SEEDSOURCE

'Developing best practice for seed sourcing of planted and natural regeneration in the neotropics'

SIXTH FRAMEWORK PROGRAMME
Call identifier: FP6-2002-INCO-DEV-1

PRIORITY A.2.1.
Managing humid and semi-humid ecosystems

SPECIFIC TARGETED RESEARCH PROJECT

Third Reporting Period
Periodic Activity Report

01/05/07 – 30/04/2008

Proposal/Contract no.:	003708
Project coordinator:	Stephen Cavers
Coordinating Institution:	CEH
Project start date:	01/05/2006
Duration:	4 years

Executive Summary - project description

The aim of SEEDSOURCE is to provide best practice policies for sourcing tree germplasm for use within a range of degraded landscapes to ensure the use of best adapted material, that maximises production, without eroding genetic and ecosystem diversity and long term adaptive potential.

Supply of appropriate germplasm is a critical factor for reforestation programmes. Use of inappropriately sourced material (due to lack of knowledge or availability) can lead to ecological and/or commercial failures, as trees die or fail to meet the particular objectives of a reforestation or restoration project. With recent interest in the conservation and restoration of native habitat, there is a growing trend towards planting trees with wider objectives than simply maximising production. Germplasm selection for production forestry is generally based on growth, form and quality criteria. In contrast, planting for ecological restoration requires an emphasis on different traits such as reproductive vigour, seed and seedling survival, and ability to compete with other species. Considerations of sustainability, ecological restoration and conservation of biodiversity also lead to promotion of 'local' seed sources for planting. However, the concept of 'local' is a relative one, depending on the scale over which adaptation occurs, and definition of 'local' seed collection zones is often arbitrary in the absence of adequate information about population delimitation.

The contrasting interests of production and ecological restoration mirror underlying scientific issues. The source of planting stock needs to be considered at both the population level and the individual level *i.e. which populations and which trees within that population?* The key scientific question is how gene flow and selection interact to influence population delimitation and reproductive fitness. The relationships between genetic diversity, habitat heterogeneity and the scale of adaptation in trees are complex, involving a variety of factors. Gene flow may counteract even fairly strong selection, preventing formation of locally adapted populations, although very strong environmental variation (hence selection pressure) may produce adaptive differences over short distances, despite continued high levels of gene flow. Since the genetic composition of seed is affected by patterns of pollen flow, the extent of localised adaptation and fitness may vary with pollen flow from differing environments. Human disturbance can thus have considerable and far-reaching genetic consequences through its effects on patterns of pollen flow. Furthermore, deforestation and other environmental changes may mean that previously well-adapted local populations become less so. In this context, the dangers of using inbred germplasm in tree species are clear. Trees generally carry heavy genetic loads (deleterious recessive alleles, *e.g.* Williams & Savolainen 1996), such that inbreeding, and in particular selfing, may lead to reduced fertility, slower growth in progeny and increased susceptibility to pests or diseases (*e.g.* Park & Fowler 1982, Sim 1984, Griffin 1991). Furthermore, deforestation and other environmental changes may reduce the adaptive potential of local populations, a dangerous scenario when coupled with altered patterns of pollen flow and reductions in genetic diversity.

A fuller understanding of population structure, delimitation and adaptation can provide a rational basis for selection of locally adapted and genetically diverse seed for planting, as well as for the management of natural regeneration. Different types of genetic studies provide input on the spatial dynamics of different genetic processes, and a full understanding requires the integration of a range of descriptive and experimental approaches. Neutral molecular markers are primarily influenced by gene flow and mating system. In contrast, adaptive differences are determined by differences in selection regime. There may be large genetic differences between

populations across the geographic range at the molecular level (*e.g.* because of genetic drift), but few significant adaptive differences (e.g. *Cedrela odorata*, Cavers *et al.* 2003b; Navarro *et al.* 2002). In other cases, field trials may reveal adaptive differences among populations where there is an absence of neutral marker differentiation (e.g. *Pinus sylvestris*, Garcia-Gil *et al.* 2003; *Araucaria araucana* Bekessy *et al.* 2003). At the regional scale, provenance trials can demonstrate the suitability of transplanted material but neutral molecular loci can also be effective markers of source areas for transplantation purposes and can provide important information about the evolutionary history of source populations.

Overall objective:

SEEDSOURCE will apply appropriate molecular and quantitative genetic tools to study both aspects of scale (populations and trees within them) in the sourcing of germplasm for varied use of widespread tree species of high socioeconomic importance in the neotropics.

Specific objectives.

1. The integration of climatic, topographic and substrate information with genetic differentiation and diversity estimates from non-coding and potentially coding genetic markers and adaptive performance from growth trials will produce appropriate translocation guidelines and seed source maps for each of the study species of the SEEDSOURCE project.

2. Appropriate application of hypervariable molecular markers will assess individual mating parameters and will be combined with quantitative assessment of the performance of seed sourced from a variety of forest landscapes (from continuous forest to remnant trees in farm land) and pollination conditions. Recommendations will be produced on the origin of germplasm to select for future tree establishment.

3. A metapopulation model will be developed to test the sensitivity of defined seed source areas/restrictions to translocations.

4. The ECOGENE model will be developed and used as tool to study genetic impacts within agroecosystems landscapes and relevant to the local environment of individual trees.

5. A combined field derived data and modelling approach will facilitate the development of informed management strategies for planting and natural regeneration for each study species.

6. Fifty of the most socio-economically important tree species within each of the Central American and South American tropics will be classified for their genetic and flowering/reproductive syndromes, and the most appropriate seed sourcing strategies identified for each under a variety of management scenarios. Dissemination of this information in a practical and relevant format will target relevant forestry and agroforestry stakeholders across tropical Latin America (e.g. policy makers, seed banks, forest management certifiers and educators).

Executive Summary - progress

Coordinating activities

The third coordination meeting was held 05/07-07/07/07 at PUCE in Quito, Ecuador, hosted by partner 7 - Dr. Renato Valencia. All partners except partner 8 attended. The meeting served primarily to monitor and review progress in all WPs and confirm and adjust plans for RP3. Minutes of the meeting follow the main text of this report.

Members of the SEEDSOURCE (SC, BD, AL) consortium also attended the Workshop 'Fingerprinting methods for the identification of timber origins', in Bonn, Germany in Oct 2007. The Workshop was jointly organised by the German Federal Ministry of Food, Agriculture and Consumer Protection and the WWF, as part of an effort to explore the application of high technology to control of trade in illegal timber. The meeting resulted in an undertaking to pursue funding for gathering of the necessary data on key target tree species and development of a framework for using that data for timber monitoring; a project which will be led by Bernd Degen of vTI - a SEEDSOURCE partner.

Activities in Work Packages

The project was structured in four core areas (CAs), each containing 3 work packages; in each CA the first work package comprises coordination duties ensuring focus across the core area. CA / WP leaders were assigned during project preparation and maintain responsibility for monitoring progress and reporting.

CA1 covers responsibilities for collections for the project as a whole, reciprocal transplant experiments and rangewide phylogeographic studies. **WP1** serves to coordinate collections for all species, but primarily the rangewide collections being used in WPs3 & 5. Substantial collections by PUCE from Ecuadorian and Peruvian populations and new Brazilian field collections by INPA of many species were prepared & exchanged. Both partners also prepared significant herbarium collections from herbaria at PUCE, INPA and EMBRAPA. A final material transfer agreement was signed and exchanged amongst all partners, and export permissions was secured from both Ecuador and Brazil. Phylogeographic progress has been substantial for **WP3**, and rangewide analyses of cpSSR and cp sequence data have been analysed for eight species. Under **WP2**, RTE sites have now been established in Costa Rica for three species and are entering their second year of assessment. In Ecuador, efforts are well advanced for two species and 12 month data should be available by the end of RP4.

CA2 examines patterns of diversity at the landscape scale and the implications of landscape context for reproductive performance. In **WP5**, during RP3 candidate gene markers have been extensively tested in several species and will result in publishable data during RP4. Microsatellite development in target species in now largely complete, with most of the new markers fully optimised and in many cases, published. Full surveys of microsatellite variation are complete in several species, and are underway in most others, except where markers optimsation remains to be finalised. In **WP6**, several of the target assessments of mating system have been completed and final preparations are in place for completion of the rest during RP4. Assessments of progeny trialswill continue until late in RP4.

CA3 will use new and developed simulation models to study the consequences of seed sourcing strategy and germplasm movement on gene flow and genetic diversity, and synthesise outputs from CA1 and 2. Suggestions for a 'data compatibility template' have been presented under the **WP7** report in this document to facilitate synthesis of results in downstream analysis,

covering sample labelling. Under **WP8**, meta-analysis efforts are underway comprising 4 synthetic publications plus consolidation of data from previous projects. The EcoGene (**WP9**) model has undergone further refinement in preparation for simulation analysis in the second half of the project resulting new publications.

CA4 functions to consult the end-user community as to their requirements and expectations from the project, in terms of information and formats, and refine the target audience such that the effectiveness of message delivery is maximised. During RP3, the most significant activity under **WP10** has been joint initiative with Bioversity International (IPGRI) to develop stand-alone training materials suitable for tertiary-level educators: the package will provide long-term legacy from the project and, through collaboration with Bioversity, achieve a wider geographic reach than possible through SEEDSOURCE alone. Following the thrid coordination meeting a workshop was held in Ecuador (**WP11)**, similar to those held previously in Central America: similar issues were identified. The workshop in Ecuador also provided the possibility to refine formats and content of dissemination materials and prepare plans for information gathering exercises to be carried out during RP4 to deliver the dissemination materials expected by the project (**WP12**).

Deviations

The major deviations to the workplan are consequences of earlier delays (reported in RP1, 2) related to late initiation of the project arising from funding delays and consequent collecting problems, such as missing fruiting season in some species. To a large extent the workplan has been amended to take account of these problems but, for example, completion of datasets in RTE trials may only be partial relative to what was anticipated in the technical annex. In most cases, delayed to molecular analyses arising from incomplete collections have been overcome and full screening is mostly underway.

Partner list

1. **CEH**: Stephen Cavers, Katherine Walker, Sam Davies
2. **OFI**: David Boshier, Paul Rymer, Jesus Cordero, Stephen Harris, Sarah Rendell
3. **INRA**: Henri Caron, Ivan Scotti, Caroline Scotti-Saintagne, Antoine Kremer, Remy Petit
4. **CNR**: G.G:Vendramin, A. Buonamici, F. Sebastiani, M.L. Racchi
5. **INPA**: Maristerra R. Lemes, Rogério Gribel, Christopher Dick, **Students:** Tatiana Menecucci, Gabriela S. Farias, and Carolina Braga Medeiros, **Technicians:** Mahatma S. Porto, José Ribeiro and Jakes Câmara.
6. **CATIE**: Carlos Navarro, Bryan Finegan, Carolina Cascante, Gustavo Hernandez
7. **PUCE**: Renato Valencia, Galo Buitrón, Juan Iglesias , and Álvaro Pérez.
8. **UFRJ**: Rogerio Margis, Marcia Margis
9. **BFH**: Bernd Degen, Alexandre Sebbenn

Associated Institutions

UFRGS: Rogerio Margis, Marcia Margis
University of Adelaide: Andrew Lowe

Periodic activity report

Workpackage progress for period 2.

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	1	***Collection and exchange of materials and methods***
Work Package	2	***Quantitative performance for replanting***
Work Package	3	***Evolutionary history and developing regional markers for species***
CORE AREA	**2**	**Diversity, reproductive performance & recruitment at the landscape scale**
Work Package	4	***Ensuring focus of quantitative and genetic studies***
Work Package	5	***Gene dynamics & quantitative seed performance in relation to landscape***
Work Package	6	***Estimate partitioning of non-coding and coding genetic diversity***
CORE AREA	**3**	**Analysis and prioritisation of regional and local sourcing strategies**
Work Package	7	***Data compatibility***
Work Package	8	***Meta-analysis of data***
Work Package	9	***Selection and definition of resource priorities***
CORE AREA	**4**	**Knowledge gathering, integration and dissemination of priorities**
Work Package	10	***Communication of biological and socio-economic information***
Work Package	11	***Knowledge gathering***
Work Package	12	***Preparation and dissemination of extension materials***

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	*1*	*Collection and exchange of materials and methods*

1. Workpackage objectives and starting point of work at beginning of reporting period

- Manage collection and exchange of materials and methods
- Draw up IPR, germplasm exchange and data management agreements between partners
- Coordinate collection activities
- Ensure export and collection permits and procedures are followed
- Construct project website

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved.

Collections summary
Sample collection has progressed significantly for all species during the third reporting period. Specific new field missions completed at the time of the report:

INPA: In total collections of 18 target species (930 samples) were done by INPA for phylogeographic and genetic diversity analyses by INPA in Brazil (Tables 1.1 and 1.3).

- Collections of samples from natural populations were also done by Chris Dick in Panama (12 species, 228 samples) for phylogeographic analysis. He also collected leaf tissues from *Virola sebifera* (201 individuals), *Virola multiflora* (18 individuals) and *Virola surinamensis* (20 individuals) in Barro Colorado Island, Panama. (Table 1.2)
- We collected leaves of 55 mother trees and 457 progenies (55 families) from three populations of *B. excelsa* established in the common garden experiments for WP6.
- Plant tissues of 13 tree species (647 samples) and DNA (66 samples of *Minquartia guianensis*) collected in Brazil were sent to the SEEDSOURCE partners.

Table 1.1 - Samples collected by INPA from natural populations of 11 target species for phylogeographic and genetic diversity analyses in WP3 and 5.

Species	No Pops.	No. ind.	Locations	WP
Bertholletia excelsa	2	50	(Boca do Acre, AM; Tefé, AM), Brazil	3, 5
Carapa guianensis	2	35	(Boca do Acre, AM; Tefé, AM), Brazil	3, 5
Cedrela odorata	1	30	(Boca do Acre, AM), Brazil	3, 5
Ceiba pentandra	2	24	(Boca do Acre, AM; Tefé, AM), Brazil	3
Hymenaea courbaril	1	31	(Boca do Acre, AM), Brazil	3, 5
Jacaranda copaia	2	11	(Boca do Acre, AM; Tefé, AM), Brazil	3, 5
Minquartia guianensis	2	84	(Manaus, AM; Tefé, AM), Brazil	3, 5
Schizolobium amazonicum	1	31	(Boca do Acre, AM), Brazil	3
Socratea exorrhiza	2	41	(Corumbiara, RO; Tefé, AM), Brazil	3, 5
Symphonia globulifera	1	31	(Boca do Acre, AM), Brazil	3, 5
Vochysia ferruginea	1	6	(Tefé, AM), Brazil	3
TOTAL	**17**	**374**	-	-

Table 1.2 - Samples collected by Chris Dick from natural populations of 12 target species for phylogeographic analysis (WP 3) (Period: 2007 – 2008).

Species	No Pops.	No. ind.	Locations
Bombacopsis quinata	01	03	Weckso, Panama
Carapa guianensis	01	10	Esmeraldas, Ecuador
Ceiba pentandra	02	11	BCI, Weckso, Panama
Cordia alliadora	03	30	El Cope, Caldera Chiriqui, Pipeline, Panama
Hymenaea courbaril	03	19	Farallon; Gualaca; Caldera; Panama
Jacaranda copaia	02	20	Pipeline; Santa, Panama
Minquartia guianensis	01	02	Yasuni, Ecuador
Ochroma pyramidale	10	48	Campana; BCI; Pipeline; IA highway; Weckso; Changuinola; Azuero; Sherman Esmeraldas (Panama), Yasuní, Ecuador
Schizolobium parahyba	02	03	Yasuní, Ecuador; Cochabamba, Bolivia
Simarouba amara	04	31	Pipeline; Cerro Jefe; Weckso; Santa Rita, Panama
Socratea exorrhiza	03	41	BCI; Campana; Weckso, Panama
Vochysia ferruginata	02	10	Altos de Piedra, Panama
TOTAL	**34**	**228**	-

IPR & material exchange agreement (all partners)
The final text of the Material Transfer Agreement was signed by all partners and copies including signatures of all partners have now been distributed.

Export permissions
INPA: Permits were obtained for plant collection and export from Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (IBAMA) in Brazil and Autoridad Nacional del Medio Ambiente (ANAM) in Panama.

Herbaria.
Sub-samples of the SEEDSOURCE target species were collected at INPA and EMBRAPA´s herbaria for analyses within WP 3. Table 1.2 shows the number of accessions collected per each species in each of the herbarium. In total we have collected 556 samples from 18 species in the two herbaria.

Table 1.3 – Accessions of target species collected at INPA and EMBRAPA herbaria

Species	EMBRAPA	INPA
	No. accessions	No. accessions
Bertholletia excelsa	-	28
Bombacopsis quinata	-	01
Carapa guianensis	25	33
Cedrela odorata	07	32
Ceiba pentandra	06	07
Cordia alliadora	-	18
Hymenaea courbaril	07	24
Jacaranda copaia	27	32
Minquartia guianensis	24	13
Ochroma pyramidale	-	09
Schizolobium parahyba	-	02
Schizolobium amazonicum	-	01
Socratea exorrhiza	2	23
Simarouba amara	12	51
Swietenia macrophylla	07	06
Symphonia globulifera	35	-
Virola sebifera	34	48
Vochysia ferruginea	08	34
TOTAL	**194**	**362**

TOTAL (EMBRAPA + INPA) = 556

3. Deviations from the project workprogramme, and corrective actions taken/suggested

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)

5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	*2*	*Quantitative performance for replanting*

1. Workpackage objectives and starting point of work at beginning of reporting period

- Assess the scale of local adaptation under conditions of natural regeneration in four study species at seed germination, seedling establishment and the relation to genetic, environmental and geographic distance.
- Compare the patterns of adaptation under conditions of natural regeneration with those in plantation provenance trials.
- Relate evidence for the scale of adaptation to existing seed sourcing practices.

Modifications:

The WP will finally focus on the following study species: *Cedrela odorata*, *Cedrela tonduzzi* and *Ulmus mexicana* in Costa Rica; *Cedrela odorata* L. (Spanish cedar) and *Ochroma pyramidale* (cav. ex Lam) Urb. (balsa), two widely distributed species in different habitats and forest types in Ecuador and *Cedrela odorata* and *Ochroma lagopus* in Ecuador. The activities for objective 1 consist in establishing Reciprocal Transplant Experiments (RTEs) for three selected tropical forest trees species in Costa Rica and two species in Ecuador.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Activities in WP2 are primarily undertaken by partners CATIE and PUCE, reports on progress in RTE establishment and preliminary analysis are given below. The three experiments (three species x 12 sites) planned for CATIE have been established, PUCE is progressing with plans and, despite problems with asynchrony of fruiting for *Cedrela odorata*, the activity will be completed during 2008 including the RTE of *Ochroma pyramidale*.

1. CATIE Activities. CATIE developed RTEs for three species: *Cedrela odorata, Cedrela tonduzii, and Ulmus mexicana.* The experiments were developed in six regions for *C.odorata*, four for *Cedrela tonduzii* but Llano Grande was severely affected by a hail storm and three regions for *Ulmus mexicana*. Table 2.1 gives the locations of collection zones for RTEs of *C.odorata, C.tonduzii* and *U.mexicana*. Table 2.2 gives locations of planting sites for all the species evaluated in the project.

The three experiments for *Cedrela odorata, Cedrela tonduzzi*, and *Ulmus mexicana* have been evaluated already; results for *C. odorata* were prepared in the previous reporting period and those for *C. tonduzii* are currently being prepared. Here we report statistical analysis for *Ulmus mexicana*.

Table 2.1. Locations of collecting zones for Reciprocal Transplant Experiments.

Specie/Population	**Longitude**	**Latitude**	**Altitude**	**Location**
Cedrela odorata				
Guanacaste	84.98000	10.25000	150	Las Juntas of Abangares
Pérez Zeledón	83.60000	09.380000	700	General Viejo
Puriscal	84.25000	09.90000	700	Puriscal, Ciudad Colón
San Carlos	84.48000	10.37000	200	Florencia
Sarapiquí	84.90000	10.23000	150	Horquetas, Puerto Viejo
Turrialba	83.70000	09.85000	700	Tucurrique, Turrialba
Cedrela tonduzzi				
Cartago	83.96700	9.70000	2000	El Empalme, Santa María of Dota.
Llano grande	83.91600	9.94400	2300	Llano Grande, Cartago
Zarcero	84.30014	10.189098	2000	Tapezco, Laguna,
Santa Cruz	83.73400	9.96800	1500	Santa Rosa, Santa Cruz
Ulmus mexicana				
Cartago	83.93200	9.80900	1400	Cervantes, Cartago
Turrialba	83.71400	9.87300	700	Chiz, Turrialba
Santa Cruz	83.44800	9.95800	1500	Santa Cruz

Table 2.2. Locations of planting sites for Reciprocal Transplant Experiments.

Specie/Population	**Longitude**	**Latitude**	**Altitudemasl**	**Location/ Proprietary**
Cedrela odorata				
Guanacaste	84.98489	10.25452	108	Las Juntas, Abangares –Manuel Bonilla
Pérez Zeledón	83.66381	9.38984	790	General Viejo- Tulio Granados
Puriscal	84.25233	9.90340	700	Ciudad Colón- Ronald Madrigal
San Carlos	84.51179	10.37587	191	Santa Clara of Florencia- Ana Lía Quirós
Sarapiquí	84.03148	10.44266	150	Cristo Rey of Puerto Viejo- Abelardo Oconitrillo
Turrialba	83.65329	9.89756	597	CATIE
Cedrela tonduzzi				
Cartago	83.93250	09.80950	1550	El Guarco – Carlos and Uriel Navarro
Zarcero	84.5000	10.2500	1600	Jaime Barrientos
Santa Cruz	83.75	9.9500	1500	La Pastora of Santa Cruz- Municipality of Turrialba
Ulmus mexicana				
Cartago	83.93250	09.80950	1550	El Guarco – Carlos and Uriel Navarro
Turrialba				CATIE
Santa Cruz	83.75	9.9500	1500	La Pastora of Santa Cruz- Municipality of Turrialba

Statistical analysis of the *Ulmus mexicana* RTEs.

The tables below show some preliminary results obtained using SAS statistical systems, they show highly significant differences between provenances in adaptation to different types of soils and climate; there are clear differences between provenances of the area of Santa Cruz with the extrusive volcanic soils of the Turrialba Volcano and the south with intrusive soils of the Cordillera of Talamanca. The tables below report the ANOVA for the trials Cartago, Santa Cruz and Turrialba for the variables: root collar diameter in millimetres (Dia0mm) and total height in cm (Alt0cm).

Analysis of Variance (SC type III) for diameter in Cartago trial

Trial	Variable	N	R^2	R^2 Aj	CV
Cartago	Dia0mm	593	0.28	0.25	33.96

S.V.	SS	df	MS	F	p-valor
Model	232.19	21	11.06	10.46	<0.0001
Block	44.20	19	2.33	2.20	0.0024
Population	189.56	2	94.78	89.68	<0.0001
Error	603.48	571	1.06		
Total	835.66	592			

SV sources of variation, SS: square sums, df degrees of freedom, MS mean square

Test of means for diameter in Cartago Trial. Santa Cruz performed better than the other populations Duncan Alfa=0.05

Error: 1.0569 gl: 571

Population	Means	n			
Turrialba	2.37	195	A		
Cartago	2.95	200		B	
Santa Cruz	3.75	198			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Analysis of Variance for height in Cartago trial (SC tipo III)

Trial	Variable	N	R^2	R^2 Aj	CV
Cartago	Alt0cm	5 92	0.30	0.27	38.55

S.V .	SS	df	MS	F	p-valor
Model	41436.69	21	1973.18	11.55	<0.0001
Block	16008.06	19	842.53	4.93	<0.0001
Population	25843.68	2	12921.84	75.67	<0.0001
Error	97337.66	570	170.77		
Total	138774.35	591			

Test means showing that Santa Cruz performed better than the other populations:Duncan Alfa=0.05

Error: 170.7678 gl: 570

Population	Means	n			
Turrialba	25.96	195	A		
Cartago	33.36	200		B	
Santa Cruz	42.19	197			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Analysis of Variance for Dia0mm in Santa Cruz trial (SC tipo III)

Trial	Variable	N	R²	R² Aj	CV
Santa Cruz	Dia0mm	540	0.20	0.17	58.22

F.V.	SS	df	MS	F	p-valor
Model	991.17	21	47.20	6.27	<0.0001
Block	668.12	19	35.16	4.67	<0.0001
Population	300.34	2	150.17	19.94	<0.0001
Error	3900.55	518	7.53		
Total	4891.72	539			

Test of means showing that Santa Cruz performed better than the other populations. Duncan Alfa=0.05
Error: 7.5300 gl: 518

Population	Means	n			
Turrialba	3.67	176	A		
Cartago	4.84	184		B	
Santa Cruz	5.48	180			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Analysis of Variance for the variable height in Santa Cruz trial (SC tipo III)

Trial	Variable	N	R²	R² Aj	CV
Santa Cruz	Alt0cm	539	0.22	0.19	52.48

F.V.	SC	gl	CM	F	p-valor
Model	103490.71	21	4928.13	6.93	<0.0001
Block	58728.42	19	3090.97	4.35	<0.0001
Population	41719.82	2	20859.91	29.34	<0.0001
Error	367587.48	517	711.00		
Total	471078.19	538			

Test of means showing that Santa Cruz performed better than the other populations :Duncan Alfa=0.05
Error: 711.0009 gl: 517

Population	Means	n			
Turrialba	38.94	175	A		
Cartago	51.48	184		B	
Santa Cruz	60.60	180			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Analysis of Variance for the site Turrialba, dependent variable diameter.(SC tipo III)

Trial	Variable	N	R²	R² Aj	CV
Turrialba	Dia0mm	579	0.58	0.56	36.33

F.V.	SC	gl	CM	F	p-valor
Model	1134.18	21	54.01	36.39	<0.0001
Block	88.57	19	4.66	3.14	<0.0001
Population	1038.86	2	519.43	350.02	<0.0001
Error	826.59	557	1.48		
Total	1960.76	578			

Test of means showing that Santa Cruz performed better than the other populations: Duncan Alfa=0.05
Error: 1.4840 gl: 557

Population	Means	n			
Turrialba	1.56	187	A		
Cartago	3.58	195		B	
Santa Cruz	4.83	197			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Analysis of Variance for Height in the Turrialba trial (SC tipo III)

Trial	Variable	N	R^2	R^2 Aj	CV
Turrialba	Alt0cm	577	0.51	0.50	43.29

F.V.	SC	gl	CM	F	p-valor
Model	218500.93	21	10404.81	27.98	<0.0001
Block	14801.77	19	779.04	2.09	0.0044
Population	202140.55	2	101070.28	271.77	<0.0001
Error	206401.62	555	371.89		
Total	424902.56	576			

Test of means showing that Santa Cruz performed better than the other populations: Duncan Alfa=0.05
Error: 371.8948 gl: 555

Population	Means	n			
Turrialba	19.24	187	A		
Cartago	48.83	194		B	
Santa Cruz	64.64	196			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Below, we present the statistical analysis of all sites together, offering information on which of the populations presented the best performance for the growth of *U. mexicana* in Costa Rica.

Analysis of variance for Diameter at root collar in *Ulmus mexicana* pooled across all trials.

For all sites planted with *Ulmus mexicana*, all variables were highly significant (SC tipo III)

Variable	N	R^2	R^2 Aj	CV
Dia0mm	1712	0.30	0.29	51.54

F.V.	SC	gl	CM	F	p-valor
Model	2542.10	23	110.53	30.91	<0.0001
Block	297.64	19	15.67	4.38	<0.0001
Trial	879.40	2	439.70	122.95	<0.0001
Population	1350.80	2	675.40	188.86	<0.0001
Error	6036.56	1688	3.58		
Total	8578.66	1711			

Test of means for diameter , showing that the trial Santa Cruz presented the best conditions for growth of the populations. Duncan Alfa=0.05
Error: 3.5762 gl: 1688

Trial	Means	n			
Cartago	3.02	593	A		
Turrialba	3.33	579		B	
Santa Cruz	4.69	540			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Test means for diameter, showing that population Santa Cruz is growing better than the Cartago and Turrialba. Duncan Alfa=0.05
Error: 3.5762 gl: 1688

Population	Means	n			
Turrialba	2.54	558	A		
Cartago	3.79	579		B	
Santa Cruz	4.72	575			C

Distinctive letters indicate significant differences between means (p<= 0.05)

Analysis of Variance for height for all the sites planted for *Ulmus mexicana,* pooled across all trials.

All the classification variables including the site of the trial were highly significant (SC tipo III)

Variable	N	R^2	R^2 Aj	CV
Alt0cm	1708	0.31	0.30	50.10

F.V.	SC	gl	CM	F	p-valor
Model	342363.84	23	14885.38	32.32	<0.0001
Block	35265.72	19	1856.09	4.03	<0.0001
Trial	81220.58	2	40610.29	88.17	<0.0001
Population	223289.97	2	111644.98	242.40	<0.0001
Error	775621.09	1684	460.58		
Total	1117984.93	1707			

Test of mean height showed that the trial Santa Cruz presented best conditions for growth. Duncan Alfa=0.05
Error: 460.5826 gl: 1684

Trial	Means	n			
Cartago	33.81	592	A		
Turrialba	44.27	577		B	
Santa Cruz	50.53	539			C

Distinctive letters indicate significant differences between means ant(p<= 0.05)

The performance in height of the three populations, showing that the population Santa Cruz is the population that for the three sites is growing better than the Cartago and Turrialba. Duncan Alfa=0.05

Error: 460.5826 gl: 1684

Population	Means	n			
Turrialba	28.05	557	A		
Cartago	44.51	578		B	
Santa Cruz	56.05	573			C

Distinctive letters indicate significant differences between means(p<= 0.05)

2. PUCE Activities. PUCE efforts in WP2 are focused on fruit collections for seed trials and seedling RTEs. PUCE is working with two species:

1. Description of species and study sites:

Cedrela odorata L.
Spanish cedar or tropical cedar is a long lived secondary species. In Ecuador *C. odorata* grows in a variety of ecological gradients ranging from the Coastal plains and the Amazon lowlands to the Andean slopes up to 2500 m altitude. At higher elevations (>1800 m altitude), the species grows sympatricaly with *C. montana*, a sister species relatively common in the highlands of Ecuador. In the Coast we found a population at Las Mercedes (MER), a pre-montane forest in the western foothills of the Andes; according to the nearest climate record its climate is wet, with only one dry month (Table 2.3). MER is the highest study site in the Coast. In Chindul (CHI) we found a remnant population of the species in a semi-deciduous forest, an almost vanished forest type in Ecuador. CHI is the driest *C. Odorata* study site, with a dry season of 5 months (Table 2.3). On the other hand, our Amazonian localities, Puerto Napo-Ahuano road (NAR) and the Yasuni Scientific Station (YAS), shelter large populations of *C. odorata*, probably the largest in the country. The density of *C. odorata* in YAS primary forest (saplings and adults = 135 stems) is illustrated in Figure 2.1. In NAR *C. odorata* trees are relatively frequent in pastures and secondary forests (Figure 2.2; Table 2.3). Populations of this species are rare in the Coastal plains (see Appendix II - Annex 1 and 2 for distribution and conservation status).

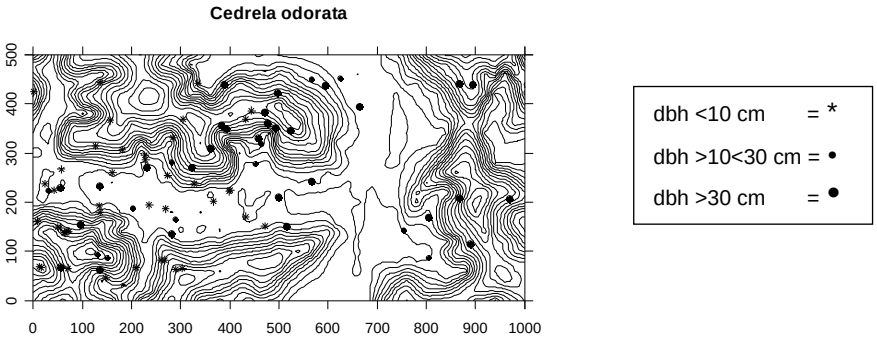


Figure 2.1. Saplings and adult trees of *Cedrela odorata* in a large 50-ha primary forest plot (1000 × 500 m) at the Yasuni Scientific Station (YAS).

Ochroma pyramidale (cav. ex Lam) Urb.
Balsa is a South American pioneer tree that grows frequently in large light gaps and road sides. In Ecuador it grows in disturbed areas in the Coastal lowlands and the Amazon regions, from sea level up to 1,500 m of elevation (Jørgensen & León-Yánez 1999). Although it is a pioneer species without much economic importance in most countries where it grows, in Ecuador it has been planted and managed for decades. Since the early 1940's private companies plant and export Balsa around the world. Currently, the species is widely planted in Ecuador. For instance, one single company cultivated ~7000 ha until year 2006 (http://www.alcanbaltek.com). It prefers the humid lowlands but it can grow naturally as high as 1,500 m asl in the Andean slopes, according to our SEEDSOURCE project records. The species is frequent in road sides and secondary forests. Farmers from the Coast leave balsa trees in pasturelands since timber companies buy the best Balsa logs. Identifying suitable populations of this species was not a difficult task, however, trees in the coast did not fructify synchronically. Because of that we completed the seed collection in March 2007. Up to now, we have measured fruits and extracted and measured seeds of all provenances (two in the Coast and two in the Amazon region; Figure 2.2 and Table 2.3).

Site descriptions for Amazon Lowland sites (Pto. Napo - Ahuano Road (NAR) Yasuní Scientific Station (YAS) Archidona (ARC)) were presented in the previous reporting period. For Coastal plain sites, Chindul (CHI) and Las Mercedes (MER) descriptions were previously presented but the following new sites were added:

Pedro Vicente Maldonado (PVM) (0° 7' 3.4'' N; 79° 3' 34.5'' W, 450–600 m asl) is located 120 km north-west of Quito, in the western slopes of Andes. Close to PVM there are several forest remnants, including the Rio Silanche Forest Reserve, which is a 85-ha reserve of primary forest surrounded by timber plantations of several species (e.g., *Schizolobium parahyba*, *Parkia multijuga*, *Jacaranda copaia*). The climate is tropical wet without dry months. The vegetation is tropical premontane rainforest with a canopy of 40 m high. Common trees in the reserve are *Carapa guianensis*, *Virola dixonii*, *Brosimum utile*, etc. A detailed description of Rio Silanche Flora was published by Jorgensen & Ulloa (1989). Balsa trees were found inside of the reserve and in adjacent farmlands and secondary forest in roadsides.

Pedernales (PED) (0 ° 1' 29.6'' N ; 79° 56'' 17.5'' W; 105–330 m asl) represents a dry forest site located close to the Pacific Ocean. The population of *Ochroma pyramidale* grows in secondary forests and pastures. In secondary forests around Pedernales we recorded trees of *Vernonia* sp., *O.* pyramidale, *Cecropia* sp., *Erythrina* sp. and introduced species as *Tectona grandis*.

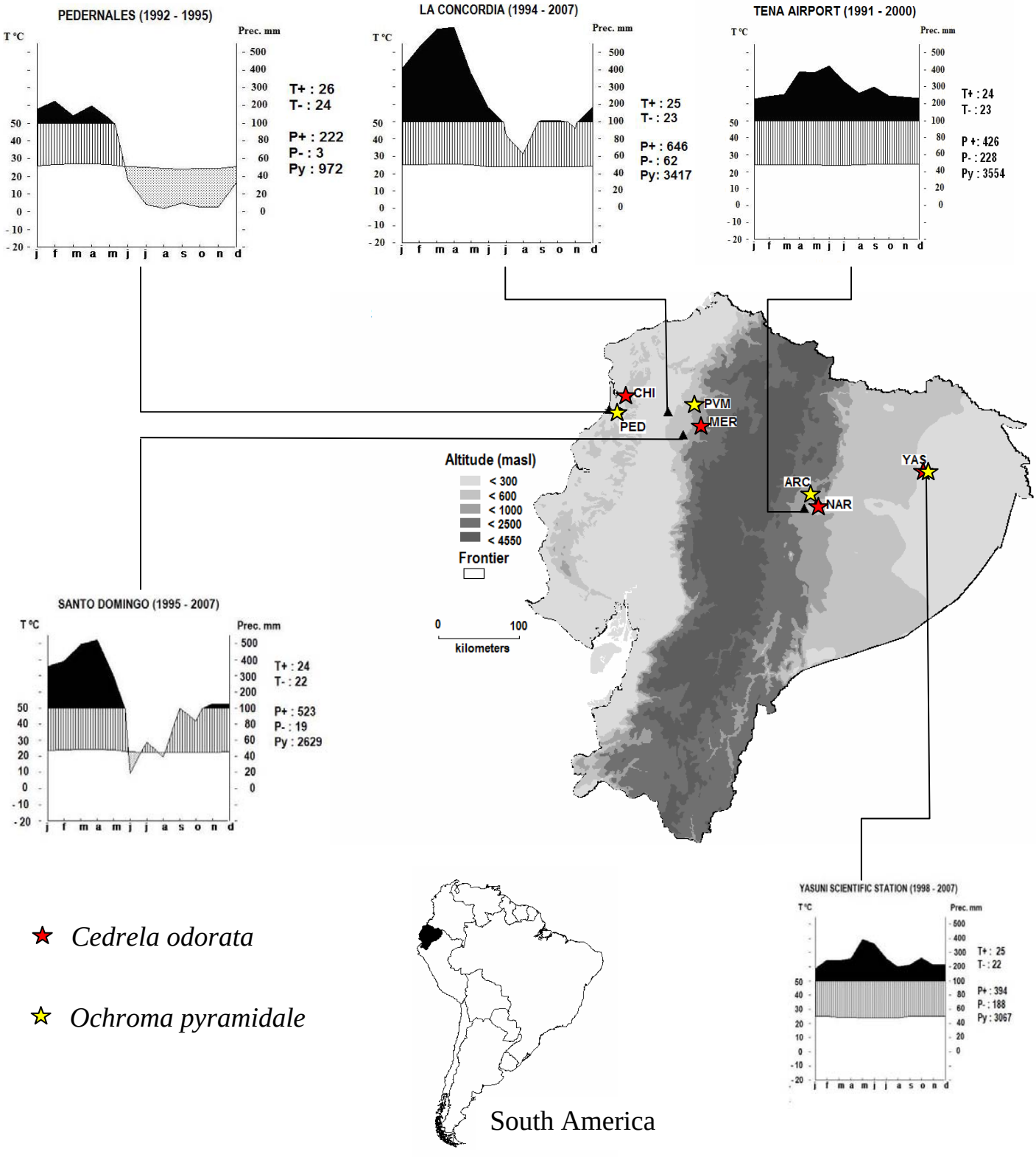


Figure 2.2. Map of selected sites for RTE's showing climatic diagrams of the nearest climatic stations.

Table 2.3. Characteristics of selected sites to carry out RTE's in Ecuador. TPR = Tropical Pre-montane rainforest; TSF = Tropical Semi-deciduous forest; TER = Tropical Evergreen Rainforest; TDF = Tropical dry forest. A map of these localities is presented in Figure 2.2.

Region	Locality	Elevation (m asl)*	Climate	Rainfall (mm/yr)	Temp. °C	Vegetation type	Mother trees	Soil type	Species
Coast	Las Mercedes (MER)	590–790	Tropical Wet	2800 (1)	22	TPR	21	Volcanic	*C. odorata*
Coast	Chindul-La Quinta (CHI)	20–450	Tropical Dry	890 (5)	24	TSF	21	No data	*C. odorata*
Amazonia	Pto. Napo-Ahuano (NAR)	410–515	Tropical Wet	3554 (0)	25	TER	22	Sedimentary	*C. odorata*
Amazonia	Yasuní Scientific Station (YAS)	210–275	Tropical Wet	3000 (0)	25	TER	31	Clay	*C. odorata*
Amazonia	Yasuní Scientific Station (YAS)	225–275	Tropical Wet	2750 (0)	25	TER	21	Clay	*O. pyramidale*
Coast	Pedro Vicente Maldonado (PVM)	450–600	Tropical Wet	5545 (0)	21	TER	30	Volcanic	O. pyramidale
Coast	Pedernales (PED)	105–330	Tropical Dry	750 (7)	24	TDF	31	No data	*O. pyramidale*
Amazonia	Archidona (ARC)	520–860	Tropical Wet	6316 (0)	23	TPR	20	Sedimentary	*O. pyramidale*

Research status with *Cedrela odorata*

Phenology and fruit collections. Detailed information about the timing for reproductive events in *Cedrela odorata* is scarce in Ecuador. In order to plan seed collections we carried out monthly phenological observations of 18 to 29 individuals at each site. Monitoring observations were not systematical and parallel between study sites (see Figures 3 and 4), in particular, because we followed the phenology only until we collected the necessary fruits for the experiments. However, phenological comparisons are still possible between study sites.

In year 2007, results reveal time differences in the occurrence of flowering and fruiting between eastern localities in the Amazon lowlands (YAS and NAR) and western localities in the Coastal plains (CHI and MER; Figures 3 and 4). The flowering episode in YAS and NAR ended five months earlier than in CHI and MER during this year. The fruiting period in the Amazon (YAS and NAR sites) was also longer and more frequent than in Coastal sites (CHI and MER). By August 2007 we collected all the necessary fruits for the experiments and stop the phenological observations in the Amazonian study sites.

Starting in September 2007, we only follow phenology in Coastal localities, where we still need seeds for the experiments. The flowering time between the two Coastal localities overlap but, in MER the event started one month later (November vs. October) and trees flowered more frequently and for a longer period (it will probably continue flowering in May) than in CHI. Flowering failure in MER was high in 2007 and low in 2008; the peak of activity in June 2007 is <10%. Likewise, fruiting in MER was almost none existing in 2007 and conspicuous in 2008 (Figures 3 and 4).

Figure 2.3. Flowering occurrence recorded for *Cedrela odorata* in four localities (abbreviations of localities in Table 2.3). Values show the proportion of active individuals in each study site in different months. Upper colored lines represent monitoring periods.

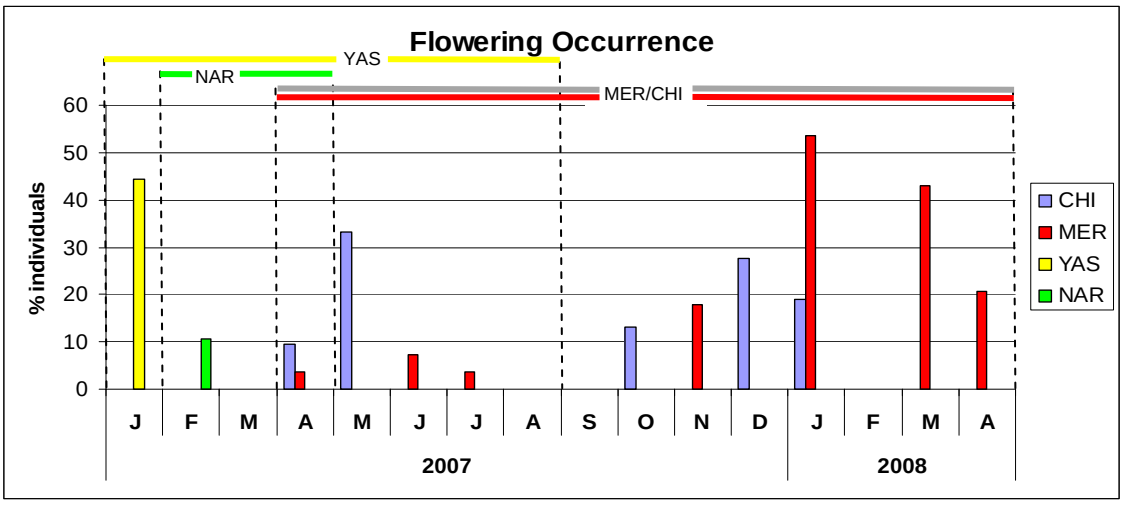
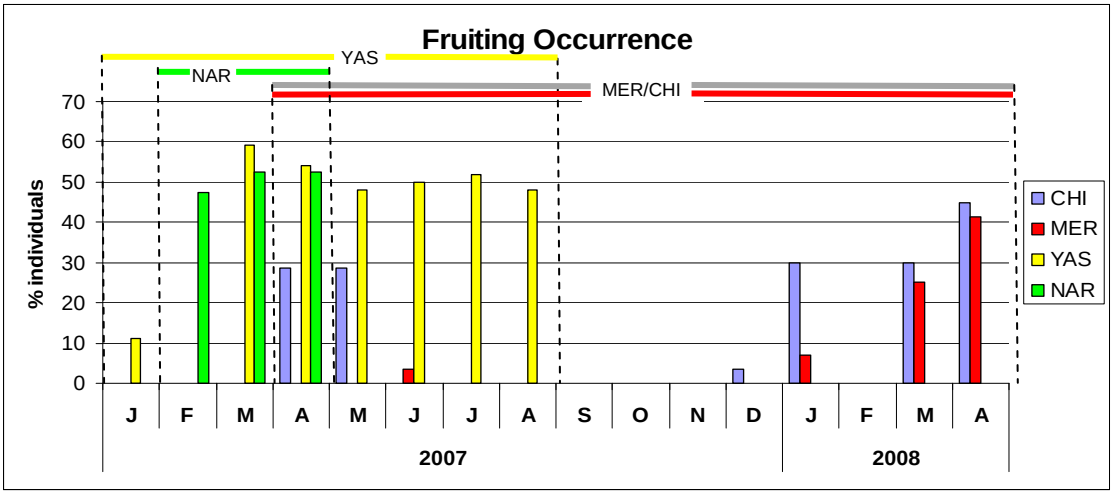


Figure 2.4. Fruiting occurrence recorded for *Cedrela odorata* in four localities (abbreviations in Table 2.3). Values show the proportion of active individuals in each study site in different months. Upper colored lines represent monitoring periods.



Up to date, we have collected seeds of all (20) mother trees in the Amazon lowland study sites and seven mother trees in each locality of the Coastal plains. We hope to complete the collection of fruits by June 2008 (we are monitoring ~60 trees in the Coastal localities). Nonetheless, in MER, the large majority of fruits we observed were damaged by *Hypsipyla grandela* before they mature and we decided to collect less infested or non-infested young fruits with a formed embryo to ensure the seed lots from this locality. A summary of fruit characteristics of *Cedrela odorata* is presented in Table 2.4.

Table 2.4 Morphometric measures of fruits and seed number per fruit of *Cedrela odorata*. Fifteen capsules were measured per each mother tree.

			Average of fruit size and weight			
Region	Population localities	Mother trees	**length (cm)**	**wide (cm)**	**weight (g)**	**Seeds per fruit**
Coastal plains	Las Mercedes (MER)	7	-	-	-	-
	Chindul (CHI)	7	3.4 ± 0.7	2.2 ± 0.5	2,7 ± 1.0	21 ± 11
Amazon lowlands	P. Napo-Ahuano (NAR)	20	4.3 ± 0.5	2.3 ± 0,3	4.5 ± 2.8	35 ± 10
	Yasuni SS (YAS)	20	3.5 ± 0.5	1.9 ± 0.3	2.4 ± 0.6	25 ± 8

Germination capacity test for seed lots of Cedrela odorata. Since collection time differs between localities (Table 2.5), the Amazonian seed lots have been stored for 8 to 23 months (in dark conditions and ~15 °C) and we are still waiting for collections of fruit/seeds in the Coast, we decided to carry out a test of seed viability. Seed tests are developed to evaluate germination capacity rates among seed lots of different mother

trees from NAR and YAS, with the corresponding storage time. Germination capacity of seed lots is recorded daily in four repetitions of ten seeds each and results are assessed for individual mother tree. The tests have been performed under laboratory conditions; paper was used as substrate. Preliminary results of the first repetitions show that seeds from NAR germinated precociously (Figures 5 and 6). The experiment is not finished and we do not have data about the variation of these results.

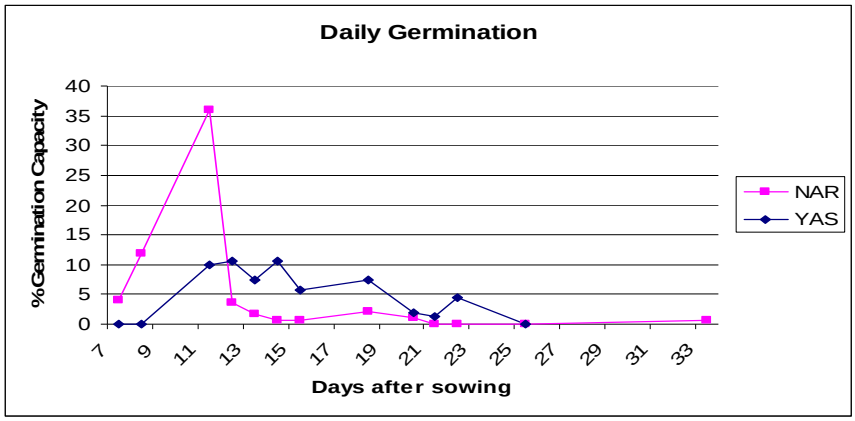


Figure 2.5 Mean daily germination capacity rates for two *Cedrela odorata* Amazonian localities: NAR (14 individuals) and YAS (16 individuals).

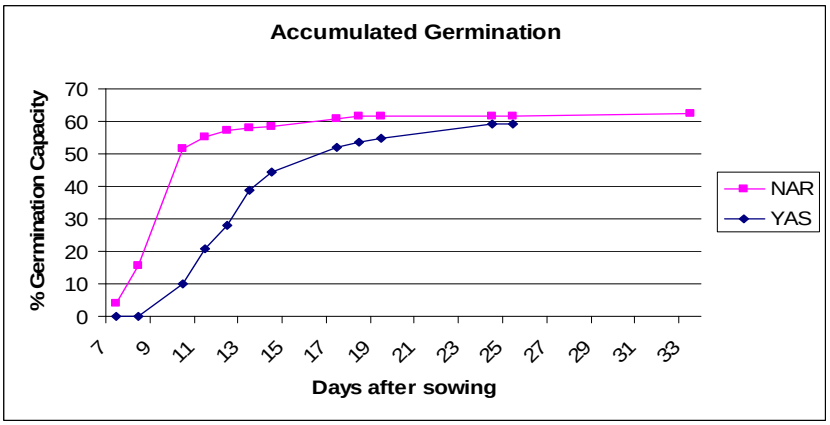


Figure 2.6 Average accumulated germination capacity rates for two *Cedrela odorata* Amazonian localities: NAR (14 individuals) and YAS (16 individuals).

Table 2.5 Collection period of fruits/seeds for RTE's in two species.

Species	Region	Study site	Collection season
Cedrela odorata	**Coastal plains**	CHI	Apr–May 2007
		MER	Apr–May 2008
	Amazonian lowlands	NAR	May 2006 and May–Jul 2007
		YAS	Jul–Aug 2007
Ochroma pyramidale	**Coastal plains and W slopes**	PED	Oct 2007
		PVM	Jun-Oct 2007
	Amazonian lowlands	ARC	Jan 2007
		YAS	Jan 2007

Research status with *Ochroma pyramidale*

We collected in year 2007 all the fruits needed for RTE's in the Amazonian localities (YAS and ARC), and had a large but incomplete collection from the Coastal sites where not all mother trees produced fruits. A monthly monitoring schedule was set until trees produce fruits. Because monitored trees were logged or, in other case, did not produce fruits for a long time, we collected trees from two new localities where fruits were available. The new study sites in the Coastal plains as well as the sites in the Amazon region are described in Table 2.3 and Figure 2.2 (see also Table 2.5).

Seed collection and characteristics in Ochroma pyramidale. Collections of seeds in the Amazon region (i.e., ARC and YAS) were completed in early 2007. In the Coast, we collected seeds from 12 mother trees in Camarones (by November 2006) and seeds from 7 mother trees (by April 2007) in Rio Silanche Reserve. Starting in January 2007 we carry out monthly visits to record phenology in these localities. In Camarones, however, nine of 16 monitored trees were logged and we had to move into a new locality: Pedernales (Figure 2.2 and Table 2.3). By October 2007 we completed a collection of fruits from 20 trees per population in each Coastal locality. Seed lots were stored in paper bags and kept in dark conditions at room temperatures (~15 °C).

Seed trials in Ochroma pyramidale. In mid January 2008 we finished seed extractions of *O. Pyramidale* fruits and the seed trial started in late February 2008. To date (30.04.08) we have completed three censuses of seed germination and seedling mortality.

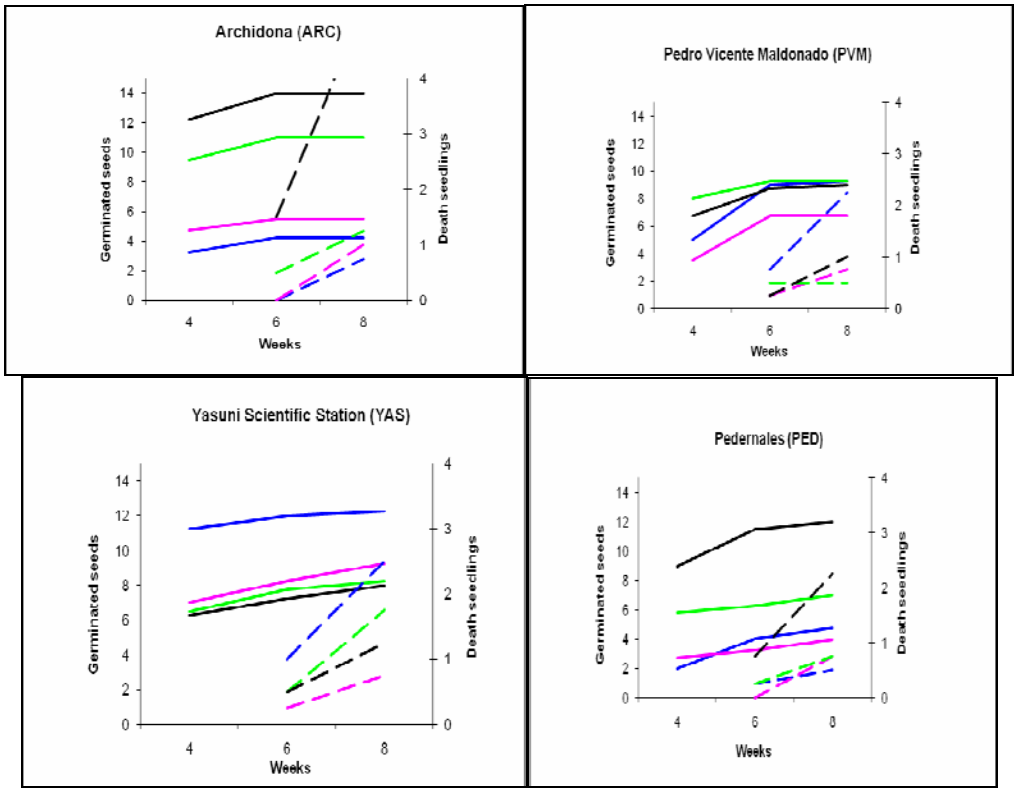
The most frequent finding is that the highest seed germination and the highest seedling mortality occur in the same treatment (i.e., soil type), except for PVM provenance. It is remarkable that the highest germination of a provenance occurred frequently in a different soil than in its own soil, except in ARC that grew better in its own soil and PVM that grew better in soils of YAS, PVM and ARC. However, the results are preliminary and careful analyses need to be done to see if variations between treatments are significant. Preliminary results are presented in Figure 7.

RTEs with Ochroma pyramidale. We built two nurseries and sowed seeds in plastic bags in the Amazonian localities in February 2008. We plan to start sowing in the Coastal sites in August 2008 to plant seedlings in the rainy season (around December).

Table 2.6 Morphometric measures of fruits and number of seeds per fruit of *Ochroma pyramidale* in seven Ecuadorian sites. PED, PVM, ARC and YAS are used in RTE's.

				Average of fruit size and weight			
Region	Population localities	Mother trees	Collected fruits	**length (cm)**	**wide (cm)**	**weight (g)**	**Seeds per fruit**
Coastal plains	Camarones	12	51	19,9 ± 5.0	2.5 ± 1.0	34.7 ±12	959 ± 294
	Pedernales (PED)	20	135	21.4 ± 2.6	2.8 ± 0,2	40.9 ± 6.9	118 ± 161
Inter-andean Valley	Salinas de Ibarra	5	16	17.7 ± 1.3	2.4 ± 0.9	29.7 ± 2.8	652 ± 124
Western slopes	Lita	4	19	15.9 ± 0.9	2.7 ± 0.4	31.2 ± 5.5	645 ± 142
	PV Maldonado (PVM)	20	94	19.74± 3.25	2.9 ± 0.4	40.5 ± 13.9	793 ± 323
	Archidona (ARC)	20	40	23.6 ± 3.0	3.0 ± 0.5	32.8 ± 7.9	631 ± 266
Amazon lowlands	Yasuni SS (YAS)	20	50	19.2 ± 6.8	2.4 ± 0.9	27.2 ± 12.5	554 ± 332

Figure 2.7 Seed germination (lines) and seedling mortality (dashed lines) found in a seed trial carried out in four provenances. Each line corresponds to different treatment: seeds of each provenance tested were sowed in home soil type and in the soils of the three other provenances. The color of each treatment is constant (PVM is blue; YAS is green; ARC is black; PED is pink). Note scales for seeds and seedlings are different.



3. Deviations from the project workprogramme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

CATIE : The chronogram of activities was affected by asynchrony in the phenological fruiting period, caused mainly by atmospheric changes. This affected both *Carapa guianensis* and *Vochysia ferruginea*, and meant that it was impossible to get seeds from different populations at the same time; additionally, those species have recalcitrant seeds, which cannot be stored in the seed bank, so collection of different populations at different times cannot be achieved.

PUCE: We have had great difficulties finding *C. odorata* trees in the Coast and then finding enough fruiting trees to collect seeds for RTEs. We are still monitoring trees in the Coast and plan to finish fruit collection in late June 2008. In case we do not obtain enough fruits, we will do the experiments with the Amazonian populations alone. The *O. pyramidale* seed trial is underway and we have started to sow seeds for RTEs in the Amazon sites. We will start RTEs in the Coast during August 2008 to coincide seedling transplants with the rainy season.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**1**	**Adaptive variation & genetic differentiation at rangewide scale**
Work Package	*3*	*Evolutionary history and developing regional markers for species*

1.Workpackage objectives and starting point of work at beginning of reporting period

- Evaluate chloroplast and nuclear nucleotide polymorphism and construct phylogeographic maps for the study species
- Evaluate DNA extraction methods from herbarium and woods samples for genotyping in comparison to DNA extraction of living tissues (for study species)
- Evaluate the possibility to identify species and range of species of interest based on genetic sequence data

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Objective 1: Evaluate DNA extraction methods from herbarium and woods samples for genotyping in comparison to DNA extraction of living tissues (Partner3)

During this 3^{d} period, we extracted DNA from herbarium samples collected by other partners. Although extraction kits were used, the quality of extracted DNA remains uneven between individuals according to the conditions of drying and conservation (chemical treatments) of herbarium samples.

Objective 2: Evaluate chloroplast and nuclear nucleotide polymorphism and construct phylogeographic maps for the study species

Screening of variation at cpDNA loci to identify 'universal phylogeography locus

- **Screening of variation at cpSSR loci (Partner 4)**

In addition to the samples analysed during the second year:

- 78 samples of *Socratea exorrhiza* from French Guyana (Partner 3);
- 42 samples of *Virola sebifera* from French Guyana (Partner 3);
- 10 samples of *Jacaranda copaia* from French Guyana (Partner 3)
- 30 samples of *Swietenia humilis* from 6 populations (Honduras, Nicaragua, El Salvador, Costa Rica) (Partner 2)
- 36 samples of *Bombacopsis quinata* from 7 populations (Honduras, CostaRica, Colombia, Nicaragua) (Partner 2)

during the third year we analysed:

- 116 samples of *Cordia alliodora* from 42 populations (**collaboration with Partner 2**).
- 1480 samples of *Cedrela odorata* (**in collaboration with Partner 1**)

Primers developed by Weising and Gardner (1999) (ccmp1, ccmp2, ccmp3, ccmp4, ccmp5, ccmp6, ccmp7, ccmp10) were used to amplify genomic DNA. Amplified products were run using a MegaBace 96 capillaries automatic sequencer, according to what reported in Heuertz *et al.* (2006).

Phylogeographic analyses concerning *Cordia alliodora and Cedrela odorata*

Cedrela odorata (**in collaboration with Partner1**) We amplified 480 samples with the primers ccmp 2, 3, 4, 5, 6, 10 and genotyping is in progress. Data are expected to be available within May 2008

Cordia alliodora (**in collaboration with Partner2**) : We analysed a total of 42 populations (116 individuals). Ccmp 1 and 4 resulted monomorphic. Ccmp 2, 3, 5, 6, 7 and 10 displayed 5, 2, 4, 2, 4, 2 variants respectively, combining into 18 different haplotypes. (Fig 3.1)

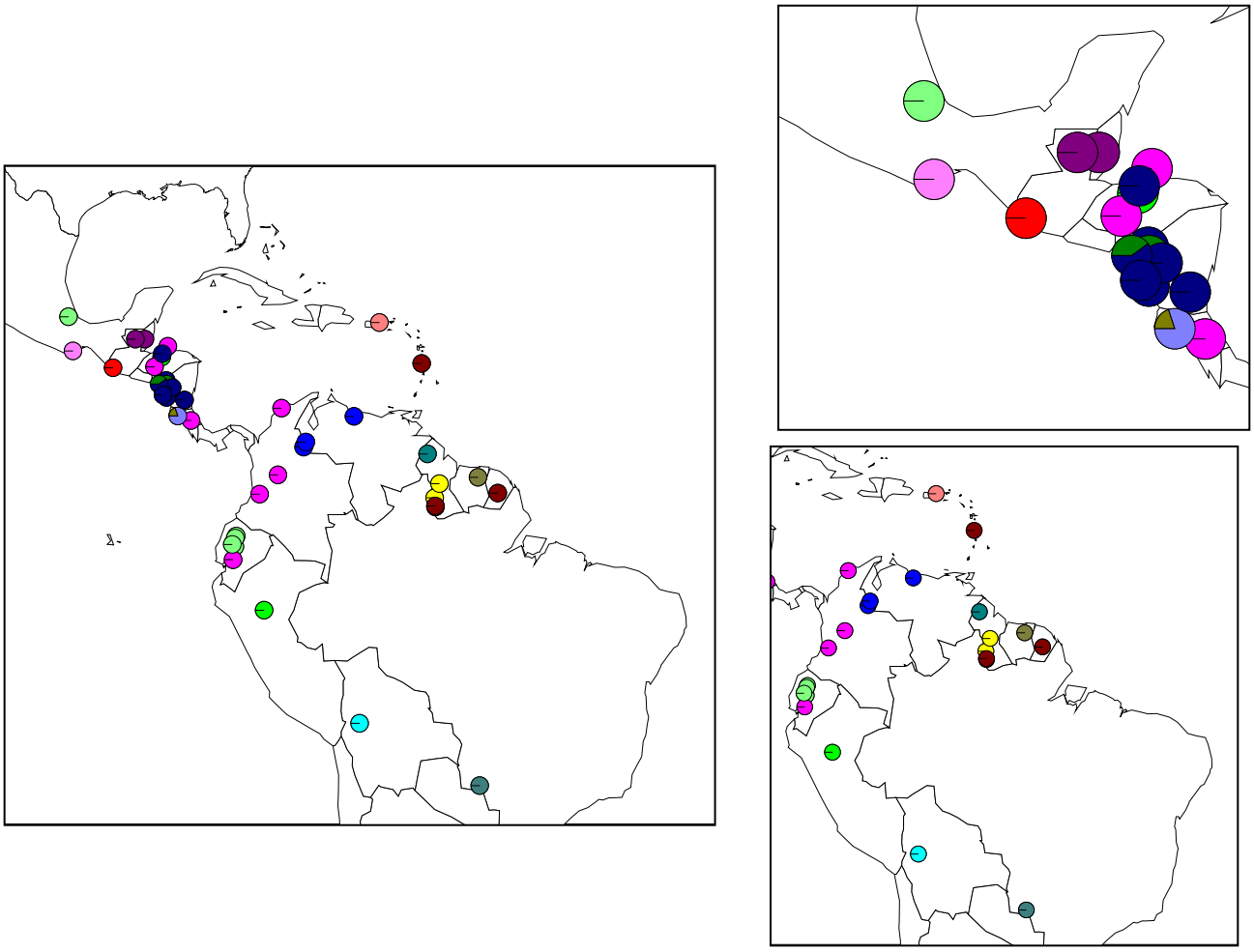


Figure 3.1: Phylogeographic analysis of *Cordia alliodora*, using cpSSRs.

Screening of variation at cpDNA loci by sequencing (Partner 3)

During the third meeting in Quito it has been recommended to use a common cp locus for all species: the intergenic sequence ***trnC-ycf6*** (see Shaw *et al* 2005).
In addition, the sequencing of **ITS** region was proposed.

We sequenced the ***trnC-ycf6*** fragment for 5 samples of each of the 11 species. We could observe 2 to 5 haplotypes in each species;

Tests for ITS amplification have been done. Specific PCR protocol seems to be required for each species in order to get single band amplification. The multiband pattern does not permit sequencing and needs PCR optimization. The study is in progress and results will be available in the next months

Phylogeographic analyses concerning *Simarouba, Carapa, Jacaranda*

- **At chloroplast regions**
  We selected two intergenic regions to conduct the phylogeography analysis : TrnCYcf6 and TrnHpsbA.
  For the three species, TrnHpsbA is the more polymorphic region due to the presence of microsatellites. Inconvenient of microsatellites is the difficulty to obtain good sequences. Taq polymerase mistakes often lead to sequence overlapping after microsatellite patterns.

Design of new primers for Herbarium Amplification
We designed new primers based on the polymorphism at TrnCYcf6 and TrnH-PsbA regions which was detected in individuals extracted from fresh material. These primers permit amplification of shorter sequences containing informative polymorphic sites.

Table 3.1 : List of newly designed primers:

	Jacaranda copaia		*Carapa sp*
TrnCYcf6	700 base pairs	TrnCYcf6	750 base pairs
HB_Jc_TrnCYcf6_1	148	HB_Csp_TrnCYcf6_1	136
HB_Jc_TrnCYcf6_2	193	HB_Csp_TrnCYcf6_2	100
TrnHPsbA	380	TrnHPsbA	500
HB_Jc_TrnHPsbA_1	150	HB_Csp_TrnHPsbA_1	141
HB_Jc_ TrnHPsbA _2	100	HB_Csp_ TrnHPsbA _2	135
		HB_Csp_ TrnHPsbA _3	182

The two sets of primers that were designed for *Jacaranda* herbarium have been already tested and PCR optimization is done. Primers for Carapa herbarium have still to be tested and optimized for PCR. Primers for *Simarouba* herbarium will be designed in the next weeks.

Phylogeography analysis at the continental scale

Phylogeographic study of *Jacaranda copaia* is almost done and will be published this year. Additional analyses are necessary for *Carapa sp* and *Simarouba amara*.

Table 3.2 : Number of haplotypes observed per population.

	(Number of individuals) and Number of haplotypes		
	Simarouba amara	***Carapa sp***	***Jacaranda copaia***
BG		ND	(1) 1
BO		ND	(2) 2
BRA	(2) 2	ND	(21) 8
CO		ND	(2) 2
CR	(2) 2	(1) 1	(9) 1
DG		(7) 1	
E	(2) 1	(8) 3	(10) 1
FG	(6) 3	(83) 5	(106) 4
PAN	(2) 1		(9) 1
PER	(1) 1		(4) 1
VEN		(3) 1	(1) 1
Global	(15) 8	(102) 10	(165)14

Jacaranda copaia

Polymorphism at the two intergenic regions revealed the presence of two lineages in the Amazonian Basin. Lineage A is located in the oriental part (Guyana shield, North of Brazil) and lineage B is located in the occidental part (E, PER, BO, CO,PAN, CR). The pattern suggests an independent history between the two lineages which could correspond to two events of colonization after a founder event.

Previsions for next month:
Sequences of Herbarium samples have to be completed in several populations (BRA, VEN, BG,DG,DG)

Simarouba amara:
The study is still in progress. A first test based on fifteen individuals from four populations revealed polymorphism (8 haplotypes) at TrnCYcf6 and TrnHPsbA. New primers will be designed in the next days to amplify herbarium samples.

Carapa sp
The study is still in progress. The phylogeography of Carapa is more complicated to conduct since there are two species in the Guiana shield: *Carapa procera* and *C. guyanensis*. The species classification needs a preliminary test of assignation based on polymorphism at seven microsatellite nuclear markers as published by Dumenil et al., 2006. Ten haplotypes have been observed in totality; five in French Guiana and three in Ecuador. As for *Jacaranda copaia* , the strong divergence between individuals from Guiana shield and Ecuador suggests two lineages with independent evolutionary history.

INPA: Screening of markers and analysis of variation at non-coding regions of chloroplast genome for phylogeographic analysis in *Minquartia guianensis*.

DNA was extracted from 155 samples from natural populations of *Minquartia guianensis* populations in Ecuador, Peru, Costa Rica and Brazil (Table 4). PCR conditions were optimized for amplification of four non-coding regions of the chloroplast genome of *Minquartia guianensis*. Table 5 shows the cpDNA markers optimized and the characteristics.

Table 3.3 – Natural populations of *M. guianensis* sampled for analyses in WP 3 and 5

Location	No. pops	No. individuals
Brazil	2	84
Ecuador	1 (?)	38
Costa Rica	1 (?)	11
Peru	1 (?)	22
Total	5	155

Table 3.4 – Characteristics of the chloroplast non-coding markers analysed for *M. guianensis*

cpDNA Region	Sequence	T_A (ºC)	Fragment size (bp)	Reference
psbA - trnH	CGAAGCTCCATCTACAAATGG ACTGCCTTGATCCACTTGGC	56	600	Hamilton, 1999
trnG - trnS	GAACGAATCACACTTTTACCAC GCCGCTTTAGTCCACTCAGC	57	600	Hamilton, 1999
psbB - psbF	GTTTACTTTTGGGCATGCTTCG CGCAGTTCGTCTTGGACCAG	63	850	Hamilton, 1999
trnL-F	CGAAATCGGTAGACGCTACG GGGGATAGAGGGACTTGAAC	56	850	Taberlet, 1999

Individuals from each of the three populations (Ecuador = 8, Peru = 8, Costa Rica = 10) of *M. guianensis* were sequenced for the four regions considering both directions using forward and reverse *primers*. Table 6 shows molecular diversity measures based on variation found in sequences of the non-coding region *psbB/psbF* of the chloroplast genome for the three populations of *M. guianensis.* For the other three markers tested *trnH/psbA*, *trnS/trnG*, and *trnL/trnF* no sequence polymorphism was found for the same individuals analysed from the three *M. guinanesis* populations.

Table 3.5 - Molecular diversity based on variation at *psbB/psbF* region for three populations of *M. guianensi*s using Arlequin software (Schneider *et al.*, 2001).

Variation	Peru	Costa Rica	Equador	Mean
Transitions	0	0	0	0.000
Transversions	1	0	1	0.667
Substitutions	1	0	1	0.667
Indels	1	1	0	0.666
No of observed sites	626	626	626	-
No. of usable sites	610	612	613	-

An Analysis of Molecular Variance (AMOVA) was performed based on the varitaion found at the *psbB/psbF* region for the three populations of *M. guianensi*s analysed (Table 7). The results

showed that most of the genetic variation was significantly found within rather than among populations.

Tabela 3.6 - Distribution of genetic variation in *M. guianensis* populations based on AMOVA (Excoffier *et al*., 1992).

Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	0.987	0.02458	8.03
Within populations	23	6.475	0.28152	91.97
Total	25	7.462	0.30610	

F_{ST}: 0.08029. P = 0.04594. Test: (1023 permutations).

Median Joining network analysis was performed based on haplotypes found by seqeuncing the region *psbB/psbF* of the chloroplast genome of *M. guianensis* (Figure 4).

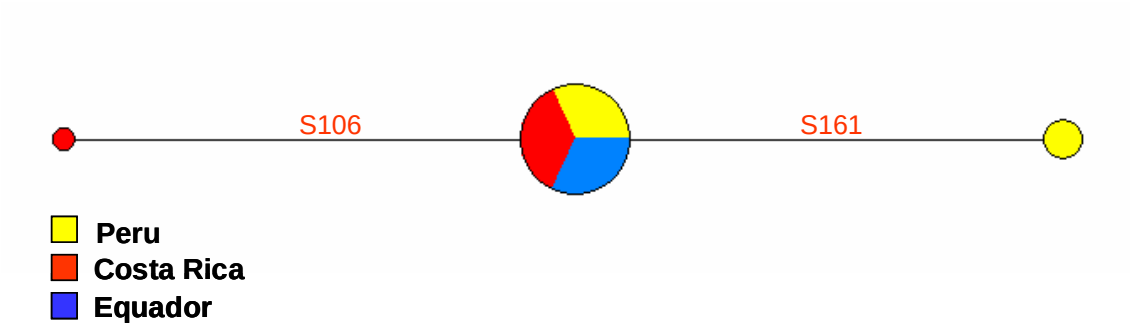


Figure 3.2 – Median-Joining network analysis based on *psbB/psbF* haplotypes observed for *Minquartia guianensis*.

Analysis of variation at non-coding regions of chloroplast genome for phylogeographic analysis in *Swietenia macrophylla*.

We performed sequencing analysis for three (psbA-trnH, trnG-S, ans psbB-F) out of four non-coding regions of the cpDNA of *Swietenia macrophylla***.** Four individuals from each of the six poplations (P. Bueno, RO, Brazil, Pontes e Lacerda, MT, Brazil, Chupinguaia, RO, Brazil, Mukwas, Nicaragua, Pocosol, Costa Rica, and Tonosi, Panama) analysed were sequenced in both directions using forward and reverse *primers* for the three markers. Sequences of *psbA-trnH* region showed very bad quality due to presence of many microsatellites. For the other two markers, no single variation was found for all sequences analysed.

Optimization of PCR conditions for amplification of chloroplast microsatellite loci in *Minquartia guianensis*.

We screened 14 cpDNA microsatellite markers, ten markers previously developed for *Nicotiana* and teted in other angiosperms (Weising and Gardner, 1999), and four developed for *Eucalyptus* by Steane *et al.* 2005. PCR conditions and annealing temperature were optimized for eight out of 14 loci tested for *Minquartia guianensis* (Table 8; Figures 5 and 6) for micrsosatellite analysis using fluorescent dyes.

Table 3.7 – Characteristics of chloroplast microsatellite loci optimized for *M. guianensis*

cpSSR Locus	Sequence 5'-3'	cpDNA region	Ta (ºC)	Fragment size (bp)
EMCRC67	6-FAM-CATCCTCAAATCCGTCCT TATTGCTTAGTCTGGCTTTTATG	atpF-atpH intergenic	56ºC	237
EMCRC74	6-FAM-GGC CGT GTA CGA GAA GTC AA CCA AGG GCT ATA GTC ATA GTG ATC C	trnT-psbD intergenic	57ºC	145
ccmp 1	GGCCGTGTACGAGAAGTCAA TET-CCAAGGGCTATAGTCATAGTGATCC	trnK	57 ºC	146
ccmp 3	CAGACCAAAAGCTGACATAG HEX-GTTTCATTCGGCTCCTTTAT	trnG *intron*	57 ºC	95
ccmp 4	AATGCTGAATCGAYGACCTA TET-CCAAAATATTBGGAGGACTCT	atpF *intron*	56 ºC	120
ccmp 5	TGTTCCAATATCTTCTTGTCATTT 6-FAM-AGGTTCCATCGGAACAATTAT	3' - rps2	56 ºC	104
ccmp 6	CGATGCATATGTAGAAAGCC 6-FAM-CATTACGTGCGACTATCTCC	ORF 77-ORF 82	58 ºC	102
ccmp 10	TTTTTTTTTAGTGAACGTGTCA HEX-TTCGTCGDCGTAGTAAATAG	rpl2-rps19	56 ºC	107

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**2**	**Diversity, reproductive performance & recruitment at the landscape scale**
Work Package	*4*	*Ensuring focus of quantitative and genetic studies*

1.Workpackage objectives and starting point of work at beginning of reporting period

- Ensure that criteria are agreed upon for design of sampling and experiments, and that response variables to be measured in order to characterize gene flow, genetic diversity, progeny performance and other response variables, are agreed upon by all partners in the project planning meeting.
- Prepare clear written guidelines for the research on the basis of agreements reached during the planning meeting, to be circulated to all partners and made available on the project website.
- Ensure that all WP leaders report regularly on progress and lessons learned to the leader of this CA, and that this information is shared among all those concerned.
- Try to make contact with other projects/institutes using similar genetic tools to quantify germplasm material.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

WP4 functions as a coordinating activity and as such progress reported in WPs 5 & 6 represents output for WP4. See following WP summaries for progress.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	2	Diversity, reproductive performance & recruitment at the landscape scale
Work Package	*5*	*Estimate partitioning of non-coding and coding genetic diversity*

1.Workpackage objectives and starting point of work at beginning of reporting period

- Development and optimisation of neutral and adaptive molecular DNA markers, with main emphasis on chloroplast and nuclear microsatellites and Single Nucleotide Polymorphism in candidate genes related to water stress responses and photoperiod induced phenology.
- Analysis of distribution of genetic diversity within species using neutral molecular markers
- Comparison of diversity in natural populations and in provenance trials for identifying hot spots of genetic diversity
- Analysis of diversity of candidate coding markers, and association between SNPs and Quantitative Traits Loci (QTL)

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved.

Development and screening of SNP markers

During the third year of the project, attention was focussed on the screening of SNPs in aquaporin genes.

Isolation of PIP genes from several tropical tree species, based on public sequence information, was successfully performed. This approach has allowed the cloning of 1-5 different genes per species, and the sequence information thus obtained was used for the development of gene-specific and species-specific primer pairs for the amplification of gene portions (see Figure 1). The PCR primer pairs developed in the first phase were applied to the amplification of genomic DNA from natural samples of the target species (*Carapa guianensis*, *Virola sebifera*, *Bombacopsis quinata*, *Eperua falcata*) in order to evaluate the potential of such gene fragments to provide sufficient genetic variation (**Partner 2, 3 and 4**). The PCR products obtained were sequenced and the sequences aligned within species. Table 2 shows the amount of polymorphism shown by each gene fragment and each species. Polymorphism was identified in all species, although its extent varies according to the gene and the species. In one case (PIP2.4) the same primer pair was used to amplify two congeneric species (*C. guianensis* and *C. procera)*, thus providing a direct comparison between orthologs. In addition the *Virola sebifera* primer pair was successfully transferred to *V. surinamensis, V. michelii* and *V. kwatae*. The extensive amount of sequence polymorphism detected was found to be evenly distributed across coding and non-coding regions (Table 1), and between synonymous and non-synonymous sites. This allowed preliminary tests on the demographic/selective significance of the observed polymorphism patterns. Preliminary results are displayed in Table 2. For some species, two subgroups of samples were generated in order to obtain genetically homogeneous populations (which is a prerequisite of Tajima's *D* and Fu's F_s tests; displayed as "pop 1" and "pop 2" in Tables 1 and 2). It was thus possible to compare populations having undergone different demographic and selective events.

Three genes show significant results for selection tests. The second population of *Carapa guianensis* contains only two haplotypes that lead to positive and significant Tajima's *D* and

Fu's F_S. These results could be interpreted as a contraction of population size (in terms of demographic event) or balanced selection (in terms of selective event). It is interesting to note that the eastern samples have been collected from a hybridisation zone between *C. guianensis* and the congeneric *C. procera*. The result of Bayesian assignment methods applied to independent loci (SSRs) shows, however, that these trees belong to *C. guianensis* populations, therefore pointing to a stable and selectively advantageous introgression of *C. procera* genes into a *C. guianensis* genetic background.

For *Bombacopsis quinata*, only Fu's F_S is positive and significant, and the same assumptions as for *Carapa* (*i.e.* contraction of population size or balanced selection) can be made. Although F_S is negative and significant for PIP1.1 gene in population 1 of *Eperua falcata*, it is in fact significantly higher than the expected values. Indeed, under neutral conditions *i.e.* considering only mutations and genetic drift but not recombination, simulated values of F_S are centered on zero, but when recombination rate is taken into account, the simulated values become negative. So the interpretation of this test is the same as for the previous ones. In conclusion, all departures from neutral expectations show the same trend, but we can't so far say if they are due to demographic or selective events. Later comparisons with neutral markers will distinguish selective and demographic events.

This study proves the validity of the method chosen for the isolation of gene fragments and demonstrates that the sequences thus obtained are useful for the detection of sequence polymorphism. The observed genetic variation was sufficient for the application of population-genetic analytical methods, in spite of the relatively small size of the selected gene fragments.

Table 5.1 Description of Polymorphism for each gene region.

Genes	Nr of sequences	Sequence Length(bp)	Nr of SNPs			
			Total	Intron	Exon	
					synonymous	non synonymous
Cg PIP1.5 pop 1	60	673	-	-	-	-
Cg PIP1.5 pop 2	12	673	8	7 (3 indels)	1	-
Vs PIP2.5	46	627	10	7 (2 indels)	2	1
Bq PIP2.5	32	513	16	10	6	-
Ef PIP1.1 pop 1	104	459	12	10 (2 indels)	2	-
Ef PIP1.1 pop 2	50	459	12	9 (2 indels)	3	-
Ef PIP1.2	206	521	11	8 (1 indel)	1	2
Ef PIP2.1	166	572	5	2 (1 indel)	1	2
Eg PIP2.1	194	671	14	7 (1 indel)	2	3

Gene names = first letter of genus and species names (Cg: *Carapa guianensis*, Vs: *Virola sebifera*, Bp: *Bombacopsis quinata*, Ef: *Eperua falcata*), followed by the name of the closest *Arabidopsis thaliana* Blast match. No polymorphism was observed in *C. guianensis* population 1.

Table 5.2 Genetic diversity and selection tests results for each gene.

Genes	Nr of sequences	Nr of haplotypes	θ_π	θ_S	Haplotypic Diversity *Hd*	Tajima's *D*	Fu's F_S
Cg PIP1.5 pop 1	60	1	0	0	0	-	-
Cg PIP1.5 pop 2	12	2	4,24	2,65	0,53	2,4 ***	7,51 ***
Vs PIP2.5	46	17	2,72	2,27	0,89	0,56	- 7,48
Bq PIP2.5	32	7	4,63	3,97	0,79	0,56	2,76 *
Ef PIP1.1 pop 1	104	19	3,27	2,30	0,87	1,11	- 4,20 **
Ef PIP1.1 pop 2	50	12	3,48	2,68	0,86	0,89	- 1,00
Ef PIP1.2	206	15	1,56	1,86	0,71	- 0,39	- 4,70
Ef PIP2.1	166	6	1,37	0,88	0,70	1,1	1,14
Eg PIP2.1	194	10	1,21	2,40	0,23	-1,24	- 2,11

Recombination rate was taken into account in the analyses. Test significance (P value) is indicated by asterisks (*: 5%, **: 1% and ***: 0.1%). (Cg: *Carapa guianensis*, Vs: *Virola sebifera*, Bp: *Bombacopsis quinata*, Ef: *Eperua falcata*)

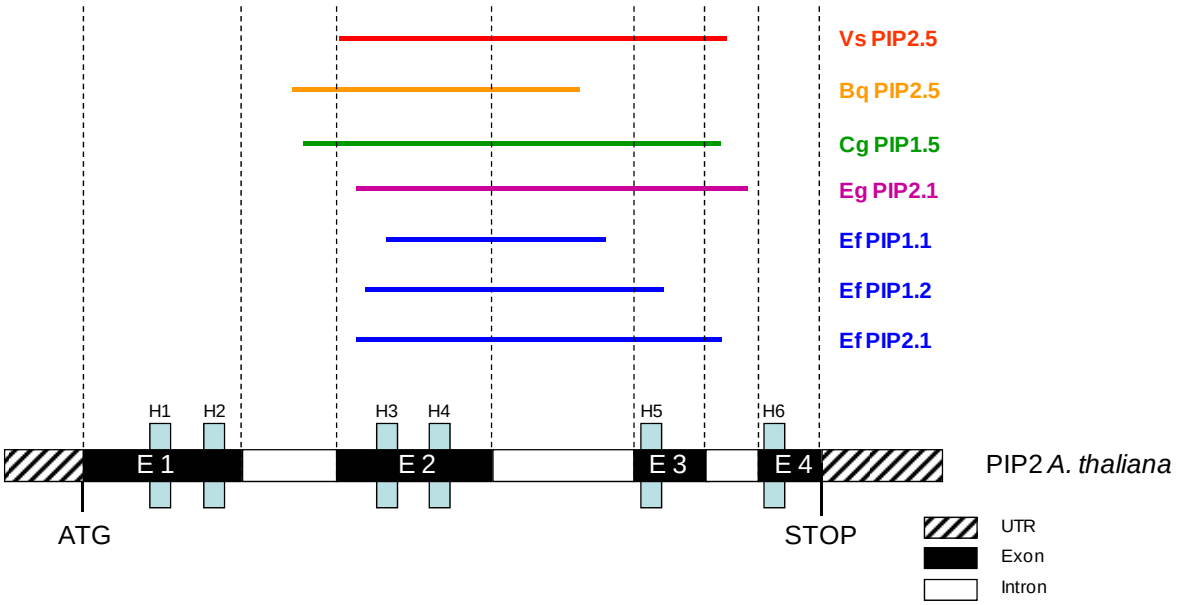


Figure 5.1 Representation of different fragment localities compared to Arabidopsis gene. Blue rectangles, located on the diagram of the Arabidopsis gene structure, represent the six transmembrane helices.

For *Cedrela odorata* three primer pairs are available to amplify three different aquaporin genes CodPIP2.1, CodPIP2.2, CodPIP2.3 (**Partner 1 and 4**). CNR screened variation in three samples per each of the following populations were analysed: Honduras, Nicaragua, Costa Rica, and two populations from Mexico. Amplified products were sequenced using a MegaBace 96 capillary automatic sequencer. Sequences were analysed with CodonCode Aligner to check for SNPs. CEH prepared sequence data for each of the three loci for further populations including Cuba, Peru, Brazil, yielding around 150 sequences. Datasets will be synthesised following the annual meeting in July and data analysis will be completed.

Development of nuclear microsatellite markers
Microsatellite enriched (di- , tri- and tetra-nucleotide repeats) libraries for 6 different species (*Cedrela odorata, Hymenaea courbaril, Ochroma pyramidale, Minquartia guianensis, Schizolobium parahyba, Drimis)* were constructed and screened (Partners 1, 2 and 4). Primers were designed and tested for quality and polymorphism in different populations (**Partners 1, 2, 4 and 8**). New nuclear microsatellites are available for the following species.

Cedrela odorata

24 primers were designed by **Partner 4** using the software Primer 3 and sent to **Partner 1** for test for quality and polymorphism. Nine gave clear, interpretable band patterns and were polymorphic. Polymorphism was tested in 487 individuals belonging to 12 populations distributed across Mesoamerica. The results are reported in a paper which is published in as: Hernandez G., Buonamici A., Walker K., Vendramin G.G., Navarro C. and Cavers S. (2007) Isolation and characterization of microsatellite markers for *Cedrela odorata* L. (Meliaceae), a high value neotropical tree. *Conservation Genetics*. 9 (2): 457-459.

Hymenaea courbaril

A library enriched in microsatellite was constructed by **Partner 4** following the method described by Edwards et al. (1996). A total of 181 clones were sequenced of which about 49% contained a microsatellite (SSR). More than half of them (about 70%) were discarded since the SSR stretches were too short or too complex or too close to the vector or because the clones showed sequence redundancy. In total 28 primer pairs were designed using the computer program PRIMER3 (Rozen & Skaletsky 2000). Eleven primer pairs generated easily scorable amplification products of the expected size while the others showed no amplification, multi-banding patterns, or too pronounced stutters.

Two populations samples in French Guyana and in Panama were analysed to detect polymorphism. The results are reported in a paper which is published online in *Molecular Ecology Resources* (Buonamici A., Cavers S., Vendramin G.G. Microsatellite loci isolated from the tropical tree Hymenaea courbaril L. (Fabaceae) Microsatellite loci isolated from the tropical tree *Hymenaea courbaril L.* (Fabaceae)).

Ochroma pyramidale

A library enriched only in GT and GA repeats was constructed also for this species (**Partner 4**): 12 primer pairs were tested, 4 worked, among which 2 displayed variation. In addition, a library enriched only in tri and tetra nucleotide repeats was prepared and two polymorphic tetra microsatellite markers were developed. Four polymorphic markers for *Ochroma pyramidale* are available so far. Work to identify an additional set of polymorphic SSR is in progress.

Minquartia guianensis

A library enriched for di- (GA, GT, AT, GC), tri- (CAA, ATT, GCC) and tetra-nucleotide (CATA, GATA, ATAG) of *Minquartia guianensis* was constructed according to Edwards et al. (1996) (**Partner 4**). Ten primers were designed and test to identify polymorphism is in progress.

Schizolobium parahyba

Two libraries enriched for di- (GA, GT, AT, GC), tri- (CAA, ATT, GCC) and tetra-nucleotide (CATA, GATA, ATAG) of *Schizolobium parahyba* and *Drimis* were constructed according to Edwards *et al*. (1996) (**Partner 4**).
For *Schizolobium parahyba*, 14 primer pairs were designed (**Partner 4**) and sent to **Partner 8** who tested them for their quality and variation. The results are reported in the Figure:

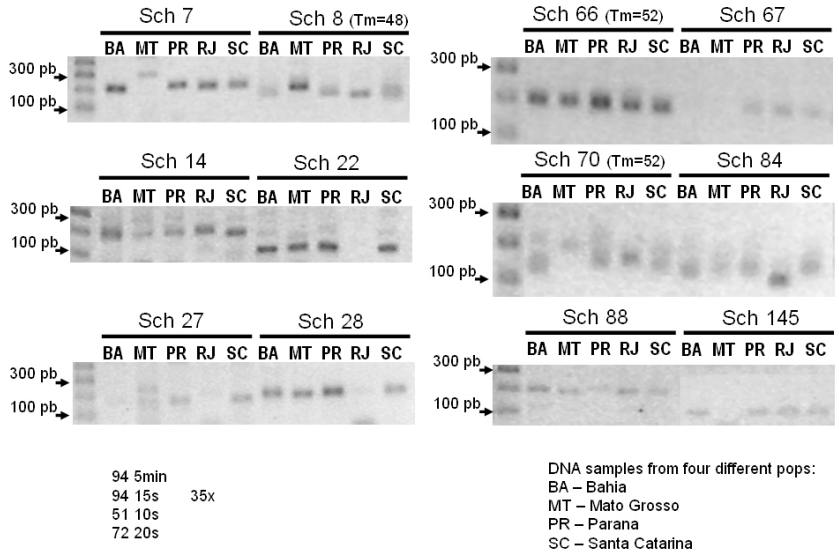


Figure 5.2: Validation of 12 new Schizolobium parahyba SSRs by PCR amplification

Genotyping of SSRs

***Hymenaea courbaril* (CNR)**
The eleven newly developed SSR primers were used to amplify 184 *Hymenaea courbaril* individuals. DNA was extracted and genotyping is in progress (**Partner 1 and 4**).

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**2**	**Diversity, reproductive performance & recruitment at the landscape scale**
Work Package	*6*	*Gene dynamics and quantitative seed performance in relation to landscape*

1.Workpackage objectives and starting point of work at beginning of reporting period

- Using polymorphic molecular markers compare the level of inbreeding for seeds, seedling recruits and adult trees for populations of three selected species occurring in primary forests and different man-made landscapes.
- Determine how landscape changes may affect the demography and the pollination system and their consequences for the mating system parameters.
- Determine how changes in local landscapes may affect the seed/fruit output of remnant adult trees and the population's recovery capacity.
- Relate how the mating system may contribute to shaping and maintaining any genetic structure of the populations
- Describe the implications of habitat disturbance and degradation on the long term conservation and management of selected tropical tree species
- Assess the impact of the mother trees local environment on seed set and progeny performance
- Assess the performance of selfed progeny relative to outcrossed progeny in species that show mixed mating
- Assess the impact of pollination distance on the quality of seed

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

CATIE:
Objective 1
During the current project period, characterisation and collection of foliage and/or cambium samples, begun during the previous period, was completed for twenty mother trees for all six of the study species except *C. guianensis,* for which only seven mothers could be located. So far, samples have been packed and labelled for two species, *D. panamensis* and *M. guianensis*. Processing of the respective permits required under national legislation for export of specimens to partners is in progress. The geographical location of all mother trees and the three or four closest conspecifics was determined for all species.

Objective 6
Species were assigned to one of the two study locations – Bijagual (Ultisols) and El Roble (Inceptisols) according to their soil preferences in natural forests of the landscape. A pasture and a forest fragment site are being used for the experiment at each location. Seedlings of *C. guianensis, M. guianensis, S. amara* (Inceptisols) *V. sebifera* and *D. panamensis* (Ultisols) were planted during this project period. A complete randomised block experimental design is being used with the individual seedling as the experimental unit. In pastures, planting distance is 1.5 m with seedlings being planted in such an arrangement that 3 m is maintained between conspecifics and 1.5 m between individuals of different species. In the forest sites, planting

distance is 2 m x 2 m due to lack of planting space and the expectation that growth will be slow.

Table 6.1 Study species and ecological characteristics most relevant to the present research.

Species and guild	Family	Sexual system	Seed dispersal	Seed viability	Phenology	
					Flowering	Fruiting
Non-pioneer						
Carapa guianensis	Meliaceae	Monoecious	Terrestrial vertebrates	Recalcitrant	Jan-Apr	Mar-Sep
Minquartia guianensis	Olacaceae	Hermaphrodite	Flying and arboreal vertebrates	Recalcitrant	irregular	Feb-Apr
Virola sebifera	Myristicaceae	Dioecious	Flying and arboreal vertebrates	Recalcitrant	Jan-Mar	
Pioneer						
Dipteryx panamensis	Fabaceae	Monoecious	Bats	recalcitrant	Currently being determined	Currently being determined
Simarouba amara	Simaroubaceae	Dioecious	Flying and arboreal vertebrates	Recalcitrant	Oct-May	Nov-Apr
Vochysia ferruginea	Vochysiaceae	Hermaphrodite	Wind	Recalcitrant	Apr-Jun Dec-Jan	Jun-Jul Set- Oct

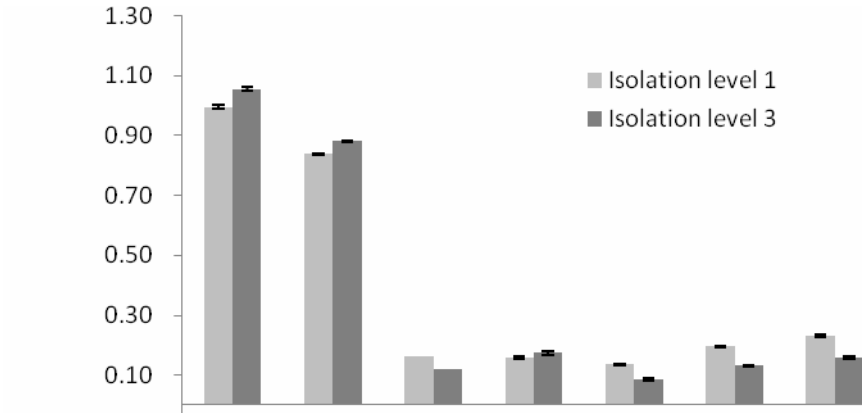
Measurements of seedling growth and survival were implemented at three-monthly intervals, and at the time of writing one measurement of *M. guianensis* has been completed, and three for each of the other species. Variables evaluated are root collar diameter, height (at apical meristem), tree form, condition and leaf number. In the forest sites tree crown illumination was assessed during the first measurement using an adaptation of the well-known five-category scale. Observations include recording of herbivore attack or other damage. Maintenance operations have been carried out in parallel with the measurements with chemicals being used only in the case of *S. amara*, which was attacked by a lepidopteran larva that caused severe damage to foliage and meristem in the pasture site. Seedlings of *V. ferruginea* are currently in the nursery awaiting planting.

Table 6.2 Number of mother trees collected and number of progenies planted, per species and habitat type.

Species	Forest		Pasture	
	N mother trees collected	N progeny planted	N mother trees collected	N Progeny planted
Carapa guianensis	2	122	6	182
Dypterix panamensis	17	619	20	625
Minquartia guianensis	20	695	20	708
Simarouba amara	20	451	20	468
Virola sebifera	17	0	16	362
Vochysia ferruginea	17	pending	20	pending

Cedrela odorata

Gustavo Hernandez (MSc student, CATIE) completed analysis of genotype data from progeny arrays of *C. odorata*, maintained in trials in Costa Rica. He analysed nearly 600 progeny seedlings in 70 families from mother trees in either forest or isolated landscape contexts. Small but significant differnces were found between isolation categories for the degree of correlated paternity although no differences were observed in terms of levels of outcrossing, or level of biparental inbreeding. Data are currently being united with growth data from earlier analyses to determine whether or not the analysed populations display differences in performance.



	tm	ts	ss	tm-ts	F	rp	rs
Isolation level 1	1.00	0.84	0.16	0.16	0.14	0.20	0.23
Isolation level 3	1.06	0.88	0.12	0.17	0.08	0.13	0.16

Figure 6.1: Mating system estimates for *Cedrela odorata*, from progeny trials in Costa Rica.

***Bombacopsis quinata* (OFI)**

Paul Rymer carried out DNA extraction, PCR, sequencing and scoring for 5 SSR loci on 24 seed from 20 trees in each of 2 populations in Costa Rica (dry forest – Lomas Barbudal Biological Reserve, pasture – Stewart property). DNA extraction from seed coats was tested as an aid to genotyping more parents for paternity analysis in the Lomas Barbudal population but failed to provide reliable maternal genotypes. David Boshier and Paul Rymer completed mating pattern, gene flow, Twogener and Neighbour analysis and are preparing a manuscript for publication.

An MSc student, Jared Honeycutt, genotyped progeny arrays (24 seed) of 19-21 mothers from three hills and isolated trees of *B. quinata* within the fragmented landscape in Punta Raton (Honduras) using 5 SSR loci. Mating pattern, gene flow, Twogener, and Neighbour analysis were completed and a manuscript is in preparation in conjunction with Paul Rymer and David Boshier.

An existing trial of *B. quinata* in Honduras was thinned and controlled F2 generation crosses carried out in Feb/March 2007, to examine the existence, scale and nature of outbreeding depression in this species. Fruit was collected, germinated and grown in the La Soledad (Honduras) nursery in May, and the common garden field trial established in July 2007. However the seedling failed to establish showing approx 90% mortality, due to a variety of reasons.

While seed material held at Oxford was not germinable, data collected by a previous PhD student, Martin Billingham, is being assessed for its use in providing estimates of relative fitness.

Stake material from the original trial was established in a clonal orchard to make repetition of the outbreeding depression study much easier .it wil however not be possible to carry this out within the timeframe of the project (i.e. Feb/March 2010).

Swietenia macrophylla (CATIE, UoA), Vochysia ferruginea (CATIE, CEH) Cedrela odorata (CATIE)

For *Swietenia macrophylla*, open-pollinated progeny arrays from a range of mother trees within different forest contexts (from intact forest to trees isolated in pasture landscapes) have been collected across Central America. Dr Carlos Navarro (CATIE) visited the University of Adelaide during October/November 2007 and undertook molecular marker analysis on this material together with Dr Mike Gardner and Prof Andy Lowe. The list of samples analysed in the table below. Seven previously developed microsatellites loci were used to assess variation in this progeny array and to calculate; outcrossing rate, selfing; biparental inbreeding and correlation of paternity. The data are currently being collated and analysed and results will be presented at eh 4th coordination meeting.

In addition, data already exist either from populations with different densities and logging regimes (CEH, *Swietenia macrophylla*), differential density post-logging site (INPA, *Swietenia macrophylla*), or differential densities and colonisation phase (CATIE and CEH, *Vochysia ferruginea*) and are being re-analysed for synthesis and publication as part of this WP.

Work that has examined the growth rate of progeny sampled from mother trees across a range of Central American populations and from different isolation and forests contexts has been analysed and written up for two publications by Drs Navarro (CATIE), Cavers (CEH) and Lowe (UoA). The first paper has been accepted in the *Journal of Sustainable Forestry*:

> **Abstract:** *Swietenia macrophylla* and *Cedrela odorata* are globally and locally important tree species because of their high quality wood. Both species have been severely exploited for export and internal markets and many populations are already extinct. Central America has been highly deforested for agriculture and other human activities and efforts to restore forest and increase connectivity between remnant fragments of forest are currently of widespread interest. A series of progeny trials were evaluated for variation due to region of collection and the forest context of the mother trees from which seed were sourced. Region of collection accounts for a large component of the variability indicating the importance of local adaptation. Forest context was not highly significant, but further studies on these areas with increased numbers of families in different forested and non-forested conditions are recommended. The results also indicate that mother trees (seed producers) that were isolated by more than 500 meters from the nearest conspecific tree produce progeny that perform less well than those that have pollen donors at shorter distances. This important result has substantial implications for future seed collections, i.e. isolated sources should be avoided. Furthermore, where possible, progeny trials should be re-evaluated taking the isolation level of the source trees into account. Such actions should also help prioritise efforts to prevent extreme fragmentation and isolation of trees, boost survival capacity of restored forests and avoid problems resulting from a dependency on isolated trees for restoration of degraded areas.

The second has been submitted as a short note to *Ecology*.

> **Abstract:** Forest fragmentation has a significant impact on the mating system and gene flow of many neotropical trees. In particular, the practice of retaining valuable trees in pastures can cause extreme isolation, with significant potential impacts on progeny performance. To address this issue, which has significant implications for tree restoration and plantation, the growth performance of progeny in relation

to tree isolation was assessed for two neotropical timber species. Overwhelmingly, progeny collected from isolated trees performed less well than those sourced from trees clustered with conspecifics, indicating that collectors need to be mindful of tree isolation when sourcing seed.

Dr Sam Davies, together with Drs Stephen Cavers, Bryan Finegan, Andy White and Andy Lowe have written up and submitted work on *Vochysia ferruginea* to *Molecular Ecology* (see below).

Abstract: In forests with gap disturbance regimes, pioneer tree regeneration is strongly linked to canopy disturbance, and is typically abundant following stand-replacing disturbances, whether natural or anthropogenic. Differences in pioneer tree density, linked to disturbance regime, can influence pollinator behaviour and so impact gene flow dynamics within pioneer populations. Here we investigate the consequences of secondary forest colonisation on mating system, pollen-mediated gene flow and progeny fitness for the long-lived pioneer tree, *Vochysia ferruginea*, at two Costa Rican sites. Five microsatellite loci were employed in a screen of adult and seed cohorts from primary forest with a low density of the species, secondary forest with high density, and isolated individuals in pastures. All forest contexts sampled exhibited predominant outcrossing (average *tm* 0.9468) and a low frequency of selfing (average *rs* 0.073), although secondary forest populations exhibited elevated levels of biparental inbreeding (average *tm-ts* 0.105 compared to 0.0605 in primary forest). There was evidence that pollen dispersal distances are shorter in dense secondary forest populations, with estimates of mean dispersal up to 12.85 m compared to 2431.3 m in primary forest, suggesting that biparental inbreeding is likely to be a permanent feature of dense secondary forest populations of this species. The degree of biparental inbreeding was negatively correlated with stem diameter growth rate>=10 cm dbh, though not significantly. Our results provide evidence that this pioneer species may not be sensitive to biparental inbreeding effects, due to repeated genetic purging events resulting from colonisation bottlenecks experienced as part of *V. ferruginea*'s pioneer lifestyle.

Provenance Trial of Brazil-nut tree (*Bertholletia excels*a): Growth monitoring and data collection (diameter and height, survival).

- Survival count gathered showed low mortality after one year of plantation (Figure 13). Oriximiná provenance has the highest survivorship (98.1%), followed by Itacoatiara (95.1%), and Costa Marques (94.6%). The differences were statistically non-significant.

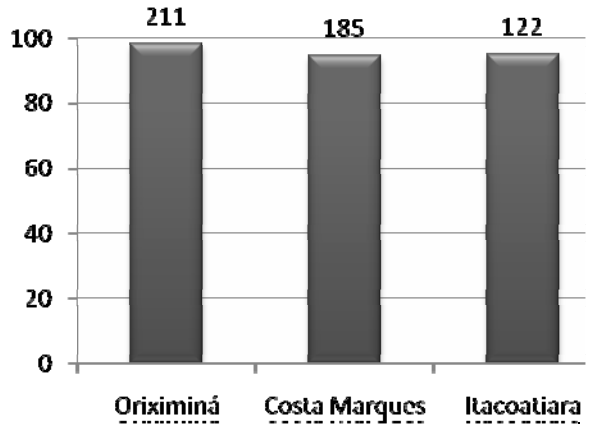


Figure 6.2 – Seedling survival (%) for three provenances of *Bertholletia excelsa* in a two-years-old plantation. Values above bars denote number of seedlings planted per provenance.

- Among the three provenances, Costa Marques obtained the higher performance in terms of height (4.67 m) and diameter (2.1 cm) growth (Figure 14a and b).

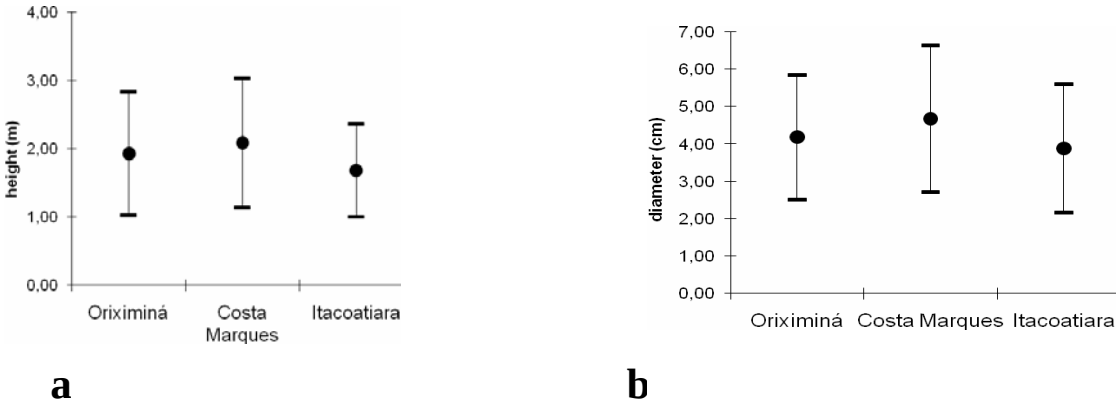


Figure 6.3 – Height growth (a) and diameter growth (b) for three provenances of *Bertholletia excelsa*. Two-years–old plantation. Vertical bars represent ± one standard deviation.

Genetic analysis: Mating system of *B. excelsa*

Sampling – Leaves were collected from 55 mother trees and 730 progenies (mean of 16 sedlings per mother tree) perfoming a total of 55 families established in the field for the provenance trail experiments. The material was collected from three locations in the Brazilian Amazon (Itacoatiara, AM, Costa Marques, RO and Trombetas, PA), as especified in the previuos annual report (2006 – 2007). These families were sampled for the mating system analysis of *B. excelsa.* **DNA extraction –** Total genomic DNA was successfuly extracted for 49 mother trees and 457 progenies using standard CTAB protocol. Figure 15 shows the quantification and quality of the extracted DNA´s in agarose gel for three families (mother tree + 16 progenies) of *B. excelsa* from different locations. **Microsatellite analysis.** We have optimized a high throughput genotyping system by multiplexing seven microsatellite loci for mating system analysis of *B. excelsa* (Figure 16). Figure 17 shows electropherograms with allele resolution for homozygous and hetereozygous individuals of B. excelsa for seven microsatellite loci analysed.

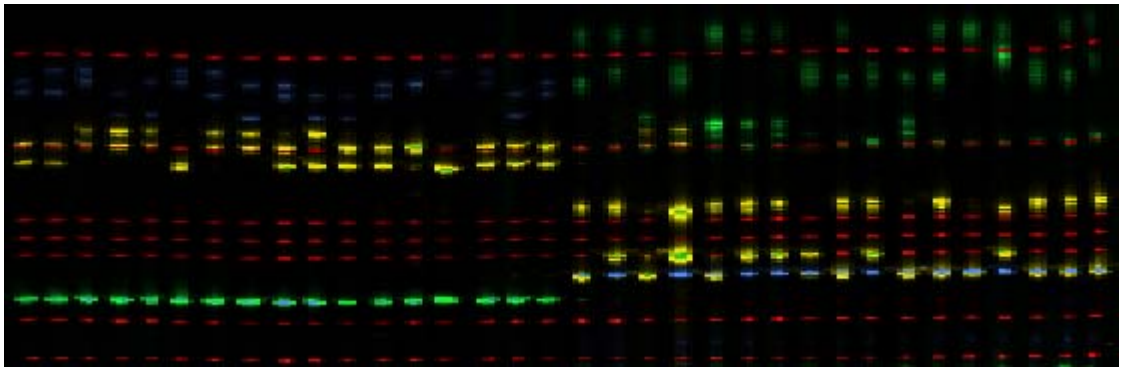


Figure 6.4 - Multiplex system showing alele resolution for seven microsatellite loci simultaneously analysed for one family of *B. excelsa* in a single gel.

Genotyping - Table 10 shows allele size range and number of allles found for seven SSR loci after genotyping of *B. excelsa* families. Up to now 136 individuals of *B. excelsa* from eight

families (Costa Marques, RO) were genotyped for seven SSR loci. The total number of alleles found was 50 and number of alleles per locus ranged from 2 to 12.

Table 6.3 – Allele size range and number of alleles (A) found per SSR locus after genotyping individulas of eight families of *B. excelsa* from Costa Marques, Ro, Brazil.

SSR locus	A	Size range (bp)
BEX01	10	218 - 250
BEX02	4	112 - 118
BEX12	4	200 - 208
BEX22	11	127 - 169
BEX32	2	128 - 142
BEX33	12	203 - 261
BEX37	7	189 - 209
Total	50	-

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

CATIE: Carolina Cascante´s contract expires on May 31st 2008, and as a substantial amount of work remains to be done for WP 6, B. Finegan is identifying funding sources for her to be contracted as a consultant in order to complete the work.

Unpredictable flowering and fruiting in the study landscape required an additional change in the study species, and the definitive list of six species is shown in Table 1. *Vochysia ferruginea* was reinstated to the group of pioneer species as it proved impossible to collect sufficient seed of *Jacaranda copaia*, while *V. ferruginea* flowered and fruited with sufficient intensity to make seed collection following the WP´s sampling design possible.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**3**	**Analysis and prioritisation of regional and local sourcing strategies**
Work Package	*7*	*Data compatibility*

1.Workpackage objectives and starting point of work at beginning of reporting period

- to coordinate information acquisition of molecular and phenotypic data (WP1-WP6), prior to data analysis (WP8).
- to organize data flow among partners through appropriate tools, for estimating relevant genetic, ecological parameters prior to modelling (WP10)

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Data compatibility issues were discussed, and solutions agreed on, at the previous meeting. The proposals submitted to the partnership have been accepted, with the implicit assumption that any pending proposal for protocol modification would be submitted to general discussion. To this date, no modification has been submitted and therefore the proposal is considered as accepted. However, data sharing and the actual implementation of these guidelines have been blocked so far due to discussions on the partnership agreements on material transfer and data sharing. Most of the partners have thus retained the information concerning the samples they have provided and the feasibility of a common frame for data handling could not be directly verified.

The following guidelines were adopted by the partnership:

1. Collecting cross-coherent sets of data in like experiments, with special reference to sample collection for genotyping and seed collection for reciprocal transplants and seedling performance experiments.

2. Recording data using a common coding standard, with special reference to units of measure for seedling performance experiments and sample collections and to genotypes and DNA sequence positions for informative polymorphisms in within- and between-species phylogeographic comparisons and PCR primer sites for gene sequencing.

3. Making the above conventions compatible with the needs of modelling actions, that is, recording the data in the appropriate digital format and following the most appropriate layout for data bases.

Decisions made on **point (1)** confirmed previously discussed strategies, which are summarised here.

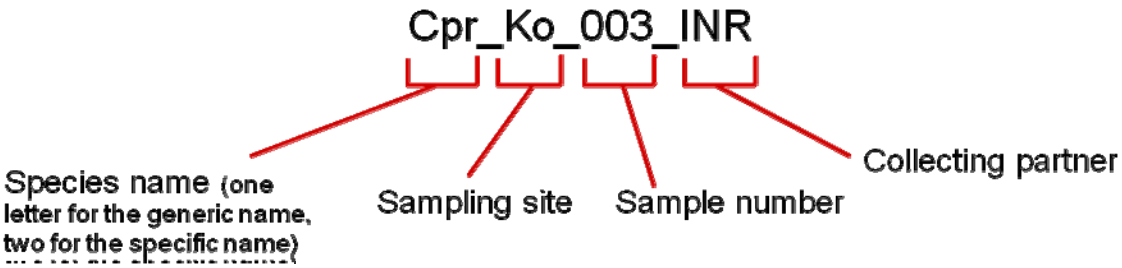
- The following data should be recorded for each field sample (wood and leaf tissues, seeds from a mother tree):
  - Exact GPS position
  - DBH
  - Distance to nearest 3 trees of same species
  - Foliage projective cover (canopy density) around tree
  - Basal area around tree
  - Fruit output

It will not be possible to have all the data for all samples, but this should be the reference data list.

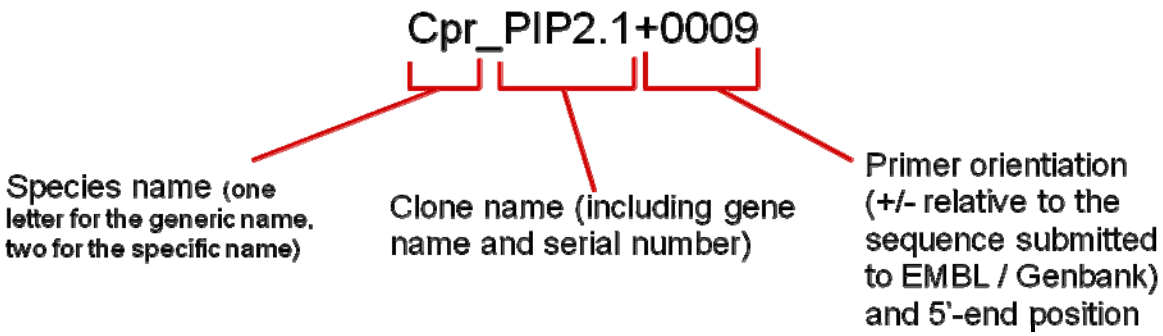
- Additional data that should be collected for each sampling site:
  - Pedology
  - Hydrology

As for **point (2)**:

- Units of Measure shall be the same for all partners; conversions should be applied prior to data transfer. The proposed units are those of the International System (m, s, g, N…), or the internationally agreed standard for any single quantity, if it exists.
- Sample names: the following naming pattern is adopted in order to take into account species, sample, provenance, and operator identity:



- Coherence of genotyping and sequencing data:
  - Microsatellite data: alleles to be recorded as fragment sizes (and not as repeat numbers).
  - Chloroplast PCR-RFLP data: genotyping profiles shall be recorded as fragment lists with molecular sizes for each fragment, within the precision limits imposed by the chosen detection system (agarose gels, acrylammide gels, automated gel slab or capillary sequencers).
  - Chloroplast sequence data: recorded as whole sequence and/or as SNP position with the associated nucleotide identity. For the latter case, SNP positions will be provisionally recorded relative to the position of the “forward” priming site as identified in the sequences that will be submitted to GenBank / EMBL prior to publication (as required by all publishers). It is recommended that the long term, stable coding convention for these sequences is to refer them to a reference chloroplast sequence (e.g. *Arabidopsis* or tobacco complete chloroplast sequence).
  - Nuclear sequence data: recorded either as diploid sequence or as SNP sites. The position of SNP sites is coded as a reference relative to the unique clone of the gene that has been used to define PCR primers for fragment amplification and sequencing, and that will be submitted to EMBL / GenBank prior to publication. The following convention is applied to the naming of gene-based primers:



- For both chloroplast sequence and gene sequence polymorphism data, when coded as SNP polymorphisms, the international standard for the description of mutant haplotypes will be applied.

For **point (3)**: in the absence of actual data sets to submit to the model for parameter estimation, only general guidelines are issued. Partners should record their data in the spreadsheet format, of their choice.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**3**	**Analysis and prioritisation of regional and local sourcing strategies**
Work Package	*8*	*Meta-analysis of data*

1.Workpackage objectives and starting point of work at beginning of reporting period

To analyse data produced in WP2 – 6 in a way that will be productive towards the goals of the project and useful for dissemination purposes, as identified in core area 4

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Building on syntheses of the available literature a review was prepared and has been published by Dick et al, on the spatial scale of seed and pollen-mediated gene flow in tropical forest trees, and will be disseminated to partners during the 4th coordination meeting.

> **Abstract:** Gene flow via seed and pollen is a primary determinant of genetic and species diversity in plant communities at different spatial scales. This paper reviews studies of gene flow and population genetic structure in tropical rain forest trees and places them in ecological and biogeographic context. Although much pollination is among nearest neighbors, an increasing number of genetic studies report pollination ranging from 0.5-14 km for canopy tree species, resulting in extensive breeding areas in disturbed and undisturbed rain forest. Direct genetic measures of seed dispersal are still rare; however, studies of fine scale spatial genetic structure (SGS) indicate that the bulk of effective seed dispersal occurs at local scales, and we found no difference in SGS (Sp statistic) between temperate (N = 24 species) and tropical forest trees (N = 15). Our analysis did find higher genetic differentiation in tropical trees (FST = 0.182; N = 42) than in temperate forest trees (FST = 0.116; N = 82). This may be due to the fact that tropical trees experience low but significant rates of self-fertilization and bi-parental inbreeding, whereas half of the temperate tree species in our survey are wind pollinated and are more strictly allogamous. Genetic drift may also be more pronounced in tropical trees due to the low population densities of most species.

The following paper was resubmitted to *Ecological Applications* as it was considered too long for the first journal it was submitted to, *The Journal of Applied Ecology*: Linda M. Broadhurst, Andrew J Lowe et al., on maximising evolutionary potential in broadscale restoration.

> **Abstract**: To combat broadscale environmental degradation, large areas of land need to be restored, at scales ranging from 100's - 1000's of ha. to entire river catchments. This broadscale restoration practice demands collection and deployment of large quantities of seed (germplasm, propagules). Sourcing of this material raises important questions related to seed quality, sustainable harvest, provenance selection, and genetic pollution and hybridisation. Restricting collections to local sources in an attempt to maximise local adaptation can confine collectors to small remnants where there is limited seed of poor genetic quality while imposing significant economic costs and high harvesting impacts. And while there is scientific evidence that some species benefit from local seed sourcing, application principles are often overly restrictive (contained to a few km), and recent evidence indicates that for restoring degraded landscapes, issues pertaining to seed quality may be far more important. Moreover, selection regimes in landscapes most needing restoration have been modified by habitat destruction, fragmentation, altered disturbance regimes, invasive species, changed soil resources and climate change. This negates the role of local adaptation. For three specific topic areas; 1. appropriateness of using 'local' seed, 2. sample sizes required to capture sufficient genetic diversity to establish self-sustaining populations, and, 3. the impact of over-harvesting source populations, this review examines; i). current collection guidelines, ii). the scientific evidence supporting these guidelines, iii). whether the guidelines can be improved in light of more recent evidence, iv). the consequences of not improving the guidelines, and iv). where knowledge gaps exist. In addition, where sufficient information exists, we review the potential impacts of climate change. Based on this review, we find that local seed sourcing practises will in many cases lead to poor restoration outcomes, particularly for broad-scale initiates. We suggest that seed sourcing should focus less on local collection

and more on increasing the quantity available and maximising genetic quality of seed so as to maximise the adaptive potential of restoration plantings to current and future environmental conditions and change.

Components of the composite provenancing strategy for the above paper were prepared for a separate paper and submitted to *Wingspan* as: Lowe AJ. Composite provenancing – progressing the 'local is best' paradigm for seed sourcing.

GENEO-TROPECO meta-analysis

A final analysis of the GENEO-TROPECO AFLP data has been prepared. During the 4th meeting partners will hold a workshop session to present the data and work out life history traits for correlation analysis.

Publications arising from work in WP8

Dick CW, Jones FA, Hardy OJ, Petit R (2008) Spatial scales of seed and pollen-mediated gene flow in tropical forest trees. *Tropical Plant Biology* 1: 20-33.

Broadhurst LM, Lowe AJ, Coates D, Cunningham S, McDonald M, Vesk P, Yates C (submitted) Maximising evolutionary potential in broadscale restoration. *Ecological Applications.*

Lowe AJ (submitted) Composite provenancing – progressing the 'local is best' paradigm for seed sourcing. *Wingspan.*

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**3**	**Analysis and prioritisation of regional and local sourcing strategies**
Work Package	*9*	*Selection and definition of resource priorities*

1.Workpackage objectives and starting point of work at beginning of reporting period
Computer simulation studies will be done:

- to estimate the impact of different strategies of natural and artificial regeneration on the genetic structure of a tree population,
- to predict the impact of gene flow and local selection on the genetic structure of tree populations of adapted (autochthonous) and non adapted origin
- to design optimal seed harvesting strategies that maintain the genetic diversity of the original stands in the regeneration material.
- to estimate the impact of transfer of seed between different ecozones as defined by the quantitative assessment of provenance material and reciprocal transplant experiments (WP2) and genetic diversity and gene dynamic estimates (WP3 and 4).

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

The only contribution to WP9 came as planned from the BFH. The main objective of this WP is to perform a further development and application of the simulation model Eco-Gene and to develop a new model for simulations on a larger spatial scale.

Deliverable 23: During the third 12 months of the project additional changes have been made on the simulation model Eco-Gene. Modules to simulate genetic controlled self-incompatibility systems and to simulate vegetative regeneration have been added.

The manuscript submitted during the second report period on the application of Eco-Gene to estimate the impact of selective logging on the genetic diversity and demographic structure of tropical tree species in the in Brazil has been improved according to the reviewers suggestions. The paper is accepted now

Main output of these activities is the paper:

Alexandre M. Sebbenn ab, Bernd Degen b, Vânia C.R. Azevedo c, Marivana B. Silva d, André E.B. de Lacerda e, Ana Y. Ciampi c, Milton Kanashiro e , Francimary da S. Carneiro e , Ian Thompson e, Marilyn D. Loveless (2008): Modelling the long-term impacts of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. Forest Ecology and Management (254), 335-349

Deliverable 24: The work on the new multi-species, multi-scale version of Eco-Gene which is linked the Geographic Information System ArcView has been continued. The main advance that has been made together with Alexandre Seebben is the integration of a new data-generation-engine into the large scale multi-species version of Eco-Gene. Now it is possible to use aggregated data on landscape structure, species abundance, diameter distribution and allele frequencies to generate the needed complex input data for simulations with this model.

As next and remaining steps has to be listed:

- Inclusion of functions on the competition among species/ species groups
- Module on seed harvesting strategies
- Module on landscape management
    - deforestation and reforestation
    - plantations with improved seed material
    - selective logging
    - clear cuts

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**4**	**Knowledge gathering, integration and dissemination of priorities**
Work Package	*10*	*Communication of biological and socio-economic information*

1.Workpackage objectives and starting point of work at beginning of reporting period

- To act as a promotion pathway for the outputs of the research.
- To facilitate feedback from end-users to ensure the relevance of the research, and that the implications of the research can be incorporated as realistic management practices.
- To ensure the production of technically accurate extension materials

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

The Central American survey carried out in the first year identified critical issues with respect to the sustainability of past dissemination efforts related to forest genetic resources. It was agreed that Seedsource emphasis should be on tertiary education teachers where there is greater stability of personnel and a higher probability that any materials and training produced by the project will be utilised beyond the project's funding timeframe. Allied with the yearly influx of fresh students, teachers offer greater prospects for sustainability and outreach in disseminating messages on forestry genetics conservation and diversity, compared to fellow forestry professionals in both public and private organisations. This has led to a clearer targeting of efforts within WPs 11 and 12 (see these WPs for more details). The joint initiative with Bioversity International (formerly IPGRI) to develop stand-alone training support materials will also ensure that the materials have both a longer lasting and wider geographical uptake than would have been possible working alone.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**4**	**Knowledge gathering, integration and dissemination of priorities**
Work Package	*11*	*Knowledge gathering*

1.Workpackage objectives and starting point of work at beginning of reporting period

- Produce list of potential species from which 12 will be chosen for case study in the project and 50 will be chosen for Central and South America for guideline dissemination according to analytical results of the project.
- To identify how germplasm collection and management practices vary with respect to species' regeneration characteristics, socio-economic importance, and the agroecosystems/forest types in which they occur.
- To provide feedback from end-users on research scope, methods and outputs to ensure that it is fine-tuned to the issues they face.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

The workshop in Ecuador was held in July 2007 in conjunction with WP 12 and followed the same format as those in Central America. Project personnel from CATIE (Carlos Navarro) and OFI (David Boshier) assisted those from PUSC (Renato Valencia, Galo Buitrón, Juan Iglesias, Daniela Cevallos, María Dolores Proaño) in facilitating the workshop with the participation of 13 tertiary educators and seed collectors from Ecuador (see WP12 report for more details). The workshop highlighted similar issues to the workshops held in Central America. In particular the lack of access to suitable materials (appropriate level of writing and language) for many university teachers was raised, along with differences between forestry and biology courses in the coverage of forest genetic resources.

In Ecuador Seedsource personnel attended meetings of the Biological Society and the National Forest Inventory Strategy, and explained the project's activities. Carlos Navarro (CATIE) participated in a roundtable discussion on the role of conservation and adequate use of genetic resources within forest management (in conjunction with Bioversity Internacional) in the IUFRO Latin American Congress in Mérida.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

None

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

CORE AREA	**4**	**Knowledge gathering, integration and dissemination of priorities**
Work Package	*12*	*Preparation and dissemination of extension materials*

1.Workpackage objectives and starting point of work at beginning of reporting period

- To produce and disseminate extension materials based on the projects (and other relevant research) results in a range of formats and levels, appropriate to a range of target end users.

2. Progress towards objectives – tasks worked on and achievements made with reference to planned objectives, identify contractors involved

Description of work

A 1 day workshop was organized (July 2007) in Ecuador, with 2 main objectives:

- to understand the information needs of tertiary education teachers and germplasm collectors on Ecuadorian native tree germplasm, and to explore different ways to present information from research, to thus obtain guidelines for future dissemination materials;
- to draw up a strategy to establish sustainable dissemination pathways, and to discuss the collaboration and task sharing necessary to implement the proposed strategy.

As such the workshop covered activities in WPs 10-12, but is reported here for convenience. Three project members (David Boshier, Carlos Navarro, Renato Valencia) facilitated the workshop with some 13 teachers/seed collectors attending from the main relevant educational institutions in Ecuador. The workshop was divided into 4 sessions with participatory activities: presentations, individual surveys, revision of didactic materials, and plenary and group discussions.

The results showed ample scope for dissemination of existing and future Seedsource research results through a variety of current courses: Ecology, Natural Resources Conservation, Endangered Native Species Conservation, Silviculture, Forestry Seeds and Nurseries. Forest Genetics Conservation and Forest Genetic Improvement were considered as the most appropriate courses, but they are only offered in post graduate degree programmes. Students have access to computing and the Internet, but resources are often insufficient, not free, out of date, or simply do not work. Teachers enjoy better access to these resources. There is in general free access for all to libraries and documentation, although the offer is somewhat limited and outdated. Teachers use a wide range of aids, according to individual institutional resources. In general, no differences were found between institutions with respect to the courses and educational levels offered, computing and documentation access, or teaching aids. However, a huge resource gap was found between individual institutions.

The participants outlined the principal ideal characteristics of forestry related didactic materials for students and documentation for teachers. They grouped real examples of seed collection and management materials by their utility to students and/or teachers, setting some guidelines for future Seedsource research results dissemination materials. This identified strengths and weaknesses for transferring information as viewed by users.

A consensus was reached on teaching forestry genetics resources conservation and diversity in curricula across a range of courses, under the individual initiative of teachers or departments, introducing concepts in specific lectures or seminars within courses or modules. It was not deemed essential to create specific courses on this topic, or to include it within official degree programmes. Higher importance was given instead to a wider and far reaching dissemination of information, together with some sort of teacher training. Participants expressed willingness to participate in an informal network, to meet fellow teachers and professionals working on this topic, exchange information, discuss topics, and as an access point to forestry genetics conservation and diversity resources. In practical terms, the following actions were proposed and actioned:

- use the Seedsource users' forum via the project's website;
- make materials available on the website that were considered by teachers as the most relevant and appropriate as teaching aids– it will involve scanning of existing material;
- development of case studies on a variety of topics related to Forest Genetic Resources. These will be suited for use in classes and include teacher notes, powerpoint and other materials suitable for use in presentations. David Boshier started development of these case studies during 2007-8, in conjunction with Bioversity International's Understanding and Managing Biodiversity Programme. Draft materials on 'species conservation strategies' and 'trees on farms' will be ready by June 2008 and will be tested in a workshop in Ethiopia in July. Further revision and development of case studies on more subjects (e.g. logging impacts, seed collection and genetic diversity) will follow in the remainder of 2008-09.

In response to feedback at the workshops, a bulletin board was set up, using the YaBB opensource software. The *Foro de participantes en la Red Seedsource de diseminacion de resultados* was opened from July 2007 and contains various sections:

- Pregunte al experto
- Noticias globales
- Documentacion disponible para descargar o donde se puede localizer
- Ayuda/General/Contactos (contactar a miembros de la red para solicitar ayuda o colaboraciones especificas, avisos de reuniones, conferencias, talleres, etc).

To date, use has been poor, and probably reflects the fact that people are now awaiting the availability of new materials that they can trial, before they are likely to be enthused to participate in such fora.

Carlos Navarro included information in postgraduate courses and in an international training course *Forestería Análoga y Diversidad Genética* held in Mérida, April 2008 for 44 people from 22 countries. In the session *Conservation of genetic diversity for the perpetuation of Latin American forest species in the 21st century*, he covered the value of genetic diversity for its role in the improvement of productive forest systems and restoration.

3. Deviations from the project work programme, and corrective actions taken/suggested: identify the nature and the reason for the problem, identify contractors involved

With the change in the principal target audience for dissemination materials to university and technical school teachers, the type of dissemination materials to be produced also altered. Initial focus in 2007-08 was on making available on the website existing materials that were

assessed in the workshops as being of immediate use. Seedsource will look at running training workshops in Nicaragua and Honduras for teachers on the use of this material towards the end of 2008 or early in 2009. David Boshier also will further trial materials in a course taught jointly with Bioversity International in Latin America in 2009.

4. List of deliverables, including due date and actual/foreseen submission date (Table, p57)
5. List of milestones, including due date and actual/foreseen achievement date (Table, p59)

Table A: Deliverables List

List all deliverables, giving date of achievement and any proposed revision to plans.

No.	Deliverable name	WP no.	Date due	Actual/Forecast delivery date	Estimated indicative person-months *)	Used indicative person-months *)	Lead contractor
1	Initial project website established	1	3	7	2	2	OFI / CEH
2	Guideline of collection and exchange priorities	1	6	12	6	5	CEH
3	Reciprocal transplant experiments established	2	21	21	36	24	CATIE
4	Data from seedling reciprocal transplant experiments to WP7	2	36				CATIE
5	Paper on scale of adaptation: plant fitness in relation to genetic, environmental and geographic distance and in comparison to production variables	2	45	45	-	-	CATIE
6	Protocols for DNA extraction and analysis of herbariums and wood samples	3	36	24	4	4	INRA
7	Phylogeographic maps of study species	3	42	42	2+8	2+3	INRA
8	Database of DNA sequences to identify range and study species available	3	42	42	6	3	INRA
9	Written guidelines on standard project research procedures for CA2	4	9	18	1	1	CATIE
10	Enriched libraries in microsatellites for the selected species	5	12	12	9+61.5	9+30	CNR-IGV
11	List of new neutral (chloroplast and nuclear) and SNP markers for the selected species	5	18	18	1	1	CNR-IGV
12	Map of distribution of neutral diversity within and across natural populations	5	42	42	4.5	1.5	CNR-IGV
13	Method for tracing origin of plant material based on molecular markers assessed	5	42	42	-	-	CGR-IGV
14	Method for linking neutral and adaptive markers assessed	5	42	42	-	-	CNR-IGV
15	Database of molecular markers polymorphism	5	42	42	-	-	CNR-IGV

16	DNA collection from open pollinated progenies, seedling recruits, and mapped adult trees within range of landscapes	6	18	30	34.5+4	23	INPA
17	Common garden experiments established	6	21	21	36	12	INPA
18	Seed from outbreeding depression controlled pollinations	6	21	21	6	2	INPA
19	Gene dynamic parameters estimated for study species under different environmental conditions	6	42	42	24	6	INPA
20	Development of guidelines for data acquisition	7	18	18	1	1	INRA
21	Integrated species maps showing quantitative and molecular genetic discontinuities and variability	8	45	45	-	-	CEH
22	Correlations of gene dynamic and progeny performance parameters with biological and landscape features	8	45	45	-	-	CEH
23	Development of Eco-Gene model	9	45	45	-	1	BFH
24	Development of a GIS based Meta-population model	9	45	45	-	1	BFH
25	Reports on workshops covering survey results and draft extension materials	10	7	21/27	4.5	3+1	OFI
26	Reports on workshops covering project results	10	40	40	-	-	OFI
27	Report on germplasm collection & management practices	11	7	12/24	9.5	3.5	OFI
28	Trial extension materials based on existing research papers	12	4	36	6.5	2	CATIE
29	Extension materials produced	12	36	36	15	4+2	CATIE
30	Extension materials disseminated	12	48	48	15	4	CATIE

Table B: Milestones List

List all milestones, giving date of submission and any proposed revision to plans.

No	Milestone name	WP no.	Date due	Actual/Forecast delivery date	Lead contractor
1.1	Updated website	1	3	3	CEH
1.2	Reference collection and exchange guidelines	1	6	6	CEH
2.1	Determination of the scale of local adaptation in three neotropical species during seed germination and seedling establishment, and how this relates to: (a) genetic, environmental and geographic distance; (b) existing seed sourcing practices for ecological restoration; (c) population variation in plantation production traits.	2	45	45	CATIE
3.1	Access to herbarium and wood samples	3	12	30	INRA
3.2	Access to population samples of widely distributed populations	3	18	30	INRA
3.3	Detection of variable regions in study species genomes	3	12	24	INRA
4.1	Definitive guidelines circulated among all partners and available on project website.	4	9	9	CATIE
4.2	Progress reports and modifications to guidelines agreed upon on the basis of experience during the execution of the project.	4	12	12	CATIE
4.3	End of planning meeting – draft guidelines prepared.	4	18	18	CATIE
4.4	Annual meetings – progress reports and updated guidelines circulated to all partners and available on project website.	4	12, 24, 36	12, 24, 36	CATIE
5.1	New microsatellite markers provided	5	12	12	CNR
5.2	List of candidate stress related genes as putative target for SNP detection provided, list of primers for their amplification prepared	5	12	12	CNR
5.3	Sequences of the identified candidate genes and of SNPs provided	5	18	18	CNR
5.4	Organisation of a technical meeting among WP4 participants	5	12	12	CNR
5.5	Populations diversity screened with microsatellites and SNPs	5	36	36	CNR
5.6	Assignment tests performed: organisation of a technical meeting among WP4 participants	5	45	45	CNR
5.7	Association tests with QTL performance and selected candidate genes performed	5	45	45	CNR
6.1	The impact of local environment of on seed set and progeny performance in all study species;	6	45	45	INPA

6.2	the impact of selfing on progeny performance in mixed mating species;	6	45	45	INPA
6.3	the extent of fitness loss through population mixing with respect to both geographical and ecological distance of the pollinating trees from mother trees.	6	45	45	INPA
6.4	How different local habitat condition affect the mating system and pattern of gene flow in selected tropical forest trees, the results of this WP will influence the decisions on management strategies in WP9 and the communication and dissemination activities of WP11 & 12.	6	45	45	INPA
7.1	Definition of genetic and ecological parameters to be estimated and analysed in the meta-analysis (WP8)	7	6	6	INRA
7.2	Ensure project-long data compatibility	7	45	45	INRA
8.1	Useable maps of distribution of variation within species for a range of quantitative, phylogenetic, diversity and coding loci.	8	45	45	CEH
8.2	General overview of how biology and landscape of species impacts on gene diversity and progeny performance	8	45	45	CEH
9.1	Thresholds for each studies species on the minimum number and spatial distribution of reproductive trees for forest management based on natural regeneration	9	45	45	INRA
9.2	A map of ecozonation across the range of each case study species which indicates areas within which seed transfer can occur but between which there are some performance consequences to transfer	9	45	45	INRA
9.3	Recommendations for the way of harvesting and the minimum number of individual trees that seed should be collected from to ensure maximum progeny performance	9	45	45	INRA
10.1	Documentation on current utilisation practices across the range of case study species	10	12	24	OFI
11.1	The survey is expected to reveal key factors in germplasm collection & management practices	11	12	24	OFI
11.2	Workshops held with stake holders and scientists in each of the regions	11	12	24	OFI
12.1	Workshops held with stake holders and scientists in each of the regions (months 5-6)	12	12	24	CATIE
12.2	Workshops held with stake holders and scientists in each of the regions (months 40)	12	5-6	18	CATIE

Plan for using and disseminating the knowledge

The preparation of dissemination materials began early in the project. Recent research on neotropical trees (from previous EU and other projects) are being revised for their implications for seed collection and other genetic resource management issues. Draft extension materials will be prepared based on key implications from this revision and trialled at initial workshops within the context of the survey (see WP 11). As analyses from Core Area 3 are produced and assimilated, results will be incorporated into extension materials. The presentation of extension materials (format, scope, style, coverage) and dissemination of resource management priorities will be informed initially by output from WP11 and through stakeholder workshops at various phases of the project. A variety of media (pamphlets, books, CDs, the web) will be used, depending on the target audience of each material and input from stakeholders. The academic community will be targeted by scientific papers and articles. Materials will be prepared in the languages appropriate to the intended geographical coverage of the material (e.g. Spanish, Portuguese, French, English for wide coverage materials, while only one of these for locally targeted material).

Extension materials will cover the following:

- Issues on genetic resource management with respect to land use and establishment systems (named species examples given within them).
- For species identified as of very high socioeconomic importance and where genetic resource management will vary with ecosystem and management scenario a detailed pamphlet containing life history and utilisation information will be prepared.
- A check-list will be produced for the 50 most socio-economically important species in each of Central and South America, detailing their current use, biology and recommendations for seed transfer and source collections (includes ecozonation across the range of each case study species)

Dissemination/extension materials will be circulated at a country and regional scale to a range of end users (government agencies, non-government organisations, foresters/agroforesters, teachers, extension workers) across the neotropics. Workshops will be organised with stakeholders at various stages of the project at locations within Central America and South America to disseminate results to both the resource management and scientific communities. The project will work through existing dissemination networks.

Dissemination of knowledge

A fundamental basis of the project is that research on seed source is directly applicable to issues of production, conservation and sustainability – both of ecosystems and human welfare. Thus an equal emphasis on dissemination and research along with involvement of a wide range of stakeholders will ensure a broad spread of the information, far beyond the immediate research community. Working through existing dissemination networks will ensure that the project outputs are placed within a much broader context and reach a wider public than would more normally be the case. Similarly the feedback process will ensure that the research is centred within that broader context. We anticipate that the approach and information developed and used in the project will be applicable to other developing country regions.

Table C : Cumulative activities in dissemination of knowledge

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
15/04/05	*Conference*	*Higher education*	*Students from Honduras, Nicaragua, Bolivia, Perú, Costa Rica, Mexico,Colombia*	*15*	*6*
10/2006	*Workshops*	*Academic*	*international*	*100*	*6 and 2*
Ongoing	*Project web-site*	*Research/Higher education*	Neotropical		*OFI (J. Cordero)*
1-4/10/2006	*Poster*	*Research*	Worldwide	*~ 150*	*OFI (D Boshier, P Rymer)*
Ongoing	*Flyers*	*General Public*	Ecuador		*PUSC (R. Valencia)*
Ongoing	*Didactic game*	*School Children*	Neotropical		*CATIE (C. Navarro)*
30/11-1/12/2006	*Workshop*	*Higher education*	Nicaragua	*22*	*OFI (D. Boshier, J. Cordero) CATIE (C. Navarro)*
4/12/2006	*Workshop*	*Higher/Secondary education*	Honduras	*13*	*OFI (D. Boshier, J. Cordero) CATIE (C. Navarro)*
7/12/2006	*Workshop*	*Higher/Secondary education*	Costa Rica	*25*	*OFI (D. Boshier, J. Cordero) CATIE (C. Navarro, C. Cascante)*
30/05/07	*Conference*	*Higher education*	*Students from, Mexico,Colombia and Italy*	*5*	*6*
16/07/2007	*Conference*	*Research*	Neotropical	*Approx 100*	*OFI (D Boshier)*
planned	*Workshops*	*End-users*	*Central / South America*	*>1000*	*OFI, INPA, CATIE, PUCE, UFRJ*
planned	*Extension materials*	*Universities, teachers*	*Central / South America*	*100-1000*	*All partners*
05/04/2008	*MSc Thesis 'Genetic diversity and mating system analysis of Cedrela odorata L. (Meliaceae) populations under different human dominated landscapes and primary forests; paper on CATIE website*	*Biologists and foresters working with Tropical Species*	Latin America	*>100*	*CATIE/CEH/University of Adelaide*
20/12/2007	*Conference*	*Research*	European	*Approx 150*	*OFI (P Rymer, D Boshier)*
06/07/2007	*Workshop*	*Higher education*	Ecuador	*17*	*OFI (D. Boshier) CATIE (C. Navarro) PUCE (R. Valencia)*

Table C : Cumulative activities in dissemination of knowledge (contd.)

Actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
2-5/04/2008	*Latin American Forest Congress*	*Forest engineers and other professionals*	Venezuela, and the public was for Latin American institutions	*42*	*CATIE*
04/2008	*Paper in Journal. Seed sourcing recommendations for forest restoration: tree isolation impacts progeny performance of cedar and mahogany in the neotropics*	*Forest engineers and other professionals*	International Journal of Sustainable Forestery		*Carlos Navarro, Stephen Cavers , Andrew Lowe. CATIE, CEH, University of Adelaide*
02/2008	*International Training Course on Analog Forestry*	*Farmers and professionals*	Farmers and professionals of 14 countries	*32*	*CATIE, Carlos Navarro*
04-06/2008	*Master degree Course on Introduction to Silviculture*	*MSc Students of CATIE*	Six countries of Latin America	*8*	*CATIE, Carlos Navarro*

Publications arising from SEEDSOURCE activities during RP3

1. André, T., Lemes, M. R., Grogan, J. & Gribel, R. (2008). Post-logging loss of genetic diversity in a mahogany (*Swietenia macrophylla* King, Meliaceae) population in Brazilian Amazonia. *Forest Ecology and Management* 255: 340-345.
2. Buonamici A, Cavers S, Vendramin GG (2008) Microsatellite loci isolated from Hymenaea courbaril L. (Fabaceae). *Molecular Ecology Notes.*
3. Broadhurst LM, Lowe AJ, Coates, D., Cunningham, S., McDonald, M., Vesk, P. and Yates, C. (submitted) Maximising evolutionary potential in broadscale restoration. *Ecological Applications*
4. Davies SJ, Cavers S, Finegan B, White A, Lowe AJ. (submitted) The impact of forest disturbance regime on mating system and pollen-mediated gene flow for a neotropical pioneer tree, *Vochysia ferruginea* Mart. *Molecular Ecology*
5. Davies S, Cavers S, Lowe AJ The genetic diversity consequences of reforestation and habitat restoration, *Genes, Genomes & Genomics.* In Prep.
6. Dick, C. W., Bermingham, E., Lemes, M. R. & Gribel, R. (2007) Extreme long distance dispersal of the lowland rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics. *Molecular Ecology* 16: 3039-3049.
7. Dick, C. W., F. A. Jones, O. J. Hardy, R. Petit (2008) Spatial scales of seed and pollen-mediated gene flow in tropical forest trees. *Tropical Plant Biology* 1: 20-33.
8. Dick, C. W. (2008) New interpretations of fine scale spatial genetic structure *Molecular Ecology* 17: 1873-1874.
9. Dick, C. W., M. Heuertz (in review), Mountains, monkeys and marine currents: The role of biogeographic history in the evolution of a widespread tropical rainforest tree species. *Evolution*
10. Dick CW, Bermingham E, Lemes MR, Gribel R (2007) Extreme long-distance dispersal of the lowland tropical rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics. Molecular Ecology. 16 (14): 3039–3049.
11. Dick, C. W., Bermingham, E., Lemes, M. R., Gribel, R. (2007). Extreme long distance dispersal of a lowland rainforest tree (*Ceiba pentandra*: Malvaceae) across the Neotropics and Africa.
12. Gribel, R., Lemes, M. R., Bernardes, L. G., Pinto, A. E. & Sheppard, G.(2007) Phylogeography of Brazil-nut tree (*Bertholletia excelsa*, Lecythidaceae): evidence of human influence on the species distribution
13. Hernandez G, Buonamici A, Walker K, Vendramin GG, Navarro C, Cavers S (2008) Isolation and characterization of microsatellite markers for Cedrela odorata L. (Meliaceae), a high value neotropical tree. *Conservation Genetics*. 9 (2): 457-459
14. Lemes, M. R., Grattapaglia, D., Grogan, J., Proctor, J. & Gribel, R. (2007) Flexible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened neotropical tree species. *Plant Ecology* 192: 169–179.
15. Lemes M. R., Martiniano, T. M., Reis, V. M., Faria, C. P. & Gribel, R (2007). Cross-amplification and characterization of microsatellite loci for three species of Theobroma (Sterculiaceae) from the Brazilian Amazon. *Genetic Resources and Crop Evolution* 54: 1653–1657.
16. Lemes MR, Grattapaglia D, Grogan J, Proctor J, Gribel R (2007) Felixible mating system in a logged population of *Swietenia macrophylla* King (Meliaceae): implications for the management of a threatened neotropical species. Plant Ecology. DOI 10.1007/s11258-007-9322-9
17. Lemes, M. R., Alencar, R. R., Coley, P. D., Farias, G. S., Kursar, T., Porto, M. S. A. & Pennington, R. T. (2007). Phylogenetic relationships among *Inga* (Fabaceae) species from Manaus, Amazon, Brazil.
18. Lowe AJ (submitted) Composite provenancing – progressing the 'local is best' paradigm for seed sourcing. *Wingspan*.
19. Martins R. L. & Gribel, R. (2007). Polinização de *Caryocar villosum* (Aubl.) Pers. (Caryocaraceae), uma árvore emergente da Amazônia Central. *Revista Brasileira de Botânica* 30: 35-43.

20. Navarro C, Cavers S, Lowe AJ (Accepted) Seedsourcing recommendations for forest restoration: Isolation impacts progeny performance in the neotropics. *Journal of Sustainable Forestry*

21. Navarro C, Cavers S, Lowe AJ. (submitted) Isolation impacts progeny fitness in trees: for reforestation, forest trees are superior seed sources. *Ecology*

22. Petit, R. J., F. S. Hu, C. W. Dick (2008). Forests of the past: a window to future changes. *Science* 320: 1450 - 1452.

23. Pennington, R. T. & C. W. Dick (in press) Diversification of the Amazonian flora and its relation to key geological and environmental events: a molecular perspective. In C. Hoorn, H. Vonhof and F, Wesselingh (eds) Neogene history of Western Amazonia and its significance for modern biodiversity. Blackwell Publishing, Oxford, UK.

24. Reis, A. M. M., Braga, A., Lemes, M. R., Gribel, R. & Collevatti, R. G. Development and characterization of microsatellite markers for the Brazil nut tree *Bertholletia excelsa* Humb. & Bonpl. (Lecythidaceae) (submitted to *Molecular Ecology Resources*).

25. Sebbenn ab, A.M. Bernd Degen, Vânia C.R. Azevedo, Marivana B. Silva, André E.B. de Lacerda, Ana Y. Ciampi, Milton Kanashiro, Francimary da S. Carneiro , Ian Thompson, Marilyn D. Loveless (submitted): Modelling the long-term impacts of selective logging on genetic diversity and demographic structure of four tropical tree species in the Amazon forest. Forest Ecology and Management

26. Sinclair, E., Byrne, M., Coates, M., Dixon, K., Hammersley, L., Hobbs, R., Hopper, S., Koch, J., Krauss,S., Lowe, A., Venning, D., Vlahos, S., Yates, C. Ecological Restoration Genetics – from Generalities to Practicalities. For *Conservation Biology*

Theses

1. Hernandez G (2008) Genetic diversity and mating system analysis of *Cedrela odorata* L. (Meliaceae) populations under different human dominated landscapes and primary forests. MSc Thesis, CATIE, Costa Rica.

Communications /talks

1. Degen, B. and Sebbenn, A.: Large-scale, multi-species model Eco-Gene, Workshop EMBRAPA, Belem, Brasil, 11-16/02/2006, addressed countries: Brazil, Size of the audience 20

2. Degen B. Impact of selective logging on genetic composition and demographic structure of seven tropical tree species in French Guiana and Brazil, 01.03.2007, Kourou, French Guiana, addressed countries: France / French Guiana.

3. Degen, B.: Contribution to the organisation of an international workshop on "Fingerprinting methods for the identification of timber origins", 8-9 October 2007 in Bonn, Germany; other partners involved S. Cavers, A. Lowe, Size of the audience: 50, addressed countries: tropical countries in Latin-America, Africa Asia own talk at the workshop: "Use of DNA-markers for identification purposes in forestry" 11-16/02/2006.

4. Gribel, R., Lemes, M. R., Bernardes, L. G., Pinto, A. E. & Sheppard, G.(2007) Phylogeography of Brazil-nut tree (*Bertholletia excelsa*, Lecythidaceae): evidence of human influence on the species distribution . ATBC Meeting, Morelia, Mexico

5. Dick, C. W., Bermingham, E., Lemes, M. R., Gribel, R. (2007). Extreme long distance dispersal of a lowland rainforest tree (*Ceiba pentandra*: Malvaceae) across the Neotropics and Africa. ATBC Meeting, Morelia, Mexico.

6. (3) Lemes, M. R., Alencar, R. R., Coley, P. D., Farias, G. S., Kursar, T., Porto, M. S. A. & Pennington, R. T. (2007). Phylogenetic relationships among *Inga* (Fabaceae) species from Manaus, Amazon, Brazil. ATBC Meeting, Morelia, Mexico.

Dick, C.W. (University of Michigan, USA):

5. Invited keynote speaker, Association for Tropical Biology and Conservation, Surinam
6. Invited Biology Dept. Seminar speaker, Bowler Green University, OH
7. Invited Biology Dept. Seminar speaker, Eastern Michigan University, MI
8. Invited keynote speaker, 9th International Pollination Symposium, Ames, Iowa.
9. Invited lecture, Association for Tropical Biology and Conservation, Morelia, Mexico.
10. Invited Tupper Seminar, Smithsonian Tropical Research Institute, Panama City, Panama
11. Poster, Association for the Taxonomic Study of the Flora of Tropical Africa, Cameroon
12. Annual meeting of the Association for Tropical Biology and Conservation, Morelia, Mexico.

APPENDIX I:

SEEDSOURCE

Minutes of the third co-ordination meeting

PUCE
Quito, Ecuador
05 - 07 July 2007

Participating Institutions

CEH	Centre for Ecology and Hydrology, Natural Environment Research Council
OFI	Oxford Forestry Institute, University of Oxford
INRA	Instit Nacional de la Recherche
CNR	Consiglio Nazionale delle Ricerche
INPA	Instituto Nacional de Pesquisas da Amazonia
CATIE	Centro Agronómico Tropical de Investigación y Ensenanza
PUCE	Pontificia Universidad Catolica del Ecuador
BFH	Institute of Forest Genetics
UA	University of Adelaide
UM	University of Michigan

Members present

CEH	Stephen Cavers (SC), Katherine Walker (KW)
OFI	David Boshier (DB), Paul Rymer (PR)
INRA	Henri Caron (HC), Ivan Scotti (IS)
CNR	Beppe Vendramin (BV)
INPA	Rogerio Gribel (RG), Maristerra Lemes (ML)
CATIE	Carlos Navarro (CN)
PUCE	Renato Valencia (RV), Álvaro Pérez (AP)
BFH	Bernd Degen (BD)
UA	Andrew Lowe (AL), Melita de Vries (MdV)
UM	Chris Dick (CD)

Meeting Agenda

Tuesday	(03/07/2006)	Arrival of participants / acclimatisation
Wednesday	(04/07/2006)	Arrival of participants / acclimatisation
Thursday	(05/07/2006)	SEEDSOURCE Coordination meeting
Friday	(06/07/2006)	SEEDSOURCE Coordination meeting
Saturday	(07/07/2006)	SEEDSOURCE Coordination meeting / excursion

SEEDSOURCE Coordination Meeting

THURSDAY (05/07/2007)
- General business & opening - project deadlines, reporting etc (SC)
- Overall project review (SC)

WP1 Collection and exchange of materials and methods (SC)

WP2 - Quantitative performance for replanting (CN)
Progress at CATIE - (CN)
Progress at PUCE - (RV)

WP3 - Evolutionary history and developing regional markers for species (HC)
DNA extraction methods, phylogeography: multiple species - (HC)
cpSSR development in 4 species - (BV)
Marker development in 3 species - (PR)
Cp and nDNA Markers in Swietenia macrophylla / B. excelsa - (ML)
Which cpDNA loci are useful for phylogeographic analysis ? - (AL)
Phyloegeography of Ceiba / Symphonia - (CD)

WP5 - Estimate partitioning of non-coding and coding genetic diversity (BV)
Development of nSSRs in 4 species / development of SNPs in 6 species - (BV)
nSSRs in Carapa species - (HC)
Analysis of 3 species - (ML)
Development of nSSRs and SNPs in 2 species - (PR)

FRIDAY (06/07/2007)
WP6 - Gene dynamics & quantitative seed performance in relation to landscape (AL)
Review and summary - (AL)
Progress with Bombacopsis - (PR)
Progress with six species in Costa Rica - (CN)
Progress with Bertholletia excelsa - (RG)

WP7 - Data compatibility (IS)

WP8 - Meta-analysis of data (AL / CD)
Restoration genetics-myths developed (AL)
AFLP meta-analysis and other life history/genetic structure correlations (AL)

WP9 - Selection and definition of resource priorities (BD)

WP10 & 11 - Communication of biological / economic information & Knowledge gathering (DB)

WP 12 - Extension materials and dissemination of resource management priorities (CN)
Development of an educational game for schools and colleges - (CN)
Review of dissemination with Theobroma - (RG)

Any Other Business (SC)

ACTIONS AND UPDATES
Project Level: Consortium business, management structures and reporting

- Project reporting structure was reiterated as below: it was emphasised that WP leaders are responsible for reporting on their WP and will be expected to submit this for preparation of annual reports.

WP No	Workpackage title	Lead contractor No
CA1	**Adaptive variation and genetic differentiation at a range-wide scale**	**CEH**
1	Collection and exchange of materials and methods	CEH – SC
2	Quantitative performance for replanting	CATIE - CN
3	Evolutionary history and developing regional markers for species	INRA - HC
CA2	**Diversity, reproductive perf. and recruitment at the landscape scale**	**CATIE**
4	Ensuring focus of quantitative and genetic studies	CATIE - BF
5	Estimate partitioning of non-coding and coding genetic diversity	CNR-IGV – BV
6	Gene dynamics and quantitative seed performance in relation to landscape characteristics	UA – AL
CA3	**Analysis and prioritisation of regional and local sourcing strategies**	**INRA**
7	Data compatibility	INRA – IS
8	Meta-analysis of data	UM – CD / UA - AJL
9	Selection and definition of resource priorities	BFH – BD
CA4	**Knowledge gathering, integration and dissemination of priorities**	**OFI**
10	Communication of biological and economic information	OFI – DB
11	Knowledge gathering	OFI – DB
12	Preparation of extension materials and dissemination of resource management priorities	CATIE - CN

- Reporting was completed much faster this year and at the time of the meeting, annual report had been completed and sent to EC.

- Cost statements still not completed, likely to mean delays in payments again. It was emphasised that prompt reporting is the only way to make this happen faster. [RV, CN both raised concerns about rate and lack of detail in advance regarding payments to partners; SC accepted that there are issues but said there is little to be done beyond returning cost statements on time. SC to follow up with EC for more information.]

- The text of the Material Transfer Agreement governing the exchange and use of samples between partners within the project was agreed by all partners. A final text for signing was in circulation - a final complete signed copy will be forwarded by SC once all signatures are in.

Workpackage 1 (CEH): Collection and exchange of materials and methods

- Significant progress has been made on collections for all species. New field collections have been made for multiple species in Costa Rica (CATIE), Ecuador & Peru (PUCE), Peru (IIAP via CEH), Cuba (IFS via CEH) and Panama (UM / INPA). Over 1000 herbarium samples have been collected from 10 herbaria worldwide. SC is coordinating collections where data is sent; many samples forwarded without complete data or without coordinates.

Action: Coordinate data of samples to be sent to SC **(all partners)**

- New collecting trips planned in 2007/08: CD (student in sth Peru); IS (Guiana, Suriname, VZ), AL (Guyana); DB (Columbia & Honduras).

- Export permissions. ML reported that Brazilian export permission is due in August 2007. RV reported that export permission has been obtained for samples collected by PUCE.

Workpackage 2 (CATIE): Quantitative performance for replanting

- WP2 is progressing well with RTEs currently being established in Costa Rica and collections completed for Ecuador. All partners involved in establishment are happy with progress and plans and are maintaining close contact. In Costa Rica, the species list has been altered for practical reasons to *Cedrela odorata, C. tonduzii* and *Ulmus mexicana*.

Action: Consider incorporation of *C. tonduzii* and *U. mexicana* into other WPs. **(SC / CN)**

Workpackage 3 (INRA): Evolutionary history and developing regional markers for species

- Methods optimised for each spp for both DNA extractions and cpSSRs, resulting in recommended procedures for dealing with samples (particularly herbarium samples) and markers / regions for phylogeographic studies.

1. DNA extraction: Recommend - Invisorb Spin Plant Mini kit for genomic DNA followed by cleanup using GenElute cleanup kits.

2. Loci for phylogeographic analysis: Recommend

- cpSSR loci as identified during optimisation stages
- sequencing of common cp locus for all species: *trnC-ycf6* (see Shaw *et al* 2005)
- sequencing of ITS region
- RFLP markers if necessary

Workpackage 3 Collections made for phylogeographic study, including herbarium material collected: update from third meeting.

Responsible partner*		CD/CN	CD/CN	CD/CN	CN	CN	CN	CN	STRI	DB	PUCE	PUCE	DB/INRA	INRA	CD	INPA/UFRJ/Andy/Chris	
Species		**ME**	**BE**	**EL**	**GU**	**HO**	**NI**	**CR**	**PA**	**CO**	**EC**	**PE**	**VE**	**GY**	**BO**	**BR**	Total
Bertholletia excelsa	INPA													h		19	30
Carapa guianensis	INRA							6	2	h	5	h	h	24		2	45
Cedrela odorata	CEH	x	x	x	x	x	x	x	x		4+3	1+3	h	1+h	h	3+2	50
Hymenaea courbaril	CATIE	h		h		h	h	h	3	h		1	h	4	h	3+h	20
Jacaranda copaia	INRA							1	2+h	h	2+h	3	h	6+h	h	2+h	33
Minquartia guianensis	INPA							1	h	h	3+h	1	h	1+h	h	2+h	30
Simarouba amara	INRA			h				1	2	h	5+h	2	h	9+h	h	1+h	33
Swietenia macrophylla	INPA	4	3	2	2	2	3	5	2				h			12+h	50
Symphonia globulifera	INPA	x	x	.	x	x	x	x	x	x	4	3	x	1	x	h	30
Virola sebifera	PUCE							1		h	4+h	4+h	h	1+h	h	h	30
Vochysia ferruginea	CATIE							11	1	h	1	1		h		2+h	19
Ceiba pentandra	INPA							2	2		5	3		1		10	39
Ochroma pyramidale	PUCE							1	7		9+h	2+h	h		h	h	36
Cordia alliodora	OFI	h	h	h	h	3	2	5	2	h	h	h	h	1+h	h	h	30
Socratea exorrhiza	PUCE							1	3		4	4+h	h	1+h	h	h	30
Bombacopsis quinata	OFI					1	3	4	1	1			1			h	30
Schizolobium parahyba	UFRJ				h				h		3+h	h			h	1+9	30

existing sample herbarium sample only

? - indicates uncertainty as to whether or not collection can be made / need to access other sources than direct collection

People and partners responsible for getting contacts in particular target countries to facilitate collection indicated in first row, partner responsible for overall collection in column 2.

Workpackage 5 (CNR): Estimate partitioning of non-coding and coding genetic diversity

- nSSRs have now been developed or are in advanced preparation for all species.

- SNP development. Primers specific to the aquaporin family of genes have been developed and tested for seven species. In addition, other loci (PepC, Proline carboxylate synthase) have been tested in some species.

- Screening of markers will be undertaken once collections are completed.

Workpackage 6 (UA): Gene dynamics and quantitative seed performance in relation to landscape

- Progress is underway on multiple species in field collection, trial establishment, phenological observation and molecular analysis.

Workpackage 7 (INRA): Data compatibility

- IS presented options for improving data compatibility: inc sample labelling, minimum data requirements and formats. The possibility of devising a data handling agreement analogous to the material transfer agreement was discussed, although this may possibly already be covered by either consortium agreement or MTA.

Action: IS / SC - review possibilites / necessity for data sharing agreement.

Workpackage 8 (UA – AJL / UM - CD): Meta-analysis of data

- The key questions were reviewed by AL (see presentation).

Workpackage 9 (BFH): Selection and definition of resource priorities

- BD: latest developments of the Eco-GENE model were presented, including the possibility to conduct simulations on large scales. BD also reviewed some case studies for devising seed zones in N. American species, pointing out the intensity of sampling undertaken where rigourous frameworks had been established. Given the low density of sampling likely to be achieved by SEEDSOURCE it was generally agreed that the aim of seed zone description was likely be beyond the scope of the project.

Workpackages 10 (OFI): Communication of biological and economic information

Workpackage 11 (OFI): Knowledge gathering

Workpackage 12 (CATIE): Preparation of extension materials and dissemination of resource management priorities

- DB reviewed progress in WPs 10, 11, 12: particularly the outcomes from reviews of past efforts and the workshops held in Central America.

- The new forum area on the website for forest genetic questions and central dissemination of publications, and Spanish-language versions of the project website were presented. See:

www.seed-source.net

- CN presented, as an example, the development of a board game designed around the conservation of genetic resources in tree species, for dissemination to schools and colleges as an educational tool. The game was devised as one of the outputs from a previous INCO project.

- RG presented some dissemination activities that were undertaken in previous studies for *Theobroma grandiflorum*.

Workpackage 6 Collections made and ongoing: update from third meeting.

Species	impact or issue tested	Plot design	no samples popXtreeXprog	Collectio	tree data min/max	phenolo	Seedling rearing	germ./ fitness	Repro manips	Genotype	mating system	Gene flow	Ref?
Bertholletia excelsa	density	1-gradient	1x40x20(40)	INPA	min	no	INPA	yes	no	INPA	yes	exclusion	
Bertholletia excelsa	inbreeding	5-populatio	5x20x20	INPA	no	no	INPA	yes	yes?	INPA	yes	no	
Swietenia macrophylla	density/logg	1-gradient	1x30x20(x2 loggi	INPA	min	no	INPA	yes	no	INPA	yes	exclusion	
Carapa guianensis	fragmentatio	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	INRA	yes	neighbour	Dec-07
Jacaranda copaia	fragmentatio	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	CNR	yes	neighbour	Dec-07
Minquartia guianensis	fragmentatio	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	UoA	yes	neighbour	
Simarouba amara	fragmentatio	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	INRA	yes	neighbour	
Virola sebifera	fragmentatio	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	UoA	yes	neighbour	
Dipterix panamensis	fragmentatio	1-gradient	1x40x20(40)	CATIE	max	yes	CATIE	yes	no	CNR	yes	neighbour	
Cedrela odorata	tree isolation	10 frag pops	10x10x10	CATIE	min	no	CATIE	yes	no	CEH	yes	no	Gustavo
Swietenia macrophylla	tree isolation	20 frag pops	20x10x10	CATIE	min	no	CATIE	yes	no	UoA	yes	no	Navarro
Schizolobium parahyba	fragmentatio	1-gradient	1x40x20(40)	UFRJ	min	?	UFRJ	yes	no	UFRJ	yes	neighbour	?
Carapa guianensis	fragmentatio	1-gradient	1x40x20(40)	PUCE	max	yes	PUCE	yes	no	CNR/UoA	yes	neighbour	
Jacaranda copaia	fragmentatic	1-gradient	1x40x20(40)	PUCE	max	yes	PUCE	yes	no	UoA/CNR	yes	neighbour	
Symphonia globulifera	density/logg	1-gradient	1x40x20(40)	INRA	max	?	INRA	yes	no	INRA	yes	neighbour	
Bombacopsis quinata	fragmentatio	1-gradient	1x40x20(40)	OFI	min	yes	OFI	yes	no	OFI	yes	neighbour	no germ
Ommited													
Cedrelinga cataeniformis				PUCE?			PUCE?			UoA/CNR			
Cedrela odorata				PUCE?			PUCE?			UoA/CNR			
Ochroma pyramidale				PUCE?			PUCE?			UoA/CNR			
Previous INCO analysis													
Ceiba pentandra	density	1-gradient	1x6x40	INPA	min	yes	no			INPA	yes	exclusion	Gribel
Swietenia macrophylla	density/post	1-gradient	1x25x16	INPA	min	no	planned to start			INPA	yes	exclusion	Lemes
Swietenia macrophylla	density/logg	4-plots	4x400x20	CEH	min	no	no			CEH	yes	exclusion	Cavers
Vochysia ferruginea	fragmentatio	1-gradient	1x40x20(40)	CATIE	max	yes	no			CEH	yes	two gener	Davies
Symphonia globulifera	density/logg	1-gradient	1x40x20(40)	INRA	max	no	no			INRA	yes	exclusion	Degen
Cordia alliodora	high density	1 site	1x44x20	OFI	min	yes	no			OFI	yes	exclusion	Boshier
Swietenia humilis	density	1-gradient	1x44x20	OFI	min	?	no			OFI	yes	exclusion	White

APPENDIX II

Process for RTE site identification undertaken in Ecuador.

Annex 1. Herbaria records of *C. odorata* in Ecuador. Acronyms follow the Index Herbariorum (Holgren et al. 1990): MO = Missouri Bothanical Garden Herbaria, NYB = New York Botanical Garden, QCA = *Herbario de la Pontificia Universidad Católica del Ecuador*.

Province	Locality	Specimens	Herbarium
Carchi	Maldonado Road	1	QCA
Cotopaxi	Illinizas	1	MO
Esmeraldas	Bilsa*+	1	MO
	Awa Reserve*+	1	MO
Guayas	Cerro Blanco	1	MO
	Uncertain	1	MO
Loja	Podocarpus, Loja Zamora Road	3	QCA, MO
Manabí	Santa Rosa	1	MO
Morona Santiago	Cordillera del Cóndor	1	MO
	Sangay	2	MO
	Macas	1	QCA
	Uncertain locality	6	MO
	Centro Ashuar	5	NYB
Napo	Tena-Ahuano Road & Jatun Sacha Reserve*+	5	QCA, MO
	Antisana Ecological Reserve*+	2	MO
	Sumaco National Park	3	MO
	Oyacachi	1	QCA
	Cayambe Coca Ecological Reserve*	1	MO
	Uncertain locality	10	MO
Orellana	Yasuni National Park*+	11	QCA, MO
	Chiruisla*	1	QCA, MO
	Coca*	1	QCA
Pastaza	Kapawi Lodge	1	MO
	Uncertain	5	MO
Pichincha	Empalme Road & Guajalito Forest Reserve*+	3	MO, QCA
Sucumbíos	Cuyabeno Faunistic Reserve	1	QCA
	Lumbaqui	1	QCA
	Shushufindi*	1	QCA
	Uncertain	5	MO
Tungurahua	Uncertain	1	MO
Zamora	Podocarpus National Park	1	MO
Total		**79**	

* Populations surveyed in the field by PUCE, SEEDSOURCE project.

Annex 2. Search of Cedrela *odorata* and notes about its conservation status in Ecuador.

In February 2006 we started our work analysing herbaria records of the species (Annex 1). The information showed six potential localities for our RTEs work (in the Coast: Lita-San Lorenzo road, Awa Reserve, El Empalme Road, and in the Amazon region: Baeza, Ahuano-Tena Road, Yasuní National Park). In two Amazonian localities we found the number of trees needed for the experiments: Tena - Jatun Sacha Biological Reserve and the Yasuní Research Station. Although we found *C. odorata* in fifteen localities and collected DNA samples in most of them, we hardly found trees of the species in the historical localities of the Coast. We searched

for *C. odorata* populations in the western slopes of the Andes, in localities like Guajalito Forest Reserve (~2000 m altitude in March 2007) and Bilsa Biological Station (~700 m in September 2006) in the Coastal range of Mache-Chindul, but never found a population of twenty mother trees after several days of exploration. During our search for populations at mid elevations, we had difficulties to tell apart *C. odorata* and its sister species *C. montana* that grows sympatrically in Guajalito.

In March 2007, after exploring several localities, we finally found two populations in the Coast. A remnant population was located in the western slope of the Mache-Chindul ridge (this population is a new distributional record for the species in semi deciduous forest of Manabí). And twenty remnant trees were located at Las Mercedes, in the road between Sto. Domingo and Los Bancos. Since March 2007 to date we are monitoring these population to collect fruits. Only part of the trees produced flowers and fruits in Chindul and Las Mercedes in 2007. In Las Mercedes we have detected that all of the selected trees are attacked by *Hypsiphyla grandela* and, although the trees produce flowers, most fruits are damaged before they are riped.

According to our observations, *C. odorata* is critically endangered in the Coast of Ecuador. Spanish cedar (*C. odorata*) is one of the most frequently extracted timbers in natural forests of Ecuador. Outside national parks or private reserves of the Coastal region it is scarce and in remote areas, particularly in the northern portion were we explored more intensively. In Amazonian protected areas, were the species is relatively common, like in Yasuni National Park, it is the most commonly and illegally extracted timber. The timber is usually sent to markets in Colombia. This species is cited as an endangered species in Ecuador by the CITES (Apendix III, CITES) and it is critically endangered in the neighbouring Colombia and Peru (CITES, 2005).