A 125

COLDTREE March 15, 2000

The application of cDNA microarray technology for unravelling molecular events underlying dormancy and cold hardiness in forest tree seedlings.

A first step towards the development of molecular diagnostic tests for cost efficient reforestation and nursery logistics.

PART A

Thematic priorities: QOL-2000 -5.3.1
QOL-2000 - 5.1.1
QOL-2000 - 8.2

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For guidelines see in relevant "Guide for Proposers"

Proposal submission forms for financial support from the EC for shared-cost RTD actions: research and technological development projects, demonstration projects, and combined projects

If possible, these forms should be prepared using the Proposal Preparation Tool (ProTool), which is available via the Commission Internet site http://www.cordis.lu/fp5/protool or on CD-ROM. Use of the Proposal Preparation Tool is preferred by the Commission. However applicants may also use the forms in the Guide for Proposers. Using the ProTool, forms may be submitted electronically, or printed out and returned on paper.

Information on the Proposal [1]				
Proposal Full Name	The application of cDNA microarray technology for unraveling molecular events underlying dormancy and cold hardiness in forest tree seedlings.			
Proposal Acronym [5]	COLDTREE		**Proposal No [6]**	
Call Identifier [3]	1999/C 361/06			
Research Programme(s) [2]	QOL-2000	QOL-2000	QOL-2000	
Thematic priorities [2]	QOL-2000-5.3.1	QOL-2000-5.1.1	QOL-2000-8.2	

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Post stamp ☐☐ / ☐☐ / ☐☐☐☐ **Reception date** ☐☐ / ☐☐ / ☐☐☐☐

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

Thematic priorities [2]	QOL-2000-5.3.1	QOL-2000-5.1.1	QOL-2000-8.2	
Type of Action [4]	RS			
Proposal Full Name	The application of cDNA microarray technology for unraveling molecular events underlying dormancy and cold hardiness in forest tree seedlings.			

Contact person(s) of the proposal(s)

Title (Dr, Prof., ...)	Dr.		Gender [8]	F	X	M	
Family Name	van Wordragen						
First Name	Monique F.						
Organisation Legal Name [9]	Agricultural Research Department Agrotechnological Research Institute						
Department / Institute Name [10]	Business unit Agro-Industrial Production Chains						
PO Box [11]	17						
Street Name and Number	Bornsesteeg 59						
Post Code [12]	6700 AA	Cedex [13]					
Town/City	Wageningen						
Country Code [14]	NL	Country Name [14]	Netherlands				
Telephone No [15]	(31-317)475000	Fax No [15]	(31-317)475347				
E-mail	m.f.vanwordragen@ato.wag-ur.nl						

Proposal abstract (maximum 1000 characters) [16]

Reforestation logistics and forest nursery management depends on the physiological status of the trees. Lifting and cold storage of insufficiently hardened trees leads to reduced vitality, frost damage and desiccation. Identification of molecular processes underlying winter hardiness will allow for the development of rapid and reliable tools to determine seedling condition. The powerful cDNA microarray technology will be employed to analyse gene expression patterns in economically and ecologically important forest tree species in combination with physiological and morphological screening. This multidisciplinary approach will result in a detailed picture of molecular events involved in the onset and release of dormancy and hardiness. Genes with a strong predictive value will be selected for future implementation in molecular hardiness tests that will aid in improving forestation planning and forest tree nursery management.

Duration (in Months) [17]	48	Total Eligible Cost (in euro) [18]	728331	EC Contribution requested (in euro) [19]	364164
Keywords [20]	cDNA microarrays	forestry	cold hardiness		

Have you or any of your partners, previously or currently, submitted this proposal or one similar in content to any Community Programme? If yes, please give details of the proposal [21]					Y	X	N
Programme Name	FFP, theme 1	Year	1999	Proposal No	QLRT 1999 30274		

Duly authorised by the consortium partners to send this proposal to the Commission, I certify that the description of this proposal and the information on forms A1, A2, A3 and A4 is accurate and agreed to by the consortium partners and that the consortium collectively agrees to carry out a project as described herein.

Date (DD/MM/YYYY)	15/03/2000
Signature of person authorised to submit a proposal in the co-ordinating organisation	DLO-Agrotechnological Research Institute ATO-DLO P.O. Box 17 6700 AA Wageningen The Netherlands

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Objectives (maximum 1000 characters)

To ensure seedling vitality for cost-effective forestation, and for efficient management of forest tree nurseries, it is important to have accurate knowledge of the physiological status of the trees during autumn, winter, and cold storage. For this it is necessary to unravel the molecular events underlying dormancy and frost hardiness. Therefore, our objectives are:
- To identify genes and molecular pathways involved in the onset and release of winter hardiness and dormancy in trees using cDNA microarrays and to define a conceptual model describing the molecular events underlying these processes.
- To select a set of key genes, of which the expression patterns can be used to describe the various stages of dormancy and hardiness,
- To evaluate the merits of these key genes as molecular diagnostic tool for nursery practice and improved forestation

Description of the work (maximum 2000 characters)

The workplan to this project can be divided in three parts. In the first phase Pinus and Fagus seedlings will be cultured in controlled environment to generate plant material in various, well-defined, stages of dormancy and hardiness. Screening of a cDNA microarray with RNA from these plants, will result in a set of 20-30 Pinus transcripts representing putative dormancy or hardiness genes. The clones will be sequenced and characterised. Beech homologues to these pine genes will be isolated, sequenced, and characterised. For all selected genes RT-PCR-primers will be designed.
In the second phase, the transcriptional regulation of the selected genes in field-grown seedlings, will be analysed in more detail in different organs and various environmental conditions, using quantitative RT-PCR assays and fluorescent detection. Correlations will be studied between gene expression, climatic conditions, geographical position, provenance and physiological parameters, such as root/shoot electrolyte leakage, root/shoot growth potential, electrical conductivity of the stem and frost tolerance. This will reveal a subset of key genes, that can be used as accurate and general molecular descriptors for dormancy and hardiness.
In the third phase, all data will be combined and used for the definition of a general hypothesis on the molecular events underlying the onset and development of dormancy and cold hardiness in woody species. Additionally, in this phase the operational evaluation of the selected makers in a commercial setting will be undertaken and a demonstration workshop will be organized to communicate the results to the forestry sector.

Milestones and expected results (maximum 500 characters)

The result will be reached through 6 milestones:
M1. Initial set of relevant pine genes selected (Month 20)
M2. Initial set of relevant beech genes selected (M23)
M3. Subset of pine and beech key genes selected (M38)
M4. Usefulness of key genes as molecular diagnostic tests evaluated (M43)
M5. Integrated database available (M43)
M6. Conceptual model on molecular processes underlying dormancy/hardiness defined

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A3. Participant Profile/Information (1 form per participant) [23]

Legal information on the participating organisation

Participant Role [24]	CO	Participant No [25]	1	Assistant to Contractor No [26]	
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Agricultural Research Department Agrotechnological Research Institute				
Short Name [29]	ATO-DLO			Legal Registration No [30]	09098104
Activity Type [31]	REC	Legal Status [32]	PNP	If 'PRC', Specify [33]	
Business Area [34] (NACE)	73	User/Supplier [35] (U / S)	S	Cost Basis [36] (FC / FF / AC)	FC

Organisation details [37]

Annual turnover [38]	T2	Annual Balance Sheet Total [39]	B2	Number of employees [40]	S5

Is Your Organisation independent [41]?	Y	X	N	
If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]				
Is Your Organisation affiliated to any other participant(s) in the proposal [43]?	Y	X	N	

If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]	2	PRI-DLO	I
	0		
	0		

Address of the main department carrying out the work [45]

Department/ Institute Name [10]	Agrotechnological Research Institute		
PO Box [11]	17		
Street Name and Number	Bornsesteeg 59		
Post Code [12]	6700 AA	Cedex [13]	
Town/City	Wageningen		
Country Code [14]	NL	Country Name [14]	Netherlands

Authorised person [46]

Title (Dr, Prof., ...)	Dr.		Gender [8]	F		M	X
Family Name	Franken						
First Name	Anton						
Telephone No [15]	(31-317)475000	Fax No [15]	(31-317)475347				
E-mail	a.a.j.m.franken@ato.wag-ur.nl						

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	30/05/1999
Signature of authorised person	

DLO-Agrotechnological Research Institute ATO DLO
P.O. Box 17
6700 AA Wageningen
The Netherlands

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A3. Participant Profile/Information (1 form per participant) [23]

Legal information on the participating organisation

Participant Role [24]	CR	Participant No [25]	2	Assistant to Contractor No [26]	
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Plant Research International, as part of DLO, a foundation according to the Dutch law.				
Short Name [29]	PRI	Legal Registration No [30]	09098104		
Activity Type [31]	RPN	Legal Status [32]	PNP	If 'PRC', Specify [33]	
Business Area [34] (NACE)	73	User/Supplier [35] (U / S)	S	Cost Basis [36] (FC / FF / AC)	FC

Organisation details [37]

Annual turnover [38]	T3	Annual Balance Sheet Total [39]	B2	Number of employees [40]	S6

Is Your Organisation independent [41]?	Y	X	N	
If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]				
Is Your Organisation affiliated to any other participant(s) in the proposal [43]?	Y	X	N	

If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]	1	ATO-DLO	I

Address of the main department carrying out the work [45]

Department/ Institute Name [10]	Plant Research International		
PO Box [11]	16		
Street Name and Number	Droevendaalsesteeg 1		
Post Code [12]	6700 AA	Cedex [13]	
Town/City	Wageningen		
Country Code [14]	NL	Country Name [14]	Netherlands

Authorised person [46]

Title (Dr, Prof., ...)	Dr.			Gender [8]	F		M	X
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First Name	Nic							
Telephone No [15]	(31-317) 477001	Fax No [15]	(31-317) 418094					
E-mail								

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	01/03/2000
Signature of authorised person	

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A3. Participant Profile/Information (1 form per participant) [23]

Legal information on the participating organisation

Participant Role [24]	CR	Participant No [25]	3	Assistant to Contractor No [26]	
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Dalarna University				
Short Name [29]	DU			Legal Registration No [30]	
Activity Type [31]	REC	Legal Status [32]	GOV	If 'PRC', Specify [33]	
Business Area [34] (NACE)	02	User/Supplier [35] (U / S)	S	Cost Basis [36] (FC / FF / AC)	FC

Organisation details [37]

Annual turnover [38]		Annual Balance Sheet Total [39]		Number of employees [40]	S5

Is Your Organisation independent [41]?	Y [X]	N [ ]
If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]		
Is Your Organisation affiliated to any other participant(s) in the proposal [43]?	Y [ ]	N [X]

If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]			

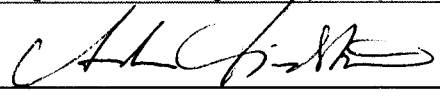
Address of the main department carrying out the work [45]

Department/ Institute Name [10]	Forest Industry and Technology		
PO Box [11]			
Street Name and Number	Herrgaardsvaegen 122		
Post Code [12]	776 98	Cedex [13]	
Town/City	Garpenberg		
Country Code [14]	S	Country Name [14]	Sweden

Authorised person [46]

Title (Dr, Prof., ...)	Assoc Prof	Gender [8]	F [ ] M [X]
Family Name	Lindstroem		
First Name	Anders		
Telephone No [15]	+46 225 26 191	Fax No [15]	+46 225 26 100
E-mail	ali@du.se		

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	2000-03-06
Signature of authorised person	

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A3. Participant Profile/Information (1 form per participant) [23]

Legal information on the participating organisation

Participant Role [24]	CR	Participant No [25]	4	Assistant to Contractor No [26]	
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Danish Institute of Agricultural Sciences				
Short Name [29]	DIAS			Legal Registration No [30]	DK 17 88 24 49
Activity Type [31]	RPU	Legal Status [32]	GOV	If 'PRC', Specify [33]	
Business Area [34] (NACE)	73	User/Supplier [35] (U / S)	S	Cost Basis [36] (FC / FF / AC)	FC

Organisation details [37]

Annual turnover [38]	T3	Annual Balance Sheet Total [39]	NA	Number of employees [40]	S6

Is Your Organisation independent [41]?	Y	X	N	
If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]				

Is Your Organisation affiliated to any other participant(s) in the proposal [43]?	Y		N	X
If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]				

Address of the main department carrying out the work [45]

Department/ Institute Name [10]	Danish Institute of Agricultural Sciences, Department of Ornamentals		
PO Box [11]	102		
Street Name and Number	Kirstinebjergvej 10		
Post Code [12]	DK-5792	Cedex [13]	
Town/City	Aarslev		
Country Code [14]	DK	Country Name [14]	Denmark

Authorised person [46]

Title (Dr, Prof., ...)	Scientist			Gender [8]	F		M	X
Family Name	Brønnum							
First Name	Peter							
Telephone No [15]	(-45) 63904343	Fax No [15]	(-45) 63904393					
E-mail	p.bronnum@agrsci.dk							

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	03/03/2000
Signature of authorised person	

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A3. Participant Profile/Information (1 form per participant) [23]

Legal information on the participating organisation							
Participant Role [24]	AC	Participant No [25]	5	Assistant to Contractor No [26]		4	
Registration No with the European Commission's Research Programmes [27]							
Organisation Legal Name [28]	Research station for Nursery Stock						
Short Name [29]	RSNS			Legal Registration No [30]		41172281	
Activity Type [31]	REC	Legal Status [32]	GOV	If 'PRC', Specify [33]			
Business Area [34] (NACE)	01	User/Supplier [35] (U / S)		Cost Basis [36] (FC / FF / AC)		FF	
Organisation details [37]							
Annual turnover [38]	T1	Annual Balance Sheet Total [39]	B2	Number of employees [40]		S3	
Is Your Organisation independent [41]?					Y X	N	
If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]							
Is Your Organisation affiliated to any other participant(s) in the proposal [43]?					Y	N X	
If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]							
Address of the main department carrying out the work [45]							
Department/ Institute Name [10]	Research Station for Nursery Stock						
PO Box [11]	118						
Street Name and Number	Rijneveld 153						
Post Code [12]	2770 AC	Cedex [13]					
Town/City	Boskoop						
Country Code [14]	NL	Country Name [14]	Netherlands				
Authorised person [46]							
Title (Dr, Prof., ...)	Ir.			Gender [8]	F	M X	
Family Name	Dolmans						
First Name	Nico						
Telephone No [15]	(31-0172)236700	Fax No [15]	(31-0172)236710				
E-mail	N.G.M.Dolmans@bpo.agro.nl						

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	07/03/2000
Signature of authorised person	

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Part B - Participant Profile (organisation information)

Legal information on the participating organisation

Participant Role [24]	AC	Participant No [25]	6	Assistant to Contractor No [26]	4
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Hedeselskabets Planteskole				
Short Name [29]	HSP			Legal Registration No [30]	82257055
Activity Type [31]	OTH	Legal Status [32]	PRC	If 'PRC', Specify [33]	
Business Area [34] (NACE)	02	User/Supplier [35] (U / S)	U	Cost Basis [36] (FC / FF / AC)	FF

Organisation details [37]

Annual turnover [38]	T1	Annual Balance Sheet Total [39]	B1	Number of employees [40]	S3

Is Your Organisation independent [41]?	Y		N	X

If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]	Hedeselskabet

Is Your Organisation affiliated to any other participant(s) in the proposal [43]?	Y		N	X

If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]			

Address of the main department carrying out the work [45]

Department/ Institute Name [10]	Hedeselskabets Planteskole, Brøndlundgaard		
PO Box [11]			
Street Name and Number	Brøndlundvej 2, Gabøl		
Post Code [12]	DK-6500	Cedex [13]	
Town/City	Vojens		
Country Code [14]	DK	Country Name [14]	Denmark

Authorised person [46]

Title (Dr, Prof., ...)	Nursery Director	Gender [8]	F		M	X
Family Name	Thybo Møller					
First Name	Leo					
Telephone No [15]	(-45) 74871600	Fax No [15]	(-45) 74871543			
E-mail	lml@hedeselskabet.dk					

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	03/03/2000
Signature of authorised person	Thybo Møller

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A3. Participant Profile/Information (1 form per participant) [24]

Legal Information on the participating organisation

Participant Role [25]	CR	Participant No [26]	7	Assistant to Contractor No [28]	
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Forestry Commission Research Agency				
Short Name [29]	FC (FR)		Legal Registration No [30]		
Activity Type [31]	REC	Legal Status [32]	GOV	If 'PRC', Specify [33]	
Business Area [34] (NACE)	02	User/Supplier [35] (U / S)	S	Cost Basis [36] (PC / FF / AC)	FC

Organisation details [37]

Annual turnover [38]	T2	Annual Balance Sheet Total [39]	B2	Number of employees [40]	S5

Is Your Organisation independent [41]?	Y	X	N	
If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]				
Is Your Organisation affiliated to any other participant(s) in the proposal [43]?	Y	X	N	
If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (P / I) [44]		FE Nurseries		1

Address of the main department carrying out the work [45]

Department/ Institute Name [10]	Forestry Commission Research Agency		
PO Box [11]			
Street Name and Number			
Post Code [12]	EH25 9SY	Cedex [13]	
Town/City	Roslin, Midlothian, Scotland		
Country Code [14]	UK	Country Name [14]	United Kingdom

Authorised person [46]

Title (Dr, Prof., ...)	Mr	Gender [8]	F		M	X
Family Name	Dewar					
First Name	James					
Telephone No [15]	(044-131)4452176	Fax No [15]	(044-131)4455124			
E-mail	j.dewar@forestry.gov.uk					

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	06/03/2000
Signature of authorised person	J Dewar

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A3 Participant Profile/Information (1 form per Participant)

Legal information on the participating organisation

Participant Role [24]	AC	Participant No [25]	8	Assistant to Contractor No [26]	7
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Forestry Commission				
Short Name [29]	FE Nurseries	Legal Registration No [30]			
Activity Type [31]	OTH	Legal Status [32]	GOV	If 'PRC', Specify [33]	
Business Area [34] (NACE)	02	User/Supplier [35] (U / S)	U	Cost Basis [36] (FC / FF / AC)	FC

Organisation details [37]

Annual turnover [38]	T2	Annual Balance Sheet Total [39]	B1	Number of employees [40]	S4

Is Your Organisation independent [41]?	Y	X	N	

If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]	

Is Your Organisation affiliated to any other participant(s) in the proposal [43]?	Y	X	N	

If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]			
	5	FC (FR)	I
	0		
	0		

Address of the main department carrying out the work [45]

Department/ Institute Name [10]	Plant and Seed Supply Branch, Forest Enterprise		
PO Box [11]			
Street Name and Number	231 Corstorphine Road		
Post Code [12]	EH12 7AT	Cedex [13]	
Town/City	Edinburgh, Scotland		
Country Code [14]	UK	Country Name [14]	United Kingdom

Authorised person [46]

Title (Dr, Prof., ...)	Dr	Gender [8]	F		M	X
Family Name	Morgan					
First Name	John					
Telephone No [15]	(044-131)3340303	Fax No [15]	(044-131)3146316			
E-mail	john.morgan@forestry.gov.uk					

I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	06/03/2000
Signature of authorised person	

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A3 Participant Profile/Information (1 form per participant)

Participant identification

Participant Role [24]	AC	Participant No [25]	9	Assistant to Contractor No [26]	7
Registration No with the European Commission's Research Programmes [27]					
Organisation Legal Name [28]	Alba Trees PLC				
Short Name [29]	Alba Trees		Legal Registration No [30]		
Activity Type [31]	OTH	Legal Status [32]	PRC	If 'PRC', Specify [33]	Ltd.
Business Area [34] (NACE)	02	User/Supplier [35] (U / S)	U	Cost Basis [36] (FC / FF / AC)	FF

Organisation details [37]

Annual turnover [38]	T1	Annual Balance Sheet Total [39]	B1	Number of employees [40]	S3
Is Your Organisation independent [41]?				Y: X	N:
If No, please indicate legal name(s) of owner(s) who own 25 % or more [42]					
Is Your Organisation affiliated to any other participant(s) in the proposal [43]?				Y:	N: X
If Yes, please indicate Participant No, Short Name(s) and character of affiliations(s) (D / I) [44]					

Address of the main department carrying out the work [9]

Department/ Institute Name [10]	Alba Trees PLC		
PO Box [11]			
Street Name and Number	Lower Winton		
Post Code [12]	EH33 2AL	Cedex [13]	
Town/City	Gladsmuir, East Lothian, Scotland		
Country Code [14]	UK	Country Name [14]	United Kingdom

Authorised person [45]

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I certify that the above information is accurate and that my organisation has agreed to participate in this proposal.

Date (DD/MM/YYYY)	06/03/2000	
Signature of authorised person		

Shared Cost RTD Proposal Form – Form A4 (1/2)

EUROPEAN COMMISSION
RESEARCH DIRECTORATES
GENERAL
SHARED COST
RTD PROPOSAL FORMS

EN E 2 FP5RTD

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Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A4 Cost Summary in euro [47] (part 1/2)

Participant Role [24]	Participant No [25]	Assistant to Contractor No [26]	Participant Short Name [31]	Number of person/months [52]	Personnel Costs [53]	Durable Equipment [54]	Consumables [55]	Travel and Subsistence [56]	Computing [57]	Subcontracting [58]	Subtotal part 1/2 [59]
CO	**1**	[48]	ATO	57	238575	7350	46000	12000	2000	8500	314425
CO	**1**	[49]	**Co-ordination**	9	26675	0	0	6000	1500		34175
CO	**1**	[50]	**Total co-ordinator costs**	66	265250	7350	46000	18000	3500	8500	348600
CR	2		PRI	42	164766	0	115000	17000	0	0	296766
CR	3		DU	20	95018	0	28000	16000	0	0	139018
CR	4		DIAS	55	216644	0	58057	16108	0	0	290809
AC	5	4	RSNS	11	52575	0	8000	7250	0	0	67825
AC	6	4	HSP	3	11434	0	5380	2015	0	0	18829
CR	7		FC(FR)	65	275763	0	38400	12202	6400	0	332765
AC	8	7	Fe-nurs	1	3428	0	2600	1800	0	0	7828
AC	9	7	ALBA	1	4606	0	2600	1800	0	0	9006
TOTAL [66]				264	1089484	7350	304037	92175	9900	8500	1511446

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EUROPEAN COMMISSION
RESEARCH DIRECTORATES GENERAL
SHARED COST RTD PROPOSAL FORMS

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FOR COMMISSION USE ONLY

Proposal Acronym [5]	COLDTREE	Proposal No [6]	

A4. Cost Summary in euro [47] (part 2/2)

Participant Role [24]	Participant No [25]	Assistant to Contractor No [26]	Participant Short Name [51]	Subtotal of part 1/2 [59]	Other Specific project Costs [60]	Protection of knowledge [61]	Overhead Costs [62]	Total Costs [63]	Costs Basis : FC/FF/AC [37]	% Requested from the Community [64]	Requested Contribution from the Community [65]
CO	**1**	[48]	ATO	314425	1800	5000	262700	583925			291962
CO	**1**	[49]	**Co-ordination**	34175	15000	5000	0	54175			27087
CO	**1**	[50]	**Total co-ordinator costs**	348600	16800	10000	262700	638100	FC	50	319049
CR	2		PRI	296766	19898	0	180603	497267	FC	50	248633
CR	3		DU	139018	11000	0	133226	283244	FC	50	141622
CR	4		DIAS	290809	0	0	203814	494623	FC	50	247311
AC	5	4	RSNS	67825	0	0	59927	127752	FF	50	63876
AC	6	4	HSP	18829	0	0	2288	21117	FF	50	10558
CR	7		FC(FR)	332765	0	0	203635	536400	FC	50	268200
AC	8	7	Fe-nurs	7828	0	0	747	8575	FC	50	4287
AC	9	7	ALBA	9006	0	0	997	10003	FF	50	5001
TOTAL [66]				1511446	47698	10000	1047937	2617081			1308537

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Appendix 1

WAGENINGEN

STICHTING DLO

European Commission
Rue de la Loi 200
B-1049
Brussel
Belgie

DATE
13 January 2000

SUBJECT
change # institutes

OUR REFERENCE
00/0001013

HANDLED BY
A.D. de Leur

DIRECT [TELEPHONE] LINE
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E-MAIL
A.D.de.Leur@co.dlo.nl

Until 1999 the DLO-organisation was a foundation of thirteen individual institutes.

On the 1st of januari this year however there has been a change of number and names of the institutes.

The following institutes:
- Research Institue for Agribiology and soil Fertility (AB)
- Centre for Plant Breeding and Reproduction Research (CPRO)
- Research Institute for Plant Protection (IPO)

Become together a new institute with the name:
- Plant Research International (PRI)

The following institutes:
- Institute for Forestry an Nature Research (IBN)
- Winand Staring Centre for Integrated Land, Soil and Water Research (SC)

Become together a new institute with the name:
- Alterra Green World Research

With kind regards,

Ir.K.J. van Ast
Deputy General Director DLO

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A 126

COLDTREE March 15, 2000

The application of cDNA microarray technology for unravelling molecular events underlying dormancy and cold hardiness in forest tree seedlings.

A first step towards the development of molecular diagnostic tests for cost efficient reforestation and nursery logistics.

PART B

Thematic priorities: QOL-2000 -5.3.1
QOL-2000 - 5.1.1
QOL-2000 - 8.2

Part B: description of scientific/technological objectives and workplan

B1. Title page.

The application of cDNA microarray technology for unravelling molecular events underlying dormancy and cold hardiness in forest tree seedlings.

A first step towards the development of molecular diagnostic tests for cost efficient reforestation and nursery logistics.

Acronym: COLDTREE

March 15, 2000

B2. Table of Contents

B3. Objectives and expected achievements

Sustained yield from Europe's commercially exploited forests requires a supply of millions of seedlings annually. The planting stock for reforestation and urban horticulture, almost 1.7 billion tree seedlings and ornamental woody plants comprising a total value of about 2 billion Euro, is mainly produced by European forest tree nurseries. These nurseries rely on a tight scheduling of operations, to be able to deliver vital seedlings, with a high regrowth potential, to the planting site. A critical step in a modern nursery production chain is the transfer of seedlings to cold or frozen storage; plants survive periods of low temperature because their natural preparation for winter includes the activation of biological mechanisms for cold tolerance.
The cold storage period is required for several reasons:

1) In most of Europe climatic conditions are optimal for planting in spring or early summer, while seedlings are ready for delivery in late autumn. The winter intervening is a hazardous period in the nursery production chain. If left outside, harsh winter temperatures will often damage the young seedlings, especially when severe frost is not accompanied by snowfall. Containerised seedlings, which are not sheltered by the soil, are particularly vulnerable to winter damage. The problem is exacerbated by the fluctuating and unpredictable winter climate in Northern Europe. For example; during the winter of 98/99 in central Sweden, the temperature dropped from +5 °C to -20 °C in two days. Young seedlings can not adapt to such rapid fluctuations.
2) In cold stores nursery stock can be maintained in an inactive condition: a prerequisite for successful planting. The planting season may thus be lengthened into April and May. By then, plants left in outdoor beds will have resumed active growth and be no longer suitable to plant.
3) The consequence of the recent trend towards increased centralisation and large-scaled nurseries is that each nursery provides plants for a wide area. This leads to situations in which forest conditions are suitable for planting, but the conditions at the nursery are not suitable for lifting; the ground could be waterlogged, frozen or under snow. This problem is even more acute when forest tree seedlings are exported. A reservoir of plants in cold storage ensures plant supply.

Indoor storage has therefore become common practice, but poses a new dilemma for nurserymen. Efficient management requires that the handling of seedlings, such as transfer to cold storage, be carried out at the earliest possible time. However, lifting and storage of insufficiently hardened plants reduces vitality and may lead to cold damage, dehydration and fungal infection. To prevent this kind of damage, and its adverse economic effects on nurseries and end-users, it is of vital importance to be able to determine accurately when the seedlings have reached the peak physiological condition for lifting or transfer.
Despite the economic importance of such decisions, nurserymen still predominantly rely on traditional (morphological) methods to identify this moment. Recently, several physiological measurement techniques have been proposed, and some of them have been used operationally [8,14,15,18]. However, the number of nurseries in all Europe utilising these techniques is limited, because the methods are either unreliable, labour intensive or technically complicated and a minimum test period of 2 or 3 days is required. In nursery practice, where lifting opportunities can be severely limited by rainfall, frost and snow, a two-day delay may significantly reduce the number of plants lifted at peak physiological condition.

In addition to the onset of dormancy, dormancy release is also of economic importance. If plants growing out in the nurseries are put into cold storage too late in spring, they show evidence of damage, particularly to the root system. In spring, plants start reversing the processes that protect them during winter before there is a visible sign of regrowth [17,18]. So, efficient forestation and cost-effective nursery management require tools for rapid and reliable determination of the physiological condition of forest tree seedlings. To develop such tools, a thorough understanding of the cellular and molecular processes underlying cold hardiness is required. Unravelling the gene expression pattern as a seedling acquires the hardened state will reveal key processes that can be used as landmarks to describe the physiological condition of the tree. Eventually, this will result in molecular tests based on the presence or absence of specific messenger RNA's or proteins, that will allow a rapid evaluation of the physiological state and will facilitate forestation logistics. Such 'techniques of tomorrow' are not yet available to forest tree nurseries and in this respect the forestry sector lags behind on horticulture and agriculture.

Winter hardiness is closely correlated to endodormancy, the state of inactivity in which plants spend the winter. From a biological point of view winter buds, just like seeds, are a means to overcome hostile environmental conditions: the influence of climatic conditions on the process is strong. Not much is known on the molecular nature of the intertwined processes of dormancy and hardiness in woody species. In order to identify molecular mechanisms involved in winter hardening in woody plants we will employ the powerful technology of cDNA micro-arrays, also known as DNA chips, which has recently been developed and allows the monitoring of thousands of mRNA's simultaneously [10,12]. The technique will be used to detect transcripts characteristic for the dormant or active phase in Scots pine *(Pinus sylvestris)* and common beech *(Fagus sylvatica)*. These two economically important forest trees were chosen as model species to represent the coniferous and deciduous European trees. Relevant mRNAs will be selected and characterised to unravel molecular pathways involved in the process of hardening. Seedlings will be grown in climate-controlled environments for the initial identification of relevant genes. Outdoor trials will be performed to detect the effect of climatic conditions, geographical position and provenance on the underlying molecular processes. Plant material (buds, roots, needles) collected during these trials will be analysed both physiologically and for gene expression, employing cDNA microarrays and PCR technology. Together, these data will allow the creation of a detailed picture of molecular events involved in the onset and release of cold hardiness and dormancy and the effect of environmental factors on these processes. Furthermore, the research will result in a selected set of genes with a strong predictive value for cold acclimation in the tested plant species. These genes can be used for the future development of a rapid hardiness test that will support nursery management decisions and facilitate forestation logistics.

Summarising, our objectives are:

- *To identify* genes and molecular pathways involved in the onset and release of winter hardiness and dormancy in woody species using cDNA microarrays and to postulate a conceptual model describing the molecular events underlying these processes.
- *To select* a set of key genes, of which the expression patterns can be used to describe the various stages of dormancy and hardiness,
- *To evaluate* the merits of these key genes as molecular diagnostic tool for nursery practice and improved forestation.

B4. Contribution to programme/specific action objectives

The European forestry sector lags behind agriculture and horticulture in the application of new technologies for solving practical problems and in order to obtain better insight in the molecular and cellular processes underlying those problems. The present project aims at making up for those arrears, which negatively influence the competitiveness of European forestry companies (**general aim Key-Action 5**).
Molecular monitoring of forest tree seedling quality will aid in the development of improved forestation and planning techniques. Adequate tools for predicting and measuring the physiological condition of tree seedlings will facilitate the management of forest tree nurseries. Efficiently operating nurseries will ensure a steady supply of high quality forest tree seedlings, which is required for the sustained-yield use of commercially exploited forests and cost-effective reforestation. (**Key Action 5.3**).
At present, it is not uncommon for 25% of the seedlings in new plantations to die. Poor establishment is often caused by frost damage or desiccation, during storage of insufficiently hardened plants. Therefore, better characterisation of the seedling level of cold hardiness will also ensure an enhanced quality of planting stock (**Key-Action 5.1**).
Apart from these applied results, the present project will also enhance our knowledge of the fundamental cellular and molecular processes underlying winter dormancy and cold hardiness development in woody species. The cDNA microarray technology provides a new experimental tool for global searches on the function of genes, and is just starting to penetrate the field of plant molecular biology. The technique is pre-eminently suited for the unravelling of functional networks and interlinked molecular pathways that determine complex physiological processes. The present project will allow for the correlation of functional and genomic data concerning bud dormancy and cold tolerance (**Generic Activity 8.2**).

B5. Innovation aspects

State of the art

While the physiological stages of induction, completion and release of the interlinked processes of cold hardiness and dormancy in woody species are well-described [6,16,22], up to date information on the effect of different climatic regimes on hardiness development in woody plants is scarce. In general, shortening of daylength triggers the onset of dormancy, but the development of cold tolerance requires the extra stimulus of exposure to low temperature [21]. Prolonged exposure to gradually lower temperatures increases the tolerance of plants during winter. Different parts of the plant acquire different levels of cold hardiness: the shoot is generally more resistant than the root system. Most plants require a certain period of cold (the coldsum) before they are able to resume growth. At this stage the plants pass from endodormancy into ecodormancy; the plant is still quiescent, but on 'stand-by'. Favourable environmental conditions will trigger active growth. Evidence is accumulating that already during ecodormancy plants begin to loose part of their winter hardiness. Actively growing plants are no longer cold resistant [13].

Molecular data on the events that constitute dormancy and winter hardiness in trees are very limited. Research in this field has mostly been done on herbaceous plants [13]. Recent investigations show that the onset of dormancy is accompanied by an increase in the concentration of the plant hormone ABA [4,19,20,26]. This hormone is also involved in seed dormancy and in protection against desiccation by triggering the expression of defence genes. From a biological point of view winter buds, just as seeds, are a means to overcome environmental stress conditions of various nature, such as cold and drought. This fits well with recent findings, including our own, that general defence genes, such as dehydrin and active oxygen scavengers, are constitutively expressed in dormant buds [1,2,5,9,11,23,25,27]. Dormancy release is marked by the enhanced expression of cell division related proteins, such as β-tubulin [3]. There are indications that some of the events that accompany the release of seed dormancy are also important in the resumption of growth after winter dormancy. Though a considerable number of genes related to cold tolerance have been identified in herbaceous species [13] not much is known on the molecular genetics of hardiness in trees. In addition, the separation between dormancy and hardiness is usually unclear: the picture is far from complete. For instance, the effect of varying climatic conditions on the molecular events related to the preparation for winter is largely unknown.

Expected innovations

In order to describe and ultimately manipulate the development of cold hardiness in woody species it is necessary to gain extensive information on the molecular events underlying this process. Such insights can only be gained by linking genetic expression profiles to biological function. Using standard molecular biological techniques, which allow for the monitoring of five to ten genes simultaneously, the molecular unravelling of a largely unexplored mechanism such as hardiness, would be extremely laborious and time consuming. However, with the recently developed cDNA microarrays or gene-chips it has become possible to analyse the expression of thousands of genes in one assay [10,12]. Using cDNA microarrays the sequential changes in gene expression over time can be followed as the cell reacts to external or internal stimuli. That in turn will reveal key genes or pathways that can be used as indicators for the physiological status of the organism.

Microarrays are made by positioning tiny droplets of solubilised cDNA, each representing a gene, in an ordered pattern on a glass slide or nylon membrane. This is done by a robotic device, based on ink-jet technology, with a resolution of several thousands of DNA fragments per square cm. The DNA chip is challenged with fluorescently labelled (anonymous) DNA or RNA, derived from the tissue to be tested. The fragments on the chip function as highly accurate molecular tweezers, gripping only those pieces of DNA that are an exact match to their own sequence. Matching fragments are retained on the chip; the rest is washed away. Subsequent analysis of the fluorescence pattern will reveal what transcripts were present in the sample. Probing the chip with RNA derived from, for example, a tissue sample taken from a plant that has been subjected to low temperature, will reveal genes that respond to cold stress. Some will show an enhanced expression, others will be down regulated. After identification of the genes involved, an overall picture will emerge of how the organism deals with low temperatures. Adaptation processes can be studied by examining samples taken at different time points, after different climatic regimes or from stress treatments with increasing severity.

Improved knowledge of the responsible cellular mechanisms will allow the development of accurate diagnostic tools and methods to manipulate the physiological path towards the acquisition of stress tolerance (hardening). Such tools will support management decisions and facilitate forestation planning and nursery logistics. In contrast to current tests, a molecular assay will provide nurserymen and forest managers with a fast result; depending on the type of test only one hour to one day will be required. As a first step towards implementation and to allow for in depth gene expression analysis of a few selected key genes the novel Molecular Beacon markers [24] will be employed in a RT-PCR based assay, for which the protocol will be developed during the project. Using the test, nursery managers will be able to respond quickly to changing weather conditions. In addition the research will provide molecular markers characteristic for the different phases of winter dormancy and cold tolerance, that can be used as starting point for marker aided breeding, for future improvement of planting stock via genetic modification or for certification.

In the next decade, DNA microarrays are expected to force a breakthrough in functional genomics. Pre-made gene-chips are already used in the medical sector as a diagnostic tool to determine many characteristics of an infectious agent or tumour in one test. However, the new technique has hardly penetrated the field of plant biotechnology. To understand how genes work, they must be compared in many individuals at different times of life and under different conditions. Doing such comparisons by the thousand is simply not possible with the current gene-analysing techniques - it requires a high throughput technique such as the DNA chip. In 1998 partner 2 was amongst the first European Research institutes to employ the new technology.

B6. Project workplan

Introduction:
To allow for the identification of key genes and pathways involved in the onset, development and release of winter dormancy and stress tolerance in economically and ecologically important woody species, the extremely powerful technology of cDNA micro-arrays will be employed. The arrays enable the monitoring of thousands of mRNA species simultaneously. The technique can be used to detect specific or general mRNAs characteristic for dormancy or hardiness. Relevant mRNAs will be selected and characterised to reveal molecular pathways involved in the process of hardening.

Outline
The workplan is designed around two economically important forest tree species: *Pinus sylvestris*, the primary model, and *Fagus sylvatica,* the secondary model. Two models have been chosen to have both coniferous and broad-leaved species represented. Pine mRNA, derived from seedlings grown under controlled conditions, will be used for screening a cDNA microarray that will carry clones from a cDNA expression library, made from dormant pine buds. The array will be supplemented with conserved genes from other species that are expected to be of importance in the development of bud dormancy; such as *Arabidopsis* cell cycle genes and dehydrin genes. To identify relevant clones, the expression information will be compared with data derived from thorough physiological and morphological analysis of the seedlings. The selected clones will be sequenced and homologous genes from beech will be isolated and checked against beech mRNA, also derived from trees cultured in controlled environments. For rapid detection of the selected genes RT-PCR assays will be developed. Employing quantitative detection (via real-time monitoring of accumulating fluorescence) these assays will be used to obtain detailed information on the expression profile of the selected genes in field conditions.

Scots pine and common beech will be grown in climate rooms with three different regimes - decreasing daylength, decreasing temperature and control (both constant)- in order to discriminate between the processes of dormancy and hardiness development as much as possible. Dormancy is thought to be triggered by shortening of daylight, while cold tolerance needs the (additional) trigger of low temperature. The trials will be sampled at regular intervals for physiological and morphological determination of the level of dormancy and hardiness and for mRNA isolation. The next season these data will be extended with the results from climate room trials aimed at investigating the combined effect of daylength reduction and fluctuating temperature.
In outdoor field trials two provenances of the model species will be subjected to various growing conditions in order to detect the effect on dormancy and cold hardiness development and on the molecular events determining those processes. Effective functional genomics -the coupling of genes to biological function- requires analysis of gene expression under many different conditions. Therefore, the outdoor trials will be aimed at the production of a broad range of plant material, covering different growing conditions. Special emphasis will be given to the effects of provenance, climate and seedling age. These factors are known to influence dormancy/hardiness development and will be studied in additional trials. Plant material (buds, roots, leaves) derived from the regular sampling of all trials will be analysed physiologically, morphologically and molecularly.

Data obtained from this multidisciplinary approach will be used to assemble an integrated database that will reveal a detailed picture of the molecular events involved in the onset and release of winter dormancy and hardiness and its interaction with environmental factors. A set of genes with a strong predictive value in the tested plant species will be selected for future development of highly accurate and rapid hardiness and dormancy tests based on mRNA or the corresponding proteins. As a first step towards implementation of the results in nursery practice the predicitve value of the selected marker genes on plant material cultivated at commercial nurseries will be evaluated. The link with nursery practice is strengthened even further by the assessment of the correlation of gene expression profiles and performance of pine seedlings in cold storage.

Description of the work:

The present proposal describes a four-year project, with the assumed starting date March 2001. Since field experiments can only be performed in autumn and winter this will allow for three trials. In the first and second trial season, climate room experiments will be performed. Controlled field trials will be carried out in year 1, 2, and 3. Repetition is necessary to discriminate between genes reproducibly involved in dormancy and hardiness development, and noise caused by the fluctuating environmental conditions in outdoor trials. The various field trials will produce data on the influence of climatic and geographical conditions on the expression of the key genes. Additonally, in the second and third season the use of selected key genes as a molecular diagnostic tool for the determination of hardiness or dormancy status in commercial settings, will be evaluated.

The work in this project can be divided into three phases.
In the first phase, a two-year period, screening of a cDNA microarray will result in a set of 20-30 *Pinus* transcripts representing putative dormancy or hardiness genes. The clones will be sequenced and characterised. Beech homologues to these pine genes will be isolated, sequenced, and characterised. For all selected genes RT-PCR-primers will be designed.
In the second phase, that may start while the first phase is still going on and will take 18 months, the transcriptional regulation of the selected genes will be analysed in more detail in different organs and various environmental conditions, using quantitative RT-PCR assays and fluorescent detection. Correlations will be studied between gene expression, climatic conditions, geographical position, provenance and physiological parameters, such as root/shoot electrolyte leakage, root/shoot growth potential, electrical conductivity of the stem and frost tolerance. This will reveal a subset of key genes that can be used as accurate and general molecular descriptors for dormancy and hardiness.
In the third phase, that will take one year, all data will be combined and used for the definition of a general hypothesis on the molecular events underlying the onset and development of dormancy and cold hardiness in woody species. Additionally, in this phase the operational evaluation of the selected markers in a commercial setting will be undertaken.

Kick-off
The kick-off meeting of the project will be combined with a Molecular Analysis Workshop in which all partners will be trained in RNA-isolation and RT-PCR, according to standard protocols. Also, a standard protocol for sampling the plant material will be agreed upon during this workshop. This is necessary to avoid deviant results caused by differences in

laboratory practice. Especially the RNA used for screening the microarray should be of uniform, high quality.

Phase 1

As a first step in the identification of dormancy and cold tolerance related transcripts climate room experiments will be conducted by partners 4 and 7, aimed at the production of pine and beech seedlings in which the processes of dormancy and cold tolerance development are separated. To this end three climate regimes will be used:

- constant daylength and decreasing temperature
- constant temperature and decreasing daylength
- constant growth-permissive temperature and constant daylength (control)

Partner 4 will focus on beech and partner 7 on pine, but in both cases the experimental set-up will be the same, except for the daylength and temperature values that will differ for pine and beech. In the second trial season climate room experiments will be used for assessing the combined effect of decreasing and fluctuating temperature and decreasing daylength. In a series of regimes, natural occuring fluctuations in temperature will be mimicked. The experiments will last from the beginning of September until the beginning of January. The number of sampling dates will be eight, the first date will be in the beginning of September. If room available, additional sampling data can be incorporated in the individual experiments. Each partner will do at least one dormancy test (RGP and/or terminal budbreak) and one hardiness test. (frost tolerance, REL/SEL of excised root and shoot parts). The assays will be performed with 4 replicates, each sample consisting of material from 5 different plants. Thus, at each sampling date 20 different plants will be analysed. Regrowth will be monitored in randomised trials consisting of 5 plots, of 10 plants each. Results will be analysed statistically using analysis of variance calculations

For analysis of gene expression the partners will prepare total RNA from bud and root samples taken at each time point, according to a common protocol. The RNA will be dissolved in a buffer containing 0.1% SDS, to ensure stability, and shipped to partner 2, who will use it for the preparation of microarray probe.

Concurrently, partner 1 will pre-select 500 putative dormancy related genes from a pine cDNA expression library via differential cold-plaque screening and isolate the corresponding inserts. Partner 2 will use the 500 cDNAs, several known conserved genes and a set of controls, to construct a cDNA microarray, for the analysis of gene expression in pine seedlings derived from the climate room experiment described above. A total of 100 independent pine samples will be used to challenge the microarray. In addition, 5 beech mRNA samples will be analysed to test homology, with respect to sequence and expression pattern. From each sample two probes will be made, using different fluorescent dyes. The screenings data will be analysed in comparison to the physiological data obtained in the climate room trials to identify a set of 20 - 30 transcripts presumably involved in either dormancy, frost hardiness, or both. The selected pine transcripts will be sequenced and characterised and the information will be used to isolate homologous genes from the beech genome. This task will be performed co-operatively by partners 1 and 2.

Phase 2

As soon as sequence information from the selected pine and beech transcripts comes available, it will be used to design PCR primers for the development of RT-PCR assays with fluorescent detection markers (Molecular Beacon, Taqman). For detailed expression analyses of a limited number of genes, microarrays are less suitable, because the costs per analysed

gene become too high. The PCR-based assays allow for the rapid and reliable detection of specific gene expression and will be carried out by all partners to gain in-depth information on the expressional regulation of the selected genes in plants grown in a variety of environmental conditions. For these analyses field trials, using multiple provenances from pine and beech, will be performed in three subsequent trial seasons, by partners 4 and 7 and their assistants. The replication (both in time and in location) is necessary to be able to identify genes whose expression is correlated to the traits of interest, independent of climatic variance, environmental conditions or geographic position. Field trials will mostly be done in the second and third trial season, but some initial experiments will already start in year 1. Most outdoor trials will be carried out using containerised seedlings to allow comparison with the controlled environment experiments. Additionally, bare-rooted plants will suffer damage to the rootsystem when lifted and this might compromise the results. Assessment of dormancy and hardiness development will start in September and proceed until January. Individual experiments may proceed until spring to evaluate dormancy release as well. In general, samples will be taken every two weeks for physiological analyses and regrowth assessment as described for the climate room trials, and for RNA isolation. In RNA samples the presence of relevant transcripts will be monitored using PCR assays. Part of the RNA will be used for detailed expression studies using quantitative PCR. Eventually, these data will aid in the selection of about ten key genes that are descriptive of the physiological condition of pine and beech with respect to dormancy and cold hardiness. Additionally, the development of PCR-assays is a first step towards implementation of the knowledge gained within the project in the development of an operational test.

Phase 3

The key genes selected in phase 2 will be evaluated in the practical setting of forest tree nurseries. This task will be performed by partners 3 and 5, for pine and beech respectively. In addition, partner 3 will investigate the predicitve value of the selected genes for cold storage performance. These trials will reveal whether the future development of molecular diagnostic tests for hardiness and dormancy, based on the expression of these genes, is feasible.

All data derived from the physiological and morphological analysis of the seedlings will be collected by partner 7 and combined in one database. The expression information for each spotted clone, derived from the screenings of the microarray, will be stored and made accessible using specialised software. Specific adaptations will be made to allow linkage of physiological, expressional, functional, and sequence information into an integrated and searchable database.

This combination of data in the final project phase will lead to a profound insight in the molecular pathways involved in the onset and release of winter hardening in *Pinus sylvestris* and *Fagus sylvatica*. Information on the influence of climate, environment and provenance on the expression of the genes concerned will become available, and will contribute to the definition of a general hypothesis on the molecular events underlying the onset and development of dormancy and cold hardiness in woody species.

Finally, the third phase will also be used to communicate the project results to the sector, via a demonstration workshop for nurserymen. In this workshop the main focus will be on the significance of the results for facilitating nursery management.

Project planning

Tasks and milestones

The project planning is laid down in a Gantt chart and a corresponding tasks list (Table 1), which together give an overview of the time scheduling within the project. The assumed start date of the project is March 2001.

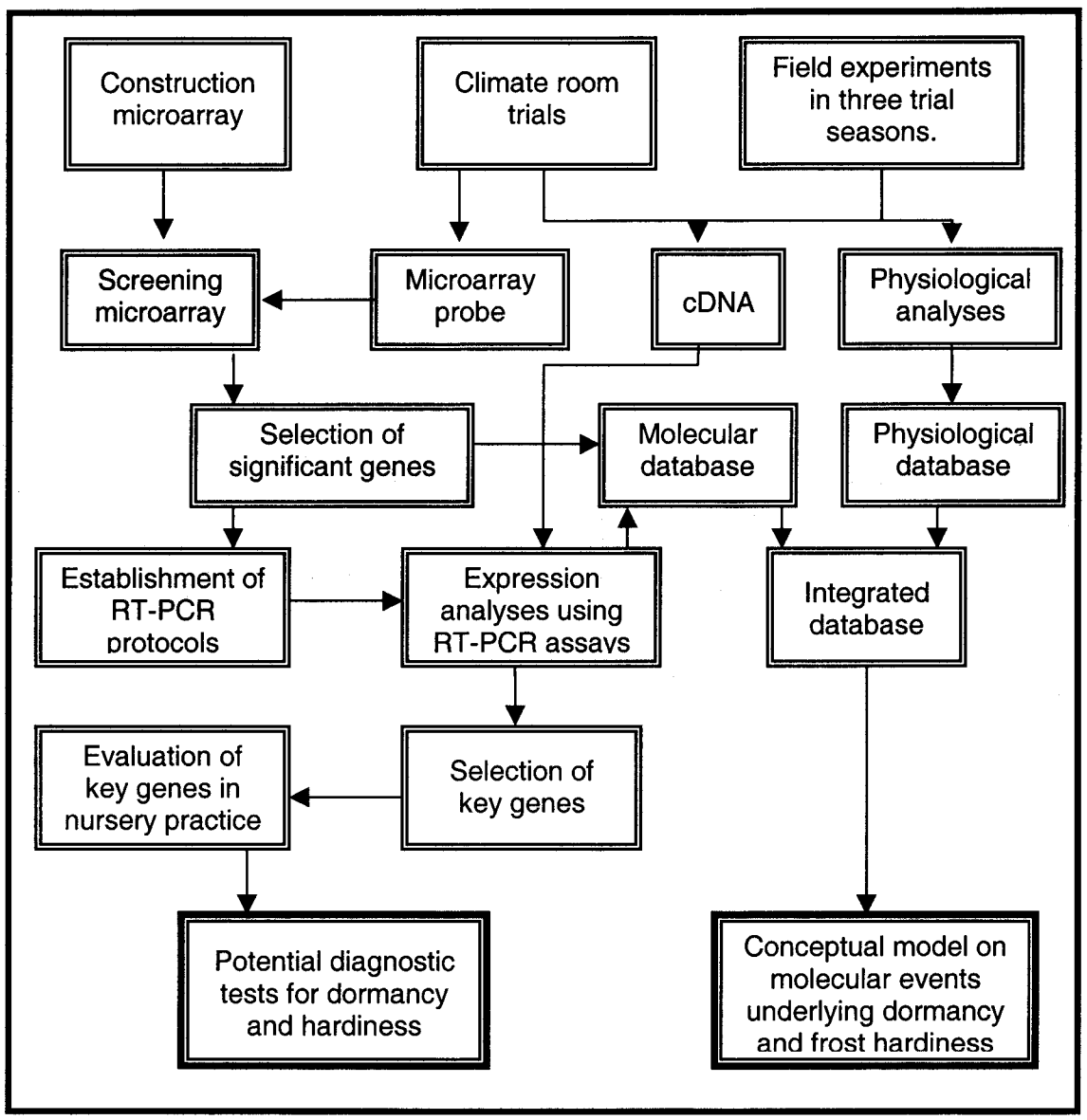
Table 1. List of Tasks and Milestones

Task number	Start month	Task name	Special identifiers
1	0	**Workshop Molecular techniques**	
		Climate room experiments pine	WP1
2	0	Pine trials year 1: day vs. temp	
3	6	Physiological testing and regrowth	
4	6	RNA isolation	
5	12	Pine trials year 2: temp. effect	
6	18	Physiological testing and regrowth	
7	22	RNA isolation	
		Climate room experiments beech	WP2
8	0	Beech trials year 1: day vs. temp	
9	6	Physiological testing and regrowth	
10	6	RNA isolation	
11	12	Beech trials year 2: temp. effect	
12	18	Physiological testing and regrowth	
13	22	RNA isolation	
		Field experiments pine	WP3
14	0	Outdoor trials pine yr1	
15	6	Physiological testing and regrowth	
16	12	RNA isolation	
17	12	Outdoor trials pine yr2	
18	18	Physiological testing and regrowth	
19	24	RNA isolation	
20	24	Outdoor trials pine yr3	
21	30	Physiological testing and regrowth	
22	36	RNA isolation	
		Field experiments beech	WP4
23	0	Outdoor trials beech yr1	
24	6	Physiological testing and regrowth	
25	12	RNA isolation	
26	12	Outdoor trials beech yr2	
27	18	Physiological testing and regrowth	
28	24	RNA isolation	
29	24	Outdoor trials beech yr3	
30	30	Physiological testing and regrowth	
31	36	RNA isolation	
		Microarray	WP5
32	0	Differential screening cDNA library	
33	2	Assembling microarray	
34	3	Adaptation microarray software	
35	8	Screening microarray	
36	12	Analysis screeningsdata/selection relevant genes	
	20	*Initial set of relevant pine genes selected*	MILESTONE 1

		Beech homologues	WP6
37	1	Construction beech cDNA library	
38	11	Selection homologous genes	
39	13	Checking expression pattern against climate room material	
	23	*Initial set of relevant beech genes selected*	MILESTONE 2
		RT-PCR assays	WP7
40	20	Development RT-PCR assay for key genes	
41	24	Application of RT-PCR for detailed expression analysis	
	38	*Subset of pine and beech key genes selected*	MILESTONE 3
		Effect of provenance, age and climate	WP8
42	0	Sowing three pine and beech provenances, yr1	
43	12	Sowing three pine and beech provenances, yr2	
44	18	First Physiological evaluation of provenance,age and climate effect	
45	24	Sowing three pine and beech provenances, yr 3	
46	30	Physiological evaluation of provenance, age and climate effect	
47	30	Sampling pine and beech from commercial nurseries	
48	30	Physiological evaluation of nursery effect	
49	38	Testing for presence of key genes via RT-PCR	
		Prediction of storage performance	WP9
50	18	Storage experiments pine, first season	
51	18	Physiological testing and regrowth, first season	
52	30	Storage experiments pine, second season	
53	30	Physiological testing and regrowth, second season	
54	38	Testing for presence of key genes via RT-PCR	
	43	*Usefulness of key genes as molecular diagnostic tests evaluated*	MILESTONE 4
55	44	Demonstration workshop	
		Integrated database	WP10
56	39	Assembly physiological database	
57	38	Assembly molecular database	
58	41	Integration of databases	
	43	*Integrated database available*	MILESTONE 5
59	43	Definition of conceptual model on molecular processes underlying dormancy/hardiness	
	46	*Conceptual model defined*	MILESTONE 6
60	48	REPORTING	

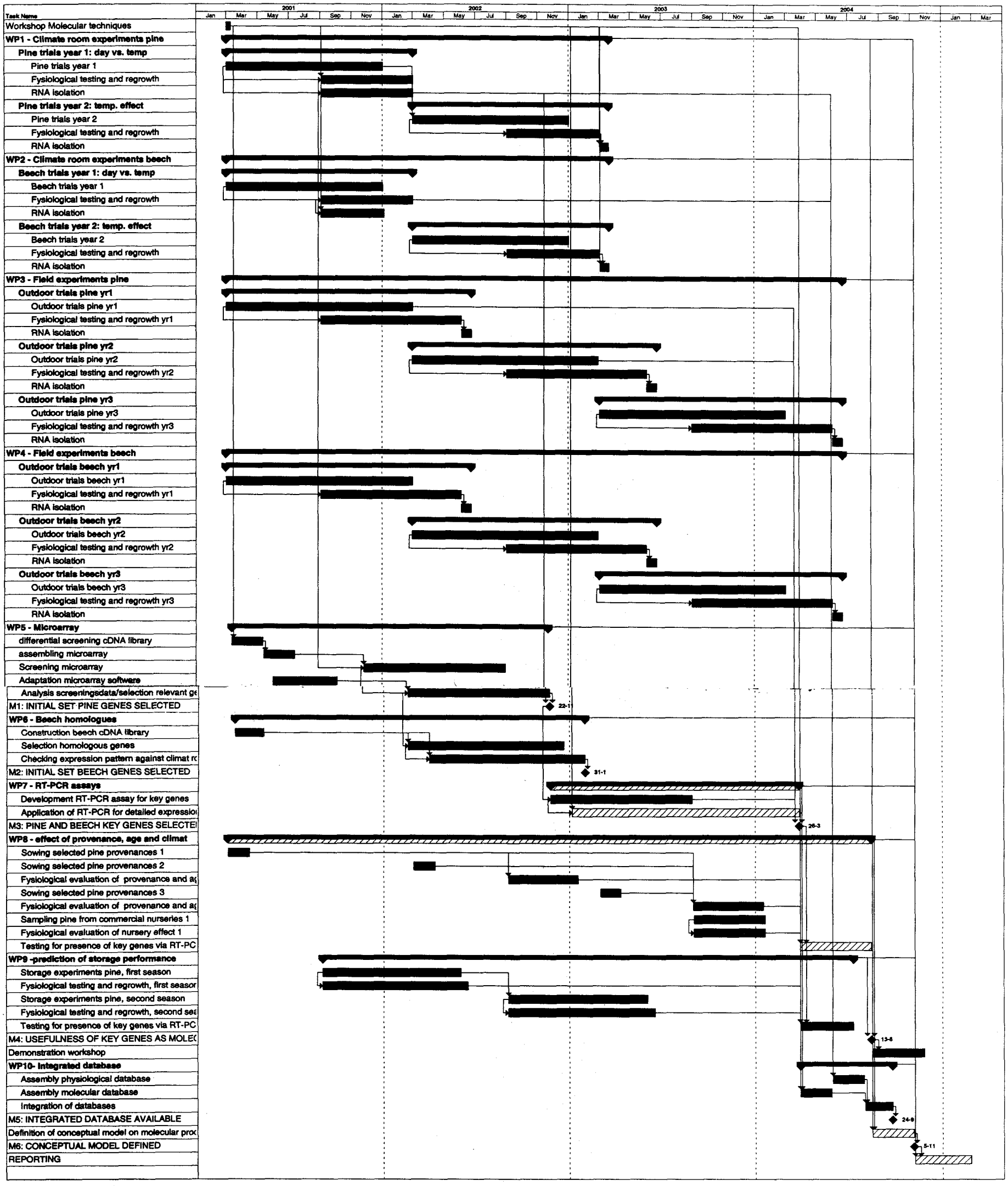
Pert Diagram

The Pert diagram below shows the relations between the tasks in the present project. Only global tasks are mentioned; a detailed enumeration of tasks is given in Table 1.



Gantt Chart

The course of the project in time is represented in the Gantt chart below. The tasks and milestones are also described in Table 1. Bold is used to identify workpackages, capitals indicate a milestone.



Detailed project description broken down in workpackages.
The project is divided in 10 workpackages, listed below and described in detail in WP2 forms.
The assumed start date of the project is March 2001 (Month 0)..

WP1.	Workpackage list

Work-package No.	Workpackage title	Responsible participant N°.	Person months	Start month	End month	Deliverables N°.
WP1	Climate chamber experiments pine	7	22	0	24	DL1, DL2, DL4, DL24, DL25
WP2	Climate chamber experiments beech	4	24	0	24	DL3, DL4, DL24, DL25
WP3	Field experiments pine	7	35	0	38	DL4, DL5, DL24 DL25
WP4	Field experiments beech	4	27	0	38	DL4, DL6, DL24, DL25
WP5	Production and screening of a pine cDNA microarray	2	28	0	20	DL7, DL8, DL9, DL10, DL24, DL25
WP6	Isolation of beech homologues to the selected pine genes	1	23	1	23	DL11, DL12, DL24, DL25
WP7	Development and application of RT-PCR assays	1	41	20	38	DL13, DL14, DL15, DL24, DL25
WP8	The effect of provenance, climate and age	5	23	0	43	DL16, DL18, DL23, DL24, DL25
WP9	Prediction of storage performance	3	12	18	43	DL17, DL18, DL23, DL24, DL25
WP10	Set up of an integrated physiological and molecular database	7 and 2	20	39	46	DL19, DL20, DL21, DL22, DL24, DL25
	TOTAL		255			

Deliverables
The project will yield 25 distinct deliverables, listed below. These results vary from publications and conceptual models to items such as genes and primers. The delivery date is given as a month in the course of the project, in which the starting month is 0.

DL.	Deliverables list

Deliverable N°	Deliverable title	Delivery date	Nature	Dissemination level
DL1	RNA for preparing microarray probe	12,24	O	CO
DL2	Physiological data on dormancy and hardiness development in pine in controlled environments	12,24	O	CO

DL3	Physiological data on dormancy and hardiness development in beech in controlled environments	12,24	O	CO
DL4	RNA for RT-PCR	12,24,36	O	CO
DL5	Physiological data on dormancy and hardiness development in pine in outdoor situations	12,24,36	O	CO
DL6	Physiological data on dormancy and hardiness development in beech in outdoor situations	12,24,36	O	CO
DL7	cDNA microarray carrying dormancy related pine cDNAs and relevant conserved genes from other species	4	O	CO
DL8	Expression profiles during dormancy and hardiness development of the clones on the microarray	20	O	CO
DL9	Set of ca. 30 genes whose expression is indicative for dormancy or hardiness in pine	20	O	CO
DL10	Primers for PCR amplification of ca. 30 genes whose expression is indicative for dormancy or hardiness in pine	20	O	CO
DL11	Set of ca. 30 genes whose expression is indicative for dormancy or hardiness in beech	23	O	CO
DL12	Primers for PCR amplification of ca. 30 genes whose expression is indicative for dormancy or hardiness in beech	23	O	CO
DL13	RT-PCR protocols for the amplification of dormancy/hardiness related genes from pine or beech	30	O	CO
DL14	In depth expressional information on ca. 60 genes from pine and beech, in relation to dormancy and hardiness development in varying environmental conditions	38	O	CO
DL15	A subset of ca. 15 highly informative key genes, descriptive of the physiological state of the tree seedling with respect to dormancy and frost hardiness	38	O	CO
DL16	Information on the influence of provenance, climatic conditions and age on the expression of dormancy/hardiness related genes in pine and beech	43	O	CO
DL17	An assessment of the usefulness of the selected key genes as predictors for performance of pine seedlings during cold storage.	43	O	CO
DL18	An assessment of the use of the selected key genes as molecular diagnostic tool to predict the physiological state of tree seedlings in a commercial setting	43	O	CO
DL19	A physiological database combining all information on growth conditions and physiological parameters obtained during the project	41	O	CO
DL20	A molecular database combining all expressional and sequence data, including PCR primers, obtained during the project	41	O	CO
DL21	An integrated searchable database combining the physiological and molecular databases	43	O	CO
DL22	A conceptual model describing the molecular events and pathways underlying the development of winter dormancy and frost hardiness in pine and beech seedlings.	46	O	CO
DL23	Trade demonstration workshop aimed at communication of the results to the forestry sector (nurserymen, foresters)	46	D	PU
DL24	Scientific publications in peer-reviewed journals	Several	R	PU
DL25	Publications in trade journals	Several	R	PU

WP2. Climate room experiments using pine

Workpackage number: WP1
Start date or starting event: Start of project
N° of the partner responsible: 7
N°s of other partners involved:
Person-months per partner: partner 7: 22 months

Objectives

- To assess seedling response to the two prime environmental factors of daylength and temperature, particularly fluctuating temperature
- To produce, by manipulating daylength and temperature in an otherwise constant environment, pine seedlings at various stages of dormancy and frost hardiness. The plant material will be subjected to thorough physiological analyses and will be used for preparing microarray probe and RT-PCR template.

Description of work

Climate chamber experiments in the first trial season will be designed to separate the processes of dormancy and frost hardiness development as far as possible, to allow for the selection of genes specific for the two phenomena. The experiments will be performed with the *P. sylvestris* provenance A70, derived from a Brittish seed orchard which is composed of tested clones with good progeny performance.

Set-up: Three climate regimes will be applied:

1. Fixed daylength: 16 h. Decreasing temp: 15 -> 5 °C
2. Fixed temperature 15°C,. Decreasing daylength: 16 h -> 6 h
3. Fixed daylength: 16 h. Fixed temperature: 15 °C.

The second trial season will be devoted to the interaction of temperature and daylength.

Set-up: Five climate regimes will be applied:

1. Decreasing daylength: 16 h -> 6 h. Decreasing temp: 15 -> 5 °C
2. As 1, but with an intermittent cold spell of 1 week starting week 40
3. As 1 but with an intermittent cold spell of 2 weeks starting week 40
4. As 1 but with an intermittent warm spell of 1 week starting week 46
5. As 1, but with an intermittent warm spell of 2 weeks starting week 46

Sampling dates will be in week number 34, 36, 40, 41,42, 43, 46,47, 48, 49 and 52. Physiological tests will be aimed at evaluating dormancy depth (RGP and/or terminal budbreak) and hardiness status (frost tolerance, REL/SEL of excised root and shoot parts). The assays will be performed with 4 replicates, each sample consisting of material from 5 different plants. Thus, at each sampling date 20 different plants will be analysed. Regrowth will be monitored in randomized trials consisting of 5 plots, of 10 plants each. Results will be analysed statistically using analysis of variance calculations. For analysis of gene expression the partners will prepare total RNA from bud and root samples taken at each time point, according to a common protocol.

Deliverables

DL1 RNA for preparing microarray probe
DL2 Physiological data on dormancy and hardiness development in pine in controlled environments
DL4 RNA for RT-PCR
DL24 Scientific publications in peer-reviewed journals
DL25 Publications in trade journals

Milestones and expected results

This workpackage will yield physiologically well described pine seedlings at various stages of dormancy and hardiness, of which RNA can be used for screening the microarray, and as RT-PCR template for selecting relevant genes.

Relevant milestone:

M 1 Initial set of relevant pine genes selected

WP2. Climate room experiments using beech

Workpackage number: WP2
Start date or starting event: Start of project
N° of the partner responsible: 4
N°s of other partners involved:
Person-months per partner: partner 4: 24 months

Objectives

- To assess seedling response to the two prime environmental factors of daylength and temperature, particularly fluctuating temperature
- To produce, by manipulating daylength and temperature in an otherwise constant environment, pine seedlings at various stages of dormancy and frost hardiness. The plant material will be subjected to thorough physiological analyses and will be used for preparing microarray probe and RT-PCR template.

Description of work

Climate chamber experiments in the first trial season will be designed to separate the processes of dormancy and frost hardiness development as far as possible, to allow for the selection of genes specific for the two phenomena. The experiments will be performed with the romanian *F.sylvatica* provenance Maramures.

Set-up: Three climate regimes will be applied:

4. Fixed daylength: 14 h. Decreasing temp: 15 -> 0 °C
5. Fixed temperature 15°C,. Decreasing daylength: 14 h -> 6 h
6. Fixed daylength: 14 h. Fixed temperature: 15 °C.

The second trial season will be devoted to the interaction of temperature and daylength.

Set-up: Five climate regimes will be applied:

1. Decreasing daylength: 14 h -> 6 h. Decreasing temp: 15 -> 0 °C
2. As 1, but with an intermittent cold spell of 1 week starting week 40
3. As 1, but with an intermittent cold spell of 2 weeks starting week 40
4. As 1, but with an intermittent warm spell of 1 week starting week 46
5. As 1, but with an intermittent warm spell of 2 weeks starting week 46

Sampling dates will be in week number 34, 36, 40, 41, 42, 43, 46, 47, 48, 49,and 52. Physiological tests will be aimed at evaluating dormancy depth (RGP and/or terminal budbreak) and hardiness status (frost tolerance, REL/SEL of excised root and shoot parts). The assays will be performed with 4 replicates, each sample consisting of material from 5 different plants. Thus, at each sampling date 20 different plants will be analysed. Regrowth will be monitored in randomized trials consisting of 5 plots, of 10 plants each. Results will be analysed statistically using analysis of variance calculations. For analysis of gene expression the partners will prepare total RNA from bud and root samples taken at each time point, according to a common protocol.

Deliverables

DL1 RNA for preparing microarray probe
DL3 Physiological data on dormancy and hardiness development in beech in controlled environments
DL4 RNA for RT-PCR
DL24 Scientific publications in peer-reviewed journals
DL25 Publications in trade journals

Milestones and expected results

This workpackage will result in physiologically well described beech seedlings at various stages of dormancy and frost hardiness, of which RNA can be used for screening the microarray and as RT-PCR template for selecting relevant genes.

Relevant milestone:

M2 Initial set of relevant beech genes selected

WP2.	Field trials using pine

Workpackage number:	**WP3**
Start date or starting event:	**Start of project**
N° of the partner responsible:	**7**
N°s of other partners involved:	**4**
Person-months per partner:	**partner 4: 9 months, partner 7: 26 months**

Objectives

- To assess, in field trials, the effect of climatic conditions, geographical position and provenances on the development of dormancy and hardiness
- To produce *P. sylvestris* plant material for studying the expression of genes involved in these processes

Description of work

General trials, to be performed by partners 4 and 7:
Containerized pine seedlings, 1 or 2 year old, will be grown in field trials at two different locations in North-Western Europe. Both a standard provenance (the same as used in WP1) and a local one will be used. Samples will be lifted in bi-weekly intervals starting from September 1 until January. The samples will be subjected to physiological analyses, comparable to the analyses in WP1 and results will be processed using statistical methods. Additionally, the material will be used for the isolation of mRNA. Part of the RNA will be shipped to partner 1 and 2, who will use it for quantitative PCR. Partly the RNA will be used as a template for RT-PCR assays. The trials will be repeated three times to capture the effect of year to year climatic variation and to allow for the selection of genes that are least influenced by these fluctuations.

Specific trials, to be performed by individual partners:
Several other parameters will be assayed in additional small-scale field trials by individual partners. The trials are aimed at assessing the predictive value of selected genes in circumstances occuring in nursery practice, not covered by the general trial. Topics to be evaluated are: containerized vs. bare-root plants, effect of plant age on key gene expression, assessment of dormancy release, the effect of cold storage parameters, etc. The set-up of these trials, with respect to sampling and performed tests will be the same as for the general trials.

Deliverables

DL4	RNA for RT-PCR
DL5	Physiological data on dormancy and hardiness development in pine in outdoor situations
DL24	Scientific publications in peer-reviewed journals
DL25	Publications in trade journals

Milestones and expected results

This workpackage will produce physiologically well-described plant material of which RNA analysis will reveal the effect of climatic conditions, geographical position and provenances on the expression of a specific set of selected genes.
Relevant milestone:

M3	Subset of pine and beech key genes selected

WP2.	Field trials using beech

Workpackage number:	**WP4**
Start date or starting event:	**Start of project**
N° of the partner responsible:	**4**
N°s of other partners involved:	**6, 7**
Person-months per partner:	**partner 4: 15 months, partner 6: 8 months, partner 7: 9 months**

Objectives

- To assess, in field trials, the effect of climatic conditions, geographical position and provenances on the development of dormancy and hardiness.
- To produce *F. sylvatica* plant material for studying the expression of genes involved in these processes

Description of work

General trials, to be performed by partners 4 and 7:
Containerized beech seedlings, 1 or 2 year old, will be grown in field trials at two different locations in North-Western Europe. Both a standard provenance (the same as used in WP2) and a local one will be used. Samples will be lifted in bi-weekly intervals starting from September 1 until January. The samples will be subjected to physiological analyses, comparable to the analyses in WP1 and results will be processed using statistical methods. Additionally, the material will be used for the isolation of mRNA. Part of the RNA will be shipped to partner 1 and 2, who will use it for quantitative PCR. Partly the RNA will be used as a template for RT-PCR assays. The trials will be repeated three times to capture the effect of year to year climatic variation and to allow for the selection of genes that are least influenced by these fluctuations.

Specific trials, to be performed by individual partners:
Several other parameters will be assayed in additional small-scale field trials by individual partners. The trials are aimed at assessing the predictive value of selected genes in circumstances occuring in nursery practice, not covered by the general trial. Topics to be evaluated are: containerized vs. bare-root plants, effect of plant age on key gene expression, assessment of dormancy release, the effect of cold storage parameters, etc. The set-up of these trials, with respect to sampling and performed tests will be the same as for the general trials.

Deliverables

DL4	RNA for RT-PCR
DL6	Physiological data on dormancy and hardiness development in beech in outdoor situations
DL24	Scientific publications in peer-reviewed journals
DL25	Publications in trade journals

Milestones and expected results

This workpackage will produce physiologically well-described plant material of which RNA analysis will reveal the effect of climatic conditions, geographical position and provenances on the expression of a specific set of selected genes.
Relevant milestone:
M3 Subset of pine and beech key genes selected

WP2. Production and screening of a pine cDNA microarray

Workpackage number:	**WP5**
Start date or starting event:	**Start of project**
N° of the partner responsible:	**2**
N°s of other partners involved:	**1**
Person-months per partner:	**partner 2: 21 months, partner 1: 7 months**

Objectives

- Production and screening of a cDNA microarray carrying dormancy/hardiness related genes from *P. sylvestris*.
- Selection of genes involved in dormancy/frost hardiness development in pine.

Description of work

Partner 1 will perform an initial differential screening on a previously constructed cDNA expression library from dormant pine buds. Several hundreds of preselected clones will be used for the assembly of a cDNA microarray. Partner 2 will construct and screen the array, that will be supplemented with a number of genes from other species (*Arabidopsis*), presumably involved in dormancy and hardiness. Examples are: cell-cycle genes, dehydrins, ABA-regulated genes

The arrays will be screened with probes derived from pine seedlings grown under controlled conditions (WP1) to reduce the number of positives, due to non-specific variation. A total of 200 screenings will be performed, and each probe will be analysed twice, alternating the fluorescent dies. This results in the analysis of 100 independent samples, divided amongst the three climate chamber regimes and the 8 sampling moments. In addition five probes from beech will be used, to analyse homology and cloning feasibility.

In combination with the physiological data obtained in WP1, the expression analyses will be used to identify genes putatively involved in the processes of dormancy and cold hardiness in *Pinus sylvestris* seedlings, using statistically sound correlations.

Selected genes will be sequenced and characterized.

Deliverables

DL7	cDNA microarray carrying dormancy related pine cDNAs and relevant conserved genes from other species
DL8	Expression profiles during dormancy and hardiness development of the clones on the microarray
DL9	Set of ca. 30 genes whose expression is indicative for dormancy or hardiness in pine
DL10	Primers for PCR amplification of ca. 30 genes whose expression is indicative for dormancy or hardiness in pine
DL24	Scientific publications in peer-reviewed journals
DL25	Publications in trade journals

Milestones and expected results

This workpackage will result in the identification of *P. sylvestris* genes, whose expression is consistently correlated to the development of dormancy and/or frost hardiness

Relevant milestone:

M 1 Initial set of relevant pine genes selected

WP2. Identification and isolation of beech homologues

Workpackage number:	**WP6**
Start date or starting event:	**Identification of relevant pine genes**
N° of the partner responsible:	**1**
N°s of other partners involved:	**2**
Person-months per partner:	**Partner 1: 14 months, partner 2: 9 months**

Objectives

To identify *F. sylvatica* genes that are involved in dormancy and/or frost hardiness development by isolating homologues to the selected pine genes (M1) and verifying their expression pattern in RNA isolated from controlled environment grown beech seedlings.

Description of work

To allow for the isolation of beech genes, homologous to the pine genes selected in WP5, a cDNA library will be made from *F. sylvatica* dormant buds. Via non-stringent hybridisation, the library will be screened for the presence of homologues to the pine genes selected as relevant for dormancy and hardiness development.
In addition, PCR based strategies will be used when feasible. These will employ degenerate primers, based on the *Pinus* sequence to amplify the corresponding beech gene fragment. Isolated genes will be sequenced and characterized.
The expression pattern of the identified genes in beech will be checked against cDNA derived from the climate room experiments from WP2. This will reveal whether the isolated homologue is also relevant for the processes under investigation in beech. The experiments will start when the first results from the cDNA microarray screenings are available.

Deliverables

DL11 Set of ca. 30 genes whose expression is indicative for dormancy or hardiness in beech
DL12 Primers for PCR amplification of ca. 30 genes whose expression is indicative for dormancy or hardiness in beech
DL24 Scientific publications in peer-reviewed journals
DL25 Publications in trade journals

Milestones and expected results

This workpackage will result in a set of beech genes whose expression is correlated with the development of dormancy and/or winter hardiness.
Relevant milestone:
M2 Initial set of relevant beech genes selected

WP2.	Development and application of RT-PCR assays

Workpackage number:	**WP7**
Start date or starting event:	**Identification of relevant pine and beech genes**
N° of the partner responsible:	**1**
N°s of other partners involved:	**2, 4, 7, 8, 9, 6**
Person-months per partner:	**partner 1: 25 months, partner 2: 8 months, partner 4: 3 months, partner 7: 3 months, partner 8: 1 month, partner 9: 1 month**

Objectives

- To develop straightforward and rapid tests for the presence of dormancy/hardiness keygenes
- To use those tests for detailed analysis of the expression profiles of the selected genes.
- To assess the diagnostic value of the tests in a commercial setting

Description of work

Partners 1 and 2 will design RT-PCR primers, based on the sequence of the selected keygenes. The primers will be used by partners 1 and 2 in a quantitative PCR assay to refine the expression profile of the selected genes using previously untested material from the climate room experiments (WP1 and WP2) and material from the WP3 and WP4 outdoor trials, which will reveal the effects of annual and geographical climatic variation, growth conditions and provenance. These experiments will start as soon as the first relevant genes have been selected and will continue to the end of the project. They will result in the selection of a sub-set of highly informative key genes, descriptive and predictive of dormancy and frost hardiness state. Partners 4 and 7 will evaluate this sub-set of primers in specific field trials and, aided by partners 8 and 9, in a commercial setting. Trials will be designed to test whether the PCR assays can be used as diagnostic tests to predict the level of hardiness or dormancy. Primers showing optimal predictive properties will be tested in a commercial setting to evaluate their usefulness as practical diagnostic test for forest tree nurseries.

Deliverables

DL13	RT-PCR protocols for the amplification of dormancy/hardiness related genes from pine or beech
DL14	In depth expressional information on ca. 30 genes from pine and beech, in relation to dormancy and hardiness development in varying environmental conditions
DL15	A subset of ca. 15 highly informative key genes, descriptive of the physiological state of the tree seedling with respect to dormany and frost hardiness
DL18	An assessment of the use of the selected key genes as molecular diagnostic tool to predict the physiological state of tree seedlings in a commercial setting
DL24	Scientific publications in peer-reviewed journals
DL25	Publications in trade journals

Milestones and expected results

This work will result in a detailed insight in the expressional regulation of genes involved in the development of dormancy and/or hardiness. A subset of genes whose expression pattern is highly descriptive of dormancy and/or hardiness status in pine and beech will be selected and evaluated in a commercial setting

Relevant milestone:

M3	Subset of pine and beech key genes selected
M4	Usefulness of key genes as molecular diagnostic tests evaluated

WP2. Provenance effect on gene expression patterns

Workpackage number:	**WP8**
Start date or starting event:	**March 2001**
N° of the partner responsible:	**5**
N°s of other partners involved:	**3, 1**
Person-months per partner:	**partner 3: 10 months, partner 5: 11 months, partner 1: 2 months**

Objectives

To evaluate the correlation of key gene expression and dormancy/hardiness status, under conditions were the hardening process might interfere with plant specific genetic properties and with changes in physiological conditions caused by age and climatic conditions.

Description of work

The aim of this trial is to evaluate the expression of the selected genes under conditions were the hardening process might interfere with plant specific genetic properties and with changes in physiological conditions caused by age and climatic conditions. These are situations that are common in commercial situations. In controlled trials the effect of provenance, age, and climate conditions on the predictive value of marker gene expression will be evaluated. In addition the diagnostic value of key gene expression will be studied in material from commercial nurseries. Gene expression profiles will be compared with relative root and shoot freezing tolerance ($REL_{diff\text{-}10}$ and $SEL_{diff\text{-}10}$) and with stem conductance (EC). The trials will involve beech (partner 5) and pine (partner 3).

Set-up provenance effect:

Seeds of 3 geographic provenances will be sown in three subsequent years, starting in 2001. One of the provenances will be identical to the standard provenance used in WP3 and WP4, respectively. In trial season 2002/2003 $REL_{diff\text{-}10}$, $SEL_{diff\text{-}10}$ and EC of 0.5 and 1.5 year old seedlings of the 3-4 provenances will be measured bi-weekly from autumn to mid winter (6 sampling dates). Material will be collected for mRNA analysis and send to partner 1. In the season 2003/2004 this experiment will be repeated with seedlings from all three sowing seasons (0.5, 1,5 and 2,5 years old). At each sampling date 40 plants per provenance-age combination are used for $REL_{diff\text{-}10}$, $SEL_{diff\text{-}10}$ and EC analyses (4 replicates of 5 seedlings at the two temperatures). For mRNA analysis 50 plants are used.

Set-up commercial testing:

In year 2003 samples of beech and pine seedlings will be taken tree times from the onset of hardening in September to completely hardened in December from 3 commercial nurseries. The root and shoot freezing tolerance will be measured as $REL_{diff\text{-}10}$ and $SEL_{diff\text{-}10}$ using 40 seedlings (4 replicates with 5 seedlings in each for each temperature) at each sampling date. Material for mRNA analyses will be collected at the same occasions and sent to partner 1. The results will be compared with that of the provenance trial.

Deliverables

- DL16 Information on the influence of provenance, climatic conditions and age on the expression of dormancy/hardiness related genes in pine and beech
- DL18 An assessment of the use of the selected key genes as molecular diagnostic tool to predict the physiological state of tree seedlings in a commercial setting
- DL23 Trade demonstration workshop
- DL24 Scientific publications in peer-reviewed journals
- DL25 Publications in trade journals

Milestones and expected results

This workpackage will result in insight in the effect of provenance, climatic conditions and age on the expression of the dormancy/hardiness key genes. This will facilitate the identification of broadly applicable genes for future implementation in diagnostic tests.

Relevant milestone:

M4 Usefulness of key genes as molecular diagnostic tests evaluated

WP2.	Relating storage performance to key gene expression

Workpackage number:	**WP9**
Start date or starting event:	**September 2002**
N° of the partner responsible:	**3**
N°s of other partners involved:	**1**
Person-months per partner:	**partner 3: 10 months, partner 1: 2 months**

Objectives

To study the expression of selected markers in relation to storage performance of pine and to evaluate their predictive value for this application

Description of work

The first storage trial (season 2001-2002) will focus on frozen (below 0°C) storage. One and two-year old seedlings of three different provenances, one of which is the standard provenance used in WP1 and WP3 will be stored in frozen storage when physiological freezing tolerance tests show that the seedlings are storable (approx in September-October). The root and shoot freezing tolerance will be evaluated 6 times during storage (at transfer to storage, mid-Oct, mid-Dec, mid-March and early in May). At these occasions, tissue samples will be yaken and stored at -80°C for future mRNA screening by partner 1.

The second trial (season 2002-2003) will be focused on cool (above 0°C) storage and the results will be compared to the frozen storage. One- and 3-year-old seedlings of three different provenances will be used to be able to evaluate the effect of seedlings age and provenance. The root and shoot freezing tolerance will be evaluated 6 times during storage (at transfer to storage, mid-Oct, mid-Dec, mid-March and early in May). At these occasions, tissue samples will be yaken and stored at -80°C for future mRNA screening by partner 1. These screenings will be performed once the key genes have been selected.

Deliverables

DL17	Evaluation of the predictive value of the selected keygenes with respect to storage performance of pine
DL18	An assessment of the use of the selected key genes as molecular diagnostic tool to predict the physiological state of tree seedlings in a commercial setting
DL23	Trade demonstration workshop
DL24	Scientific publications in peer-reviewed journals
DL25	Publications in trade journals

Milestones and expected results

This workpackage will result in information of the applicability of the selected keygenes as predictors of storage performance of pine, in relation to age, provenance and storage conditions.

Relevant milestone:

M4 Usefulness of key genes as molecular diagnostic tests evaluated

WP2. Development of an integrated database

Workpackage number:	**WP10**
Start date or starting event:	**May 2004**
N° of the partner responsible:	**2 and 7**
N°s of other partners involved:	**1, 4**
Person-months per partner:	**Partner 1: 7 months, partner 2: 4 months, partner 4: 4 months, partner 7: 5 months**

Objectives

- To develop an integrated searchable, cross-referenced database that combines all data obtained in this project. For each clone on the microarray physiological, morphological, expressional and sequence information should be accessible.
- To define an hypothesis describing the molecular pathways involved in dormancy and hardiness in Scots pine and common beech.
- To communicate these results to the sector in a demonstration work shop

Description of work

Partner 7 will combine all physiological and morphological data obtained during the project in one database that includes information on growth conditions. Partner 2 will purchase specific software for storing and manipulating microarray data and will adapt this to allow linkage with the physiological database. The database will be used to reveal coordinated expression patterns linked to the development of dormancy and cold tolerance in pine and beech and will thus aid in the definition of an hypothesis or conceptual model on the molecular events underlying these processes. In addition the database can be used for the selection of keygenes whose expression pattern is tightly linked to the development of hardiness or dormancy and not subject to aspecific fluctuations. These genes will be a good basis for the future develeopment of a diagnostic test. The information available in the integrated database and the conclusions drawn from it will be presented to the sector in a demonstration workshop that will be organized at the end of the project. This workshop will be focused on the significance and implementation of the results in forest tree nurseries.

Deliverables

DL19	A physiological database combining all information on growth conditions and physiological parameters obtained during the project
DL20	A molecular database combining all expressional and sequence data, including PCR primers, obtained during the project
DL21	An integrated searchable database combining the physiological and molecular databases
DL22	A conceptual model describing the molecular events and pathways underlying the development of winter dormancy and frost hardiness in pine and beech seedlings.
DL23	Trade demonstration workshop aimed at communication of the results to the forestry sector (nurserymen, foresteers)
DL24	Scientific publications in peer-reviewed journals
DL25	Publications in trade journals

Milestones and expected results

This workpackage will result in a detailed insight in the molecular processes involved in adaptation to winter conditions, and in communication of these results and their applications to the sector

Relevant milestone:

M5 Integrated database available

M6 Conceptual model defined