 1980; Si-Mohamed 1983; Barnola et al 1986; Jacques *et al.* 1989; Balandier *et al.* 1993; Ricaud 1995; Falusi & Calamassi 1997 b, 2003; Bonhomme *et al.* 2005; Mazzitelli *et al.* 2007; Pang *et al.* 2007). In temperate climates, this delay diminishes from December on and becomes quite weak in January. Later on, until bud-burst in natural conditions, the buds reach a quiescent phase during which growth is restricted only by low temperatures.

Vegis (1964) regarded dormancy as an inhibition state during which the effective temperature range for growth is reduced, and dormancy release as a progressive widening of this range. The narrower this range, and the slower its widening, the greater is the initial dormancy (Figure 3). Buds undergoing true dormancy cannot resume growth even when subjected to favourable temperature conditions. Thus true dormancy protects buds by ensuring that growth won't be resumed until the stable return of favourable conditions in spring. When the following post-dormancy stage is reached, growth may resume and bud burst may occur in a

narrow temperature range. With time, the temperature range progressively widens until growth is fully renewed. During late dormancy, temperatures exceeding the effective range may induce a state of secondary dormancy.

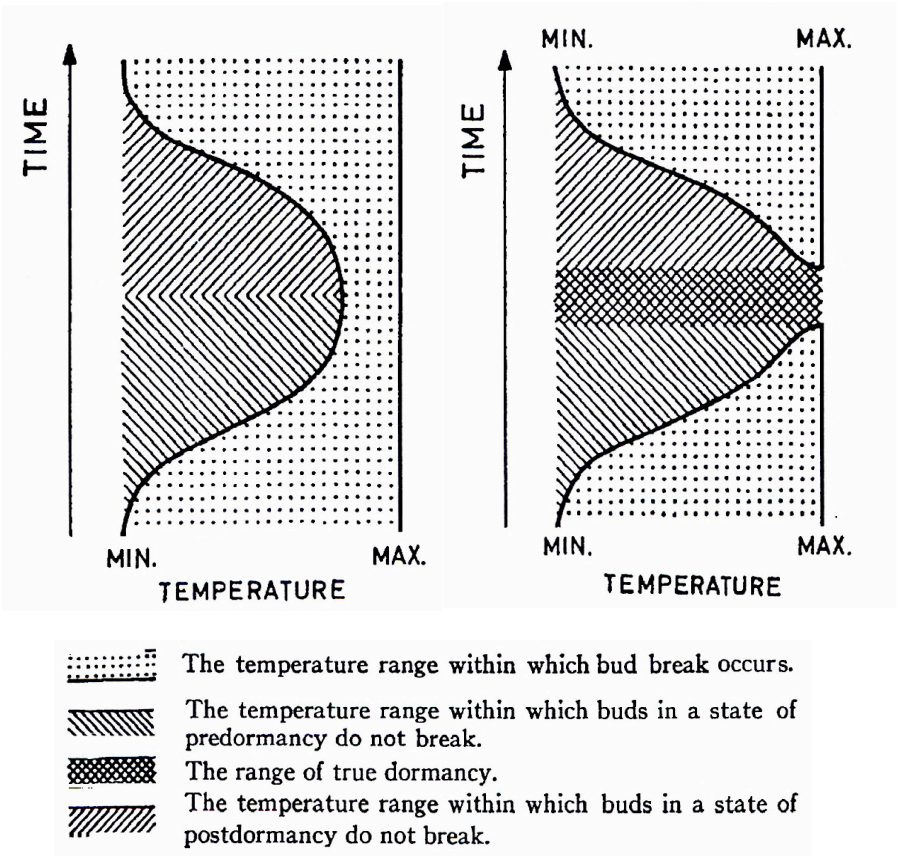

Figure 3. The narrowing and widening of the temperature range for growth in temperate trees coincides with pre- and post-dormancy phases, respectively. Species with true dormancy (right) have a period in which growth is totally restricted irrespective of temperature. Species with no true dormancy (left) resume growth in a narrow temperature range even in the period of maximum growth inhibition. Adapted from Vegis (1964).

Environmental signals involved in dormancy induction

The first step in dormancy induction is the cessation of apical growth. Light (mainly photoperiod but also light intensity) and temperature both play a significant role in these phenomena, although other environmental factors such as nutrient or drought stress may be involved (Perry 1971; Noodén & Weber 1978). Change in photoperiod seems to be the predominant environmental cue for growth cessation and onset of dormancy in many temperate and boreal trees (Vaartaja 1954, 1959; Down & Borthwick 1956; Wareing 1956; Nitsch 1957; Heide 1974 a; Håbjørg 1978; Li *et al.* 2003). Latitudinal variation in the critical photoperiod for growth cessation is documented in many species. Typically, as a consequence of local adaptation, provenances from high latitudes have shorter critical nightlength

for growth cessation, as shown for instance in *Picea abies* (Dormling 1973; Clapham *et al.* 1998), *Salix* spp. and *Betula* spp. (Junttila 1980; Viherä-Aarnio *et al.* 2006), and *Populus tremula* (Ingvarsson *et al*. 2006). In some trees, in which growth cessation is induced by photoperiod, high temperatures during dormancy induction may delay dormancy development and increase dormancy deepness, as shown in *Betula* spp., *Alnus glutinosa* and *Picea abies* (Heide 1974b, 2003; Junttila *et al.* 2003; Søgaard *et al.* 2008). However, some trees, such as *Malus pumila*, *Pirus communis* and *Prunus avium*, do not respond to photoperiod for growth cessation and dormancy induction (Wareing 1956; Nitsch 1957). Although dormancy onset in these species has long been considered to be entirely controlled by endogenous factors (Wareing 1956; Thomas & Vince Prue 1997; Battey 2000), recent evidence indicates that low temperature alone controls growth cessation and dormancy induction in apple and pear (Heide & Prestud 2005).

Almost nothing is known about the environmental control of growth cessation and dormancy induction in elms. According to the only study in controlled conditions, these responses are scarcely affected by photoperiod in *U. americana* seedlings (Downs & Borthwick 1956). However, results obtained with seedlings in common garden experiments suggest a shorter critical night length for growth cessation in northern versus southern populations in *U. glabra* (Myking & Skrøppa 2007), *U. laevis* (Whiteley *et al.* 2003) and *U. pumila* (Geng 1989).

Temperature and light control of dormancy release

There is substantial evidence from controlled-conditions experiments and from cultivation of temperate trees in warm climates that a lack of chilling during winter causes anomalies in the opening of the buds, in the growth of the shoots, and in the development of the whole plant (Saure 1985; Falusi & Calmassi 1990; Champagnat 1993). Buds must be subjected to low temperatures for a certain period (chilling requirement) for dormancy release to take place, thus winter chilling is the most important environmental factor for the release of dormancy. Sufficient chilling in a cool winter causes a quick decrease in true dormancy and a subsequent state of quiescence is reached. Insufficient chilling in warm winters causes true dormancy to decrease slowly and to extend. When there is no chilling, true dormancy is not released, which leads to prolonged dormancy. The course of dormancy as influenced by internal and external factors under different winter conditions has been clearly illustrated by Saure (1985) (Figure 4).

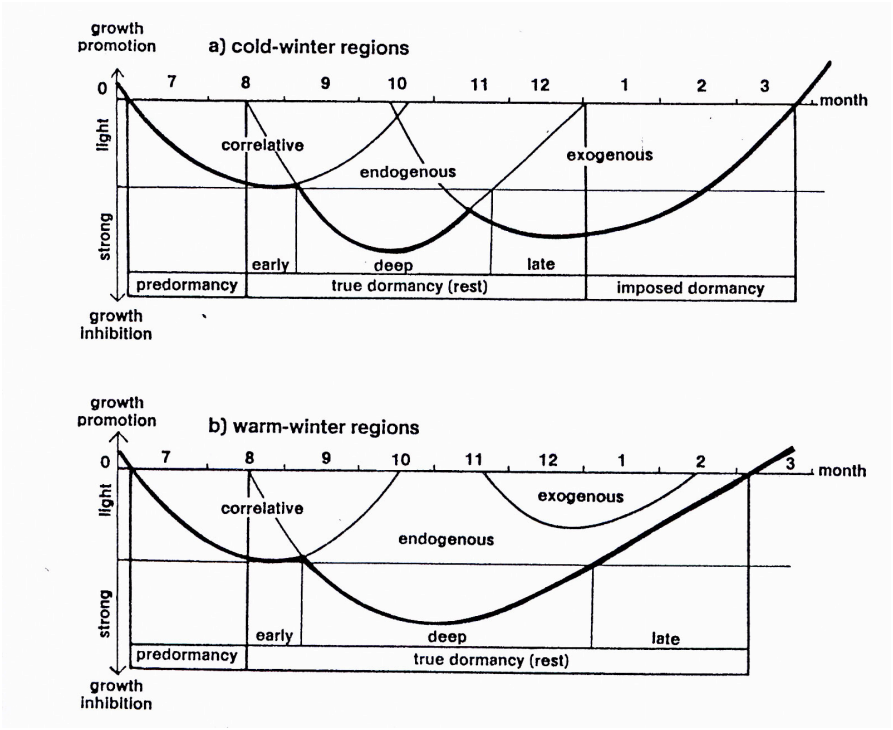

Figure 4. Progression of dormancy under climates with contrasting winter conditions. In cold-winter regions (a), sufficient chilling quickly decreases the intensity of true dormancy. Subsequently, a state of quiescence (imposed dormancy) is reached. In warm-winter regions (b), insufficient chilling causes true dormancy to decrease slowly and to extend over a longer period. From Saure (1985).

Temperatures between 0°C and 15°C are effective for releasing dormancy, and the optimum temperature range is generally considered 3-7°C, lower and higher temperatures being less effective (Perry 1971; Saure 1985; Champagnat 1993). There is evidence for some trees that the effect of chilling is not irreversible and that high temperatures during winter may partly negate previous chilling, thereby inducing a deeper condition of dormancy (Vegis 1964; Erez *et al.* 1979; Couvillon 1995; Tamura *et al.* 1995).

Besides chilling, light conditions may affect dormancy release. Long photoperiods are able to release dormancy and promote bud burst in pre-dormancy or post-dormancy phases (Wareing 1953; Downs & Borthwick 1956; Falusi & Calamassi 1990; Heide 1993 a, b; Linkosalo & Lechowicz 2006). Moreover, in many trees the effect of chilling can be partly replaced by long photoperiods when the chilling requirement is not satisfied (Downs & Borthwick 1956; Roberts & Main 1965; Cannell & Smith 1983; Garber 1983; Heide 1993 a, 1993b; Falusi & Calamassi 1996, 1997 b; Myking & Heide 1995; Myking 1997).

Under natural conditions the chilling requirement is generally met early in the winter, at least in cold regions with stable climate. The duration of true dormancy

is inversely related to duration and severity of the winter (Saure 1985). As a broad trend, chilling requirement can be considered to increase from southern to northern regions, winter dormancy being the result of adaptation to cold winters. Within species, at a narrower geographic scale, shorter chilling requirements have been found in northern than in southern provenances (see references in paper III). Moreover, at the same latitude, longer chilling requirements are typically found in maritime than in continental provenances (Nienstaedt 1967; Campbell & Sugano 1979; Farmer & Reinhold 1986; Myking & Heide 1995; Myking 1997).

When true dormancy is released, the buds start to elongate at a rate directly related to temperature (Arnold 1959; Hari *et al.* 1970; Sarvas 1972; Cannell & Smith 1983). At this stage bud burst can still be delayed for a long time if temperature remains too low. Assuming that a thermal unit is equivalent to a growth unit, i.e. that there is a linear relationship between temperature and growth, bud burst can be considered to occur after the accumulation of a specific temperature sum above a threshold (thermal time to bud burst). For many trees there is evidence that, within limits, the longer the buds are exposed to chilling, the greater is the rate at which they will grow when afterwards exposed to warm temperatures (reviewed in Cannell & Smith 1983; Battey 2000). Thus, chilling decreases the thermal time to bud burst down to a level where the chilling requirement is fully met.

Timing of bud burst

Timing of budburst in spring results from adaptation to the local climate (Lockhart 1983; Lechovicz 1984). Optimal timing of bud burst allows trees to begin growth early enough to maximize the use of favourable spring conditions, but late enough to minimize the risks of being damaged by late frosts (Levitt 1969; Lockhart 1983; Hänninen 1990). Timing of bud burst is important for the long term survival of species and is considered a major determinant of trees' distribution limits in northern regions (Chuine & Beaubien 2001).

Timing of bud burst is triggered by environmental factors, mainly winter chilling and spring temperatures (Linkosalo *et al.* 2006; Hänninen & Kramer 2007). These signals have synergistic effects on bud burst by releasing dormancy and promoting development of buds, respectively. In some species, light conditions (photoperiod, i.e. day or night length and spectral composition) may be an additional trigger of bud burst (Heide 1993b; Linkosalo & Lechowicz 2006), especially in sites and years with warm winters (Campbell & Sugano 1975). Moreover, high temperatures during dormancy induction in autumn affect the timing of bud burst in some trees, enhancing the chilling requirement for dormancy release and delaying bud burst (Heide 1974b, 2003; Junttila *et al.* 2003; Søgaard *et al.* 2008). This interplay among environmental triggers provides the adaptive plasticity needed to survive yearly climatic fluctuations (Kramer 1995; Hänninen & Kramer 2007).

As a result of local adaptation, timing of bud burst typically displays geographic variation along climatic gradients (Wright 1976; Morgenstern 1996; Eriksson *et al.*

2006). Since elevation and latitude largely determine the seasonal course of temperature, the timing of bud burst generally varies along altitudinal and latitudinal gradients. However, more complicate trends are found where different climatic gradients owing to maritime influence or to local topography (Lee & Baumgartner 1966) overlap with the general latitudinal or altitudinal trends (Campbell & Sugano 1979; Jensen 2000). Moreover, geographic trends in bud burst may follow rainfall distribution and summer drought which, in addition to temperature, strongly constrain the length of growing season (Campbell & Sugano 1979; Geng 1989; Fernandez-Lopez 2005; Paper III).

A general trend of earlier bud burst in northern/highland vs. southern/lowlland populations is documented for many trees in common garden experiments, as for instance *Betula alleghaniensis* and *B. lenta* (Sharik & Barnes 1976), *Populus balsamifera* (Farmer & Reinhold 1986), and *Alnus rubra* (Hamann *et al.* 2001). The opposite trend was observed in *Quercus petraea* (Doucousso *et al.* 1996), *Juglans nigra* (Bei 1973), *Juglans regia* (Germain 1992), *Fagus sylvatica* (Von Wuehlisch *et al.* 1995; Chmura & Rozkowski 2002), *Prunus padus* (Baliuckas *et al.* 2005) and *Castanea sativa* (Fernandez-Lopez *et al.* 2005). Moreover, earlier bud burst in inland compared to coastal provenances is reported in some trees (Myking & Heide 1995; Myking 1997; Hamann *et al.* 2001). Furthermore, the order of bud burst may be inverted when the same provenances are grown in sites with either warm or cold winters, as reported for *Acer saccharum* (Kriebel & Wang 1962). At sites where chilling requirement is fully satisfied for all studied provenances, the bud burst ranking most likely depends on differences in the specific threshold temperature for growth. This threshold was found to be lower in northern/highland than in southern/lowland provenances (Worrall & Mergen 1967; Worrall 1983; Myking & Heide 1995). At warm-winter sites, where chilling is not sufficient, the observed ranking in bud burst reflects differences in chilling requirement for dormancy release (see discussion in Paper III).

Large within-population and clonal variation in timing of bud burst was observed in broadleaves and in conifers (Worral & Mergen 1967; Dietrichson 1969; Worrall 1975; Rönnberg-Wästljung & Gullberg 1999; Baliuckas *et al.* 2001; Rousi 2005, 2007). As for other growth traits with adaptive significance, large within-population differences in timing of bud burst are probably due to ample gene flow (pollen and seed exchange) among populations. Consistent with this hypothesis, large within-population variation in growth traits is generally found in wind-pollinated species with large and continuous distributions (Myking 2002, Savoilanen *et al.* 2007).

Modelling bud burst

Various models using climatic variables such as temperature and photoperiod have been developed for prediction of vegetative bud burst timing (reviewed in Chuine 2000; Chuine *et al.* 2003). These models are developed using phenological data series and meteorological data as input variables and they assume certain cause-effect relationships between the biological processes underlying bud burst and the driving environmental factors (Hänninen & Kramer 2007). Since the regulation of

bud burst is only partially understood and various theories describing dormancy and growth promotion during quiescence have been formulated, several bud burst models based on different ecophysiological assumptions have been developed. According to Hänninen & Kramer (2007), models of bud burst can be classified into 'E-models', where the response to the triggering environmental factors is constant over time, and 'ES-models', where the response changes over time according to the course of bud development (dormancy phases).

E-models for bud burst are the simplest models as they don't address the phenomenon of dormancy. The promoting effect of temperature on bud development and growth is considered to start from an arbitrary date in winter or early spring when dormancy is considered released. Bud burst is assumed to occur when a certain number of accumulated growth units, expressed as heat units, is reached (high temperature requirement for bud burst). The relationship between temperature and growth assumed by the model may be linear, with a threshold (Arnold 1959), exponential (Hari *et al.* 1970) or sigmoid (Sarvas 1972).

ES-models of bud burst are more complex and address dormancy. Consequently, they include physiological assumptions about: 1) the effect of chilling in releasing dormancy; 2) the effect of temperature in promoting bud growth in absence of dormancy; 3) the effect of the actual state of dormancy on limiting growth response to temperature, i.e. the relation between 1) and 2). Dormancy release is assumed to occur when a certain number of accumulated dormancy breaking units, expressed as chill units, is reached (chilling requirement for dormancy release). The relationship between temperature and dormancy release assumed in the model can be variously shaped to fit experimental results. The effect of temperature in promoting bud growth with no limitation due to dormancy is modeled as in the E-models. On the basis of the relations assumed between these two effects, ES-models are further distinguished as: 1) "sequential models" when the two effects are separated in time, and temperature can promote growth only when dormancy release is complete and 2) "parallel models" when a growth promoting effect of temperature is possible during dormancy but varies in intensity according to the dormancy phase.

In this thesis we used a model (see equation [1] in Materials and Methods) developed by Cannell & Smith (1983), the so called "alternating model" (Kramer 1995). This model can be classified as an "ES-parallel model" because it assumes that, within limits, the longer the buds are exposed to chilling, the greater is the rate at which they will grow when exposed to warm temperatures. Chilling thus increases the slope of the relationship between bud growth rate and temperature, thereby decreasing the thermal time to budburst. The model also assumes a linear relationship between bud elongation rate and temperature above a threshold, so that accumulated growth units are expressed as thermal time (day degrees > threshold temperature). According to this model, thermal time for budburst decreases exponentially with increasing chill days down to a level where the chilling requirement is fully met. This allows prediction of the budburst date by using only day degrees and chill days. Moreover, this model is easily parameterised as it contains only three parameters: 1) the maximum thermal time

required when no efficient chilling occurred; 2) the thermal time required when chilling requirement is fully satisfied; 3) the velocity of decrement. This model proved efficient in modelling chilling requirement for dormancy release (Hannerz *et al.* 2003) and in predicting climate warming-dependent changes in budburst date in several tree species (Cannell & Smith 1986; Murray *et al.* 1989, 1994).

In the last three decades, tree phenology has received increasing attention in studies on global warming. Phenological models simulating the timing of bud burst and bud set are used for predicting increased risk of frost damage (Hänninen 1991), changes in the length of growing season and variation in species composition of forest ecosystems following global warming (for reviews see Hänninen 1995; Chuine *et al*. 2003; Cleland *et al.* 2007).

Models based on quantitative effects of temperature on dormancy and bud burst have not always proved to be reliable in making predictions on a global scale. Nevertheless, they have shown a certain degree of accuracy in predicting bud-burst dates of different species on a local scale. For this reason, they could be successfully used on a local scale for predicting response of populations or clones to changes in environmental conditions. For the purpose of elm conservation and breeding, phenological models could be useful for predicting the response of European elms to increased temperatures due to climate change and the behaviour of selected clones when reintroduced into the natural environment.

Aims of this study

The general aim of this thesis is to study the timing of bud burst in elms and its environmental control with regard to susceptibility to DED. The starting hypothesis is that the susceptibility is related to spring phenology and growth rhythm.

The main questions addressed were:

1. Can the variation in bud burst date of the European elm species observed in *ex situ* clone collections across sites and years be explained by a phenological model based on temperature? (I)

2. Is there any variability in susceptibility to Dutch elm disease among elms conserved in the Italian clone collection? If so, is it possible to reveal any relationship between disease susceptibility and spring phenology? (II)

3. Is there a relationship between timing of bud burst and geographic origin in *Ulmus minor*? If so, can the observed differences among origins be attributed to differences in chilling requirements for dormancy release? (III)

4. What is the effect of temperature (I, III, IV) and photoperiod (IV) on dormancy release and control of bud burst in *Ulmus* spp.?

Materials and methods

Plant material

The field and growth chamber experiments in this thesis used plant material of six European *ex situ* elm clone collections established for conservation purposes (Figure 5). Most of the clones were obtained by agamic propagation from young individuals of natural populations across Europe, while a few clones were obtained from adult trees which had survived DED epidemics. The field experiments (Paper I, II and III) were part of the EU-project RESGEN CT96-78.

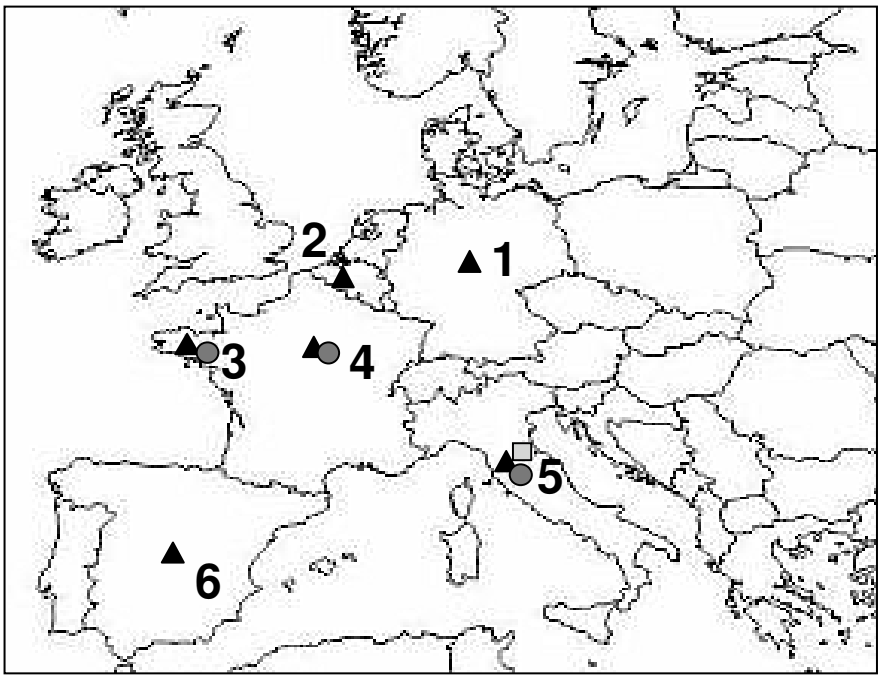

Figure 5. Geographic location of the *ex situ* clone collections of elms studied in this thesis. Triangles: field studies in Paper I. 1 = Hann Münden (51°26′ N, 9°38′ E, Germany). 2 = Geraardsbergen (50°46′ N, 3°55′ E, Belgium). 3 = Nogent sur Vernisson (47°51′ N, 2°45′ E, France). 4 = Guémené-Penfao (47°38′N, 1°50′ W, France). 5 = Antella (43°43′ N, 11°22′ E, Italy). 6 = Puerta de Hierro (40°28′ N, 3°45′ W, Spain). Circles: field studies in Paper III. Squares: field studies in Paper II and growth chamber studies in Paper IV.

A temperature model for bud burst date in European elms (I)

Bud burst phenology was assessed from February to May, at least once a week, on 386 *U. minor*, 386 *U. glabra* and 103 *U. laevis* clones for one to three years (2000-2002) in six clone collections in Central and Southern Europe (Figure 5). For each species the bud burst date in each site and year was defined as the modal value of date distribution expressed in Julian days. Daily mean temperature records were used to calculate chilling and thermal time with three threshold temperatures (2°,

5° and 7°C) and two methods: 1) day degrees/chill days above/below the daily mean temperature; 2) hour degrees/chill hours above/below the hourly mean temperature. November 1st and February 1st were chosen as arbitrary stating dates for chilling and thermal time accumulation respectively. Approximated mean hourly temperatures simulated with a sine-logarithmic model based on minimum and maximum daily temperature records and day-length (Linvill 1990) were used to calculate thermal time and chilling with method 2. The relationship between bud burst date and thermal time to bud burst in all sites and years was analysed graphically for each species, assessing thermal time by each of the methods described above. The effectiveness of different regressions (based on alternative estimates of thermal time and chilling) to explain the observed variation of the bud burst date among sites and years was evaluated for each species. Accuracy was expressed as percentage of variance explained by the relative functions fitted to data.

In order to describe bud burst date variation as a function of thermal time and chilling, the inverse exponential model [Equation 1] (Cannel & Smith 1983) was fitted to the data.

[Equation 1] $$DD = a + b\, e^{(r\,CD)}$$

where DD is the day degrees > 5°C; CD is the number of chill days with mean temperature ≤ 5°C; 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. 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.

Susceptibility to DED and relation to spring phenology (II)

In order to assess DED susceptibility and to investigate its relationship with bud burst phenology, a field trial was established in 1998 at Antella. The experimental planting was carried out with rooted cuttings of four elm species (*U. minor, U. glabra, U. laevis, U. pumila*) and natural hybrids (*U. minor x U. glabra* and *U. minor x U. pumila*) from the Italian and the French clone collections (Figure 5). The trial contained 101 clones planted in a randomised block design with two blocks, each block containing four individuals per clone. Two Dutch hybrid clones 'Lobel' and 'Commelin' were added as benchmarks for relatively high and low disease resistance, respectively. Bud burst was recorded weekly between mid March and early May 2001. Inoculations with a well-known tester isolate of *Ophiostoma novo-ulmi* (H328), which proved to be very aggressive in previous tests, were conducted on May 17 2001, a time of the year when elm trees are generally very susceptible to the disease in the local area. Percentage defoliation was assessed on three dates during the summer and percentage dieback of the crown was recorded twice during the following spring. Data were analysed using one-way analyses of variance, with taxonomic group or latitudinal origin of *U. minor* clones as fixed effect. Regressions were calculated relating bud burst date to disease symptoms.

Geographic variation in bud burst date in *U. minor* (III)

Relations between bud burst date and geographic origin of the clones in *U. minor* were studied using one (2002) and four years (2000, 2001, 2003, 2004) of bud burst records in Nogent-sur-Vernisson and Antella collections respectively. The trials contained individuals from wide latitudinal and altitudinal ranges (36°54′ to 57°22′ N; 0 to 1200 m above sea level) within the species distribution area. Simple (Nogent-sur-Vernisson 2002) and multiple (Antella 2000, 2001, 2003, 2004) regressions were calculated relating bud burst date and thermal time (accumulated day degrees > 5°C) to latitude and altitude of origin. Chill days ≤ 5°C were calculated for each year and site combination, and results were discussed with regard to chilling duration. The variation of the bud burst date among sites and years, and the effect of chilling duration on thermal time to bud burst were studied in four groups of clones from different latitudes (Northern France, Southern France, Northern Italy and Southern Italy) at Guèmenè-Penfao and Nogent-sur-Vernisson (years 2000 and 2001) and at Antella (years 2000, 2001, 2004) by means of factorial ANOVAs.

Photoperiod and temperature effects on bud dormancy release in *Ulmus* spp. clones (IV)

Dormancy release in vegetative buds of elm was studied under controlled forcing conditions in two growth chamber experiments. The experiments were established using one year old twigs from one clone each of *U. glabra, U. minor, U. macrocarpa, U. parvifolia, U. pumila* and *U. villosa*. Twigs were sampled from four healthy trees per clone in the elm clone collection of Antella (Italy) (Figure 1) during two years, once a month from October 15 to March 15, at varying degrees of outdoor chilling. During autumn and winter 2002-2003 (Experiment 1) the effect of two photoperiod treatments (16h and 8h) on percentage bud burst and thermal time to bud burst (day degrees >0°C) in twigs of all clones was tested under constant 21°C temperature. During autumn and winter 2003-2004 (Experiment 2), the effect of two temperature treatments (14°C and 26°C constant temperature) on the same variables in whole twigs and single-node cuttings was studied in four clones (*U. glabra, U. minor, U. parvifolia and U. pumila*) under 16h photoperiod. Data were subjected to factorial analyses of variance with intake date and photoperiod (Experiment 1) or temperature (Experiment 2), and their interactions as fixed effects. In order to describe the variation in thermal time as a function of previous chilling (chill days (CD) < 5°C from October 1st to onset of forcing), the inverse exponential model formerly used to explain timing of bud burst under field conditions in European elms (Paper I), was fitted to the data of Experiment 1. The point where the required thermal time becomes less than 5% higher than the minimum thermal time was calculated [Equation 2] and used as an estimate of chilling requirement for dormancy release in each clone.

[Equation 2] $CD = |r|\text{-}1(\ln b - \ln 0.05a)$

Main results and Discussion

A temperature model for bud burst date in European elms (I)

The variation of bud burst date among clone collections and years could not be explained by thermal time alone in any of the European elm species, whichever method (day degrees or hour degrees) or threshold temperature (2, 5 or 7°C) was used for calculating it. When chilling was included, a significant portion of the observed variance could be explained. In all species, thermal time to bud burst (day degrees > 5°C) decreased with increasing winter chilling (chill days ≤ 5°C) according to an inverse exponential function. Hence, a phenological model including the effect of winter chilling may be considered for predicting bud burst in European elms under a large range of environmental conditions. Calculating thermal time as hour degrees and chilling as chill hours did not improve the relationships. This result suggests that, as found for other species, the use of hourly weather data as input in bud burst models does not improve their accuracy (Cannell & Smith 1983). Regressions were slightly improved by using 2°C as threshold temperature for *U. glabra* and 7°C for *U. minor* and *U. laevis,* hence the use of other species-specific thresholds than the most commonly used (5°C) for quantifying temperature effects may be considered in future modelling work in elms. The 'alternating model' (Cannel & Smith 1983) [equation 1 in Materials and Methods] efficiently explained the variation in bud burst in *U. laevis* (89.0% explained variance) and *U. glabra* (83.2%), whereas for *U. minor* the fitting was relatively poor (62.9%). The fitted curves are shown in Figure 6.

The same model efficiently explained bud burst variation in *Picea sitchensis* (Figure 7 left, 1; Cannel & Smith 1983) and in several European trees (Figure 7 left, 2, 3 and 4; Murray *et al.* 1989). Over the chilling range considered here, the regression curves of European elms were similar to those of trees defined by Murray *et al.* (1989) as low-dormancy species (*Sorbus aucuparia*, *Betula pendula*, *Corylus avellana*, *Sambucus nigra*, *Salix viminalis*, *Prunus avium*). Hence, European elms have relatively low chilling requirements for dormancy release, thermal time to bud burst tending to become steady when chilling exceeds 100 chill days (Figure 7 right).

This model has also been successfully used for predicting bud burst of trees under changing temperature (Murray *et al.* 1989, 1994). Although no simulations were done in this thesis, we hypothesise that in a climate warming scenario, elms would react as the other low-dormancy species in Murray *et al.* (1989), thus anticipating bud burst in most parts of Europe.

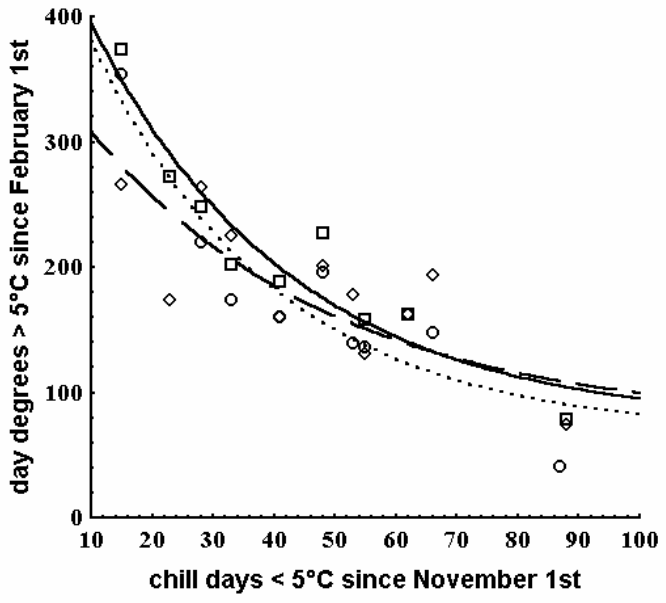

Figure 6. Fitted curves of the model DD = *a* + *b* exp (*r* CD) for *Ulmus glabra* (○ dotted line; DD = 66.9 + 439.3 exp (-0.033 CD)), *U. laevis* (□ continuous line; DD = 74.0 + 434.0 exp (-0.030 CD)) and *U. minor* (Δ broken line; DD = 74.6 + 299.8 exp (-0.025 CD) clones. Each point represents the modal value of bud burst date at a collection site in a given year. DD is day degrees > 5°C. CD is the number of chill days ≤ 5°C.

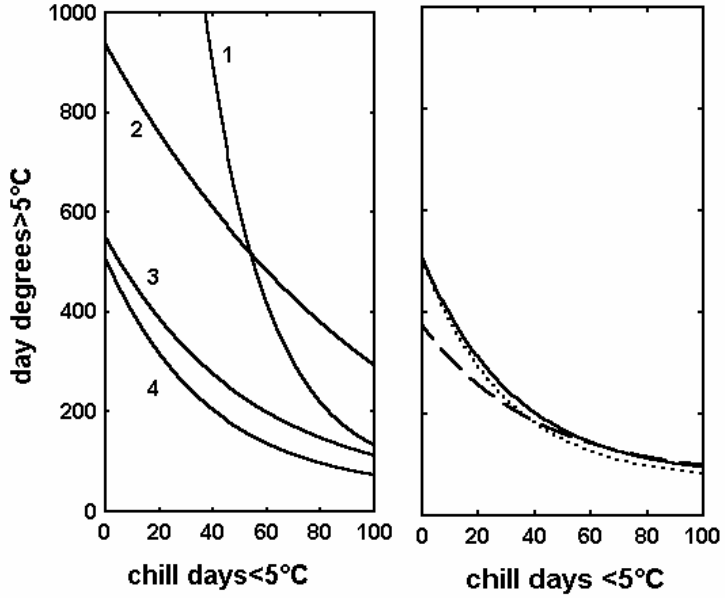

Figure 7. Curves obtained by fitting the model in equation 1 (M & M) to bud burst data of some European trees. Left: 1 *Picea sitchensis* (Cannell & Smith 1983); 2 *Fagus sylvatica* (Murray *et al.* 1989), 3 *Sorbus aucuparia*, *Betula pendula*, *Corylus avellana*, (Murray *et al.* 1989); 4 *Sambucus nigra*, *Salix viminalis*, *Prunus avium* (Murray *et al.* 1989). Right: *Ulmus glabra* (dotted line), *U. minor* (broken line), *U. laevis* (solid line) (Paper I).

In Central and Northern Europe, chilling requirements of elms are fully met and no variation in TT is expected following the predicted rise in winter temperatures. Hence, elms can be expected to advance their bud burst date, as shown for many early flushing species (Cleland *et al.* 2007), with increased risk of late frost damage. In Southern and Western Europe, chilling requirements are not fully met, thus a significant increment in TT is expected following the predicted rise in winter temperatures. However, according to the fitted curves, the increased TT requirement is low and likely to be easily compensated by the rising spring temperature. Thus, even in the warmer part of the distribution range, bud burst of elms will occur earlier following climatic warming.

Variability in timing of bud burst within collections was generally large for all species, especially in case of accessions from a wider geographic range and in *U. minor*. This suggested that budburst date and dormancy in elms varies with geographic origin, which was studied in Paper III. Geographic differentiation in dormancy was probably the main source of unexplained variance in *U. minor*, which justifies the poor fitting of the model in this species. The model could be improved by fitting it to data from different origins or single clones, as indicated by results obtained for a few clones that were present in all collections (Ghelardini *et al.* unpublished data).

The year-to-year variation in bud burst date within sites was very low for all species despite large differences in winter and spring temperatures. Strong bud burst date stability among years with different temperatures is reported for trees with a dual control of dormancy by temperature and photoperiod (Heide 1993 a, b). Our results suggested that photoperiod is involved in the control of bud burst. For this reason, we studied the effect of photoperiod on dormancy release in *Ulmus* clones (Paper IV).

Susceptibility to DED and relation to bud burst date (II)

Significant differences in susceptibility to DED were found between elm taxonomic groups. Natural hybrids between *U. minor* and *U. pumila* were the most resistant and *U. glabra* the most susceptible species. Highly susceptible clones showed a steady progression of symptoms through the growing season, while relatively resistant clones showed a decrease in symptoms because of flushing of dormant secondary buds. In the second year, recurrence of disease symptoms was frequent in clones with extensive defoliation in the previous year, but it also occurred occasionally in other more resistant clones. The highest susceptibility found for *U. glabra* confirms this species as the most susceptible in Europe (Townsend 1971; Brasier 1977; Solla *et al.* 2005 a). High resistance in *U. minor* x *U. pumila* hybrids is likely to be an effect of the introduction of resistance genes from *U. pumila* (Smalley & Guries 2000). Natural hybridisation between *U. minor* and *U. pumila* in Europe is well documented (Mittempergher & La Porta 1991; Cogolludo-Agustín *et al.* 2000; Goodal-Copestake *et al.* 2005). Natural hybrids with high DED resistance and good capability to adapt to the Mediterranean climate (Santini *et al.* 2007b) may represent a threat for the genetic integrity of the native species in southern Europe. At the same time these hybrids could represent a

source of resistant plant material for breeding purposes and for genetic research on DED resistance.

Within *U. minor,* the best-represented species in the Italian collection, there were significant differences in susceptibility, some clones being even more resistant than 'Lobel'. This was the most promising result since the presence of genotypes with a good level of resistance is the indispensable prerequisite for selecting and breeding resistant elms of European species. A positive relationship was found in *U. minor* between latitudinal origin and susceptibility, southerly clones showing fewer disease symptoms than more northerly ones (Figure 8 left) and there was a significant direct relationship between disease severity and bud burst date (Figure 8 right). In paper III we report that southern clones at Antella flushed significantly earlier in the inoculation year than northern ones. Considering that all clones were inoculated on mid-May, i.e. the time generally favourable for the disease to develop in the Florence area, the above results may be explained with the hypothesis that at the inoculation date, southern early flushing clones had already passed the period of maximum susceptibility. The period of maximum susceptibility in European elms starts 40-50 days after bud burst and lasts a variable number of days depending on environmental conditions and genotype (Tchernoff 1965; Smalley & Kais 1966; Santini & Ghelardini unpublished data). We can assume that the southern early flushing clones had completed formation of their earlywood vessels and were starting to lay down latewood, a transition related to the seasonal decrease of susceptibility (Pope 1943; Solla *et al.* 2005 b; Santini, Ghelardini & Falusi unpublished data).

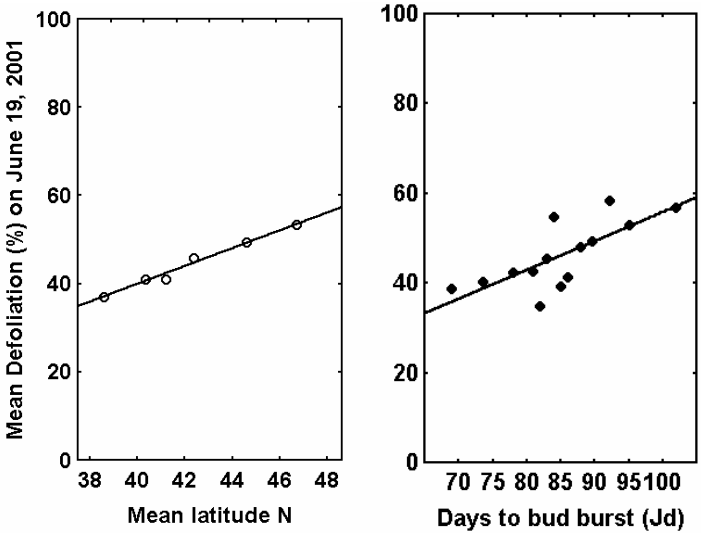

Figure 8. Relationship between symptoms (% defoliation) and latitudinal origin (left) and bud burst date (right) in *Ulmus minor* clones at Antella (Italy) in spring 2001. There was a positive relationship between latitude and susceptibility ($R = 0.99$, $p < 0.001$), southerly clones showing fewer defoliation than northerly ones. Defoliation and bud burst date were significantly related ($R = 0.74$, $p < 0.001$), early flushing clones showing fewer defoliation than late flushing ones. Jd = Julian days (days from January 1st).

Thus, the selection of early flushing clones may be included in breeding programs to obtain elms avoiding natural infection through an asynchrony between the host's susceptibility period and the *Scolytus* vector's phase of disease transmission. This approach grounds on the fact that the host's phenology and the emergence of *Scolytus* vectors in spring are regulated by temperature (Sinclair & Campana 1978; Sengonca & Leisse 1984).

Geographic variation in bud burst date in *U. minor* (III)

In all study years we observed a large clonal variation in the bud burst date of *U. minor* in the Italian and the French collections. Clones from more northerly latitudes and higher elevations flushed significantly later and required more thermal time to bud burst at both sites in all years. Conflicting results on latitudinal and altitudinal trends of bud burst in trees have been previously reported from provenance trials. North to south and high to low elevation trends of bud burst are more common, but opposite trends are also found. To date, reports on the geographical variability in bud burst within European elm species are scarce. Whiteley *et al.* (2003) reported a north to south latitudinal trend in bud burst of five *U. laevis* populations from Central and North Europe in a common garden trial in Sweden. Myking & Skrøppa (2007) reported a similar latitudinal trend of bud burst of five Norwegian *U. glabra* populations grown in Norway.

We explained the observed trends in bud burst date and thermal time in *U. minor* at the mild-winter climate sites as an effect of parallel trends in chilling requirements. Since the difference in thermal time to bud burst among geographic origins diminished in colder winters (Figure 9), we conclude that chilling requirement for dormancy release in *U. minor* increases with latitude and altitude.

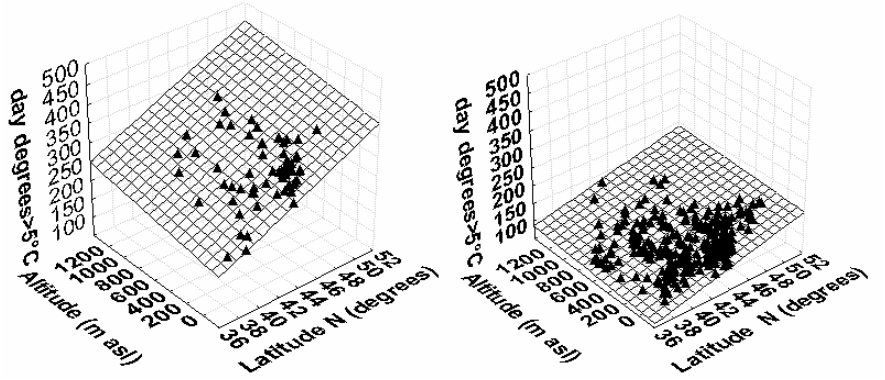

Figure 9. Multiple regressions of thermal time (day degrees > 5°C) to bud burst on latitude and altitude in clones of *Ulmus minor* in field conditions at Antella (Italy) in two years with different winter conditions. Left: mild winter in 2001 (chill days < 5°C = 15), R=0.58 p < 0.01. Right: cold winter in 2004 (chill days < 5°C = 63), R = 0.50 p < 0.01.

The hypothesis that chilling requirement increases with latitude is supported by the results of the ANOVA performed on groups of clones from different latitudes in

the Italian collection. Differences in thermal time to bud burst among groups were largest in years with very short chilling duration. With increasing duration of chilling, thermal time to bud burst decreased earlier in southerly clones and became steady after about 50 chill days, whereas in northerly clones it continued to decrease over the entire chilling range (Figure 10a). Chilling requirements were thus fully met only in clones of Southern Italy.

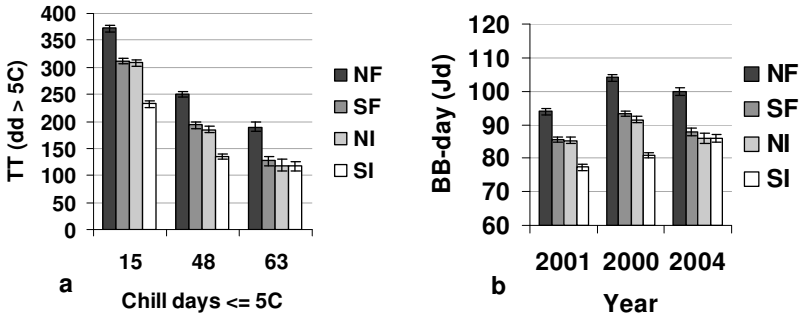

Figure 10. Variation in thermal time (TT) to bud burst (± SE) (a) and bud burst date (BB-day) (± SE) (b) in four groups of *Ulmus minor* clones from different latitudes (NF=Northern France; SF=Southern France; NI=Northern Italy; SI=Southern Italy) at Antella (Italy) among years with different winter temperature conditions (2001, 15 chill days; 2000, 48 chill days; 2004, 63 chill days). Chill days are the days from November 1st with mean daily temperature below 5°C. Jd = Julian days (days from January 1st).

The order of bud burst of clones from different latitudes was maintained over a wide range of temperature conditions (Fig 10b). This was due to differences in dormancy among origins. This result is especially important for exploiting bud burst earliness in breeding programs for selection of DED resistant clones. Our results also show that the shift in bud burst date among years, although dependent on the interplay between chilling and thermal time (Figure 10a), does not necessarily reflect it (Figure 10b), but largely depends on the course of temperature in spring (data not shown). The effect of insufficient chilling, i.e. an increase in thermal time requirement, can be compensated by a rapid accumulation of thermal time in a warm spring leading to an early bud burst (year 2001). Similarly, the increase in chilling, i.e. a decrease in thermal time to bud burst, can be hidden in a cool spring so that no advancement in bud burst is observed (year 2004). These phenological data are consistent with phenological trends reported for other species of the genus *Ulmus* (Sparks & Carey 1995; Chuine *et al*. 2000).

Photoperiod and temperature effects during bud dormancy release in *Ulmus* spp. clones (IV)

This study showed that photoperiod had no effect on dormancy release or bud burst in *Ulmus* clones from different latitudes and species. Photoperiodic effects on dormancy release and bud burst have been reported for many trees in the literature (Heide 1993 b; Falusi & Calamassi 1997 a; references Introduction and paper IV).

Roberts & Main (1965) reported long photoperiod to substitute for chilling in *U. americana* seedlings. This is the only information on this subject in elms.

Photoperiodic effects on dormancy release may vary with the plant's ontogenetic stage (Partanen *et al.* 2005). Hence, our experiment on twigs of ontogenetically old trees does not allow to exclude photoperiodic effects on bud burst in seedlings.

According to the phenological model we fitted to bud burst data from field trials (paper I), European elms have low chilling requirements. This growth chamber study confirmed low dormancy in the clones of two European species (*U. minor* and *U. glabra*) and found dormancy to be even lower in the clones of four Asian species (*U. pumila*, *U. parvifolia*, *U. macrocarpa*, *U. villosa*). Experiment 1 showed that dormancy was highest in mid-October and that it was alleviated earlier in the Asian than in the European clones, although there was variation within each group. In the elm clones studied, thermal time to bud burst (day degrees > 0°C) decreased exponentially with increasing outdoor chilling, tending to reach a stable level more rapidly in the Asian than in the European clones. Chilling requirement was less than 35 CD in *U. pumila*, *U. parvifolia* and *U. macrocarpa*, about 70 CD in *U. villosa*, and more than 100 CD in *U. glabra* and *U. minor.*

Experiment 2 confirmed the ranking in dormancy among the clones. In all clones, bud burst started first at high temperature (26°C) and occurred earlier in Asian (mid-Oct. to mid-Nov.) than in the European (mid-Dec.) clones. From mid-November to mid-January, the capacity to bud burst was regained at low temperature (14°C) with the same time-sequence among clones. Widening of the temperature range in which bud burst occurs is a classic index of dormancy release and the rapidity of widening is proportional to the initial intensity of dormancy (Vegis 1964; Champagnat 1993; Crabbè & Barnola 1996). *U. pumila* flushed, although in a narrow temperature range, even at the time of the strongest growth inhibition, which suggests that this clone was not in a state of true dormancy.

Endodormancy (Lang 1987), as measured by the single-node cuttings test (Crabbé & Barnola 1996), was shallow and short in all clones compared with data from other temperate trees studied with the same technique (Bailly & Mauget 1989; Jacques *et al.* 1989; Falusi & Calamassi 1997 b). In the *U. pumila* and *U. parvifolia* clones, the buds flushed in less than 10 days at the first intake, which is considered the limit defining endodormancy (Balandier *et al.* 1993). This suggests that these clones had no endodormancy.

Since dormancy release was studied in one clone per species, and dormancy traits typically vary within species as a result of adaptation to the local environment (Saure 1985), the results obtained in this paper cannot be generalised. In *U. minor,* a north to south trend in chilling requirement is suggested by our phenological data (paper III) and is confirmed by preliminary results from experiments under controlled conditions (Ghelardini & Black-Samuelsson unpublished data). Further studies would be needed to investigate geographic differentiation in dormancy.

Conclusions and future perspectives

Data on dormancy in elms are scarce until now. This thesis provides new knowledge on the subject. Dormancy in elms proved to be generally low. This resulted from species-specific thermal time-chilling curves obtained under field conditions for European elms (paper I) and from experiments under partially controlled conditions for both European and Asian clones (paper IV).

Timing of budburst in *Ulmus minor* displayed a large intra-specific variation with geographic origin in *ex situ* collections. Date of bud burst was directly related to latitude and altitude, and trends were explained by larger chilling requirements in northern/highland than in southern/lowland origins.

This thesis also provided new information on susceptibility to Dutch elm disease (DED). The results of Paper II suggest that earliness of bud-burst relates to DED avoidance. In *U. minor* disease severity was related to bud burst date, early flushing clones showing fewer disease symptoms than late flushing ones. This relationship was paralleled by a geographic trend in bud burst, southerly clones showing fewer disease symptoms than more northerly ones. It can be hypothesized that disease avoidance (asynchrony between maximum susceptibility and time of infection) in early flushing clones is partly determined by an early transition from spring to late wood. However, this hypothesis needs to be tested in field and controlled conditions.

Disease avoidance owing to earliness in flushing may be exploited for selection and breeding of DED-resistant clones. This could be achieved through a combination with other types of resistance such as true resistance, which can be accumulated through recurrent breeding in European elms, and avoidance owing to unattractiveness for *Scolytus* vector's feeding, which proved to be an efficient mechanism in the case in *Ulmus laevis*. Early flushing clones could be selected either from southerly populations, provided that they maintain earliness compared to local elms, or from local populations, given the large within-population variability which can be expected in wind-pollinated species. Moreover, in doing this we should not forget that these clones may be susceptible to frost. Hence, testing and selection for frost resistance should be included in breeding programs.

Besides the above mentioned transition to late wood, other factors such as growth rhythm and leaf expansion are very likely to be involved in the seasonal course of susceptibility. It would therefore be interesting to study in controlled conditions how growth rhythm influences susceptibility to DED in clones with different earliness and origin.

As the genetic basis of host resistance to DED is still poorly understood, it would be challenging to identify genomic regions (QTLs) involved in DED resistance. In elms, the first step to prepare a QTL study would be the development of appropriate genomic tools, i.e. a sufficient number of molecular markers and a

reference genetic map. Identification of QTLs for DED resistance may be a valuable tool for breeding new cultivars by marker assisted selection (MAS).

Because of their fast growth, their adaptability to highly different soil conditions, their resistance to wind, to pruning and root damage, to city conditions in general, today, after the market release of resistant clones, elms are again sought-after as urban and landscape trees. As a consequence, the tree planting community waits for more clones to be developed, so as to have enough genetic variability to cope with other environments and diseases. Therefore, exploring new approaches to study resistance to DED and to select new clones is of considerable importance.

References

Arora, R., Rowland, L. & Tanino, K. 2003. Induction and release of bud dormancy in woody perennials: a science comes of age. *HortScience* 38(5), 911-921.

Arnold, C.Y. 1959. The determination and significance of the base temperature in a linear heat unit system. *Proceedings of the American Society for Horticultural Science* 74, 430-445.

Bailly, O. & Mauget, J.C. 1989. Physiological correlations and bud dormancy in the apple three (*Malus domestica* Borkh.). *Annals of Forest Science* 46 (Suppl.), 220-222.

Balandier, P., Gendrad, M., Rageau, R., Bonhomme, M., Richard, J.P. and Parisot, E. 1993. Bud break delay in single-node cuttings and bud burst capacity for nucleotide accumulation as parameters for endo- and paradoemancy in peach trees in a tropical climate. *Scientia Horticulturae* 55, 249-261.

Baliuckas, V., Lagerstrom, T. & Eriksson, G. 2001. Within population variation in juvenile growth rhythm and growth in *Quercus robur* L. and *Fagus sylvatica* L. *Forest Genetics* 8(4), 259-269.

Banfield, W.M. 1941. Distribution by the sap stream of spores of three fungi that induce vascular wilt diseases of elm. *Journal of Agricultural Research* 62, 637–681.

Banfield, W.M. 1968. Dutch elm disease Recurrence and recovery in American elm. *Phytopathologische Zeitschrift* 62, 21-60.

Barnola, P. 1976. Recherches sur la dormance et la morphogenèse de quelques espèces buissonnantes. *Thèse de Doctorat d'État, Université de Clermont-Ferrand II*, Clermont-Ferrand, 173 p.

Barnola, P., Lavarenne, S. & Gendraud, M. 1986. Dormance des bourgeons apicaux du Frene (*Fraxinus excelsior* L.): évaluation du pool des nucléosides triphosphates et éventail des températures actives sur le débourrement des bourgeons en période de dormance. *Annales des Sciences Forestières* 43(3), 339-350.

Battey, N.H. 2000. Aspects of seasonality. *Journal of Experimental Botany* 51, 1769-1780.

Bei, C. F. 1973. Growth of black walnut trees in eight Midwestern states. A provenance test. *USDA Forest Service, Research Paper* 99, 1-7.

Bhardwaj, D.R. & Mishra V.K. 2005. Vegetative propagation of *Ulmus villosa*: effects of plant growth regulators, collection time, type of donor and position of shoot on adventitious root formation in stem cuttings. *New Forest* 29, 105-116.

Binz, T.G. & Canevascini, G. 1996. Xylanases from the Dutch elm disease pathogens *Ophiostoma ulmi* and *Ophiostoma novo ulmi*. *Physiological and Molecular Plant Pathology* 49, 159-175.

Bonhomme, M., Rageau, R., Lacointe, A. & Gendraud, M. 2005. Influence of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batch.) *Scientia Horticulturae* 105, 223-240.

Bonsen, K.J.M., Scheffer, R.J. & Elgersma D.M. 1985. Barrier zone formation as a resistance mechanism of elms to Dutch elm disease. IAWA Bulletin 6(1), 71-77.

Brasier, C.M. 1977. Inheritance of pathogenicity and cultural characteristics in *Ceratocystis ulmi*. Hybridisation of protoperithecial and non-aggressive strains. *Transactions of the British Mycological Society* 68, 45–52.

Brasier, C.M. 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of the current Dutch elm disease pandemics. *Mycopathologia* 115, 151-161.

Brasier C.M. 2000. Intercontinental spread and continuing evolution of the Dutch elm disease pathogens In: *The Elms: Breeding Conservation and Disease Management.* C.P. Dunn ed. Kluwer Academic Publisher, Boston, USA, pp. 61-72.

Brasier, C.M. & Kirk, S.A. 2001. Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. *Mycological Research* 105, 547-554.

Buchel, A. 2000. The species of the genus *Ulmus* L. In: *The Elms: Breeding Conservation and Disease Management* Kluwer Academic Publisher, Norwell Massachusetts USA.

CAB International. 2005. *Forestry Compendium*. CAB International Wallingford UK.

Campbell, R.K. & Sugano, A.I. 1975. Phenology of bud burst in Douglas-fir related to provenance, photoperiod, chilling, and flushing temperature. *Botanical Gazette* 136, 90-98.
Campbell, R.K. & Sugano, A.I. 1979. Genecology of bud burst phenology in Douglas-Fir: response to flushing temperature and chilling. *Botanical Gazette* 140(2), 223-231.
Cannell, M.G.R. & Smith R.I. 1983. Thermal time, chill days and prediction of bud burst in Picea sitchensis. *Journal of Applied Ecology* 20, 951-963.
Cannell, M.G.R. & Smith R.I. 1986. Climatic warming, spring bud-burst and frost damage on trees. *Journal of Applied Ecology* 23, 177-191.
Champagnat, P. 1983a. Bud dormancy, correlation between organs, and morphogenesis in woody plants. *Soviet Plant Physiology* 30, 458-471
Champagnat, P. 1983b. Quelques réflexions sur la dormance des bourgeons des Végétaux ligneaux. *Physiologie Végétal* 21, 607-618.
Champagnat, P. 1989 Rest and activity in vegetative buds of trees. In : Proceedings of the Forest Tree Physiology International Symposium, INRA, Université de Nancy, France 1988/09/25-30, *Annales des Sciences Forestières* 46 (Suppl.), 9s-26s.
Champagnat, P. 1993. Dormance des bourgeons chez les végétaux ligneux In: *Les végétaux et le froid.* Come D. ed. Herman, Paris, pp. 203-262
Chmura, D.J. & Rozkowski, R. 2002. Variability of beech provenances in spring and autumn phenology. *Silvae Genetica* 51, 123-127.
Chouard P. 1956. Dormance et inhibition des graines et des bourgeons. Préparation au forcage et au thermopériodisme. *Centre de Documentation Universitaire*, Paris.
Chuine, I. 2000. A unified model for budburst of trees. *Journal of theoretical biology* 207, 337-347.
Chuine, I. & Beaubien, E.G. 2001. Phenology is a major determinant of tree species range. *Ecology Letters* 4, 500-510.
Chuine, I., Camion, G., & Comtois, P. 2000. Scaling phenology from the local to the regional level: advances from species specific phenological models. *Global Change Biology* 6, 943-952.
Chuine, I., Kramer, K., & Hänninen, H. 2003. Plant development models In: *Phenology: an integrative environmental science* (M.D. Schwartz ed.) Kluwer Academic Publishers, Dordrecht pp. 305-333.
Clapham, D., Dormling, I., Ekberg, I. Eriksson, G., Qamaruddin, M. & Vince-Prue, D. 1998. Latitudinal cline for requirement for a far-red light for the photoperiodic control of bud set and extension of growth in *Picea abies*. *Physiologia Plantarum* 102, 71-78.
Clapham, D., Ekberg, I., Norell, L. & Vince-Prue, D. 2001. Circadian timekeeping for the photoperiodic control of bud set in *Picea abies* (Norway spruce) seedlings. *Biological Rhythm Research* 32, 479-487.
Cleland, E.E., Chuine, I., Menzel, A., Mooney H.A. & Schwarz M.D. 2007. Shifting plant phenology in response to global change. *Trends in Ecology and Evolution* 22(7), 357-365.
Collin, E. 2002. Strategies and guidelines for the conservation of the genetic resources of *Ulmus* spp. In: *«Noble Hardwoods» network: Report of the fourth and fifth meetings*, Sept. 1999 and May 2001 (Borelli S. ed.) International Plant Genetic Resources Institute, Rome, pp. 50-67.
Collin, E. 2003a. European elms (*Ulmus* spp.) genetic resources conservation strategy. http://www.bioversityinternational.org/Networks/Euforgen/Networks/Scattered_Broadleaves/NHStrategies/UlmusSppConsStrategy.htm (Accessed 19 December 2007).
Collin, E. 2003b. EUFORGEN Technical Guidelines for genetic conservation and use for European white elm (*Ulmus* laevis). International Plant Genetic Resources Institute, Rome, Italy. http://www.bioversityinternational.org/publications/Pdf/921.pdf (Accessed 19 December 2007)
Collin, E., Rusanen, M., Ackzell, L., Bohnens, J., de Aguiar, A., Diamandis, S., Franke, A., Gil, L., Harvengt, L., Hollingsworth, P., Jenkins, G., Meier-Dinkel, A., Mittempergher, L., Musch, B., Nagy, L., Paques, M., Pinon, J., Piou, D., Rotach, P., Santini, A., Vanden Broeck, A. & Wolf H. 2004. Methods and progress in the conservation of elm genetic

resources in Europe. *Investigación agraria: Sistemas y Recursos Forestales* 13(1), 261-272.
Cogolludo-Agustín, M.A., Agúndez, D. & Gil, L. 2000. Identification of native and hybrid elms in Spain using isozyme gene markers. *Heredity* 85, 157-166.
Couvillon, G.A. 1995. Temperature and stress effects on rest in fruit trees: a review. *Acta Horticulturae* 395, 11-20.
Crabbè, J. 1968. Evolution annuelle de la capacité intrensèque de débourrement des bourgeons successifs de la pousse de l'année chez le pommier et le Poirier. *Bulletin de la Société Royale De Botanique de Belgique* 101, 195-204.
Crabbè, J., & Barnola P. 1996. A new conceptual approach to bud dormancy in woody plants. In: *Plant dormancy: physiology, biochemistry and molecular biology* (Lang GA ed.) Wallingford UK: CAB International, 83-113.
Cronquist, A. 1981. *An integrated system of classification of flowering plants*. Columbia University Press, New York, 1262 pp.
Dennis, F.G. 1994. Dormancy – What We Know (and Don't Know). *HortScience* 29(11), 1249-1255.
Dethier, V.G. 1954. Evolution of feeding preferences in phytophagous insects. *Evolution* 8 (1), 33-54.
Dietrichson, J. 1969. Growth rhythm and yield as related to provenance, progeny and environment. *Second World Consultation on Forest Tree Breeding*, IUFRO, Washington DC, pp. 1-15.
Dixon, E.B. 1964. Attack response of the smaller European elm bark beetle, *Scolytus multistriatus*, in confinement. *Journal of Economic Entomology* 57(1), 170-172.
Doorenbos, J. 1953. Review of the literature on dormancy in bud of woody plants. *Medelingen van de Landbouwhogeschool te Wageningen*/Nederland 53, 1-24.
Dormling, I. 1973. Photoperiodic control of growth and growth cessation in Norway spruce seedlings. International symposium on Dormancy in Trees. IUFRO division 2, working party 2.01.4, Kornic, Poland.
Downs, R.J. & Borthwick, H.A. 1956. Effects of photoperiod on growth of trees. *Botanical Gazette* 117: 310-326.
Duchesne, L.C. 1988. Resistance mechanisms to Dutch elm disease: a review. *Naturaliste Canadien* 115, 163-167.
Duchesne, L.C. 1993. Mechanisms of resistance: can they help to save susceptible elms? In: *Dutch elm disease research: Cellular and molecular approaches.* (M.B. Sticklen & J.L. Sherald eds.) New York: Springer-Verlag, pp. 239-254.
Duchesne, L.C., Jengand, R.S. & Hubbes, M. 1985. Accumulation of phytoalexins in *Ulmus americana* in response to infection by a nonaggressive strain of *Ophiostoma ulmi*. *Canadian Journal of Botany* 63, 678–680.
Ducousso, A., Guyon, G.P., & Kremer A. 1996. Latitudinal and altitudinal variation of bud burst in western populations of sessile oak *Quercus petraea* (Matt.) Liebl. *Annales des Sciences Forestières* 53, 775-782.
Dunn, C. P. 2000. *The elms: breeding, conservation and disease management.* Kluwer Academic Publishers, Boston, 384 p.
Elgersma, D.M. 1970. Length and diameter of xylem vessels as factors in resistance of elms to *Ceratocystis ulmi*. Netherlands Journal of Plant Pathology 76, 179-182.
Elgersma, D.M. 1982. Susceptibility and possible mechanisms of resistance to Dutch elm disease. In: *Proceedings of Dutch elm disease symposium and workshop.* (Kondo E.S., Hiratsuka Y. & Denyer W.B.G. eds.) Manitoba Department of Natural Resources, Winnipeg, Canada pp. 169-177.
Ellmore, G.S. & Ewers, F.W. 1985. Hydraulic conductivity in trunk xylem of elm, *Ulmus americana*. *IAWA Bulletin* 6, 303-307.
Erez A., Couvillon G.A. & Hendershott, C.H. 1979. Quantitative chilling enhancement and negation in peach buds by high temperatures in a daily cycle. *Journal of the American Society for Horticultural Science* 104, 536-540.
Eriksson, G. 2001. Conservation of noble hardwoods in Europe. *Canadian Journal of Forest Research* 31, 577-587.

Eriksson, G., Ekberg, I., and Clapham, D. 2006. *An introduction to forest genetics*. Genetic Center, Department of Plant Biology and Forest Genetics, SLU, Uppsala, Sweden.

Et-Touil, A., Rioux, D., Mathieu, F.M. & Bernier, L. 2005. External symptoms and histopathological changes following inoculation of elms putatively resistant to Dutch elm disease with genetically close strains of *Ophiostoma*. *Canadian Journal of Botany* 83, 656-667.

Falusi, M., & Calamassi, R. 1990. Bud dormancy in beech (*Fagus sylvatica* L.). Effect of chilling and photoperiod on dormancy release of beech seedlings. *Tree Physiology* 6, 429-438.

Falusi, M. & Calamassi, R. 1996. Geographic variation and bud dormancy in beech seedlings (*Fagus sylvatica* L.). *Annales des Sciences Forestières* 53, 967-979.

Falusi, M., & Calamassi, R. 1997a. Bud dormancy in *Fagus sylvatica* L. I. Chilling and photoperiod as factors determining budbreak. *Plant Biosystems* 131 (1), 67-72.

Falusi, M. & Calamassi R. 1997b. Bud dormancy in *Fagus sylvatica* L. II. The evolution of dormancy in seedlings and one-node cuttings. *Plant Biosystems* 131(2), 143-148.

Falusi, M. & Calamassi, R. 2003. Dormancy of *Fagus sylvatica* L. buds III. Temperature and hormones in the evolution of dormancy in one node cuttings. *Plant Biosystems* 137(2), 185-191.

Farmer, R.E. & Reinholt, R.W. 1986. Genetic variation in dormancy relations of balsam poplar along a latitudinal transect in Northwestern Ontario. *Silvae Genetica* 35, 38-42.

Fernandez-Lopez, J., Zas, R., Diaz, R., Villani, F., Cherubini, M., Aravanopoulos F.A., Alizoti, P.G., Eriksson, G., Rosa, E. & Monteiro, A.A. 2005. Geographic variability among extreme European wild chestnut populations. *Acta Horticulturae* 693, 181-186.

Fu, L. & Yquin, X. 2000. Elms of China. In: *The Elms: Breeding, Conservation and Disease Management,* (C.P. Dunn ed.), Kluwer Academic Publishers, Boston, USA, pp. 3-22.

Fu, L., Xin, Y. & Whittemore, A. 2002. *Ulmus* In *Flora of China, Vol. 5 (Ulmaceae* through *Basellaceae).* Wu, Z. & Raven, P. (eds.). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, USA, pp. 1-10.

Garber, M.P. 1983. Effects of chilling and photoperiod on dormancy release of container-grown loblolly pine seedlings. *Canadian Journal of Forest Research* 13, 1265-1270.

Geng, M.A. 1989. A provenance test of with elm (*Ulmus pumila* L.) in China. *Silvae Genetica* 32(2), 37-44.

Germain, E. 1992. Le noyer In: *Amélioration des espèces végétales cultivées, objectifs et critères de sélection.* A. Gallais, H. Bannerot, eds. INRA Editions, Paris, pp. 620-632.

Greenidge, K.N.H. 1955. Observation on the movement of moisture in large woody stems. *Canadian Journal of Botany* 33, 202-221.

Gibbs, J.N. 1978. Intercontinental epidemiology of Dutch elm disease. *Annual Review of Phytopathology* 16, 287-307.

Goodal-Copestake *W.P., Hollingsworth, M.L., Hollingsworth,* P.M., Jenkins G.I. & Collin E. 2005 Molecular markers and ex situ conservation of the European elms (*Ulmus* spp.) *Biological Conservation* 122, 537-546.

Guries R.P. 2001. Elms: past, present, and future In: *Proceeding of national conference on wilt diseases of shade trees* (C.L. Ash ed.) St. Paul, Minnesota 25–28 August 1999, USA, APS Press, pp. 29–36.

Hamann, A., Namkoong, G. & Koshy, M.P. 2001. Multiple population breeding for uncertain climatic future with *Alnus rubra*: ecological genetics and selection experiments. In: *Forestry Sciences Vol. 70. Genetic response of forest systems to changing environmental conditions* (G. Müller-Starck & R. Schubert eds.) Kluwer Academic Publisher, London, UK pp. 321-331.

Hannerz, M., Ekberg, I. & Norell, L. 2003. Variation in chilling requirements for completing bud rest between provenances of Norway spruce. *Silvae Genetica* 52 (3-4), 161-168.

Hari, P., Leikola, M. & Räsänen, P. 1970. A dynamic model of the daily high increment of plants. *Annales Botanici Fennici* 7, 375-378.

Hänninen, H. 1990. Modelling bud dormancy release in trees from cool and temperate regions. *Acta Forestalia Fennica* 231, 47

Hänninen, H. 1991. Does climatic warming increase the risk of frost damage in northern trees? *Plant Cell & Environment* 14, 449-454.
Hänninen, H. 1995. Effects of climatic change on trees from cool and temperate regions: en ecophysiological approach to modelling of bud burst phenology. *Canadian Journal of Botany*. 73, 183-199.
Hänninen, H. & Kramer, K. 2007. A Framework for modelling the annual cycle of trees in boreal and temperate regions. *Silva Fennica* 41(1), 167-205.
Håbjørg A. 1978. Photoperiodic ecotypes in Scandinavian trees and shrubs. *Meldinger fra Norges Landbrukshøgskole* 57, 1-20.
Heide, O.M. 1974a. Growth and dormancy in Norway spruce ecotypes (Picea abies). I. Interaction of photoperiod and temperature. *Physiologia Plantarum* 30, 1-12.
Heide, O.M. 1974b. Growth and dormancy in Norway spruce ecotypes. II. After-effects of photoperiod and temperature on growth and development in subsequent years. *Physiologia Plantarum* 31, 131-139.
Heide, O.M. 1993a. Daylength and thermal time responses of budburst during dormancy release in some northern deciduous trees. *Physiologia Plantarum* 88, 531-540.
Heide, O.M. 1993b. Dormancy release in beech buds (*Fagus sylvatica*) requires both chilling and long days. *Physiologia Plantarum* 89, 187-191.
Heide, O.M. 2003. High autumn temperature delay spring bud burst in boreal trees, counterbalancing the effect of climate warming. *Tree Physiology* 23, 931-936.
Heide, O.M. & Prestud, K. 2005. Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. *Tree Physiology* 25, 109-114.
Herms, D.A. & Mattson, W.J. 1992. The dilemma of plants – To grow or defend. *Quarterly Review of Biology* 67 (4), 478-478.
Heybroek, H.M. 1957. Elm breeding in the Netherlands. *Silvae Genetica* 6, 112-117.
Heybroek, H.M. 1979. *Elms in agroforestry.* Dorschkamp Research Institute for Forestry and Landscaping, Wageningen, The Netherlands.
Heybroek, H.M. 1993. The Dutch elm breeding program, pp. 16-25. In: *Dutch Elm Disease Research: Cellular and Molecular Approaches*. Sticklen and Sherald Eds., Springer-Verlag, New York.
Heybroek, H.M. 2000. Los olmos en la historia y la cultura de centroeuropa, pp 23-48. In : *Los olmos ibéricos. Conservación y mejora frente a la grafiosis*. L. Gil, A. Solla, & S. Iglesias eds. Serie Tecnica Ministerio de Medio Ambiente. Organismo Autonomo Parques Nacionales, Madrid.
Horvarth, D.P., Anderson J.V., Chao, W.S. & Foley, M.E. 2003. Knowing when to grow: signals regulating bud dormancy. *Trends in Plant Science* 8(11), 534-540.
Hubbes, M. 2004. Induced resistance in the control of Dutch elm disease. *Investigación agraria: Sistemas y Recursos Forestales* 13, 185-196.
IAWA Committee. 1989. IAWA list of microscopic features for hard- wood identification. *International Association of Wood Anatomists New Series* 10, 219-332.
Ingvarsson, P.K., García, M.V., Hall, D., Luquez, V. & Jansson, S. Clinal variation in phyB2, a candidate gene for day-length-induced growth cessation and bud set, across a latitudinal gradient in European aspen (*Populus tremula*). *Genetics* 172, 1845-1853.
Jacques, M., Bonnetmasimbert, M., Cornu, D. & Miginiac, E. 1989. Development of budding, longitudinal-growth and branching potentials of isolated *Pseudotsuga menziesii* (Mirb.) buds during winter dormancy. *Annals of Forest Science* 46 (Suppl.), 223-227.
Jeng, R.S., Alfenas, A.C., Hubbes, M. & Dumas, M.T. 1983. Presence and accumulation of fungitoxic substances against *Ceratocystis ulmi* in *Ulmus americana*: possible relation to induced resistance. *European Journal of Forest Pathology* 13, 239–244.
Jensen, J.S. 2000. Provenance variation in phenotypic traits in *Quercus robur* and *Quercus petraea* in Danish provenance trials. *Scandinavian Journal of Forest Research* 15, 297-308.
Jourdan, J. 1980. Variations saisonnières de la morphogenèse et de la croissance des systèmes aériens et souterraines chez les peuplier: leur relations avec les glucides et les transferts minéraux. *Thèse de Doctorat d'État, Université scientifique et médicale Grenoble*.

Junttila, O. 1980. Effect of photoperiod and temperature on apical growth cessation in two ecotypes of *Salix* and *Betula*. *Physiologia Plantarum* 66, 409-412.
Junttila, O., Nilsen, J. & Igeland, B. 2003. Effect of temperature on the induction of bud dormancy in ecotypes of *Betula pubescens* and *Betula pendula*. *Scandinavian Journal of Forest Research* 18, 208-217.
Kozlowsky, T.T. & Pallardy, S.G. 1997. *Physiology of woody plants*, Academic Press, San Diego 410 p.
Kramer, K. 1995. Phenotypic plasticity of the phenology of seven European tree species in relation to climatic warming. *Plant Cell and Environment* 18, 93-104.
Kriebel, H.B. & Wang, C.W. 1962. The interaction between provenance and degree of chilling in bud break of sugar maple. *Silvae Genetica* 11, 125-130.
Lang, G.A. 1987. Dormancy: a new universal terminology. *HortScience* 22, 817-820.
Lechowicz, M.J. 1984. Why do temperate deciduous trees leaf out at different times? Adaptation and ecology of forest communities. *American Naturalist* 124, 821-842.
Lee, R. & Baumgartner, A. 1966. Topography and isolation of a mountainous forest area. *Forest Science* 12(3), 258-267.
Lester, D.T. & Smalley E.B. 1972. Response of *Ulmus pumila* and *Ulmus pumila×rubra* hybrids to inoculation with *Ceratocystis ulmi*. *Phytopathology* 62, 848–852.
Levitt, J. 1969. Growth and survival of plants at extreme temperature – a unified concept In: Dormancy and Survival, *Symposia of the Society for Experimental Biology*, XXIII (H.W. Woolhouse ed.). Cambridge University Press, pp. 395-448.
Li, C., Junttila, O., Ernstsen, A., Heino, P. & Palva, T. 2003. Photoperiodic control of growth and dormancy development in silver birch. *Physiologia Plantarum* 117, 206-212.
Linkosalo, T. & Lechowicz, M. 2006. Twilight far-red treatments advances leaf bud burst of silver birch (*Betula pendula*). *Tree Physiology*. 26, 1249-1256.
Linkosalo, T., Häkkinen R. & Hänninen H. 2006. Models of the spring phenology of boreal and temperate trees: is there something missing? *Tree Physiology*. 26 1165-1172
Linvill, D.E. 1990. Calculating chilling hours and chill units from daily maximum and minimum temperature observations. *HortScience* 25(1), 14-16.
Lockhart, J.A. 1983. Optimum growth initiation time for shoot buds of deciduous plants in a temperate climate. *Oecologia* 60, 34-37.
Mazzitelli, L., Hancock, R.D., Haupt, S., Walker, P.G., Pont, S.D.A., McNicol, J., Cardle, L., Morris, J., Viola, R., Brennan, R., Hedley, P.E. & Taylor, M.A. 2007. Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus* L.) buds. *Journal of Experimental Botany* 58, 1035-1045.
MacHardy W.E. & Beckman C.H. 1973. Water relations in American Elm infected with *Ceratocystis ulmi.* Phytopathology 63 (1), 98-103.
Martin, J.A., Solla, A., Woodward, S. & Gil, L. 2005. Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. *Tree Physiology* 25, 1331-1338.
McNabb H.S., Heybroek H.M. & MacDonald, W.L. 1970. Anatomical factors in resistance to Dutch elm disease. *Netherlands Journal of Plant Pathology* 76, 196-204.
Melville, R. & Heybroek, H.M. 1971.The elms of the Himalaya. *Kew Bulletin* 26, 5-28.
Mittempergher, L. & La Porta, N. 1991. Hybridization studies in the Eurasian species of elm (*Ulmus* ssp.). *Silvae Genetica* 40, 237-243.
Mittempergher, L. & Santini, A. 2004. The history of elm breeding. *Investigación agraria: Sistemas y Recursos Forestales* 13, 161-177.
Morgenstern E.K. 1996. *Geographic variation in forest trees*. UBC Press Vancouver, B.C. 209 p.
Murray, M.B., Cannell, M.G.R. & Smith, R.I. 1989. Date of budburst of fifteen tree species in Britain following climatic warming. *Journal of Applied Ecology* 26, 693-700.
Murray, M.B., Smith, R.I., Leith, I.D., Fowler, D., Lee, H.S.J., Friend, A.D. & Jarvis, P.G. 1994. Effect of elevated CO_2 nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage. *Tree Physiology* 14, 691-706.
Myking, T. 1997. Dormancy, budburst and impacts of climatic warming in coastal-inland and altitudinal *Betula pendula* and *B. pubescens* ecotypes In: *Phenology in Seasonal*

Climates (I.H. Lieth & M.D. Schwartz eds.), Backhuys Publishers, Leiden, The Netherlands, pp. 51-66.
Myking, T. 2002.Evaluating genetic resources of forest trees by means of life history traits – a Norwegian example. *Biodiversity and Conservation* 11, 1681-1696.
Myking, T. & Heide, O.M. 1995. Dormancy release and chilling requirement of buds of latitudinal ecotypes of *Betula pendula* and *B. pubescens*. *Tree Physiology* 15, 697-704.
Myking, T., & Skrøppa, T. 2007. Variation in phenology and height increment of northern *Ulmus glabra* populations: implications for conservation. *Scandinavian Journal of Forest Research* 22, 369-374.
Neely, D. 1968. Twig inoculations on American elm with *Ceratocystis ulmi*. *Phytopathology* 58, 1566-1570.
Neely, D. 1970. Dutch elm disease symptom progression. *Plant Disease Report* 54, 127-129.
Newbanks, D., Bosh, A. & Zimmermann, M.H. 1983. Evidence for xylem dysfunction by embolization in Dutch elm dsease. *Phytopatology* 73, 1060-1063.
Nienstaedt, H. 1967. Chilling requirements in seven *Picea* species. *Silvae Genetica* 16, 65-68.
Nitsch, J.P. 1957. Photoperiodism in woody plants. *Proceedings of the American Society for Horticultural Science*. 70, 526-544.
Noodén, L.D. & Weber J.A. 1978. Environmental and hormonal control of dormancy in terminal buds of plants In: *Dormancy and developmental arrest.* Clutter M.E. ed. Academic Press, New York. pp 221-268
Ouellette, G.B. & Rioux, D. 1992. Anatomical and physiological aspects of resistance to Dutch elm disease In: *Defense mechanisms in woody plants against Fungi* (A. Blanchette & R. Biggs eds.) Springer-Verlag Berlin, pp 257-305.
Ouellette, G.B., Rioux, D., Simard, M. & Cherif, M. 2004 a. Ultrastructural and cytochemical studies of host and pathogens in some fungal wilt diseases: retro- and introspection towards a better understanding. *Investigación agraria: Sistemas y Recursos Forestales* 13(1), 119-145.
Ouellette, G.B., Rioux, D., Simard, M., Chamberland, H., Cherif, M. & Baayen, R.P. 2004 b. Ultrastructure of the alveolar network and its relation to coating on vessel walls in elms infected by *Ophiostoma novo-ulmi* and in other plants affected with similar wilt diseases. *Investigación agraria: Sistemas y Recursos Forestales* 13(1), 147-160.
Pajares, J.A. 2004. Elm breeding for resistance against bark beetles. *Investigación agraria: Sistemas y Recursos Forestales* 13(1), 207-215.
Pang X., Halaly, T., Crane, O., Leilin, T., Kenen-Keiserman, A., Ogrodovitch, A., Galbraith, D. & Or, E. 2007. Involvement of calcium signalling in dormancy release of grape buds. *Journal of Experimental Botany* 58(12), 3249-3262.
Partanen, J., Hänninen, H., and Häkkinen, R. 2005. Bud burst in Norway spruce (Picea abies): preliminary evidence for age-specific rest pattern. *Trees* 19: 66-72.
Perry, T.O. 1971. Dormancy of trees in winter. *Science* 171, 29-37.
Pinon, G., Husson, C. & Collin, C. 2005. Susceptibility of native elm clones to *Ophiostoma novo-ulmi*. *Annals of Forest Science* 62, 689-696.
Pomerleau, R. 1965. The period of susceptibility of *Ulmus americana* to *Ceratocystis ulmi* under conditions prevailing in Quebec. *Canadian Journal of Botany* 43, 787-792.
Pomerleau, R. 1966. Relation of early cambial activity in white elm and infection by *Ceratocystis ulmi* (Buism.) C. Moreau. *Canadian Journal of Botany* 44, 109-111.
Pomerleau, R. 1968. Progression et localisation de l'infection par le Ceratocystis ulmi dans l'orme d'Ameérique. *Phytopathologische Zeitschrift*. 63, 301-327.
Pope, S.A., 1943. Some studies on the Dutch elm disease and the causal organism. *Doctoral Thesis, Cornell University*.
Ricaud, S., Alaoui-Sossé, B., Crabbé, J. & Barnola, P. 1995. Dormance et croissance des bourgeons du platane hybride (*Platanus acerifolia*) en milieu urbain. *Canadian Journal of Botany* 73, 130-140.
Richards W.C. 1993. Cerato-ulmin: a unique wilt toxin of instrumental importance in the development of Dutch elm disease In: *Dutch elm disease research: cellular and*

molecular approaches (M. Sticklen & J. Sherald eds.) Springer Verlag, New York, USA, pp. 89-151.
Richens, R.H. 1983. *Elm*. Cambridge University Press, Cambridge, UK, 347 p.
Rioux, D. 1996. Compartmentalization in trees: new findings during the study of Dutch elm disease. In: *Histology, ultrastructure and molecular cytology of plant-microorganism interactions.* (M. Nicole & V. Gianinazzi-Pearson eds.) Kluwer Academic, Dordrecht, The Netherlands, pp. 211-225.
Rioux, D. & Ouellette, G.B. 1989. Light microscope observations of histological changes induced by *Ophiostoma* ulmi in various nonhost trees and shrubs. *Canadian Journal of Botany* 67, 2335-2351.
Rioux, D. & Ouellette, G.B. 1991a. Barrier zone formation in host and nonhost trees inoculated with *Ophiostoma ulmi*. I. Anatomy and histochemistry. *Canadian Journal of Botany* 69, 2055-2073.
Rioux, D. & Ouellette, G.B. 1991b. Barrier zone formation in host and nonhost trees inoculated with Ophiostoma ulmi. II. Ultrastructure. *Canadian Journal of Botany* 69, 2074-2083.
Rioux, D., Nicole, M., Simard, M. & Ouellette G.B. 1998. Immunocytochemical evidence that secretion of pectin occurs during gel (gum) and tylosis formation in trees. *Phytopathology* 88(6), 494-505.
Roberts, B.R., & Main, H.V. 1965. The effect of chilling and photoperiod on budbreak in American elm. *Journal of Forestry* 63, 180-181.
Rohring E. 1996. Elms in Europe: ecology and Dutch elm disease. *Forstarchiv* 67, 179–198
Rönnberg-Wästljund, A. & Gullberg, U. 1999. Genetics of breeding characters with possible effect on biomass production in *Salix viminalis* (L.). *Theoretical and Applied Genetics* 98, 276-282.
Rousi, M. & Pusenius, J. 2005. Variations in phenology and growth of European white birch (*Betula pendula*) clones. *Tree Physiology* 25(2), 201-210.
Rousi, M. & Heinonen, J. 2007. Temperature sum accumulation effects on within-population variation and long-term trends in date of bud burst of European white birch (*Betula pendula*). *Tree Physiology* 27(7), 1019-1025.
Rudinsky J.A. 1962. Ecology of Scolytidae. *Annual Review of Entomology* 7, 327-348.
Sacchetti, P., Tiberi, R. & Mittempergher, L. 1990. Preference of *Scolytus multistriatus* (Marsham) during the gonad maturation phase between two species of elm. *Redia* 73, 347-354.
Samish R.M. 1954. Dormancy in woody plants. Annual Review of Plant Physiology 5, 183-203.
Santamour F.S. 1979. Resistance of Himalayan small-leaved elm to Dutch elm disease. *Journal of Arboriculture* 5, 110-112.
Santamour, F.S. & Bentz, S.E. 1995. Updated checklist of elm (*Ulmus*) cultivars for use in North America. *Journal of Arboriculture* 21(3),122-131.
Santini, A., Fagnani, A., Ferrini, F. & Mittempergher, L. 2002. 'San Zanobi' and 'Plinio' elm trees. *HortScience* 37(7), 1139-1141.
Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L. & Mittempergher, L. 2007a. 'Fiorente'and 'Arno' elm trees. *HortScience* 42(3), 712-714.
Santini, A., La Porta, N., Ghelardini, L. & Mittempergher, L. 2007 b. Breeding against Dutch elm disease adapted to the Mediterranean climate. *Euphytica* DOI 10.1007/s10681-007-9573-5.
Sarvas, R. 1972. Investigations on the annual cycle of development of forest trees. Active period. *Communicationes Instituti Forestalis Fenniae* 76(3), 110 p.
Saure, M.C. 1985. Dormancy Release in Deciduous Fruit Trees. *Horticultural Reviews* 7, 239-300.
Savolainen, O., Pyhajarvi, K., & Knurr T. 2007. Gene flow and local adaptation in trees. *Annual Review of Ecology, Evolution and Systematics* 38, 595-619.
Schwarz M.B. 1922. Das Zweigensterben der Ulmen, Trauerweiden und Pfirsichbäume. *Mededelingen uit et Phytopatologish laboratorium "Willie Commelin Scholten"* 5, 1-73.

Sengonca, C. & Leisse, N. 1984. Significance of bark beetles (Col. *Scolytidae*) in the spread of the Durch Elm Disease in the area of Euskirchen. *Journal of Applied Entomology* 98 (4), 413-423.
Sharik, T.L. & Barnes, B.V. 1976. Phenology of shoot growth among diverse populations of yellow birch (*Betula alleganiensis*) and sweet birch (*B. lenta*). *Canadian Journal of Botany* 54, 2122-2129.
Shigo A.L., 1982. Dutch elm disease: a CODIT perspective In: Proceedings of Dutch elm disease symposium and workshop. (E.S. Kondo, Y. Hiratsuka & W.B.G. Denyer eds.) Manitoba Department of Natural Resources, Winnipeg, Canada. pp. 151-168.
Shigo A.L., 1984. Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology* 22, 189-214.
Shigo, A. & Tippet, J.T. 1981. Compartmentalization of America elm tissues infected with *Ceratocystis ulmi*. *Plant Disease* 65, 715-718.
Si-Mohamed, C. 1983. Germination, rythmes de la croissance et morphogenèse des jeunes plants chez *Castanea sativa* Miller. *Thèse de Doctorat Université de Clermont-Ferrand II, Clermont-Ferrand*, 201 p.
Sinclair, W.A. & Campana R.J. 1978. Dutch elm disease, perspectives after 60 years. Cornell University Agricultural Experiment Station, *Search (Agriculture)* 8, 1-52.
Sinclair, W.A., Zahand, J.P. & Melching, J.B. 1975. Anatomical markers for resistance of *Ulmus americana* to *Ceratocystis ulmi*. *Phytopathology* 65, 349-352.
Singh, R.V. 1982. Fodder trees of India. Oxford and IBH Publishing Co. Ptv. Ltd. New Delhi, India, 663 p.
Smalley, E.G. 1963. Seasonal fluctuations in susceptibility of young elm seedlings to Dutch elm disease. *Phytopathology* 53, 846–853.
Smalley, E.B. & Guries, R.P. 1993. Breeding elms for resistance to Dutch elm disease. *Annual Review of Phytopathology* 31, 325–352.
Smalley, E. B. & Guries, R. P. 2000. Asian elms: source of disease and pest resistance In: *The Elms: Breeding, Conservation, and Disease Management* (C. Dunn ed.) Kluver Academic Publishers, Boston, pp. 215-230.
Smalley, E.B. & Kais, A.G. 1966. Seasonal variation in the resistance of various elm species to Dutch elm disease In: *Breeding Pest-Resistant Trees* (H.D. Gerhold, E.J. Schreiner, R.E. Mc Dermott, J.A. Winieski eds.) Pergamon Press, Elmsford, pp. 279–292.
Solla, A. & Gil, L. 2002. Xylem vessel diameter as a factor in resistance of *Ulmus minor* to *Ophiostoma novo-ulmi*. *Forest Pathology* 32, 123-134.
Solla, A., Bohnens, J., Collin, E., Diamantis, S., Franke, A., Gil, L., Burón, M., Santini, A., Mittempergher, L., Pinon, J. & Vanden Broek, A. 2005 a. Screening European elms for resistance to *Ophiostoma novo-ulmi. Forest Science* 51(2), 134-141.
Solla, A. Martin J.A., Corral P. & Gil, L. 2005 b. Seasonal changes in wood formation of *Ulmus pumila* and *U. minor* and its relation with Dutch elm disease. *New Phytologist* 166(3), 1025-1034.
Sparks, T.H. & Carey, P.D. 1995. The responses of species to climate over two centuries: an analysis of the Marsham phenological record, 1736-1947. *Journal of Ecology* 83, 321-329.
Søgaard, G., Johnsen, Ø., Nilsen, J., and Junttila O. 2008. Climatic control of bud burst in young seedlings of nine provenances of Norway spruce. *Tree Physiology* 28, 311-320.
Sticklen, M.B., Bolyard, M.G., Hajela, R.K. & Duchesne L.C. 1991. Molecular and cellular aspects of Dutch elm disease. *Phytoprotection* 72, 1-13.
Stipes R.J. & Campana R. 1981. *Compendium of Elm Diseases.* American Phytopathological Society, St Paul MN, USA. 66 p.
Sutherland, M.L., Pearson S. & Brasier C.M. 1997. The influence of temperature and light on defoliation levels of elm by Dutch elm disease. *Epidemiology* 87(6), 576-581.
Takai, S. & Kondo E.S. 1979. Seasonal development of Dutch elm disease on white elms in Central Ontario, Canada. I. Following wound inoculation. *Canadian Journal of Botany* 57, 341–352
Tamura, F., Tanabe, K., & Itai A. 1995. Effect of interruption of chilling on bud break in Japanese pear. *Acta Horticulturae* 395, 135-140.

Tchernoff, V. 1965. Methods for screening and for the rapid selection of elms for resistance to Dutch elm disease. *Acta Botanica Neerlandica* 14, 409-452.
Temple, B. & Horgen, P.A. 2000. Biological roles for cerato-ulmin, a hydrophobin secreted by the elm pathogens, *Ophiostoma ulmi* and *O. novo-ulmi*. *Mycologia* 92 (1), 1-9.
Thomas, B. & Vince Prue, D. 1997. *Photoperiodism in plants.* 2nd edition. Academic Press, London.
Townsend, A.M. 1971. Relative resistance of diploid *Ulmus* species to *Ceratocystis ulmi. Plant Disease Report*er 55, 980-982.
Townsend, A.L. 2000. USDA genetic research on elms In: *The Elms: Breeding, Conservation and Disease Management* (C.P. Dunn ed.) Kluwer Academic Publishers, pp. 271-278
Vaartaja, O. 1954. Photoperiodic ecotypes of trees. *Canadian Journal of Botany*. 32, 392-399.
Vaartaja, O. 1959. Evidence of photoperiodic ecotypes in trees. *Ecological Monographs* 29, 91-111.
Van deer Veen, R. 1951. Influence of daylength on the dormancy of some species of the genus *Populus*. *Physiologia Plantarum* 4, 35.
Vegis, A. 1964. Dormancy in higher plants. *Annual Review of Plant Physiology* 15, 185-224.
Viherä-Aarnio, A., Häkkinen R. & Junttila, O. 2006. Critical night length for bud set and its variation in two photoperiodic ecotypes of *Betula pendula*. *Tree physiology* 26, 1013-1018.
Von Wuehlisch, G., Krusche, D. & Muhs H.J. 1995. Variation in temperature sum requirement for flushing of beech provenances. *Silvae Genetica* 44, 343-346.
Ware, G. 1995. Little-known elms from China: landscape tree possibilities. *Journal of Arboriculture*. 21, 284-288.
Wareing, P.F. 1953. Growth studies in woody species. 5. Photoperiodism in dormant buds of *Fagus sylvatica* L. *Physiologia Plantarum* 6 (4), 692-706.
Wareing, P.F. 1956. Photoperiodism in woody plants. *Annual Review of Plant Physiology* 7, 191-214.
Webber, J.F. 2000. Insect vector behaviour and the evolution of Dutch elm disease. In: *The elms: breeding, conservation and disease management.* (C.P. Dunn ed.). Kluwer Academic Publishers, Boston, pp. 47-60.
Webber, J.F. 2004. Experimental studies on factors influencing the transmission of Dutch elm disease. *Investigación agraria: Sistemas y Recursos Forestales* 13(1), 197-205.
Webber J.F. & Brasier, C.M. 1984. The transmission of Dutch elm disease: a study of the process involved In: *Invertebrate-Microbial Interactions*. (J.M. Anderson, A.D.M. Rayner & D. Walton eds.) Cambridge University Press, Cambridge UK, pp. 271-306.
Wiegrefe, S.J., Sytsma, K.J. & Guries, R.P. 1994. Phylogeny of elms (*Ulmus*, Ulmaceae): molecular evidence for a sectional classification. *Systematic Botany* 19(4), 590-612.
Whittaker, R.H. & Feeny, P.P. 1971. Allelochemics – Chemical interaction between species. *Science* 171 (3973), 757-770.
Whiteley, R.E., Black-Samuelsson, S. & Jansson, G. 2003. Within and between populations variation in adaptive traits in *Ulmus laevis* Pall., the European white elm. *Forest Genetics* 10(4), 313-323.
Worrall, J. 1975. Provenance and clonal variation in phenology and wood properties of Norway spruce. *Silvae Genetica* 29, 2-5.
Worrall, J. 1983. temperature-bud-burst relationships in amabilis and subalpine fir provenance tests replicated at different elevations. *Silvae Genetica* 32, 203-209.
Worrall, J. & Mergen, F. 1967. Environmental and genetic control of dormancy in *Picea abies*. *Physiologia Plantarum* 20, 733-745.
Wright, J.W. 1976. Introduction to forest genetics. *Academic Press*, N.Y., 463 pp.
Zimmermann, M.H., & McDonough , J. 1978. Dysfunction in the flow of food. In: *Plant disease – an advanced treatise. Vol III. How plants suffer from disease* (J.G. Horsfall & E.D. Cowling eds.), Academic Press, New York, pp. 117-140.

Acknowledgements

This thesis was made possible by Paolo Raddi, Director of the Institute for Plant Protection of the Italian National Research Council (CNR), who supported my work at SLU in Uppsala. For this I wish to thank him sincerely.

I am most indebted to Sanna Black-Samuelsson and Alberto Santini, who took the first agreements, outlined the whole thing and both became supervisors of my thesis. Dear Sanna, your kindness, your availability and your support until the end were so precious for the accomplishment of the thesis and even besides work. Dear Alberto, without your friendship and help I would not have done all this. I hope you will keep on providing me your priceless mix of scientific competence and jokes.

My work over all these years was accompanied and assisted by Mauro Falusi. I owe to Mauro inspiration and most of my scientific knowledge. My thankfulness goes far beyond this thesis and scientific work to all what we enjoyed together back at the Faculty, 'alle Cascine'.

I am very grateful to Tor Myking, supervisor of this thesis, who brought in many precious comments and corrections even during the Christmas holidays. Dear Tor, without your confidence I would have given up.

Lorenzo Mittempergher who with his long and precious work on elms at the Institute for Plant Protection built the foundations of my work is greatly acknowledged.

I am especially indebted to Eric Collin for inspiration, encouragement, friendship and good wine tasting. Un très grand merci du fond du mon coeur, Eric!

I also thank all the other partners of the EU-RESGEN project for sharing their data.

A warm thank you goes to Ignazio Graziosi, who provided this thesis some beauty.

During my stay at the Department of Plant Biology and Forest Genetics in Uppsala I was rescued by the kindness of David Clapham, who provided me practical help and - what is most - the warmness I really needed in my first and lonely days in Sweden. David also kindly corrected the English of this thesis in the very last days. Thank you so much.

For their indispensable contribution during the fieldwork I warmly thank Fabio Ferrini, Alberto Fagnani and Abdellah Dahmany. Dear Abel thank you for sharing with me your special wisdom and for all the excellent lunches you prepared!

I also wish to thank my colleagues at the Institute for Plant Protection in Italy and those at the Department of Plant Biology and Forest Genetics in Uppsala for the

nice time we had, thus for their indirect but so important support to this thesis. I am especially grateful to the present Director of the Institute for Plant Protection, Tullio Turchetti, who encouraged me during the last phases of this work. Special thanks also to Nurun Nahar, Birgitta Eriksson, Björn Nicander, Urban Pettersson, Anki Wästljung and Anna Westerbergh.

I am also very grateful to Mats Hannerz for useful comments, suggestions and encouragement.

Thank you so much Anna and Vittorio for your love and support.

Francesco thanks for all the thoughts, the words and songs and fun during my dark and my shining days.

Thanks for being so close even when so far to Antoni, Giampiero, Alitukiriza, José, Marcin, Barbara, Ercole, Checco, Mascia, Danilo.

I have no words to express my gratefulness to my all life friend and love Lorenzo. I hope that all the mornings still to live will be enough to thank him for his endless patience and tenderness and devotion.

The work comprised in this thesis was funded by the EU through the RESGEN CT-96-78 project, by the Ente Cassa di Risparmio di Firenze, and by the Swedish Institute.