

Pollen2013

2nd International APLE-APLF Congress



Pollen 2013
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XIX Symposium Asociación de Palinólogos de Lengua Española, APLE
XXIII Symposium Association des Palynologues de Langue Française, APLF

***Pollen Biotechnology, Diversity and Function in
a Changing Environment***

Madrid
17th-20th September 2013

Consejo Superior de Investigaciones Científicas, CSIC
Auditorium
(117 Serrano Street, Madrid)

Organized by
APLE & APLF

Edited by
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Invitation

We are pleased to announce and to invite you to participate in the 2nd International APLE-APLF Congress on “**Pollen Biotechnology, Diversity and Function in a Changing Environment**” which will be held in Madrid from 17th to 20th September 2013, in the CSIC campus of the city centre, in the historic building of the CSIC headquarters of the Serrano Street.

The congress is promoted by the Spanish (APLE) and French (APLF) palynology societies and organized by palynologists of the National Research Council, CSIC (Consejo Superior de Investigaciones Científicas) and the Complutense University of Madrid (UCM). The Spanish and French Palynological Societies, APLE (Asociación de Palinólogos de Lengua Española) and APLF (Association des Palynologues de Langue Francaise), will join for their XIX and XXIII Symposia, respectively, in Madrid.

The Congress includes 3 Plenary Lectures, a Workshop on New Methodological Advances on Palynology Research and Imaging and 7 Scientific Sessions and Posters Sessions covering a wide spectrum on hot topics on palynology research: Applied palynology, bee pollination and diseases, Paleooceanography, dinocysts and acritarchs, Paleoenvironment and paleoclimate, Pollen morphology, development and germination, Pollen Biotechnology and Genetics, Airborne pollen and spores: environmental indicators and allergens, Landscape change and human-environment interactions. Each session will start with 1-2 lectures of relevant experts in the field followed by several oral communications selected from abstracts. Internationally recognized scientists will participate as invited speakers.

As a complement to the scientific sessions and discussions, Madrid and its environs offer unique opportunities for the visitors. We invite you to explore the tourist information about the city in the corresponding link of the Congress page.

We encouraged all of you, researchers on any pollen area, newcomers and veterans of APLE and APLF, especially young scientists, to attend the congress.

Hoping to meet you in Madrid for the 2nd International APLE-APLF Congress, with best wishes

Pilar S. Testillano
President of the Organizing Committee

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Program Timetable

Session list

Session 1a, 1b
Applied palynology, bee pollination and diseases

Session 2
Paleoceanography, dinocysts and acritarchs

Session 3
Pollen morphology, development and germination

Session 4a, 4b
Paleoenvironment and paleoclimate

Session 5
Pollen biotechnology and genetics

Session 6a, 6b, 6c
Airborne pollen and spores: environmental indicators and allergens

Session 7a, 7b, 7c
Landscape change and human-environment interactions

Time	Tuesday 17th September	Wednesday 18th September	Thursday 19th September	Friday 20th September
8,30-10,00h		Session 1a, 1b Applied palynology, bee pollination (room A) Session 2 Paleoceanography (room B)	Session 6a Airborne pollen and spores (room A) Session 7a Landscape change (room B)	Session 6c Airborne pollen and spores (room A) Session 7c Landscape change (room B)
10,00-10,30h		<i>Coffee break</i>	<i>Coffee break</i>	<i>Coffee break</i>
10,30-11,30h		Plenary Lecture <i>Iñaki Hormaza</i> (room A)	Visit to Posters	Closing Plenary Lecture <i>Donatella Magri</i> (room A)
11,30h	Registration and Documents	Visit to Posters		Closing ceremony
12,45h		<i>Lunch</i>	<i>Lunch</i>	<i>Lunch</i>
14,30-15,00h	Opening ceremony	Session 3 Pollen morphology and development (room A) Session 4a Paleoenvironment and paleoclimate (room B)	Session 6b Airborne pollen and spores (room A) Session 7b Landscape change (room B)	Saturday 21th September Post-Congress Excursion
15,00-16,00h	Opening Plenary Lecture <i>Thomas Servais</i> (room A)			
16,00-16,30h		<i>Coffee break</i>	<i>Coffee break</i>	
16,30-17,30h	Workshop New Methodological Advances in Palynology Research and Imaging (room A)	Session 5 Pollen biotechnology and genetics (room A) Session 4b Paleoenvironment and paleoclimate (room B)	Visit to Posters	
17,30-18,30h			Assembly APLE (room A) Assembly APLF (room B)	
19,00h	Welcome cocktail		Congress Dinner	

Scientific Program

TUESDAY, 17th September

Room A: Auditorium of the CSIC Central Building

Opening Plenary Lecture

15,00h-16,00h:

PLENARY LECTURE (PL-01)

The evolution of the phytoplankton during the Phanerozoic: relation to sea-level, climate and CO₂

Thomas Servais (CNRS-Univ. Lille 1, France)

16,30h- 18,30h: **Workshop**

New Methodological Advances in Palynology Research and Imaging

Chairpersons: Cristina Pardo (UCM, Madrid, Spain), José M. López-Cepero (Univ. Cádiz, Spain)

16,30h-17,00h:

WORKSHOP LECTURE (W-L-01)

New frontiers in paleopalynology

Donatella Magri (Sapienza Univ. Rome, Italy)

17,00h-17,30h:

WORKSHOP LECTURE (W-L-02)

Cryoimmobilization techniques in TEM for in situ molecular recognition in pollen studies

Carmen López Iglesias (CCiT Univ. Barcelona, Spain)

17,30h-18,00h:

WORKSHOP LECTURE (W-L-03)

Optical and digital image processing applied to pollen analysis. Problems and challenges

Gabriel Cristóbal (Inst. Óptica, CSIC, Madrid, Spain)

18,00h-18,30h:

WORKSHOP LECTURE (W-L-04)

Quantitative approaches to pollen analysis: the role of super-resolution microscopy, machine learning, and bioimage informatics

Surangi W. Punyasena (Univ. Illinois, USA)

WEDNESDAY, 18th September

Room A: Auditorium of the CSIC Central Building

8,30h-10,15h

Session 1: Applied palynology, bee pollination and diseases

Session 1a

Chairpersons: Yves Loublier (INRA, France), Carmen Gómez Ferreras (UCM, Madrid, Spain)

8,30h-8,55h: INVITED LECTURE (S1-L-01)

A practical example of a multidisciplinary collaborative work to understand the honey bee colony losses: from palynology to bee pathology

Raquel Martín-Hernández (Agronomic Center of Marchamalo, Spain)

8,55h-9,10h: ORAL COMMUNICATION (S1-O-01)

Pollen diversity associated to olive orchards-visiting bees

Alché JD, Fernández-Sierra ML, Rodríguez-Sánchez A, García-Quirós E, M. Campos

Session 1b

Chairpersons: Raquel Martín (Agronomic Centre of Marchamalo, Spain), Marzia Boi (Univ. Islas Baleares, Spain)

9,10h-9,35h: INVITED LECTURE (S1-L-02)

Assessing the impact of declining pollinators on world agriculture

Bernard Vaissiere (INRA, Avignon, France)

9,35h-10,00h: INVITED LECTURE (S1-L-03)

Pollen grain as bioindicator

José María Moreno (Tech. Univ. Cartagena, Spain)

10,00-10,15h: ORAL COMMUNICATION (S1-O-02)

The importance of pollen morphology in the Christian relic of the Shroud of Turin

Marzia Boi

Room B: Auditorium of Rocasolano Institute

8,30h-10,00h

Session 2: Paleoceanography, dinocysts and acritarchs

Chairpersons: Thomas Servais (CNRS-Univ. Lille 1, France), Frédérique Eynaud (Univ. Bordeaux 1, Talence, France)

8,30h-9,00h: INVITED LECTURE (S2-L-01)

Worldwide paleogeography of Aptian and Albian dinoflagellate cysts:
Implication for sea-surface temperature gradients and paleoclimate
Edwige Masure (CNRS-Univ. Paris, France)

9,00-9,15h: ORAL COMMUNICATION (S2-O-01)

Toxic dinoflagellates and their benthic cysts: the revival strategy! A review for
western European and Mediterranean margins
Eynaud F, Penaud A, Castera M-H.

9,15-9,30h: ORAL COMMUNICATION (S2-O-02)

Deglacial changes over the last 20 000 years in the Equatorial Atlantic: a high-
resolution dinocyst analysis
William Hardy, Aurélie Penaud, Fabienne Marret-Davies, Laurence Droz, Tania
Marsset, Bernard Dennielou

9,30-9,45h: ORAL COMMUNICATION (S2-O-03)

Tethyan Albian dinoflagellate cyst assemblages in coastal and fully marine
environments (Portuguese basin and DSDP site 398D).
Raquel Sánchez-Pellicer, Edwige Masure

9,45-10,00h: ORAL COMMUNICATION (S2-O-04)

Last-glacial oceanic conditions south of the Faeroe Shetland Gateway
Wary Mélanie, Eynaud Frédérique, Rossignol Linda, Zaragosi Sébastien,
Malaizé Bruno, Michel Elizabeth, Penaud Aurélie, Caulle Clémence, Zumaque
Jena

Room A: Auditorium of the CSIC Central Building

Plenary Lecture

10,30h-11,30h: PLENARY LECTURE (PL-02)

Pollen development and pollen-pistil interactions in the early-divergent
angiosperm *Annona cherimola*
Iñaki Hormaza (EE La Mayora, CSIC, Málaga, Spain)

Room A: Auditorium of the CSIC Central Building

14,30h-16,00h

Session 3: Pollen morphology, development and germination

Chairpersons: Maribel Rodríguez (EEZ, CSIC, Granada, Spain), Mari Carmen
Fernández (Univ. Granada, Spain)

14,30h-15,00h: INVITED LECTURE (S3-L-01)
AGPs cross-talk in Arabidopsis pollen-pistil interactions
Silvia Coimbra (BioFig, Univ. Porto, Portugal)

15,00h-15,15h: ORAL COMMUNICATION (S3-O-01)
Electrophoretic profiling and immunocytochemical detection of pectins and arabinogalactan proteins during olive pollen germination and pollen tube growth
Krzysztof Zienkiewicz, Antonio Jesús Castro, Cynthia Suárez, Juan de Dios Alché, Agnieszka Zienkiewicz, María Isabel Rodríguez-García

15,15h-15,30h: ORAL COMMUNICATION (S3-O-02)
New insights into the early steps of lipid body mobilization during pollen germination
Agnieszka Zienkiewicz, Krzysztof Zienkiewicz, Juan de Dios Alché, María Isabel Rodríguez-García, Antonio Jesús Castro

15,30h-15,45h: ORAL COMMUNICATION (S3-O-03)
The phosphorylated pathway of serine biosynthesis is essential for Arabidopsis pollen maturation.
Walid Toujani, María Flores-Tornero, Armand D. Anoman, Sara Rosa-Tellez, Jesús Muñoz-Bertomeu, Roc Ros

15,45h-16,00h: ORAL COMMUNICATION (S3-O-04)
RX microtomography in studies of floral development in plants
Ana T. Romero, Samira Ben-Menni Schuler, Víctor Suárez Santiago, Dolores Garrido, Javier Alba Tercedor

Room B: Auditorium of Rocasolano Institute

14,30h-16,00h
Session 4: Paleoenvironment and paleoclimate
Chairpersons: María Fernanda Sánchez-Goñi (Univ. Bordeaux 1, France),
Donatella Magri (Sapienza Univ. Roma, Italy)

Session 4a

14,30h-15,00h: INVITED LECTURE (S4-L-01)
Long continental pollen sequences: a state of the art
Jacques-Louis de Beaulieu (CNRS-Univ. Aix-Marseille, France)

15,00h-15,15h: ORAL COMMUNICATION (S4-O-01)
European climate optimum and enhanced Greenland melt during the Last Interglacial
María Fernanda Sánchez-Goñi, Pepijn Bakker, Stéphanie Desprat, Anders E. Carlson, Cédric J. Van Meerbeeck, Odile Peyron, Filipa Naughton, William J. Fletcher, Frédérique Eynaud, Linda Rossignol, Hans Renssen

15,15h-15,30h: ORAL COMMUNICATION (S4-O-02)
Cedrus atlantica in the Middle Atlas Morocco: an interglacial refugium
Cheddadi, R. Nourelbait, M., Fady, B., Rhoujjati, A.

15,30h-15,45h: ORAL COMMUNICATION (S4-O-03)
Responses of a neotropical interglacial microrefugium to climate instability
Vincent Montade

15,45h-16,00h: ORAL COMMUNICATION (S4-O-04)
Progress in the identification of Pinus pollen grains at a specific level in the
Iberian Peninsula records
Stéphanie Desprat, Pedro Manuel Díaz Fernández, María Fernanda Sánchez-
Goñi, Luis A. Gil Sánchez, Tabatha Coulon, Leila Ezzat, Julien Pessarossi-
Langlois, Ludovic Devaux

Room A: Auditorium of the CSIC Central Building

16,30h-19,00h

Session 5: Pollen biotechnology and genetics

Chairpersons: María Carmen Risueño (CIB-CSIC, Madrid, Spain), José María
Seguí-Simarro (COMAV, UPV, Valencia, Spain)

16,30h-17,00h: INVITED LECTURE (S5-L-01)
Engineering male sterility as a tool to decrease pollen-caused allergies
José Pío Beltrán (IBMCP, CSIC-UPV, Valencia, Spain)

17,00-17,30h: INVITED LECTURE (S5-L-02)
Drive in favour of a parasite chromosome during pollen mitosis and effects on
histone H3 methylation pattern
María Jesús Puertas (Univ. Complutense, Madrid, Spain)

17,30h-17,45h: ORAL COMMUNICATION (S5-O-01)
Pollen embryogenesis in *Citrus sinensis* L. Osbeck cv. Moro, a blood orange
cultivar
Ahmed Abdelgallel, Benedetta Chiancone, Orietta Lain, Raffaele Testolin,
Ahmed-Abdalla El Tantawy, María-Carmen Risueño, Pilar S. Testillano, María
Antonietta Germanà

17,45h-18,00h: ORAL COMMUNICATION (S5-O-02)
Arabinogalactan proteins expression and distribution patterns, early markers of
microspore embryogenesis
María-Teresa Solís, Ahmed-Abdalla El-Tantawy, Mario Da Costa, Silvia
Coimbra, María Carmen Risueño, Pilar S. Testillano

18,00h-18,15h: ORAL COMMUNICATION (S5-O-03)

Massive autophagy and excretion-based cytoplasmic cleaning during *Brassica napus* microspore embryogenesis

Jose M. Seguí-Simarro, Patricia Corral-Martínez, Verónica Parra-Vega

18,15h-18,30h: ORAL COMMUNICATION (S5-O-04)

Induction of the sporophytic pathway in pollen from *Quercus Ilex* l. Anther cultures

Beatriz Pintos, Nieves Sánchez M^a Ángeles Bueno, Rafael M^a Navarro, Jesús Jorrín, José Antonio Manzanera, Aranzazu Gómez-Garay

18,30h-18,45h: ORAL COMMUNICATION (S5-O-05)

Gametophytic-to-esporophytic switch in microspores of sweet orange genotypes

Jean C. Cardoso, Rodrigo R. Latado, Maria A. Germanà, Adriana P. Martinelli

18,45h-19,00h: ORAL COMMUNICATION (S5-O-06)

A multiplex system to analyse the expression of allergens and other gene products of interest in the olive pollen.

Sonia Morales Santana, Antonio Jesús Castro López, José Carlos Jiménez López, Adoración Zafra Álvarez, M^a José Jiménez Quesada, José Fernando Florid, María Isabel Rodríguez García, Juan De Dios Alché Ramírez

Room B: Auditorium of Rocasolano Institute

16,30h-18,30h

Session 4: Paleoenvironment and paleoclimate

Chairpersons: María Fernanda Sánchez-Goñi (Univ. Bordeaux 1, France),
Donatella Magri (Sapienza Univ. Roma, Italy)

Session 4b

16,30h-17,00h: INVITED LECTURE (S4-L-02)

Climate and vegetation change during the Holocene in southern Iberia
Gonzalo Jiménez Moreno (Univ. Granada, Spain)

17,00h-17,15h: ORAL COMMUNICATION (S4-O-05)

The climate of the Mediterranean basin during the Holocene from terrestrial and marine pollen data: A model/data comparison

O. Peyron, N. Combourieu-Nebout, S. Goring, D. Brayshaw, M. Magny, I. Dormoy, S. Joannin, J.-L. de Beaulieu, E. Brugiapaglia, S. Desprat, W. Fletcher, U. Kotthoff, K. Kouli, J. Pross, L. Sadori, M.F. Sánchez-Goñi

17,15h-17,30h: ORAL COMMUNICATION (S4-O-06)

Past environmental changes in the western Rif mountains, Morocco: A new pollen record

O. Bouaissa, A. Merzouki, A. Rhoujjati, R. Cheddadi

17,30h-17,45h: ORAL COMMUNICATION (S4-O-07)

Impact of climatic change and human activities on vegetation dynamics in Coastal Syria

Mathis M., Sorrel Ph., Geyer B., Goiran J-Ph., Marriner N.

17,45h-18,00h: ORAL COMMUNICATION (S4-O-08)

Past environmental changes and their impacts on the Lebanese Cilician fir
Lara D.N. Awad, Bruno Fady, Carla Khater, Rachid Cheddadi

18,00h-18,15h: ORAL COMMUNICATION (S4-O-09)

Holocene rapid climate changes as recorded in northwestern France (Grande Vasière): high resolution dinocyst analysis over the last 9000 years.

Axelle Ganne, Aurélie Penaud, William Hardy, Samuel Toucanne, Filipa Naughton, Frédérique Eynaud, Jean-François Bourillet

18,15h-18,30h: ORAL COMMUNICATION (S4-O-10)

Palynology in fluvio-estuarine environment: the example of Nantes (Northwestern France)

Axelle Ganne, Chantal Leroyer, Aurélie Penaud, Evelyne Goubert, Valérie Lecadre, Rémy Arthuis

THURSDAY, 19th September

Room A: Auditorium of the CSIC Central Building

8,30h-10,00h

Session 6: Airborne pollen and spores: environmental indicators and allergens

Chairpersons: Delia Fernández (Univ. León, Spain), Carmen Galán Soldevilla (Univ. Córdoba, Spain)

Session 6a

8,30h-9,00h: INVITED LECTURE (S6-L-01)

Factors involved in pollen germination could have a role also in allergic sensitization

Stefano del Duca (Univ. Bologna, Italy)

9,00h-9,15h: ORAL COMMUNICATION (S6-O-01)

Hourly dynamics of airborne pollen in the southeast of Castilla-La Mancha
Consolación Vaquero del Pino, Ana Rapp Benito, Jesús Rojo Úbeda, Rosa Pérez
Badia

9,15h-9,30h: ORAL COMMUNICATION (S6-O-02)

A semi-automatic pollen recognition system for aerobiological monitoring
Estela Díaz López, Consolación Vaquero del Pino, Ana Rapp Benito, Jesús Rojo
Úbeda, Rosa Pérez Badia, Mariano Rincón Zamorano

9,30h-9,45h: ORAL COMMUNICATION (S6-O-03)

Detection of Ole e 1 protein, major allergen of *Olea europaea* L., in rainwater.
J. García-Sánchez, M.M. Trigo, M. Recio, B. Cabezudo

9,45h-10,00h: ORAL COMMUNICATION (S6-O-04)

Relationship between ornamental Pinaceae trees density and their airborne
pollen in an urban environment
J. M. Maya, S. Fernández, I. Silva, Á. Gonzalo, R. Tormo, L. Fernández, J. V.
Alfonso, P. Vaquero, M. L. Pérez, P. Cosmes, R. M. Blanco, C. Domínguez

Room B: Auditorium of Rocasolano Institute

8,30h-10,00h

Session 7: Landscape change and human-environment interactions

Chairpersons: Blanca Ruiz Zapata (Univ. Alcalá, Spain), Walter Finsinger
(CNRS, Montpellier, France)

Session 7a

8,30h-9,00h: INVITED LECTURE (S7-L-01)

From pristine landscape to agriculture: vegetation history and human impact in
South Greenland during the last millennium
Emilie Gauthier (Univ. Franche-Comté, Besançon, France)

9,00h-9,15h: ORAL COMMUNICATION (S7-O-01)

Local hominin mobility in response to middle pleistocene environmental
dynamics in central and southern Italy
R. Orain, V. Lebreton, E. Russo Ermolli, A.-M. Sémah, S. Nomade, Q. Shao, J.-J.
Bahain, U. Thun Hohenstein, C. Peretto

9,15h-9,30h: ORAL COMMUNICATION (S7-O-02)

Holocene changes in pollen-based vegetation composition in N-NW Europe:
why does quantitative reconstruction matters?
Laurent Marquer, Marie-José Gaillard, Shinya Sugita, Anneli Poska, Anna-Kari
Trondman, Florence Mazier, Anne Birgitte Nielsen, Ralph Fyfe, Data
Contributors

9,30h-9,45h: ORAL COMMUNICATION (S7-O-03)
Pollen and NPP analysis of the Holocene palaeoenvironmental sequence of Qallimiut (SW Greenland)
Currás, A., Guillemot, T., Massa, C., Bichet, V., Richard, H., Gauthier, E.

9,45h-10,00h: ORAL COMMUNICATION (S7-O-04)
Persistence of cold-adapted species through the Holocene in the Spanish Central System
D. Abel Schaad, F.J. Pulido Díaz, J.A. López Sáez, S. Pérez Díaz, F. Alba Sánchez, F. Franco Múgica, B. Ruiz Zapata, M.J. Gil García, M. Dorado Valiño

Room A: Auditorium of the CSIC Central Building

14,30h-16,00h
Session 6: Airborne pollen and spores: environmental indicators and allergens
Chairpersons: Delia Fernández (Univ. León, Spain), Carmen Galán Soldevilla (Univ. Córdoba, Spain)

Session 6b

14,30h-15,00h: INVITED LECTURE (S6-L-02)
Pollen counts and aeroallergen quantification in the atmosphere: overview and current status
María del Mar Trigo (Univ. Málaga, Spain)

15,00h-15,15h: ORAL COMMUNICATION (S6-O-05)
Study of floral phenology of the olive tree (*Olea europaea* L.) using geostatistical techniques
Jesús Rojo Úbeda, Consolación Vaquero del Pino, Ana Rapp Benito, Rosa Pérez Badía

15,15h-15,30h: ORAL COMMUNICATION (S6-O-06)
New statistical approaches in long term pollen trend analysis: application to the olive pollen series in South Spain
Yaezel, L., García-Mozo, H., Oteros, J., Galán, C.

15,30h-15,45h: ORAL COMMUNICATION (S6-O-07)
The first airborne pollen calendar of Mexico City (2008-2013)
M.C. Calderón, F. Fuentes, T. Robledo, C. Guerrero, F. Tellez, I. López, B. Martínez, A. Martínez, L. Quiroz, A. Guidos, M.M. Trigo

15,45h-16,00h: ORAL COMMUNICATION (S6-O-08)
Fraxinus airborne pollen in the atmosphere of Mexico City
M.M. Trigo, T. Robledo, I. López, C. Guerrero, F. Tellez, L. Quiroz, A. Martínez, M. Recio F. Fuentes, M. Olive, M.C. Calderón

Room B: Auditorium of Rocasolano Institute

14,30h-16,00h

Session 7: Landscape change and human-environment interactions
Chairpersons: Blanca Ruiz Zapata (Univ. Alcalá, Spain), Walter Finsinger
(CNRS, Montpellier, France)

Session 7b

14,30h-15,00h: INVITED LECTURE (S7-L-02)

Landscape history studies: an integration of pollen analyses, multi-proxy records and archaeohistorical data
Santiago Riera (Univ. Barcelona, Spain)

15,00h-15,15h: ORAL COMMUNICATION (S7-O-05)

On the origins of highly human-transformed landscapes: the case of the Castilian Plateau (NW Spain)
César Morales-Molino, Mercedes García-Antón, Carlos Morla

15,15h-15,30h: ORAL COMMUNICATION (S7-O-06)

Prehistorical hunting and pastoral activities in the remote and marginal karstic landscape of the “Silberer Plateau” (Muotathal Alps, Canton of Schwyz, Switzerland)
Jean Nicolas Haas, Notburga Wahlmüller, Thilo Kappelmeyer, Benjamin Dietre, Irka Hajdas, Urs Leuzinger, Walter Imhof

15,30h-15,45h: ORAL COMMUNICATION (S7-O-07)

The Silvretta Massif in Switzerland: A hot-spot for the reconstruction of climatic and human impact on the European Alps by palynological means
Benjamin Dietre, Irina Anich, Thilo Kappelmeyer, Christoph Walser, Karsten Lambers, Thomas Reitmaier, Irka Hajdas, Jean Nicolas Haas

15,45h-16,00h: ORAL COMMUNICATION (S7-O-08)

The contribution of non-pollen palynomorphs to retrace reconstruct past pastoral dynamics in the western Pyrenees mountains (Ossau valley and Basque Mountains), France
Cugny C, Mazier F, D. Rius, Galop D.

FRIDAY, 20th September

Room A: Auditorium of the CSIC Central Building

8,30h-10,00h

Session 6: Airborne pollen and spores: environmental indicators and allergens

Chairpersons: Delia Fernández (Univ. León, Spain), Carmen Galán Soldevilla (Univ. Córdoba, Spain)

Session 6c

8,30h-8,45h: ORAL COMMUNICATION (S6-O-09)

Relationship between trends in airborne pollen series in Catalonia (NE Spain) and the NAO and WeMO regional teleconnections
Marta Alarcon, Jordina Belmonte, Cristina Periago

8,45h-9,00h: ORAL COMMUNICATION (S6-O-10)

An integrated olive-crop yield-forecast model for the Mediterranean area.
Jose Oteros, Fabio Orlandi, Herminia García-Mozo, Fátima Aguilera, Ali Ben Dhiab, Tommaso Bonofiglio, Mounir Abichou, Luis Ruiz-Valenzuela, M^a del Mar Trigo, Consuelo Díaz de la Guardia, Eugenio Domínguez-Vilches, Monji Msallem, Marco Fornaciari, Carmen Galán

9,00h-9,15h: ORAL COMMUNICATION (S6-O-11)

Phenology and Aerobiology of the Maidenhair tree (*Ginkgo biloba*): a living fossil for modern cities
Paloma Cariñanos, Alberto Amores, Damián Iglesias, Marcos Maroto, Cristina Orihuela, Consuelo Díaz de la Guardia

9,15h-9,30h: ORAL COMMUNICATION (S6-O-12)

Seasonality of *Penicillium* species in outdoor air in Badajoz
Santiago Fernández Rodríguez, Rafael Tormo Molina, José María Maya Manzano, Inmaculada Silva Palacios, Ángela Gonzalo Garijo

9,30h-9,45h: ORAL COMMUNICATION (S6-O-13)

The pollen content of the atmosphere on the Abomey campus during the dry season in Benin
Tossou G. Monique

9,45h-10,00h: ORAL COMMUNICATION (S6-O-14)

Aerobiological and ecological study of the potentially allergenic ornamental plants in South Spain.
Velasco-Jiménez M.J., Alcázar P., Trigo M.M., Valle A., Minero F., Domínguez-Vilches E., Galán C.

Room B: Auditorium of Rocasolano Institute

8,30h-10,00h

Session 7: Landscape change and human-environment interactions

Chairpersons: Blanca Ruiz Zapata (Univ. Alcalá, Spain), Walter Finsinger (CNRS, Montpellier, France)

Session 7c

8,30h-8,45h: ORAL COMMUNICATION (S7-O-09)

Human management and microregional landscape variability of high altitude Eastern Pyrenees during historical times: the upper valleys of Ter and Tet
Santiago Riera, Yannick Miras, Yolanda Llergo, Ramon Julià, Aude Beauger, Ana Ejarque, Josep Maria Palet, Hèctor A. Orengo, Itxaso Euba, Arnau García

8,45h-9,00h: ORAL COMMUNICATION (S7-O-10)

Pollen data and other archaeobotanical remains from the middle ages in Wallonia (Southern Belgium): a review
Mona Court-Picon, Sidonie Preiss

9,00h-9,15h: ORAL COMMUNICATION (S7-O-11)

Environmental changes during the past 1000 years: a high-temporal-resolution multiproxy record from a mire in northern Finland
Walter Finsinger, Kristian Schoning, Sheila Hicks, Andreas Lucke, Tomasz Goslar, Friederike Wagner-Cremer, Heidi Hyyppa

9,15h-9,30h: ORAL COMMUNICATION (S7-O-12)

Climate and land-use changes during the four last millennia in Southern Alps (Italy) recorded at Lake Ledro
Sébastien Joannin, Michel Magny, Odile Peyron, Boris Vannière, Didier Galop

9,30h-9,45h: ORAL COMMUNICATION (S7-O-13)

Focus on humans and landscapes during the last millennium in a mid-level mountain area (Lake Remoray, Jura Mountains, France): a multi-proxy approach including historical and palae
Murgia Laurie

9,45h-10,00h: ORAL COMMUNICATION (S7-O-14)

Highlighting environmental and climate changes in Vanuatu in connection with the early settlement of the Pacific - palynological study of Holocene deposits
Combettes Claire

Room A: Auditorium of the CSIC Central Building

Closing Plenary Lecture

11,00h-12,00h: PLENARY LECTURE (PL-03)

Quaternary disappearance of tree taxa from Europe: timing and trends
Donatella Magri (Sapienza Univ., Roma, Italy)

Posters communications

Posters Session 1:

Applied palynology, bee pollination and diseases

POSTER COMMUNICATION S1-P-01

Pollen conservation tests over time: changes in quality, nutritious and healthy parameters, and microbiology analysis

Amelia Virginia González Porto, Cristina Pardo Martín

POSTER COMMUNICATION S1-P-02

Studying the daily harvesting of pollen by *Apis mellifera* L. (Galicia – NW Spain)

Sandra Armesto-Baztán, M^a Pilar de Sá-Otero

POSTER COMMUNICATION S1-P-03

Patterns of uncorrespondance between the palynological profile and the specifications of a Protected Designation of Origin: PDO “Miel de Granada” A case study.

S. Manzano, D. González, J.C. Bravo, A. Gallego, R.M. Garcinuño, C. Gómez Ferreras

POSTER COMMUNICATION S1-P-04

Tracing the geographical origin of chestnut honeys with protected designation of origin “Miel de Granada” based on mineral content and melissopalynological profile

D. González, S. Manzano, J.C. Bravo, A. Gallego, R.M. Garcinuño, C. Gómez Ferreras

POSTER COMMUNICATION S1-P-05

Pollen characterisation of honeydew honeys from NW Spain and their contribution as health food

Rodríguez-Flores M. Shantal, Escuredo Olga, Seijo M. Carmen

POSTER COMMUNICATION S1-P-06

In vitro pollen germination: effects of low and high temperature stress and pollen viability of GM and non-GM maize lines.

Alicia Tabasco, Pedro M. Díaz-Fernández, Aranzazu Gómez-Garay

POSTER COMMUNICATION S1-P-07

The investigation of morphologic and humidity analysis of the pollen grains collected by honeybees in Ardahan region of Turkey between 2010 and 2011
Deniz Canlı, Kadriye Sorkun

POSTER COMMUNICATION S1-P-08

Molecular methods: a new antifraud methodology on bee pollen
Ana Lumbreras, Alberto Benavent, Amelia Virginia González Porto, Cristina Pardo

Posters **Session 3:**

Pollen morphology, development and germination

POSTER COMMUNICATION S3-P-01

Immunolocalization of arabinogalactan proteins (AGPs) and pectins in cork oak male flower and pollen
Amorim, M.I., Costa, M.L., Silva, R., Sobral R., Coimbra, S.

POSTER COMMUNICATION S3-P-02

PsPMEP a pollen-specific protein of pea (*Pisum sativum* L.) with homology to pectin methylesterases
Begoña Renau, María Dolores Gómez, Edelín Roque, Julio Polaina, José Pío Beltrán, Luis A. Cañas

POSTER COMMUNICATION S3-P-03

Pollen tube culture medium induces variations of cell wall associated proteins in *Arabidopsis thaliana*.
Costa, M., Pereira, L.G., Coimbra, S.

POSTER COMMUNICATION S3-P-04

Immunocytochemical localization of allergenic proteins in *Plantago lanceolata* pollen grains
Z. González Parrado, A.M. Vega Maray, R.M. Valencia Barrera, C.R. Fuertes Rodríguez, R. Monsalve, D. Fernández González

POSTER COMMUNICATION S3-P-05

How methods, state, and source of the material can influence on discernibility of exine layers in *Quercus* pollen?
Tekleva M.V., Polevova S.V.

POSTER COMMUNICATION S3-P-06

A comparison of the tapeta within the family Papaveraceae s.l.: the presence and absence of Ubisch bodies.

M.C. Fernández, M.A. Pérez-Gutierrez, V.N. Suarez-Santiago, A.T. Romero

POSTER COMMUNICATION S3-P-07

Pollen morphology, ultrastructure and viability in *Vriesea ensiformis* (Vell.) Beer (BROMELIACEAE)

Sandra Santa Rosa, Monica Lanzoni Rossi, Adriana Pinheiro Martinelli

POSTER COMMUNICATION S3-P-08

Pollen grain morphology and viability in five *Eucalyptus* species

Mônica Lanzoni Rossi, Everton Hilo de Souza, Adriana Pinheiro Martinelli

POSTER COMMUNICATION S3-P-09

Pollen morphology, germination and viability in Bromeliaceae

Everton Hilo de Souza, Monica Lanzoni Rossi, Renan Machado Packer, Fernanda Vidigal Duarte Souza, Adriana Pinheiro Martinelli

POSTER COMMUNICATION S3-P-10

Pollen Morphology of Some Endemic *Silene* L. (Caryophyllaceae) taxa

Burcu Yilmaz Çitak, Hüseyin Dural, Yavuz Bağcı

POSTER COMMUNICATION S3-P-11

Comparative palynomorphological study of the genus *Euonymus* (Celastraceae)

Gavrilova O.A., Tarasevich V.F., Grigorieva V.V., Britski D.A., Pozhidaev A.E., Leunova V.M.

Posters **Session 4:**

Paleoenvironment and paleoclimate

POSTER COMMUNICATION S4-P-01

Dynamics of cedar forests in the northern part of the Middle Atlas Mountains, Morocco

Tabel, J., C. Khater, A. Rhoujjati, R. Cheddadi

POSTER COMMUNICATION S4-P-02
OLEA-DP: a new application used to plot pollen.
T. Martín Arroyo, M.B. Ruiz Zapata, M.J. Gil García

POSTER COMMUNICATION S4-P-03
Pollen record during the Eemian from the Fuentillejo maar-lake sequence
(Ciudad Real, Spain).
M.J. Gil-García, M.B. Ruiz-Zapata, J. Vegas, A. García-Cortés, L. Galán, J.E.
Ortiz, J.M. García Rincón

POSTER COMMUNICATION S4-P-04
Migrations and climatic changes in South East Asia and Pacific areas
AM Sémah

POSTER COMMUNICATION S4-P-05
Carya, pollen indicator of quaternary refuge area in mediterranean Europe
R. Orain, V. Lebreton, E. Russo Ermolli, N. Combourieu-Nebout, A.-M. Sémah

POSTER COMMUNICATION S4-P-06
Holocene vegetation and climate changes in central Mediterranean inferred
from a high-resolution marine pollen record (Adriatic Sea)
N. Combourieu-Nebout, O. Peyron, V. Bout-Roumazielles, S. Goring, S. Joannin

POSTER COMMUNICATION S4-P-07
Palaeoenvironmental changes in Turonian-Coniacian Cerro de la Mesa
sequence (Sierra de Guadarrama, Madrid, Spain) based on the palynological
data analysis.
M^a Teresa Fernández Marrón, J.Fernando Fonollá Ocete, Javier Gil Gil

POSTER COMMUNICATION S4-P-08
Pollen analysis of three sequences in the Riologo peat bogs, Leon NW of Spain
M^a Amor Fombella Blanco, Emilio Puente García, Elena García-Rovés
Fernández, Ruth Rodríguez Pastor, Laura García Parada, Fco. Javier Ezquerro
Boticario

POSTER COMMUNICATION S4-P-09
Pollen analyses of surface samples obtained from different sedimentary
environments in the Vilaboa salt-marsh (Ría de Vigo).
Iria García Moreiras, José María Sánchez, Soledad García-Gil, Castor Muñoz
Sobrino

POSTER COMMUNICATION S4-P-10

Lateglacial early Holocene transition in SW Europe: a high resolution multiproxy record from Laguna de la Roya (NW Iberia).

García-Moreiras I., Heiri O., Hazekamp M., van der Velden D., Kirilova E.P., Lotter A.F., Muñoz Sobrino C.

POSTER COMMUNICATION S4-P-11

Paleoclimatic reconstruction based on archaeopalynological data: a novel approach

F. Alba Sánchez, J.A. López Sáez T. Nájera Colino, F. Molina González, S. Pérez Díaz, L.M. Quinzo Ortega

Posters Session 5:

Pollen biotechnology and genetics

POSTER COMMUNICATION S5-P-01

Anatomical anther development and anther culture in *Brachiaria* sp..

Andréa D. Koehler, Diva M. A. Dusi, Mônica L. Rossi, Renan M. Packer, Vera T. C. Carneiro, Adriana P. Martinelli

POSTER COMMUNICATION S5-P-02

ROS and differential stress responses of antioxidative systems during in vitro *Quercus suber* microspore embryogenesis.

Cristina Parra, Beatriz Pintos, José Antonio Manzanera, Pilar S. Testillano, Luisa Martín, Aranzazu Gómez-Garay

POSTER COMMUNICATION S5-P-03

Histological evidence for reprogramming of pollen grains in the cultured cassava anthers

P.I.P. Perera, C.A. Ordoñez, B. Dedicova

POSTER COMMUNICATION S5-P-04

DNA methylation and MET1a-like expression are regulated during pollen development and pollen embryogenesis

María-Teresa Solís, Manuel-Jesús Soriano, María C. Risueño, Pilar S. Testillano

POSTER COMMUNICATION S5-P-05

Early common markers of microspore and somatic embryogenesis in *Quercus suber*

Héctor Rodríguez-Sanz, José Antonio Manzanera, María-Carmen Risueño, Pilar S. Testillano

POSTER COMMUNICATION S5-P-06

Effect of various factors on microspore embryogenesis induction in two tunisian olive cultivars

M. Mtimet, B. Chiancone, M. Mars, A. Ferchichi, M.A. Germanà

Posters **Session 6:**

Airborne pollen and spores: environmental indicators and allergens

POSTER COMMUNICATION S6-P-01

“Región of Murcia” Aerobiological Network REaReMur. Preliminary data
Elvira-Rendueles B., Moreno JM, García-Sánchez A., Moreno-Grau S.

POSTER COMMUNICATION S6-P-02

Comparison between two adhesives in Hirst spore traps in a controlled environment

José María Maya Manzano, Santiago Fernández Rodríguez, Inmaculada Silva Palacios, Ángela Gonzalo Garijo, Rafael Tormo Molina

POSTER COMMUNICATION S6-P-03

Quercus airborne pollen tendencies in the south of Iberian Peninsula, its correlation with meteorological trends and possible effect of the climatic change in mediterranean forests

Marta Recio, Maria Del Mar Trigo, Herminia García-Mozo H, Carmen Galán, Consuelo Díaz-De La Guardia, Luis Ruiz, Silvia Docampo, Baltasar Cabezudo

POSTER COMMUNICATION S6-P-04

Pollen Calendar of the atmosphere of Tetouan (NE Morocco): 2008-2011

N. Aboulaich, M.M. Trigo, H. Bouziane, M. Recio M. Kadiri, B. Cabezudo, H. Riadi, M. Kazzaz

POSTER COMMUNICATION S6-P-05

One year aeropalynological analysis of atmospheric pollens in Ankara, Turkey
Aydan Acar, N. Münevver Pinar, Talip Çeter, Yavuz Türkmen, Derya Şimşek

POSTER COMMUNICATION S6-P-06

Aerobiological survey in the Biosphere Reserve “Sierras de Francia y Béjar”
(MW Spain)
David Rodríguez de la Cruz, Estefanía Sánchez Reyes, Alberto Martín Baz, José Sánchez Sánchez

POSTER COMMUNICATION S6-P-07

Relationship between aeroallergen Pla a 1, Platanus pollen and air pollutant
De Castro-Alfageme S., Fernández-González D., Valencia-Barrera R., Vega-
Maray A., González-Parrado Z., Sánchez-Reyes, E., Mandrioli P.

POSTER COMMUNICATION S6-P-08

Incidence of fungi spores in the atmosphere of Tetouan (NW Morocco) and
effect of the meteorological parameters
F. Bardei, H. Bouziane, M.M. Trigo Pérez, N. Ajouray, F. El Haskouri, F. Filali
Ben Sidel, R. Abiri, M. Kadiri, M. Kazzaz, H. Riadi

POSTER COMMUNICATION S6-P-09

Olive tree genetic background is a major cause of profilin (Ole e 2 allergen)
polymorphism reflected in functional and allergenic variability
Jose Carlos Jiménez-López, María Isabel Rodríguez-García, Juan de Dios Alché

POSTER COMMUNICATION S6-P-10

Air pollen on the Island of Menorca (Spain) during the period 2006-2010
José Luís Frontera, Gloria Florit, Marzia Boi, Leonardo Llorens

POSTER COMMUNICATION S6-P-11

Proteomics analysis of Holm oak pollen
José Valero Galván, Sekvan Demir, Jesus V. Jorriñ Novo

POSTER COMMUNICATION S6-P-12

Relationship between the dynamic of atmospheric olive pollen and the flowering
phenology in diverse geographic locations of the Jaén province
Luis Ruiz Valenzuela, Fatima Aguilera Padilla & Concepción Muñoz Quesada

POSTER COMMUNICATION S6-P-13
Allergenic Pleosporales in home environments in Barcelona
Álvaro Rúa-Giraldo, Jordina Belmonte, Jorge Martínez

POSTER COMMUNICATION S6-P-14
Incidence of Cupressaceae pollen in the atmosphere of Málaga (Spain): 1992-
2013
M. M. Trigo, L. López-Serrano, J. García-Sánchez, M. Recio, S. Docampo, M.
Melgar, B. Cabezudo.

POSTER COMMUNICATION S6-P-15
Airborne detection of Pla a1 allergen, an important source of allergy in the cities
Rodríguez-Rajo, F.J., Vara, A., Fernández-González, M., Guedes, A., Abreu, I.

POSTER COMMUNICATION S6-P-16
A model to predict the concentration of airborne Platanus pollen in Central
Spain
Silvia Sabariego Ruiz, Ana Rapp Benito, Consolación Vaquero del Pino, Jesús
Rojo Úbeda, Veronica Bouso Muñoz, Rosa Pérez Badia

POSTER COMMUNICATION S6-P-17
Seasonal atmospheric pollen variations in Montevideo city: general trends of a 2
year survey.
Tejera, L., Beri, A., Martínez-Blanco, X.

POSTER COMMUNICATION S6-P-18
Aeroallergens cross reaction detection among the Oleaceae family
Vara, A, Fernández-González, M, Jato, V, Suárez-Cervera, M, Aira, MJ,
Rodríguez-Rajo, FJ

POSTER COMMUNICATION S6-P-19
Annual Atmospheric Polen Calendar of Gümüşhane, Turkey
Yavuz Türkmen, N. Münevver Pinar, Talip Çeter, Aydan Acar, Derya Şimşek

Session 7:

Landscape change and human-environment interactions

POSTER COMMUNICATION S7-P-01

Palaeoenvironmental and vegetation changes during the Holocene in the NW sector of the Iberian Range (Cuenca del río Añamaza, NE Spain)

A.Gauthier, A. Luzón, A. Pérez, A. Muñoz, M.J. Mayayo

POSTER COMMUNICATION S7-P-02

All about yew: on the trail of *Taxus baccata* in SW Europe by means of integrated archaeobotanical studies

Uzquiano, P., Allué, E., Antolín, F., Burjachs, F., Piqué, R., Ruiz-Alonso, M., Zapata, L.

POSTER COMMUNICATION S7-P-03

Expansion and decline of yew (*Taxus baccata* L.) in the Basque Mountains (Northern Iberian Peninsula) during the Holocene

Pérez-Díaz, S., López-Sáez, J. A., Ruiz-Alonso, M. Zapata, L.

POSTER COMMUNICATION S7-P-04

Pollen content in Muslim vessels found in Mallorca

Marzia Boi, José Luís Frontera, Gloria Florit, Jaume Deyà, Pablo Galera

POSTER COMMUNICATION S7-P-05

La Carisa roman site (Asturias) pollen data.

M.B. Ruiz Zapata, M.J. Gil García, T. Martín Arroyo, Ángela Sánchez, M. Jiménez Sánchez

POSTER COMMUNICATION S7-P-06

Scots pine (*Pinus sylvestris*) forests in the Spanish Central System.

Phytosociological and paleopalynological considerations.

López Sáez, J.A., Sánchez Mata, D., Alba Sánchez, F., Abel Schaad, D., Gavilán, R.G., Pérez Díaz, S.

POSTER COMMUNICATION S7-P-07

Nomadic societies and pastoral pressure during the last 2000 years in the mountains of Arkhangai, Mongolia

I. Jouffroy-Bapicot, B. Vannière, D. Etienne, J. Magail

POSTER COMMUNICATION S7-P-08

Holocene vegetation, fire and climate interactions in western Spain: El Maíllo
mire

César Morales-Molino, Mercedes García-Antón, José M. Postigo-Mijarra, Carlos
Morla

Abstracts by sessions

Plenary Lectures

Thomas Servais, CNRS-Univ. Lille 1, France

“The evolution of the phytoplankton during the Phanerozoic: relation to sea-level, climate and CO₂”

Iñaki Hormaza, EE La Mayora, CSIC, Málaga, Spain

“Pollen development and pollen-pistil interactions in the early-divergent angiosperm *Annona cherimola*”

Donatella Magri, Sapienza Univ. Rome, Italy

“Quaternary disappearance of tree taxa from Europe: timing and trends”

PLENARY LECTURE

**The evolution of the phytoplankton during the Phanerozoic:
relation to sea-level, climate and CO₂**

Thomas Servais

UMR 8217 Géosystèmes CNRS-Univ. Lille1

The modern oceans display three major groups of marine phytoplankton: the calcareous (coccolithophores, etc.), siliceous (diatoms, etc.) and organic-walled (dinoflagellates, etc.) phytoplankton. The fossil record indicates that elements of the organic-walled microphytoplankton were most probably present in the oceans since the Precambrian. However, the presence of calcareous and siliceous phytoplankton in the Precambrian and Palaeozoic is not clearly established, coccolithophorids and diatoms only appear in the fossil record in the early Mesozoic.

In the Palaeozoic the fossil record of the acritarchs can be used as a proxy for the larger marine organic-walled microplankton (> 20 µm), the smaller fractions of the phytoplankton, the picoplankton and the bacterioplankton having usually not been documented. Although the artificial group of the acritarchs is, by definition, polyphyletic, many of the Palaeozoic acritarchs most probably represent the cysts of phytoplanktonic organisms, similar to modern organic-walled dinoflagellates.

The diversity changes of the Palaeozoic acritarchs at the species and genus level indicate some major palaeoecological trends and allow us to redraw partly the evolution of the marine phytoplankton from the Cambrian to the Permian. Here we present a summary of the evolution of the Palaeozoic organic-walled phytoplankton in major steps: a long-term Cambrian-Ordovician radiation, most probably triggering the 'Ordovician plankton revolution', possibly following a 'pulse of atmospheric oxygen' in the Late Cambrian; fluctuating diversities in the Silurian and Early Devonian, leading to the 'Devonian nekton revolution'; a dramatic decrease during the Late Devonian-Early Carboniferous interval; a period with apparently low diversities during the 'Late Palaeozoic phytoplankton blackout'.

Similarly to Mesozoic-Cenozoic phytoplankton diversities, the acritarch diversity in the Lower and Middle Palaeozoic can roughly be correlated with sea levels. Moreover, the Palaeozoic diversity curve can also partly be compared with the evolution of the atmospheric pCO₂. However, the fossil record of the Palaeozoic phytoplankton is far from being complete and most interpretations remain highly speculative.

PLENARY LECTURE

Pollen development and pollen-pistil interaction in the early-divergent angiosperm *Annona cherimola*

I. Hormaza ¹, J. Lora ², M. Herrero ²

¹Instituto de Hortofruticultura Subtropical y Mediterránea “la Mayora” CSIC, 29750 Algarrobo-Costa, Málaga, Spain

²Dept. Pomology, Estación Experimental Aula Dei, CSIC, Apdo. 202/ 50080. Zaragoza, Spain

Studies of reproductive biology in ancient angiosperm lineages are beginning to throw light on the early evolution of flowering plants, but comparative studies are still restricted by fragmented and insufficient species representation in most of these angiosperm clades. In this work pollen development and the progamic phase, from pollination to fertilization, are described in cherimoya (*Annona cherimola* Mill.) a member of the Annonaceae, the largest living family among the magnoliid group of early-divergent angiosperms. Flowers of *A. cherimola* are hermaphrodite, but self-fertilization in the same flower is hindered by a protogynous dichogamous system where female and male structures do not mature simultaneously. At anther dehiscence *A. cherimola* releases a variable ratio of bicellular and tricellular hydrated pollen aggregated in groups of four depending on temperature. Examination of pollen cell number during pollen development showed that this coexistence was due to a late mitosis starting shortly prior to pollen shedding. The presence of both types of pollen at anthesis is an uncommon characteristic in angiosperms making *A. cherimola* an excellent model to study the effect of variable environmental conditions during the final stages of pollen development on subsequent pollen performance. After pollen germination on the stigma the pollen grains compete in the stigma-style interface to reach the narrow secretory area that lines the margins of a semi-open stylar canal, which shows a continuous secretory papillar surface along the carpel margins, which run from the stigma down to the obturator in the ovary. This plesiomorphic gynoeceium hosts a simple pollen-pistil interaction that results in a support-control system of pollen tube growth. This extramural pollen tube competition contrasts with the intrastylar competition predominant in more recently derived lineages of angiosperms.

PLENARY LECTURE

Quaternary disappearance of tree taxa from Europe: timing and trends

Donatella Magri

Dip. Biologia Ambientale, Sapienza University, P.le Aldo Moro, 5, 00185 Roma, Italia

The long-term behaviour of tree populations in Europe during the last 2 Ma has been very different from taxon to taxon: many tree populations have undergone shifts in their geographical distribution; many tree genera have persisted in selected regions of Europe for millions of years; several tree taxa have disappeared from part or the whole of the European territory. In S Europe, from where many Early and Middle Pleistocene pollen records are available, a later disappearance of tree taxa with respect to the central and northern European countries is generally recorded, with some regional differences: in the Italian and Balkan Peninsulas, several tree genera that are currently absent (e.g. *Pterocarya*, *Tsuga*, *Cedrus* and *Zelkova*), were more abundant during the late Early Pleistocene and persisted longer during the Middle Pleistocene than in Spain and France. By contrast, *Engelhardia*, *Eucommia*, *Parthenocissus*, and *Aesculus* are found in both Spain and France during the late Early Pleistocene, at a time when they were already absent from central Italy. *Picea*, which was everywhere abundant in Europe during the Early Pleistocene, markedly declined in central Italy and Spain during the Middle Pleistocene, disappearing at the end of the Late Pleistocene. This suggests that, contrary to what is commonly believed, there are tree populations that undergo progressive reductions in their distributions, eventually leading to regional disappearance, starting from the Mediterranean countries. This is the case, for example, for *Buxus* and *Abies*, which show progressive fragmentation in their southern geographical distribution during the Late Quaternary. Mapping the temporal processes of the decline and disappearance of tree populations, and detecting the European territories with high extinction rates in the past, and conversely the regions with high persistence chance, may significantly help planning new biodiversity conservation policies.

Abstracts by sessions

Workshop
“New Methodological Advances in Palynology Research and Imaging”

Workshop

“New Methodological Advances in Palynology Research and Imaging”

Donatella Magri (Sapienza University of Rome, Italy)

“New frontiers in paleopalynology”

Carmen López Iglesias (CCiT Univ. Barcelona, Spain)

“Cryoimmobilization techniques in TEM for *in situ* molecular recognition in pollen studies”

Gabriel Cristobal (Inst. Óptica, CSIC, Madrid, Spain)

“Optical and digital image processing applied to pollen analysis. Problems and challenges”

Surangi W. Punyasena (Univ. Illinois, USA)

“Quantitative approaches to pollen analysis: the role of super-resolution microscopy, machine learning, and bioimage informatics”

WORKSHOP “New Methodological Advances in Palynology Research and Imaging”

New frontiers in paleopalynology

Donatella Magri

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The earliest pollen analysis was published almost one century ago. Since then, a considerable development of palaeopalynological studies has allowed reconstructions of past vegetation dynamics from all over the world. Recent scientific developments show that traditional pollen analysis, based on pollen morphology, may be profitably supplemented with innovative methodological approaches, paralleling the advances of biological research. Three such novel approaches include genetic, isotopic and biochemical techniques, respectively. Pioneering studies on the ancient DNA from tree pollen grains, linking the information on extant genetic features and the history of fossil plant populations, have demonstrated the interest of studying genetic changes through time and the role that glacial refuge areas may have had in shaping the modern genetic diversity (cf. Magyari et al., 2011; Parducci et al., 2012). Carbon isotopic analysis on pollen grains is an emerging field of research allowing reconstruction of the abundance of C₄ grasses in past landscapes (Nelson et al., 2008), assessment of the palaeophysiology of C₃ plants, and more in general inferences about past plant physiological processes (Nelson, 2012). A recent paper by Willis et al. (2012) has opened a new path in the assessment of past UV-B fluxes in the environment through the analysis of UV-B-absorbing compounds in fossil pollen grains from Holocene sediments in Norway. The comparison of pollen records and reconstructed UV-B fluxes at different latitudes may help to determine the regional response of natural terrestrial ecosystems to variations in UV-B radiation through time. Another future prospect in this field of research is the possibility to elucidate whether causal relationships exist between changes in UV-B flux and extinction, origination and turnover rates of plant species, thus contributing to a better knowledge of biological evolution.

WORKSHOP “New Methodological Advances in Palynology Research and Imaging”

Cryoimmobilization techniques in TEM for *in situ* molecular recognition in pollen studies

C.López-Iglesias, L.Delgado, G. Martínez & M. Suárez-Cervera

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A major objective of modern structural biology is to decipher the cellular organization by elucidating the spatial arrangement of macromolecular complexes within a cell. Three-dimensional studies combined with cryo-preparation methods in electron microscopy enable the study of biological specimens in a quasi *in vivo* “hydrated” and “three-dimensional” state. To achieve the close-to-native state, samples have to be cryo-immobilized, either by plunge freezing, impact freezing or high pressure freezing (HPF). The goal is to obtain “vitreous” or amorphous ice after ultra-rapid freezing, without destroying cell structures and preserving a precise moment of their interactions, which are rapidly changing *in vivo*. Vitrification allows spatial and temporal resolution of cellular events.

Once the cells are immobilized by freezing, two strategies can provide the three-dimensionality of the structures of interest: Single particle analysis and electron cryo-tomography. Single particles are small isolated structures not needing sectioning and the procedure carried out is the reconstruction from the different orientations of the single particle. Electron cryo-tomography refers to the three-dimensional reconstruction of bulk structures acquiring images at low temperature and in as many directions of the electron beam as possible. Two approaches for cryo-thinning samples for cryo-tomography are now being used and improved: vitreous cryo-sectioning and cryo-FIB-SEM thinning.

On the other hand, freeze substitution, together with other techniques, like Tokuyasu and the new rehydration and VIS2FIX methods, can also be combined with on-section immunogold labelling for protein localization studies with reliable morphology. In pollen grains, we compared three different strategies to immunolocalize the major allergens, soluble proteins, before their exit: HPF-freeze substitution, chemical fixation-cryo-sectioning, and chemical fixation-freeze substitution.

WORKSHOP “New Methodological Advances in Palynology Research and Imaging”

Optical and digital image processing applied to pollen analysis. Problems and challenges.

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Manual identification of pollen samples is a time consuming process, requiring the presence of highly trained palynologists. Routine palynological studies often require analysing thousands of individual pollen grains, most frequently using transmitted brightfield microscopy (e.g. 40X magnification). Therefore, there is a need for an automated classification system which can provide an accurate analysis of the samples. The two main problems that are difficulting the current progress in this field are a) the extraction of knowledge from expert palynologists and b) a limited access to open pollen databases with a large number of reference pollens per taxa. Pollen analysis can be accomplished through different stages: acquisition, pre-processing, segmentation, feature extraction and classification. Robust automatic segmentation methods are needed for avoiding dust and pollution or the presence of aggregates (e.g. by clump splitting). The depth of field of a typical optical microscope only allows visualizing part of the specimen in focus and therefore the use of a multifocus stack could eventually provide more details about the pollen surface. However, how to gather such multiple information from the stack is still an open issue. Previous work in the area of aero-palynology (ASTHMA EU project) used multifocus stacks and reported recognition rates around 77% for 30 pollen types. In this paper, a large dataset of features taken both from the exine and from the inner part of the pollen (texture) have been extracted from few representative examples of european bee pollen. In particular 10 taxa with 120 reference pollen grains per taxa (Apifresh EU project) were analyzed. The accuracy reached around 94% of recognition of the pollen grains using the leave-one-out validation model. In the future, we plan to extend the current study with a large number of taxa and to investigate more robust methods for segmentation, multifocus fusion and texture analysis of the pollen grains.

WORKSHOP “New Methodological Advances in Palynology Research and Imaging”

Quantitative approaches to pollen analysis: the role of super-resolution microscopy, machine learning, and bioimage informatics

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Although fossil pollen and spores comprise one of the most abundant and temporally continuous histories of terrestrial vegetation changes over time, there are key limitations to current approaches. Firstly – our inability to quantify much of the morphological differences among taxa has led to conservative identifications and limited taxonomic resolution. Secondly – the rate at which samples can be analyzed is significantly slower than many other geological proxies. This has meant that a fraction of the palynological data are analyzed, limiting and potentially biasing our assessments of past plant diversity and compositional turnover.

I present results from three veins of collaborative research that attempt to address the limitations. The first applies a bias-optimized, layered machine-classification model that uses instance-based learning to the classification of pollen images. The resulting supervised system not only automates the classification of individual grains, it provides explicit, quantitative assessments of classification confidence, allowing the inherent uncertainty within any given classification to be propagated in analyses that rely on pollen data, such as many climate-vegetation models.

Our second vein of research is in the development of new algorithmic measures of morphology. We demonstrate that one of the most challenging classification problems – the discrimination of grass species – can be achieved by quantifying subtle differences in texture evident below the diffraction limit of light. Our model achieves 75% accuracy in the classification of 12 grass species.

Our third approach is the development of a shared pollen image database that seeks to incorporate machine learning and new algorithmic features into a “search by example” format. The intention is to develop a communal knowledge base of pollen types. This database is currently in development for the Miocene palynomorphs of tropical South America.

Abstracts sessions 1-7

Lectures & Oral communications

Session 1:

Applied palynology, bee pollination and diseases

Session 2:

Paleoceanography, dinocysts and acritarchs

Session 3:

Pollen morphology, development and germination

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Pollen biotechnology and genetics

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Airborne pollen and spores: environmental indicators and allergens

Session 7:

Landscape change and human-environment interactions

Session 1: Applied palynology, bee pollination and diseases
INVITED LECTURE

A practical example of a multidisciplinary collaborative work to understand the honey bee colony losses: from palynology to bee pathology

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Honeybee colony collapse is a sanitary and ecological worldwide problem. The features of this syndrome are an unexplained disappearance of adult bees, a lack of brood attention, reduced colony strength, and heavy winter mortality without any previous evident pathological disturbances. This problem is thought to be related to the effects of pathogens or pesticides on honey bees, although to what extent these factors are implicated is still not clear.

A collaborative multidisciplinary group of palinologists, chemists and bee pathologists has been working to study the causes of bee mortality in Spain. In this way, different works were performed in samples coming from experimental colonies or professional apiaries collected either under active or passive surveillance programs. In all of them, the anamnesis with the beekeeper, clinical and laboratory examinations of bee samples, and palinological and chemical analyses on pollen stored in the colonies were carried out.

The crops more usually linked with harming bee pesticides are sunflower and corn. For that reason, the palinology was used to determine if those crops were present in stored pollen and if they were in fact foraged. Results confirmed that the most of pollen samples analyzed were from wild vegetation. The palinological analysis also highlighted certain inconsistencies in the beekeepers' perception of the flora the honey bees really visit and the unreliability of their information. All the analysis demonstrated also the pollen availability in colonies, its diversity and the presence of more than enough stored pollen at the time of breakdown. The presence of pesticides (phenilpyrazols and neonicotinoids) was not significantly correlated with the collapse of the colonies.

Finally, the results of all the analyses demonstrated that the colony collapse is related to the infection by *Nosema ceranae* (Microsporidia) an emerging pathogen of *Apis mellifera*. No other significant pathogens or pesticides were detected.

Session 1: Applied palynology, bee pollination and diseases
INVITED LECTURE

Assessing the impact of declining pollinators on world agriculture

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Pollinator populations, especially bees, are declining all over the world, often in overall abundance and always in diversity. I will examine the consequences of this decline on our food supply and more generally on the world agriculture and environment. In 2007, we estimated that 68% of the 57 crop species that provide nearly 95% of our food supply benefited from insect pollination and these crops provided 37% of the human diet (Proc. R. Soc. B. 274:303-313). Yet the reliability of these estimates remained in question, and subsequent studies have suggested that the impact of pollinator decline has yet to become apparent in world statistics and that it will remain marginal at worst as various pollination strategies will alleviate the impact of pollinator decline. I will review the evidence for both sides of this argument, and use recent results to show how research on pollen and pollination holds essential keys to provide answers to these questions and alleviate both pollinator decline and its impact on world agriculture.

Session 1: Applied palynology, bee pollination and diseases
INVITED LECTURE

Pollen grain as bioindicator

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We would like to quote the delightful foreword of Knut Faegri in the 1992 book "Pollen et Spores" by Ranault-Mikovsky and Petzold:

"Pollen-pollen-everywhere.... In laboratories, medicals are looking for allergens, policemen for criminals, geologists for oil, botanist for the story of the plant world of times passed, and archeologists for intimate glimpses of life, food and customs".

As we can see from the previous paragraph the applications of Palynology are ample. In this presentation, after a brief summary of the various applications of Palynology, just look at the title of this session, we will focus our attention in the use of the pollen grain as a bio-indicator for dust intrusions coming from the African deserts of Sahara and Sahel.

Air quality surveillance in Spain is regulated by the Royal Decree 102/2011 that was transposed from the EU directive 2008/50/EC, under the law 34/2007. This law states that both PM₁₀ and PM_{2,5} fractions of atmospheric aerosols have to be monitored, and a procedure has been established to distinguish natural episodes. Querol et al. 2010, highlights the negative effects of particulate matter on human health, climate and the ecosystems, indicating that a rise in the number of African dust intrusions over the Iberian Peninsula, could be a sign of climate change

In this point is where our group considers that Aero-Palynology plays a key-role, as a complimentary tool to air quality surveillance networks. And will convince the administration to support the Aerobiology networks as an essential tool to illustrate these natural events.

Session 1: Applied palynology, bee pollination and diseases
ORAL COMMUNICATION

Pollen diversity associated to olive orchards-visiting bees

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Plant vegetation cover provides many benefits for olive orchards, among which are the enhancement of the populations of honeybees and wild pollinators, as it creates favourable habitats for their conservation. This topic is currently of great interest since both the abundance and the diversity of these important pollinators have declined in many agricultural landscapes.

The number of flowers present and their type will affect the number of bee visitors. This study aims to know the plant species visited by each different group of bees and to determine the number and type of pollens associated to such groups. As an innovation, confocal laser scanning microscopy has been used to assess pollen morphology and content. *Calendula officinalis* and *Echium vulgare* have been identified as the most visited plant species by mining bees and megachilid bees, while the honey bee (*Apis mellifera*) showed a noticeable preference for *Borago officinalis*. Mining and megachilid bees typically carried pollens from two-to-three different flower species on their bodies, corresponding to those species taking part of the plant vegetation cover, as well as pollen from *Olea europaea* itself.

This study was supported by the company Syngenta Agro S.A. (Spain).

Session 1: Applied palynology, bee pollination and diseases
ORAL COMMUNICATION

The importance of pollen morphology in the Christian relic of the Shroud of Turin

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In the 70's and 80's, the criminalist Max Frei studied the pollen of the Shroud of Turin, which he collected using a simple piece of sticky tape applied directly onto the surface of the relic. At the beginning of the 90's, Danin & Baruch analyzed the same tapes, and a total about 313 palinomorphs were discovered, but only 204 were identified to species or family level; the others were in undetermined categories.

The most abundant pollen, as previous studies testify, corresponds to entomophilous species. The most numerous species is the Irano-Turaniana desert plant of *Gundelia turnefortii*. The presence of the various different pollen types led to the deduction that the geographical origin was Asia minor, and that the relic had travelled through Europe and had had direct contact with flower debris.

Although there are not many publications about the pollen in the Shroud, this study shows the mistakes made in the identification of the most abundant types. An example is that of *Gundelia turnefortii*, which corresponds in reality a *Helichrysum* spp type. *Helichrysum* spp. is plant that was highly valued during ancient history for its use in oil and ointments. The presence of its pollen, therefore, along with another type found in the relic, confirms the ethnocultural significance and the use of ointment during the preparing of the body, so its gives a possible explanation as to why it was conserved in the fabric.

Session 2: Paleoceanography, dinocysts and acritarchs
INVITED LECTURE

Worldwide distribution of mid-Cretaceous dinoflagellates: method for palaeoceanographic reconstructions of warm Southern Ocean and impact of northern paleogeography

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The sea surface temperatures (SST) are the most problematic aspects of the Cretaceous greenhouse period. 32 Aptian and 38 Albian dinoflagellates selected from 36 and 70 papers relating studies located in the northern hemisphere and 14 and 17 involving with the southern hemisphere have been mapped using a Geographical Information System (GIS) to precise their worldwide distribution along palaeolatitudes. Distribution from 40°N to 70°S of subtropical Tethyan species demonstrates same asymmetric Aptian and Late Albian SST gradients, stronger in the northern hemisphere than in the southern one. Warm Southern Ocean is now well established by dinoflagellates biogeography and estimated temperatures from $\delta^{18}O$ of organisms or TEX86. The narrow mixing belts of subtropical and subpolar dinocysts in the northern hemisphere (35-40°N) and the broad ones in the southern hemisphere (30 -70°S and 50- 70°S) suggest a dissimilarity in the heat transport on both hemispheres. We suggest that the proto-North Atlantic and the Greenland-Norwegian seaway might have been barriers for oceanic Tethyan subtropical dinoflagellates stopped either by the shallow water column, temperature and/or salinity. The northern heat transport was stopped by continental masses while southern one was not so limited. The estimation of temperature requirements of dinoflagellates is modelled by combining the estimated temperatures from $\delta^{18}O$ or TEX86 ratios related to latitudes, with the latitudinal distribution of the dinocysts. The Southern Ocean could have been warm as 20°C at 60°S in the Late Albian. The mixing of Tethyan and Austral dinoflagellates along 20° suggest the co-occurrence of southern warm and cold currents. We would have to imagine that Tethyan and Austral species lived in different water masses suggesting a structure of the ocean different from the current structure. The ocean circulation would be accomplished through mesoscale eddies which could carry warmth to the southern polar regions.

Session 2: Paleoceanography, dinocysts and acritarchs
ORAL COMMUNICATION

Toxic dinoflagellates and their benthic cysts: the revival strategy! A review for western European and Mediterranean margins

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Over the last two decades, harmful algal blooms (HAB) have been reported from increasingly diversified and numerous places in the world. Global changes (sea-surface warming especially), together with man-made local factors such as coastal pollution and eutrophication, have been identified as the main forcing controls of these massive blooms. In parallel, intensified commercial shipping with increasing ballast tank discharges contribute to disperse non-indigenous species over the world.

Organisms involved in massive HAB phenomena are mostly dinoflagellates. Their complex life cycle, involving several steps, both sexual and asexual, motile and non-motile, with especially the genesis of an organic-walled cyst during the sexual reproduction which temporarily settles to sediment, make them atypical and somewhat opportunistic marine species. For harmful dinoflagellates, these cysts constitute a true "benthic seed". Their concentration pulses, fossilized in the sediments, offer the opportunity to consider the variability and the magnitude of their blooms in past-times.

Fossil dinoflagellate toxic blooms have in this way already been identified in several coastal areas around Europe. For this region, only few species produce toxins and are at the origin of severe perturbations in aquaculture (e.g. oyster farming) activities, they are: species from the genus *Dinophysis* and *Alexandrium*, and *Gymnodinium catenatum* (an endemic species from the Moroccan margin, newly recorded along Cantabrian coasts).

Our study proposes a review of recent knowledge acquired along the south-western European margins. We will focus on the Bay of Biscay thanks to a 2 ka sedimentological archive retrieved in front of Capbreton (core MD03-2693, 43.5°N, -1.7°E, 400 water depth, mean sedimentation rate of 1 cm/year).

Session 2: Paleoceanography, dinocysts and acritarchs
ORAL COMMUNICATION

Deglacial changes over the last 20 000 years in the Equatorial Atlantic: a high-resolution dinocyst analysis

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The Congo turbidite system (Congo Fan), a giant sedimentary accumulation at the base of slope of the Congo-Angola margin, is particularly well suited to address the fundamental question of forcing factors and their impact on deep marine sedimentation. In this context, hemipelagic cores have been retrieved during Zaiango (Ifremer/Total collaboration, 1998-2003) and Repezai (2011) cruises.

Here, we present a new dinocyst-assemblage analysis from the equatorial African margin performed on KZAI-01 core (Zaiango cruise).

Over the last 21 ka, dinocyst and pollen analysis are combined so as to depict past hydrological regimes as well as synchronous ocean-vegetation changes in the equatorial and subtropical eastern Atlantic Ocean.

We show higher upwelling recurrence from Heinrich Stadial 1 (HS1) to the Holocene optimum, and weaker upwelling conditions during the Last Glacial Maximum (LGM) and the late Holocene. This is probably linked with a southern position of the ITCZ and stronger trade winds during colds periods coupled to weaker monsoons and drought regimes on the continent. This pattern explains the dominance of heterotrophic cysts during cold periods. Regional dinocyst reconstructions of paleo sea-surface temperatures and productivities were also obtained with the new transfer fonction using the modern dinocyst database for the tropical Atlantic.

Session 2: Paleoceanography, dinocysts and acritarchs
ORAL COMMUNICATION

Tethyan Albian dinoflagellate cyst assemblages in coastal and fully marine environments (Portuguese basin and DSDP site 398D).

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During the Albian the Portuguese basin and DSDP site 398D were about 30°N in paleolatitude, receiving influence from the Tethys Ocean. Masure and Vrielynck (2009) demonstrate that Cretaceous extinct species of dinoflagellate cysts show a geographical distribution along paleolatitudes which follow climatic belts, as function of their sensibility to temperature. For the Northern Hemisphere, they described four climatic belts during de Late Albian. By combining the sea surface temperatures estimated for each latitude from fish teeth and planktonic foraminifera $\delta^{18}O$ (Pucéat et al., 2007) with the dinoflagellate climatic belts, they have established the temperature range limits for extinct dinoflagellate cysts. The site area is located near the boundary between the warmest and warmer climatic belts. By applying the climatic belt distribution proposed by Masure and Vrielynck (2009) we can attribute to both sites a temperature of approximately 28°C.

The main aim of our study is to analyze the Albian dinocyst assemblages from the Portuguese basin and those from DSDP site 398D, in order to identify the species restricted to coastal and fully marine environments and to test the suitability of dinocysts as a proxy of shoreline distance. We are dealing with their paleoecology to pursue salinity variations. Previous work reveals diversity trends which are interpreted as an indicator of coastal distance. In this way we are trying to evaluate the information provided by both dominant and rare species to attempt to improve the use of diversity index.

MASURE, E. & VRIELYNCK, B. 2009. Late Albian dinoflagellate cyst paleobiogeography as indicator of asymmetric sea surface temperature gradient on both hemispheres with southern high latitudes warmer than northern ones. *Marine Micropaleontology*, 70(3-4): 120-133.

PUCÉAT, E., et al, 2007. Fish tooth $\delta^{18}O$ revising Late Cretaceous meridional upper ocean water temperature gradients. *Geology*, 35(2), 107-110.

Session 2: Paleoceanography, dinocysts and acritarchs
ORAL COMMUNICATION

Last-glacial oceanic conditions south of the Faeroe Shetland Gateway

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This work investigates the response of oceanic paleoenvironments to the last glacial (LG) climatic variability through the study of a high resolution marine archive (core MD99-2281, 60°21'N; 09°27'W; 1197 m water depth) retrieved off SW Faeroes. This area constituted a nodal point regarding paleoceanography during the LG, as it was influenced by warm Atlantic water penetration towards polar basins, overflow of deep water from Nordic Seas, and a progressive deterioration of sea-surface conditions due to the development of proximal ice-sheets (i.e.: British Irish & Fennoscandian Ice-Sheets). This period offers thus an exemplary case study of natural climate variability and atmosphere-ocean-ice coupling that our record permits to investigate at sub-millennial scales (mean sedimentation rate of 80 cm/ka).

A multiproxy approach (coupling geochemical and micropaleontological analysis including dinocysts) was conducted on the first 21 meters of core MD99-2281 (10-45 ka BP), focusing on the reconstruction of past hydrological conditions, qualitatively as well as quantitatively. Our study documents a very sensitive response of the subpolar north Atlantic region to infra-millennial abrupt events (i.e. Dansgaard-Oeschger and Heinrich Events) and points out a coupled oceanic and cryospheric dynamics. Sea-surface paleosalinity and sea-ice cover reconstructions provide a way to assess hydrological changes over the Faeroe Shetland sill.

The Last Glacial Maximum (sensu Mix et al., 2001, i.e. 19 – 23 ka cal BP) experienced hydrological variations of lesser amplitude than those recorded during the preceding Marine Isotopic Stage – MIS 3, especially marked by strong seasonal contrasts of temperature

Session 3: Pollen morphology, development and germination
INVITED LECTURE

AGPs cross-talk in Arabidopsis pollen-pistil interactions

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We have been interested in arabinogalactan proteins (AGPs) for some time, due to the fact that some of these molecules are molecular markers for gametophyte development during sexual plant reproduction. AGPs are a distinctive type of proteins, highly glycosylated that go into post-translation modifications which includes the addition of the glycosidic moieties and of a GPI anchor, both characteristics of important signalling molecules.

AGP6 and AGP11 are two Arabidopsis genes which are strongly and specifically expressed in pollen grains and pollen tubes. We have recently concluded that AGP6 and AGP11 are necessary for the proper pollen tube growth as well as for preventing untimely pollen grain germination.

We performed microarray experiments in the double null *agp6agp11* mutant pollen tube as well as yeast-two hybrid assays for these two proteins, in order to clarify the biological way of action of this ubiquitous class of plant proteoglycans. We ended up with a lot of candidate genes involved in several biological functions, namely signaling, vesicle trafficking, and of course, cell wall development.

After pollen tube arrival at the pistil, signal transduction cascades are initiated. The identification of key molecules involved in pollen tube guidance, possibly down-stream of Ca²⁺ signalling for the tip-growth will help to clarify such complex and dynamic mechanisms.

In the present work we show the different distribution of specific AGP genes throughout the Arabidopsis female reproductive tissues along the pathway followed by the pollen tube during its journey to reach the embryo sac. The specific and differential presence of these proteins was observed in the stigmatic cells, the transmitting tissue, the funiculus and the integuments that surrounds the embryo sac and in the female gametophytic cells. The expression pattern of these AGPs in the female reproductive tissues brings new and important evidences for the involvement of AGPs in sexual plant reproductive processes.

Session 3: Pollen morphology, development and germination
ORAL COMMUNICATION

Electrophoretic profiling and immunocytochemical detection of pectins and arabinogalactan proteins during olive pollen germination and pollen tube growth

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Cell wall pectins and arabinogalactan proteins (AGPs) are important for pollen tube growth. In this study, immunoblot profiling analyses combined with immunocytochemical techniques were used to analyze the temporal and spatial dynamics of four pectin and two AGP epitopes in the olive pollen during *in vitro* germination. All pectin and AGP epitopes increased their levels during germination. In the mature pollen, galactans localized in the cytoplasm of the vegetative cell, the pollen wall, and the apertural intine. After germination, galactans localized in the pollen tube wall, particularly at the tip, and formed a collar-like structure around the germinative aperture. Arabinans were mainly present in the pollen tube cell wall, forming ring-shaped deposits at regular intervals in the subapical zone. As expected, the pollen tube wall was rich in highly methyl-esterified pectins at the apex, while the cell wall mainly contained de-esterified pectins in the shank. The wall of the generative cell was labeled with arabinans, highly methyl-esterified homogalacturonans and JIM13 epitopes. In addition, the extracellular material coating the outer exine layer was rich in arabinans, de-esterified pectins and JIM13 epitopes. Our results show that pectins and AGPs are newly synthesized in the pollen tube during pollen germination, being their synthesis and secretion temporally and spatially regulated. We suggest that galactans might provide mechanical stability to the pollen tube, reinforcing those regions that are particularly sensitive to tension stress and/or mechanical damage. On the other hand, arabinans and AGPs might be important in recognition and adhesion phenomena of the pollen tube and the stylar transmitting cells, as well as the egg and sperm cells.

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Session 3: Pollen morphology, development and germination
ORAL COMMUNICATION

New insights into the early steps of lipid body mobilization during pollen germination

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In oleaginous plants, the pollen grain is an active site in storage lipid biosynthesis. This energy supply might allow the pollen tube to grow autonomously at the onset of germination. Therefore, storage lipids are a key factor for the success of pollination. This fact contrasts with the lack of knowledge regarding the molecular mechanisms that regulate lipid body (LB) mobilization in the male gametophyte. In this work, four enzymes involved in early steps of LB catabolism were functionally characterized for the first time. The effects of extracellular sugars on pollen performance and LB dynamics were also analyzed. Pollen LBs showed lipase (LIP), phospholipase A (PLA), lipoxygenase (LOX) and peroxygenase (POX) activities on their surface. Removal of extracellular sugars from the germination medium did not affect pollen performance but increased all enzyme activity rates and sped up LB mobilization. Inhibition of these enzymes seriously hampered pollen germination and pollen tube growth, leading to a characteristic accumulation of LBs in the germinative aperture. These results point to the conclusion that, in the olive, the early pollen tube growth is mainly fuelled by its own reserves. Moreover, it can be inferred that two different pathways are likely involved in mobilization of storage lipids in the pollen grain. Fatty acids released from triacylglycerols (TAGs) by the action of a LIP may be further degraded via β -oxidation in peroxysomes. Alternatively, linoleate moieties may be first oxygenated by a LOX and then released from TAGs and reduced by the peroxygenase activity of caleosin before entering the β -oxidation pathway. Extracellular sugars may modulate the function of these enzymes, while PLA may promote their access to the TAG matrix.

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Session 3: Pollen morphology, development and germination
ORAL COMMUNICATION

The phosphorylated pathway of serine biosynthesis is essential for *Arabidopsis* pollen maturation.

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In plants, serine is synthesized through different metabolic pathways. However, the biological significance of the coexistence of several serine biosynthetic pathways in plants is not understood yet. In this work, we have addressed the functional characterization of the phosphorylated pathway of serine biosynthesis (PPSB). To date, no evidence has been provided on the molecular, genetic and physiological significance of this pathway. The characterization of mutants of the first (*eda9*) and the last enzyme (PSP) of PPSB showed that both mutants were lethal in normal growth conditions. The transmission of the *eda9* and *psp* mutations through the male and female gametes was normal. These results ruled out gametophytic defects of mutant alleles, suggesting a lethal phenotype of homozygous mutant embryos. We could complement the embryo lethal phenotype of *eda9* and *psp* mutants with a *EDA9* cDNA or *PSP* cDNA under the control of their natives promoters but the mutant transformation with an *EDA9* cDNA or a *PSP* cDNA under the control of the 35S promoter, which is poorly expressed in *Arabidopsis* anthers, did not complement the mutant fertility. Observation of anthers of homozygous *eda9* and *psp* complemented with the 35S:*EDA9* and 35S::*PSP* by transmission electron microscopy revealed a clear disorganization of the tapetum cell layer at early developmental stages. They also revealed that the pollen grains displayed collapsed forms and were unable to germinate in vitro. Expression of *EDA9* and *PSP* genes analysed by Promotor- β -glucuronidase (GUS) fusions and confocal microscopy detected gene expression in both pollen and tapetal cells. DAPI experiments showed an arrested pollen development at polarized microspore stage. In this work, we provide the first evidence of the essential role of PPSB genes in both pollen grain and embryo development in *Arabidopsis*.

Session 3: Pollen morphology, development and germination
ORAL COMMUNICATION

RX microtomography in studies of floral development in plants

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To establish floral developmental stages in plant species it is necessary to manipulate flower buds from the start of development to opening. Studies on species of large flowers allow floral bracts dissections or other parts of the perianth under binocular microscope and subsequent treatment for SEM viewing. However, in small flower species, manipulation of flower buds and dissection becomes an almost impossible mission. The use of microtomography is shown as a useful technique to recognize the different stages of development of the androecium and gynoecium, with minimal sample handling, even with live samples. Flower buds were fixed in 3% glutaraldehyde in cacodylate buffer for 24 hours and post-fixed with osmium tetroxide to 2% for 1 hour, then they were dehydrated in alcohol series and immersed in hexamethyldisilazane for 24 hours or we used the technique of "critical point".

For visualization of the samples we used scanning electron microscopy and high resolution micro-CT SkyScan 1172 with the following parameters: image pixel size = 1,4 µm, Source Voltage = 52 kv and source current = 80 µA. Images were reconstructed with ©NRecon software, with a smoothing kernel = 2 (Gaussian). Later on reconstructed images were "cleaned" with ©CT-Analyser by running a Custom Processing Task List (Thresholding, Despeckle, ROI-Shrink-wrap, Reload, Bitwise operations and Save bitmaps), obtaining a new series of reconstructed images. Images of the resulting series were corrected in their position with a ©Data viewer, and saved as a definitive series, and finally the volume reconstruction images were obtained with the volume rendering software ©CTvox (for further information on micro-CT and software go to <http://www.skyscan.be>. More information: Verdú, Alba-Tercedor & Jiménez-Manrique 2012. PLoS ONE 7(3): e33914. doi:10.1371/journal.pone.0033914). The comparison of samples analyzed with SEM and micro-tomography, shows the versatility of the second technique compared to the first as it allows three-dimensional orientation and partial removal of the shells of the perianth, thus displaying the androecium and gynoecium inside the pollen sacs and the ovarian cavity.

Verdú JR, Alba-Tercedor J, Jiménez-Manrique M (2012) Evidence of Different Thermoregulatory Mechanisms between Two Sympatric Scarabaeus Species Using Infrared Thermography and Micro-Computer Tomography. PLoS ONE 7(3): e33914. doi:10.1371/journal.pone.0033914.

Session 4: Paleoenvironment and paleoclimate
INVITED LECTURE

Long European Pleistocene pollen sequences: a state of the art.

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Since the pioneer works by Wijmstra (1) on Tenaghi Philippon (Greece) a collection of key continental sequences have contributed to the understanding of vegetation and climate changes along the Pleistocene cycles. Then a series of European sites confirmed common trends in the answers of vegetations to the orbital forcing during the last 400 Ka (2). More recently pollen analyses of marine sequences offshore Iberian coast and from Alboran Sea have allowed direct correlation between continental and marine climatostratigraphies (3). In 2006, a revision of the Tenaghi Philippon sequence (1,35 Ma) (4) gave new lights on the lower and middle Pleistocene. In this domain the site of Leffe (north Italy) must be mentioned, which clearly shows great differences in the glacial/interglacial cycles before and after the “Mid Pleistocene revolution” around the Brunhes/Matuyama limit (5). Nowadays, in the context of global warming, a key question in the building of predictive models is “which past interglacial is the closest analogue to the present?”. In terms of orbital forcing Isotopic stage 11 appeared first as a good candidate but MOS 19 is now preferred (6). Unfortunately they are very few pollen records of this interglacial.

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Session 4: Paleoenvironment and paleoclimate
INVITED LECTURE

Climate and vegetation change during the Holocene in Southern Iberia

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High-resolution pollen analysis has been carried out on several sediment cores taken from high-elevation alpine lakes and bog areas located in Sierra Nevada, southern Spain. The earliest part of the record, from 8200 to about 7000 cal yr BP, is characterized by the highest abundance of arboreal pollen and *Pediastrum*, indicating the warmest and wettest conditions in the area at that time. The pollen records show a progressive aridification trend since 7000 cal yr BP through a decrease in forest species and the increase in xerophytes. The progressive aridification is punctuated by millennial-scale periodically enhanced droughts that coincide in timing and duration with well-known dry events in the Mediterranean and other areas. A relatively humid period occurred during the Roman Humid Period. The alternation of persistent North Atlantic Oscillation modes probably played an important role in controlling these humid–arid cycles. Since 1200 cal yr BP, several changes are observed in the vegetation that probably indicate the high-impact of humans in the Sierra Nevada, with pasturing leading to nutrient enrichment and eutrophication of the wetlands, *Olea* cultivation at lower elevations and *Pinus* reforestation.

Session 4: Paleoenvironment and paleoclimate
ORAL COMMUNICATION

European climate optimum and enhanced Greenland melt during the Last Interglacial

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The Last Interglacial climatic optimum, ~128,000 years ago (128 ka), is the most recent climate interval significantly warmer than present, providing an analogue, albeit imperfect, for ongoing global warming and the effects of Greenland Ice Sheet (GIS) melting on climate over the coming millennium. While some climate models predict an Atlantic meridional overturning circulation (AMOC) strengthening in response to GIS melting, others simulate weakening, leading to cooling in Europe. Here, we present evidence from new proxy-based palaeoclimate and ocean circulation reconstructions that show that the strongest warming in Western Europe coincided with maximum GIS meltwater runoff and a weaker AMOC early in the Last Interglacial. By performing a series of climate model sensitivity experiments including enhanced GIS melting, we were able to simulate this configuration of the LIG climate system and infer quantitative information on AMOC slowdown and related climate effects. These experiments suggest that GIS melt inhibited deep convection off the southern coast of Greenland, cooling local climate and reducing AMOC by ~24% of its present strength. GIS melt did not perturb, however, overturning in the Nordic Seas, leaving heat transport to - and thereby temperatures in - Europe unaffected.

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ORAL COMMUNICATION

Cedrus atlantica in the Middle Atlas Morocco: an interglacial refugium

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Pollen records from lakes Tigalmamine and Sidi Ali from the Middle Atlas, Morocco, showed that *Cedrus atlantica* appeared roughly after 7000 years BP. A pollen record from lake Ifrah, located also in the Middle Atlas, shows that the Atlas cedar was present throughout the glacial period but had in fact regressed during the early Holocene. One hypothesis is that the winter temperature was too high during the early Holocene, which had "prevented" its expansion.

In parallel to these palynological data, a genetic study of several populations of Atlas cedar in the Rif, the Middle and the High Atlas suggests that there is significant weak genetic structuring with low gene flow between populations. These DNA data may suggest that Atlas cedar did not propagate from a specific area or from a common ancestor, but they have rather persisted in different mountain areas at least since the last glacial period.

A new coring collected in the Middle Atlas confirms that the Atlas cedar was present continuously in the area during the last glacial period and the Holocene.

This data-set of pollen records and modern DNA suggest that Atlas cedar populations have persisted in the Middle Atlas probably over the Quaternary period. Unlike in the case of European and north American tree species, Atlas cedar range seems to have been even more extended during the last glacial period than during the Early Holocene and the fragmented modern distribution seems to reflect rather a case of an interglacial refugium than an optimal geographical range.

The persistence of the Atlas cedar populations in the same area through an altitudinal adaptation of its range to past climate changes may explain the weak genetic structure, the low recent gene flow and the lack of a common ancestor that may have survived in a refugium (or few refugia) which have recolonised the modern range.

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ORAL COMMUNICATION

Responses of a neotropical interglacial microrefugium to climate instability

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Under dry conditions and frequent droughts in the northeastern Brazil, the understanding of climate mechanisms and responses of natural ecosystems represent a crucial interest in the context of future climate change. Here we present a high resolution pollen record of a microrefugium located in a mountainous area in northeastern Brazil covering the mid- to late Holocene. Past vegetation dynamics revealed three main ecological successions evidenced by major changes in rain forest assemblages. The dense ombrophilous forest present at the base of the record was abruptly replaced by heliophilous early successional tree taxa at 4275 cal. yr BP. This forest disturbance took ~100 years to occur and lasted ~750 years up to 3525 cal. yr BP. Then, for ~550 years, the progressive expansion of early to secondary successional tree taxa allowed the recovery of ombrophilous forest taxa from 2965 cal. yr BP. However, between the beginning and the end of our pollen record, the early dominant plant assemblages associated with high moisture conditions shifted towards species adapted to drier soil conditions and stronger fog uplift, suggesting intermediate moisture conditions. The environmental change at ~4200 cal. yr BP was associated with a global climate event probably linked to changes in sea surface temperatures and El Niño-Southern Oscillation variability that altered annual precipitation in this region. Our results show that tropical rain forest microrefugia are subject to millennial and multidecadal time scale climate variability and respond by recruitment of key species among their large stock of different species. Consequently the high species richness in these microrefugia is a necessary condition for the rain forest to change rapidly depending on the different forcings. Short-term climate variability enhances strong competition between the frequently challenged species and results in the best association for rain forest resilience.

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ORAL COMMUNICATION

Progress in the identification of *Pinus* pollen grains at a specific level in the Iberian Peninsula records

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Pollen records show that pine is a major component of the Iberian vegetation of the last 70 000 years, in particular during the last glacial period. Because Iberian pine species have distinct ecological requirements, lack of differentiation of pine pollen at a specific level restrains our understanding of natural distribution, history and dynamics of pine forests as well as paleoclimate reconstructions from pollen records.

We present a study aiming to differentiate pollen grains from different *Pinus* species in Iberian pollen records on the basis of pollen morphological trait measurements. We have, first, generated datasets of measurements on modern pollen from Spanish pine stands of *P. pinaster*, *P. pinea*, *P. halepensis*, *P. nigra* and *P. sylvestris* (22 individuals, 2052 measured grains in equatorial view, 1757 grains in polar view). These datasets have been analyzed using classification and regression tree (CART) models which have recently proven to be efficient to distinguish North American *Pinus* and *Alnus* species pollen (Barton et al., 2011; May and Lacourse, 2012). Obtained CART trees allow pollen classification of the mountain species *P. sylvestris* and *P. nigra* with 80 to 70% accuracy. Probability in classifying pollen of both species in the mountain pine group increases up to 90%. The model exhibits a lower accuracy in classifying the thermophile Mediterranean species pollen (*P. pinea*, *P. halepensis* and *P. pinaster*). However, the probability of misclassify Mediterranean species pollen in the mountain pine group is less than 25 %. The obtained CART trees have been used to predict pine species from Iberian fossil pollen data. Morphological trait measurements have been performed on pine pollen grains from selected samples of the MD95-2042 core located off the Tagus river mouth, covering the last 140 000 years. Predictions shows that southwestern Iberian pine forests of the last glacial period (MIS4 to 2) were mainly composed of *P. nigra* and *P. sylvestris* with *P. nigra* as the dominant species. They also display an impact of the glacial millennial-scale climatic variability on the different pine species expansion: thermophile Mediterranean pine proportions were higher during Greenland Interstadials than during Greenland Stadials and in particular Heinrich Stadials. Our results also confirm that thermophile Mediterranean pines expanded at the beginning of the Holocene. However, they reveal that *P. nigra* remained the most important pine species in the region.

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ORAL COMMUNICATION

The climate of the Mediterranean basin during the Holocene from terrestrial and marine pollen data: A model/data comparison

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In the Mediterranean area, the Holocene climate is characterized by a complex pattern with strong spatial and temporal variability. Lake-levels and pollen-inferred climate reconstructions from Italy suggest a North–South climatic partition in the central Mediterranean through the Holocene. This pattern shows strong similarities with the climate changes reconstructed in the Aegean Sea and eastern Mediterranean, suggesting a more regional climate signal. Here, we test this hypothesis at the scale of the Mediterranean basin, particularly in East and West Mediterranean regions.

This study aims to provide a synthesis of the Holocene climate in the Mediterranean region based on: (1) Four high-resolution pollen records taken from lakes located along a latitudinal gradient from the northern Italy (Lakes Ledro and Accesa) to the south Italy (Lakes Trifoglietti and Pergusa) and one pollen record from Greece (Tenaghi Philippon); (2) Six high-resolution pollen records taken from marine cores located along a longitudinal gradient from the Alboran Sea to the Aegean Sea (cores ODP 976, MD95-2043, MD90-917, MDO4-2797, SL152, and NS14). (3) A multi-method approach applied to each marine and terrestrial pollen sequence to better assess the error of reconstruction inherent in pollen-based climate predictions.

The use of the marine cores will help to test this hypothesis at the scale of the Mediterranean basin, particularly in East and West Mediterranean region to better understand the main climate forcings.

These precipitation estimates are compared with regional-scale climate modelling simulations

Session 4: Paleoenvironment and paleoclimate
ORAL COMMUNICATION

Past environmental changes in the western Rif mountains, Morocco: A new pollen record

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After the last glacial period, the global temperature increased significantly which impacted the ecosystems and their dynamics. The aim of this study is to understand and possibly to contribute to better managing the impact of past climate change on two emblematic plant species which are the Atlas cedar (*Cedrus atlantica*) and Moroccan fir (*Abies maroccana*). The latter is an endangered species according to the IUCN Red List.

We will present the results of an 8m20 fossil record collected in the western part of the Rif Mountains. The age model of this record is based on 16 AMS ¹⁴C dates which is unique in Morocco. We have analyzed the pollen content, the charcoal remains, the particle size and some geochemical elements using X-Ray-Fluorescence. Besides the geological coring we have collected a series of surface samples starting from the fir forest in the Talassemtane National park towards our coring site. The aim of this modern transect is to better comprehend the pollen transport from within the fir forest to the fossil site.

The analysis of these modern samples shows that fir pollen is transported on very short distances as they do not reach the site where we have collected the coring. The latter is located at less than 5kms from the fir forest. The record was collected in a bog that is surrounded by deciduous oaks (*Quercus pyrenaica* and *Q. canariensis*). Nowadays, there are no firs or cedars in the vicinity of the site. The pollen record shows that Atlas cedar was strongly present since the very early part of the Holocene. The decline of cedar forests is recorded after 6000 cal. BP when a more drought-tolerant evergreen species such *Q. ilex/coccifera* and Cupressaceae began to expand. After 2000 cal. BP the Atlas cedar disappears from the studied site probably because of the additional human disturbance. The latter is depicted through the presence of *Olea* and *Cistus*. The Moroccan fir is not recorded during the Holocene except few pollen grains during the last 500 years which may suggest that it was transported by the wind from some populations not far from the studied site.

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ORAL COMMUNICATION

Impact of climatic change and human activities on vegetation dynamics in Coastal Syria

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Pollen grain studies were performed on a sediment core from Tell Soukas in central coastal Syria. We cover the past 7400 years BP with a 420 cm long core dominated by grey clays. Following global warm conditions during the early Holocene, a substantial increase in pollen grains of *Olea* was detected at 6500 cal BP suggesting the onset of the culture of olive trees in this region. Coevally, *Pinus halepensis* decreased. Surprisingly at ca. 4000 cal. BP a sudden decrease in *Olea* abundances is recorded (from 50% to <1%) along with the nearly disappearance of *Pinus*, whereas *Poaceae*, *Cichorioideae* and *Chenopodiaceae* show an increasing trend. Since no significant variation of pollen grain concentrations are inferred at that time, we assume that such a shift may indicate a change in the vegetation dynamics (from crops and forests to steppe-meadow environments). The diminution of pine tree taxa might be linked with the forest clearance by Early Bronze Age populations (Yasuda et al., 2000). However, the impact of climatic change cannot be dismissed. Indeed, climatic changes induce changes in land-use activities (Weiss et al., 2011; Kaniewski et al., 2012).

Besides, Tell Soukas is a famous archeological site where first human settlements date back to the Neolithic period, and we can, therefore, expect a strong anthropogenic influence in the pollen grain record, especially during the Early to middle Bronze Age. Hence, one main challenge in the Levantine Basin, and more specifically along coastal Syria, is to understand the genuine impact of climate and human activities and their interaction on palaeoenvironmental change in this region. This study brings new data to decipher this issue.

Session 4: Paleoenvironment and paleoclimate
ORAL COMMUNICATION

Past environmental changes and their impacts on the Lebanese Cilician fir

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The Cilician fir populations reach their southernmost distribution in Lebanon. They are currently limited to 15 remnant populations sheltered between ca. 1000 and 1800 masl on the western slopes of Mount Lebanon. To investigate the response of these populations to changing environmental conditions, we have analyzed the pollen content of a new sediment core covering the last 14,000 years and performed genetic analyses on modern Cilician fir DNA samples. The founder effect that promoted the existence of these populations partly dictated their genetic diversity. Slight pattern of isolation by distance and a concomitant lack of phylogeographic signals confirmed their most probable recent isolation. While anthropogenic habitat fragmentation might be a possible triggering effect in their isolation, environmental changes might have played a major additional role. Palaeoecological and genetic data showed that these populations have most probably persisted, on the long-term, by altitudinal shifts allowing them to cope with changing environmental conditions.

Session 4: Paleoenvironment and paleoclimate
ORAL COMMUNICATION

Holocene rapid climate changes as recorded in northwestern France (Grande Vasière): high resolution dinocyst analysis over the last 9000 years.

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New dinocyst data obtained on a 2.7m length core (VK-03-58 bis) enable to discuss paleohydrological changes (temperature, salinity, productivity) affecting NW France at very high resolution over the last 9000 years. This core, retrieved on the “Grande Vasière” mud shelf, has already been studied at lower resolution for pollen analysis (Naughton et al, 2007, The Holocene). Here, we present new palynological data allowing us to discuss the Holocene long-term variability as well as rapid climatic changes. Our dinocyst data confirm the footprint of the suborbital climate variability (1500 years cycles) superimposed on a long-term cooling trend. The long-term cooling is evidenced by increases of cysts of *P. dalei* relative abundances through the record, while warm-cool oscillations are emphasized by *S. mirabilis*-*S. lazus* alternances. Quantitative reconstructions of sea surface temperatures based on dinocyst assemblages strengthen palynological data (Naughton et al., 2007) by showing decreasing seasonality over the Holocene, linked with decreasing summer insolation. Furthermore, rapid paleoproductivity changes, highlighted by *S. quanta* and *P. nudum* relative abundances, give evidence of variations in nutrients supply and therefore on fluvial discharges or current reorganization. This is also enhanced by an increase of *L. machaerophorum*, suggested as being allochthonous (estuarian species) in our studied core.

Session 4: Paleoenvironment and paleoclimate
ORAL COMMUNICATION

Palynology in fluvio-estuarine environment: the example of Nantes (Northwestern France)

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Several deep drillings coming from the Loire estuary upriver and Loire tributaries at Nantes (northwestern France) are studied throughout multi-proxy analysis (pollens and spores, NPPs, microcharcoals and dinocysts). The aim is to reconstitute vegetation evolutions since 10 ka BP linked with climatic changes, holocene transgression and anthropogenic impacts. First palynological results, correlated with some radiocarbon dates, indicate an Early Holocene age for the silty-sandy bottom of the cores. These sequences are covered by fluvio-marine clayey Middle Holocene sediments. Reworking and sedimentary hiatuses could be shown by sedimentary changes, radiocarbon dates reversals and palynological assemblages including reworked and pre-Quaternary pollens. Several indicators, as *Pinus* and *Chenopodiaceae* pollens, foraminifera and cysts of marine algae, allow us to identify and quantify marine inputs through time. The anthropogenic impact on the vegetative environment is really noticeable only on upper Late Holocene levels, with hemp and walnut-tree cultures taken place on open-deforested landscape.

Session 5: Pollen Biotechnology and Genetics
INVITED LECTURE

Engineering male sterility as a tool to decrease pollen-caused allergies

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Allergies are illnesses mediated by the production of IgE antibodies causing respiratory, skin and intestinal disorders and affecting one of each four humans living in developed countries. Airborne particles such as pollen grains released by angiosperms are among the most important allergenic sources that reach the human body by inhalation. While the molecular characterization of allergens inducing IgE production and activity seems to be the best strategy for accurate diagnosis and immunotherapy of patients, the possibility exist of using biotechnological tools to eliminate pollen allergens and when possible to avoid pollen production itself.

PsEND1 is a pea anther-specific gene that displays expression in the epidermis, connective and parietal cells of the anther primordium. Taking advantage of END1 temporal pattern of expression (from stamen primordial to anthesis), we fused the PsEND1 promoter to the cytotoxic barnase gene to induce specific ablation to produce male-sterile plants. Expression of the chimaeric PsEND1::barnase gene in two Brassicaceae (*Arabidopsis thaliana* and *Brassica napus*), two Solanaceae (*Nicotiana tabacum* and *Solanum lycopersicon*) species and two ornamental plants (*Kalanchoe blossfeldiana* and *Pelargonium* spp.) impairs anther development from very early stages and produced 100% male-sterile plants. The chimaeric construct arrests the microsporogenesis before the microspore mother cells differentiation and no viable pollen grains are obtained. Genetic ablation using the tightly regulated PsEND1 promoter constitutes an useful tool to avoid angiosperm pollen formation in allergenic plant species especially in urban environments. Currently we are characterizing another gene from pea (PsPMEP) coding for a pectin methylesterase whose expression is pollen specific(see poster by Renau et al. presented in this congress) The promoter of Ps PMEPE driving the expression of a cytotoxic gene might be also used to avoid pollen development in allergenic plant species.

García Sogo et al., (2010) Efficient transformation of *Kalanchoe blossfeldiana* and production of male-sterile plants by engineered anther ablation. *Plant Cell Rep.* 29:61-77.

García Sogo et al., (2012). Production of engineered long-life and male sterile *Pelargonium* plants. *BMC Plant Biol.*12: 156-

Medina et al., (2013). Early anther ablation triggers parthenocarpic fruit development in tomato. *Plant Biotech.Journal* doi: 10.1111/pbi.12069

Session 5: Pollen Biotechnology and Genetics
INVITED LECTURE

Drive in favour of a parasite chromosome during pollen mitosis and effects on histone H3 methylation pattern.

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B chromosomes (Bs) are dispensable components of the genome that do not confer any advantage on the carrier organism. Rye Bs are parasitic because they strongly decrease fertility. In rye and other Poaceae, the Bs undergo directed nondisjunction during first pollen mitosis. FISH with rye B-specific probes revealed that B-nondisjunction is likely caused by cohesion of the B sister chromatids during first pollen anaphase. Because of unequal first pollen mitosis, nondisjoined B chromatids preferentially become located toward the generative pole; this results in the accumulation of Bs in the generative nucleus and hence in the sperm nuclei, ensuring B transmission at a higher than Mendelian rate to the next generation. High numbers of Bs produce alterations on pollen development, mainly lack of B-nondisjunction, formation of micronuclei and pollen inviability.

The histone modifications H3K4me3 and H3K27me3 were studied by immunolabelling in 0B and +B rye plants during microgametogenesis, and in root tips for comparison. In root tips H3K4me3 signals are homogeneous along the chromosome arms of the normal complement, with the exception of the pericentromeric and subtelomeric regions. On the contrary, the strongest signal on the B chromosomes is located on the subtelomeric B-specific D1100/E3900 region of heterochromatin. H3K27me3 signal was observed on the subtelomeric heterochromatin of all chromosomes and in some interstitial heterochromatic bands as well.

During first pollen mitosis the immunosignals are similar to those observed in root tips in both 0B and +Bs plants; however the patterns in +Bs plants differ later on. The vegetative nucleus shows diffuse H3K4me3 signal, whereas the generative nucleus is strongly labelled and shows β -tubuline around it. After second pollen mitosis both sperm nuclei show H3K4me3 and β -tubuline signals. When the Bs are lost as micronuclei, they neither show β -tubuline nor does H3K4me3 signal.

After first pollen mitosis the nuclei show H3K27me3 signal in 0B plants. Interestingly, in +B plants only the vegetative nucleus appears uniformly labelled. Consequently, neither second pollen anaphase nor sperm nuclei show H3K27me3 in +B plants. This indicates that B chromosomes have the ability to change both H3K4me3 and H3K27me3 pattern during pollen development.

Session 5: Pollen Biotechnology and Genetics
ORAL COMMUNICATION

Pollen embryogenesis in *Citrus sinensis* L. Osbeck cv. Moro, a blood orange cultivar

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In vitro tissue culture represents a useful support for the advancement of Citrus breeding and propagation. Haploidy technology, that is the single-step development of complete homozygous genotypes from heterozygous parents through gametic embryogenesis, has already a huge impact on many relevant crops, representing an integral part in their breeding programmes (Germanà 2011a; 2011b). In the route to gametic embryogenesis, the immature gametophytes, opportunely induced, switch from their normal developmental gametophytic pathway towards a sporophytic one; such a process is named “gynogenesis” when it initiates from a female gamete and “pollen embryogenesis” (sometimes “androgenesis”), when it starts from a male gamete. “Pollen embryogenesis” is induced through in vitro culture of anthers or of isolated microspores and it represents a fast track to produce homozygous lines compared to the traditional self-pollination approach, that requires many generations of selfing.

Sweet orange is very recalcitrant to haploid induction and only recently, homozygous short-lived plantlets have been recovered from anthers of Rhode Red Valencia sweet orange (Cao et al. 2011). In this study, different protocols of anther culture have been compared in Moro, a blood orange cultivar that previously only produced, via anther culture, somatic embryogenic callus and plantlets. In particular, two different media and two temperature treatments were applied to the anthers in culture. This paper reports on the strong influence of the medium on the type of response of in vitro cultured anthers. Regenerants were characterized for ploidy level by flow cytometry analysis and for heterozygosity by microsatellite analysis. Moreover, structural changes and histodifferentiation of tissues were observed, with particular attention to starch accumulation.

ACKNOWLEDGEMENTS

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Session 5: Pollen Biotechnology and Genetics
ORAL COMMUNICATION

Arabinogalactan proteins expression and distribution patterns, early markers of microspore embryogenesis

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Arabinogalactan proteins (AGPs) are hydroxyproline-rich glycoproteins that are massively glycosylated, present in cell walls, plasma membranes and extracellular secretions, with a key role in several plant developmental processes. After specific stress treatments, the microspore/pollen grain cultured *in vitro* can reprogram and change its normal gametophytic developmental pathway towards embryogenesis producing embryos.

In this work, the dynamics of AGPs was studied during the pollen gametophytic development and the pollen embryogenesis in *Brassica napus*, by performing dot-blot, immunofluorescence and confocal analysis with several monoclonal antibodies for AGPs (JIM13, JIM14, MAC207, LM2) and arabinans (LM6), AGP components also present in pectins. The expression pattern of the BnAGP Sta 39-4 gene was also analyzed by quantitative qPCR during different developmental stages. Results showed the developmental regulation and defined localization of the studied AGP epitopes during the two microspore developmental pathways, revealing different distribution patterns for AGPs with different antigenic reactivity. Increases in the expression of the Sta 39-4 gene, JIM13 and JIM14 epitopes, found specifically in two-four cell stage embryo cell walls, suggested that AGPs are early molecular markers of microspore embryogenesis. Later, LM2 and LM6 antigens increased progressively with embryo development. These results give new insights into the involvement of AGPs as potential regulating/signalling molecules in microspore reprogramming and embryogenesis.

El-Tantawy AA, Solís MT, Costa ML, Coimbra S, Risueño MC, Testillano PS (2013) Arabinogalactan protein profiles and distribution patterns during microspore embryogenesis and pollen development in *Brassica napus*. *Plant Reproduction*. DOI: 10.1007/s00497-013-0217-8. Published on line: June 2013.

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Session 5: Pollen Biotechnology and Genetics
ORAL COMMUNICATION

Massive autophagy and excretion-based cytoplasmic cleaning during *Brassica napus* microspore embryogenesis

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Induction of embryogenesis from isolated microspore cultures is a complex experimental system where microspores undergo dramatic changes in developmental fate. After approximately 40 years of application of electron microscopy to the study of the ultrastructural changes undergone by the induced microspore, there is still room for new discoveries. In this work we used High Pressure Freezing and Freeze Substitution (HPF/FS), the best procedures known to date for ultrastructural preservation, to process *Brassica napus* microspore cultures covering all the stages of microspore embryogenesis. Analysis of these cultures by transmission electron microscopy revealed massive processes of autophagy exclusively in embryogenic microspores, but not in other microspore-derived structures also present in cultures, such as pollen-like structures or non-embryogenic structures. Instead of following a conventional lytic pathway, a significant part of the autophagosomal cargo was not recycled, but transported out of the cell, producing numerous deposits of extracytoplasmic fibrillar and membranous material. In this work, we demonstrated that induction of embryogenesis implies both massive autophagy, and excretion of the removed material. We hypothesize that autophagy would be related to the need for a profound cytoplasmic cleaning, and excretion would be a mechanism to avoid excessive growth of the vacuolar system. Together, our results highlight the potential of HPF/FS to the study of the androgenic switch, and point to it as the best option currently available to identify the complex and dramatic ultrastructural changes undergone by induced microspores. In addition, this work provides significant insights to understand the cellular basis of induction of microspore embryogenesis, and open a new door for the investigation of this intriguing developmental pathway.

Session 5: Pollen Biotechnology and Genetics
ORAL COMMUNICATION

Induction of the sporophytic pathway in pollen from *Quercus ilex* L. anther cultures

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The objective of this work is to induce the sporophytic pathway of pollen development using anther cultures of holm oak (*Quercus ilex* L.). Mastering of this process will permit the regeneration of haploid and doubled-haploid (DH) embryos. This result provides an attractive biotechnological tool for developing homozygous lines from heterozygous parents, which is important in breeding programs, as well as in genetic studies. As a consequence, protocols to produce homozygous plants have a significant impact on plant systems. In the initial experiments, anthers were subjected to different temperature treatments for embryo induction: a cold pre-treatment (4° C) between 3 to 7 days was carried out at the beginning, followed by a heat shock (33° C) between 2 to 5 days. Most anthers responding to these stress treatments contained vacuolated microspores, indicating that this developmental stage is responsive to embryogenesis induction in holm-oak microspores. In all cases, embryos grew from the interior of the anthers, breaking through the degenerating anther walls. Under these conditions, embryo formation occurred in 31 anthers between 46 to 95 days after culture initiation. Embryo analysis performed with flow-cytometry and DNA-microsatellite markers showed haploid profiles and/or spontaneous doubling of the chromosomes during early regeneration stages. This is, to our knowledge, the first published report on gametic embryogenesis in holm oak.

Session 5: Pollen Biotechnology and Genetics
ORAL COMMUNICATION

Gametophytic-to-esporophytic switch in microspores of sweet orange genotypes

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Obtaining of haploid and double haploid plants from gametic cells is valuable for genetic and biological studies, as well as breeding programs. The switch from gametophytic to sporophytic generation is observed in the pollen nucleus and this has not been reported in sweet orange (*Citrus sinensis*). We aimed to evaluate different factors affecting gametic embryogenesis in sweet orange and report microspore developmental changes using microscopical observations. We evaluated in vitro anther culture of 100 genotypes of sweet orange from the Germplasm Bank of Centro de Citricultura Sylvio Moreira, Cordeirópolis, SP, Brazil. Flower buds, 5-7 mm in length, at the microspore uninucleate stage of development, were collected and anthers were excised and cultivated in semi-solid N6 media with NN vitamins for 10 months. Ten genotypes that produced embryogenic callus were submitted to microscopic analysis through staining with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) solution (1 mg mL⁻¹) followed by microspore observations under a fluorescent microscope after 10 months in culture medium. Most genotypes (95%) produced either swollen anthers, or anthers with two types of calli: cream-friable type and a hard whitish-green callus. Only 15% of sweet orange genotypes produced embryogenic callus. The determination of ploidy by flow-cytometric analysis showed that embryogenic callus were mixoploid, a mixture of haploid and diploid callus, with sweet orange microspores showing the ability to switch from gametophytic to sporophytic status. An evidence that supports this hypothesis is the large numbers of multinucleated pollen grains in all 10 genotypes that produced embryogenic callus from anthers, indicating the capacity of microspores to change their developmental pathway from gametophytic to sporophytic. One of the first evidence of this change is the symmetric division of the microspore nucleus followed by the observation of multinucleated microspores.

Session 5: Pollen Biotechnology and Genetics
ORAL COMMUNICATION

A multiplex system to analyse the expression of allergens and other gene products of interest in the olive pollen.

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Pollen allergy is an important health issue in numerous Western societies. More than 25% of the population displays symptoms compatible with allergy and this is considered an important challenge, particularly because untreated allergy often progresses to severe forms of the disease, which may include asthma and others.

Specific immunotherapy (SIT) represents one of the major advances in the treatment of allergy patients, and it is considered by most allergologists the election procedure to improve patient's quality of life besides other treatments, which may mitigate their symptoms.

SIT requires the use of well defined, adequate and personalized protein extracts for each patient, and this is one of the critical parameters for its success.

We have developed a specific system to analyse the allergenic components present in the protein extracts. The system is able to simultaneously detect and quantify the most clinically relevant allergens present in the olive pollen. It is based on the use of multiplex immunoassays over transference membranes, by combining secondary antibodies conjugated to fluorophores with different ranges of excitation wavelengths, together with chemiluminiscence. The method can be easily adapted to detect new olive pollen allergens and to explore protein extracts from allergenic sources other than olive pollen. Moreover, it also allows simultaneous analysis of the IgE reactivity of patient's sera.

Because most olive pollen allergens also play important biological roles, we use this method to assess their expression and those of other gene products of interest under many different physiological and cellular situations, including cell division, pollen-pistil interaction, pollen hydration, pollen tube growth and others.

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Session 6: Airborne pollen and spores: environmental indicators and allergens
INVITED LECTURE

Factors involved in pollen germination could have a role also in allergic sensitization

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In pollen tubes, cytoskeleton proteins are involved in many aspects of pollen germination and growth, from the transport of sperm cells to the asymmetrical distribution of organelles to the deposition of cell wall material. These activities are based on the dynamics of the cytoskeleton. Transglutaminases catalyze the post-translational conjugation of polyamines to different protein targets, but also the cross-linking between endo-glutamine and -lysine protein residues; they are suggested to have a role in the interaction between pollen tubes and the extracellular matrix during fertilization and a specific role during the self-incompatibility response. In such processes, the activity of transglutaminase is enhanced, leading to the formation of cross-linked products; moreover, it could act as regulator of cytoskeleton dynamics.

Different factors related to pollen germination and tube growth could be also involved in allergic sensitization. Among them, the phospholipase (PL), involved in the apex growth of the elongating pollen tube, is an integrator of the tip molecular signalling. The presence of phospholipase A2 has been recently described in pollen; being PLA2 able to generate pro-inflammatory factors, this enzyme could participate in the activation of the allergenic inflammatory cascade. It is also known that stimulation of PLA2 activity occurs when it is post-translationally modified by transglutaminase. Profilin is a known pan allergen and also a key factor to control actin filament organization, indeed the pollen tube growth. In some pollens has been observed that secretion of pro-inflammatory eicosanoid-like substances precedes allergens release from pollen grains in the initiation of allergic sensitization. Also tip-localized ROS were needed to sustain the normal rate of pollen tube growth as ROS disruption induced the pollen to programmed cell death. More studies are necessary to better know these factors and their importance in allergic sensitization.

Session 6: Airborne pollen and spores: environmental indicators and allergens
INVITED LECTURE

Pollen Counts and aeroallergen quantification in the atmosphere: overview and current status

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Traditionally, the allergenic potential of the atmosphere has been estimated on the basis of pollen counts. Different devices have been used for this, Durham, Hirst and Rotorod being the most popular. However a certain quantity of the aeroallergens are not enclosed in pollen grains, but are present in the atmosphere in the form of micronic particles, the size of these airborne particles being inversely proportional to its ability to penetrate the respiratory tract and cause allergies. Their allergen content can be highly variable, which is conditioned by the time of residence in the atmosphere, transport and environmental conditions. On the other hand, pollen counts sometimes are not enough to completely explain the symptoms of pollinosis detected in the population, since the allergenic potential of the pauci-micronic and sub-micronic particles is not take into account. So, in the last decades several authors have been carried out studies about the activity of different aeroallergens in the atmosphere of different cities.

Regarding the study of airborne aeroallergens, a very important issue to take into consideration is to select the sampling methods and the appropriate protocol for processing the samples. In the last decades different sampling devices have been used, some of them modifications of those previously existing, however, it seems to have imposed the cyclonic type samplers. On the other hand, it is necessary to isolate and preserve the antigens as much as possible, the ELISA tests being the more used today due to its high sensitivity, specificity and reproductibility. Moreover, the selection of the more suitable extraction buffer is a very important point to consider, according to the allergen or set of allergens object of study.

An overview of the current status of this kind of studies about detection of pollen allergens in the atmosphere and its relationships with pollen counts is presented in this survey.

Session 6: Airborne pollen and spores: environmental indicators and allergens
ORAL COMMUNICATION

Hourly dynamics of airborne pollen in the Southeast of Castilla-La Mancha

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Hourly pollen count dynamic lets us know the airborne pollen concentration at different times of the day. Mainly, the highest concentrations occur during daylight hours and the minimum overnight. Hourly pollen concentrations reported on the location of the emission sources of pollen and, therefore, its origin. The knowledge of the hourly distribution of airborne pollen allows to alert patients to avoid the time of highest allergenic pollen concentrations in the atmosphere.

The aim of this study is to analyze the hourly variation of Cupressaceae, Chenopodiaceae-Amaranthaceae, Olea, Plantago, Poaceae, Quercus and Urticaceae, the 8 most important airborne pollen types in Albacete, located in the southeast of autonomous community of Castilla-La Mancha (central Spain). The pollen concentration was continuously monitored from 2008 to 2010 using a Hirst-type volumetric sampler. We followed the methodology recommended by the Spanish Aerobiological Network.

The results indicate that pollen types Cupressaceae, Chenopodiaceae-Amaranthaceae and Plantago show maximum values at midday (at 12:00 Cupressaceae and Plantago and at 14:00 Chenopodiaceae-Amaranthaceae). The pollen types Olea, Poaceae y Urticaceae obtained similar values along day, and they do not show a clear maximum pollen peak. The Platanus pollen type presents the highest concentrations at 18:00 hours. Finally, Quercus pollen type shows only a slight increase between 8:00-10:00 hours in the morning and a decrease in the early afternoon (16:00 hours). Correlation analyses (Spearman test) between hourly pollen concentrations and the main hourly meteorological variables (temperature and precipitation), turn out to be significant and positive for pollen types and temperature, and negative for precipitation.

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ORAL COMMUNICATION

A semi-automatic pollen recognition system for aerobiological monitoring

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Analysis of biological air pollution based on the study of pollen and spores particles is of great interest to sectors as medicine (allergies), pharmacy (drugs) or agronomy (forecast of crops). Knowing the physical characteristics of each particle and the variables that model its appearance is very important to develop control and prediction systems. The methodology used so far is based on the manual counting of samples by a human expert, which is a complex, very time consuming and quite expensive task. The evolution of computer systems and data storage allow the digitization of the samples, together with the development of semiautomatic systems based on artificial intelligence methods, such as machine vision or data mining, enable the detection, classification and counting of different types of particles. All this saves time and permits the possibility of carrying out multiple studies on the characteristics of the samples: shape, repetition, timing, quantity, etc.

This paper presents the pollen analysis system which main goal is the automatic identification and counting of pollen. The results of scanning the sample series collected by the Castilla-La Mancha Aerobiology Network (AEROCAM, <http://aerocam.uclm.es>) since 2005 in six aerobiological stations: Toledo, Guadalajara, Cuenca, Albacete, Ciudad Real and Talavera de la Reina are also presented. Pollen samples were prepared manually and the scanning was carried out with a motorized microscope with Nikon's NIS-Elements imaging software. In the analysis system different tools were used: 1) image processing and graphical user interface was developed with MATLAB and 2) machine learning methods for classification were developed in WEKA.

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ORAL COMMUNICATION

Detection of Ole e 1 protein, major allergen of *Olea europaea* L., in rainwater.

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A phenomenon well known in Aerobiology is the washing effect that the rain water has on the atmospheric particle content, which causes the precipitation of pollen grains and micronic particles with allergenic capacity. When the rain is very abundant or lengthy in time, the washing is fully carried out and, therefore, these airborne particles achieve null values.

For demonstrating aeroallergen washing out, we gathered rain water in a rain gauge, between 2008 and 2009, during olive pollen season, as well as, during the olive harvest periods for trying to analyse and quantify Ole e 1, the major allergen of *Olea europaea* pollen.

Rain water was collected on several rainy days during winter and spring and lyophilized, followed by a protein extraction protocol and analysed by ELISA. Additionally, Ole e 1 quantifications and *Olea europaea* pollen counts, were carried out by means of Cyclone and Hirst samplers, respectively, during the same period, in order to determine whether or not the aeroallergen and pollen grains were present in the atmosphere. The relationship between them and between them and rainfall data were studied by means of correlation analysis with the aid of SPSS program.

The results showed the presence of Ole e 1 in the rain water during the pollen season as well as during the olive harvest periods, this being the first time in which the presence of aeroallergens are detected in rainfall and, therefore, demonstrating its washing effect on the airborne particles..

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ORAL COMMUNICATION

Relationship between ornamental *Pinaceae* trees density and their airborne pollen in an urban environment

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Background. Ornamental Pinaceae includes mainly species of *Pinus* and *Cedrus*. The aim of our study was to evaluate the amount of Pinaceae airborne pollen in relation to the number of trees planted in five sampling points.

Method. Airborne pollen was monitored in four cities in South West of Spain: Badajoz (2 places: Agrarian Engineering School – AES, and Faculty of Science - FS), Plasencia, Don Benito and Zafra, in 2012 using Hirst spore traps. Ornamental Pinaceae trees were counted and located in a map. Main pollen season was calculated with the 5-95% range. No *Pinus* cultures or woods were located around the cities studied.

Results. Three species of ornamental *Pinus* and two of *Cedrus* were identified: *P. pinea*, *P. halepensis*, *P. pinaster*, *C. deodara* and *C. atlantica*. Their urban density by hectare was 0.7 in Plasencia and Zafra, 1.0 in Badajoz, and 1.7 in Don Benito. Spring pollen season lasted 42 days in FS, 68 days in Plasencia, 74 days in Don Benito and Zafra and 75 days in AES, from March 17 to June 1. Sum of daily pollen concentrations was 249 (AES), 317 (Zafra), 849 (Don Benito), 998 (Plasencia), and 3584 (FS). Spring peaks of pollen concentration recorded in grains/m³ were 48 (13/5 Plasencia), 47 (14/5 Don Benito), 18 (11/5 Zafra), 18 (15/4 AES), 715 (12/5 FS). The number of Pinaceae trees around spore traps in a radius of 100 m were 0 (Plasencia), 2 (AES, *C. deodara*), 4 (Zafra, *P. pinea*), 6 (Don Benito, 2 *P. pinea*, 4 *C. deodara*), and 15 (FS, *P. pinea*). Autumn pollen season were insignificant except for Don Benito, with a peak of 48 grains/m³ (14/11).

Conclusion. Pinaceae total airborne pollen recorded by spore traps may be in consonance with tree density in an urban environment. Nevertheless the proximity of pollen sources play an important role in the amount of pollen captured, even more in relation with maximum peaks. *Cedrus* airborne pollen recorded seems to be more dependent on sources proximity that *Pinus* one.

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ORAL COMMUNICATION

Study of floral phenology of the olive tree (*Olea europaea* L.) using geostatistical techniques

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The application of geostatistical techniques for spatial analysis of environmental variables together with the use of Geographic Information Systems (GIS), allow us to model and obtain from a limited number of sampling points, continuous maps of the distribution of the studied variables.

The aim of this study was to obtain maps of the olive tree flowering in the province of Toledo (central Spain) as well as to identify the sources of pollen in every moment of the olive pollen season.

The field work was carried out along the years 2010, 2011 and 2012 and it has included phenological and aerobiological samplings. Phenological samplings were performed following the BBCH scale, in 80 olive trees belonging to eight sampling stations located at different altitude. Airborne pollen levels, recorded in a Hirst-type pollen trap located in the city of Toledo, were compared with the phenological results of the percentage of opened flowers.

Phenological maps obtained by kriging and Geographic Information Systems (GIS), show the sequence of the flowering of the olive trees, beginning in the olive groves located in the valley of the Tagus River and ending in the olive groves situated on the slopes of the Montes de Toledo, located at higher altitude. The results allow us to interpret the curve of airborne olive tree pollen as well as to know from which olive groves comes the pollen during the pollen season. The results also allow us to know that some variables such as wind direction and precipitation influence the amounts of pollen recorded.

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ORAL COMMUNICATION

New statistical approaches in long term pollen trend analysis: application to the olive pollen series in South Spain

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Long term airborne pollen observations offer the possibility of studying fluctuation patterns in the air, especially in the case of anemophilous plants. These studies provide valuable information applied to different disciplines, i.e. crop forecasting in agronomic species or pollen forecast, as a preventive measure on pollinosis. On the other hand, these data can be considered as a valuable bio-indicator of recent climate change. In literature it can be noticed that trends on airborne pollen or phenological data are usually analyzed by applying linear regression trend analysis. However, a linear analysis is not the ideal method for certain taxa, i.e. olive tree, because its pollen season shows a biannual cycle.

In the present study it has been analyzed the trends and olive pollen season fluctuations' during the last 30 years (1982-2011) at Córdoba (South Spain), where olive is an important and extensive crop and the main cause of pollen allergy. Mean features of olive pollen season, Annual Pollen Index (PI), Starting Dates (SD), Peak Dates (PD) and Pollen Season Duration (PSD) were analyzed. For this purpose it has been performed three trend statistical analyses: Linear Regression, Time Series Seasonal Decomposition and ARIMA trend model. Trend results from Seasonal Decomposition and Lineal Regression have shown rising trends in PI and PSD, whereas the phenological features SD and PD are in advance, probably influenced by the increasing temperature in the area. Seasonal decomposition technique allows us a better understanding of olive behavior due to this species usually shows a biannual cycle together with other stochastic fluctuations.

Trying to build a trend forecasting for future olive reproductive behavior, it has been developed ARIMA models for SD and PI projection until 2020. Results have shown significant adjustment degrees and better future projections than those obtained by lineal regression models.

This study concludes that olive tree is undergoing major changes in their reproductive cycle during last years. Seasonal decomposition improves the effectiveness of lineal regression in time series analysis, whereas ARIMA models provide reliable trend projections for the next years taking into account time series fluctuations.

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ORAL COMMUNICATION

The first airborne pollen calendar of Mexico City (2008-2013)

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The incidence and exposure to aeroallergens seems to have a large impact on the health of 20-30% of the world population, affecting to susceptible individuals which develop allergic problems by interacting with the environment.

Mexico currently ranks seventh in morbidity caused by allergies to pollens. Therefore, in 2008 was founded the Mexican Network of Aerobiology (REMA) to monitor and detect pollen allergens (pollen grains) dispersed in the atmosphere of Mexico City and determine their potential impacts on human health.

Continuous monitoring of airborne pollen grain was held in the southern of Mexico City. Sampling of airborne pollen grain was conducted from August 2008 to August 2013, using a Hirst spore trap, which operates continuously 365 days a year.

The methodology followed, regarding samplings and counts, was the proposed by the Spanish Aerobiology Network. With the information generated during these five years has been possible to create the first airborne pollen calendar for Mexico City. The results showed that the Annual Pollen Index (API) of airborne pollen grains has its lowest value (67 242) during 2011-2012, while the maximum (114 451) occurred in 2009-2010. There were about 45 different pollen types, trees being the most abundant followed by herbaceous. The types of pollen that reached more than 1% in descending order were: Fraxinus, Cupressaceae, Alnus, Poaceae, Pinus, Myrtaceae, Liquidambar, Quercus, and Urticaceae.

Pollens from trees showed their highest concentrations from December to March, while the herbaceous were present throughout the year in low concentrations. The admission of patients to the National Institute of Respiratory Diseases by allergic rhinitis, allergy, and asthma caused by pollen exposure was directly related to the increase in the air of pollens from trees, mainly of Fraxinus. The environmental conditions were closely related to the phenology and occurrence of airborne pollen grain of Mexico City.

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ORAL COMMUNICATION

Fraxinus airborne pollen in the atmosphere of Mexico City

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The incidence and seasonal behaviour of *Fraxinus* pollen, the most abundant pollen type in the atmosphere of Mexico D.F., has been studied for a period of four years (October 2008 to September 2012) using Hirst type volumetric spore-traps. The study, generated by the Mexican Aerobiology Network, the REMA, includes the data obtained by four sampling stations: Coyoacán, Miguel Hidalgo, Iztapalapa and Cuajimalpa, situated in different points of the city. The methodology followed, regarding samplings and counts, was the proposed by the Spanish Aerobiology Network, silicone fluid being used as adhesive substance and glycerine jelly as mounting mean. The results obtained showed that the main pollen season goes from November to mid April, with annual pollen index up to maximum values of almost 60,000 and peak days of more than 2,700 pollen grains/m³ in Coyoacán and Miguel Hidalgo sampling stations respectively, both records occurring in the seasonal period 2009-2010. This is due to the massive presence of *Fraxinus* trees in these areas, mainly *Fraxinus uhdei*, a very resistant species to atmospheric pollution. The peak days normally took place in January or February, depending on the year and the sampling stations. The lower values were detected in Iztapalapa and Cuajimalpa where *Fraxinus* trees are less frequent. Due to the results obtained, *Fraxinus* pollen must be taken into account as one of the more important aeroallergens in the Federal District of México.

Comunicación subvencionada por la Universidad de Málaga (Campus de Excelencia Internacional Andalucía Tech)

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ORAL COMMUNICATION

Relationship between trends in airborne pollen series in Catalonia (NE Spain) and the NAO and WeMO regional teleconnections

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The aim of this study is to explore the influence of NAO and WeMO climatic variability modes on the airborne series of 20 pollen types recorded at six stations of the Aerobiological Network of Catalonia during the period 1994-2011. The climatology of the Iberian Peninsula is strongly influenced by the North Atlantic Oscillation (NAO, index values from the Gibraltar-Iceland dipole). The NAO positive phase is characterized by an intensification of the 'westerlies' over Europe and by moisture transport across the N Atlantic, and results in rainfall reduction over S Europe and the Mediterranean. The NAO negative phase produces high-pressure blocking in the NE Atlantic with more southern circulation and wetter conditions in the W Mediterranean. Moreover, the W Mediterranean is under the influence of the recently defined Western Mediterranean Oscillation (WeMO) accounting for the E Iberian Peninsula regions, which are weakly or unrelated to the NAO pattern. The WeMO is defined using the dipole San Fernando (Cadiz, Spain) – Padua (Italy). The positive phase corresponds to high pressures over the Azores and SW Iberian Peninsula and low pressures in the Liguria Gulf and results in rainfall being more abundant in the Cantabrian coast and lower in the Mediterranean one. In the negative WeMO phase wetter conditions occur over the E Iberian coast and the Ebro basin.

Preliminary results show negative correlations of most of the taxa with both the NAO and the WeMO indices, which are consistent with the wetter conditions associated with the negative phase of the indices in the eastern peninsular fringe, comprising Catalonia. Positive significant correlations are obtained only for *Corylus*, which could be due to a major influence of the long/medium range transport from Pyrenees and France. Poaceae and Chenopodiaceae/ Amaranthaceae presented significant positive correlations, but only in the NE stations (Girona, Barcelona, Bellaterra), also exposed to northern long range transport.

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ORAL COMMUNICATION

An integrated olive-crop yield-forecast model for the Mediterranean area.

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Olive oil is one of the main economic resources of the Mediterranean region. Early and effective crop forecasting is very important from the standpoint of environmental, economic and social development in a changing environment. The present study was designed to identify the main factors that influence olive fruit production in the Mediterranean region by analysing data from three of the main olive-producing countries of the world: Spain, Italy and Tunisia. From 1993-2012, 17 aerobiological sampling points were analysed. Seven forecasting models are developed at different scales (from growing area, to country, to Mediterranean basin) to provide accurate models that are adapted to the different characteristics. The pollen index and water availability in spring are significant factors for all of these models, and they are essential for optimal crop production. Temperature is another parameter that is significant across most of the models, although the temporal decoupling in the phenology means that the air temperature effects are not equal across all of the study growing areas. Some of the growing areas show clear positive effects of temperature prior to flowering and during autumn. There are also clear negative effects of temperature during winter, during the process of fruit setting, and during summer. These models demonstrate highly predictive values according to the main parameters for olive crop production in the Mediterranean basin.

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ORAL COMMUNICATION

Phenology and Aerobiology of the Maidenhair tree (*Ginkgo biloba*): a living fossil for modern cities

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Ginkgo biloba is a dioecious gymnosperm native to China that has become widespread in many European cities because of its good adaptability to urban climate conditions, moreover its spectacular autumn golden-leaves. The unpleasant butyric-acid smell given off by the fruit has caused botanical sexism against female promoting the planting of male-sex individuals. Its pollen has some references as allergen, but the limited information on their aerobiological behavior is what has led us to set the goal of this work to study the phenology and aerobiology of the *Ginkgo biloba* populations existing in the city of Granada (Spain). For this study, four populations distributed in parks and streets of the metropolitan area, with different ecological and environmental conditions, have been considered. During three years (2011-2013) the phenology and aerobiological behavior in the different populations have been monitored.

The results show that the reproductive structures begin to develop when the trees have reached a maturity of 20 years. The development of the male inflorescence takes place on brachyblasts in early spring. In the studied populations this development has been strongly influenced by the microclimatic conditions prevailing in each area. The pollen production per anther is very high, on the order of 1.8×10^3 pollen grains, what is estimated to lead to a production of 1.350×10^6 pollen grains per individual. This pollen production occurs when the tree has reached reproductive maturity, estimated in about 20 years. Sampling aerobiological at short (<100m) and medium distance (<1000m) of the populations reveal high pollen emission, higher than 1,000 pollen grains/m³/hour, with great capacity of dispersal, similar to other gymnosperms. It can be concluded that the pollen of *Ginkgo biloba* is of aerobiological interest due to both its growing presence in the atmospheric pollen spectrum and its possible involvement in the development of new allergic sensitization.

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ORAL COMMUNICATION

Seasonality of *Penicillium* species in outdoor air in Badajoz

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Introduction. *Penicillium* fungal spores are important in human health and food contamination. The objective of this work was to study their seasonal abundance in outdoor air using viable methods.

Material and Methods. Sampling was carried out in the city of Badajoz (SW Spain), University of Extremadura, for two years, between March 2009 and June 2011, using two portable samplers simultaneously: a Burkard personal sampler, with a funnel device (BPSf) or with a sieve (BPSs) and a Sampl'air AES Chemunex (AES). SDA (Sabouraud Dextrose Agar) and MEA (malt extract agar) were used as culture media. Data were provided in colonies forming units per cubic meter (CFU/m³).

Results. 394 colonies of *Penicillium* were recorded. The daily average was 11 CFU/m³. 25 taxa of species were identified (*P. arenicola*, *P. aurantiogriseum*, *P. bilaiae*, *P. brevicompactum*, *P. camemberti*, *P. chrysogenum*, *P. citrinum*, *P. decumbens*, *P. digitatum*, *P. duclauxii*, *P. expansum*, *P. fellunatum*, *P. funiculosum*, *P. glabrum*, *P. hirsutum*, *P. implicatum*, *P. islandicum*, *P. italicum*, *P. janczewki*, *P. olsoni*, *P. purpurogenum*, *P. sclerotium*, *P. spinulosum*, *P. thomii*, *P. variable*). The highest concentrations of *Penicillium* were recorded on 5th March 2010 in BPSf (283 CFU/m³). Species more frequent showed a strong seasonality, in autumn (*P. aurantiogriseum*, *P. spinulosum*) and in winter (*P. chrysogenum*, *P. citrinum*, *P. expansum*, *P. glabrum*, *P. sclerotium*, *P. thomii*). Others did not show seasonality (*P. italicum*). *Penicillium* concentration was higher in winter (20 CFU/m³) and lowest in summer (6 CFU/m³). There were found statistically significant differences between the samplers ($\chi^2=28.130$, $p<0.001$), between BPSf and BPSs ($Z=-3.282$, $p<0.001$) and between AES and BPSs ($Z=-3.512$, $p<0.001$). However, correlations statistically significant were found only between AES and BPSs ($r=537$, $p<0.001$).

Conclusions. Most of conidia of *Penicillium* showed an evident seasonality with a strong increase in winter and lowest concentrations in summer, although some species did not follow this trend. The differences in the results obtained with the two samplers can be explained at least partly by the technical characteristics of each.

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ORAL COMMUNICATION

The pollen content of the atmosphere on the Abomey campus during the dry season in Benin

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Introduction

The scientific study of Aerobiological content has started since after the Second World War. However aeropalynological studies are rare in Africa in general and Benin in particular. Among the few published researches in this discipline, we can cite those of Charbert (1971), El-Gharbi (1976) Kortebi (1977) and Chafai-Ketfi and boughediri (2004) in North Africa and Ybert (1980) in Côte d'Ivoire and Chad. The purpose of this work is to determine the qualitative and quantitative composition of the pollen air during a large part of the dry season. Indeed, a better knowledge of meteorological factors that influence the production, release and dispersal of pollen grains is more essential to the variability of pollen scores that attempt to predict the organic content of the air (Kaaidi and al., 1997). Annual pollen counts will later correlate the clinical manifestations and flowering time of plants to establish a pollen calendar as in developed countries.

Study area

The Campus of Abomey-Calavi is located in the town of Abomey-Calavi which belongs to the Department of the Atlantic. Infrastructures are diverse and range from small rooms, auditoriums and relatively tall buildings such as residence Hassan II with 10 m high. The Abomey-Calavi campus has a sub-equatorial climate with two dry seasons (one long and one short) and two rainy seasons (one long and one short). The vegetation man-made and is composed of species that are mostly planted.

Materials and methods

According to Burden et al., (2003), the general principle of pollen counts is to collect the pollen with a sensor whose role is to trap in impacting on the "adhesive backing" and analyze the optical microscope to recognize the nature of pollen collected (qualitative analysis) and then determine the number of pollen grains of each taxon (quantitative analysis). Pollens are captured using a microscope slide covered with glycerin gelatinized deposited on the support device and Durham recorded every 24 hours. The blades used as trap is stored in a slide box. The data were analyzed with Excel software.

Results and Discussion

A total of 250 pollen are counted during the three months of experimentation. They belong to different families, the most important is the Leguminosae. The most common pollen is that of *Acacia auriculiformis* exotic species used for reforestation of large areas in southern Benin. The number of pollen grains varies from 1 to 50. According to Besancenot (1989) and Burden et al. (2003), gravimetric devices have a relatively low yield of recovery. Pollen analyzes show that large pollen are more likely to be trapped than the small ones. According to Besancenot (1989) and Laaidi et al. (2003), location largely determines the harvest. The sensor traps the tree pollens when placed in elevated stands and grass pollen when placed on ground surface. Indeed the sensor in this study is placed on the roof of a building of 10 m high.

Conclusion

Results from this aeropalynology study that is firstly carried out in Benin shows that the campus environment is also responsible for pollen allergies. Pollens being collected only during a portion of the long dry season, however, give an idea of the pollen content of the air on campus. The result of the experiment will provide information to help establish an exquisite pollen calendar Calavi

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Session 6: Airborne pollen and spores: environmental indicators and allergens
ORAL COMMUNICATION

Aerobiological and ecological study of the potentially allergenic ornamental plants in South Spain.

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The present study sought to analyse the incidence of potentially-allergenic ornamental species in the cities of Seville, Cordoba, Granada and Malaga (southern Spain), examining a number of ecological and aerobiological variables. The information will undoubtedly be of value when designing new urban green areas and assessing their health impact on the local population. Complete lists of ornamental flora growing in the urban green areas of each city were compiled from previously-published papers, local council, university databases and in situ observation. The variables to be examined were: a) biological form, using Raunkiær's major classes, b) geographical origin, c) Ellenberg's ecological values. Airborne pollen count databases were obtained from sampling stations located in the cities, which belong to the Spanish Aerobiology Network (REA)/Andalusia Aerobiology Network (RAA). Sampling was performed from 2006 to 2009, using Hirst-type spore traps and following the methodology designed by the REA. Allergenic ornamental species in these cities have shown slight differences in the ecological characteristics. Seville and Malaga count with a higher proportion of terophytes and geophytes, a greater abundance of tropical species and higher luminosity and temperature values that probably reflects the milder climate. By contrast, the continental climate of Cordoba and Granada would account both greater presence of European species and the absence of tropical species. Analysis of airborne pollen counts for all four cities indicated that not all studied species were actually likely to suppose a health risk for allergy-sufferers. Some pollen types tended to be negligible, either because these were entomophilous species or because they were represented by few individuals. Valid future strategies include using zoophilous plants or dioecious plants, ensuring predominance of female plants, and promote greater species diversity when designing new gardens.

Session 7: Landscape change and human-environment interactions
INVITED LECTURE

From pristine landscape to agriculture: vegetation history and human impact in South Greenland during the last millennium

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The Norse colonization of Greenland, beginning ca AD 985, lasted more than 400 years. It resulted in the introduction of a northwest European-style of pastoral farming to a 'pristine' environment (Dugmore et al. 2005). The arrival of settlers and their domesticated animals (sheep, goats, cattle and horses) in the so called eastern settlement can be traced in the palaeoenvironmental record using biological (Pollen, NPPs, Diatoms, Chironomids, molecular biomarkers such as fecal sterols and bile acids) and sedimentological proxies (geochemistry and isotopes). Lakes and peat deposits from the Eastern settlement were investigated in order to get information on farming impact during the medieval time as well during the modern period, with the reintroduction of sheep in 1920. The settlement of Norse farmers, by the very end of the 10th century AD, is concomitant to the decrease in *Betula pubescens* and *Juniperus*, the introduction of non indigenous taxa (*Rumex acetosa-t*) and the rise in coprophilous fungi and molecular biomarker related to dung (Gauthier, 2010 ; Massa et al. 2012b). This colonization phase is followed by a period of decreasing human impact as soon as the end of the 13th century/ beginning of the 14th century, with cooling evidence and a decrease in coprophilous fungi suggesting a reduced grazing pressure. After the 15th, the re-growth of shrubs and the disappearance of coprophilous fungi demonstrate the abandonment of the settlement, until the development of contemporary agriculture in the 20th century. Recent agricultural activities (1920-1980 AD) had the same impact than Norse agriculture. However, the biological and sedimentological response to the last 30 years of modern sheep farming is marked, with drastic changes in diatom taxa, NPPs, diatoms and C and N isotopes (Perren et al. 2012).

Session 7: Landscape change and human-environment interactions
INVITED LECTURE

Landscape history studies: an integration of pollen analyses, multi-proxy records and archaeological data

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During the initial stages of the Palaeopalynology, pollen analysis was mainly used to establish chronologies in Northern European peatbogs. Later on, pollen research focused on palaeoecology, analyzing plant evolution, palaeobiogeography and palaeoclimatology. During the 40's and 50's, human societies were acknowledged as a significant agent of environmental change and pollen sequences used to track anthropic activities. This idea represented the starting point of Modern Pollen analyses and during the following 70's and 80's decades, the history of Cultural Landscapes became a central research topic for Central and Northern European Palynology. In 1981, the publication of the first list of "pollen human indicators" evidenced that pollen could be used not only to reconstruct past landscapes but also to infer human practices. Both, the Ystad project (18986) and the publication of the Past, present and future landscape (1988) definitely placed Palynology as a core discipline in the study of long-term landscape history. This allowed the European expansion of pollen research applied to landscape history during the 80's.

Such pollen studies shifted their study goal from palaeovegetation and palaeoclimatology to landscape history, a fact that implied both the "lost of centrality of Palynology" and a completely different approach:

- 1) Being landscape an eco-socio-cultural product, the socio-historical perspective becomes essential
- 2) The integration of palaeoenvironmental, archaeobiological, archaeological and historical data is needed in order to understand landscape changes and the socio-economical-cultural triggers
- 3) High resolution time models are required in order to integrate different nature datasets
- 4) Landscape diversity is related to different land-uses and, thus, local and micro-local scale, as well as high resolution analyses are indispensable.
- 5) Multiproxy analyses allow to identifying a wide range of human activities.

Session 7: Landscape change and human-environment interactions
ORAL COMMUNICATION

Local hominin mobility in response to middle pleistocene environmental dynamics in Central and Southern Italy

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During the “Mid-Pleistocene Transition (MPT)”, from about 1 to 0.6 Ma, the transition from 41-ka to 100-ka dominant climatic oscillations, occurring within a long-term cooling trend, was associated with an aridity crisis which strongly modified the ecosystems. At this time, the palaeobotanical records of Early Palaeolithic sites from Western Europe indicate that hominins settled in different types of environments, suggesting an important ecological plasticity of early hominins to temperate environments.

Since the MPT, the more favorable climate of central and southern Italy provided propitious environmental conditions for long-termed and sustainable human occupations even during the glacial times. In fact, the human strategy of territory occupation was certainly driven and constrained by the availabilities of both floral and faunal resources. Prehistoric sites such as Notarchirico (ca. 680–600 ka, OIS 15), La Pineta (ca. 600–620 ka, OIS 15), Fontana Ranuccio (ca 460 ka, OIS 13), or Guado San Nicola (ca. 380–350 ka OIS 11-10) testify to a preferential occupation of the central and southern Apennines valleys during interglacial phases, while later interglacial occupations were oriented towards the coastal plains, as attested by the numerous settlements of the Roma basin (ca. 300 ka OIS 9). Faunal remains retrieved in these sites indicate that human subsistence behaviors benefited of a diversity of exploitable ecosystems, from semi-open to closed environments.

In central and southern Italy, several palynological records have already illustrated the regional and local scale vegetation dynamic trends. During the Middle Pleistocene climate cycles, mixed mesophytic forests developed during

the interglacial periods and withdrew in response to increasing aridity during the glacial episodes.

Pollen data from the Boiano basin (Molise, Italy) bring new details concerning the regional evolution of vegetation and climate between OIS 13 and 9 (ca. 500 to 300 ka). In this basin, the physiography and the persistence of high edaphic humidity, even during the glacial phases, could have allowed the establishment of a refuge area for the arboreal flora, providing subsistence resources for the animal and hominin communities during the Middle Pleistocene. This could have constrained human groups to migrate into such a propitious area, in order to keep access to a large diversity of ecosystems. Regarding to the local climate evolution during the glacial episodes, the supposed displacement from these sites could be linked to the environmental dynamics solely due to the aridity increase rather than directly to the global climate changes.

Orain, R., Lebreton, V., Russo-Ermolli, E., Sémah, A.-M., Nomade, S., Shao, Q., Bahain, J.-J., Thun Hohenstein, U., Peretto, C., 2013, Hominins responses to environmental changes during the Middle Pleistocene in Central and Southern Italy, *Clim. Past*, 9, 687-697.

Session 7: Landscape change and human-environment interactions
ORAL COMMUNICATION

Holocene changes in pollen-based vegetation composition in N-NW Europe: why does quantitative reconstruction matters?

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This study aims at pollen-based reconstruction of the spatiotemporal dynamics of the N-NW European regional vegetation and land cover in the Holocene. We apply the REVEALS model (Regional Estimates of VEgetation Abundance from Large Sites) for reconstruction using fossil pollen records from eighteen sites currently distributed in five different biomes in the region. Principal Components Analysis results of the spatiotemporal dynamics of the past vegetation and land cover are classified into four time-trajectory types in both REVEALS-based vegetation (RVs) and pollen percentage (%pollen) changes. Those four types are better separated using the RV-based trajectories than using %pollen-based ones. Further, our results indicate that the REVEALS- and %pollen-based estimates of timing of shifts and rates of change in vegetation and land-cover can be significantly different. Then, the REVEALS-based reconstruction gives new insights into the timing and magnitude of human disturbances on Holocene regional vegetation, which are critical to understand the human impact on landscape and climate in the past.

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ORAL COMMUNICATION

Pollen and NPP analysis of the Holocene palaeoenvironmental sequence of Qallimiut (SW Greenland)

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With the aim to provide an in-depth understanding on the land-use, human impact and environmental evolution of the Southern Greenland fjords area, the study of Holocene lakes records has been recently addressed.

We present the results of the pollen and NPP analysis of a 118 cm depth core retrieved in Lake Qallimiut (24 m depth), a lake located in the Vatnahverfi region, under suboceanic conditions (Low Arctic zone), 1 km away from the coastline. Surroundings of Lake Qallimiut have been surveyed and Norse ruins of two sites (Ø77 and Ø77a) have been found. Modern sheep farms and fields linked to the activity of two current families of farmers are located between the coast and the lake.

Pollen and NPP analyses performed on Qallimiut record enables an in-depth study of the comparative effects of medieval (985-1450 AD) and modern (since 1920) agricultural activities upon an otherwise pristine subarctic landscape.

The geographical setting of Vatnahverfi region enables the comparison of the Qallimiut palaeoenvironmental record with Lake Igaliku sequence, located 30 km North and characterised by the dominance of subcontinental subarctic climate conditions (Subarctic zone). Lake Igaliku is situated circa 2 km northwest from the medieval site of Garðar, which is known to be among the largest medieval farms of Greenland. A modern sheep farm was built on its shore in the 1980's.

As both lakes show the same size and depth, the correlation and comparison between these two lacustrine records provides a reflection of the diverse land management at a local scale during the Norse period and the modern time.

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ORAL COMMUNICATION

Persistence of cold-adapted species through the Holocene in the Spanish Central System

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Persistence of cold-adapted forest species in Mediterranean environments is increasingly a challenging task, specially facing current global change. The Holocene climate variability and, more recently, anthropogenic impacts have led many populations to fragmentation and isolation and even to extinction. Anyway, some of them have persisted to the present day. The understanding of their evolution needs long-term studies, in which pollen analysis appears as a key approach, both to shape hypothesis and support results from other disciplines. On the other hand, mountain ranges have played, and still do, an essential role as both glacial and interglacial refugia during the Quaternary.

The Iberian Central System harbors an exceptional diversity due to its geographical position and complex topography. Some cold-adapted species find here a significant proportion of their southernmost populations, likely displaying remarkably adaptations to changing environments. In this presentation, a comprehensive review of palynological investigations developed along the Spanish Central System evidences the distribution of genera like *Taxus*, *Betula*, *Fagus*, *Carpinus* and *Tilia* through the Holocene, and also highlights the scarcity of well-dated and high-resolution works, which may aid a better understanding of their recent and future evolution.

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ORAL COMMUNICATION

On the origins of highly human-transformed landscapes: the case of the Castilian Plateau (NW Spain)

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A new palaeoecological record (pollen, charcoal, dung fungal spores) from a mid-altitude site of NW Iberia is presented. The main aims were to describe vegetation changes since the late-glacial in response to climatic changes and disturbances and how human activities have contributed to alter the original vegetation cover. In this context, last deglaciation was mainly characterized by steppic grasslands and heathlands with an almost absent fire activity. The spread of pines apparently took place during the Younger Dryas and the onset of the Holocene. Pines continued dominating for several millennia at the beginning of the Holocene showing an inertial development favoured by the enhanced seasonality during the early Holocene and the inland location. Climatic amelioration promoted *Betula* and deciduous *Quercus* spread, taking part of mixed stands with pines. Later, mid-Holocene presented a highly forested landscape where deciduous trees dominated (*Alnus*, *Betula*, deciduous *Quercus*). Human impact is detected quite early at this site, as dung fungal spores and *Plantago* started continuous curves around 6000 cal yr BP and a sharp rise in fire activity occurred at 6400-6200 cal yr BP. However, forests seemed to cope with these disturbances and deforestation was unimportant until the Chalcolithic ca 4500 cal yr BP. Fire could be used to clear the forest and promote the establishment of pasturelands and heathlands as the period of highest fire activity since the last glaciation was comprised between 4500 and 2700 cal yr BP. The final step in landscape transformation was during the Iron Age (ca 2700 cal yr BP onwards), when land-use intensification occurred marked by the establishment of cereal crops, higher grazing pressure and the spread of shrublands.

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ORAL COMMUNICATION

Prehistorical hunting and pastoral activities in the remote and marginal karstic landscape of the “Silberer Plateau” (Muotathal Alps, Canton of Schwyz, Switzerland)

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Palaeoecological research including the analysis of pollen, spores, non-pollen palynomorphs and macrofossils was performed on peat sediments from the small Schattgaden-Bog (1890 m a.s.l.) in the remote Alpine valley of Muotathal, Switzerland. Given the calcareous and karstic geological setting of this research area (famous for its huge and impressing stalagmite caves), the presence of a mire on top of this geological feature is exceptional. The 130 cm long, high resolution radiocarbon dated peat core revealed mire initiation during the Late Glacial, and a subsequent Early Holocene flora and vegetation dominated by open woodlands of pine (*Pinus*), hazel (*Corylus avellana*) and elm (*Ulmus*) at and/or near the timberline. Local archaeological findings and several bones of wild forest animals from near-by rock-shelters and caves support a Mesolithic to Bronze Age landscape used for hunting and gathering activities. Rather exceptionally for the Central Swiss Alps, this remote area seems not to have been used as pastoral land for livestock until the beginning of the Iron Age (ca. 750 BC), probably because of its poor vegetation cover in this karstic environment. Palynological evidence for heavy pastoral activity since the Iron Age does therefore manifest the prehistorical utilization of marginal Alpine land resources, which were unimportant before, possibly as a result of rising human population pressure in the surrounding valleys. Interestingly, a major shift in the mire flora and upland herb diversity after 1000 AD may have been related to rising nutrient values of the local soils due to changing livestock preferences of the local summer farmers on the “Silberer Plateau”.

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ORAL COMMUNICATION

The Silvretta Massif in Switzerland: A hot-spot for the reconstruction of climatic and human impact on the European Alps by palynological means

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Getting accurate knowledge of human activities in high altitude environments has become a major matter of concern in understanding the dynamics of populations and their impact on ecosystems. The Silvretta Massif in the Central Alps offers a large range of archaeological sites from Mesolithic Times to the Medieval Period, such as rock shelters, huts and enclosures, thereby revealing the human and livestock presence in the according valleys. Here we present the history of climate and human impact on flora and vegetation composition as well as on the landscape development for the Holocene thanks to palynological data (pollen, spores, non-pollen palynomorphs, and macrofossils) from three peat bogs of the Silvretta Massif. The bogs were selected on a North-South transect from the Fimber Valley (Northern Silvretta, Switzerland) to the Lower Engadin Valley (Southern Silvretta, Switzerland). This transect was likely to emphasize the differences and similarities of the data sets collected in relation to their context (i.e. elevation, vegetation zones, proximity to archaeological sites, etc.). Vegetation historical reconstruction show fire events during the Mesolithic Times which might have been anthropogenically induced, as well as first subalpine livestock impact since the Neolithic Period. Massive grazing impact was revealed for the Bronze and Iron Age (mainly by sheep and goat) with a more pronounced effect in the Lower Engadin, where cereal cultivation in the valleys was predominant. During the Medieval Period and Modern Times grazing pressure (mainly by cows) reached maximal values as indicated by high values of spores from coprophilous fungi, which might even reveal the grazing activity on a bog surface itself.

Session 7: Landscape change and human-environment interactions
ORAL COMMUNICATION

The contribution of non-pollen palynomorphs to retrace reconstruct past pastoral dynamics in the Western Pyrenees mountains (Ossau valley and Basque Mountains), France

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The expansion of studies on quaternary non-pollen palynomorphs (NPPs) leads to continuous discovery of new microremains, which in turn gives rise to new questions on the identification, indicator value, distribution or spatial resolution of both known and new NPP Types. Among the fungal microremains, spores of dung-related Ascomycota (coprophilous s.l.) are used to assess past grazers' presence and abundance. Based on the current knowledge on the ecology of coprophilous Ascomycetes, once can wonder if their fossil spores can be used to better quantify and qualify the past faunas?

A dataset of 40 modern NPP assemblages was acquired in summer mountain pastures (Ossau Valley) in order to provide spatial and ecological indicator values of NPPs for reconstructing past pastoral activities using four peat cores from the Ossau valley and the Basque Mountains. The relationship between 266 modern NPP Types and 51 environmental and pastoral variables was evaluated using direct ordinations (redundancy and canonical correspondence analyses). Pastoral indicators were searched within a set of 51 spore-Types assigned to dung-related Ascomycete taxa among Pleosporales, Sordariales, Melanosporales and Xylariales. The NPP fossil data contribute significantly to emphasize different trends of evolution of past grazing activities between the Ossau and the Basque Mountains since Neolithic times and over the last 2000 years in particular.

The typology and the nomenclature used to classify and name the Types will also be presented.

Session 7: Landscape change and human-environment interactions
ORAL COMMUNICATION

Human management and microregional landscape variability of high altitude Eastern Pyrenees during historical times: the upper valleys of Ter and Tet

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Interambar and Carambar projects aim determining the landscape history of the Eastern Pyrenees' upper mountain areas-above 2000 m- during the last 2000 years. These projects are mainly focused on the role of past human activities in the process of landscape shaping. One of the main features of the Mediterranean Eastern Pyrenees is the prevailing different Northern/Southern slopes' environmental conditions, which are mainly due to different orography and water availability. According to this, one of the main goals in this study is to detect similarities and dissimilarities in human management and landscape trajectories between both slopes which have similar cultural and historical context, that could contribute to explain current differences in landscape configuration. To achieve this objective and to characterize highland landscape variability, palaeoenvironmental and archaeological studies have been carried out at high spatial and temporal resolution in the neighbouring upper valleys of Ter (Spain) and Tet (France) rivers. As a result, a set of peat bogs located between 2100 and 2500 m have been sampled and analyzed. The multiproxy approach include pollen analyses, non-pollen palynomorphs (mainly coprophilous fungal spores), macrocharcoal particles, diatoms, mineralogy and geochemistry. In addition, palaeoenvironmental data sets have been integrated with archaeological and archaeobotanical series. Archaeological data has been obtained from intensive total-recovery fieldwork, excavation, radiocarbon dating and charcoal analysis of archaeological levels. Obtained results show four main phases of human use and highland landscape shaping during Roman, Late Antiquity, Early Medieval and Modern periods. In addition, palaeoenvironmental records reveal that major trends in land use and landscape history are synchronous in both the Northern and Southern slopes of the Eastern Pyrenees. However, recorded divergences suggest that distinct human occupation and land-use of upper mountains differ between different oriented slopes, probably depending on specific environmental conditions and the availability of certain natural resources.

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ORAL COMMUNICATION

Pollen data and other archaeobotanical remains from the middle ages in Wallonia (Southern Belgium): A review

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Since the mid-20th century several pollen analyses have been carried out in Wallonia (Southern Belgium) mainly from natural deposits but also from archaeological sediments. However, although these previous studies have outlined the general vegetation features for the Holocene, temporal resolutions of the sequences stay low and not suited to catch short changes as anthropogenic disturbances may be. Moreover, none focused on recent periods to elucidate the different forms of economies in historical times. From an archaeological point of view, sites investigated are very scarce, despite their importance in tracing the consumption patterns and exploitation strategies of plant resources. The pollen studies were furthermore rarely employed in combination with other proxy data and published.

In this context, a new multi-disciplinary team in bioarchaeology has been established in 2012 at the Royal Belgian Institute of Natural Sciences, involving specialists in archaeozoology and archaeobotany (fruit/seeds, wood charcoal, phytoliths, starch grains and pollen). This team works on all archaeological sites excavated in this region in order to fill the gap in knowledge of land-uses and living conditions from the Upper Paleolithic onwards.

The purpose of this presentation is to review all existing pollen data in Wallonia for the Middle Ages, which is up to now the less documented period, both from natural sites and archaeological contexts. New archaeological sites recently explored by the team will be also added. The aim is here to investigate landscape changes and land-uses within the area during the medieval period, but also to try to reconstruct the lifestyle and dietary patterns of the populations. Is palynology able to provide evidence of different strategies of plant acquisition, food/agricultural production, storage, processing, trade or exchange? On what do we have to focus in a next future to provide a more accurate insight in interactions between plants and people?

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ORAL COMMUNICATION

Environmental changes during the past 1000 years: a high-temporal-resolution multiproxy record from a mire in Northern Finland

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CBAE (UMR5059 CNRS)

We present a record of peatland development in relation to climate changes and human activities from the Palomaa mire, a remote site in northern Finland. We used fine-resolution and continuous sampling to analyse several proxies including pollen (for vegetation on and around the mire), testate amoebae (TA; for mire-wetness changes), oxygen and carbon isotopes from Sphagnum cellulose ($\delta^{18}O$ and $\delta^{13}C$; for humidity and temperature changes), peat-accumulation rates and peat-colour changes. In spite of an excellent accumulation model (30 ^{14}C dates and estimated standard deviation of sample ages <1 year in the most recent part), the potential to determine cause-effect (or lead-lag) relationships between environmental changes and biotic responses is limited by proxy-specific incorporation processes below the actively growing Sphagnum surface. Nevertheless, what emerges is that mire development was closely related to water-table changes rather than to summer temperature and that water-table decreases were associated with increasing peat-accumulation rates and more abundant mire vegetation. A rapid fen-to-bog transition occurred within a few years around AD 1960 when the water table decreased beyond the historical minimum, supporting the notion that mires can rapidly shift into bogs in response to allogenic factors.

Session 7: Landscape change and human-environment interactions
ORAL COMMUNICATION

Climate and land-use changes during the four last millennia in Southern Alps (Italy) recorded at Lake Ledro

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In the vast debate considering respective climate and anthropogenic influence over environmental and societal changes, we investigate the deep core (LLO81) of the Lake Ledro (652 m a.s.l., Southern Alps, Italy). Holocene environmental changes reconstructed through multiproxy analysis [pollen-inferred vegetation, Magnetic Susceptibility, lake-level, flood frequency and XRF measurements (Magny et al., 2012; Joannin et al., 2013; Vannière et al., 2013)] are focused on climate and land-use changes recorded during the Late Holocene (since ca. 4000 cal. BP).

For this time interval, the Lake Ledro records higher mean water table and increasing amount of pollen-based precipitation and more erosive conditions. This argues for a more humid Late Holocene scenario, southern of the Alps, having the potential to reinforce the forest presence, but pollen-based anthropogenic activities locally change the impact of this regional scenario. Land-use activity (farming, arboriculture, pastoralism and forest clearance) opens the large vegetated slopes in the catchment of the Lake Ledro, which in turn magnified the erosion related to the change in the precipitation pattern.

The record of an almost continuous human occupation for the last 4000 cal. BP however provides accelerating and decreasing of land-use phases. On the one hand, forest redevelopments on abandoned or less cultivated area appear to be climatically induced as they occurred in relation with well-known events such as the 2.8 kyr and the Little Ice Age. On the other hand, climatically-independent land-use changes are observed, such as the Bronze Age lake-dwellings abandonment, the population migration at ca. 1600 cal. BP and the period of the Black Death and famines at 600 cal. BP.

Session 7: Landscape change and human-environment interactions
ORAL COMMUNICATION

Focus on humans and landscapes during the last millennium in a mid-level mountain area (Lake Remoray, Jura mountains, France): a multi-proxy approach including historical and palae

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Over the past three decades, many palaeoenvironmental studies were performed in the Jura Mountains, rich in peat and lake deposits, with the aim to reconstruct large-scale landscape change and human-environment interactions. Settlement areas of mid-level mountains are particularly sensitive to changes that marked medieval and modern periods. From the ninth century, several areas of Jura Mountains show a succession of agro-pastoral retreats and expansions induced by demographic fluctuations and climatic variations. We focus now, on a lake with a little catchment area in the High Jura chain, Lake Remoray (850 m) which offer the interest to record local perturbation. A multi-proxy approach is started, with the aim to clarify and spatialize the last millennium landscape and land use of this area. Based on a precise chronological setting of sedimentary sequences, sedimentological analyzes document the local soil erosion. The vegetation cover and land use are restored from the determination of pollen, spores and non-pollen palynomorphs. The regressive method uses the counting of the historical archives which include agricultural inventories, maps, cadaster and various representations of landscape.

Session 7: Landscape change and human-environment interactions
ORAL COMMUNICATION

Highlighting environmental and climate changes in Vanuatu in connection with the early settlement of the Pacific - palynological study of Holocene deposits

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My research turns towards the reconstruction of environment and climate changes during the latter half of the Holocene in Vanuatu, using pollen analysis. Here, I present the results of the work done during my Master and my first thesis year. The interest of this analysis is to combine results of palynological and archaeological studies. Indeed, Vanuatu has a multitude of archaeological sites, and some of them are among the most ancient sites, dating back to the first human migrations, ca. 3500 BP. These migrations have been dependent on climatic factors. After settlement, these human groups have also had an impact on the natural environment of the islands. Thus, Vanuatu is a key area to the understanding of initial human colonization of Remote Oceania.

The first results of this high-resolution study cover specific sections of a sediment core collected at Emaotfer (Efate, Vanuatu), Tfero6. This analysis concerns two sedimentary levels with facies changes. The most ancient samples were dated ca. 3900 – 3500 BP, and the most recent ca. 1700 – 1300 BP. In addition, one of the most ancient Austronesien archaeological site, Teouma cemetery, (3200 – 3000 BP), is located nearby Emaotfer. Archaeological and isotopic studies have already been done on this site.

The most ancient samples correspond to a sedimentary transition, from a black clay-rich organic sediments to a pinkish gel rich in cyanobacteria. The pollen analysis shows a significant decrease in mangrove taxa, indicates a falling sea level. The substitution of tropical moist forest by open forest and marshy vegetation also illustrates a sudden drier regional climate. Moreover, taxa easily found today in anthropic areas, such as *Macaranga*, *Homalanthus* and *Mallotus*, were observed in these sediments. Those evidences probably indicate the arrival of the first Melanesians on Efate Island.

The most recent samples correspond to another sedimentary transition, from the pinkish bacterial gel to a peat deposit. The pollen analysis shows a replacement of open forests by mixed vegetation, composed of open forest and tropical moist forest taxa. This change indicates a return to wetter conditions. Results of the high-resolution study of the whole sedimentary core will be completed by pollen analysis done on others sedimentary cores, from different geomorphological contexts, collected on Efate and others Vanuatu islands. This will help to differentiate between local and regional data.

Abstracts sessions 1-7

Posters communications

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

Pollen conservation tests over time: changes in quality, nutritious and healthy parameters, and microbiology analysis

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Conservation of bee pollen (BP) properties is related to the collection of BP. BP collected adequately retains nutritional and healthier values better. Right BP collection implies good methodology (avoiding raining days) and not pollute the samples.

The aim of the study is analyze variations over time in dry and frozen BP in the following parameters: physicochemical (moisture, water activity, pH and acidity), nutritional (proteins and sugars) and healthy (antioxidants: polyphenols and vitamin C) The study has been carried on 10 pattern samples of European BP (Spain, Italy, Turkey and Greece).

Moisture doesn't shows a significant decrease after 18 months, either in frozen neither in dried samples. Water activity doesn't shows a significant decrease after 12 months, in dried samples. However, in frozen samples, water activity decrease sharply. Fresh samples with an initial pH higher than 5 experiment a further decline over the time. However, samples with lower pH are more stable. Acidity shows in both, fresh and dried samples, an almost general decline after 15 months. Protein content doesn't exhibit variations over time. Ratio Fructose/Glucose increases at 12 months or 15 months, depending samples. After six months storage, there is a notable decrease in monosaccharides (glucose and fructose). Glucose content decreased more. Polyphenols content in both, fresh and dry samples, increases slightly over time because, as reducing water activity then antioxidants concentration is increased. In fresh samples, Vitamin C shows a great variability of results and is very difficult to draw conclusions. However, although there is a very slight decrease over time, it can be said that freezing BP allows certain stability. Vitamin C is very sensitive to drying, showing a significant loss in dried samples. In dry samples the microorganisms content decreases over time. In frozen samples, yeast and fungi tends to remain or descend except in samples with high humidity and low acidity.

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

Studying the daily harvesting of pollen by *Apis mellifera* L. (Galicia – NW Spain)

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It was done an hourly pursuit of the quantities and origin of harvested pollen by four *Apis mellifera* hives located in Galicia South (NW Spain).

For this, samples of corbicular pollen were taken, by pollen traps, at different hours of the day at successive days. The results show the hive foraging behavior on the different pollinic sources in its action radius. Analyzing the data of each pollinic source we obtained several models of pollen foraging:

-Morning, the higher percentages of pollen harvesting are registered at the morning, to the midday -*Consolida ajacis*, wild Graminae group, *Oenothera stricta*, *Ranunculus peltatus*-t.-

- At central hours of the day, the higher relevance of those pollen sources are registered at 12h and 16h intervals -*Erica arborea*, *Raphanus raphanistrum*-t., *Sedum acre*-.

- To 16h, they are sources exploited mainly at morning and early afternoon with a following fall -*Cytisus scoparius*-t., *Eucalyptus globulus*, cultivated Graminae, *Halymium alyssoides*, *Plantago lanceolata*, *Quercus pyrenaica*, *Q. robur*, *Salix fragilis*-.

-From 16h, the higher percentages are registered after 16h. Those sources are important at the end of the journey. Any dominant sources (over 45%) had got this foraging model.

-Any sources are exploiting at midday with a tendency to crease its percentages about afternoon -*Castanea sativa*, *Erica umbellata*, *Rubus ulmifolius* -t.-.

-Variable -*Anthemis arvensis* -t., *Echium vulgare* -t., *Scandix pecten-veneris* -t.-.

All described models were found in all studies hives.

Sometimes, the hourly model of determinate pollen source in one determined hive is not maintained in the other hives. The same source can be forage following different patterns by different honey bee colonies.

This poster is centered in the data of those taxons more represented (with percentages over 45%, at least one sample). Each of those pollen sources, important in the studied area, describe the same foraging model on the part of studied colonies, with same exception.

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

Patterns of uncorrespondance between the palynological profile and the specifications of a Protected Designation of Origin: PDO “Miel de Granada” A case study.

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The designation of European figures that promote and protect quality food names have been widely applied to different products. This protection figures such as PDO (Protected Designation of Origin) cover agricultural products and foodstuffs, which are produced, processed and prepared in a given geographical area using recognised know-how.

In the case of Spanish honeys only two PDOs have been declared so far: PDOs “Miel de La Alcarria” and “Miel de Granada”. Each of them is regulated by a Regulatory Council that certifies that the characteristics of honey are in accordance with, and thus can be sold under the PDO specifications.

The designation of this public trademarks is directly linked to its geographical origin, in the case of honey, palynological analysis is used in order to discriminate both the botanical origin and the fitness of the palynological profile into the honey types established by the Regulatory Council of the PDO. This asserts the geographical origin and therefore the autenticity and quality of the product.

This work examines honey samples sold under the PDO “Miel de Granada” framework through classic palynological analysis in order to establish if the commercial designations are in accordance to the pollen profile and fit into the criteria stipulated by the PDO.

Our results pose interesting questions as how frequently should stocks be analysed, how much deviation from the legal values can be tolerated and whether a combination of multiproxy characters should complement the current set of analysis in order to establish multiparameter ranges rather than unimodal threshold values.

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

Tracing the geographical origin of chestnut honeys with protected designation of origin “Miel de Granada” based on mineral content and melissopalynological profile

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The European Union (EU) promotes and protects quality food names via the system known as PDO (Protected Designation of Origin). The link with the geographical area is an essential requirement in the case of PDO honeys, and it must demonstrate why a honey is associated with one particular area and not another.

The authenticity of attributes of honey guarantees quality and economic value, together with many other properties of the product. Thus, the quality control of honey has two principal purposes, to verify its authenticity regarding the honey production (to reveal possible frauds and adulteration), and to determine its authenticity in respect of descriptions. “Miel de Granada” is a highly quality product with protected designation of origin (PDO), which contains six unifloral and two multifloral honeys. Two types of chestnut honeys are included in this PDO: “Chestnut honey” and “Mountain range honey”, containing more than 75% and 20 % of *Castanea sativa* M. pollens, respectively.

In the last years great attention and effort have been focused to correlate pollen analysis and other identification parameters to establish geographical and botanical origin of honeys by means of statistical studies. The content of metals in honey, for instance, is affected by the soil composition (geographical origin) and floral type (botanical origin). Although the honey mineral content can differ within a wide range, metal elements are some of the parameters that have been researched to determine the botanical and geographical origin of honeys.

Thus, the aim of this work was to establish the correlation between the metal content characteristic and the melissopalynological profile of chestnut honeys labelled as PDO “Miel de Granada”. Different statistical tools as cluster analysis (CA) and principal component analysis (PCA) were used for this purpose. Our research could be applicable for the authentication of honeys and contribute to the groundwork studies in order to determine geographical origin of Spanish and European honeys.

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

Pollen characterisation of honeydew honeys from NW Spain and their contribution as health food

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Honey is a health food, traditionally used as an alimentary supplement to which are attributed many biological properties. The antioxidant effect of the honey depends of the phenolic content, mainly of the flavonoids. The honeybees collect important quantity of pollen, nectar and frequently honeydew. The honeydew comes mainly from sweetened non-floral secretions of oak and cork and green parts of plants.

The aim of this work was to study the pollen spectrum of 48 honeys from NW Spain and the most important healthy compounds containing this food. The honeys were characterised as honeydew honeys according to the physicochemical and melissopalynological characteristics. The identification of the pollen types was performed by optical microscopy with a count close to 800 pollen grains on an aliquot of 100 μ L and the results were expressed in percentages. The phenolic content was determined by Folin-Ciocalteu method, using gallic acid as reference phenol. The flavonoid content was analyzed by the Dowd method, using the quercetin as reference standard.

The pollen types *Castanea sativa*, *Rubus*, *Cytisus* type, *Erica*, and *Quercus* were present in more than 80% of the studied samples. In the pollen spectra were found as dominant pollen (more than 45%): *Castanea sativa*, *Rubus* and *Cytisus* type. As accompanying pollen (15-45%) were present *Frangula alnus*, *Cynoglossum* and *Sesamoides*; and as important pollen (3-15%) were found *Echium*, *Eucalyptus* and *Crataegus monogyna* type. The mean phenol content was of 126.8 mg/100g, with a range between 78.1 and 217.3 mg/100g. The mean flavonoid content was of 9.2 mg/100g, with a range between 6.6 and 14.3 mg/100g. Comparing the results with the reported by other researchers, the phenolic content was higher in honeydew honeys than in floral honeys; therefore, this honey type has an important biological value.

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

***In vitro* pollen germination: effects of low and high temperature stress and pollen viability of GM and non-GM maize lines.**

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The aim of this work is to assess pollen viability using *in vitro* germination with different concentrations of sucrose, H₃BO₃ and CaCl₂ in genetically modified (GM) and non-GM *Zea Mays*, contributing to the knowledge of their management and utilization. To study the influence of temperature on the germination ability pollen grains were culture at 12 °C, 22 °C and 36 °C. The culture medium for pollen germination was solidificated by the addition of 3 g/L Phytigel™ combined with 0 (control), 0.1, 0.5, and 1.0 M of sucrose; 0 (control), 1.0, 2.0, and 4.0 mM of boric acid and 0 (control), 1.0, 2.0, and 4.0 mM of calcium chloride. The data were submitted to analysis of variance at 5% probability. The rate of formation of pollen tubes was higher in medium with 0.5 M of sucrose for maize pollen because the trend is that the sucrose concentration increases the supply of carbon, changes the osmotic potential and inhibits the formation of pollen tube *in vitro*. The results indicated that germination of pollen is affected by temperature. Both groups, GM and non-GM varieties, showed higher and faster germination at 12°C, and exhibited a threshold maximum temperature around 36°C, above which germination is avoided or extremely low and delayed.

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

The investigation of morphologic and humidity analysis of the pollen grains collected by honeybees in Ardahan region of Turkey between 2010 and 2011

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The aim of this study was to investigate which types of pollen resources were preferred by honeybees and used for brood feeding in Ardahan region that considerably available for beekeeping.

To this end, between June and August in 2010 and 2011, 47 samples collected from 6 different districts of Ardahan (Ardahan, Göle, Damal, Hanak, Çıldır, Posof) and pollen loads separated manually according to their colors. Then, a total of 400 color-depend subsample slides were obtained by Wodehouse method. Pollen family identifications were made by using light microscopy and compared with the reference slides. And the most common pollen types analyzed for water content by Karl-Fisher. As a result of this research, the honeybee foragers concentrate on few plant species and the most frequent plant families that preferred by honeybees were Fabaceae, Brassicaceae, Cistaceae, Asteraceae, Dipsacaceae, Rosaceae and Boraginaceae, although the rich floral diversity of the region.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

Immunolocalization of arabinogalactan proteins (AGPs) and pectins in cork oak male flower and pollen

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The evergreen cork oak (*Quercus suber*) is a dominant monoecious tree species in the forests of Southern Iberian Peninsula. This Fagacea tree species has a socio-economic, a cultural and an environmental extreme importance for Portugal. Studies on the sexual reproduction of cork oak are essential to understand the molecular mechanisms of fertilization and identify the difficulties associated with seed production.

AGPs are a superfamily of highly glycosylated hydroxyproline-rich glycoproteins that occur throughout the plant kingdom and are found on the plasma membrane, in the cell wall, in the apoplastic space and in secretions. In reproductive tissues, the expression of AGPs is associated with the sporophyte–gametophyte transition. Pectins are galacturonate-rich, acidic polysaccharides, abundant in angiosperm pollen tubes. Our own previous work has shown a specific AGP expression pattern during plant gametogenesis in *Arabidopsis thaliana* and in *Trithuria submerses*.

To investigate the distribution of AGPs in cork oak male flower developmental stages and in the pollen germination development a set of monoclonal antibodies (mAbs JIM8, JIM13, JIM16, MAC207 and LM2) against glycosyl moieties specific to AGPs were used. To correlate AGPs and pectins, immunocytochemical localizations were performed with mAbs JIM5, JIM7 and LM7 which are specific to homogalacturonan epitopes. JIM7 and JIM5 were used to discriminate between methyl-esterified and unesterified pectins. The labeling obtained with anti-AGP and anti-pectins in cork oak male flowers from different phenological stages and in pollen showed a temporal and spatial specific pattern of distribution of those sugar epitopes. Anti-pectin epitopes showed a very uniform labeling present in almost all cell types including the pollen tube cell walls. Intense labeling was obtained with some anti-AGP epitopes in the anther, pollen intine wall and pollen tube cell wall.

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Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

PsPMEP a pollen-specific protein of pea (*Pisum sativum* L.) with homology to pectin methylesterases

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Pectin methylesterases (PMEs) produced by plants are a multigene family of enzymes involved in diverse physiological processes associated with both vegetative and reproductive development, including microsporogenesis, pollen germination and pollen tube growth. PsPMEP is a pea (*Pisum sativum* L.) pollen-specific gene that encodes a typical PME sequence. Analysis of PsPMEP showed that it is a group 2 PME composed of a PME inhibitor domain (PMEI) and a catalytic domain. PsPMEP three-dimensional structure has been modelled using carrot PME (44% sequence identity) as a template. The resulting structure shows a single domain of parallel beta-strands folded into a right-handed cylinder. The putative catalytic residues have been identified. Northern blot and in situ hybridization analyses showed that PsPMEP begins to be specifically expressed in the anthers (pollen maturation) at 4-5 days before anthesis till anther dehiscence and in pollinated carpels after anthesis. In the PsPMEP promoter region we have identified three CArG boxes showing the consensus motif C[A/T]8G, which is preferentially bound by the MADS domain of the transcriptional regulator AGL15, and two conserved cis regulatory elements (AATTGA and AGAAA) that have been described to be responsible of pollen-specific expression in other plant promoters. Functional analysis of the PsPMEP promoter fused to the uidA reporter gene in transgenic *Arabidopsis thaliana* plants showed a very similar expression pattern when compared with pea, indicating that this promoter is also functional in a non-leguminous plant. GUS expression was detected into the pollen sacs of the anther (pollen maturation), during pollen germination in the stigmatic tissue, during pollen tube penetration and elongation along the stylar transmitting tract and in the ovules when the pollen tube reaches the embryo sac.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

Pollen tube culture medium induces variations of cell wall associated proteins in *Arabidopsis thaliana*

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The pollen tube has for long been used as a model for apical growth and asymmetrical expansion. Easy pollen grain access and pollen tube culture makes it an excellent model for the study of this type of growth. Many pollen tube culture media are described in the literature which offers fairly good germination rates. A large amount of transcriptome data on pollen and pollen tube is now available which provides a good starting point to perform gene studies on pollen tube growth associated processes. Most of the described pollen tube media have the same fundamental components. However the comparison of pollen tube array data using different growth media shows some divergences in gene expression and level of expression patterns. After preliminary studies on *Arabidopsis* pollen tube growth in several highly referenced culture media, a significant variation in pollen tube phenotypes, as well as gene expression patterns, could be observed.

Among the genes with altered expression induced by the composition of the growth media are the arabinogalactan proteins (AGPs). AGPs are a large and complex family of cell wall associated glycoproteins, which possess a hydroxyproline rich protein backbone, highly glycosylated and GPI-anchored. AGPs are ubiquitous in tissues and organs of plants and show a specific expression pattern during plant development. Studies have shown the association of AGPs to multiple processes of cell differentiation, organ development and strong evidence suggest their involvement in plants reproduction. They also have been implicated in stress responses and programmed cell death (PCD). We observed a significant expression level alteration of pollen tube AGPs as one of the effects induced by the growth media. Other cell wall associated genes were also affected in different degrees, suggesting that the growth media and culture parameters can have an impact on the transcriptome and therefore the available transcriptome data on *Arabidopsis* pollen tubes should be viewed with caution.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

Immunocytochemical localization of allergenic proteins in *Plantago lanceolata* pollen grains

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Ultrastructural studies with pollen grains have shown that during the hydration, soluble proteins are released to the outside through the wall. Most of them are involved in the pollen-stigma recognition. Same process occurs when pollen grains penetrate into respiratory mucous of sensitive individuals. The majority allergen of *Plantago lanceolata* is Pla l 1, a polymorphic protein with two bands (17 kDa and 20 kDa).

Material and methods

Hydration and activation of *P. lanceolata* pollen grains were induced in vitro using agar germination medium. We have used two methods for immunolocalization:

- A.-Using Spur resin: Sections of pollen grains were incubated on drops of monoclonal antibody Pla l 1 (1:10). After washes, the sections were incubated with a goat anti-mouse IgG antibody coupled to 18-nm gold particles (1:50).
B.-Freeze-Substitution: Samples were frozen in liquid propane and stored in liquid nitrogen. Then, samples were embedded in Lowicryl. For immunogold labelling, samples were incubated with monoclonal antibody Pla l 1 (1:15). After washes, sections were incubated with a goat anti-mouse IgG antibody coupled to 12-nm gold particles (1:40).

Results

Equivalent immunolabelling patterns were obtained with the two protocols used. A lightly increase in labelling was observed in the material exuded from the pollen grain and in the wall. So, we can noticed an increase in the number of gold particles in all organelles, mainly in starches, vacuoles, lipid bodies, rough endoplasmic reticulum and in Golgi stacks and associated vesicles. So, we founded a light increase in the labelling in vegetative nucleus and sperm cells.

Conclusions

The existence of allergenic proteins in sperm cells of *P. lanceolata* confirms the existence of common allergenic proteins between them and the vegetative cell, so that both would be involved in pollen-stigma recognition, as in the production of allergen.

This study was supported by grant CGL2006-15103-C04-03 and grant LEO44A07.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

How methods, state, and source of the material can influence on discernibility of exine layers in *Quercus* pollen?

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Pollen morphology and ultrastructure of modern *Quercus robur* L. were studied using several methods. The fresh material was fixed on middle and late posttetrad stages using cacodilate buffer with and without tannines, and phosphate buffer. The herbarium material was fixed directly with osmium tetroxide (Os). The protocol for the material and then pollen sections includes either all standard chemicals (Os, uranyl acetate (Ur), lead citrate (Pb)) or only some of them. Pollen fixed in different buffers showed that in case of phosphate buffer membrane structures of the protoplast were weakly seen and the contrast between ect- and endexine was rather low. Pollen fixed in cacodilate buffer showed well distinguished membrane structures and clearly discernable exine layers. The cacodilate buffer with tannins resulted in even more clearly seen membrane structures while the border between ect- and endexine was masked. The border of ect- and endexine in pollen studied without Pb or Ur was visible, but the contrast was rather low. Mature pollen from herbarium without buffer showed the best contrast when all standard chemicals were used, and a somewhat weaker contrast when only some of the chemicals were applied.

Dispersed pollen from Holocene sediments of Sea of Japan were studied, using different combinations of standard chemicals. The best contrast was also observed for the pollen contrasted with the whole set of chemicals, while even in non-contrasted pollen the border between ect- and endexine was sometimes quite clear. This could be due to fossilization conditions. Thus, the complex study of fossil and modern fresh and herbarium material of *Quercus* pollen using several methodic approaches showed differences in the exine contrast. Obtained results are important for correct interpretation of fossil material and pollen on different ontogenetic stages; they also can help to determine the most appropriate way of studying material depending on its peculiarities.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

A comparison of the tapeta within the family *Papaveraceae* s.l.: the presence and absence of Ubisch bodies.

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The tapetum is the most metabolically active layer of the anthers and also undergoes one of the most important cytological changes. There are two major types of tapetum: secretory (primitive) and amoeboid. Both secrete nutrients into the anther locule, where the microspores reside and develop. Microspore development is sustained by the tapetum. Sporophytic support continues until the programmed degeneration of the tapetum during the advanced stages of microspore development, when its contents are released into the locule, usually in the form of Ubisch bodies.

Ubisch bodies originate as pro-orbicular bodies within the tapetum but their definitive function is still to be fully understood. Some authors consider them to be a product of tapetal metabolism whilst others have suggested such functions as the transport of sporopollenin. Ubisch bodies are not always present and some tapetal lipids and proteins are released in the form of tapetosomes and elaioplasts.

In this continuation of our studies into the *Papaveraceae* family we have found that its species have different types of tapeta, but we intend to focus primarily on describing the presence/formation or not of Ubisch bodies. Their presence or absence appears to be related to the ultrastructure and ontogeny of the pollen wall. We therefore conducted an electron-microscope study of the ontogenetic anthers of several species of this family. On the basis of our results we try to answer the question of why some plants seem to require Ubisch bodies for the development of their exine pollen wall whilst others do not.

This work was supported by the Spanish Ministry of Science and Innovation Project CGL2008-01554/BOS.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

Pollen morphology, ultrastructure and viability in *Vriesea ensiformis* (Vell.) Beer (BROMELIACEAE)

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Vriesea ensiformis (Vell.) Beer is an ornamental bromeliad, restricted to the Atlantic Forest biome, with high rates of extractivism, as many other bromeliad species, and potential for the generation of ornamental hybrids. *V. ensiformis* is an epiphyte, with green rosulate leaves with many scales. The inflorescence develops in a rigid scape with red scapal and floral bracts. The fertility of pollen grains is indispensable for classical breeding, with data on viability and development are essential for reproductive studies and breeding success. Furthermore, the morphological and ultrastructural characterization provides valuable data for the taxonomy of bromeliads. This study aimed to describe the floral morphology in *V. ensiformis*, emphasizing the pollen morphology, and evaluating pollen fertility by colorimetric viability and pollen grain germination in different culture media. Pollen characterization was done by observations on acetolyzed pollen grains and scanning and transmission electron microscopy. Pollen grains are of medium size, elliptical, with bilateral symmetry, monocolpate, oblate, exine is eutectate, with a reticulate ornamentation and heterobrocate granulate lumina, from rounded to polygonal. Pollen grain viability was assessed by acetic carmine staining (2%), with approximately 93% viability. For germination, pollen grains were cultured in vitro in four culture media (BM, BKM, SM and MBKM). Best results of germination percentages were obtained in BKM (82.44%) and MBKM (85.88%), more similar to the viability percentages. Germination in SM medium was approximately 45% and less than 1% in BM medium, not consistent with the colorimetric results for viability. These results contribute to the reproductive studies in *V. ensiformis*, being useful for breeding, taxonomical characterization and conservation studies of Bromeliaceae.

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POSTER COMMUNICATION

Pollen grain morphology and viability in five *Eucalyptus* species

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The genus *Eucalyptus* is the most cultivated forest species in the world. Studies of pollen viability have fundamental importance for optimizing breeding and genetic improvement, contributing to basic and applied studies. Pollen morphology offers important data for taxonomic analysis, assisting in the definition of genus and species. The aim of this work was to investigate pollen grain morphology, germination and viability of *E. benthamii*, *E. brassiana*, *E. dunnii* and *E. grandis* and *E. urophylla*. Pollen grains were collected at anthesis and maintained at -20°C for further use in pollination experiments. Morphology and ultrastructure were assessed by scanning and transmission electron microscopy. In vitro pollen germination was evaluated in two culture media (BA and BK) and pollen viability with three staining methods: fluorescein diacetate (FDA), Alexander stain (1969) and acetocarmine. In vitro germination percent and pollen tube growth, after 24 h in BK, averaged 75 % and 0,50 mm for *E. benthamii*, *E. dunnii* and *E. grandis*; 29,54 % and 0,44 mm for *E. urophylla*; 0,71 % 0,10 mm for *E. brassiana*. Pollen grains did not germinate in BA medium. Pollen viability in FDA and Alexander showed results similar to pollen germination in BK. All species revealed acetocarmine viability above 10 % pollen in vitro germination, with the exception of *E. urophylla* with viability above 40 % pollen in vitro germination. Pollen grains were characterized as small (ranging from *E. benthamii* $20,09 \pm 0,99 \mu\text{m}$ to *E. urophylla* $24,38 \pm 1,28 \mu\text{m}$) triangular, oblate, with radial symmetry, tri-parasyncolporate, with a distinctive apocolpial field. Exine surface is regulate, thickness ranging from *E. benthamii* $0,60 \pm 0,11 \mu\text{m}$ and *E. dunnii* $0,87 \pm 0,15 \mu\text{m}$. Pollenkitt deposition was observed in all species, especially in *E. grandis*. Pollen grain characterization contributes to reproductive and breeding studies and taxonomy. Acknowledgements: CNPq, Suzano Papel e Celulose, FuturaGene Brasil Tecnologia Ltda.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

Pollen morphology, germination and viability in *Bromeliaceae*

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Pollen morphology characterization is important in taxonomy, conservation and breeding and viability studies support genetic improvement through the production of hybrids. This study investigated pollen morphology and male fertility in 18 species of Bromeliaceae belonging to the genera *Aechmea*, *Alcantarea*, *Ananas*, and *Vriesea*. For morphological characterization, pollen grains were prepared by lactic acetolysis (ACLAC 40) and observed on the light microscope. Characterization of exine was done using scanning and transmission electron microscopy. Pollen germination was analyzed in vitro in four different media, and viability was done comparing three histochemical tests (Alexander, acetic carmine and Sudan IV).

Species belonging to *Aechmea* and *Ananas* genera presented medium size pollen grains, except for *Ae. fasciata* and *An. macrodontes*, with large pollen grains. *Al. nahoumii* and the *Vriesea* species analyzed showed large pollen grains. Pollen grains of *Aechmea* and *Ananas* presented bilateral symmetry, ovate shape, biporate, exine, varying from tectate (*Ae. bicolor*, *Ae. bromeliifolia*, *Ae. distichantha*) to semitectate (*Ae. fasciata*, *Ae. nudicaulis* and *Ananas*). *Al. nahoumii* and *Vriesea* species presented pollen grains with bilateral symmetry, elliptical shape, monocopate; exine is semitectate, with reticulate ornamentation, heterobrochate lumina, from round to polygonal. Germination percent and tube growth were higher in SM and BKM media than in BM and MBKM, for all species. Histochemical tests showed pollen viability above 70% for all species, except for *Ananas* sp. with viability below 40%. Pollen morphology characterization is important in the identification of genera and species, especially in this family with a large number of species with still some controversies in its taxonomy. High rates of pollen germination and viability favor fertilization and consequently seed production, essential for efficient hybrid production and species conservation.

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

Pollen morphology of some endemic *Silene* L. (*Caryophyllaceae*) taxa

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In this study pollen morphology of four endemic taxa of *Silene* genus were studied with light microscope and scanning electron microscopes. Pollen slides made according to Wodehouse technique. Palynological definitions for each taxa were made. Pollens are periporate. The shapes of the pollens are spheroidal. The ornamentation of pollens is microechinate and microperforate. Such as the diameter of pollens and pores, the number of pores, distance between two pores, spinule length and spinules base width showed differences between taxa. The results and evaluations of the study indicated that the pollen morphology of *Silene* taxa were different.

Keywords: *Silene*, *Caryophyllaceae*, Pollen, Endemic, Morphology

Session 3: Pollen morphology, development and germination
POSTER COMMUNICATION

Comparative palynomorphological study of the genus *Euonymus* (Celastraceae)

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Pollen of 62 species from the genus *Euonymus* (Celastraceae) were studied using light (SM), confocal laser scanning (KSLM) and scanning electron (SEM) microscopes. This is about a half of the species composition of the taxon. Pollen grains of 25 species has already been described but mainly with CM. Previously KSLM has not been used to identify structural features of the pollen. The genus as the whole family is quite homogeneous and is represented by 3-colporate pollen type, on the other hand some species and (or) group of species could be segregated considering the details of structure (apertural, exine, ornamentation). We have found 3-colporate pollen type in all investigated taxa, and 19 species (7 of 15 species from subgenus *Kalonymus* and 12 of 47 species from subgenus *Euonymus*), along with 3-colporate pollen grains have zonocolporate, 2 - 4 or 6-colporate deviated grains with different colpa arrangement. Their structure have been studied more detailed with a 3-D reconstruction (KSLM). Grains are isopolar, ellipsoidal or spheroidal, medium sized, from 17 to 41 mkm in diameter. Colpa are long, spired at the ends. Endoaperture (ora) range from poorly-defined, rounded or slightly elongated along the colpus up to well-defined, with sharp edges, elliptical, elongated along the equator. CM-observing show H-shaped aperture in 17 species. KLSM data demonstrate that they are constructed from orus bridge and exine thinnings on both sides parallel colpus. The exine is tectate, collumelate, with well-defined columella layer. Exine thickness is from 1.5 to 5.1 mkm. The ornamentation varies from reticulate to microperforate. The species groups divided according to their palynomorphological characteristics generally do not coincide with the the genus subdivision onto subgenus and sections. The taxonomic significance of various palynological characteristics are discussed. The investigations were carried out in Komarov Botanical Institute RAS.

Session 4: Paleoenvironment and paleoclimate
POSTER COMMUNICATION

Dynamics of cedar forests in the northern part of the Middle Atlas Mountains, Morocco

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Climate changes since the last glacial period had an impact on Mediterranean ecosystems such as the cedar forests and their dynamics. The purpose of this study is to reconstruct the impact of postglacial warming on these forests in the Middle Atlas.

We performed a new coring of 5m depth in a swamp located in the northern part of the distribution of cedar forests of the Middle Atlas. The record is dated with 14 ¹⁴C dates and the age/depth model indicates that it covers the last 16.000 millennia.

The preliminary palynological analyzes show that cedar forest dynamics is quite similar to that reconstructed from other available records in the Middle Atlas. Thus, as observed in the palynological record from lake Ifrah, cedars were present in the Middle Atlas during the last glacial period. During the early Holocene, the pollen records from lakes Tigalmamine and Sidi Ali show that cedar was virtually absent in the area until 6 ka where it began to settle and expand. Its expansion reached an optimum around 3ka. One of the main differences we observe in the new pollen record is the strong presence of *Pinus* during the early Holocene instead of the evergreen oak as observed in Tigalmamine, Sidi Ali and Ifrah.

As a first conclusion, these preliminary results confirm the presence in the Middle Atlas cedar forests during the glacial period and its noticeable re-expansion after ca. 6 ka. These results will contribute to better delineate the possible extension of cedar forests in the Middle Atlas.

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POSTER COMMUNICATION

OLEA-DP: a new application used to plot pollen.

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The continuous innovations in Software, provide us tools to facilitate analysis of statistical and graphic data.

We tried to develop a tool that takes advantage of all this great functionality offered by the new software packages and solve the big problem that has led to the inability to continue using Tilia-Software, due to recent changes in the operating system of the computers.

The tool presented, called Olea-DP by us is specially created to meet the graphics and statistics needs of the Palynology, but can be used for a wide variety of stratigraphic, geologic, and ecological data.

Developed under the philosophy of Tilia-Software, it has been generated in an more universal and powerful environment like Microsoft Excel, in order to take advantage of all the power and versatility of such software and make it serve for different areas.

The tool presents several sheets, each of them performs a specific function; In addition to a toolbar that provides access to all functions. All of this easy to use and a great potential.

Basic features of Tilia-Software, are included, such as the calculation of percentages, absolute frequencies, etc.. In addition to the realization of the pollen diagrams with a control precise. For example: graphs that can be represented as curves, histograms, or presences, which can be represented in different colors and patterns.

We also, have created other new features such as the direct count, while reading of the microscope, calculation of rarefaction or and represent all taxa with the same ecological requirements together.

The usage of Excel format for data, streamlines the management with any other graphic or statistical software.

In short, it is a modern, functional and powerful tool that facilitates the work to the palynologists.

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POSTER COMMUNICATION

Pollen record during the Eemian from the Fuentillejo maar-lake sequence (Ciudad Real, Spain).

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The Fuentillejo maar is located in the Central Spanish Volcanic Field of Campo de Calatrava (Ciudad Real). Fuentillejo maar-lake is a closed system covering over 142 m depth of lacustrine sediments; it is one of the best examples of long and continuous cores at terrestrial site from the Iberian Peninsula. Palynological, mineralogical and sedimentary facies analysis were performed to characterize the sedimentary record during the Last Interglacial. In core FUENT-1 this period (dated in 133 ka-120 ka) is detected between 45,90-56,90 m depth. Sedimentology point of view is characterized by develop of lacustrine facies, finely laminated black-brown dolomicrite mud and sapropel layers (Sedimentary Units 16,6-17-18).

The vegetation is characterised by high pollen taxa diversity (around 50 pollen taxa of terrestrial types, 5 pollen taxa of aquatic types, spores and 9 types of non palynological microfossils-NPM) together with a high content in the Mediterranean and mesic forest components (*Quercus* evergreen, Oleaceae, *Quercus* decidous and *Corylus*), typical of warm and humid conditions, and a few content on *Artemisia*, *Pinus* and *Juniperus* taxa (typical of cold or warm arid phases). The scarce forest development can be interpreted from the pollen record of mesophilus and thermophilous vegetation of the FUENT-1 sequence, in which only 40-50% of total pollen come from arboreal associations. These values for arboreal pollen content are low compared with other European pollen sequences.

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POSTER COMMUNICATION

Migrations and climatic changes in South East Asia and Pacific areas

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Migrations and climatic changes in South East Asia and Pacific areas
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Pollen analyses have emphasized, in the South East Asia and Pacific areas, correlations between population's movements and climatic changes. The oldest human migration to the Indonesian archipelago has been possible only due to the land bridges formations during important glaciations.

So, the Javanese *Homo erectus* became the first Islander, approximately 1.5 Million years ago.

Many other migrations followed on the occasion of glacial cycles during the Quaternary period and especially during the MIS 22, 800 ky ago.

The Sangiran Dome, in Central Java, is particularly rich in *Homo erectus* fossil specimens. Different groups have been discovered and identified during the Quaternary and these groups lived in different environments (from the rain forest to the savannah).

The last Glacial Maximum, 12 ky ago, corresponds, in a cave record (Song Terus), to the arrival of a new population, probably linked with coastal traditions (mollusk tools). In this record (15 meters depth) which relates 300 ky of story, *Homo erectus* is replaced by *Homo sapiens*. The arrival of *Homo sapiens* is estimated at c. 150/200 ky and could correspond to the MIS 8. An important migration, the Austronesian one, originating from Taiwan, start c. 5000 years BP (equivalent to the "petit âge glaciaire"). This migration spread to the Pacific area.

The invention of more and more sophisticated crafts, from the monoxylic pirogue to outriggers, allows the transportation of numerous people and the progressive settlement of the South East Asia and, further, of the Pacific islands, c. 3500 years BP.

The successive waves of migration, associated with the navigation progress, depend on many parameters, like wars, demographic pressure and climatic changing. Sailors brought with them their subsistence, essential and traditional. So, they modify and often enrich the vegetal diversity.

The last migration wave is linked with the climatic and global warming with the rising of sea level. The inhabitants of lower islands are obliged to move and find new refuges.

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POSTER COMMUNICATION

Carya, pollen indicator of quaternary refuge area in Mediterranean Europe

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Carya, a tree genus of the Juglandaceae family, has nowadays a limited repartition, mainly constrained within the southwestern North America and local environments of continental Southeastern Asia. It is characterized by strict ecological requirements and is a relatively weak and slow competitor. However, *Carya* emerge and spread in Europe since the Paleogene, and reached a wide extension towards the northern hemisphere during the Neogene. This extension reduced in response to increasing cooling and aridity along the Pliocene and Quaternary. Among the Early and Middle Pleistocene palaeobotanical records, *Carya* is considered as relict taxa, decreasing and disappearing under the pressure of the climate cyclicity.

Quaternary volcano-sedimentary basins of Central and Southern Italy constitute suitable archives for pollen sequences, and thus, local and regional palaeoenvironmental studies. Numerous pollen records describe a part of the scenario of the global vegetation changes occurring in the Italian Peninsula, and also highlight local palaeoecological singularities. Among them, the lacustrine and fluvio-palustrine sequence of the Boiano basin (Molise) recorded a Middle Pleistocene sedimentary filling, with a chronological framework based on several tephra layers provided a description of the environmental changes between OIS 13 and 2. Boiano's biotope, characterized by a continuous humidity, favored the persistence of hygrophilous trees. Furthermore, *Carya* is still present during OIS 9, its latest occurrence in Western Europe. Thus, the Boiano basin could have constituted an ecological refuge for humidity demanding trees facing the climate pressure during the Middle Pleistocene. In fact, its position, physiography and orientation certainly softened the impact of the climate peioration, especially in term of atmospheric and edaphic aridity during the glacial episodes.

This late *Carya* occurrence, supposed extinct at this time, lead to the reconsideration of its precise ecological requirements, in order to constitute a strong palaeoecological indicator within the Quaternary palaeoenvironmental reconstructions, and to attest of potential vegetation refuge areas in Western Eurasia.

Orain, R., Lebreton, V., Russo-Ermolli, E., Combourieu Nebout, N., Sémah, A.-M., 2013, *Carya* as marker for tree refuge in southern Italy (Boiano, Molise) at the Middle Pleistocene, *Palaeogeogr., Palaeocl.*, 369, 295-302

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POSTER COMMUNICATION

Holocene vegetation and climate changes in central Mediterranean inferred from a high-resolution marine pollen record (Adriatic Sea)

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To understand the effects of future climate change on the ecology of the central Mediterranean we studied the impacts of long-term, millennial to centennial-scale climatic variability on vegetation. Pollen data from the Adriatic Marine core MD 90-917 allowed us to reconstruct vegetation and regional climate changes over the south central Mediterranean during the Holocene. Clay mineral ratios from the same core reflect the relative contributions of riverine (illite and smectite) and eolian (kaolinite) contributions to the site.

Pollen record shows the vegetation response to the late-Glacial Preboreal oscillation, most likely driven by changes in precipitation. Pollen-inferred temperature declines during the early-mid Holocene, but increases during the mid-late Holocene, similar to southern-western Mediterranean climatic patterns during the Holocene. Several short climatic events occur in the pollen record, indicating the sensitivity of central Mediterranean vegetation to millennial-scale variability.

Reconstructed summer precipitation shows a regional maximum between 8000 and 7000 cal yrs BP similar to the general pattern across southern Europe. Two important shifts in vegetation occur at 7700 and between 7500 and 7000 years. They are linked to changes in seasonal precipitation and are correlated to increased river inputs respectively from the north (7700 event) and/or from the central Adriatic borderlands (7500-7000 event). These results reinforce the strengths of multi-proxy analysis and provide a deeper understanding of the role of precipitation and particularly the seasonality of precipitation in mediating vegetation changes in the central Mediterranean during the Holocene.

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POSTER COMMUNICATION

Palaeoenvironmental changes in Turonian-Coniacian Cerro de la Mesa sequence (Sierra de Guadarrama, Madrid, Spain) based on the palynological data analysis

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This study is a result of new samplings that has been carried out in grey and black clayey levels situated between sand layers that appear in the left margin of the river Guadalix. These sediments belong to the lower siliclastic set (Utrillas Fm) of Cerro de la Mesa succession.

The palynological information obtained has been compared with those samples obtained in the upper carbonated set of this succession that crops out above. These upper level samples have been published before (Alvarez Ramis, Kedves y Fernández Marrón 1994, 1998) in order to know the flora evolution through the sequence as a result of the paleoenvironmental changes in the sedimentary succession. The analysis of the palynological assemblages from successive levels has allowed to elaborate a diagram with the percentage of selected groups that reveals the variations in the pollen-spore composition related to environmental changes. In the pollen diagram a sudden decrease of the algae since the first lower level can be appreciated being constant in percentage along the sequence. In the lower level of the series, dinoflagellate cysts are noteworthy, whereas in the top of the section freshwater algae, as *Botryococcus* appear.

The spores are scarce along the whole section. The Gymnosperm pollen grains are abundant and represent more than 40 % of the palynological assemblages. In the lower levels (CM-1 and CM-2) *Classopollis* are predominant. Together with the significant presence of *Dinocysts* would suggest a coastal saline environment since the *Classopollis* pollen type is considered indicator of marginal marine deposits. In the organic matter levels interbedded between calcarenites the percentage of *Classopollis* pollen (about 7 %) decrease dramatically in favour of other Conifers, especially *Taxodiaceae/Cupresaceae*, and *Angiosperms*, many of them with *Normapolles* pollen type. The presence and proliferation of *Normapolles* seems to be linked to conditions of soil salinity and more or less disturbed environmental conditions. The association between *Normapolles*, *Classopollis* and spores of *Schizeaceae* and *Gleicheniaceae* seems to be related to climate and/or eustatic changes. The palynological assemblage seems to indicate a euryhaline coastal environment where the palynomorphs transported by wind and water from emerged zones are deposited.

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POSTER COMMUNICATION

Pollen analysis of three sequences in the Riolago peat bogs, Leon NW of Spain

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Three new palaeoecological sequences have been studied as a contribution to the knowledge on the Holocene vegetation dynamics in northwest Iberia. Pollen analysis of three peat-bogs have been carried out in the western area of the Cantabrian range at Riolago (León province, Spain). The study focuses in an area named Babia, a Natura 2000 site (as SPA and SCI) designed as Biosphere Reserve and proposed for Natural Park. The results reveal the stage of maximum forest development of the Middle Holocene, where *Pinus* and *Betula* formed the main forest communities and the fluctuations of pollen percentage between them could indicate changes in the environmental conditions. These boreal taxa were accompanied by other deciduous trees like *Corylus*, *Alnus*, *Fagus* and *Fraxinus*, which were more abundant during the period of forest regression of the Late Holocene. Identification of *Pinus uncinata* record in two sequences is a remarkable fact, its pollen percentages being higher than the registered ones in Laguillín, the first sequence in the Leon province where this taxa have been found. Presence of *Fagus* before 4.590 ± 40 B.P at Riolago means a new refuge area than those previously cited by other authors. The record of *Dacrydium* in the sequences of Riolago, Laguillín and Los Rajaos during the Middle Holocene indicate the existence of a glacial refuge area for this Tertiary coniferous, from where a little population should restart a dispersion taking advantage of the thermal increase but with no success at last. At the other side, in this same area, *Juniperus thurifera* open forests have remained nowadays as a relict community from the Tertiary times in some nearby places of the Cantabrian Range, probably because of their better adaptation to the Holocene changes.

Key words: peat-bogs, Holocene, refuge areas, *Fagus*, relict taxa, *Dacrydium*, Riolago

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POSTER COMMUNICATION

Pollen analyses of surface samples obtained from different sedimentary environments in the Vilaboa salt-marsh (Ría de Vigo)

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Salt marshes are high valuable climatic-sensitive coastal ecosystems where vegetation distribution is mainly determined by physical and chemical gradients. Notable differences in the pollen assemblages of the sediments may also exist between marsh habitats, which could be related to their unequal inputs of palynomorphs and local preservation conditions. Identifying subtidal, intertidal and upper marsh sediments by pollen analyses may be especially relevant for palaeoenvironmental reconstructions in coastal regions.

In this research, pollen and non-pollen palynomorphs (namely fungal and algal spores, dinocysts and others) percentages and concentrations distribution were analyzed in 27 surface sediment samples. They were obtained from different vegetation habitats distributed along a topographic gradient, from upper marsh to subtidal, in the Vilaboa salt-marsh (Ría de Vigo, NW Spain). The aim of this study is to determine whether marsh zones can be characterized by their pollen spectra, and also to evaluate how the abiotic gradient and the local and extra-local inputs contribute to the pollen fingerprint.

Our results suggest a relevant contribution of the pollen produced by the local vegetation to the final pollen assemblage. Besides, we found that both regional inputs and local preservation conditions also determine the pollen composition and distribution in the salt-marsh. Thus, several marsh zones can be distinguished by their pollen fingerprint. Particularly, the best differentiated pollen patterns are those from the sediments in the upper marsh dominated by *Phragmites australis*, and those from the subtidal sediments.

This study provides a number of data which may be useful as references to improve the interpretation of stratigraphic samples obtained at the bottom of the Ría de Vigo.

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Session 4: Paleoenvironment and paleoclimate
POSTER COMMUNICATION

Lateglacial early Holocene transition in SW Europe: a high resolution multiproxy record from Laguna de la Roya (NW Iberia)

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Laguna de La Roya (Sanabria, NW Iberia), is a small lake formed in an over-deepened glacial basin, at 1608 m asl, in a very climatic-sensitive position at the eastern North Atlantic margin. The prevailing oceanic climate, the dominant siliceous bedrock, the mountainous orography, and the relative well-preserved natural state of this area have being key factors contributing to preserve the postglacial sedimentation in this site.

High-resolution pollen analyses were performed on a 223 cm section of organic sediments accumulated in La Roya basin, in order to study the main climatic shifts during the Lateglacial-early Holocene transition, and to assess their influence on local aquatic and terrestrial ecosystems. Radiocarbon dates and pollen correlations were used to develop an age-depth model, being estimated the age of the section of sediments analysed as ranging from ca. 15,600 to 8,800 cal. yr BP. We studied changes in pollen and non-pollen palynomorphs (namely fungal, algal spores, chironomids and others) which allow us to establish a number of inferences related with variations in the local conditions and the productivity of the lake. Furthermore, our results enable the reconstruction of the local and regional vegetation dynamics, and also the main oscillations for the vegetation belts may be deduced. Therefore, this study contributes to a better understanding of the end of the last glaciation and the beginning of the postglacial period in SW Europe.

Acknowledgements

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Session 4: Paleoenvironment and paleoclimate
POSTER COMMUNICATION

Paleoclimatic reconstruction based on archaeopalynological data: a novel approach

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Palaeoenvironmental information is often extracted from pollen records using quantitative methods. We proposed the application of quantitative techniques (WAPLS and MAT) to archaeopalynological data in order to provide local insights on how Holocene climatic events were the main forcing for rapid cultural changes. The pollen record came from the archaeological site of La Motilla del Azuer. This Bronze Age site of La Mancha (2200-1350 cal BC) is one of the most remarkable prehistoric settlements of the Iberian Peninsula known as motillas, artificial mounds with concentric lines of fortification located in plains or valleys. This fortification contains an extraordinary hydraulic structure to facilitate obtaining water from the phreatic level. So these fortified settlements played an important function in the control and management of a critical resource, the water, particularly limited in La Mancha. From a climate point of view, our results could be framed within two periods. The first one (2200-1600 cal BC) is characterized by a deforested landscape enriched with xerophilous and thermophilous elements, under a dry and warm climate related to the 4,0 ka cal BP event. Livestock grazing and agriculture are detected at this moment. This aridification process forced the Chalcolithic societies to change their subsistence strategies, creating motillas. The Bronze Age begins in La Mancha ca. 2300 cal BC. In this climatic period, a sharp decline in rainfall and temperature was recorded (2000-1800 cal BC), probably related to the Neoglacial episode. So, the results show a decline in the riparian forests as well as the hydro-hygrophytes, in addition to the slowdown of productive activities. Roughly 1600-1400/1350 cal BC another climate shift was observed, characterized by a trend to wet and cooling conditions in contrast to the above period. Probably this wet phase caused the abandonment of motillas, since the water resource control was not necessary during a rainy period.

Session 5: Pollen Biotechnology and Genetics
POSTER COMMUNICATION

Anatomical anther development and anther culture in *Brachiaria* sp.

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Brachiaria, an important forage crop in tropical countries, presents many tetraploid and apomictic commercial varieties, hindering breeding programs. Induction of haploids is of great interest for the generation of new genotypes with potential use in intraspecific crosses. A key factor for the success of this technique is identifying adequate microspore developmental stages for efficient embryogenesis induction. Thus, knowledge of the microsporogenesis and microgametogenesis steps and sporophytic tissues composing the anther is critical for identifying the ontogenesis of callus and somatic embryos. Also, morphological markers, can help the choice of the best explant. In this work, anther and pollen grain developmental stages were histologically analyzed, and associated with morphological markers. Anther development was divided into 13 cytological stages, from the establishment of bilateral symmetry to anther dehiscence. The expression of SERK genes was analyzed by in situ hybridization during anther development in vivo and in vitro. Transcripts were detected in the pollen grain mother cells and tapetum, and after microsporogenesis only in tapetum cells, with no hybridization signal in microspores. *B. brizantha* and *B. decumbens* anthers were inoculated in different culture conditions. Callus formation was observed in all treatments, however only in a few anthers, with low proliferation ability. Histological analysis of cultured anthers revealed the presence of microspores with a symmetrical division, an important response observed in other species, and SERK transcripts were detected in these microspores showing symmetrical division and also in uninucleate microspores in cultured anthers, suggesting a possible modulation in the expression of these genes in the inducing conditions and a possible initiation of embryogenesis. However no continuity in development of these microspores was observed. Acknowledgements: CNPq.

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POSTER COMMUNICATION

ROS and differential stress responses of antioxidative systems during *in vitro* *Quercus suber* microspore embryogenesis.

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Embryogenesis was induced from microspores of cork oak (*Quercus suber* L.) through anther (gametic) culture, and mature cotyledonary embryos were formed. Among the microspore-derived embryos produced in culture, some spontaneous diploid embryos were found. Chromosome duplication may occur spontaneously in anther cultures producing doubled-haploid (diploid, in cork oak $2n=24$) microspore-derived embryos which are fully homozygous. To analyze the response of developing embryo cells in culture, Reactive Oxygen Species (ROS) levels and activities of antioxidant enzymes including catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) were evaluated in three different stages of microspore embryogenesis. According to the obtained results, some proteins and antioxidant enzymes have been regarded as markers for phase change during the embryogenesis process.

Session 5: Pollen Biotechnology and Genetics
POSTER COMMUNICATION

Histological evidence for reprogramming of pollen grains in the cultured cassava anthers

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Cassava is a basic dietary component for millions of people in the world. It is of utmost importance to improve this crop both in terms of its nutritional value and its productivity. The production of homozygous lines in cassava will significantly impact and accelerate the production of new hybrids. Anthers and isolated pollen grains are the most common sources to produce the doubled haploid plants. Use of anthers is the simplest technique whereas the isolation of pollen grains employs a number of steps that could potentially affect the efficiency of embryogenesis. However, anthers contain both haploid microspores/ pollen grains and diploid somatic cells that make confusion about the origin of the anther-derived structure. Thus, a histological analysis was conducted to examine the potential changes occur within the pollen grains of the cultured anthers. Anthers were sampled in two, eight and sixteen weeks after culture initiation and were subjected to a histological analysis. The pollen grains followed three pathways: many were immediately arrested or died; a few continued towards becoming mature pollen by accumulating starch; and a few others were induced to divide. The characteristics of pollen undergoing the third pathway showed the signs of re-programming of the developmental path with a significant enlargement, re position of the nucleus to the cell center and the breaking of a large vacuole into smaller. After symmetrical nuclear division occurred, the cells were initially formed in the non-vacuolated region and then grew to fill the entire space of the enlarged pollen grain forming the multicellular structures. The results clearly showed that the induced pollen grains have shifted their developmental pathway from gametogenesis to embryogenesis under the given conditions for cultured anthers.

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POSTER COMMUNICATION

DNA methylation and *MET1a-like* expression are regulated during pollen development and pollen embryogenesis

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DNA methylation, accomplished by DNA methyltransferases, constitutes a prominent epigenetic modification of the chromatin fibre which regulates gene expression, and changes during plant cell differentiation and proliferation. After a stress treatment, in vitro cultured microspores are reprogrammed and change their gametophytic developmental pathway towards embryogenesis. In this work, the dynamics of global DNA methylation levels and distribution patterns and the expression of BnMET1a-like DNA methyltransferase have been analyzed during pollen development and pollen embryogenesis in *Brassica napus* L. Quantification of DNA methylation by high performance capillary electrophoresis and in situ localization by immunofluorescence and confocal analysis, showed an epigenetic reprogramming after microspore embryogenesis induction. This regulation involved a decrease of global DNA methylation with the change of developmental program. In contrast, global DNA methylation increased with pollen and embryo differentiation. Levels and in situ distribution of BnMET1a-like transcripts were analyzed by RT-PCR and fluorescence in situ hybridization (FISH), results highly correlated with variations in DNA methylation. Preliminary results of treatments with azacytidine, to block DNA methylation, showed the increase of the embryogenesis induction rate in treated microspore cultures. Results suggested the involvement of DNA methylation in regulating microspore embryogenesis induction and the participation of MET1a-like proteins in the epigenetic control of the two pollen developmental programs, gametophytic and embryogenic.

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Session 5: Pollen Biotechnology and Genetics
POSTER COMMUNICATION

Early common markers of microspore and somatic embryogenesis in *Quercus suber*

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The search for molecular and cellular markers during early stages of pollen embryogenesis and further embryo development constitutes an important goal in the identification of cells committed to the embryogenesis developmental programme as opposed to those cells which are non-responsive to the embryogenic pathway, as well as in the elucidation of the cellular mechanisms underlying *in vitro* embryo progression. Tree breeding strategies has focused on ways to reduce cycle time and improve the efficiency of selection; here, propagation of selected trees by somatic embryogenesis and genetic engineering approaches applied to haploids and double-haploid plants produced in short-times by *in vitro* pollen embryogenesis have a high potential.

In this work, we approach a comparative analysis of the two *in vitro* pathways, pollen and somatic embryogenesis, in *Quercus suber*, cork oak, in order to characterize common markers of both pathways. Somatic embryos were induced from immature zygote embryo cultures, and pollen embryos were induced on anther cultures. Samples were obtained at different culture stages, fixed and processed for further cytochemical and immunocytochemical techniques at light and confocal microscopy. Rearrangements of the structural organization of cells and tissues, changes in cell wall polymers, nuclear organization and epigenetic marks were analyzed in developing embryos of both *in vitro* systems.

Results showed that markers that distinguished embryogenic cells show similar localization patterns in both somatic and pollen embryogenesis. The level and nuclear pattern of DNA methylation, the proportion of esterified and non-esterified pectins in cell walls, and the endogenous auxin distribution were different in embryo cells in comparison with explant and callus cells. The results give new insights into the identification of the cellular mechanisms involved in the *in vitro* embryogenesis induction and progression in woody species, in which there is scarce information.

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POSTER COMMUNICATION

Effect of various factors on microspore embryogenesis induction in two Tunisian olive cultivars

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Haploid and homozygous doubled haploid plants are of interest for genetic and developmental studies as well as for plant breeding. In fact, they can be employed in selection, mutation research, genetic analysis and genetic transformation (Germanà., 2006; 2011). Whereas the production of doubled haploids reached very good results in many crops (Maluszynski et al. 2003), fruit species, where the process is of special interest (Bueno et al, 2004, 2005), are recalcitrant regarding haploid production. In *Olea europaea* L., due to the prevailing auto sterility, the long juvenile phase and reproductive cycle that characterize this species, the use of haploids and doubled haploids is of unquestionable importance (Rugini. and Gutiérrez Pesca, 2006). The most effective way of producing haploids is pollen embryogenesis, through anther or isolated microspore cultures. Genotype, physiological state and conditions of growth of donor plants, stage of pollen development, pretreatment of flower buds or anthers and in vitro culture medium and conditions, together with their interactions, are all factors that greatly affect the response of explants in pollen embryogenesis.

In this study, the influence of the medium composition, genotype and pretreatments on microspore embryogenesis induction in two Tunisian genotypes of olive cultivars "Chemlali" and "Zalmati" was evaluated. Microspore viability was determined by FDA staining. Symmetrical divisions of microspores were observed using DAPI staining. "Swollen anthers" and callus derived from anther culture were scored. Our results confirmed remarkable effect of examined factors on microspores embryogenesis induction via anther and microspore culture. The results obtained can give a contribution to set up an efficient protocol for gametic embryogenesis and haploid production in olive.

Keywords: microspores embryogenesis, microspore culture, anther culture, Olive.

Session 6: Airborne pollen and spores: environmental indicators and allergens
POSTER COMMUNICATION

“Región of Murcia” Aerobiological Network REaReMur. Preliminary data.

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Aerobiological studies in our region began in Cartagena during the 80's of last century. Was in the year 1993 when a Hirst sampler was installed in Cartagena. We had to wait until the year 2009 to have a second sampler installed in the city of Murcia, and soon followed a third one in the city of Lorca in the year 2010. According to this chronology, we have data from the 3 samplers for the years 2011 and 2012. In this presentation we will depict those data sets, describing briefly the remarkable aspects of each sampling station, going to the similarities among the three bioaerosols, to finally describe the distinctive features of each city.

The first distinctive feature is the significance differences among the total pollen counts found in Cartagena from those of Lorca and Murcia. While in Cartagena for years 2011 and 2012 the total pollen counts (Pollen Index, PI) are 18142 and 16964 grains/m³ respectively, in Murcia were 38324 and 28092 grains/m³, and for Lorca 38025 and 28047 grains/m³. The figures show a decrease in the PI for year 2012 in the three sampling points. Again Cartagena showed the lower readings but proportionally the reduction was less marked.

Seventy pollen types have been identified in the Region of Murcia. Thirteen out of seventy are considered mayor pollen types, because at least in one of the stations the PI are higher than 500 grains/m³, Cupressaceae, *Olea*, *Casuarina*, *Artemisia*, *Quercus*, *Pinus*, Chenopodiaceae-Amaranthaceae, *Platanus*, Urticaceae, Poaceae, Arecaceae, *Morus* and *Zygophyllum*. We will considered minor types those whose PI are between 50 and 500 grains/m³ in any of the stations, *Plantago*, *Schinus*, Myrtaceae, *Betula*, *Ulmus*, Asteraceae *tubiflorae*, Brassicaceae, *Populus*, *Rumex*, *Acer*, Theligonaceae, *Tamarix*, *Typha* y Apiaceae. And scarce those that do not reach 50 grains/m³ in any of the stations, *Mercurialis*, Cyperaceae, *Alnus*, *Fraxinus*, *Sideritis*, *Salix*, *Ligustrum*, Ericaceae, *Ricinus*, Ligulifloras, *Thymelaea*, *Efedra*, Cucurbitaceae, *Echium*, *Juglans*, *Cannabis*, etc.

Session 6: Airborne pollen and spores: environmental indicators and allergens
POSTER COMMUNICATION

Comparison between two adhesives in Hirst spore traps in a controlled environment

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Background. Different types of adhesives are used in Hirst spore traps. The comparison among them should take into account the differences between spore traps and environmental influences. The aim of this work was to compare the adhesives Petrolatum white and Silicone diluted with carbon tetrachloride in a controlled environment avoiding outdoor differences between samplers and heterogeneous distribution of pollen on slides..

Method. Two Burkard spore traps three years old were placed in a closed room. Intake holes were separated 45 cm each one. Plants at starting pollination of *Rumex bucephalophorus* and branches of *Acer negundo*, *Platanus hispanica* and *Quercus rotundifolia* were used as pollen sources between spore traps and a fan than dispersed the pollen. They were homogeneously distributed in water pots in a table 2 m long. Drums were coated with Petrolatum white and Silicone diluted with carbon tetrachloride. To avoid differences between spore traps drums were interchanged between spore traps each 24 h. 6 gravimetric sampling points were located around the sources and close to spore traps intake holes. To avoid heterogeneous distribution of pollen on slides only pollen in center of slides were counted. Sampling was taken in March 2013. An automatic weather station inside the room was used..

Results. Temperature ranged 10-33 °C, relative humidity ranged 40-84 %, and sun radiation ranged 0-15.8 w.m-2. Plants showed a free shedding of pollen. Gravimetric results showed a homogenous total distribution of pollen. No statistical significant differences were found between both halves of the sources distribution and spore trap location. Paired statistical test showed no significant differences between results for both samplers in *Quercus* and *Platanus* but differences were found in *Rumex* and *Acer*.

Conclusion. Both Petrolatum white and Silicone diluted did not show statistically significant differences as adhesive in Hirst spore traps in a close environment for *Quercus* and *Platanus*, for *Rumex* more pollen were recorded with Silicone and for *Acer* more pollen with Petrolatum.

Session 6: Airborne pollen and spores: environmental indicators and allergens
POSTER COMMUNICATION

***Quercus* airborne pollen tendencies in the south of Iberian Peninsula, its correlation with meteorological trends and possible effect of the climatic change in Mediterranean forests**

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This study analyzes the aerobiological tendencies of the more representative and important native species of the Mediterranean forest. Using pollen registered in the atmosphere of Malaga during 1992-2012 and their relationship with meteorological parameters, have been possible to observe very interesting results concerning annual trends, that could explain possible effects of climatic change. We have observed significant trends to increase the spring pollen index, the number of days with pollen counts, to advance the beginning of the pollen season and to extend the duration of the pollination period. At the same time, significant annual tendencies for some meteorological parameters have been showed. Not only there is a tendency to increase the temperature, but also to diminish the relative humidity and to increase the sunshine hours. All this suggests that a greater environmental dryness is taking place. It could affect to the formation and development of the floral buds that occur in winter, interesting aspect to investigate. At least in Malaga, and only for this taxon, we have observed that every four years takes place an important peak of pollen index, which in addition coincides with the more dry years. This led us to investigate and to verify if it happened the same in other localities in the south of the Iberian Peninsula. During spring, the humidity is a negative factor for the bouyancy of the pollen grains in the air, therefore its tendency to fall would make increase the pollen index. Using Spearman correlation analysis, we have obtained significant associations between all the studied variables. On the other hand, there is a tendency to advance the chilling period, as well as to increase the accumulated maximum temperature from December to late February, both factors are probably being the cause of the advance of the beginning of the pollen season, since there was a significant correlation between both variables.

Finally, there was a significant trend for the wind speed to increase.

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Session 6: Airborne pollen and spores: environmental indicators and allergens
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Pollen Calendar of the atmosphere of Tetouan (NE Morocco): 2008-2011

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In this work, a 4-year pollen calendar of the atmosphere of Tetouan is presented, this being the first pollen calendar made for the Mediterranean south. The study was carried out with the aid of a Hirst type volumetric pollen-trap situated on the plain roof of the Science Faculty, University of Tetouan. The methodology followed for sampling, mounting and counting the pollen grains was the proposed by the Spanish Aerobiology Network (Galán et al., 2007). During the period 2008-2011 a total of 52 pollen types were detected in the atmosphere. The most abundant (>1% of the annual total) being: Cupressaceae (44.63%), *Urtica membranacea* (12.09%), Poaceae (7.82%), *Olea europaea* (6.84%), *Cannabis* (4.33%), *Quercus* (3.75%), *Parietaria* (3.04%), *Plantago* (2.30%), *Mercurialis* (2.23%), Chenopodiaceae-Amaranthaceae (1.52%), *Pinus* (1.52%), *Rumex* (1.42%) and *Morus* (1.24).

During the studied period, 89.5% of the annual total pollen occurred from March to June (both inclusive), March (27.55%) and February (25.99) being the months with highest percentages. On the contrary, November (1.00%), September (0.91%), December (0.75%) and August (0.72%) were the months with the lowest levels of atmospheric pollen.

In general, the pollen calendar of Tetouan shows a high diversity of pollen types, as well as the typical features of other Mediterranean calendars, such as pollen presence throughout the year, long pollen seasons with long tails, and typically Mediterranean elements such as *Quercus* and *Olea europaea*. Nevertheless, the pollen spectrum also presents their own features, compared with other localities of southern Spain, such as a high incidence of *Cannabis* pollen, longer seasonal pollination periods and earlier start of the main pollen seasons.

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One year aeropalynological analysis of atmospheric pollens in Ankara, Turkey

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Pollens are among the allergens groups of atmosphere. They are observed in the atmosphere most of the year due to the difference in the pollination periods of plants. Determination of concentrations of airborne pollens has importance for protection and treatment of sensitive individuals. Because pollens are the main agents those cause asthma and rhinoconjunctivitis all over the world. Burkard Volumetric Pollen Trap was used to determine the atmosphere pollen concentration of Ankara, the capital city of Turkey.

The trap was placed on the roof of a building at Ankara University. The study was carried out between June 2011- May 2012. A total of 72.835 pollen grains belonging to 50 taxa were observed during the study. Arboreal taxa are; Cupressaceae/Taxaceae, Pinaceae, Moraceae, Populus, Quercus, Platanus, Fraxinus, Acer, Betula, Alnus, Ailanthus, Ulmus, Salix, Fagus, Fabaceae, Corylus, Oleaceae, Vitis, Rosaceae, Juglans, Aesculus, Tilia, Sambucus, Myrtaceae, Rhamnaceae, Ericaceae, Castanea, Tsuga and Elaeagnus in decreasing order. Nonarboreal taxa are; Poaceae, Chenopodiaceae, Asteraceae, Brassicaceae, Rumex, Plantago, Apiaceae, Artemisia, Urticaceae, Typha, Lamiaceae, Galium, Boraginaceae, Caryophyllaceae, Carex, Ranunculaceae, Humulus, Sanguisorba, Onagraceae, Liliaceae and Fumaria in decreasing order.

As a result of the study, it is identified that 92,35% of the total concentration were the pollens of arboreal taxa, where 2,75% were pollens of nonarboreal taxa and 4,90% for the Poaceae. The highest pollen concentration was observed in April, while the lowest concentration in January. In April, May and June tree pollens were dominant. Daily mean temperature total precipitation and daily mean humidity was observed most relating meteorological factors with pollen concentrations

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POSTER COMMUNICATION

Aerobiological survey in the Biosphere Reserve “Sierras de Francia y Béjar” (MW Spain)

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This communication shows the results of monitoring the pollen and pteridophyte spores in the atmosphere of the Biosphere Reserve UNESCO-MAB (2006) «Sierras de Francia y Béjar» (MW Spain) by means of Hirst Volumetric Spore Trap during years 2011-2012 located in a ecological vineyard (Viñas del Cámbrico, S. L.; Villanueva del Conde Municipal District). The reserve forms part of the Las Batuecas-Sierra de Francia Nature Reserve and the Quilamas and Candelario nature areas, and they are included in Natura2000 Network. The landscape has steep mountains, with altitudes ranging between 360 and 2,425 metres, with green leafy forests formed mainly by oak trees (*Quercus pyrenaica* Willd.). Chestnut (*Castanea sativa* Mill.) and strawberry tree (*Arbutus unedo* L.) forests are other remarkable forests. During this period, 60 types of pollen and 2 types of pteridophyte spores (*Pteridium*, *Blechnum*) were identified in the atmosphere of the analyzed area. Airborne pollen levels of *Quercus*, *Castanea*, *Olea* and *Ericaceae*, together with low concentrations of pollen grains from ornamental species widely used in urban areas such as *Platanus*, reveal the environmental importance of this world-renowned wildlife Reserve, due to the differences detected between pollen spectrum and pollen concentration of the natural study area and the surrounding cities. In addition, intra-diurnal pattern from trees surrounding the trap presented a clear peak at midday/afternoon, as also probably occurred in the case of Early Sand-grass (*Mibora minima* (L.) Desv.).

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Relationship between aeroallergen Pla a 1, Platanus pollen and air pollutant

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Platanus acerifolia (Aiton) Willd. is an abundant ornamental plant in the world. In the town of Valladolid the different methods of pruning affect the flowering of plane tree. There are studies above the relationship or independence between the presence of Platanus pollen and the aeroallergen Pla a 1 in the atmosphere and the possible role in the allergic response of patients.

This study aims to investigate the relationship between the atmospheric concentration of Pla a 1 aeroallergen, the Platanus pollen and the different air pollutant. **Material and Methods.** The period of this study includes the Platanus main pollen season, from March to June in the years 2009, 2010 and 2011.

The pollen sampling was carried out using a Hirst-type volumetric trap (Lanzoni©) for pollen grains and a Burkard Cyclone sampler (Burkard©) for Pla a 1 allergen. Allergen were analysed following ELISA, with certain modifications. Air pollutant (PM-10, Pm-2.5, nitrogen oxide NO and NO₂, carbon monoxide, sulfur dioxide, ozone and benzene) levels have been provided by the Air Pollution Control Network of the City of Valladolid.

We have used Spearman's test through the SPSS 14.0 statistical package in order to establish potential correlations between concentrations of Pla a 1, different air pollutant and daily pollen counts.

Results. The aerobiological dynamics of Platanus pollen grains and Pla a 1 are quite alike for 2009, 2010 and 2011, particularly during the Platanus pollination period. On the other hand, the total amount of allergen detected was higher in 2011 (1479.5pg/m³) than 2009(507.6 pg/m³) and 2010 (570.5 pg/m³).

However the Seasonal Pollen Index of Platanus detected was higher in 2009 (7,565) and 2011 (6,679) than in 2010 (4,312). We got, with a level of significance of 0.01, positive correlations (0.568) between concentration of Platanus pollen and levels of aeroallergens in 2010.

Conclusions. The higher levels of Pla a 1 are produced with a high concentration of Platanus pollen. Despite of there are some specific correlations between the allergen or pollen and some atmospheric pollutants, we can not conclude that the studied air pollutant concentrations can influence allergen or pollen concentrations.

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Incidence of fungi spores in the atmosphere of Tetouan (NW Morocco) and effect of the meteorological parameters

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Fungal spores are known to cause rhinitis and asthma and are playing increasingly important role in respiratory allergy.

Objective: the aim of this work is to analyse the spore airborne fungi in the atmosphere of Tetouan in Morocco during 2009, 2010 and 2012, to determine their diversity and abundance, their interannual and seasonal variations and the effect of meteorological parameters on the incidence of the fungal spores.

Material and methods: Aerobiological sampling was performed by a Burkard volumetric spore traps located on the flat roof of the department of the Biology of the Faculty of Sciences in Tetouan. Preparations, mounting and counting of microscopical slides were made according to the Spanish aerobiological Network (REA). Daily mean concentrations were expressed as the number of spores per cubic metre of air. The daily incidence of aeromycoflora was explored by Spearman correlation test to analyse the influence of meteorological parameters.

Results: More than 80 spores categories were identified and annual spore load varied between 474867 and 615193 spores. Principal taxa represented approximately 99,5 % of the annual total, among them the most abundant were namely Cladosporium, Basidiospores, Ustilago, Alternaria, Leptosphaeria, Aspergillaceae, Uredospores and Pleospora. Most spores recorded the highest incidence from spring to autumn, however certain fungal spores showed the minimal monthly spore counts in summer. Alternaria, Cladosporium, Stemphylium, and Ustilago spores correlated positively and with a highly significance level with temperature. At the exception of Cladosporium, humidity and rainfall negatively influenced most of these spores. Ascospores and basidiospores showed negative correlations to temperature, while they were associated positively and with significant to highly significant level to humidity and rainfall.

Conclusion: Exposition of pre-sensitized individuals to high fungal spores concentrations may incite respiratory allergic diseases.

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Olive tree genetic background is a major cause of profilin (Ole e 2 allergen) polymorphism reflected in functional and allergenic variability

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Profilins, one of the major allergens (Ole e 2) of olive (*Olea europaea* L.) pollen, are broadly distributed actin-monomer-binding proteins (ABPs). They display a major regulatory role in actin cytoskeleton dynamics, mainly due to their interaction with multiple partners, i.e. actin, poly-L-proline (PLP) rich proteins and phosphoinositides. Plant profilins are a multigene family with multiple isoforms/isovariants differentially regulated. They have a central part in translating signals into cellular responses.

It is still an open question whether these profilin isoforms, generated by multiple gene sequence polymorphism, are functionally different. The role of such polymorphism in pollen allergy is also contentious, more precisely in the generation of differential epitopes, the sensitization to different profilin isoforms and the cross-reactivity among plant cultivars, and even among species.

We have investigated the role of such polymorphism in both pollen functionality and allergy. At this last regards, we have analysed the generation of differential epitopes, the sensitization to specific profilin isoforms and the cross-reactivity among cultivars, and even among species. Biochemical and cellular techniques have been used aimed to analyzing the immune-reactivity of profilins to a panel of antibodies, and their differential subcellular location.

Also, we used bioinformatic tools to characterize and label differences in conformational, B-, and T-epitopes among the profilin sequences previously cloned.

Our results suggest that the profilin family contains numerous isoforms functionally distinctive, which are differentially expressed in a spatial-temporal manner. They are differentially regulated during vegetative development, pollen maturation and pollen tube growth. Definite immune-reactivity is likely the result of both common and specific epitopes generated by the sequence polymorphism. This might explain differential cultivar specific sensitizations among the patients allergic to olive pollen, and cross-reactions between pollens from different species.

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Air pollen on the Island of Menorca (Spain) during the period 2006-2010

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The Island of Menorca, in the Western Mediterranean (Spain) is the second most important island of the Balearic archipelago and has been Unesco reserve since 1993. The aerobiology study performed an analysis with a Lanzoni VPPS spore trap from 2006 to 2010, to report on the air pollen found in the locality of Alaior.

30 pollen types were found and among the most interesting species are: *Olea europea* L. (33,3%), *Pinus* spp. (14,8%), Urticaceae (9,6%), Cupressaceae (8,2%), Poaceae (7,5%), *Quercus ilex* L. (7,3%), *Urtica membranacea* Poiret (5,9%), Chenopodiaceae/Amaranthaceae (3,5%) and *Plantago* spp. (2,3%), which represent 92,4 % of the total. The pollen calendar shows a long pollen season, but the low presence of garden species, along with a low presence of air pollen is of great interest, from the point of view of studies in allergic disease, as well as from a perspective that looks at good biological quality in air.

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POSTER COMMUNICATION

Proteomics analysis of Holm oak pollen

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Proteomics is a very valuable technique for the study of plant biological processes, from development and growth to responses to environmental factors. It can also be used for translational purposes such as food traceability, allergen detection, and proteotyping.

By using a proteomics strategy, the protein profile of Holm oak (*Quercus ilex* subsp. Ballota[Desf.] Samp.) is being characterized. The protein profile differed among provenances, therefore, the technique can be utilized in population characterization. Among the identified proteins, three of them corresponded to Que a 1 allergen, and a fourth protein matched to Cass 1 (*Castanea sativae*) allergen.

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Relationship between the dynamic of atmospheric olive pollen and the flowering phenology in diverse geographic locations of the Jaén province

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The most aerobiological studies try to prove like the presence of a pollen specie in the atmosphere it are intimately related with the distribution for the territory of the plant producing source, therefore the intensity and evolution of that pollen in the air is an accurate response of their abundance. In this work the aerobiologic behaviour of local form is studied of the olive pollen in a province where this grooving are extremely extended.

At the moment in Jaén province is concentrate the biggest extension of olive grove of the world. More that 570.000Ha is distributed for the whole territory and they is included in a large range of altitude. There are olive grove from 200m until the 1200m above sea level. After several serial years of aerobiologic pollen and flowering phenology period monitoring in different geographical localizations we show the local differences. For this, we related the dynamic of the olive pollen with the flowering in their nearer environment.

Generally we observed like the beginning the period of pollen in the atmosphere take places of almost simultaneous form in all geographic localizations. In this way this behaviour is independence with the flowering phenology of each zone and, therefore, with the altitude. On the other hand, the intensity and extension of pollen period they seem to be conditioned by the flowering in each zone and they are translated in an increase of this in the areas to more altitude, where the olive groves show a significant delay in the beginning of their flowering.

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Allergenic Pleosporales in home environments in Barcelona

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Alternaria alternata is reputed as the most allergenic fungus. Between 3,6 and 39,4% within the atopics are sensibilised to *Alternaria* and its Alt a 1 protein induces an IgE type response in more than 90% of the allergic patients. Orthologous of this protein have been identified in other fungi of the Pleosporales order. This is why our aim was to study the aerobiology of the *Alternaria* conidia as well as that of the ascospores and conidia of other Pleosporales.

We studied 60 homes in Barcelona (31 of them from patients allergic to *Alternaria*), evaluating the air and the dust on surfaces indoor and outdoor. We used viable (Microflow with malt extract agar) and non-viable (Hirst) methods. Levels of At a 1 in dust samples were quantified using ELISA (detection limit 0,5 µg).

In the air, the number of spores (Pleosporales and *Alternaria*) was always higher than the number of CFU, counts outdoor were always higher than indoor and counts in the allergy sufferers homes' were higher than in non-allergic. Surfaces showed also number of spores always higher than number of CFU and counts outdoor higher than indoor, but counts were lower in the allergy sufferers homes. Counts in the surfaces resulted always lower than airborne counts.

Between the 32 Pleosporales taxa identified it is to be mentioned (non-viable method) *Leptosphaeria*, *Pleospora* and *Venturia* type ascospores, and *Epicoccum* and *Stemphylium* conidia; (viable method) *Alternaria*, *Epicoccum* and *Phoma* conidia and none meiosporic. In 13 homes (12 from allergic patients) we found Alt a 1 concentrations varying from 0,5 to 16,9 µg/mg. No correlation was found between Alt a 1 levels and the spores counts and CFU of *Alternaria* and Pleosporales.

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Incidence of *Cupressaceae* pollen in the atmosphere of Málaga (Spain): 1992-2013

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A study about the incidence of Cupressaceae pollen in the atmosphere of different localities of Málaga (Málaga-capital, Vélez-Málaga, Nerja, Estepona and Antequera) has been carried out from 1992 to 2013 (both inclusive). For the study, Hirst-type volumetric spore traps were used, following the methodology adopted by the Spanish Aerobiology Network, the REA (Domínguez et al., 1997; Galán et al., 2007).

Cupressaceae pollen, one of the main causes of winter pollinosis in Spain, is the third pollen type on average in abundance order in Málaga, representing between the 13 and 25 % of the annual total airborne pollen (Recio, 1995; Melgar, 2010), depending on the station and the year. During the studied period, the highest pollen concentrations were detected during February and March, and the peak days occurred during the second fortnight of February or early March, with daily concentrations that can reach values of 1000 pollen grains/m³ of air (daily mean). Regarding intradiurnal distribution, the highest concentrations tend to be concentrated at midday.

During the studied years, a tendency to come early and increase the lasting of the main pollen season has been observed. However, there has been a slight decreasing in the intensity of the maximum peaks, at least in Málaga-capital, the station with a longer series.

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Airborne detection of Pla a1 allergen, an important source of allergy in the cities

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Platanus hispanica Miller ex Münchh, also known as London plane tree or sycamore, is frequently used as ornamental in parks, gardens and avenues of the cities in the south of Europe, this tree are considered an important source of airborne pollen allergens and cause diseases as rhinoconjunctivitis. The *Platanus* pollen season takes place in the North Spain between March and April; this period is greatly influenced by the meteorological conditions, which directly affect the airborne allergen Pla a₁. The objective of this study was to develop a model o predict the *Platanus* allergy risk periods as a function of the airborne pollen and allergen concentration as well as the main meteorological parameters.

The study was conducted in Ourense (NW-Spain). *Platanus* pollen quantified by using a volumetric sampler Lanzoni VPPS-2000. A Burkard multivial Cyclone Sampler was used for the atmospheric allergens collection. The Dot-Blot and the Raman spectroscopy methods were used to corroborate the results. Meteorological data were obtained from the Spanish National Institute of Meteorology.

Platanus pollen is present in the atmosphere during March and April. The pollen concentration is recorded before the aeroallergen Pla a₁ presence. These data are associated with the variations of the main weather variables. The atmospheric presence of *Platanus* aeroallergens during the tree flowering period was confirmed by other two methods, Dot-blot test and Raman spectroscopy. The regression analysis test developed including the Pla a₁ allergen and the average temperature as estimators, explained the 64.5% of variance of the pollen presence in the environment.

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A model to predict the concentration of airborne *Platanus* pollen in Central Spain

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The influence of meteorological variables on airborne *Platanus* pollen levels in Toledo (central Spain) was analyzed, and a model based on multiple regression analysis was used to predict pollen counts throughout the *Platanus* pollen season. *Platanus hispanica* Miller ex Munch (plane tree, London plane tree) is used as an ornamental tree in parks and gardens in numerous cities in Spain and it is considered an important source of airborne pollen allergens. The airborne pollen was registered uninterruptedly over eight years, from 2005 to 2012, using a Hirst volumetric spore trap. The sampling method established by the Spanish Aerobiology Network was used.

During the eight years studied, a total of 21402 *Platanus* pollen grains were collected, giving an annual mean of 2675 pollen grains. The annual total amount was lower in 2010 (1440 grains) and higher in 2011 (2503 grains). *Platanus* pollen is present in the atmosphere for a short period in spring, reaching its highest amount along the latest week of March and the first two weeks of April. In every one of those eight years the peak concentration was reached a day between 24th March and 5th April. The highest concentration was 679 grains/m³ obtained on the 1th May in 2012

Regression analysis shows that the minimum temperature, wind speed and the pollen concentration of the previous day are the best predictive variables. The prediction equation obtained for the daily *Platanus* pollen concentration showed a coefficient of $R = 0.64$.

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Seasonal atmospheric pollen variations in Montevideo city: general trends of a 2 year survey.

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Daily pollen concentration was recorded at Montevideo city from June 2011 to June 2012 and compared with data from a previous sampling period 2000-2001, using a Rotorod Model 40. Seventy-six taxa were identified, belonging to 44 trees and 32 herbaceous taxa. Poaceae was the most important pollen type accounting for 33% of total pollen in 2011-2012 and 46% total pollen in 2000-2001. Other important taxa exceeding 1% of total pollen were *Platanus* spp., Cupressaceae, *Celtis* sp., Urticaceae, Myrtaceae, *Casuarina* sp., Amaranthaceae, Cyperaceae, *Fraxinus* sp., *Schinus* type, *Ricinus communis*, Moraceae, *Myrsine* sp., *Typha* spp., Asteraceae and *Ambrosia* spp. Pollen was recorded all year around, but the main pollen period was from August to April when most of the anemophilous taxa flower, and was positively correlated with mean temperatures. Arboreal pollen dominance was observed by the end of winter until early spring, while non-arboreal pollen dominated the spectra from spring to summer. Despite a similar annual pattern between both sampling periods, some differences were recorded on weekly totals in March-April, and from October to December. Different meteorological conditions during the emission and preconditioning periods could explain such differences. Allergenic taxa were registered in important concentrations in Montevideo, information that should be taken into account for allergy sufferers and allergist. Seasonal and inter-annual differences highlight the relevance to perform long term studies in order to establish more reliable airborne pollen calendars, the factors involved in pollen production and emission, and to forecast pollen concentrations.

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Aeroallergens cross reaction detection among the *Oleaceae* family

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Among the Oleaceae family only the pollen from the Olea trees is considered as an important aeroallergen. Likewise other members of the family, largely represented in the ornamental flora of the cities (such as fresh and privet trees), are not considered an important source of airborne pollen allergens. Even though their major allergens Fra e1 and Lig v1 could present cross reactions with the olive major allergen Ole e1. The objective of this study was to ascertain whether pollen Fraxinus, Ligustrum and Olea europaea pollen concentrations and Fra e1, Lig v1 and Ole e1 allergens (detected by means the use of Ole e1 antibodies) were correlated.

The content of airborne pollen in Ourense (NW Spain) was quantified by using a volumetric sampler Lanzoni VPPS 2000 during the year 2011. The content of the atmospheric allergen was quantified by using a Burkard Cyclone sampler and enzyme-linked immunosorbent assay (ELISA) double sandwich modified technique.

Fraxinus pollination usually occurs in January and February. The maximum pollen daily concentration was the January 20th with 89 pollen grains/m³ and the maximum aeroallergen concentration the January 12th with 0.0477 ng/m³. Olea europaea pollen grains and Ole e1 allergens were detected during May and June with maximum daily concentrations the May 11th with 85 pollen grains/m³ and the May 11th with 0.0442 ng/m³ respectively. Ligustrum flowering season takes place from June to August, depending of the weather conditions, with a maximum of 7 pollen grains/m³ the June 27th and 0.0075 ng/m³ the June 26th.

We observed cross reactions between the Fra e1 and Lig v1 allergens and the olive major allergen Ole e1. The aeroallergen daily distribution curve follows very closely the variations of the Fraxinus, Olea and Ligustrum daily mean pollen concentrations. Therefore, the allergenic people sensitized to Olea pollen could present allergenic reactions in previous (January or February) and later periods (July or August) to the Olea flowering.

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Annual atmospheric pollen calendar of Gümüşhane, Turkey

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Pollens and spores in the atmosphere most of year due to the difference in the pollination periods of plants. Knowledge of concentrations of airborne pollens is important for allergists, allergy patients, systematics, agriculture and forestry studies. Also studies on pollens take importance in Forensic and Criminal investigations recently. Annual recording "Burkard Volumetric Pollen and Spores trap was used for pollen sampling. Sampled was according to the standard preparation procedures. Daily pollen count has been expressed as daily average of pollen grains per cubic meter of air sampled per day. Then the concentration of pollens were projected as weekly, monthly and annually. Atmospheric pollen studies were carried out between August-2010 July 2011 in Gümüşhane. 58 taxa were observed during the study. These taxa are; Arboreal taxa; Pinaceae, Cupressaceae/Taxaceae, Betula, Alnus, Carpinus, Corylus, Populus, Salix, Morus, Fabaceae, Ulmus, Ostrya, Quercus, Fraxinus, Juglans, Castanea, Rosaceae, Robinia, Fagus, Aesculus, Tilia, Acer, Platanus, Maclura, Ericaceae, Ailanthus, Carex, Eleagnus, Myrtaceae, Tsuga, Laurus. Nonarboreal taxa; Gramineae, Chenopodiaceae/Amaranthaceae, Compositae, Plantago, Apiaceae, Rumex, Artemisia, Ambrosia, Urticaceae, Galium, Brassicaceae, Humulus, Caryophyllaceae, Polygonaceae, Poterium, Parietaria, Papaver, Centaurea, Boraginaceae, Lamiaceae, Cistaceae, Hedera, Ranunculaceae, Liliaceae, Thypha, Campanula and Onobrychis respectively. As a result of the study, it is identified that 73,3 % of the total pollen concentration were the pollens arboreal taxa, where 10,54 % were pollens of nonarboreal taxa and 16,16 % for the Gramineae. During annual study, the highest pollen concentration was observed in June-July period while the lowest concentration observed in December-January period. Daily mean temperature total precipitation and daily mean humidity was observed most relating meteorological factor with pollen concentrations.(TUBİTAK-COST EUPOL,109S265)

Session 7: Landscape change and human-environment interactions
POSTER COMMUNICATION

Palaeoenvironmental and vegetation changes during the Holocene in the NW sector of the Iberian Range (Cuenca del río Añamaza, NE Spain)

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A multiproxy analysis of Holocene sediments north to the Añavieja Lake suggests deposition in a vegetated area with stagnant waters close to a carbonate fluvial system. Episodes of high (siliciclastics and tufa debris) and low detrital supplies (deposit of lutites) alternated. Vegetation structures preservation suggests acidic and anoxic conditions that slow down microbial activity. Such conditions favoured an exceptional preservation of pollen. The pollen record shows typical characteristics of the early Holocene: development of a Pinus forest in which Betula is well represented, expansion of deciduous Quercus, xerophilous and heliophilous grassland. The data suggest that after 8705 yrBP, increasing moisture supported open forests with deciduous (Quercus, Ulmus, Corylus) and evergreen (Quercus ilex, Pistacia) species. Pollen data, supported with ¹⁴C dating, reveal a stratigraphic discontinuity between 8500 and 4000 yrBP. From 4000 yrBP, a dominant deciduous Quercus forest with groves of Corylus, Ulmus, Acer, Fagus, Taxus expands and human activities (grazing) occur. The greatest change in vegetation is recorded from 1200 yrBP with the expansion of dry grassland due to intensive land use (agro-pastoral activities). This pattern of intensive land use persists and the end of the pollen record corresponds to the almost completely deforested plateau that surrounds the site today with the surrounding slopes covered by patchy grass with junipers groves and screes with little soil.

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All about yew: on the trail of *Taxus baccata* in SW Europe by means of integrated archaeobotanical studies

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Yew (*Taxus baccata*), a beautiful and magnificent tree, has captivated the attention of human groups since early Holocene as shown by a large number of archaeobotanical studies, pollen and plant macroremains, carried out throughout Europe.

This paper reviews current information about the history and traditional uses of *Taxus baccata* in SW Europe from a dual perspective based on the interaction of environmental and cultural factors. Different types of archaeobotanical and archaeological evidences will be considered (pollen, charcoal, plant macroremains, artifacts) as well as current phyto-toponymy. The geographical scope will cover different areas of Iberia, the Pyrenees and neighboring regions. The first archaeobotanical occurrences of *Taxus baccata* are recorded at the end of Pleistocene (ca 12 Kyr calBP) and beginnings of the Holocene (between 8000-7000 calBP) and correspond to the Epipalaeolithic and Mesolithic-Neolithic transition respectively. However, its most relevant values are found between 6000-2000 BP coinciding with its maximum pollen curve in the area of study. According to pollen records this taxon has firstly played a significant role within the mixed oak forest and then went gradually disappearing (ca. 3000 BP) from middle altitudes due to increasing human pressure. Human use can be clearly foreseen through plant macroremains from archaeological sites. The presence of *Taxus* fruits, manufactured yew tools and samples associated to livestock activities throughout the Neolithic allow us to discuss palaeoethnobotanical uses that may have contributed to its clear decrease in the late Holocene. Throughout the study area it is also very common to find specimens of yew alongside small romanesque churches, chapels or even in cemeteries. This may give us an idea of how yew has endured in the memories of humans wherever it has remained.

Session 7: Landscape change and human-environment interactions
POSTER COMMUNICATION

Expansion and decline of yew (*Taxus baccata* L.) in the Basque Mountains (Northern Iberian Peninsula) during the Holocene

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Global palaeoenvironmental reconstructions referred to Holocene chronologies have increased markedly in recent years, focusing on the evolution of the vegetal landscape, climatic variability and the increasing human influence on ecosystems. However, only a few studies pay their attention on the particular evolution of different species, trying to explain possible expansions or declines, or trying to understand their role in the current landscape configuration. In this paper we pay attention to the evolution of yew (*Taxus baccata* L.) in the Basque Mountains (Northern Iberian Peninsula) during the Holocene, according to the palaeobotanical record. We have considered all the palaeobotanical studies with any *Taxus* remains from both non-anthropogenic deposits (peat bogs and marshes) and archaeological sites. Results document a significant increase in the number of yew fossil evidences since the middle Holocene, as an expression of the extent of yew forest in mountainous areas. From ca. 3000 cal BP those evidences decreased markedly, showing the retreat of yew forests.

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POSTER COMMUNICATION

Pollen content in Muslim vessels found in Mallorca

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Almallutx is a town which formed part of the Muslim resistance during the Christian invasion by James I. This settlement is found in the Tramuntana mountain range (Mallorca, Balearic Island, Spain) and was discovered in 2011. It dates back to between the beginnings of the 13th, in the Almohad period and seems it may have been a place of refuge for three years (from 1229 to 1232).

The site is of some 60,000 metres square (equivalent to 7 football pitches) and is being studied by a group of archaeologists. The settlement has had urban planning, seen in the fact that it has been built using advanced construction techniques to provide residential areas, areas for government and for burials and also a mosque.

Among the archaeological remains with its network of streets, houses with inner central patios, a temple and two Muslim cemeteries, different pieces of Islamic pottery have been found. The town has been submerged since the middle of the past century in a reservoir. For that reason it can not be excavated very quickly, since only 30% becomes visible during the summer drought.

The preliminary study has been presented of the contents of two pots which were found sealed and with some substances inside during the 2012 dig. These vessels can help us to get closer to knowing about the inhabitants' customs and way of life. One of the containers is an urn found at the dig site (two samples) and the second is a pot with handles, from which part of the content has been extracted (four samples).

The palynological fossil method of Faegri & Iversen has been applied, with the subsequent acetolysis.

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La Carisa roman site (Asturias) pollen data

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In this work, we show pollen data of two sequences (CURS-2 and CUR-CP), located in the roman military site called “La Carisa”, built around 26 yr. BC. This area, 1728 m of altitude, near to pico la Boya (in the top of Curriechu mountain), is a natural walking way between León and Gijon bay. During roman age, this area was used for farming, mining exploitation and was a stage of battles between Roman and Astures.

The arboreal composition is dominated by *Corylus*; we detected presences of river taxa, and lower values of *Pinus*. The development of open landscapes, the increase of scrub (*Ericaceae*), associated a high values of pastures and nitrophilous plants and coprophilous non pollen microfossil (NPM) reflects the Anthropic pressure on the forest mainly due to livestock type.

However, the different origins of two sequences will allow to compare the vegetation response to anthropogenic activity. CUR-CP sequence is formed by three profiles, located in the archaeological site and CURS-2 sequence is located in a little humid area at the lower area of the archaeological site.

So to perform a multivariate analysis of main components (ACM), on whole samples, we observed two clusters, corresponding to each profile, due to a different response in: the dressing tree (higher in survival rate, which reaches up to 60%, of the total), without presence of thermophilous taxa. Above of *Pinus* is present with low values. The ruderal plant have higher values relative to grasslands and nitrophilous.

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POSTER COMMUNICATION

Scots pine (*Pinus sylvestris*) forests in the Spanish Central System. Phytosociological and paleopalynological considerations

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Fifty-eight modern pollen surface samples from different Scots pine (*Pinus sylvestris*) forest communities, in the Spanish Central System (central Spain) were palynologically and statistically analyzed (using hierarchical cluster analysis and principal component analysis) to derive correlations between pollen assemblages and environmental gradients at the sampled points. Numerical classification and ordination were performed on pollen data to assess similarities among Scots pine forest phytosociological associations. The results show a strong relationship between altitude, temperature, rainfall, arboreal cover and variations in pollen taxa percentages. The statistic discrimination of some of these forests has allowed us to propose three new phytosociological associations.

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POSTER COMMUNICATION

Nomadic societies and pastoral pressure during the last 2000 years in the mountains of Arkhangai, Mongolia

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The mountainous steppes of the aimag Arkhangai in Mongolia are rich of archaeological remains, mainly from Bronze Age and Iron Age (1200 BC-200 AD) with most often funerary and ritual structures. But less is known about the settlements, the activities and the environment of these first nomadic societies, as well as about the following periods of Pre-Mongol and Mongol times. Furthermore, Late Holocene palaeoecological high-resolution records remain scarce in Mongolia. Recently, several small lakes located along the Hanuy valley have been investigated to test their potential for palaeoecological studies. This poster presents the results of a multi-proxy approach combining sediment, pollen, non-pollen palynomorph (NPP) and micro- and macro-charcoal analyses performed on a core from the largest investigated lake, Shargyl Nuur. The results highlight the early formation of the cultural steppes landscape, at least from 2 millennia, with a persistent pastoral pressure characterized by low variations. Ancient DNA analysis is in process on the same core, to test its conservation and if possible to look for the evolutions of the livestock composition (horse, cattle, sheep, goat).

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POSTER COMMUNICATION

Holocene vegetation, fire and climate interactions in western Spain: El Maíllo mire

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A new palaeoecological sequence from the western Iberian Central Range significantly contributes to the knowledge on the Holocene vegetation dynamics in central Iberia. This sequence supports the existence of time-transgressive changes in the vegetation cover during the beginning of the Holocene over these central Iberian mountains, specifically the replacement of boreal birchpine forests with Mediterranean communities. Anthracological analyses also indicate the replacement of boreal pines (*Pinus sylvestris*) with Mediterranean ones (*Pinus pinaster*) during the early Holocene. The observed vegetation changes were generally synchronous with climatic phases previously reconstructed for the western Mediterranean region, and they suggest that the climatic trends were most similar to those recorded in the northern Mediterranean region and central Europe. Several cycles of secondary succession after fire ending with the recovery of mature forest have been identified, which demonstrates that the vegetation of western Iberia was highly resilient to fire disturbance. However, when the recurrence of fire crossed a certain threshold, the original forests were not able to completely recover and shrublands and grasslands became dominant; this occurred approximately 5800e5400 cal yr BP. Afterwards, heathlands established as the dominant vegetation, which were maintained by frequent and severe wildfires most likely associated with human activities in a climatic framework that was less suitable for temperate trees. Finally, our palaeoecological record provides guidelines on how to manage protected areas in Mediterranean mountains of southwestern Europe, especially regarding the conservation and restoration of temperate communities that are threatened there such as birch stands.

Addendum

Session 1: Applied palynology, bee pollination and diseases
POSTER COMMUNICATION

Molecular methods: a new antifraud methodology on bee pollen

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The traditional procedure for bee-pollen identification based on colour and melissopalynological analysis (optical microscopy) is a non-specific identification method because only pollen types are identified. Pollen types correspond to a plant family, a variable number of genera or a group of species. DNA sequencing is a more precise technique for botanical identification and its application recognizes species or, in a few cases, groups composed by a few number of closely related plant species.

This new molecular method is based on the PCR technique. The starting point is the selection of different monotype pollen balls from the same hive. These balls must be washed at 60° C to eliminate nectar sugars. Total DNA is extracted, and the PCR is carried out using ITS1 and ITS4 universal primers. PCR products are purified and sequenced. Finally, BLAST (Basic Local Alignment Search Tool) is used to identify the sequences obtained by comparison with database. 152 samples from different hives from Spain, Italy, Turkey, Bulgaria and Greece have been analysed and a total of 92 sequences of bee pollen have been obtained. A molecular database with genetic information about different bee pollen of European species will be created using these sequences.

The specific botanical identification of pollen composition in a sample using the PCR technique provides accurate information about the biogeographical origin of the pollen. Thus, this new molecular method can be used as an anti-fraud tool against alien bee pollen.

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