



# ABSTRACT BOOK

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Scientific Responsible:  
Prof.ssa A.M. Mercuri  
Prof.ssa E. Tedeschini

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“AEROBIOLOGIA 4.0”


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Prof. V. Patella  
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# ONE HEALTH

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**ABSTRACT  
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# HEALTH **2022**

## PAESTUM

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**POSTER**

ABSTRACT  
BOOK

## TOPIC: ARCHAEOPALYNOLOGY

# ARCHAEOPALYNOLOGY AND THE HUMAN HISTORY: A VIEW FROM ITALY

Laura SADORI

*Sapienza University of Rome, Department of Environmental Biology*

### Introduction

Archaeopalynology deals with the traces left by humans in the plant environment. It starts with the appearance of the genus *Homo*, so, in principle, it covers the whole Quaternary. As a matter of fact, the research is mainly concentrated on the last thousands of years, strictly depending on the increase of the number of investigated archaeological sites.

Detecting human presence is not always an easy task and depends on the kind of land use, changing not only according to the human species, but also on the demography and on technical skills of single populations.

### Materials and Methods

The studied materials are sediments, either deposited on-site and off-site, always taken in stratigraphic order and possibly dated, with different methods.

Pollen is extracted from the inorganic matrix following chemical procedures that include the use of strong acids and bases, heavy liquids, and sieving. Each laboratory adapts the method to the kind of material. In lucky cases (rather often at any rate) pollen grains are preserved and can be identified at the transmitted light microscope at a magnification varying from 40 to 100 X.

### Results and Discussion

Middle and Upper Pleistocene archaeopalynologically investigated Italian sites are just a few (e.g. Isernia- Accorsi et al. 1996, Grotta Romanelli-Russo Ermolli et al. 2021, Grotta Grande di Scario-Ronchitelli et al. 1998) and the main results concern the reconstruction of past environment, with very low possibility to disentangle natural changes and human fingerprints. In particular cases it is possible to highlight possible plant use for food, medicine or ritual purposes.

Since the Holocene the human fingerprint can be more easily recognized, and the human impact on the environment is becoming more and more evident (e.g. Mariotti-Lippi et al. 2014).





Humans shaped their landscape clearing the forest to get fuel and to obtain new areas for cultivation. The presence of anthropogenic indicators in pollen assemblages can be used to reconstruct the synanthropic flora that comprehends both cultivated plants and weeds. The availability of fuel is a factor that has conditioned a considerable range of needs, from daily life to such activities as the production of pottery, mostly considered in prehistory still as a domestic activity, and metallurgy, a specialized craft which needs considerable supplies of fuel of plant origin.

During the last millennia anyway disentangling climate forcing and human influence becomes more and more critical as both changes can result in forest opening, either due to clearance or to a natural reduction of the forest for the increasing aridity. In this case, whenever possible, palynology must be supported by other palaeoenvironmental analyses (Sadori et al. 2016, Zanchetta et al. 2022).

## Conclusions

Archaeopalynology starts with the presence of humans. Human populations left different traces in the pollen archives, due to the kind of settlement, of technological skills, and to demographical issues.

Since the Neolithic human populations modified flora and vegetation in three main ways: introducing or favoring edible plants, opening up woods for animal husbandry and at the same time using natural resources such as wood for heating, building, or producing metals. Each of these three ways produce a characteristic vegetation pattern, whose traces can be found in the pollen and micro-charcoal record.

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## TOPIC: ARCHAEOPALYNOLOGY

# THE CONTRIBUTION OF CAVE PALYNOLOGY FOR THE RECONSTRUCTION OF PLANT USE DURING THE PALAEOLITHIC: THE CASE OF GROTTA ROMANELLI (SOUTHERN ITALY)

Cristiano VIGNOLA <sup>1-2</sup>, Elda Russo ERMOLLI <sup>3</sup>, Alessia MASI <sup>1-2</sup>, Raffaele SARDELLA <sup>4</sup>, Laura SADORI <sup>1</sup>

<sup>1</sup> *Department of Environmental Biology, Sapienza University of Rome, cristiano.vignola@uniroma1.it, alessia.masi@uniroma1.it, laura.sadori@uniroma1.it*

<sup>2</sup> *Paleo Science and History Group, Max Planck Institute for the Science of Human History*

<sup>3</sup> *Department of Earth Sciences, Environment and Resources, University of Naples Federico II, ermolli@unina.it*

## Introduction

We present here results of the first palynological study carried out on the infilling deposits of Grotta Romanelli in Southern Italy (Russo Ermolli et al. 2021). The cave is a reference site for the Middle and Upper Palaeolithic of Italy due to the remarkable faunal assemblage and evidence of human frequentation (Sardella et al. 2019). Although the biases related to the study of fossil pollen in cave sediments, the pollen grains recovered give new insights into the chronological and palaeoenvironmental setting of the site, contributing also to the discussion of plant use by earliest humans.

## Materials and Methods

We focus on the lowermost levels so far investigated at the site, a fine unit of Aeolic origin named *Terre rosse* and till now ascribed to a warm and dry phase following the Würm acme. Seventeen sediment tubes were sampled during excavation from the exposed stratigraphical units of two sections. Samples were processed through standard treatment to extract fossil pollen grains. Pollen identification was based on atlases and reference collection. Modern material, provided by the *Herbarium Sapienza* (Rome, Italy) and recovered in the proximity of the cave, was treated with acetolysis in order to attempt a comparison with the fossil one.

## Results and Discussion

The first goal of this study is to contribute to the proper chronological attribution of *Terre rosse*. The abundant presence of *Olea* (olive), in fact, strongly suggests that these levels were deposited during

the Eemian, when a significant expansion of olive trees is recorded throughout the Mediterranean basin. This Last Interglacial dating is also consistent with the recovering of fossil bones of large herbivores from the same deposit, whose traces of exploitation clearly indicates the occupation of the cave by Neanderthals.

Despite the scarcity of pollen grains doesn't allow a statistical representation, the palaeoenvironmental information agrees with the general characterisation of the Last Interglacial vegetation in the Mediterranean. The main association of thermophilous trees (*Quercus*, *Olea* and *Pinus*) suggested at Grotta Romanelli confirms that the warm and dry climate of the Eemian fostered the expansion of xerophilous communities in Southern Italy up to middle altitudes.

In addition, some fossil pollen grains of *Terre rosse* have caught our attention since they were found in high amounts and in clusters. A comparison with modern pollen material allows us to ascribe the fossil grains to the species *Crithmum maritimum* (sea fennel) and *Muscari comosum* (grape hyacinth). The occurrence of such plants is noteworthy since their pollen is mainly dispersed by insects rather by wind and suggests transportation inside the cave by animals or humans. Moreover, the two plants are edible and/or have healing properties and many Mousterian lithic tools come from the same deposit. For this reason we propose an intentional introduction and possible use for cultural purposes by Neanderthals.

## Conclusions

The palynological study of Grotta Romanelli provides new data for the reconstruction of Mediterranean environments during the Eemian. The sequence represents, in particular, one of the earliest evidence of the use of plants in the diet and/or, more importantly, in the medicinal and ritual behaviours of the earliest humans in Italy.

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## **PALYNOLOGICAL INVESTIGATIONS IN THE ORCE ARCHAEOLOGICAL ZONE, EARLY PLEISTOCENE OF SOUTHERN SPAIN**

Juan Ochando<sup>1,2,\*</sup>, José Carrión<sup>1,3</sup>, Yul Altolaguirre<sup>4</sup>, Manuel Munuera<sup>5</sup>, Gabriela Amorós<sup>1</sup>, Gonzalo Jiménez-Moreno<sup>6</sup>, José Solano-García<sup>7</sup>, Deborah Barsky<sup>8,9</sup>, Carmen Luzón<sup>10</sup>, Christian Sánchez-Bandera<sup>8,9</sup>, Alexia Serrano-Ramos<sup>10</sup>, Isidro Toro-Moyano<sup>11</sup>, Juha Saarinen<sup>12</sup>, Hugues-Alexandre Blain<sup>8,9</sup>, Hervé Bocherens<sup>13</sup>, Oriol Oms<sup>14</sup>, Jordi Agustí<sup>15,8,9</sup>, Mikael Fortelius<sup>16</sup>, Juan M Jiménez-Arenas<sup>7,17</sup>

<sup>1</sup> Department of Plant Biology (Botany Area), Faculty of Biology, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain, [juan.ochando@um.es](mailto:juan.ochando@um.es), [carrion@um.es](mailto:carrion@um.es), [gabriela.amoros@um.es](mailto:gabriela.amoros@um.es)

<sup>2</sup> Sapienza University of Rome, Department of Environmental Biology, Italy, [juan.ochando@uniroma1.it](mailto:juan.ochando@uniroma1.it)

<sup>3</sup> Evolutionary Studies Institute, University of Witwatersrand, South Africa, [carrion@um.es](mailto:carrion@um.es)

<sup>4</sup> ROCEEH Research Centre 'The role of culture in early expansions of humans' of the Heidelberg Academy of Sciences, Senckenberg Research Institute, 60325 Frankfurt am Main, Germany, [yaltolaguirre@gmail.com](mailto:yaltolaguirre@gmail.com)

<sup>5</sup> Department of Agricultural Engineering, Polytechnic University of Cartagena, 30203 Cartagena, Spain, [manuel.munuera@upct.es](mailto:manuel.munuera@upct.es)

<sup>6</sup> Departamento de Estratigrafía y Paleontología, Universidad de Granada, Spain, [gonzaloj@ugr.es](mailto:gonzaloj@ugr.es)

<sup>7</sup> Departamento de Prehistoria y Arqueología, Universidad de Granada. Museo Primeros Pobladores de Europa 'Josep Gibert', Orce (Granada), [jgsolano@outlook.com](mailto:jgsolano@outlook.com), [jumajia2007@gmail.com](mailto:jumajia2007@gmail.com)

<sup>8</sup> IPHES-CERCA, Institut Català de Paleoecologia Humana i Evolució Social, Zona Educacional 4, Campus Sescelades URV (Edifici W3), 43007, Tarragona, Spain, [dbarsky@iphes.cat](mailto:dbarsky@iphes.cat), [csanchezbandera@gmail.com](mailto:csanchezbandera@gmail.com), [hablain@iphes.cat](mailto:hablain@iphes.cat), [jordi.agusti@icrea.cat](mailto:jordi.agusti@icrea.cat)

<sup>9</sup> Departament d'Història i Història de l'Art, Universitat Rovira i Virili, Avinguda de Catalunya 35, 43002, Tarragona, Spain, [dbarsky@iphes.cat](mailto:dbarsky@iphes.cat), [csanchezbandera@gmail.com](mailto:csanchezbandera@gmail.com), [hablain@iphes.cat](mailto:hablain@iphes.cat), [jordi.agusti@icrea.cat](mailto:jordi.agusti@icrea.cat)

<sup>10</sup> Programa de Doctorado en Historia y Artes, Universidad de Granada, [kebariense@hotmail.com](mailto:kebariense@hotmail.com), [alisera@correo.ugr.es](mailto:alisera@correo.ugr.es)

<sup>11</sup> Museo Primeros Pobladores de Europa 'Josep Gibert', Orce (Granada), [toromoyano@gmail.com](mailto:toromoyano@gmail.com)

<sup>12</sup> Department of Geosciences and Geography, University of Helsinki, [juha.saarinen@helsinki.fi](mailto:juha.saarinen@helsinki.fi)

<sup>13</sup> Faculty of Science, University of Tübingen and Senckenberg Centre for Human Evolution and Palaeoenvironment, Tübingen, [herve.bocherens@uni-tuebingen.de](mailto:herve.bocherens@uni-tuebingen.de)

<sup>14</sup> Departamento de Geología, Universitat Autònoma de Barcelona, [joseporiol.oms@uab.cat](mailto:joseporiol.oms@uab.cat)

<sup>15</sup> ICREA. Institución Catalana de Recerques Avançades. Barcelona, [jordi.agusti@icrea.cat](mailto:jordi.agusti@icrea.cat)

<sup>16</sup> Finnish Museum of Natural History - LUOMUS and Department of Geosciences and Geography, University of Helsinki, [mikael.fortelius@gmail.com](mailto:mikael.fortelius@gmail.com)

<sup>17</sup> Instituto Universitario de Investigación de la Paz y los Conflictos, Universidad de Granada, [jumajia2007@gmail.com](mailto:jumajia2007@gmail.com)

Palynological investigations in the Orce Archaeological Zone (OAZ) (Guadix-Baza Basin, Granada, Spain), Venta Micena 1 (VM1), Barranco León (BL) and Fuente Nueva 3 (FN3) are presented. This archaeological region is connected with the first *Homo* populations in Western Eurasia during the Early Pleistocene. The VM1 pollen record is characterized by *Ephedra*, and to a lesser extent, *Pinus*, *Juniperus* and evergreen *Quercus*, occasionally accompanied by *Olea*, Genisteae, *Erica*, deciduous *Quercus*, *Alnus*, *Castanea*, *Fraxinus*, *Salix* and *Phillyrea*. BL is dominated by *Juniperus*, *Olea*, *Pinus*, Poaceae, and evergreen *Quercus*. FN3 is characterized by an open Mediterranean woodland dominated by evergreen *Quercus*, *Pinus*, *Juniperus* and *Olea*, accompanied by deciduous *Quercus*, *Castanea*, *Populus*, *Salix*, *Ulmus*, *Fraxinus*, *Pistacia*, *Phillyrea*, Genisteae, *Erica*, *Cistus*, and *Ephedra fragilis*. Relic Tertiary taxa in OAZ include *Carya*, *Pterocarya*, *Eucommia*, *Zelkova*, and *Juglans*. The Early Pleistocene OAZ vegetation is a mosaic of different landscapes embracing mesophytes, thermophytes, xerophytes, xerothermophytes, and Mediterranean elements. These finds are compared with former pollen analyses in the region and beyond within the Iberian Peninsula.

## Introduction

This paper provides palynological investigations performed in the Orce Archaeo-paleontological Zone (OAZ) and discusses their implications in palaeoecological and phytogeographical contexts. Except for an attempt carried out by Jiménez-Moreno at the beginning of this century (Jiménez-Moreno 2003), previous efforts to find pollen in these localities were unsuccessful, such as is often the case with archaeological and continental paleontological sites (Carrión et al. 2009). Notwithstanding the difficulties, attempting new pollen analyses in the OAZ were worth the effort due to the exceptional nature of the OAZ and to the scarcity of palaeobotanical data of Early Lower Pleistocene in southwestern Europe (e.g. Leroy 1997; Postigo-Mijarra et al. 2007; Agustí et al. 2018; Altolaguirre et al. 2019, 2020, 2021).

## Materials and Methods

As recommended for archaeological sites (Girard 1975), palynological sampling was conducted on several vertical stratigraphic profiles for each study site (VM1, BL and FN3). For the extraction of palynomorphs, the “Classic Chemical Method” was followed (Erdtman 1969; Dumbleby 1985), with





the modifications proposed by Girard and Renault-Miskovsky (1969). The pollen count data were treated with Tilia Graph 1.7.16 in order to construct pollen diagrams.

## Results and Discussion

### Venta Micena 1 (Barranco de los Zagales)

A total of 16 VM1 samples were analysed, with 10 being polleniferous (Fig. 1) and 6 being sterile samples. A total of 2694 palynomorphs were identified, counting 2172 pollen grains and 522 spores, excluding indeterminable grains, which were lower than the 22% total palynomorph count. Spores and non-pollen microfossils were excluded from the pollen sum. The number of pollen taxa was between 11 and 22 per sample, with a total of 32 taxa being recognized.

The VM1 pollen record is characterized by the prevalence and extraordinary abundance of *Ephedra distachya-nebrodensis*, while *Pinus* and *Juniperus* are also continuously relatively abundant, with only few exceptions. Often, pines and junipers are accompanied by *Olea*, evergreen *Quercus* (especially in unit C), Genisteae, *Erica*, and to a lesser extent, deciduous *Quercus*, *Alnus*, *Castanea*, *Fraxinus*, *Salix* and *Phillyrea*. The woody component shows a moderate to low cover, but includes a combination of mesophytes (deciduous *Quercus*, *Alnus*, *Castanea*, *Fraxinus*, *Salix*), and Mediterranean taxa. The xero-heliophytic component (Poaceae, *Artemisia*, *Amaranthaceae*, *Ephedra*, *Lamiaceae*) is remarkable. The VM1 pollen record shows the higher indication of xericity, especially due to the high frequencies of *Ephedra*. The thermophilic character of the vegetation in the area can be deduced by the pollen occurrences of *Maytenus* and *Olea*.

### Barranco León

A total of 21 BL samples were analysed, of which only 5 were found to be polleniferous (Fig. 2). These successful samples came from Unit A (A5), Unit D (D1 and D2), and Unit E (E1 and E2). A total of 1265 palynomorphs were identified, including 1216 pollen grains and 49 spores, excluding indeterminable pollen grains (lower than 9%). Pollen grains of Cichorioideae, and *Cistus* were excluded from the pollen sum due to high percentages in several samples, suggesting over-representation (Carrión 1992a, b; Carrión et al. 1998). The number of pollen taxa ranges from 9 to 41 per sample, with a total of 53 taxa being recognized.

The record of BL, although not as palynologically productive as FN3, is still palaeoecologically meaningful. This sequence is characterized by the prevalence of *Juniperus*, *Olea*, *Pinus*, Poaceae, and evergreen *Quercus*, which is accompanied in several samples by other taxa such as *Cistus*, *Artemisia* and Cichorioideae with relatively high values. A diversity of trees, shrubs and herbs can also be observed. Poaceae is particularly abundant in D2 and E1; Cichorioideae dominant in D1. The woody component also shows diversity, combining mesophytes, thermophytes and Tertiary relics such as *Carya*, *Eucommia*, *Pterocarya* and *Zelkova*.

### Fuente Nueva 3

In total, from 16 samples that were collected from FN3, 15 were polleniferous (Fig. 3). A total of 4026 palynomorphs were identified, including 3379 pollen grains and 647 spores, excluding indeterminable which frequencies were lower than 5%. The number of pollen types varies between 17 and 32, with a total of 60 taxa being recognized.

In contrast, FN3 can be characteristically connected with forest ecosystems. This richer pollen record suggests the prevalence of a thermo-Mediterranean woodland dominated by evergreen *Quercus*, *Pinus*, *Juniperus* and *Olea* with a high diversity of woody plants, including deciduous *Quercus*, *Castanea*, *Populus*, *Salix*, *Ulmus*, *Fraxinus*, *Pistacia*, *Phillyrea*, *Genisteae*, *Erica*, *Cistus*, and *Ephedra fragilis*. It is worth emphasizing that the pollen record contains mesophytes, thermophytes, xerophytes, xerothermophytes, and Mediterranean elements, all indicative of summer drought, together with deciduous trees indicative of humid biotopes.

## Conclusions

A mosaic landscape has been depicted with groundwater-fed springs, Mediterranean forests and scrub, mountain mixed forests with Eurosiberian and Arctotertiary trees, and more arid biotopes including saline depressions and xerophytic scrub on marls and gypsic soils. Geophysical complexity in OAZ would come to shape ecosystems, biodiversity, and in the long term, plant geography and evolution. Thus, considering that most European regions, especially at higher latitudes, suffer climatically-induced regional extinctions of tree species earlier than in the Iberian Peninsula (Carrión and Fernández 2009; Carrión et al. 2011; Verdú et al. 2019), the Early Pleistocene would be here the continent's last moment of forest grandeur. The Iberian southeast would have been in turn, and essentially, a scenario of survival, with delayed and less dramatic extinctions, but finally disrupted by the action of humans, exceeding the macroclimatic forces of taxonomic and ecological amputation.

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## Tables

## Figures

Fig. 1. Synthetic pollen diagram of VM1 including ecological groups and the main pollen contributors.

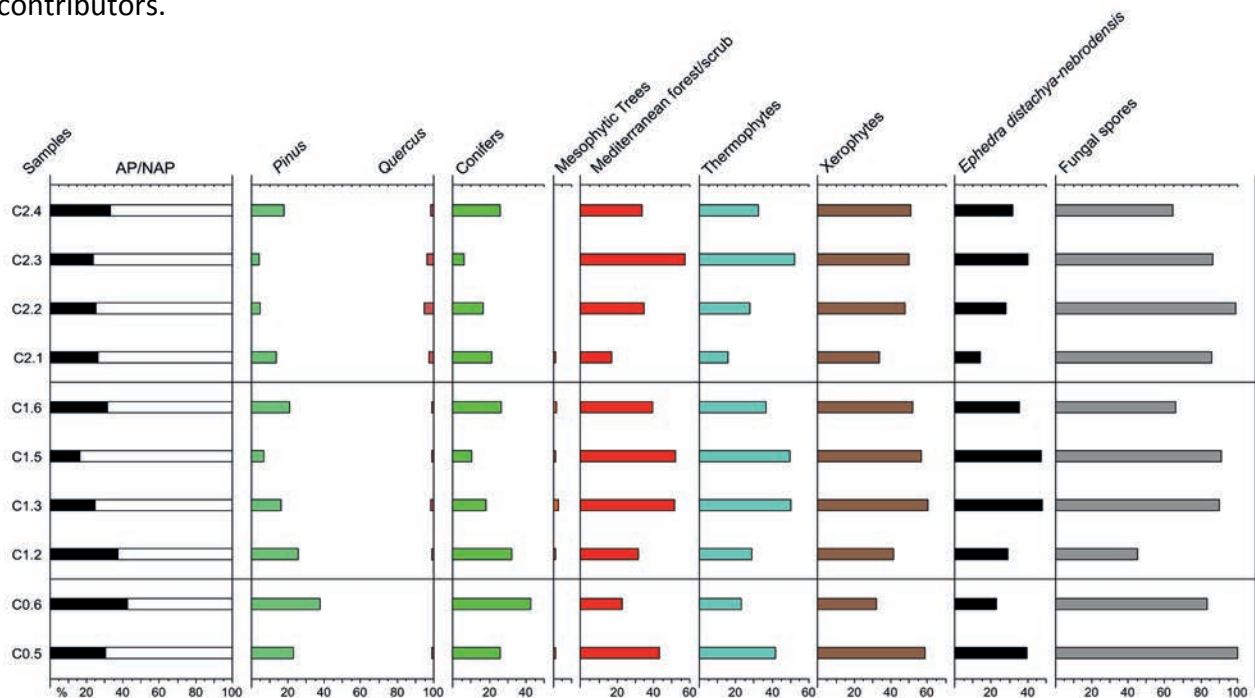


Fig. 2. Synthetic pollen diagram from BL including ecological groups and the main pollen contributors.

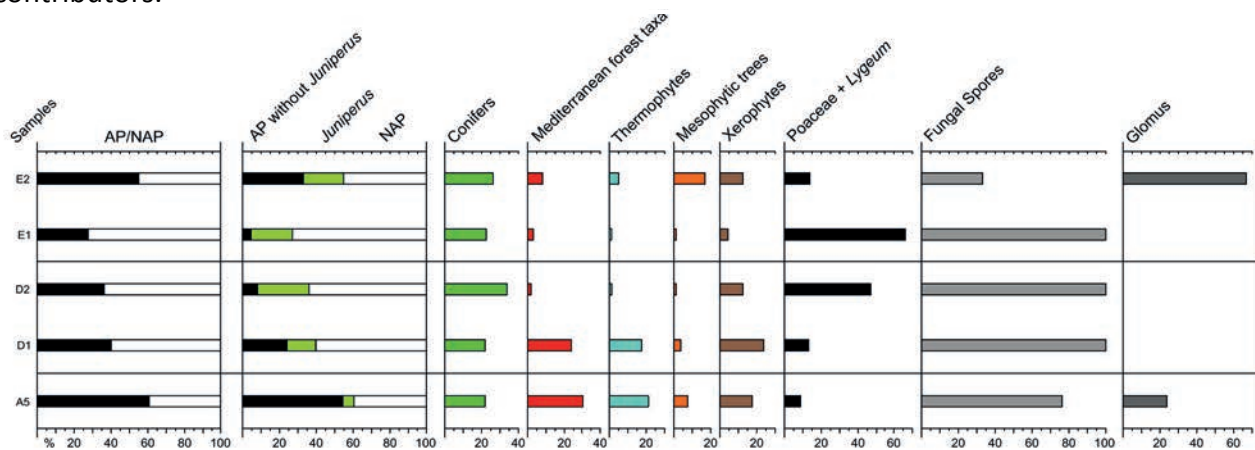
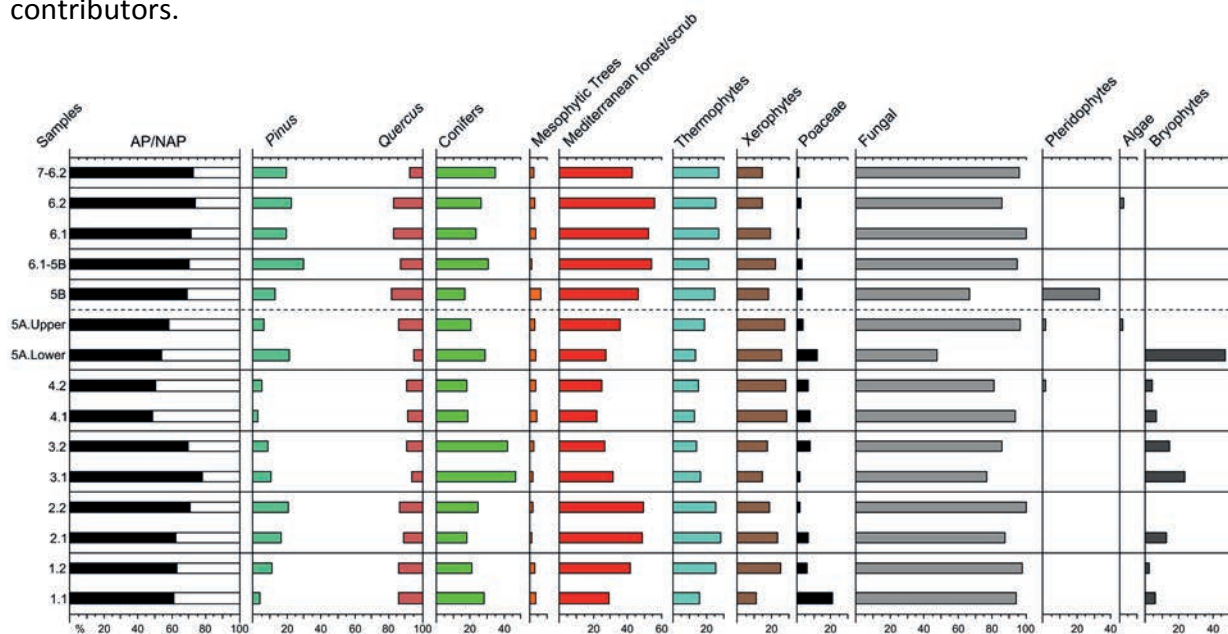


Fig. 3. Synthetic pollen diagram from FN3 including ecological groups and the main pollen contributors.





## COASTAL SOCIO-ENVIRONMENTAL TRAJECTORIES IN NORTH-BRITTANY (NW FRANCE) SINCE THE NEOLITHIC: PALYNOLOGICAL AND ANCIENT DNA MARKER RECORDS

Ophélie DAVID<sup>1,2</sup>, Muriel VIDAL<sup>1</sup>, Aneta GORCZYNSKA<sup>1</sup>, Nathan MARTIN<sup>3</sup>, Aurélie PENAUD<sup>1</sup>, Yvan PAILLER<sup>5</sup>, Morgane OLLIVIER<sup>3</sup>, Régis DEBRUYNE<sup>6</sup>, Evelyne GOUBERT<sup>2</sup>, Pierre STEPHAN<sup>4</sup>

<sup>1</sup> Univ. Brest, CNRS, Ifremer, Geo-Ocean, UMR 6538, F-29280 Plouzané, France – [ophelie.david@univ-ubs.fr](mailto:ophelie.david@univ-ubs.fr), [muriel.vidal@univ-brest.fr](mailto:muriel.vidal@univ-brest.fr), [aneta.gorczyńska@univ-brest.fr](mailto:aneta.gorczyńska@univ-brest.fr), [aurelie.penaud@univ-brest.fr](mailto:aurelie.penaud@univ-brest.fr)

<sup>2</sup> Univ. Bretagne Sud, Univ. Brest, CNRS, Ifremer, Geo-Ocean, UMR 6538, F-56000 Vannes, France – [ophelie.david@univ-ubs.fr](mailto:ophelie.david@univ-ubs.fr), [evelyne.goubert@univ-ubs.fr](mailto:evelyne.goubert@univ-ubs.fr)

<sup>3</sup> Univ. Rennes 1, Laboratoire Ecosystème, Biodiversité, Evolution, UMR 6553, F-35042, Rennes, France – [nathan.martin@univ-rennes1.fr](mailto:nathan.martin@univ-rennes1.fr), [morgane.ollivier@univ-rennes1.fr](mailto:morgane.ollivier@univ-rennes1.fr)

<sup>4</sup> CNRS, Littoral Environnement Télédétection Géomatique, UMR 6554, IUEM, F-29280 Plouzané, France – [pierre.stephan@univ-brest.fr](mailto:pierre.stephan@univ-brest.fr)

<sup>5</sup> Chaire Armerie, Univ. Brest, Inrap, Littoral Environnement Télédétection Géomatique, UMR 6554, IUEM, F-29280 Plouzané, France – [yvan.pailler@inrap.fr](mailto:yvan.pailler@inrap.fr)

<sup>6</sup> Direction générale déléguée à la Recherche, à l'Expertise, à la Valorisation et à l'Enseignement-Formation, Muséum national d'Histoire naturelle, F-75016, Paris, France – [debruyne@mnhn.fr](mailto:debruyne@mnhn.fr)

Neolithic human societies that settled in Brittany are known to have erected numerous megalithic monuments. In North-Brittany (France) the Bay of Goulven appears to be a key area to understand coastal socio-environmental trajectories thanks to an important concentration of gallery graves, erected during the Recent and Late Neolithic, testifying to a high population density. To shed new light on environmental and anthropogenic dynamics, a multidisciplinary approach mobilizing sedimentological, palynological and ancient DNA analyses was implemented on twin sedimentary cores, covering the last 7 kyrs BP and retrieved in the coastal wetland closed to Plouescat.

Preliminary results of XRF and palynological analyses display a progressive sedimentary infilling of the former maritime marsh up to a continental area totally disconnected from the marine influence at 5.4 ka BP. Superimposed to this environmental context, the first obvious, but tenuous anthropogenic markers occur from 6.8 to 5 ka BP, before disappearing up to 4.7 ka BP. This 300-years period corresponds to a potential abandonment of this coastal area before a new anthropogenic influence detected at 4.7 ka BP, and that subsequently increases reaching its maximum at 2.4 ka BP (i.e., Second Iron Age).

There are still open questions about the identification of anthropic activities in coastal environments. The low taphonomic resolution of Anthropogenic Pollen Indicators (API; i.e., ruderal, adventitious or cultivated taxa) together with the poor dispersion capacities of crops far from production areas limit the expression of anthropogenic activities in the pollen signal. The next step of this study is to combine the extracellular DNA preserved in sediments, detecting DNA markers of domestic mammals and anthropogenic plant groups, with palynological approaches to accurately reconstruct the past history of local agro-pastoral practices in northern Brittany.

**Keywords:** *North-Brittany, Neolithic, socio-environmental trajectories, palynological data, ancient DNA, agro-pastoral activities*



## TOPIC: **ARCHAEOPALYNOLOGY**

# LESSONS FROM THE PAST FOR A SUSTAINABLE FUTURE: INTERPRETATION OF NEAR-SITE PALYNOLOGICAL DATA

Eleonora CLÒ <sup>1</sup>

<sup>1</sup> *Laboratorio di Palinologia e Paleobotanica, Dipartimento di Scienze della Vita,  
Università degli Studi di Modena e Reggio Emilia, [eleonora.clo@unimore.it](mailto:eleonora.clo@unimore.it)*

### Introduction

As part of the PhD project "*Climate Change and Human Impact in a Long-Term Perspective: Palynology of Central Po Plain deposits since the Lateglacial*" (Ph.D. Course in Models and Methods for Material and Environmental Sciences - UNIMORE), the potential of the palynological method in providing information related to vegetation changes not only on a regional scale, but also by distinguishing local features in a near-site context was demonstrated. Three terrestrial cores were drilled at different distances from the Terramara S. Rosa di Poviglio in the Po Plain (N Italy), in a context that has been investigated for more than 35 years thanks to archaeological excavations directed by M. Bernabò Brea, M. Cremaschi and, in recent years, A. Zerboni (Cremaschi et al. 2018).

### Materials and Methods

Many elaborations were carried out to better understand the relationships between pollen data, the relevance of pollen taxa, and their correlation. Principal Component Analysis (PCA), Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS; de Juan and Tauler 2020), and CONstrained Incremental Sums of Squares cluster analysis are the statistical analyses considered (CONISS).

Information on spatial distribution of past vegetation has been obtained by applying the Landscape Reconstruction Algorithm (LRA; Sugita 2007a, 2007b). The lack of a list of pollen productivity estimates (PPEs) for the study area highlighted the need to test many combinations of different parameters from several papers already published for northern Europe (e.g., Abraham et al. 2014; Githumbi et al. 2021; Kuneš et al. 2019; Mazier et al. 2012; Nielsen 2004).

### Results and Discussion

A good characterization of the samples emerges from the cluster analysis that divides the sequences into large chronological intervals (four pollen zones) with different vegetation assemblages including



almost the same samples as the PCA and the MCR. Within these zones it was possible to identify pollen subzones, from which dissimilarities emerge based on local conditions.

Since the sampling points are located in the central part of the Po Plain, the study area was considered in the model as a basin that captures the airborne pollen from large distances. The three different models developed by applying the Regional Estimates of VEgetation Abundance from Large Sites–REVEALS, the first step of the LRA, reflect very similar trend among the three cores, providing a long-term picture of large-scale vegetation changes.

### Conclusions

MCR-ALS has never been applied in Palynology and the results obtained reveal the potential of this multivariate analysis for palynological investigations. All the statistical approaches have provided consistent results to support the detailed information obtained from pollen data. This result supports the initial hypothesis of considering the three sequences as single sites characterized by local differences. Nevertheless, statistical elaborations show a general similarity among the cores, which can all be divided into the same broad chronological intervals based on significant vegetation changes that occurred in the study area.

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## TOPIC: **ARCHAEOPALYNOLOGY**

# FROM ARCHEOPALYNOLOGY TO AEROPALYNOLGY: A FIRST LOOK AT THE URBAN GREEN DEVELOPMENT OF THE CITY OF REGGIO EMILIA

Elisa FURIA <sup>1</sup>, Alessandro TRAVAGLINI <sup>2</sup>, Anna Maria MERCURI <sup>3</sup>

<sup>1</sup> *Doctorate in Evolutionary Biology and Ecology, University of Rome Tor Vergata, Rome, Italy,  
elisa.furia@students.uniroma2.eu*

<sup>2</sup> *University of Rome Tor Vergata, travaglini@uniroma2.it*

<sup>3</sup> *University of Modena, mercuri.annamaria@unimore.it*

## Introduction

Allergies, including those caused by pollen grains, are on the rise due to a combination of factors, including climate change, air pollution, and new pollen allergies linked to alien plants (Damialis et al. 2019). Plants growing in urban green areas are at the same time sensible to climatic changes and in highly populated and possibly polluted areas. The possible impact plants may have on allergies and human health means it's needed to monitor these changes and study the link between climate changes, pollen and other allergens. The purpose of this study is to reconstruct the urban plants in the last millennia with a palynological perspective. One of the main target is to understand the changes that happened in the period analysed in the study-area of Reggio Emilia and what allergenic pollen spread we can expect in the near future. For this reason, moss and aeropalynological data will be collected to reconstruct the modern pollen rain as well as archaeopalynological data from two sites in the city of Reggio Emilia.

## Materials and Methods

Both archaeological and aeropalynological samples have been collected in the city of Reggio Emilia (Emilia Romagna, Italy). Archaeological samples were taken by Prof. Mauro Cremaschi (Department of Earth Science, University of Milano) during the excavation head by the Soprintendenza Archeologia Belle Arti e Paesaggio per la città metropolitana di Bologna e le province di Modena, Reggio Emilia e Ferrara (Sabap-BO) in piazza San Prospero (2020; head by Dott. Annalisa Capurso). Moreover, samples from Vittoria Park (2015-2016) were added. The samples have been treated using the method in use in the Laboratory of Palynology and Palaeobotany of Modena (Florenzano

et al. 2012). The moss sampling is based on a grid with meshes of 1 km square each. Inside each square of the grid, moss has been collected starting from parks and other green public areas.

## Results and Discussion

Preliminary results allow us to begin the description of ‘pollen green’ of urban areas in Reggio Emilia. The archaeological samples show a mostly open area (less than 20% arboreal pollen) with some trees important for the edible fruit (like *Castanea* and *Prunus*) and other important because of the know allergenic power of their pollen (like *Alnus*, *Carpinus* and *Corylus*). A greater number non arboreal pollen taxa has been found, with presence of some families that are known for being responsible for allergies (like Brassicaceae, Asteraceae and Poaceae). Moss samples contain pollen from trees growing in town like deciduous *Quercus*, *Cedrus* and *Aesculus hippocastanum*) besides many herbs mostly Poaceae and Asteraceae.

## Conclusions

The ongoing reconstruction of the taxa present in the town of Reggio Emilia has so far allowed for the start of the reconstruction of the history of plants in the urban green. Analysis of the moss samples supports the flora found in the parks in the city. We hope that with the end of the analyses it will be possible to reconstructs the evolution of urban green areas and gain more information on the evolution of taxa with known allergenic effects.

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## TOPIC: FROM ARCHAEOPALYNOLOGY TO PALAEOPALYNOLOGY

# BIG DATA PALAEOECOLOGY: A NEW APPROACH IN PALYNOLOGY TO RECONSTRUCT HISTORICAL KEY EVENTS SUCH AS THE BLACK DEATH ACROSS EUROPE

Alessia MASI<sup>1,2</sup>, Ana EJARQUE<sup>3</sup>, Assunta FLORENZANO<sup>4</sup>, Katerina KOULI<sup>5</sup>, José Antonio LÓPEZ-SÁEZ<sup>6</sup>, Reyes LUELMO-LAUTENSCHLAEGGER<sup>6</sup>, Lucrezia MASCI<sup>1</sup>, Florence MAZIER<sup>7</sup>, Anna Maria MERCURI<sup>4</sup>, Jordan PALLI<sup>8</sup>, Sebastián PÉREZ-DÍAZ<sup>9</sup>, Gianluca PIOVESAN<sup>8</sup>, Laura SADORI<sup>1</sup>, Cristiano VIGNOLA<sup>1,2</sup>, Adam IZDEBSKI<sup>2</sup>

<sup>1</sup> *Sapienza University of Rome, Italy, alessia.masi@uniroma1.it*

<sup>2</sup> *Palaeo Science and History group, Max Planck Institute, Germany*

<sup>3</sup> *ISEM, University of Montpellier, CNRS, IRD, France*

<sup>4</sup> *University of Modena and Reggio Emilia, Italy*

<sup>5</sup> *National and Kapodistrian University of Athens, Greece*

<sup>6</sup> *CSIC Madrid, Spain*

<sup>7</sup> *Jean Jaurès University, Toulouse, France*

<sup>8</sup> *Tuscia University, Italy*

<sup>9</sup> *Universidad de Cantabria, Spain*

## Introduction

In prehistorical and historical times, the connection between human impact and vegetation is extremely strong and the study of land use changes can be used as a tool for a better understanding of past human choices. Integration between palaeo- and archaeological sciences has a decadal tradition but with history a common pathway must still be built. Here we demonstrate the result of a strict integration between the palaeoenvironmental reconstruction and one of the most debated historical events of the past: the Black Death (1347–1352 AD). Above all the Black Death affected not only the demography but also the social economic history of Europe. The current knowledge of the effects of this pandemic is limited to fragmented written sources, available for some areas of Europe, and some pioneer studies on ancient DNA. The palaeoenvironmental record can produce evidence for a decisive influence on the future debate on major historical events.

## Materials and Methods

A large group of palynologists and historians applied a pioneering approach named ‘big data palaeoecology’, based on fossil pollen data, to evaluate the landscape impact of the Black Death on a regional scale across Europe (Izdebski et al. 2022). Palynological data from 261 coring sites (lakes

and wetlands), located in 19 present-day European countries, have been selected after checking quality and resolution of the chronologies. Data have been collected from available database as well as by directly contacting the owners. We evaluated whether changes in the landscape reconstructed at the time of the Black Death agree with the hypothesis that a large portion of the population, upwards of half, died within a few years in the 21 historical regions we studied.

In particular, we grouped pollen in four indices supported by Ellenberg light indicator and Niinemets and Valladares shade tolerance scale. These indices describe (1) landscape with high human pressure: Cereals, (2) evidence of pastureland: Herding, (3) abandonment of the land: Fast succession, and (4) rewilding: Slow succession.

## Results and Discussion

The assumption of our approach is that cultivation testifies the highest level of human presence in a territory. A strong reduction in population brings to the reorganization of the land as pasturelands, that require less workforce, or to the abandonment at different scales. The pollen indices have been compared before and after the pandemic arrival with different time extent. In fact, we checked for possible changes in land management occurring 50, 100 and 150 years before and after the pandemic. We found that in some regions (e.g., France, southwestern Germany, Greece and central Italy) the Black Death had a devastating impact with a clear decrease in cereal cultivation and sometime increase of rewilding (Fig. 1). On the other hand, some regions (e.g., Ireland and Iberia) had negligible or no impact with an increase both in Cereals and Herding indices. Such an inter-regional difference in the Black Death's mortality across Europe demonstrates the significance of cultural, ecological, economic, societal and climatic factors that mediated the dissemination and impact of the disease.

## Conclusions

This successful result has been the starting point for a new investigation on the Justinian plague (541–549 AD), for which the database has been improved in terms of both chronological resolution and number of sites.

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## TOPIC: FROM ARCHAEOPALYNOLOGY TO PALAEOPALYNOLOGY

# PALYNOLOGICAL ANALYSES AT GARDOLO DI MEZZO (TN): ENVIRONMENTAL TRANSFORMATIONS AND LAND USE IN A CULT AREA FROM THE COPPER AND BRONZE AGE

Jessica ZAPPA <sup>1</sup>, Paola TORRI <sup>1</sup>, Anna Maria MERCURI <sup>1</sup>, Michele BASSETTI <sup>2</sup>, Mauro ROTTOLI <sup>3</sup>, Elisabetta CASTIGLIONI <sup>3</sup>, Elisabetta MOTTES <sup>4</sup>

<sup>1</sup> *Laboratory of Palynology and Palaeobotany of Modena and Reggio Emilia*, [jessica.zappa@unimore.it](mailto:jessica.zappa@unimore.it); [paola.torri@unimore.it](mailto:paola.torri@unimore.it); [annamaria.mercuri@unimore.it](mailto:annamaria.mercuri@unimore.it); [assunta.florenzano@unimore.it](mailto:assunta.florenzano@unimore.it)

<sup>2</sup> *CORA Recherche Gardolo di Mezzo*, [michele@coraricerche.com](mailto:michele@coraricerche.com)

<sup>3</sup> *Laboratorio di Archeobiologia dei Musei Civici di Como*; [archeobotanica@gmail.com](mailto:archeobotanica@gmail.com)

<sup>4</sup> *Provincia Autonoma di Trento, Soprintendenza per i beni culturali*, [elisabetta.mottes@provincia.tn.it](mailto:elisabetta.mottes@provincia.tn.it)

## Introduction

Palynological analyses at the Copper and Bronze Age site of Gardolo di Mezzo (TN) were performed to highlight the differences in the vegetation cover and the differentiated land use adopted by local people. Gardolo di Mezzo is an important site to study ritual ceremonies and burial sites from the Copper to the Bronze Age, that are very different from the previous Neolithic period. Extensive archaeological excavations were performed at this site, carried out by the Archaeological Heritage Office of the Trento Autonomous Province (Mottes et al. 2011). The archaeobotanical research includes the study of plant macroremains and pollen. This contribution reports the palynological analyses performed on-site on a trench.

## Materials and Methods

39 samples were taken from a ca. 340 cm vertical trench located in the sector Garm 2, that corresponds to the cult area of this site. The treatment for palynological extraction was performed at the Laboratory of Palynology and Palaeobotany of Modena and Reggio Emilia on 39 samples. *Lycopodium* spores were added to calculate concentration expressed in p/g; sieving in 7 µm nylon mesh; treatment with HCl and HF; treatment with heavy liquid, particularly important in archaeological and poor sediments (Florenzano et al. 2012). Pollen counts were performed at 1000X magnification through the optical microscope on permanent slides. The identification was made with atlases, keys (Beug 2015; Moore 1991; Reille 1992) and the palynological reference collection of the laboratory. Pollen diagrams were obtained with the Tilia software (Grimm 2004).

## Results and Discussion

Four pollen zones were identified. From the bottom: pollen zone GDL 1, characterized by high percentages of AP (Arboreal Pollen). *Betula* dominates the spectra, highlighting a wide forest cover and a relatively cooler period at the end of the Copper Age. In pollen zone GDL 2, *Betula* slightly decreases in favor of elements of the mixed oakwood (*Carpinus betulus*, *Corylus avellana*, *Ostrya carpinifolia*/*Carpinus orientalis* type, deciduous *Quercus*, *Fraxinus*, *Tilia* and *Ulmus*) indicating more temperate environmental conditions. In pollen zone GDL 3 a drastic reduction of AP percentages and a strong increase in NAP (Non Arboreal Pollen) indicate an open landscape. Here, it is evident a strong presence of pollen belonging to API (such as *Artemisia*, *Centaurea nigra* type, *Trifolium* type, *Plantago*; Mercuri et al. 2013) and LPPI (such as Cichorieae, *Galium* type, Ranunculaceae; Mazier et al. 2009), that increase even more in GDL 4. The presence of these taxa indicates a stronger human presence in the site and the differentiated land use adopted by local population in the Bronze Age, both for cultivation and pastoral activities.

## Conclusions

Our palynological analyses performed at Gardolo allowed to evaluate the differences in the vegetation cover from the Copper to the Bronze Age, both quantitatively (decrease in AP percentages) and qualitatively (first *Betula* woods indicating a cooler environment, then mixed oakwood indicating a more temperate one). We also found a massive increase in API and LPPI in the spectra, coherent with the increase of human impact on the territory. A different land use also emerged that was devoted both to cultivation (cereals like *Hordeum* and *Avena/Triticum* groups) and pastoral activities.





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## TOPIC: FROM ARCHAEOPALYNOLOGY TO PALAEOPALYNOLOGY

### 7000 YEARS OF VEGETATION DYNAMICS AND AGRO-PASTORAL ACTIVITIES ON THE GLIÈRES PLATEAU (FRENCH NORTHERN ALPS)

Andréa JULIEN <sup>1</sup>, Elise DOYEN <sup>2</sup>, Charline GIGUET-COVEX <sup>1</sup>, Laurent MARQUER <sup>3</sup>, Fabien ARNAUD <sup>1</sup>,  
Erwan MESSAGER <sup>1</sup>

<sup>1</sup> *Environnements, Dynamiques et Territoires de la Montagne (EDYTEM) – Université Savoie Mont-Blanc, CNRS-UMR5204, Le Bourget-du-Lac cedex, France, [andrea.julien@univ-smb.fr](mailto:andrea.julien@univ-smb.fr) – [charline.giguet-covex@univ-smb.fr](mailto:charline.giguet-covex@univ-smb.fr) – [fabien.arnaud@univ-smb.fr](mailto:fabien.arnaud@univ-smb.fr) – [erwan.messenger@univ-smb.fr](mailto:erwan.messenger@univ-smb.fr)*

<sup>2</sup> *Paleobotalab, [el.doyen@laposte.net](mailto:el.doyen@laposte.net)*

<sup>3</sup> *University of Innsbruck, Department of Botany, Austria, [Laurent.Marquer@uibk.ac.at](mailto:Laurent.Marquer@uibk.ac.at)*

Palaeoecological studies conducted in the French Alps provide important insights into the major vegetation changes throughout the Holocene, in particular those related to historical land use. However, vegetation dynamics are not homogeneous through space and can vary from one site to another. The regional and local disparities are the consequences of a combination of factors such as geomorphology, local environmental conditions, and the impact of human activities on landscapes. In this context, there is a need to increase the numbers of pollen records at local and regional scales in order to assess the spatial and temporal changes in vegetation in the French-western Alps.

In this communication, we will present the results of a new site in our study region: Glières peat bog (1435m, Bornes-Aravis massif). Pollen, coprophilous fungi, and macro-charcoal analyses have been performed to reconstruct the past vegetation dynamics and to assess the impact of human activities on the regional landscape over the last 5200 BC. The age-depth model is based on nine radiocarbon dates (two were excluded); this age-depth model is further supported by three lead pollution peaks (Contemporary, Medieval, Antic).

Our results show a first phase dominated by fir from 5200 to 2600 BC. Then, fir declined while spruce, beech and alder increased. Like many sites in the same area, the Glières record is marked by increasing agropastoral activities during the Roman Period. At Glières, the human impact remained important until the Middle Ages. A phase of reforestation is recorded, reflecting the current agricultural decline.

These outcomes are consistent with other regional sequences, but they also highlight some local disparities within the region. This new pollen record provides additional knowledge in an altitudinal



(because it is located between a mountain and subalpine belt) and geographical area that is not well-studied yet.

Note that it is still challenging to identify local differences (at the watershed scale) in human land-use and their impacts on landscapes, because the local pollen signal is difficult to dissociate from the regional pollen load. The next step of our study is to use pollen-based modelling approaches to separate the local from the regional background of pollen, and assess the relevant source area of pollen for Glières as well as all other sites located in the region, this to produce regional and local maps of past vegetation in the French-western Alps. This modelling framework will also be introduced in our communication.

## TOPIC: FROM ARCHAEOPALYNOLOGY TO PALAEOPALYNOLOGY

### LANDSCAPE EVOLUTION AT THE PALIOURAS LAGOON IN GREECE DURING THE LAST 4000 YEARS

Lucrezia MASCI <sup>1,2</sup>, Cristiano VIGNOLA <sup>2</sup>, Adam IZDEBSKI <sup>3</sup>, Alessia MASI<sup>2,3</sup>

<sup>1</sup> *Department of Earth Science, Sapienza University of Rome, lucrezia.maschi@uniroma1.it*

<sup>2</sup> *Department of Environmental Biology, Sapienza University of Rome, cristiano.vignola@uniroma1.it, alessia.masi@uniroma1.it*

<sup>3</sup> *Max Planck Institute for the Science of Human History, Jena, Germany*

Paliouras coastal lagoon, located in the Halkidiki peninsula (Greece), is palynologically investigated to reconstruct the paleoenvironmental changes and the human impact on the landscape in the last 4000 years. The coastal feature of the Paliouras lagoon is ideal for the investigation of environmental and ecological changes in relation to the human impact (Kouli 2012, Panajiotidis and Papadopoulou 2016): the area, in fact, hosted agricultural populations that started modifying their environment since Neolithic. Moreover, its proximity to Thessaloniki led to an important urbanization that started with the Macedonia kingdom and has seen the alternation of different civilizations. The paleoclimatic reconstruction of the lagoon and its evolution has been recently traced through sedimentological, micropaleontological and molluscan (Koukousioura et al. 2019) analyses from an adjacent core. The palynological analysis of 49 samples from a 2 meters core completes and fill the information about the ecological system of the lagoon and gives information about the human impact in the area. The arboreal vegetation is mainly composed by oaks, both deciduous-semideciduous and evergreen with a strong reduction in the last millennia. The natural environment shows anthropogenic traits starting from the bottom of the sequence when the first settlements of the Classical age are recorded and expand during the fifth century BC. The most important change is recorded at the end of the Macedonian reign around 170 BC, with the increase of the arboreal vegetation and the reduction of cereals and synanthropic plants including *Rumex*, *Plantago lanceolata* and *Centaurea cyanus*. A new and different land management occurred with the Roman occupation of the area with an intensification of the agriculture both of cereals and of the cultivated trees such as *Olea*, *Juglans* and *Castanea*. The increase of Amaranthaceae and the reduction of the arboreal vegetation suggest an aridification trend around the area accelerated by the increase of the human impact during the last millennia. This study provides the first pollen sequence reconstructing the environmental dynamics





in Paliouras area offering further evidence of the human impact on the landscape in the Halkidiki peninsula.

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## TOPIC: FROM ARCHAEOPALYNOLOGY TO PALAEOPALYNOLOGY

### HISTORICAL SPRUCE ABUNDANCE IN CENTRAL EUROPE: A COMBINED DENDROCHRONOLOGICAL AND PALYNOLOGICAL APPROACH

Andrea SEIM<sup>1,2\*</sup>, Laurent MARQUER<sup>2</sup>, Ugo BISSON<sup>2</sup>, Jutta HOFMANN<sup>3</sup>, Franz HERZIG<sup>4</sup>, Raymond KONTIC<sup>5</sup>, Jutta LECHTERBECK<sup>6</sup>, Bernhard MUIGG<sup>7,8</sup>, Mechthild NEYSES-EIDEN<sup>9</sup>, Andreas RZEPECKI<sup>9</sup>, Manfred RÖSCH<sup>10</sup>, Felix WALDER<sup>11</sup>, Julia WEIDEMÜLLER<sup>4</sup>, Willy TEGEL<sup>1,8</sup>

<sup>1</sup> Chair of Forest Growth and Dendroecology, Institute of Forest Sciences, Albert-Ludwig-University of Freiburg, 79106 Freiburg, Germany (andrea.seim@iww.uni-freiburg.de)

<sup>2</sup> Department of Botany, University of Innsbruck, 6020 Innsbruck, Austria

<sup>3</sup> Jahrringlabor Hofmann & Reichle, Nürtingen 72622, Germany

<sup>4</sup> Bavarian State Department for Cultural Heritage, 86672 Thierhaupten, Germany

<sup>5</sup> Labor Dendron, Basel 4057, Switzerland

<sup>6</sup> Museum of Archaeology, University of Stavanger, Peder Klows gate 36A, PB 8600 Forus, 4036 Stavanger, Norway

<sup>7</sup> Chair of Forest History, Institute of Forest Sciences, Albert-Ludwig-University of Freiburg, 79106 Freiburg, Germany

<sup>8</sup> Amt für Archäologie, Kanton Thurgau, 8510 Frauenfeld, Switzerland

<sup>9</sup> Generaldirektion Kulturelles Erbe Rheinland-Pfalz, Direktion Rheinisches Landesmuseum Trier, Trier, Germany

<sup>10</sup> Institut für Ur- und Frühgeschichte und Vorderasiatische Archäologie, Universität Heidelberg, Sandgasse 7, 69117 Heidelberg, Germany

<sup>11</sup> Competence Center for Underwater Archaeology and Dendrochronology, Office for Urbanism, City of Zürich, Zürich 8008, Switzerland

Spruce is the most cultivated tree species in modern forestry in Central Europe, since it has the ability to grow on many soil types with profitable biomass accumulation. However, even-aged and uniform spruce forests are affected by recurring droughts and associated biotic stressors leading to large-scale diebacks across Central Europe causing controversies among foresters and nature conservationists.

We investigate the role of spruce in historical woodlands by using 15666 spruce timbers from historical buildings and on the basis of pollen-based land cover estimates using the REVEALS model from 157 pollen sites in southern Central Europe. Start and end dates of the spruce timber samples and their dendrological characteristics (age, growth rates and stem diameters) were used



to obtain information on past forest structures. Tree rings and REVEALS estimates are combined at a spatial scale of  $1^\circ \times 1^\circ$  resolution, grouped in four sub-regions, and a temporal resolution of 100-year time windows starting from 1150 to 1850 CE.

We found that spruce dominates the species assemblage of construction timber with almost 41% and that the harvest age varies little through time, whereas a declining trend in growth rates and stem diameters are observed towards times before modern forestry.

Temporal and regional differences in spruce abundance and building activity were found highlighting periods of i) land abandonment and forest expansion in the 14th century, ii) increased wood consumption during the 16th century due to population increase and beginning industrial developments, iii) a forest recovery during and after the Thirty years' war, and iv) afforestation efforts from the 1650s onwards. Furthermore, this study shows that spruce was constantly present in the study area in most studied sub-regions for the last 800 years.

We demonstrate the need of combining tree-ring and pollen data to identify spatiotemporal patterns in spruce abundance and utilization.

TOPIC: FROM ARCHAEOPALYNOLOGY TO PALAEOPALYNOLOGY

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CLIMATE CHANGES DURING THE LATEGLACIAL IN SOUTH EUROPE:  
NEW INSIGHTS BASED ON POLLEN AND BRANCHED GLYCEROL  
DIALKYL GLYCEROL TETRAETHERS (BRGDGTS) OF LAKE MATESE IN  
ITALY

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Mary Robles<sup>1,2</sup>, Odile Peyron<sup>2</sup>, Guillemette Ménot<sup>3</sup>, Elisabetta Brugiapaglia<sup>1</sup>, Sabine Wulf<sup>4</sup>, Oona Appelt<sup>5</sup>, Marion Blache<sup>2</sup>, Boris Vanni  re<sup>6,7</sup>, Lucas Dugerdil<sup>2</sup>, Bruno Paura<sup>1</sup>, Salom   Ansanay-Alex<sup>3</sup>, Amy Cromartie<sup>8</sup>, Laurent Charlet<sup>9</sup>, Stephane Gu  dr  n<sup>9</sup>, Jacques-Louis de Beaulieu<sup>10</sup>, S  bastien Joannin<sup>2,4</sup>

<sup>1</sup> Univ. Molise, Department of Agricultural, Environmental and Food Sciences, Campobasso, Italy

<sup>2</sup> Univ. Montpellier, CNRS, IRD, EPHE, UMR 5554 ISEM, Montpellier, France

<sup>3</sup> Univ. Lyon, ENSL, UCBL, UJM, CNRS, LGL-TPE, F-69007 Lyon, France

<sup>4</sup> Univ. Portsmouth, School of the Environment, Geography and Geosciences, Portsmouth, United Kingdom

<sup>5</sup> Helmholtz Centre Potsdam, GFZ German Research Centre of Geosciences, Section 3.6, Telegrafenberg, Potsdam, Germany

<sup>6</sup> Chrono-Environnement, CNRS, Univ. Bourgogne Franche-Comt  , Besan  on, France

<sup>7</sup> MSHE Ledoux, CNRS, Universit   Bourgogne Franche-Comt  , Besan  on, France

<sup>8</sup> Cornell Univ., Department of Anthropology, Ithaca, NY, USA

<sup>9</sup> Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, IRD, IFSTTAR, ISTerre, Grenoble, France

<sup>10</sup> Aix-Marseille Univ., CNRS, IRD, UMR 7263 & 237 IMBE, Aix-en-Provence, France





## Introduction

The Lateglacial (14,700-11,700 cal BP) is a key climate period marked by rapid but contrasted changes in the Northern Hemisphere. Indeed, regional climate differences have been evidenced during the Lateglacial in Europe and the Northern Mediterranean areas (e.g. [Moreno et al., 2014](#); [Heiri et al., 2014, 2015](#); [Ponel et al., 2022](#)). However, past climate patterns are still debated since temperature and precipitation changes are poorly investigated towards the lower latitudes. Lake Matese in Southern Italy is a key site in the Central Mediterranean to investigate climate patterns during the Lateglacial. This study aims to 1) establish reliable quantitative climate reconstructions based on molecular biomarkers (brGDGTs) and pollen for the Lake Matese during the Lateglacial, 2) compare the reconstructions obtained with regional climate reconstructions in Italy, 3) better understand the climate processes in Europe and Mediterranean during the Lateglacial period.

## Materials and Methods

This study uses a multi-proxy approach including magnetic susceptibility, geochemistry (XRF core scanning), pollen and molecular biomarkers like branched Glycerol Dialkyl Glycerol Tetraethers (brGDGTs) to reconstruct climate changes and their impacts at Matese. Palaeotemperatures and -precipitation patterns are quantitatively inferred from pollen assemblages (multi-method approach: Modern Analogue Technique, Weighted Averaging Partial Least Squares regression, Random Forest, and Boosted Regression Trees) and brGDGTs calibrations.

## Results and Discussion

A warm Bølling–Allerød and a marked cold Younger Dryas are revealed in all climate reconstructions inferred from various proxies (chironomids, ostracods, speleothems, pollen, brGDGTs), showing no latitudinal differences in terms of temperatures across Italy. During the Bølling–Allerød, no significant changes in terms of precipitation are recorded, however, a contrasted pattern is visible during the Younger Dryas. Slightly wet conditions are recorded south of latitude 42°N whereas dry conditions are recorded north of latitude 42°N. During the Younger Dryas, cold conditions can be attributed to the southward position of North Atlantic sea-ice and of the Polar Frontal JetStream whereas the increase of precipitation in Southern Italy seems to be linked to relocation of Atlantic storm tracks into the Mediterranean, induced by the Fennoscandian ice sheet and the North European Plain (e.g. [Moreno et al., 2014](#); [Rea et al., 2020](#)). On the contrary, during the Bølling–Allerød warm conditions can be linked to the northward position of North Atlantic sea-ice and of the Polar Frontal JetStream.

## Conclusions

This study allows to document and discuss about past climate changes in Italy and to contribute to the debate about the atmospheric processes in Southern Europe. The latitudes 40-42°N appear as a key junction point between wetter conditions in Southern Italy and drier conditions in Northern Italy during the Younger Dryas but also during the Early-Mid Holocene ([Magny et al., 2013](#)).

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## TOPIC: FROM ARCHAEOPALYNOLOGY TO PALAEOPALYNOLOGY

# PALAEOENVIRONMENTAL AND PALAEOCLIMATIC CHANGE IN THE SOUTH-WEST MEDITERRANEAN (SITE 976, ALBORAN SEA) DURING THE MIS 12/11 TRANSITION

Dael SASSOON<sup>1</sup>, Vincent LEBRETON<sup>1</sup>, Nathalie COMBOURIEU-NEBOUT<sup>1</sup>, Odile PEYRON<sup>2</sup>, Marie-Hélène MONCEL<sup>1</sup>

<sup>1</sup>*Muséum national d'Histoire naturelle, UMR 7194 Histoire Naturelle de l'Homme Préhistorique, Paris*

<sup>2</sup>*Institut des Sciences de l'Évolution de Montpellier, UMR 5554, Université de Montpellier*

### Introduction

The transition from Marine Isotope Stage (MIS) 12 to 11, also known as Termination V (400–430 kyr BP), is regarded as one of the largest shifts in climatic conditions over the last 900 kyr (Tzedakis *et al.*, 2006). This period is particularly important as it represents a phase of major innovation for Hominins, and the interglacial that follows, MIS 11, is considered the best analogue for the Holocene (McManus *et al.*, 2003; Desprat *et al.*, 2005; Moncel *et al.*, 2018). We present here a high temporal resolution pollen record that encompasses this crucial climatic shift (430–400 kyr BP), recovered from the ODP Site 976 in the Alboran Sea. This location is particularly sensitive to centennial and millennial climate, and is thus the perfect candidate to help investigate the MIS 12/11 transition (Alonso *et al.*, 1999; Combourieu-Nebout *et al.*, 1999, 2009; Marino *et al.*, 2018; Azibeiro *et al.*, 2021). This study aims to provide new insights into the response of vegetation in climatically sensitive region, and to highlight changes in seasonal aridity and precipitation in the continent during Termination V.

### Materials and Methods

This study used sections of the long marine sequence from Site 976 (36°12.3'N 4°18.8'W), recovered during Leg 161 of the Ocean Drilling Program (ODP) in 1996. This sequence represents an almost complete stratigraphy of 364 mcd (meters composite depth) drilled in the Alboran Sea, about 110 km from Gibraltar strait at 1108 m water depth (Combourieu-Nebout *et al.*, 1999, 2009). Seventy samples were selected at an average resolution of 10 cm with occasional higher resolution in specific areas of interest. A minimum of 150 pollen grains were counted for each sample, excluding *Pinus*, aquatics and spores, with a target of 20 morphotypes to ensure appropriate representation of sample composition. The adopted chronology is from von Grafenstein *et al.* (1999).

## Results and Discussion

The ODP Site 976 pollen record clearly shows the shift from glacial to interglacial, interpreted based on the transition from predominantly herbaceous and steppic taxa and a high abundance of *Pinus*, to an assemblage comprised of temperate and Mediterranean taxa. This shift from cool and arid to wet and warm conditions can be correlated with major fluctuations in planktonic  $\delta^{18}\text{O}$  records from von Grafenstein (1999) and Azibeirol *et al.* (2021). An optimum for temperate and Mediterranean taxa is observed from 420 kyr BP onwards. Changes in the signals of *Cedrus* sp., *Artemisia* sp. and *Isoetes* sp. point towards a difference in the source of pollen influx during MIS 12, possibly caused by enhanced dust input from Africa during a more arid climate (Bout-Roumazielles *et al.*, 2007), or variations in the flow of water into the Mediterranean between MIS 12 and 11.

## Conclusions

The results confirm that this region is uniquely sensitive to climatic variation, and vegetation seems to respond rapidly to changes in temperature and moisture availability. The pollen data will now be used for the quantitative reconstruction of temperature and precipitation (Kousis *et al.*, 2018) with a multi-method approach including several transfer functions (MAT, WAPLS, BRT). These results will make a substantial contribution to our understanding of changes in the eco-cultural niches of Hominins from MIS 12 to MIS 11.

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## TOPIC: FROM PALAEOPALYNOLOGY TO AEROPALYNOLOGY

### POLLEN PRODUCTIVITY ESTIMATES FOR PAST LAND COVER RECONSTRUCTION IN TURKEY

Esra ERGIN <sup>1,2</sup>, Laurent MARQUER <sup>2</sup>, Ugo BISSON <sup>2</sup>, Nüzhet DALFES <sup>1</sup>

<sup>1</sup> ITU, Eurasia Institute of Earth Sciences, Istanbul, Turkey, [esraerginn@gmail.com](mailto:esraerginn@gmail.com); [dalfes@gmail.com](mailto:dalfes@gmail.com)

<sup>2</sup> Department of Botany, University of Innsbruck, Austria, [Laurent.Marquer@uibk.ac.at](mailto:Laurent.Marquer@uibk.ac.at); [Ugo.Bisson@student.uibk.ac.at](mailto:Ugo.Bisson@student.uibk.ac.at)

Vegetation is an essential component of the Earth System. Changes in plant land cover influence the local, regional and global climate mechanisms. A long-term perspective (centuries to millennia) of land cover changes is critical to understand the sensibility of the present-day vegetation to climate and land use changes (Marquer et al., 2017). Therefore, reconstructing past land cover is crucial to improve our knowledge about the interactions between vegetation, climate and land use over time, and therefore better assess the actual environmental changes related to the ongoing climate change.

Fossil pollen collected in lake and peat sediments inform about the past vegetation composition and diversity changes which further provide insights into land use and climate changes. However, vegetation reconstructions based on pollen data have been a challenge due to the non-linear relationship between a pollen assemblage and the related plant abundance (Davis, 1963; Jackson et al., 1995; Prentice, 1988; Prentice and Webb, 1986; Sugita, 2007a). Pollen-based modelling approaches have been developed (Sugita, 2007a, 2007b) to correct this non-linear relationship. In order to use such modelling approaches, one needs to get a reliable set of relative pollen productivity estimates (RPPS). RPP is one of the critical parameters to apply quantitative pollen-vegetation models such as REVEALS and the Landscape Reconstruction Algorithm (Sugita, 2007a, 2007b). Calculating RPPs requires a collection of modern pollen samples, usually from moss polsters or lake sediment surface samples along with a detailed vegetation survey around each site, from the sample point to ca. 3-5km radii (e.g. Bunting, et al., 2013). RPP field works are quite time consuming. Most of them have been done in Europe and the northern hemisphere. Only one study has been published for the Mediterranean plant taxa (Githumbi et al., 2022). Up to now, there is neither RPPs or quantitative pollen-based modelling approaches applied in Turkey.

In the present study, we aim at estimating the relative pollen production (RPPs) for the main plant taxa in the Mediterranean area-southwestern part of Turkey, Golhisar Lake-Burdur. Modern pollen



data were collected from mosses and soils from 21 sites. These data have been compared to the modern regional and local vegetation maps to assess the pollen-vegetation relationship. The objective is to get a new set of RPPs for Turkey that can be used to run the pollen-based vegetation models such as REVEALS and the Landscape Reconstruction Algorithm to reconstruct quantitatively changes in vegetation covers through time. Our sampling sites correspond essentially to the Mediterranean oak forest, and high mountain meadow, black pine forest, steppe, juniper and cedar forests. We present here the first results and what they imply in terms of perspectives for future RPP studies in the Mediterranean region.

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## TOPIC: FROM PALAEOPALYNOLOGY TO AEROPALYNOLOGY

### POLLEN PRODUCTIVITY ESTIMATES FOR PAST LAND COVER RECONSTRUCTION IN MOUNTAIN REGIONS

Ugo BISSON<sup>1</sup>, Esra ERGIN <sup>1,2</sup>, Florence MAZIER<sup>3</sup>, Laurent MARQUER<sup>2</sup>

<sup>1</sup> Department of Botany, University of Innsbruck, Austria, [Laurent.Marquer@uibk.ac.at](mailto:Laurent.Marquer@uibk.ac.at); [Ugo.Bisson@student.uibk.ac.at](mailto:Ugo.Bisson@student.uibk.ac.at)

<sup>2</sup> ITU, Eurasia Institute of Earth Sciences, Istanbul, Turkey, [esraerginn@gmail.com](mailto:esraerginn@gmail.com)

<sup>3</sup> GEODE, UMR 5602 CNRS, Toulouse, France, [florence.mazier@univ-tlse2.fr](mailto:florence.mazier@univ-tlse2.fr)

Pollen-based land cover modelling have been applied at local, sub-continental and continental scales with a major aim to evaluate the performance of past land use scenarios and to study the vegetation, climate and land use interactions through time. However, the application of such modelling in mountain regions is more complex than for lowland ecosystems, in particular because it is a challenge to model the dispersal mechanisms of pollen in complex topographic contexts.

One of the key parameters to use pollen-based modelling schemes such as the REVEALS model and the Landscape Reconstruction Algorithm is the relative pollen productivity estimates (RPPs) for the major plant taxa. Most of the RPP studies have been done in lowlands and further researches are needed to develop these approaches in mountain regions. In particular, different pollen dispersal modelling are now available and there is a need to create a set of RPPs that can be used specifically in mountains.

The present study aims at studying the production of pollen and pollen dispersal mechanisms for the most abundant plant taxa in the Eastern part of the Alps, i.e. Germany, Austria and northernmost Italy. The performance of two approaches in pollen dispersal modelling are here evaluated, i.e. Lagrangian stochastic models and the Gaussian plume models. The objective is to create a set of RPPs including spruce, pine, larch, fir, Ericaceae, Poaceae, alder, birch, beech and hazel, as examples. For this purpose, we are assessing the pollen-plant abundance relationships in an area covering different valleys and altitudes. Thirty sites have been randomly selected in an area of ca. 50km radius. For each site, a modern pollen sample (moss polsters) was collected in the center of the site and vegetation surveys have been carried out to estimate the plant abundance for incremental distances, i.e. incremental rings of 1m from the center to 5km. Vegetation maps have been created based on GPS mapping during field work for the 0-100m and then by using satellite images and regional vegetation maps for the distances from 100m to 5km. The ERV model is used for the calculations. Based on this study, a new RPP set is created for a specific use in mountain landscapes.



## Prehistoric trajectory of the triptych climate-societies-biodiversity in semi-arid Arizona (USA): a baseline for sustainable landscape management

Yannick MIRAS <sup>1</sup>, R. Scott ANDERSON <sup>2</sup>, Ana EJARQUE <sup>3</sup>, Santiago RIERA MORA <sup>4</sup>, Emmett O. TSOSIE<sup>2</sup>

<sup>1</sup> CNRS, HNHP UMR 7194, Muséum National d'Histoire Naturelle, Institut de Paléontologie Humaine, Paris, France.

[yannick.miras@mnhn.fr](mailto:yannick.miras@mnhn.fr)

<sup>2</sup> School of Earth & Sustainability, Northern Arizona University, Flagstaff, AZ 86011, USA.

[scott.anderson@nau.edu](mailto:scott.anderson@nau.edu)

[et372@nau.edu](mailto:et372@nau.edu)

<sup>3</sup> CNRS, ISEM UMR 5554, University of Montpellier, France.

[ana.ejarque@umontpellier.fr](mailto:ana.ejarque@umontpellier.fr)

<sup>4</sup> SERP-University of Barcelona, Spain.

[rieram@ub.edu](mailto:rieram@ub.edu)

### Introduction

Palaeoenvironmental research spanning from centuries to millennia can provide relevant information applicable to biodiversity conservation and to landscape management (e.g., Birks 1996). Incorporating palaeoecological inputs in the “*conservation tool kit*” for improving decision-making (Davies and Bunting, 2010, p. 58) is challenging, especially in mid-arid and arid ecosystems (Gillson 2015), particularly exposed to the effects of global change. The palaeoenvironmental investigation of Montezuma Well, a limestone sink-hole located in the Upper Verde Valley of central Arizona (USA), was performed with this ambition. Two previous palynological studies of this site (Hevly 1974; Davis and Shafer 1992) allowed to trace the major vegetation changes under the influence of past climatic fluctuations throughout the last 9 millennia approximately. Our new palaeoenvironmental analysis provides the basis for re-assessing how climatic variations affected both plant and fungal diversities (mainly, richness, evenness and composition) and landscape organisation during this time span. Furthermore, being a main freshwater source in a region where water is scarce, Montezuma Well was very attractive to past societies through time. Indeed, the surrounding Verde Valley has been occupied by hunter-gatherers and early farming societies since c. 5500 cal BC and the 1st century AD



respectively, and sedentary societies from the Sinagua culture first settled in the Montezuma Well area since c. 650 AD. The lake is thus as a key site to analyse the potential attractiveness of the long-term landscape dynamics and diversity as a function of the subsistence practices. In the same manner, early environmental transformations induced by the past societies' natural resource management and obtained from a "pristine ecological state" have been tracked and analysed in their whole dimensions: timing, extent and potential type of land uses involved (e.g., use of wild and domesticated plant resources for foraging, horticulture and cultivation, introduction of exotic plants).

### Materials and Methods

The multi-proxy palaeoenvironmental analysis we performed is based on the analysis of different complementary indicators: pollen, non-pollen palynomorphs and sedimentary charcoal. Thanks to 17 AMS radiocarbon dates, we obtained a robust depth-age model allowing us to perform this study at a high temporal resolution. Rarefaction analysis was also undertaken in order to assess temporal changes in pollen-assemblage richness (Berglund et al. 2008). The pollen-assemblage richness has been interpreted as an approximate measure of both the vegetation floristic richness in the pollen source area and the mosaic landscape configuration degree (Birks et al. 2016). Finally, the coupling of palaeoenvironmental and archaeo-historical datasets was done.

### Results and Discussion

The specific focus on Prehistory here proposed allowed us to explore how semi-arid landscapes, apprehended as a cluster of ecosystems, responded to natural disturbances such as climate, fire or erosion. It also allowed us to trace through time landscape's taxonomic composition (vegetation and fungal diversities), landscape organisation (mosaic structure and habitat diversity) as well as landscape temporal variability, magnitude and frequency of changes. Different tipping points have been revealed throughout the last 9 millennia. Even if these threshold periods are mainly climate-induced, this study allowed assessing both the attractiveness of these landscape changes for past societies and the effects that human activities have had on the long-term shaping of landscapes in the Verde Valley. Following this, we could discuss about the "naturalness" of such mid-arid landscapes.

### Conclusions

By scrutinizing the particular features of early human impacts and by analysing how prehistoric societies adapted to changing environmental conditions in this semi-arid region, we obtained fresh insights on the long-term interplay between human adaptability, climate oscillations, and environmental evolution, a complex relationship which deepened during the historical period with the colonial Anglo-American peopling of the area. This issue is crucial in semi-desert areas such as the Verde Valley, which are highly sensitive to climatic and anthropogenic changes and thus particularly threatened by global change.



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## Figures



*Montezuma Well and cliff dwellings perched along the rim of the lake (@A. Ejarque)*





## TOPIC: FROM PALAEOPALYNOLOGY TO AEROPALYNOLOGY

# LINKING MICROCHARCOAL IN MARINE SURFACE SEDIMENTS, TO CURRENT TO RECONSTRUCT PAST FIRE REGIMES IN THE IBERIAN PENINSULA

Marion Genet<sup>1</sup>, Anne-Laure Daniau<sup>1</sup>, Florent Mouillot<sup>2</sup>, Vincent Hanquiez<sup>1</sup>, Sabine Schmidt<sup>1</sup>, Valérie David<sup>1</sup>, M. Georget<sup>1</sup>, Fatima Abrantes<sup>3,4</sup>, Pierre Anschutz<sup>1</sup>, Franck Bassinot<sup>5</sup>, Jérôme Bonnin<sup>1</sup>, Bernard Dennielou<sup>6</sup>, Frédérique Eynaud<sup>1</sup>, David A. Hodell<sup>7</sup>, Thierry Mulder<sup>1</sup>, Filipa Naughton<sup>3,4</sup>, Linda Rossignol<sup>1</sup>, Polychronis Tzedakis<sup>8</sup>, Maria Fernanda Sánchez-Gofñi<sup>1,9</sup>

*1. Univ. Bordeaux, CNRS, EPOC, EPHE, UMR 5805, F-33615 Pessac, France*

*2. UMR CEFE, Univ. Montpellier, CNRS, EPHE, IRD, 1919 route de Mende, 34293 Montpellier CEDEX 5, France*

*3. Divisão de Geologia e Georecursos Marinhos, IPMA, Avenida de Brasília 6, Lisboa, 1449-006, Portugal*

*4. CCMAR, Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, Faro, 8005-139, Portugal*

*5., LSCE/IPSL, CEA-CNRS-UVSQ, Université Paris-Saclay, 91191 Gif-sur-Yvette, France*

*6. Ifremer, Unité Géosciences Marines, Centre Bretagne - 29280 Plouzané, France*

*7. University of Cambridge, Godwin Laboratory for Palaeoclimate Research, Cambridge, United Kingdom*

*8. Environmental Change Research Centre, Department of Geography, University College London, United Kingdom*

*9. Ecole Pratique des Hautes Etudes (EPHE, PSL University), F-33615 Pessac, France*

The Mediterranean region is highly subjected to fires. Projected global warming scenarios suggest an increase in fire risk for 2100, particularly in this region (Pechony and Shindell, 2010). However, fire models developed and calibrated from recent observations do not take into account some relationships such as vegetation response to climate changes (Turco et al., 2017). Studying microcharcoal preserved in sedimentary records allows the performance of models to be tested for a variety of climate conditions, although this proxy must be calibrated. In a previous study, Daniau et al., 2007 interpreted changes in microcharcoal concentration in a long marine sedimentary record off south of Portugal as increases and decreases in burned biomass that coincide with changes in vegetation induced by climate change. However, we cannot interpret yet changes in microcharcoal concentrations directly to changes in fire regime (number of fire, fire size, fire intensity) since these changes might also depend on other parameters such as vegetation and climate but also on microcharcoal transport and deposition (winds, rivers, ocean currents, marine morphology). The objective of this work is to explore the link between microcharcoal in marine sediments and fire regimes on land. For this, we analysed microcharcoal concentrations and elongation (length to width ratio of a particle) in 102 surface sediment samples located off the Iberian Peninsula that we compared with environmental data on the adjacent continent. We observed an increase in microcharcoal concentrations from north to south that coincides with decreasing precipitation and

increasing temperature, fire size and fire intensity. We also found that high microcharcoal concentrations are associated with large, rare and high intensity fires while low microcharcoal concentrations are associated with small, frequent and low intensity fires. Comparing the elongation of microcharcoal particles to the dominant burned vegetation, we found that high concentrations of highly elongated microcharcoal are associated with the burning of more mixed and open vegetation. Conversely, low concentrations of weakly elongated microcharcoal were associated with the burning of more closed vegetation, with a greater burning of closed vegetation in the north of the Iberian Peninsula, or with the burning of cultivated vegetation in the south of the Iberian Peninsula. We used the calibration results obtained on the Iberian margin to reinterpret the results of the work of Daniau et al. (2007). We suggest that between 10000 and 8000 years ago frequent small fires of low intensity were spreading in closed vegetation while the increase in microcharcoal concentrations over the last 8000 years could reflect large and rare fires of high intensity occurring in open and fire-prone vegetation such as shrub. We aim at developing this calibration over a greater diversity of climate gradient, vegetation, and fire regimes over the western Mediterranean region in order to fully constrain the use of microcharcoal to reconstruct past fire regimes.

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## TOPIC: FROM PALAEOPALYNOLOGY TO AEROPALYNOLOGY

# CHANGES IN FIRE ACTIVITY AND BIODIVERSITY IN A NORTHEAST BRAZILIAN CERRADO OVER THE LAST 800 YEARS

Sergio Augusto Santos XAVIER <sup>1,2</sup>, Francisca Soares de ARAÚJO <sup>2</sup>, Marie Pierre LEDRU <sup>1</sup>

<sup>1</sup> ISEM, Univ Montpellier, CNRS, IRD, University of Montpellier, Place Eugène Bataillon, 34095 Montpellier, France, [sergio-augusto.santos-xavier@etu.umontpellier.fr](mailto:sergio-augusto.santos-xavier@etu.umontpellier.fr)

<sup>2</sup> Graduate Course of Ecology and Natural Resources, Department of Biology, Campus of Pici, Federal University of Ceará, Fortaleza - 60440-900, CE, Brazil

### Introduction

Understanding vegetation dynamics is essential to interpret long-term ecological changes under different precipitation regimes and land-use scenarios. In Northeastern Brazil, high-resolution pollen reconstructions are challenging due to the semiarid conditions that predominate in the region. However, when achievable, they provide insights about past landscapes in a densely populated region that is rich in both biodiversity and archaeological sites (Guidon et al., 1994; Cavalcante, 2018).

Here, we investigate the effects of climate variability and anthropic activity in a Cerrado ecotone in Northeastern Brazil by assessing the impact of conservation policies on biodiversity. Climate change and fire activity are pointed as the cause of changes in the past Cerrado environments (Cassino et al., 2020). Fire can be natural (wildfires) or human-induced (Gomes et al., 2018), what causes greater damage to the Cerrado vegetation. Anthropogenic burnings have been recorded since before the Brazilian colonial rangeland era (Sluyter and Duvall, 2016), practices that have been transformed through the centuries.

We present a high-resolution pollen, charcoal and grain-size record from a sediment core collected in the Sete Cidades National Park, in the State of Piauí, Northeastern Brazil. It hosts a rich archaeological collection and evidences of human occupation since more than 800 years (Cavalcante, 2018).

### Materials and Methods

A 100-cm core (SAC18) underwent chronology, pollen, charcoal and granulometry analyses. Eight samples were radiocarbon dated and calibrated with the SHCal20 curve (Hogg et al., 2020) in the RStudio software to before the current era/current era (BCE/CE) (Table). Subsamples (0.5 cm<sup>3</sup>) were taken at 1-cm intervals for pollen analyses (Faegri and Iversen, 1989; Santos and Ledru, 2021). At least 300 terrestrial pollen grains were counted and identified, excluding aquatic and water level-

related taxa. Subsamples (0.5 cm<sup>3</sup>) taken at 1-cm intervals were prepared for the macro charcoal analysis (Stevenson and Haberle, 2005). Particles were counted and measured under a stereoscope using WinSeedle software (Regent Instruments, Quebec, Canada). Granulometry was carried out about every 5 cm and followed the Wentworth scale for clastic sediments (Wentworth, 1922) (Figure 1).

## Results and Discussion

Our record shows that Cerrado vegetation remained dominant over the last 800 years with some reorganization of species assemblages, for instance, the dry-climate related taxa *Curatella* observed at the beginning of the record and the moisture-related tree/herbaceous taxa Myrtaceae and *Spathiphyllum*. Almost no fire activity was observed in the dry period between 1211 and 1300 CE, synchronous with the Medieval Climate Anomaly (Mann et al., 2009) (Figure). A marked increase in biomass burning between 1350 and 1500 CE was linked to anthropogenic activities, first those of Amerindians (Oliveira, 2002), then of European colonists dating back to the arrival of the cattle herders in 1650 CE (Alves, 2003), when fires suddenly stopped for one century (Figure). The second decrease in fire activity was observed shortly after the creation of Sete Cidades National Park in 1961 CE (Figure), resulting in the expansion of a moist forest cover.

## Conclusions

Here we show that vegetation in Northeastern Brazil responded to both global climate changes and anthropogenic activities, and how a national park can change the evolution and dynamics of the landscape after more than 700 years of anthropogenic pressure. Something worth considering when defining conservation policies for the 21st century.

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## Tables

Table. Radiocarbon dates of the SAC18 core with  $^{14}\text{C}$  calibrated BP ages (two standard deviations ( $2\sigma$ ) ranges) and calibrated CE ages obtained using the RStudio software.

Lab Code	Depth (cm)	$\delta^{13}\text{C}$	$^{14}\text{C}$ yrs BP	Age range (cal yrs BP) $2\sigma$	Calibrated yrs CE
SacA57449	29	-27,2	$-24 \pm 17$	-42 — -10	1975
SacA60357	44	-29,7	$99 \pm 30$	-14 — 197	1856
SacA57450	55	-26,4	$228 \pm 30$	98 — 283	1725
SacA61869	67	-29,5	$511 \pm 30$	471 — 538	1439
SacA64070	69	-24,5	$535 \pm 30$	509 — 560	1415
SacA60358	77	-24,1	$365 \pm 30$	310 — 467	1483 — 1640
SacA57451	86	-25,6	$165 \pm 30$	151 — 0	1799 — 1950
SacA57451	92	-29,1	$719 \pm 30$	660 — 880	1234

## Figures

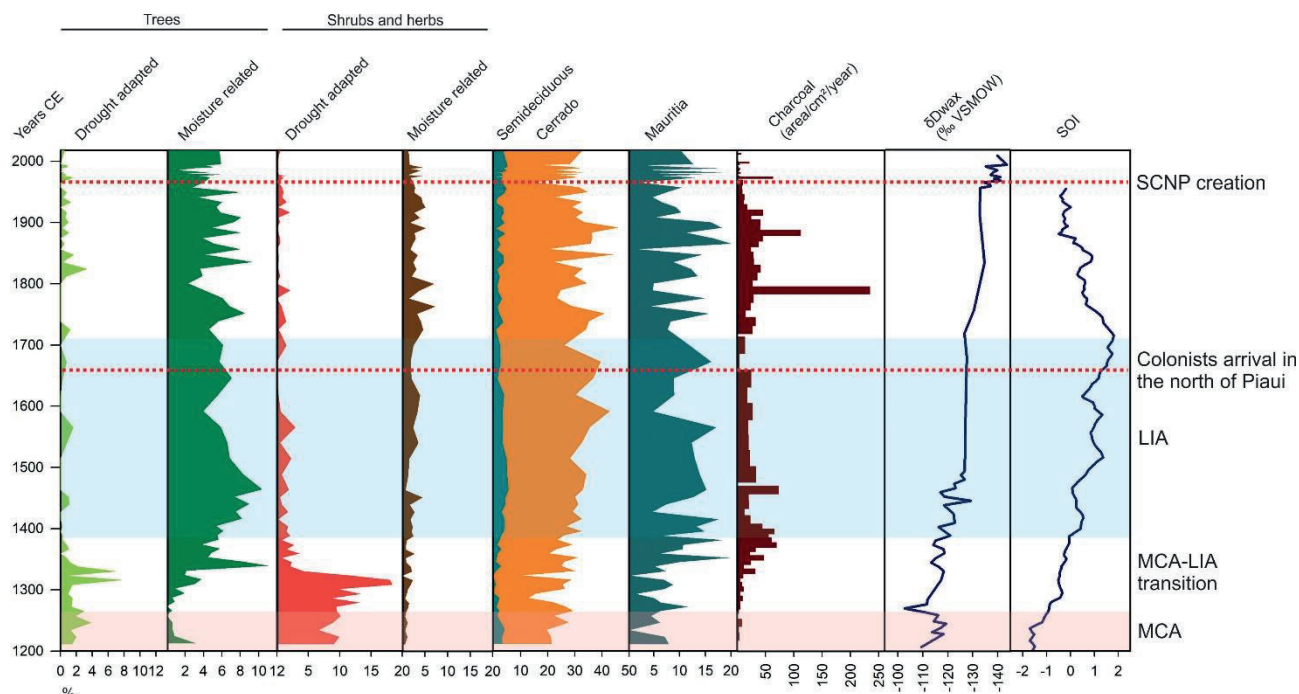


Figure. Comparison of the changes in vegetation composition and fire activity in the SAC18 record that testify to changes in precipitation in South America over the last 800 years. From left to right: 1) Drought adapted tree taxa (pale green) and moisture adapted tree taxa (dark green); 2) Drought adapted shrubs and herbaceous plants (red) and moisture adapted shrubs and herbaceous plants



(brown); 3) Semi-deciduous forest trees (blue) and Cerrado trees (orange); 4) *Mauritia* indicator of palm swamps; 5) Charcoal surface area; 6) Changes in  $\delta D_{wax}$ , a precipitation proxy from Utida et al. (2019); 7) Changes in the Southern Oscillation Index (SOI) from Yan et al. (2011). The end of the Medieval Climate Anomaly (MCA) is represented by the pink shaded area, and the Little Ice Age (LIA) by the blue shaded area. (from Xavier, in review, Anthropocene)



## TOPIC: FROM PALAEOPALYNOLOGY TO AEROPALYNOLOGY

### Global warming advances the flowering of plane tree in Malaga (southern Spain)

Antonio PICORNELL<sup>1</sup>, Rocío RUIZ-MATA <sup>2</sup>, Enrique DE GÁLVEZ-MONTAÑEZ <sup>3</sup>, Marta RECIO <sup>4</sup> M Mar TRIGO <sup>5</sup>

<sup>1</sup> Department of Botany and Plant Physiology, University of Malaga, Spain. [picornell@uma.es](mailto:picornell@uma.es)

<sup>2</sup> Department of Botany and Plant Physiology, University of Malaga, Spain. [roruizmata@uma.es](mailto:roruizmata@uma.es)

<sup>3</sup> Department of Botany and Plant Physiology, University of Malaga, Spain. [kikedegalvez@uma.es](mailto:kikedegalvez@uma.es)

<sup>4</sup> Department of Botany and Plant Physiology, University of Malaga, Spain. [martarc@uma.es](mailto:martarc@uma.es)

<sup>5</sup> Department of Botany and Plant Physiology, University of Malaga, Spain. [aerox@uma.es](mailto:aerox@uma.es)

#### Introduction

Temperature has been identified as one of the most influent meteorological variable in bud development and pollen release in many arboreal taxa (Geng et al. 2022). Consequently, global warming is affecting the flowering phenology of many species (Menzel et al. 2020). These changes have special relevance in the case of ornamental trees that produce highly allergenic pollen grains such as *Platanus orientalis* L. s.l., given that variations in the temporality of its Main Pollen Season (MPS) can affect the sensitive people living in cities (Lara et al. 2020). The aim of this study is to detect whether or not there is any significant trend in the main parameters of production and temporality of airborne *Platanus* pollen in Malaga, as well as to identify the possible causes.

#### Materials and Methods

Daily airborne pollen concentrations of *Platanus* were analysed for the period 1992-2021 in Malaga (southern Spain). 7-days moving means were calculated and the MPS was defined as the period between the first and last day of the year whose moving mean exceeded 3 pollen grains/m<sup>3</sup>, the *AeRobiology* R package being used (Rojo et al. 2019). Trends were calculated for both pollen and meteorological parameters. The thermal requirements of *Platanus* flowering were analysed by means of the *ChillR* R package, applying three different models for chilling (Utah Model, Chilling Hours and Chill Portions), as well as the Growing Degree Hours for forcing. The relevant periods for chilling and forcing accumulation were defined according to Partial Least Squares (PLS) regression analyses (Luedeling 2021).

#### Results and Discussion

There was a significant advance in the start of the MPS of *Platanus* pollen (2.5 days/decade) caused by a temperature increase during the period prior to flowering. This phenological trend has become



more pronounced since 2011 (9 days/decade). Despite there was not any significant trend detected for the chilling accumulation during the months of November and December, the global warming is increasing the forcing accumulation during January and February, so the forcing requirements of *Platanus* flowering are reached earlier and the phenophase advances. In line with these findings, the relevant dates for the chilling and forcing accumulations have advanced up to one month since 1992 according to the PLS analysis.

### Conclusions

Global warming has advanced the period of favourable temperatures for the forcing requirements of *Platanus*, so they are reached earlier and the flowering period advances.

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## TOPIC: FROM PALAEOPALYNOLOGY TO AEROPALYNOLOGY

### Comparative study of intradiurnal variations of airborne pollen in the city centre and on the outskirts of Malaga city (southern Spain)

Rocío RUIZ-MATA<sup>1</sup>, Antonio PICORNELL<sup>2</sup>, Enrique DE GÁLVEZ-MONTAÑEZ<sup>3</sup>, Marta RECIO <sup>4</sup>,María del Mar TRIGO<sup>5</sup>

<sup>1</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. roruizmata@uma.es*

<sup>2</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. picornell@uma.es*

<sup>3</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. kikedegalvez@uma.es*

<sup>4</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. martarc@uma.es*

<sup>5</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. aerox@uma.es*

#### Introduction

Airborne pollen is one of the main causative agents of allergic rhinitis in cities all over the world (Buters *et al.*, 2018). Pollen concentrations may vary throughout the day, not being equal in all the points of the city, due these concentrations are influenced by the presence of nearby pollen emission sources. Knowledge about the influence of these sources is of great interest for allergy sufferers when planning outdoor activities, especially in high populated and tourist cities such as Malaga (southern Spain).

The aim of this study was to analyse the intradiurnal variation patterns of the airborne pollen concentration both in the city centre and on the outskirts of Malaga as well as quantify the detected differences and establish the influence of the pollen emission sources present in the nearby areas.

#### Materials and Methods

The aerobiological data were obtained by means of two Hirst-type volumetric pollen trap (Hirst 1952). One of them installed on the rooftop of the Faculty of Sciences, University of Malaga (outskirts) and the other in the city centre. The study was carried out during the years 2017-2019, the most relevant pollen types being considered (those which represented more than 1% of the total pollen integral). The countings have been done hourly, considering only days without rainfall with a daily mean pollen concentration equal or greater than the mean of the main pollen season (MPS) (Galán *et al.*, 1991). Next, accumulated values of every two hours, expressed as percentages over the daily total, were calculated in order to study and compare the intradiurnal patterns.



## Results and Discussion

Despite the two sampling points are only 5 km apart, notable variations were detected in the intradiurnal patterns of some pollen types, while others showed similar behaviour. In the case of *Casuarina*, a very high peak was recorded in the outskirts while a more even daily distribution was noted in the city centre. This could be due to the vicinity of parks and gardens with ornamental *Casuarina* trees in the outskirts, which causes a pollen liberation that is directly reflected in the intradiurnal pattern. On the other hand, there are not as many *Casuarina* trees in the city centre, and the pollen recorded by the trap comes from more distant areas, making the pollen detected more homogeneously distributed. *Pinus* pollen shows similar circumstances since there are small woods near the outskirts sampling point, while the city centre has the nearest pine forests a bit further. The opposite was observed in the case of *Parietaria*: there was a pronounced peak in the city centre where the *Parietaria* sources are more abundant given the number of abandoned plots, the registers being more diffuse in the outskirts. However, for other pollen types, the intradiurnal patterns were similar in both sampling sites, such as for the *Quercus*, probably due to there are no nearby pollen sources that could specifically affect one or another sampling point.

## Conclusions

The intradiurnal patterns of the pollen types studied were different between sampling points within the same city because of the location of the nearby pollen emission sources. This reflects the need to conduct aerobiological sampling in several points of a same city in order to obtain more precise and useful information for the allergic population.

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## TOPIC: FROM PALAEOPALYNOLOGY TO AEROPALYNOLOGY

### VARIATIONS IN POLLEN CALENDARS ALONG TIME AT MALAGA (SOUTHERN SPAIN)

Enrique DE GÁLVEZ-MONTAÑEZ<sup>1</sup>, Antonio PICORNELL<sup>2</sup>, Rocío RUIZ-MATA<sup>3</sup>, Marta RECIO<sup>4</sup>, María del Mar TRIGO<sup>5</sup>

<sup>1</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. kikedegalvez@uma.es*

<sup>2</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. picornell@uma.es*

<sup>3</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. roruizmata@uma.es*

<sup>4</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. martarc@uma.es*

<sup>5</sup> *Department of Botany and Plant Physiology, University of Malaga, Spain. aerox@uma.es*

#### Introduction

Pollen calendars are graphical representations that summarize the general annual dynamics of the more abundant airborne pollen types detected in the atmosphere of a certain location. To elaborate them, it is recommended to have continuous pollen sampling data from a period of, at least, 5 years in order to such pollen calendar be representative. However, the annual pollen dynamics can suffer changes in a medium or long term due to climate and land use changes, so the pollen calendars can therefore, show variations over time.

The aim of this study was to detect whether or not changes have occurred in the pollen calendar of Malaga city (southern Spain) during a 30-year period, as well as to analyze their possible causes.

#### Materials and Methods

The aerobiological data were obtained by means of a Hirst-type volumetric pollen trap installed on the roof of the Faculty of Sciences, University of Malaga (Spain). Pollen samples were mounted and counted following the methodology proposed by the Spanish Aerobiology Network (Galán et al. 2007). A pollen calendar was elaborated for each 10-year period (i.e., 1992-2001, 2002-2011 and 2012-2021) using the AeRobiology R package (Rojo et al. 2019). This computational tool integrates the methodology developed by Spieksma et al. (1995), and the exponential classes proposed by Stix and Ferretti (1974).

#### Results and Discussion

Despite the three pollen calendars obtained were elaborated for the same sampling location, there were some important changes in the intensity and temporality of the airborne concentrations detected for some pollen types. These changes can be related to variations in the land uses near the





city and temperature variations due to climate change. For example, in the case of *Olea* pollen, a raise was observed in pollen levels detected during the last decade due to an increase in the olive crop surface in nearby areas. This pollen type has also advanced the start of its pollination period, maybe due to the temperature increase in the months prior to flowering. In other cases, such as *Pinus* and *Amaranthaceae*, their pollen concentrations decreased due to the land use changes promoted by the city growth. Moreover, an increase of the levels of *Platanus* pollen can be observed due to the ornamental trees that were planted in the west part of the city.

### Conclusions

Pollen calendars should be updated along time in order to maintain their utility, given that it is possible to find some trends in the temporality and intensity of the pollen season of some taxa that can modify substantially this graphical representation.

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## Comparison of three Swisens Poleno with Hirst

**EUMETNET AutoPollen COST ADOPT Intercomparison Campaign 2021 <sup>1</sup>,  
Reto Abt <sup>2</sup>, Elias Graf <sup>2</sup>, Cédric Spring <sup>2</sup>, Yanick Zeder <sup>2</sup>**

<sup>1</sup> *EUMETNET AutoPollen COST ADOPT Intercomparison Campaign 2021*

<sup>2</sup> *Swisens AG, sales@swisens.ch*

### Introduction

To obtain pollen concentration values in real time in a region, an automatic measurement network is needed. A central point for a measurement network is the standardization of the measurement instruments, so that the measured pollen concentrations are comparable. We show some results from the EUMETNET AutoPollen COST ADOPT Intercomparison Campaign 2021 in which Swisens AG participated with three instruments.

### Materials and Methods

The campaign was held in the Helmholtz Center Munich (Germany) from 3 March - 14 July 2021. As a reference against which the automatic instruments were compared, two to four Hirst traps were used. Volumetric sampler



*Figure 1: Measurement site*

operation and sample processing is done according to European Aerobiology Society standards (Galán et al. 2007 and 2014). The results of the following automated pollen monitors manufactured by Swisens AG are presented: SwisensPoleno Mars, SwisensPoleno Neptune, SwisensPoleno Jupiter (Figure 1). The identification algorithms only used the holography images. The evaluations were made by the members of the project group which are listed in the "About" tab of the website with the results (see references for link). For



comparison of the automated monitor with Hirst, the  $R^2$  coefficient based on averaged 3-hour periods was determined for the whole measurement period. 'Hirst' shows the mean over all Hirst traps that were working at any given time during the campaign (n=2-4). The scaling factor SF was calculated as follows: SF = (mean Hirst/mean automatic device) \* raw data.

## Results and Discussion

Table 1 shows the correlation  $R^2$  between each type of SwisensPoleno and the 'Hirst' based on 3-hourly data. All three instruments agree well with the Hirst for all varieties and show similar results. This is shown by the  $R^2$  mean as well as the sum of squared deviations in the last column which are very similar.

Instrument	Correlation factor $R^2$ of comparison 'Hirst' with SwisensPoleno based on 3-hourly averaged data						Total Pollen	$R^2$ mean	SUMQUA DABW
	Alnus	Betula	Fraxinus	Pinus	Poaceae	Quercus			
SwisensPoleno Jupiter (algorithm MeteoSwiss)	0.75	0.78	0.62	0.79	0.56	0.68	0.60	0.7	0.04
SwisensPoleno Neptune (algorithm MeteoSwiss)	0.70	0.79	0.71	0.81	0.57	0.67	0.60	0.71	0.04
SwisensPoleno Mars (algorithm MeteoSwiss)	n.A.	0.81	0.57	0.78	0.52	0.67	0.49	0.67	0.04

Table 1:  $R^2$  based on 3-hourly data Swisens Poleno compared with Hirst

## Conclusions

These results indicate that the comparability of the SwisensPoleno instruments with each other should also be very good and meet the requirements for standardisation. The planned publication of the EUMETNET AutoPollen COST ADOPT Intercomparison Campaign 2021 will confirm this.

## References

EUMETNET AutoPollen COST ADOPT Intercomparison Campaign 2021  
[https://autopollen-interactive.shinyapps.io/022\\_APP\\_AUTOPOLLEN/](https://autopollen-interactive.shinyapps.io/022_APP_AUTOPOLLEN/)

## Acknowledgements (Funding)

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## TOPIC: FROM POLLEN BIOLOGY TO MORPHOPALYNOLOGY

### COMPARATIVE TRANSCRIPTOME ANALYSIS OF MICROSPORE REPROGRAMMING TO EMBRYOGENESIS IN *BRASSICA NAPUS*

Elena CARNEROS<sup>1</sup>, Yolanda PÉREZ-PÉREZ<sup>1</sup>, Cristina RUEDA<sup>1</sup>,  
Elena CARO<sup>2\*</sup>, Pilar S. TESTILLANO<sup>1\*</sup>

<sup>1</sup>Pollen Biotechnology of Crop Plants group, Center of Biological Research Margarita Salas, (CIB) CSIC, Madrid, Spain

<sup>2</sup>Centre for Plant Biotechnology and Genomics (CBGP), UPM-INIA-CSIC, Pozuelo de Alarcón, Spain

\*Co-corresponding authors: [elena.caro@upm.es](mailto:elena.caro@upm.es) ; [testillano@cib.csic.es](mailto:testillano@cib.csic.es)

*In planta*, microspores follow the gametophytic program to produce pollen grains for fertilization. *In vitro*, by stress treatment, microspores can be reprogrammed towards embryogenesis. Microspore embryogenesis is a biotechnological process that allows to rapidly obtain doubled haploid (DH) plants to accelerate breeding programs. However, its application is limited in many crop species due to its low efficiency. Despite knowledge gained in recent years, the complex regulatory network of microspore reprogramming and embryogenesis is still far to be fully elucidated.

In this work, we carried out a comparative transcriptome analysis in microspore cultures of *Brassica napus*, model system for this process. We performed RNAseq analyses of gene expression for isolated microspores cultures, prior to embryogenesis induction (day 0) and from cultures early after induction, at proembryo stage, first morphological sign of embryogenesis initiation (day 4). Preliminary bioinformatic analyses comparing the two time points revealed 42203 differentially expressed genes (DEGs), from which around 23000 were upregulated in proembryos and 19000 were downregulated. Gene ontology analyses showed that the transition from microspores to proembryos was marked by DEGs involved in ribosome biogenesis and function, RNA processing and metabolism, carbohydrate metabolism, cell wall enzymes and regulators, and several families of transcription factors, among other pathways.

Further analyses are in progress. Results will shed light on transcriptional changes accompanying microspore reprogramming and embryogenesis initiation, information that can provide useful insights to broaden our knowledge on the molecular mechanisms governing microspore embryogenesis and its application to improve DH production in crop breeding.

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## INVOLVEMENT OF ENDOGENOUS AUXIN AND CYTOKININ DURING MICROSPORE EMBRYOGENESIS OF *BRASSICA NAPUS*

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Yolanda PÉREZ-PÉREZ<sup>1</sup>, Aurelio GÓMEZ-CADENAS<sup>2</sup>, Alfonso ALBACETE<sup>3</sup>, María-Teresa SOLÍS<sup>4</sup>,  
Pilar S. TESTILLANO<sup>1</sup>

<sup>1</sup>Pollen Biotechnology of Crop Plants group, Center of Biological Research Margarita Salas, (CIB) CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain, [yperez@cib.csic.es](mailto:yperez@cib.csic.es); [testillano@cib.csic.es](mailto:testillano@cib.csic.es)

<sup>2</sup>Dep. CC Agrarias y del Medio Natural, Univ. Jaume I, Castellón, Spain, [aurelio.gomez@uji.es](mailto:aurelio.gomez@uji.es)

<sup>3</sup>Plant Production and Agrotechnology, IMIDA, C/ Mayor s/n, 30150 La Alberca, Murcia, Spain, [albacete@carm.es](mailto:albacete@carm.es)

<sup>4</sup>Genetics, Microbiology and Physiology, UCM, Ciudad Universitaria, 28040, Madrid, Spain, [msolis03@ucm.es](mailto:msolis03@ucm.es)

*In vitro*, isolated microspores, at the responsive stage of vacuolated microspore, can be reprogrammed by stress treatments, becoming totipotent cells and producing haploid and doubled-haploid (DH) embryos and plants. This process, known as stress-induced microspore embryogenesis, is widely used in plant breeding to rapidly obtain DH plants, which represent a source of new genetic variability, fixed in complete homozygous plants in only one generation step. Production of DH lines is currently a standard method of the creation of new material in many modern breeding programs, although its application is limited in many crop species due to low efficiency. Despite knowledge gained in recent years, the complex regulatory network of cell reprogramming leading to embryogenesis is still far to be fully elucidated. Several studies have revealed the relevance of auxins and cytokinins as regulating factors for cell fate, proliferation and differentiation. However, much less is known on the involvement of endogenous auxins and cytokinins in the process of microspore embryogenesis.

In the present work we have analyzed the dynamics and possible role of endogenous auxin and cytokinins during stress-induced microspore embryogenesis in *Brassica napus* L., a model system to study this process in which no exogenous phytohormones are present in the culture media. The study was performed by a multidisciplinary approach. We use the technique of qRT-PCR to analyze the expression profile of auxin and cytokinin biosynthesis, signaling and degradation genes at different developmental stages of rapeseed microspore embryogenesis, and immunofluorescence assays to study both hormones localization by using monoclonal antibodies against the cytokinin trans-zeatin and de auxin indole-3-acetic-acid (IAA) (the most abundant cytokinin and auxin in plants, respectively). Furthermore, samples were analyzed by high performance liquid chromatography linked to tandem mass spectrometry (HPLC) in order to know the dynamic of cytokinin and auxin



levels during microspore embryogenesis progression. We also performed in vitro functional analyses with PI-55 (a compound that inhibits cytokinin signaling), Kynurenine (inhibitor of auxin biosynthesis), N-1-naphthylphthalamic acid (NPA) (inhibitor of auxin transport) and  $\alpha$ -(p-Chlorophenoxy) isobutyric acid (PCIB) (inhibitor of auxin action), to analyze the possible involvement of both fitohormones during microspore embryogenesis initiation and progression and its effects on development.

The obtained results indicate that endogenous auxins and cytokinins biosynthesis and signaling were required for the correct progression of microspore embryogenesis, playing key roles during different developmental stages of the process. Auxin is induced and necessary for cell reprogramming, embryogenesis initiation and progression, whereas cytokinin is required mainly at advanced developmental stages, during embryo differentiation.

#### Acknowledgements

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## TOPIC: FROM POLLEN BIOLOGY TO MORPHOPALYNOLOGY

# GOOD PRACTICE GUIDE. EFFECT OF BEEKEEPING MANAGEMENT ON THE QUALITY OF BEE POLLEN.

Amelia-V. GONZÁLEZ-PORTO <sup>1</sup>, Cristina PARDO-MARTIN <sup>1</sup>

<sup>1</sup> *Laboratorio de Productos de la Colmena, IRIAF-CIAPA, Camino San Martin s/n, 19180 Marchamalo, Guadalajara, Spain, [avgonzalezp@jccm.es](mailto:avgonzalezp@jccm.es), [cristinapardo52@gmail.com](mailto:cristinapardo52@gmail.com)*

### Introduction

Bee pollen is the union of pollen grains collected by bees to feed larvae and young bees, to which they add caking substances (honey, nectar, salivary secretions...) influencing on their final composition. For humans it is a highly nutritious food when consumed dry and also with healthy properties when consumed fresh (frozen). Spain is the leading producer of bee pollen in Europe and one of the largest in the world, producing between 1,000 and 1,500 tonnes, depending on annual weather conditions.

Objectives: To design a guide to good practices and to know if your application in the harvest of bee pollen influences or not at the final quality of the product.

### Materials and Methods

56 bee pollen samples have been analyzed, 33 of commercial pollen with unknown collection practices and 23 of samples collected in CIAPA apiaries following the guide to good practices developed for the project covering all the collection phases (pre-collection, collection and transport to the handling area, handling and conservation and labeling), indicating the critical points CP. The total protein content in dry pollen (40°C/4 h.) has been analyzed by the Kjeldahl Method and vitamin C by HPLC techniques with UV detection. The results obtained are presented in units of matter on a dry basis.

### Results and Discussion

Guide to good practices. A) Pre-harvest: vigor of the colony, location, placement and distance between hives and apiaries, type of pollen traps, phenology and climatology of the area. CP general hygiene and avoid rainy days. B) Collection and transport: Exposure no more than 12 hours, removing at night, no more than 15 days by hive and one day yes and two no. Using smoker only if is necessary.

Transport to the handling area always cold, do not exceed 12 hours. PC, no prolonged sun or air exposure and immediate transfer to handling area at 4°C. C) Handling and conservation: Immediate handling in hygienic conditions (beekeeper and work area), otherwise freeze until handling. Manual pollen cleaning (tweezers and/or spatulas) or by ventilation (air current). Dried pollen must be dried immediately in stoven (40°C/4 h.), packed and stored for 18 months in cool environment and out of direct sunlight. PC, avoid exposure to sunlight or air and do not store in refrigerator. Fresh pollen frozen at -20°C, quickly packed in airtight containers and stored for 6-12 months at -20°C. PC, freeze no more than 15 k and respecting the cold chain. D) Labeling: values and properties information and best before date (including time from harvest to packaging).

The average value of the protein content is 18,71 g/100g (14 to 24 %) in the commercial samples and 18.45 g/100 g (15 to 22 %) in the own samples, and that of vitamin C is 12,98 mg/100 g (5 to 21 mg/100g) and 33,84 mg/100g (14 to 54 mg/100g) respectively.

### Conclusions

A correct observation and follow-up by the beekeeper of a good practice guide is not only a critical element but also provides the possibility of obtaining a high-quality bee pollen.

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## TOPIC: FROM POLLEN BIOLOGY TO MORPHOPALYNOLOGY

# MELISSOPALYNOLOGICAL ANALYSIS OF LIGURIAN REGIONAL PARKS (ITALY) HONEYS

Davide ATTOLINI<sup>1</sup>, Martina LEONE<sup>2</sup>, Maria Angela GUIDO<sup>1</sup>, Carlo MONTANARI<sup>1</sup>

<sup>1</sup>Laboratory of Environmental Archaeology and History (DAFIST-DISTAV), University of Genova, Italy  
davide.attolini@edu.unige.it, maria.angela.guido@unige.it, carlo.montanari@unige.it

<sup>2</sup>Department for the Earth, Environment and Life Sciences (DISTAV), University of Genoa,  
martinaleone3916@gmail.com

### Introduction

Since several decades, melissopalynology has been proved to be a powerful tool for the study of the botanical and geographical origin of honey. Despite its proven utility, melissopalynological analysis still struggle to be recognized as mandatory in many countries (e.g. in Italy). Liguria (N-Italy) is a crescent shaped region characterized by an extremely heterogeneous landscape, mainly due to the close proximity of the mountains to the coast: this leads to a peculiar geomorphological diversity and a high biodiversity. Regarding honey production, the ligurian region is characterized mainly by chestnut, wildflower, and heather honey, even if many unifloral honey are produced in the area (e.g. ivy, linden, rhododendron) (Persano-Oddo et al. 2007). Chestnut cultivation in Liguria has been historically important, and the species is, to this day, still widespread. Since chestnut pollen is overrepresented in honey, it can be found in high percentage in most of the local honeys. Some alien species have been used for honey production for a long time, such as black locust (*Robinia pseudacacia*), while others only recently, e.g. glossy privet and tree of heaven (*Ligustrum lucidum*, *Ailanthus altissima*). Glossy privet and black locust, in particular, have a very abundant and noticeable flowering that attracts a lot of pollinators, thus also attracting local honey producers. This research stems from the need of a better characterization of Ligurian honey, while addressing some issues raised by honey producers joining the Ligurian parks annual honey competition ("Mieli dei Parchi della Liguria"). The study areas consist in the buffer zones of two Natural Regional Parks: Portofino Park is located on a promontory along the east Riviera, while Aveto Park consists of 3 areas, located in the mountainous interland.

### Materials and Methods

This study was carried out on 28 honey samples, 14 from the buffer area of Portofino Natural Regional Park and 14 from the buffer of Aveto Natural Regional Park. The samples were

provided by the organizers of the annual regional honey completion “Mieli dei Parchi della Liguria”, whom we thank for making this research possible. These samples were sourced by the organizers of the annual honey competition from a few local producers. They labelled them as wildflower (15), chestnut (6), heather (4), honeydew (2), and privet (1). Depending on the low quantity of available sample material, both qualitative and quantitative analyses were carried at the same time, using *Lycopodium* spores as marker. To achieve a higher detail in pollen identification, acetolysis was used. Quantitative Maurizio classes were calculated for each sample (Louveaux et al. 1978).

### Results and Discussion

Honey characterization through melissopalynological analysis mostly agrees with the designation given by the honey producers. In 21 samples, chestnut pollen reaches percentages > 83%, often exceeding 95%. This happens for chestnut honey, but also for many wildflower honeys. Privet honey, if analysed only through melissopalynological analysis, would be considered a chestnut honey due to the chestnut over-representation. Local honey producers suggest that privet has a large influence on the taste, so much as to change it in a noticeable way. Heather honey is characterized by large quantities of *Erica arborea* pollen, and variable percentages of chestnut pollen. Heather and chestnut flowering times partially overlap; thus, this difference could be possibly explained by analysing honey extraction times. The 2 analysed honeydew honeys are mainly characterized by chestnut (53% and 95%) and almost no honeydew indicators. Regarding quantitative analysis, most samples are in agreement with Maurizio classification, with very few exceptions, such as privet honey.

### Conclusions

While the taste of wildflower honey is known to change on the basis of the species foraged by bees, unifloral honeys are required to have a precise taste. Privet pollen was found in many Portofino Park samples, confirming the suspicion of local honey producers on the reason why some chestnut honeys tasted different than usual: the issue is not that the taste is unpleasant, rather that it is clearly distinguishable from chestnut honey. If the producers aimed at producing chestnut honey, they could address this issue by moving their beehives and paying close attention to privet flowering time. Nevertheless, a better characterization of this honey, through detailed melissopalynological, chemical and taste analysis, could possibly lead to a new product. This study thus restates how useful it is to carry out melissopalynological analysis and the regional characterization of honey.

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## MELISSOPALINOLOGY: THE LOUVEAUX METHOD ON HONEY SAMPLES AND THEIR ALLERGENIC POTENTIAL

Nadia TROBIANI <sup>1</sup>, Silvia BARTOLACCI <sup>1</sup>, Eva SANTOLINI <sup>2</sup>

<sup>1</sup> Marche Environmental Protection Agency (ARPAM), Ancona, Italy;  
*nadia.trobiani@ambiente.marche.it*

<sup>1</sup> Marche Environmental Protection Agency (ARPAM), Ancona, Italy;  
*silvia.bartolacci@ambiente.marche.it*

<sup>2</sup> University of Camerino (UNICAM), Camerino, Italy;  
*eva.santolini@studenti.unicam.it*

### Introduction

The research arises from the interest in investigating the pollen contained in the various honey samples analyzed. Honey is a product closely linked to the production area, the research starts from the desire to explore the complexity of the microscopic nature of pollen and to investigate its composition characteristics since they derive mainly from the type of foraged flora.

### Materials and Methods

The melissopalinoLOGY, the study of pollen in honey; the Louveaux method (Louveaux, 1978) was born in Germany with the aim of determining the botanical origin of honey, to distinguish the German one from the foreign ones; this method was then extended in the context of the definition of the botanical origin because the pollen spectrum of the sample reflects the floristic situation of the place where it was produced and essentially involves the phases of adding water and centrifugation phases, which in the complex lead to the total dissolution and elimination of sugars. The pollen content shall be observed under a microscope using the same procedure as the aerobiological analysis described in national and European standards. Although this discipline is still in the attention of a few experts, in our case we tried to obtain data that could provide useful information to verify the respect of the botanical origin declared on the label for the honeys taken on the market or declared by the local producers of our 24 samples subjected to analysis (23 of nectar honey and 1 of honeydew). Fundamental the presence of a comparison Palinoteca, that is a collection of slides after the collection of fresh pollen, taken from plants of certain identification.

For some granules the genus has been determined, for others only the family to which they belong; the obtained data have been summarized in diagrams that allowed to follow the qualitative and quantitative development of the plant elements in each honey studied. From the microscopic observation of the pollen grains it is possible to identify the plants that produced them, thus going back to a particular type of vegetation and, consequently, to the production area.

The percentage values for each nectar pollen identified have been calculated to determine the frequency classes, since it is essentially their contribution that gives the honey a precise identity in terms of botanical origin.

### Results and Discussion

Starting from an exploratory analysis, the results obtained with melissopalynology provide assessments that, combined with sensory and physical analysis, represent a useful control tool to address the growing trend of food fraud and to attribute geographical origin as an element of consumer choice. The contribution that the melissopalynological analysis could make to Italian honeys is to know the national products more accurately than the remote image with which the flora of bees of other countries is represented. The work of several researchers, has allowed to have a mapping of the main national productions; this is essential to verify the veracity of voluntary territorial indications, but also to interpret the spectra of honey in which only the assessment of national origin is required. In fact, in the interpretation of a pollen spectrum all available weapons are used, not only positive definitions, but also those of exclusion. By comparing the percentages of the pollen taxa contained in the honeys analyzed with those reported in the characterization cards, it was possible to verify the correspondence or not of the samples to their botanical origins. The samples were analyzed in duplicate to reduce operator uncertainty and to ensure identification of the largest number of species/families present and recognition of botanical families. The results provided a fairly clear picture of a first assessment of the conformity of the reference values.

In general, most of the samples examined, both those taken on the market and those of local producers, are in line with the data obtained.

Bibliographical research on cross allergies related to the presence of pollen in honey has established that allergic forms to honey are still a rare condition and the incidence does not exceed 0.001%. Studying the few reported cases, it is difficult to say that pollen proteins are the only and direct responsible for the allergic manifestations that follow the ingestion of honey; however, if we wanted to attribute the responsibility, we could refer in particular to Compositae pollens, such as *mugwort*, *ragweed* and *dandelion*.

### Conclusions

This work, while identifying many possible triggers for allergic reactions, has detected a very low number of cases for ingestion of honey; this is why the study can be a starting point for a study focusing on the allergenic potential of the pollen contained in honey and the possible etiological role of Compositae pollen in patients sensitized to the same species.

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## TOPIC: FROM POLLEN BIOLOGY TO MORPHOPALYNOLOGY

### TOWARDS THE REALIZATION OF AN ATLAS OF POLLEN OF THE MOST COMMON EXOTIC PLANTS OF WESTERN SICILY

Claudia COTTONE<sup>1</sup>, Anna ABITA<sup>2</sup>, Nicolò TIRONE<sup>2</sup>, Rossana AGAZZANI<sup>2</sup>, Daniela RINAUDELLO<sup>2</sup>,  
Gianniantonio DOMINA<sup>1</sup>

<sup>1</sup>*Department of Agricultural, Food and Forest Science (SAAF), University of Palermo, [claudia.cottone01@unipa.it](mailto:claudia.cottone01@unipa.it),  
[gianniantonio.domina@unipa.it](mailto:gianniantonio.domina@unipa.it)*

<sup>2</sup>*Regional agency for environmental protection of Sicily (ARPA Sicilia), [abita@arpa.sicilia.it](mailto:abita@arpa.sicilia.it), [ntirone@arpa.sicilia.it](mailto:ntirone@arpa.sicilia.it),  
[ragazzani@arpa.sicilia.it](mailto:ragazzani@arpa.sicilia.it), [drinaudello@arpa.sicilia.it](mailto:drinaudello@arpa.sicilia.it)*

#### Introduction

The idea of drafting a pollen atlas of the most common exotic plants found in western Sicily stems from the need to have a support tool for recognition of the different types of pollen grains present on the aerobiological sampling slides.

In Italy there are atlases for the recognition of the main allergenic species (e.g. Ciampolini and Cresti 1981, Feliziani 1986, Travaglini et al. 2017) and various palynological reports on allergenic species and native species of melissopalynological interest (Accorsi 1985 and following). Nevertheless, the operators of the monitoring centers often find necessary to identify pollen grains, even locally abundant, which are not yet included in the reference materials.

Sicily, in the center of the Mediterranean basin, hosts a very diversified exotic flora with strong sub-tropical characteristics. The American and Asian chorotypes are highly represented. To our knowledge, there are no specific atlases for southern Italy. Synoptic contributions for the Mediterranean are few (Reille 1992). The possibility of verifying the identification of species in atlases from different parts of the world (e.g. Kremp 1965) is difficult to apply in practice. Hence the need to provide a useful tool in the Mediterranean anthropogenic environment.

#### Materials and Methods

The atlas in preparation will include a worksheet for each of the taxa studied. In the sheets there is the description of the taxon and of the corresponding granule, with relative iconographic documentation.

Pollen grains were collected from species identified with certainty and photographed after the preparation of microscopy slides using glycerin gelatin with fuchsin for staining, as described in the



standardized method (Erdtman 1969). The measurements of the granules reported are obtained from Sicilian material. The identification of the species and the preparation of the slides were carried out by the authors, at the SAAF Department of the University of Palermo. The realization of this tool will require observations indifferent vegetative seasons.

### Results and Discussion

The project includes the creation of about seventy worksheets with the most commonly used species for street furniture in western Sicily (e.g. *Brachychiton acerifolius* (G.Don) F. Muell., *Ceiba speciosa* (A. St.-Hil., A.Juss. & Cambess.) Ravenna, *Koelreuteria paniculata* Laxm.) or present as naturalized aliens (e.g. *Parkinsonia aculeata* L., *Vachellia karroo* (Hayne) Banfi & Galasso). This tool is mainly aimed at operators of aerobiological monitoring centers operating in western Sicily, but its use can also be advantageous to operators of aerobiological monitoring centers operating on the national territory and for students of Aerobiology and Allergology courses in the Italian universities. The atlas will be made available online through the ARPA Sicilia website (<https://www.arpa.sicilia.it/>). The possibility of printing will also be evaluated depending on financial availability.

As suggested by Travaglini et al. (2017) even allergy doctors, and pollen allergic patients themselves can be helped in identifying the species and distinguish those responsible for allergy.

### Conclusions

The Atlas of pollen of most common exotic plants of western Sicily will be a valuable aid to operators, but certainly will not be exhaustive because will have to be integrated with other sources e.g. those for the identification of the native flora; but for this, there are already several contributions.

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## TOPIC: FROM POLLEN BIOLOGY TO MORPHOPALYNOLOGY

### IN SEARCH OF NEW TOOLS TO TRACE BACK THE HISTORY OF OLIVE CULTIVATION

Anna Maria MERCURI<sup>1</sup>, Cristina RICUCCI<sup>1</sup>, Grant SNITKER<sup>2,3</sup>, Assunta FLORENZANO<sup>1</sup>, Paola TORRI<sup>1</sup>,  
Gabriel SERVERA-VIVES<sup>1,4</sup>

<sup>1</sup> Laboratorio di Palinologia e Paleobotanica, UNIMORE (Italy):  
annamaria.mercuri@unimore.it/ serveravives@gmail.com

<sup>2</sup> Center for Forest Disturbance Science, USDA Forest Service, Southern Research Station (USA)

<sup>3</sup> Oak Ridge Institute for Science and Education, US Department of Energy (USA)

<sup>4</sup> ArqueoUIB, Departament de Ciències Històriques i Teoria de les Arts. Universitat de les Illes Balears (Spain)

#### Introduction

The olive tree (*Olea europaea* L.) is a prominent feature of current circum-Mediterranean landscapes and an essential element of modern Mediterranean agriculture. Wild olive (*Olea europaea* L. subsp. *europaea* var. *sylvestris*) is common in macchia and garrigues across the Mediterranean climate region. The early management of (wild) olive trees has often been related to village development and the “secondary products revolution” in the eastern Mediterranean (Langgut et al., 2019). The study of olive tree domestication in the Mediterranean still remains a slippery question despite recent advances from several disciplines such as DNA (Besnard et al., 2013) and macro-botanical analysis (Terral et al. 2021).

The Balearic Islands are crucial for the study of biocultural interactions during the Holocene due to their presumably late human colonisation at ca. 2500-2300 BC, compared to other Mediterranean islands. This cultural period also coincides with the expansion of *Olea* pollen and other sclerophyllous taxa in paleoenvironmental sequences, but the triggers of these changes still remain unclear. The EU-funded OLEA-project aims to focus on the drivers and timing of the spread of *Olea* macchia and olive tree cultivation as a central feature of the current Balearic mosaic landscape. In this poster we propose new ways to decipher olive tree cultivation through the development of new image analysis tools and new integrated methodological approaches.

#### Materials and Methods

In the framework of OLEA-project, four workpackages have been developed to further understand human-nature interactions and island colonization processes regarding the mosaic landscape



formation and macchia formation in the Balearics: 2 off-site paleoenvironmental sequences (WP2), 3 on-site (WP3), high-resolution pollen morphology on modern *Olea* samples (WP4) and modern pollen analogues (WP5). In this poster, we focus on WP4 dealing with the pollen morphology of ancient and modern olive tree cultivars from Mallorca to detect potential morphotypes that could be related to wild vs cultivated olive or to specific agronomic varieties of the Balearic Islands (e.g. “mallorquina”, “olivoner”, “morisca” or “olivoner”). To do so, a new image-analysis software called OLEAtool (<https://github.com/gsnitker/OLEAtool>) for morphopalynological research was developed to facilitate the morphological analysis and measurements of *Olea* pollen. OLEAtool is a macro extension for use with the open-access and freely available image-analysis software, ImageJ (Fig. 1).

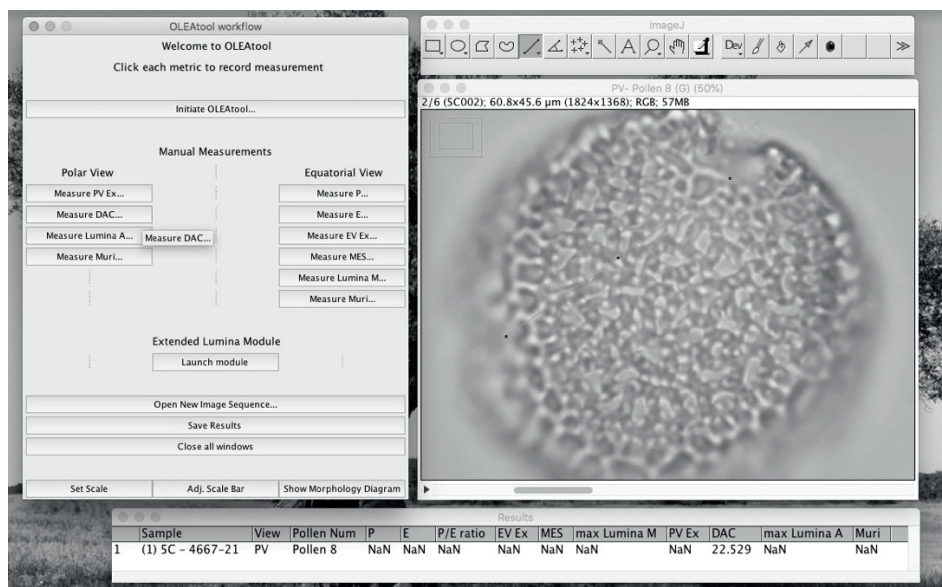


Figure 1- Screenshot of OLEAtool showing the multiple morphological parameters that can be measured using its interface.

## Results and Discussion

A total of 13,200 measurements in 1200 olive pollen grains were undertaken in 20 samples (8 cultivated olive varieties and 4 different location of wild olives). Descriptive statistics results show a) no obvious differences between wild and cultivated *Olea* pollen has been detected; b) “olivoner” and “morisca” old varieties present a clear distinction from other varieties. The similar morphology between wild and cultivated forms of olive tree highlights the resilience of *Olea europaea*, while the high intra-variability within cultivated varieties from the Balearics underlines their adaptative potential, meaning that olive trees maintained a great genetic variability (Ricucci, 2022).

## Conclusions

OLEAtool has proven to be a powerful software tool for morphopalynological analysis of *Olea* pollen by increasing the quality, quantity, and speed at which these data can be collected. New forthcoming research endeavours will include developing new statistical analysis to better discriminate wild vs cultivated olive tree pollen and between agronomic varieties. Also, the proposed morphotypes will be applied both to fossil pollen data obtained in off-site and on-site sequences from the Balearic Islands to trace back the onset of olive cultivation back in time.

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## AUTOMATED DETECTION AND CLASSIFICATION OF *VITIS* POLLEN GRAINS FROM POLLEN TRAPS

Betty GIMENEZ<sup>1</sup>, Odile PEYRON<sup>1</sup>, Céline DEVAUX<sup>1</sup>, Sébastien JOANNIN<sup>1</sup>, Doris BARBONI<sup>2</sup>, Luc BEAUFORT<sup>2</sup>, Laurent BOUBY<sup>1</sup>, Sandrine CANAL<sup>1</sup>, Nathalie COMBOURIEU<sup>3</sup>, Thibault de GARIDEL-THORON<sup>2</sup>, Yves GALLY<sup>2</sup>, Sarah IVORRA<sup>1</sup>, Angèle JEANTY<sup>1</sup>, Bertrand LIMIER<sup>1,4</sup>, Jean-Frédéric TERRAL<sup>1</sup>, Jérôme PASQUET<sup>5</sup>

<sup>1</sup> ISEM, [betty.gimenez@umontpellier.fr](mailto:betty.gimenez@umontpellier.fr)

<sup>2</sup> CEREGE, Aix-Marseille Université, CNRS, IRD, INRAE, [beaufort@cerege.fr](mailto:beaufort@cerege.fr)

<sup>3</sup> HNHP, [nathalie.nebout-combourieu@mnhn.fr](mailto:nathalie.nebout-combourieu@mnhn.fr)

<sup>4</sup> INRAE, Montpellier

<sup>5</sup> AMIS-UPV 3 and UMR TETIS, [jerome.pasquet@univ-montp3.fr](mailto:jerome.pasquet@univ-montp3.fr)

### Introduction

The identification of pollen grains provides valuable information for a diversity of fields, such as pollination ecology, paleobotany, melissopalynology or allergology. But counting and identifying pollen grains manually on a light microscope is time consuming and requires expert knowledge. Automatization of these labour-intensive tasks first emerged in the 60's and has tremendously progressed with the development of computer vision with deep-learning algorithms. Most studies so far have managed to classify images containing one single pollen grain. Prior to classification, detection of pollen grains in images containing several is recent in palynology and has been performed under ideal conditions. For example, Kubera et al. (2022) selected images with pollen grains from three taxa, showing only whole pollen grains without neither pollen fragments nor debris.

We test here how well the combination of the detection from YOLOv5 algorithm (Jocher et al. 2022) and the classification from ResNet50 algorithm (Chollet et al. 2015) performs in detecting and classifying pollen grains in images containing numerous Mediterranean pollen taxa and many different types of debris, sampled from annual pollen traps. Evaluation is two-fold, it consists in detecting and classifying (1) two pollen morphs from the European wild and cultivated grapevine, *Vitis vinifera*, and (2) all pollen taxa, in images taken from pollen traps samples. *Vitis vinifera* is of high ecological and archaeological

value as its domestication has shaped Mediterranean landscapes and social interactions since the Neolithic (Naqinezhad et al. 2018). Its pollen is tricolporate on wild male and cultivated hermaphroditic individuals, while it is inaperturate and sterile on wild female individuals. This inaperturate morph is often unidentified by palynologists, and yet potentially key for inferring stages of the *Vitis vinifera* domestication (Mercuri et al. 2021).

## Materials and Methods

Annual and monthly pollen traps were located nearby wild and cultivated individuals established in the Mediterranean Massif of Pic-Saint-Loup (Hérault, France). We trained YOLOv5 (Jocher et al. 2022) to detect pollen grains on 1,200 images in which 3,700 pollen grains were manually detected. We then applied the trained YOLOv5 on 18,000 new images and automatically detected ~53,000 pollen grains. We used the Particle Trieur software (Marchant et al. 2020) to manually classify 10,000 of these detected pollen grains, and used them for training the classification ResNet50 algorithm. Performance is evaluated for YOLOv5 and ResNet50 independently, with standard metrics (precision, recall) and from the confusion matrix (false positive and negative).

## Results and Discussion

The detection of pollen grains sampled from pollen traps by YOLOv5 resulted in less than 10% pollen grains left undetected. The classification performance of ResNet50 is currently evaluated on the 10,000 manually classified pollen grains. We will improve those results by increasing our training dataset for detection, and will soon evaluate the performance of the classification method between the two pollen morphs of *Vitis*.

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**Acknowledgements (Funds)**

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## TOPIC: FROM POLLEN BIOLOGY TO MORPHOPALYNOLOGY

### AUTOMATIC REAL-TIME SPORES MONITORING: *ALTERNARIA SPP.*, A CASE STUDY

Sophie ERB <sup>1,2</sup>, Benoît CROUZY <sup>1</sup>, Christine SALLIN <sup>1</sup>, Marie-José GRABER <sup>1</sup>, Gian LIEBERHERR <sup>1</sup>, Bernard CLOT <sup>1</sup>

<sup>1</sup> Federal Office of Meteorology and Climatology MeteoSwiss, [sophie.erb@meteoswiss.ch](mailto:sophie.erb@meteoswiss.ch)

<sup>2</sup> Environmental Remote Sensing Laboratory, École Polytechnique Fédérale de Lausanne, [sophie.erb@epfl.ch](mailto:sophie.erb@epfl.ch)

#### Introduction

Currently, we are deploying the Swiss national automatic pollen monitoring network. This network is based on the Swisens Poleno, an airflow cytometer that achieves real-time particle identification through holographic imaging and fluorescence measurements. The present network focuses on pollen monitoring but we plan on extending its capacity to include airborne fungal spores. Airborne spores, such as *Alternaria spp.*, can directly cause allergies. In Switzerland, 1-10% of the population is allergic to fungal spores (Dürr and Helbling 2012). In addition, airborne spores can have devastating effects on agriculture, where crop damage can be extensive. For example, *Alternaria* leaf blight on carrots typically leads to a yield loss of between a third and a half (Ben-Noon et al. 2001). Since automatic monitoring relies on identification through a machine learning algorithm, the crux is to get airborne spore data upon which the algorithm can be trained. Purely artificial datasets, generated by aerosolising cultured fungal spores, usually do not accurately represent reality and thus are not ideal for the development of an operational setup. An appropriate method to get representative data for fungal spores is to measure them directly as they fly nearby crops.

#### Materials and Methods

At MeteoSwiss (Payerne, Switzerland), we were able to obtain a quality dataset of *Alternaria solani* (Sorauer 1896) spores, a plant pathogen specialised in the Solanaceae family that includes notably potatoes, tomatoes and aubergine. Thanks to the harvesting of a potato field near to the measurement station, we registered a sufficient number of spores of *A. solani* in a short time period as confirmed by parallel manual measurements with a Hirst-type trap. The automatic data (holographic images) was extracted and inspected to create a dataset for algorithm training. We then evaluated the capacity of the model trained on 80% of the data to identify *Alternaria* spores



from the remaining 20% as a test set. We also assessed the reliability of the identification under operational conditions, which are inherently more complex. For this, we analysed the holographic images of all the particles measured by one Swisens Poleno over the year 2021, with our new model and extracted a time series of events labelled as *A. solani*. The validation of this time series was done by comparison with the manual Hirst measurements for the genus *Alternaria* without any distinction between species.

## Results and Discussion

The deep learning model (a convolutional neural network) trained on this dataset is able to differentiate accurately the fungal spores from a mix of 14 pollen taxa. Correlations between the manual Hirst and automatic Poleno timeseries show that both approaches agree well in terms of mean daily concentrations over the year. These results suggest that the current model, although trained on a single species (*A. solani*), is able to identify the genus in general. Further research is currently underway to better understand the specificity of this new model to *A. solani*. Since the different species of this genus are very similar, the preferred approach is to train on a larger pool of species to cover the whole genus.

## Conclusions

These first results are an important step towards the inclusion of the genus *Alternaria* as the first fungal spore monitored automatically and in real-time globally. The precise monitoring of *Alternaria spp.* would allow early warnings for allergy sufferers as well as the development of more targeted application of fungicides in agriculture, thus reducing environmental damage and costs.

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## TOPIC: AEROPALYNOLOGY

### Comparing automatic pollen monitors with Hirst-type pollen traps for total and individual pollen classification.

José M. MAYA-MANZANO<sup>1\*</sup>, Fiona TUMMON, Bernard CLOT, Benoît CROUZY, Mónica GONZALEZ-ALONSO<sup>3</sup>, Evgeny KADANTSEV<sup>4</sup>, Shigeto KAWASHIMA<sup>5</sup>, Moises MARTINEZ-BRACERO<sup>6</sup>, Predrag MATAVULJ<sup>7</sup>, Sophie MILLS<sup>8</sup>, Marina MUÑOZ-TRIVIÑO<sup>1</sup>, Gian LIEBERHERR<sup>2</sup>, David O'CONNOR<sup>9</sup>, Jose OTEROS<sup>10</sup>, Julia PALAMARCHUK<sup>4</sup>, Francis POPE<sup>8</sup>, Jesus ROJO<sup>11</sup>, Carsten SCHMIDT-WEBER<sup>1</sup>, Branko ŠIKOPARIJA<sup>7</sup>, Carsten A. SKJØT<sup>12</sup>, Mikhail SOFIEV<sup>4</sup>, Jeroen BUTERS<sup>1</sup>

1. Center of Allergy & Environment (ZAUM), Member of the German Center for Lung Research (DZL), Technical University and Helmholtz Center Munich, Munich, Germany.
2. Federal Office of Meteorology and Climatology MeteoSwiss, Chemin de l'Aérolologie, Payerne, Switzerland.
3. Department of Environmental Biology, University of Navarra, Pamplona, Spain.
4. Finnish Meteorological Institute, Helsinki, Finland.
5. Graduate School of Agriculture, Kyoto University, Kyoto, Japan.
6. Technological University Dublin, Dublin, Ireland.
7. BioSense Institute - University of Novi Sad, Novi Sad, Serbia.
8. School of Geography, University of Birmingham, Birmingham, United Kingdom.
9. School of Chemical Sciences, Dublin City University, Dublin, Ireland.
10. Department of Botany, Ecology and Plant Physiology, University of Cordoba, Cordoba, Spain.
11. Department of Pharmacology, Pharmacognosy and Botany, Complutense University of Madrid, Madrid, Spain.
12. School of Science and the Environment, University of Worcester, Worcester, United Kingdom.

\*Email: jose.manzano@tum.de



## Introduction

The importance of pollen information for allergy patients and allergists is well known. But to produce real benefits on them, pollen information must accomplish with some conditions, being important that they be both reliable and also updated, which is not possible with current manual traps. Thus, automatic pollen monitor devices are highly desired but they should be tested, ideally under the same conditions. In the framework of the European project EUMETNET AutoPollen, an intercomparison campaign, called EUMETNET AutoPollen-COST ADOPT intercomparison campaign 2021 (thereafter, the campaign) was held in Munich (Germany) from 3 March to 14 July 2021 and their results are presented in this work.

## Materials and Methods

The mean over the Hirst traps working at any given time ( $n=2-4$ ) during the full length for the campaign was compared with each automatic device running in parallel during that period. For statistical analysis, data were aggregated in 3-hourly and daily values. Five comparisons were performed, first with total pollen to allow all devices to get into the comparison and second, with four more pollen types (*Betula*, *Fraxinus*, Poaceae and *Quercus*) for some systems (device + algorithm) that allow pollen-specific classification. For the five comparisons, the  $R^2$  and slope, Mean Absolute Error (MAE) and Wilcoxon signed-rank test (paired test between Automatic device vs. Hirst) were considered.

## Results and Discussion

Overall, several systems showed excellent data availability and quality and some of them showed potential to deliver very good results for a bunch of pollen types, although frequently the best results were obtained by the same small group of systems for all pollen types. In general, 3-hourly comparisons obtained less similar concentrations to Hirst than daily comparisons. Regarding total pollen, there was a considerable spread amongst systems, with some reaching  $R^2 > 0.6$  (3-h) and  $R^2 > 0.75$  (daily), whilst other systems were not suitable to sample total pollen efficiently ( $R^2 < 0.3$ ). For *Betula*, almost all systems performed well, with high performances ( $R^2 > 0.85$  for 12 systems in daily basis). *Fraxinus* and *Quercus* were in a second step of quality for the most systems, and finally for Poaceae (with some exceptions) were obtained the lower performances, which could be due to the high variability of pollen sizes and the overlap for species within this family. The false positives outside the main pollen season period, although not being the rule of thumb, still exists for some systems and pollen types. The results for different analysis groups working in the same device showed that not only the device but also the used algorithm used is important to obtain high quality results.



## Conclusions

Except some issues with false positives that were observed outside of the main pollen season, a number of instruments perform as well as manual methods but with the extra of providing data in real-time and at high temporal resolution.

## Acknowledgements (Funds)

This article contributes to the EUMETNET AutoPollen Programme, which is developing a prototype of European automatic pollen monitoring network. The intercomparison campaign was funded by the Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL) and MeteoSwiss. We also thank financial support to the COST ADOPT action CA18226 – *New approaches in detection of pathogens and aeroallergens*.



## TOPIC: AEROPALYNOLOGY

# AUTOMATING THE SWISS POLLEN MONITORING NETWORK

Gian LIEBERHERR <sup>1</sup>, Bernard CLOT <sup>1</sup>, Benoît CROUZY <sup>1</sup>, Sophie ERB <sup>1,2</sup>, Fiona TUMMON <sup>1</sup>

<sup>1</sup> Federal Office of Meteorology and Climatology MeteoSwiss, [sophie.erb@meteoswiss.ch](mailto:sophie.erb@meteoswiss.ch)

<sup>2</sup> Environmental Remote Sensing Laboratory, École Polytechnique Fédérale de Lausanne, [sophie.erb@epfl.ch](mailto:sophie.erb@epfl.ch)

### Introduction

Monitoring pollen in the air is highly relevant as about 15 to 40% of the European population suffers from pollen allergy and asthma (Zuberbier et al. 2014). From 1969, allergists in Switzerland started to install Hirst-type pollen traps on hospital roofs to better understand the relation between pollen concentrations in the air and the symptoms of their patients. In 1993, The Swiss Federal Office of Meteorology and Climatology MeteoSwiss took over this first manual pollen monitoring network in Switzerland and developed forecast methods, climatology studies and new publication channels for the pollen data. The need for better temporal resolution and real-time data was identified together with allergists and other stakeholders. This initiated the first steps towards automation of the Swiss pollen monitoring network. In recent years, several promising methods for automated pollen measurements were developed.

### Materials and Methods

MeteoSwiss accompanied the development process of several promising instruments very closely. Among those devices, MeteoSwiss selected an airflow cytometer, the Swisens Poleno, to equip its network. The Swisens Poleno draws in ambient air and analyses the aerosols it contains. A machine-learning algorithm identifies and classifies the particles and computes a taxon-specific pollen concentration (Sauvageat et al. 2020). Currently, the Swiss pollen monitoring network is equipped with these new automated devices.

### Results and Discussion

In a proof-of-concept during the pollen season of year 2020, the particle identification, the instrument-to-instrument variability, and the temporal stability over one entire season could be successfully validated. Figure 1 shows the comparison of the daily birch pollen concentrations measured by the manual Hirst trap and by the automated Poleno measuring system.

After the successful proof-of-concept, the monitoring network runs now in a pre-operational mode for the seasons 2021 & 2022 with currently 10 measurement sites. The correlation with the manual Hirst-type traps is very high for most of the targeted pollen taxa. Further, thanks to the higher temporal resolution, the new data gives new insights into sub-daily or even sub-hourly pollen concentration patterns. However, there remain challenges for a successful automated pollen-monitoring network, such as the establishment of calibration procedures and reference standards for these new systems.

## Conclusion

The here presented results give an exclusive insight into the ongoing automation process of the pollen monitoring network of the Federal Office of Meteorology and Climatology MeteoSwiss. It presents the state of the art and the latest developments of automated pollen measurement with the Swisens Poleno system.

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## Figure

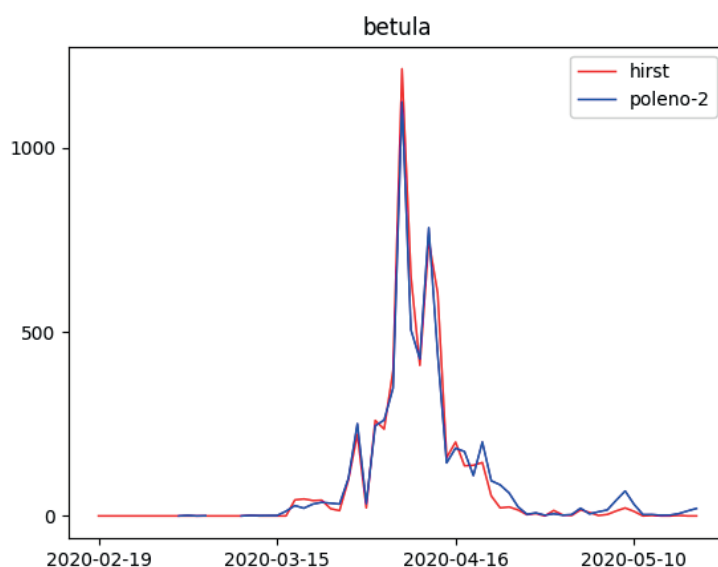


Figure 1. Comparison between manual (Hirst) measurements and the new automated method (Poleno) for the birch season 2020



## **AUTOMATIC AND REAL-TIME POLLEN MONITORING AND CLASSIFICATION USING DIGITAL HOLOGRAPHY. EXPERIENCE BASED ON SOME COMMON POLLEN TAXA OF THE MEDITERRANEAN AREA.**

Ismael LÓPEZ <sup>1,2</sup>, Yanick ZEDER<sup>3</sup>, Rut PUIGDEMUNT<sup>4</sup>, David NAVARRO<sup>4</sup>, Jordina BELMONTE<sup>1</sup>

<sup>1</sup> *Universitat Autònoma de Barcelona, jordina.belmonte@uab.cat*

<sup>2</sup> *Qualitas 4 Health, ilopez@qualitas4health.com*

<sup>3</sup> *Swisens AG, yanick.zeder@swisens.ch*

<sup>4</sup> *Universitat Autònoma de Barcelona, rut.puigdemunt@uab.cat*

<sup>5</sup> *Universitat Autònoma de Barcelona, david.navarro@uab.cat*

### **Introduction**

Experience and validation for automated monitoring of airborne pollen taxa relevant for allergic individuals based on holography is so far only available in Central Europe (Sauvageat et al. 2020). In this work, we present first results for such real-time monitoring systems in the Mediterranean area. Specifically, the performance for the common pollen taxa *Poaceae*, *Parietaria/Urticaceae*, *Olea* and *Cupressaceae* are presented, which are some of the most relevant types for allergic individuals in that region.

### **Materials and Methods**

#### *Process for obtaining, storing and using holography data for classification*

The SwisensPoleno Mars is an automatic measurement instrument for real-time bioaerosol monitoring and classification based on digital holography. For each measured aerosol particle, two holographic images are taken using two cameras offset by 90° (Figure 1). This allows the 3D shape of the particle to be estimated.

The data of one measured particle, including the two holographic images, is stored as a measurement event. If an identification algorithm is available, the classification is done directly on the instrument itself and is stored together with the data. Typically, such algorithms are based on machine learning models. These models provide a probability for an event to belong to a certain class. These probabilities are then further processed in order to derive concentration values for the available classes.

### *Procedure to obtain and qualify algorithms for the local plants*

As a reference for the qualification of the automatic identification we used the following equipment:

- a) Seven-day Hirst-type volumetric pollen trap Lanzoni VPPS 2000 (Hirst 1952).
- b) SwisensPoleno Mars manufactured by Swisens AG.

Both of them are installed (Figure 2) 3 m apart on the rooftop of the Facultat de Ciències i Biociències building of the UAB (Universitat Autònoma de Barcelona) at around 20 m height above the street level (116.8 masl).

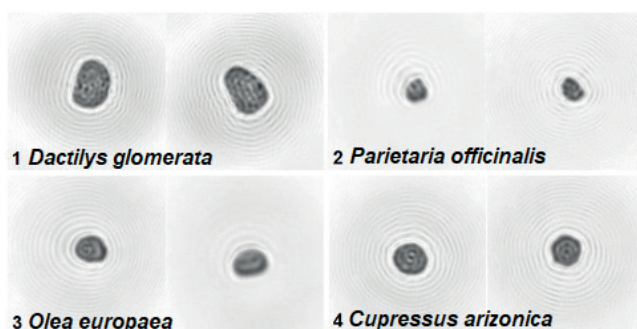
Volumetric sampler operation and sample processing (preparation, analysis, counting and computing) is done according to the Spanish and the EAS (European Aerobiology Society) standards (Galán et al. 2007 and 2014).

The first step to obtain identification algorithms for the local plants consisted of taking natural pollen samples directly from the field from different species (Mediterranean and non-Mediterranean) throughout the season. They were fed to the measurement system directly after the collection when possible or, when not, the fewer days later possible. This was done using the SwisensAtomizer particle aerosolizer. The measurement data were then submitted to a cleaning process used to make datasets containing only events from one taxa. After creating all the desired datasets, the algorithm generation was done through machine learning. To avoid confusion, the algorithm was also trained on data from Sahara dust and water droplets. The classification for the first year was then done retrospectively at the end of the measurement period with the measurement data collected on the server.

As a reference, daily Hirst pollen time series was taken from 01/05/2021 to 25/02/2022 for each pollen taxa (*Poaceae*, *Parietaria/Urticaceae*, *Olea* and *Cupressaceae*).

SwisensPoleno Mars time series resulting from the classification with the newly trained identification algorithm are scaled (differences between the sampled volume and loss-compensation) for comparison with the volumetric samplers.



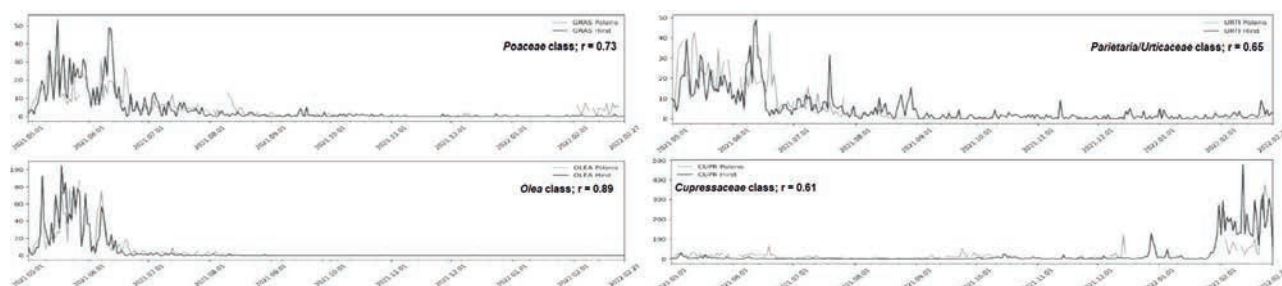


**Figure 1:** Real examples of holographic images

**Figure 2:** Devices installed in the UAB.

**Results and Discussion**

The general results for the given pollen taxa are summarized in figure 3.



**Figure 3:** Time series of Hirst pollen trap and SwisensPoleno Mars.

The time series of both measurement types (manual and automatic) has good performance as there are some deviation and differences in certain periods (more pronounced in some pollen taxa i.e. Cupressaceae), it seems they are related to false positives due to improvable datasets or lack of datasets for the whole pollen spectra.

Anyway, for SwisensPoleno Mars, pollen datasets can still be improved with new and fresher natural samples, new datasets for the whole pollen spectra (species not trained yet) and some upgrades in the classification algorithm (noise reduction, accurate dust dataset, etc.) but keeping in mind that manual measurements cannot be considered a gold standard in terms of absolute values (Tummon et al. 2021).

## Conclusions

The results of automatic and real-time bioaerosol monitoring and classification are very promising for the Mediterranean region, where the variety of pollen taxa and concentrations are highly variable throughout every season.

Efforts are still needed to acquire the maximum pollen taxa (and spores) to have a representative spectra of the particles which are known to be present in the air for every season and to improve the classification models used in particle identification.

As happened in other disciplines like in meteorology, hydrology, etc. automatic and real-time monitoring networks will be a reality because of the benefits for society (especially for the health of the population) and for science (acquiring high-resolution data in time, allowing 2D/3D and forecast modelling, etc.).

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## Acknowledgements

This experience was possible thanks to Swisens AG and Qualitas 4 Health collaboration with equipment and expertise related to the technology and data analysis.

## Figures

**Figure 1:** SwisensPoleno Mars and Hirst-type volumetric pollen trap Lanzoni VPPS 2000 installed in the UAB.

**Figure 2:** Real examples of holographic images: 1) *Dactylis glomerata*; 2) *Parietaria officinalis*; 3) *Olea europaea*; 4) *Cupressus arizonica*.

**Figure 3:** Time series of Hirst pollen trap and SwisensPoleno Mars for a) *Poaceae* class (Pearson correlation of 0.73); b) *Parietaria/Urticaceae* class (Pearson correlation of 0.65); c) *Olea* class (Pearson correlation of 0.89); d) *Cupressaceae* class (Pearson correlation of 0.61).



## **MACHINE LEARNING AND POLLEN IDENTIFICATION: CNN FOR IDENTIFICATION OF AMBROSIA AMONG LATE SEASON POLLEN**

**Dennis Reupke-Hager<sup>1</sup>, Lars Hildebrand<sup>2</sup>, Georg Meinardus-Hager<sup>1</sup>**

<sup>1</sup>*a.r.medi UG, Warendorf, Germany, email: denhag@posteo.net*

<sup>2</sup>*EXScience GmbH, Bochum, Germany, email: lars.hildebrand@exscience.de*

### **Introduction**

In the past years Convolutional Neuronal Networks (CNN) have reached a state where this software has the potential to simplify and accelerate the processing of images and data. A promising field is aerobiology, where the current standard is to sample particles and make a slide for Light Microscopic inspection by eye.

Microscope Image Analysis System (MIAS<sup>®</sup>) enables to digitize samples on a slide as a series of high quality images. A logical next step is implementing CNN for the identification of the particles. After successful tests on tree pollen the identification of *Ambrosia artemisiifolia* L. among late season pollen was the next application for our CNN.

*Ambrosia artemisiifolia* has been seen as an aggressive invasive species in Germany over the last decade and been object to ongoing surveys in Europe (Nawrath und Klingenstein 2006).

### **Materials and Methods**

The MIAS<sup>®</sup> relies on Standard methods for the sampling and staining of samples.

Operating our automatic light microscope aeroScope<sup>®</sup> simulates the routine particle counting: It produces a series of adjacent images and the details of the particles are documented by images from adjoining focus levels. Samples for the training of the CNN, the inspection of these images is supported by a tool named Marker, which enables efficiently labelling and image-related listing of structures. The CNN of MIAS<sup>®</sup> was trained to discriminate *Ambrosia* pollen against pollen from relevant late season species at an accuracy over 80%.

Results: The CNN reached an identification accuracy in comparing pollen of *Ambrosia* (99%), *Amaranthus caudatus* L. (96%), *Brassica napus* L. (95%), *Plantago lanceolata* L. (84%), *Rumex acetosella* L. (100%) and *Urtica dioica* L. (100%), using a training set of only a few hundred pollen images for each species.

### **Results and Discussion**

Using CNN in MIAS<sup>®</sup> makes the analysis of pollen objective and effective: Pollen from a daily slide are recognized in few minutes.

- Based on a series of adjacent images provided by the aeroScope<sup>®</sup>
- photographic documentation including clear relation to position on the slide is provided
- comfortable evaluation anywhere is possible

- a quality suitable for digital analysis to detect and classify distinct pollen is reached. The digitalization of aerobiological samples makes it possible to share, conserve and compare these samples.

### Conclusions

With further adjustments and training the CNN should be capable of facilitating and accelerating the analysis of large quantities of pollen imagery.

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## INTEGRATION OF DATA FROM DIFFERENT RAPID-E DEVICES SUPPORTS POLLEN CLASSIFICATION IN MORE LOCATIONS

Predrag MATAVULJ <sup>1</sup>, Antonella CRISTOFORI <sup>2</sup>, Fabiana CRISTOFOLINI <sup>2</sup>, Elena GOTTARDINI <sup>2</sup>, Sanja BRDAR <sup>1</sup>, Branko SIKOPARIJA <sup>1</sup>

<sup>1</sup> BioSense Institute, University of Novi Sad, Serbia: [matavulj.predrag@biosense.rs](mailto:matavulj.predrag@biosense.rs)

<sup>2</sup> Research and Innovation Centre - Fondazione Edmund Mach, San Michele all'Adige, Italy

### Introduction

The laser-sensing real-time bioaerosol detector Rapid-E (Plair SA) produces data prone to device-specific noise due to laser and detector sensibility, which is critical when transferring classification models between different devices (Matavulj *et al.* 2021). This study implements convolutional neural networks (CNNs) for pollen classification and tests how models trained with a combination of different datasets perform at two locations.

### Materials and Methods

The study was conducted in San Michele all'Adige, Italy, and Novi Sad, Serbia. Taxonomically determined monospecific pollen samples were collected and injected into the devices to obtain reference datasets used for classification. A multi-modal CNN architecture was applied for pollen classification on one year of Rapid-E data, obtaining daily pollen concentrations which were then compared with the concentrations obtained by light microscopy analysis on Hirst-type volumetric pollen samples. CNNs were trained with different combinations of reference datasets to test if the models were transferable from one device to the other. All networks were additionally trained with the domain adaptation technique (Ganin and Lempinsky 2015) to exclude the device-specific noise.

### Results and Discussion

Models lost performance when trained on data from one and tested on another device. We showed that data of the same pollen *taxon* from the two devices are more different than data of distinct pollen *taxa* from one device. Combining all available reference data in a single model enabled the classification of a higher number of pollen *taxa* in both study locations. The domain adaptation technique improved the performance of transferred models for several pollen *taxa*.

### Conclusions

CNNs recognize significant differences in data from the two devices which can be solved by combining reference datasets from both locations. When a reference dataset is not available, the domain adaptation can improve the performance of models trained on data from other devices.



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## TOPIC: AEROPALYNOLOGY

# ANALYSIS OF THE CORRELATION BETWEEN POLLEN AND POLLUTANTS BY TIME SLOTS IN THE CITY OF ROME

Denise DE FRANCO<sup>1</sup>, Maria Antonia BRIGHETTI<sup>1</sup>, Alessandro DI MENNO di BUCCHIANICO<sup>2,3</sup>,  
Annarosa MIRAGLIA<sup>1,2</sup>, Alessandro TRAVAGLINI<sup>1</sup>

<sup>1</sup> Department of Biology, University of Rome Tor Vergata,  
[denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com);

[maria.antonia.brighetti@uniroma2.it](mailto:maria.antonia.brighetti@uniroma2.it); [annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com); [travagli@uniroma2.it](mailto:travagli@uniroma2.it)

<sup>2</sup> PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata

<sup>3</sup> Italian National Institute for Environmental Protection and Research (ISPRA), Rome, Italy,  
[alessandro.dimenno@isprambiente.it](mailto:alessandro.dimenno@isprambiente.it)

**Introduction:** Rhinorrhoea, itchy nose and eyes, lacrimation and coughing, dyspnoea and asthenia are just some of the symptoms connected to the inhalation of allergens, such as pollen grains, dispersed every year in the atmosphere, especially during springtime.

Several studies demonstrated the existence of a connection between airborne pollen and environmental pollution (Sénéchal et al. 2015), highlighting the role of pollution as an aggravating factor in allergic and asthmatic manifestations of sensitive patients. It was therefore demonstrated an interdependence between the diffusion of the particulate matter PM<sub>10</sub> in winter and NO<sub>2</sub> in summer and the growth in concentration of pollens, especially in urban areas (D'Amato et al. 2007; Di Menno di Bucchianico et al. 2018). In these areas in particular, pollution is connected to anthropic activities, such as industrial processes, domestic heating and vehicles motors.

The objective of this research was to verify this correlation and to evaluate the consequences on public health, through the monitoring of airborne pollen.

**Material and Methods:** The aerobiological monitoring consisted of the analysis of data collected in a period of 75 total days in the years 2018-2020. This data collection was conducted in the monitoring stations RM5 and Cipro, that investigated two different areas of the city of Rome.

The present analysis involved four allergenic *taxa*, that are considered to be the most unsafe for the Italian allergic population: Urticaceae, Poaceae, Cupressaceae-Taxaceae and Betulaceae (for this last one, it was considered the species *Corylus avellana* L.).

Understating and demonstrating the correlation between allergens and pollution might bring benefits to the entire population, by reducing the exposition to allergens during the periods of massive pollen dispersion and by designing *ad hoc* and validated therapies for all the affected patients.

The collected samples were investigated through an hourly reading of the microscope slide. This method allowed a registration of the variation of the daily concentration of allergenic material in a

specific moment of the day. For the entire investigation it was utilized a Hirst volumetric sampler (Hirst 1952), denominated Pollen Trap, VPPS® 2000 model, produced by Lanzoni S.r.l.. For each sample investigated and for both the monitoring stations, it was created a report to indicate the date, hour and pollen count per hour and per day.

**Results and discussion:** The data collected in this three-years (2018-2020) concerning the pollen were then confronted with the related data to the concentration of polluting elements, in particular NO<sub>2</sub> and O<sub>3</sub>. Eventually, it was possible to confirm this correlation: a growing in the levels of pollen concentration directly corresponds to a growing in the levels of tropospheric ozone (O<sub>3</sub>), especially during the warmest hours of the day (ozone, in fact, tends to accumulate during the warmest daily hours and during spring-summer season) and in urban areas.

**Conclusions:** In conclusion, thanks to the present research and analysis, as transcribed and exposed in this dissertation, it was possible to demonstrate the existence of a synergy between the monitoring of pollen grains concentration and polluting agents, that proved to be useful to provide diagnostic and therapy indication for allergic patients, through pollen bulletins and calendar, that need to include information about the interdependence between allergens and pollution.

The results of this study represent a useful basis for future works, that might investigate additional aspects in a longer period, keeping track of other variable factors, such as climatic and meteorological information, that were not considered here.

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## TOPIC: AEROPALYNOLOGY

# AEROBIOLOGICAL CONTRIBUTION TO PARTICULATE MATTER CHARACTERIZATION

Alessandro Di Menno di Bucchianico<sup>1,2</sup>, Raffaella GADDI<sup>1</sup>, Maria Antonia BRIGHETTI<sup>3</sup>, Denise DE FRANCO<sup>3</sup>, Annarosa MIRAGLIA<sup>2,3</sup>, Alessandro TRAVAGLINI<sup>3</sup>

<sup>1</sup> *Italian Institute for Environmental Protection and Research (ISPRA), Rome, Italy;*  
[alessandro.dimenno@isprambiente.it](mailto:alessandro.dimenno@isprambiente.it); [raffaella-gaddi@isprambiente.it](mailto:raffaella-gaddi@isprambiente.it)

<sup>2</sup> *PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata*

<sup>3</sup> *Department of Biology, University of Rome Tor Vergata, Rome, Italy*  
[maria.antonia.brighetti@uniroma2.it](mailto:maria.antonia.brighetti@uniroma2.it); [denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com); [annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com);  
[travagli@uniroma2.it](mailto:travagli@uniroma2.it)

## Introduction

Although it is established that the sources of substances harmful to health are both natural and anthropogenic and that exposure to air pollution is largely a multi-polluting process, in the recently updated WHO guidelines and in the European legislation a 'single pollutant' assessment approach is still being adopted. A better approach, based on an integrated estimation of air quality, would be useful to prevent synergistic or antagonistic effects between air pollutants and allergenic pollen (Di Menno di Bucchianico *et al*, 2019).

It is known that air pollution and seasonal pollen emissions can affect human health by causing severe allergic reactions, particularly when air pollution is combined with peaks in the concentration of allergenic granules. Aerobiology can be linked to atmospheric pollution in various ways: first, the biological components can be air contaminants and produce harmful effects on health; secondly, some atmospheric pollutants can interact with the airborne biological aerosols, aggravating their effects on humans (Lebowitz M. D. and O'rourke, 1991); finally, the contribution to the mass concentrations of inhalable particulate must not be forgotten as the primary biological aerosol particles (PBAP) and, among these, the spores and pollen grains represent a not negligible share.

## Materials and Methods

This work is based on the study of the state and trend of the main allergenic pollens and the *Alternaria* spore in the city of Rome, measured from 2003 to 2019 by the Aerobiological Monitoring Center of the University of Rome Tor Vergata, and compared with the corresponding concentrations. of PM<sub>10</sub> NO<sub>2</sub> and O<sub>3</sub> from the regional air quality network (ARPA Lazio). The data analyzed refer, in particular to nine botanical families, all of significant allergological interest: pollen of Betulaceae, Asteraceae,

Corylaceae, Cupressaceae-Taxaceae, Poaceae, Oleaceae, Urticaceae and the *Alternaria* spore. The data on air concentration were analyzed in a homogeneous way, to compare series of historical data produced at different sampling points and to provide a representative assessment of urban air quality and its potential effects on human health (Di Menno di Bucchianico *et al*, 2021).

## Results and Discussion

Particles in the atmosphere can be characterized by their size, composition, shape, color, number, and gas/particle phase equilibrium. It is not useful, nor does technology exist, to measure every aspect of these properties. Previous studies of chemically characterized suspended particles in source and receptor samples from a variety of environments identify several aerosol properties related to excessive concentrations, pollution sources, visibility, and health (EPA, 1998).

Pollen grains are generally bigger than 10  $\mu\text{m}$  in size, however  $\text{PM}_{10}$  doesn't relate with Geometric diameters of particles but with their aerodynamic diameters that, depending on the density, can be significantly smaller (up to 4  $\mu\text{m}$  in DA). In this sense, pollen grain can contribute to inhalable fraction of the whole airborne particulate.

The analysis of pollen and air quality data made possible to characterize the sites under examination, highlighting the cases of influence of the meteorological conditions, the flowering calendar and local features on the representativeness of the entire territory in question and to extrapolate the coherence, apparently absent, between the trends of some taxa allergenic and traditional air pollutants. These results can be considered as an additional tool for a more comprehensive assessment of air quality and its effects on human health in an urban environment.

## Conclusions

Pollen and spores are part of the airborne particulate matter; aerobiological monitoring networks, in this sense, are peculiar speciation networks for particulate matter. The biological particulate contributes to the mass concentration of  $\text{PM}_{10}$ : it'd be useful to estimate it as for the other natural abiological contributions (i.e. desert dust). The combined assessment of the biological and abiological component of atmospheric pollution can make a useful contribution to improving the knowledge of air quality for the protection of human health.

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## AEROBIOLOGIA 4.0 NEW FRONTIERS OF AEROBIOLOGICAL MONITORING: FIRST ATTEMPT TO EVALUATE THE DIFFUSION OF SARS-CoV-2 INDOOR AND OUTDOOR AEROSOL

Emma TEDESCHINI<sup>1</sup>, Leonella PASQUALINI<sup>2</sup>, Stefano MINISTRINI<sup>3</sup>, Marco MAGGI<sup>4</sup>,  
Alessandro VALECCHI<sup>2</sup>, Roberto CALISTRONI<sup>2</sup>, Stefania PASQUALINI<sup>5</sup>

<sup>1</sup> *Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy*  
[emma.tedeschini@unipg.it](mailto:emma.tedeschini@unipg.it)

<sup>2</sup> *Department of Medicine and Surgery, University of Perugia, Italy*

<sup>3</sup> *Center for Molecular Cardiology, University of Zurich, Zurich (Switzerland)*

<sup>4</sup> *Territorial Health Agency of Valle Olona (VA) - Operational Structure of Busto Arsizio*

<sup>5</sup> *Department of Chemistry, Biology and Biotechnology University of Perugia, Italy*

**Introduction** Aerobiological monitoring or monitoring of the airborne microbiota, is a survey technique that spread around the 1950s from the need, in the health sector, to search in the bioaerosol the causes of the seasonal respiratory diseases. Following this need, a particular device for air control was performed to sucking air flow corresponding to human pulmonary ventilation. From this basic assumption, for aerobiological research arise the idea to test the air samplers like Hirst, to check the airborne diffusion of SarsCoV-2 virus in indoor and outdoor. The main purpose of this study was to verify the duration of infected droplets in suspension for understand the availability of the aerosolized and potentially respirable virus in infected indoor; secondly in outdoor, to verify a suspected link between airborne pollen, atmospheric pollution and the severity of pandemic spread (Comunian et al. 2020; Damialis et al 2021).

**Materials and Methods** During the first pandemic wave (Winter 2020) an analysis plot was carried out at the Laboratory of Palynology and Aerobiology (University of Perugia) to verify any interference between the outdoor aerobiological monitoring protocol and the diagnostic tests for the detection of SarsCoV-2. Subsequently, in collaboration with the aerobiological center of the RIMA network (AIA), located in Lombardy (Busto Arsizio-Varese), the daily samples corresponding to the pandemic peak (red weeks) were stored at -30 ° C until analysis for SarsCoV-2 detection. At the end of May 2020, the first attempt in indoor of airborne SarsCoV-2 monitoring, the aerosol in red-zone of the only covid hospital of Umbria Region was checked.

**Devices:** according to the CEN-TS2015 standard protocol, the VPPS2000 spore trap (Lanzoni) was used for outdoor monitoring, and an "easy spore trap" for daily biomonitoring (VPPS1000 Lanzoni) was applied for indoor monitoring.

The pilot study for the definition of the indoor monitoring protocol was developed during the second pandemic wave (Winter-Spring 2021), which dramatically involved the Umbria region (central Italy).

Indoor monitoring was conducted for 12 weeks between March and June 2021 in the major hospitals of Umbria region (central Italy), located in Foligno and Perugia cities.

All aerobiological samples were subjected to RTPCR (Real Time PCR) according to the standardized protocols for the identification of target genes: RNA-dependent RNA polymerase (RdRp) / helicase, Spike and SARS-CoV-2 nucleocapsid genes.

**Results and Discussion:** the results of indoor monitoring have demonstrated high specificity and sensitivity of the technology and protocol applied to SarsCovII detection; no false positives were found in the controls, and in the care settings studied the aerosolized viruses was detected also in indoor hosted by low viremic patients. While, in outdoor aerobiological monitoring samples of the weeks of pandemic peak no traces of virus were detected. Obviously, the position of spore trap (about 10 meters high) and especially the stay time (8 days) of monitoring samples in outdoor may have influenced the results obtained. Therefore, this study cannot exclude the involvement of airborne particulate in the spread of the virus.

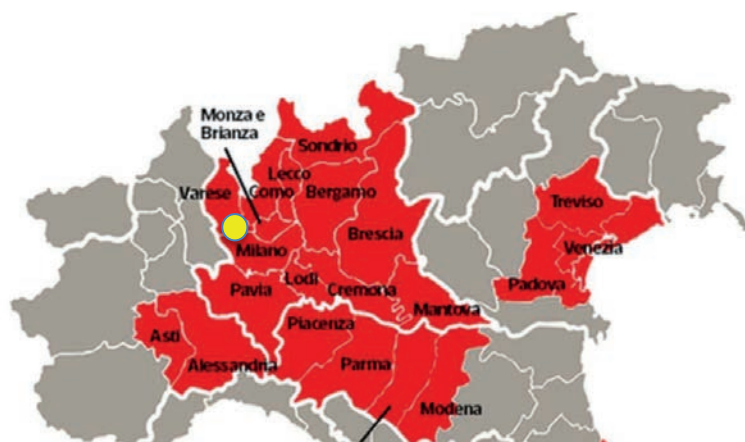
**Conclusions:** The results obtained show that the aerobiological monitoring can be involved all airborne microbiota and as already demonstrated by DNA barcoding technique, a semi-conservative experimental design (same tool and different analysis) in aerobiological monitoring are the essential transition to Aerobiology 4.0 and ecosystem health research.

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**Figures 1**

March 2020 geography of virus distribution Italy.

The red areas show the spread of the pandemic peak in the provinces of the Lombardia and Veneto regions.

Yellow dot shows the aerobiological monitoring center of the RIMA Italian network located in Busto Arsizio (Varese) .



**KEY WORDS: CAMBIAMENTO CLIMATICO VEGETAZIONE,  
RISCHIO AMBIENTALE E RISCHIO ALLERGOLOGICO**

## **Evaluation of bioaerosol in the processing environment for food of animal origin: a preliminary study**

**Beniamino Cenci-Goga<sup>1</sup>, Luca Grispoldi<sup>1</sup>, Massimo Zerani<sup>1</sup>, Emma Tedeschini<sup>2</sup>**

<sup>1</sup> *Department of Veterinary Medicine, University of Perugia, Italy*

*[beniamino.cencigoga@unipg.it](mailto:beniamino.cencigoga@unipg.it), [luca.grispoldi@unipg.it](mailto:luca.grispoldi@unipg.it), [massimo.zerani@unipg.it](mailto:massimo.zerani@unipg.it)*

<sup>2</sup> *Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy*  
*[emma.tedeschini@unipg.it](mailto:emma.tedeschini@unipg.it)*

**Introduction.** One of the main indicators of hygiene in the slaughterhouse environment is *Escherichia coli*. These bacteria are commensal of the digestive system of cattle, which, as asymptomatic diffusers, have become their main reservoir [1]. In light of this, it is inferred that skin and meat are mainly contaminated with faeces [2]. It is also conceivable that during the slaughtering contaminant particles could be aerosolized. This suggests that there may be a correlation between *E. coli* contamination and visible dirt on the skin of the slaughtered animal [3]. Therefore, to reduce or avoid carcass contamination and ensure food and slaughterhouse environment safety, the following are essential requirements: receive clean animals at the slaughterhouse and apply good hygienic practices throughout the slaughter process. The main objective of the present study is to demonstrate the possible correlation of visible contamination of carcasses and slaughterhouse aerosol with the level of hygiene, assessed by laboratory techniques.

**Materials and Methods.** In the present study, counts for total mesophilic aerobic bacteria and *E. coli* were determined on the carcasses via standard methods [4] (Figure 1A). To evaluate the visible contamination of carcasses, such as hairs and faecal specks, an innovative method was used, by using a 100 cm<sup>2</sup> sheet of transparent adhesive plastic material applied on the side of the carcass to collect all particles (Figure 1B), then counted first against a black and then against a white background. The air sampler (like Hirst) was used to determine airborne bacteria in slaughterhouse aerosol. This sampler, typically applied for monitoring of the airborne microbiota, has a suction flow of 10 L/min [5]. To evaluate three different bacterial populations in the aerosol (total mesophilic aerobic bacteria, *Enterobacteriaceae* and enterococci), three Petri dishes, with selective medium, were placed in the suction chamber of the sampler. The correlation of visible contamination of the carcass and bacterial load of the aerosol with results of the microbiological analysis was measured by a linear regression specifically prepared. In addition, a second air sampler was placed at a height of about 3 m for hourly slide deposition assessment of all airborne particulate matter (Figure 2A).

**Results and Discussion.** Two hundred forty-nine carcasses were sampled. The mean values of total aerobic bacteria and *E. coli* never exceeded the upper limit provided by the regulation. *E. coli* was present in 60 carcasses out of 249 (24%). The correlation of visual dirtiness score and aerosol with bacterial counts was statistically significant. Analysis of the hourly deposition of airborne particulate matter confirms that it is mainly deposited during the slaughtering period (Figure 2B). These results are of extreme importance in view of a reduction of controlling practices of the hygienic criteria of production processes in slaughterhouses, reducing the expensive and time-consuming routine procedures of laboratory analytical methods and, at the same time, protect the health of the slaughterhouse workers.

**Contributions:** B.C.G.: idealization, study design and analysis of results. L.G.: sampling and analysis of results; M.Z.: idealization, sampling and analysis of results; E.T.: idealization, study design, sampling and analysis of results

**Acknowledgements:** The Authors thank the Director of the Municipal Slaughterhouse of Ponte San Giovanni (PG, Italy), Dr. Luca Budelli, and the *Cooperativa Macellatori* for their willingness to carry out the present study.

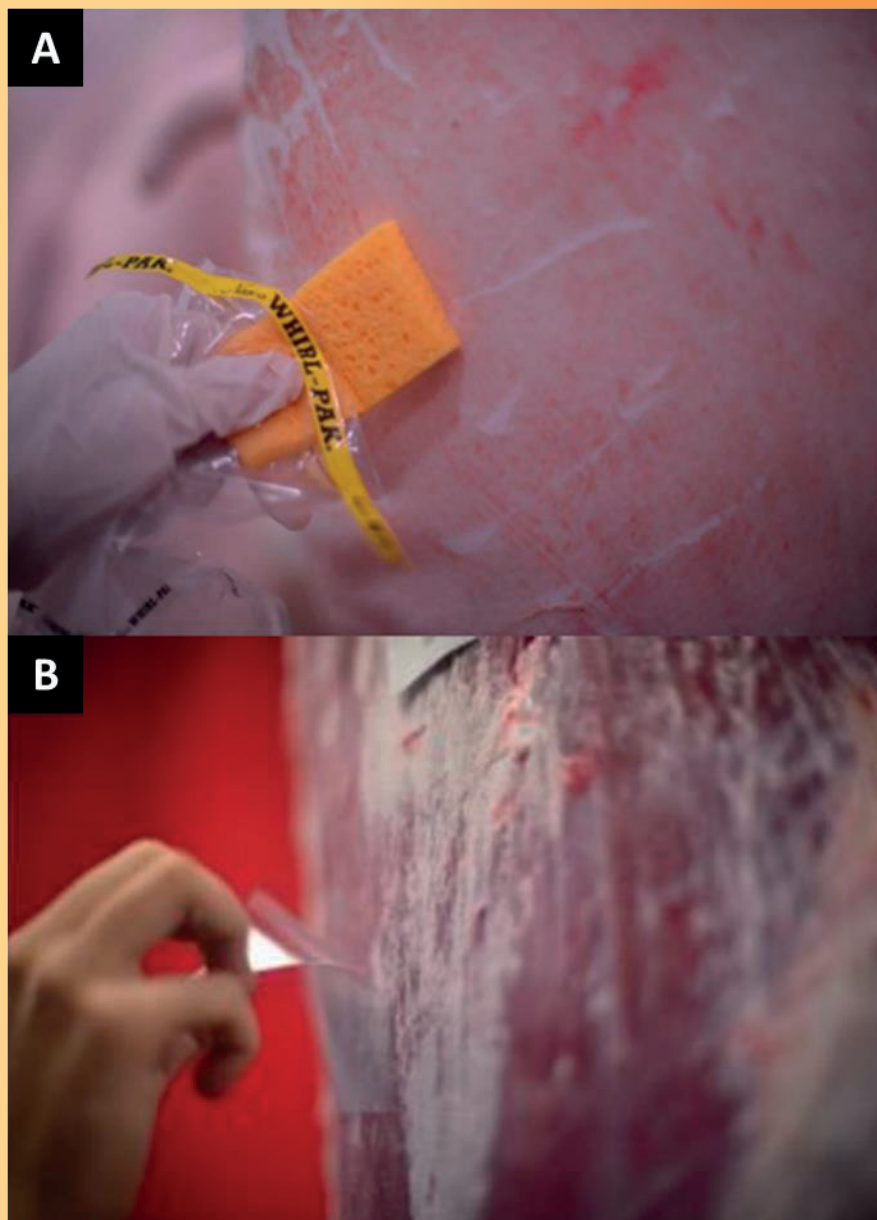
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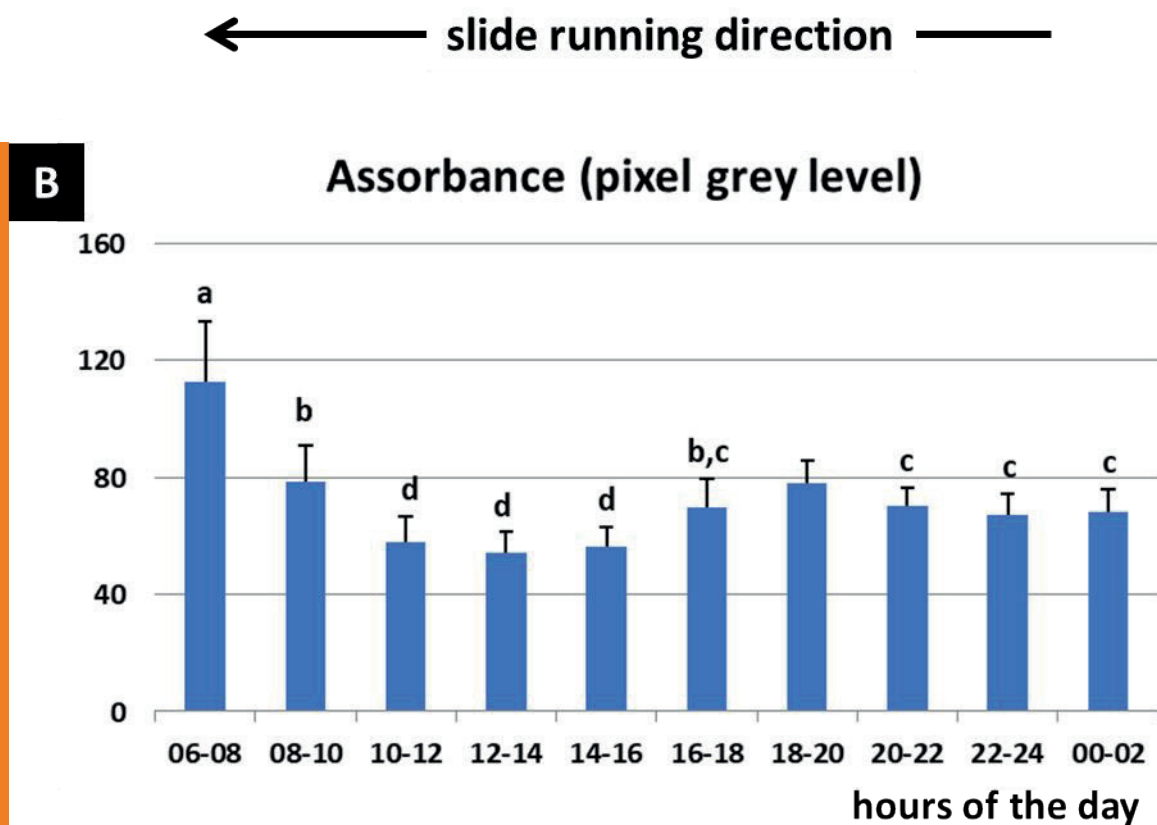


## Figures



**Figure 1.** A: carcass swabs via sponge; B: determination of visible contamination of carcasses





**Figure 2.** A: Slide with daily deposition of all airborne particulate matter; B: Variation of the daily deposition, different letters P < 0.01



**KEY WORDS: CAMBIAMENTO CLIMATICO VEGETAZIONE,  
RISCHIO AMBIENTALE E RISCHIO ALLERGOLOGICO**

## **INCREASE IN POLLEN QUANTITIES THE MOST EVIDENT SIGNAL IN 30 YEARS OF DATA IN ALPINE CONTEXT**

Fabiana CRISTOFOLINI <sup>1</sup>, Antonella CRISTOFORI <sup>2</sup>, Elena GOTTARDINI <sup>3</sup>

<sup>1</sup> *Research and Innovation Centre, Fondazione E.Mach, fabiana.cristofolini@fmach.it*

<sup>2</sup> *Research and Innovation Centre, Fondazione E.Mach, antonella.cristofori@fmach.it*

<sup>3</sup> *Research and Innovation Centre, Fondazione E.Mach, elena.gottardini@fmach.it*

### **Introduction**

The ongoing changes in climate have wide-range impacts on ecosystems and human health. One of the verified impacts of climate change on plants affects the phenology of flowering, and pollen release. The analysis of pollen trends often highlights changes in quantity and seasonality and underlines the role of temperature and precipitation (Schramm 2021). The study of pollen and its changes is important also for the allergenicity of many airborne pollens; the prevalence of pollen allergy is currently estimated to be up to 40% at European level (D'Amato 2007).

### **Materials and Methods**

The modifications of airborne pollen were analyzed on 30 years (1989-2018) of daily data collected at Fondazione E. Mach in San Michele all'Adige (Latitude 46.19 N, Longitude 11.13 E, 220 m a.s.l.). Airborne pollen was sampled by a Hirst-type trap, processed, and analyzed following conventional techniques and standardized protocols (UNI EN 16868:2019). Seasonal indicators were calculated for 24 pollen taxa (16 arboreal, AP, and 8 non arboreal (NAP) pollen taxa), representing 95% of the entire spectrum. Time trends and correlation to the meteorological parameters were analyzed. To minimize the effect of interannual fluctuations in pollen production and to maximize relevant changes during the timespan, total pollen and number of high pollen days (number of granules/m<sup>3</sup> >100) were analyzed, clustering arboreal (AP) and non-arboreal pollen (NAP) in three decadal blocks.

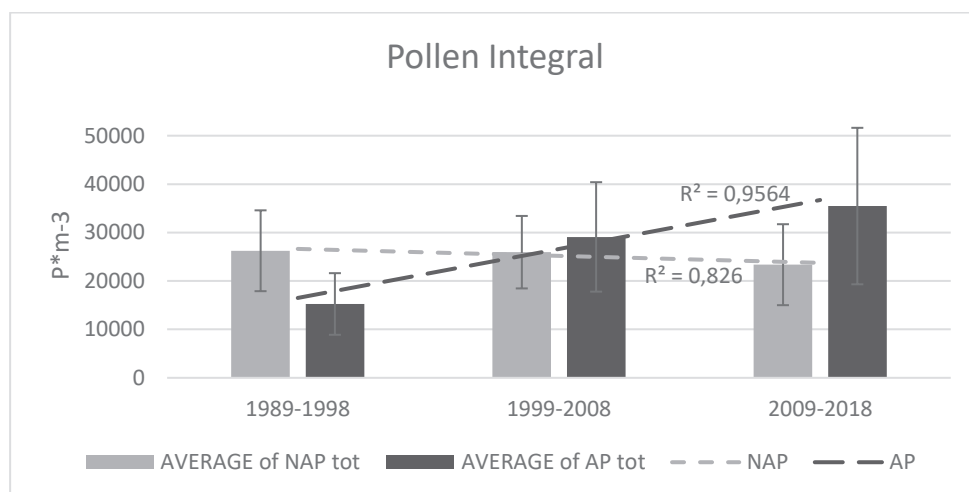
### **Results and Discussion**

The most evident and coherent signal is the increase in peak and total pollen, mainly ascribable to AP. The analysis in three decadal blocks shows that the increase in pollen load (figure) and number of high pollen days are significant. As for seasonal descriptors, an anticipation of the start date, mainly for herbaceous taxa (NAP) is recorded. On average, start date shows a trend of advance of 2,8 days

every 10 years. Correlations with meteorological parameters are more frequent with AP seasonal descriptors than pollen load (in agreement with Schramm et al. 2021).

## Conclusions

Our thirty-year pollen analysis shows a significant earlier start date, especially for NAP, and a significant increase in pollen load for AP. Our case study in the Alpine biogeographical region is consistent with findings for other geographical areas and time spans. Temperature is the meteorological descriptor mainly related with changes in seasonal descriptors. The increase in pollen quantities, not consequent to land use changes at our site, may be read in a context of global change, where the increase in CO<sub>2</sub> can play a relevant role. Detrimental effects on human health may result from pollen load changes, observed in the past decades or happening in the future.



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**KEY WORDS: CAMBIAMENTO CLIMATICO VEGETAZIONE,  
RISCHIO AMBIENTALE E RISCHIO ALLERGOLOGICO**

## AIRBORNE POLLEN IN ALPINE SITES

Elena GOTTARDINI <sup>1</sup>, Antonella CRISTOFORI <sup>2</sup>, Fabiana CRISTOFOLINI <sup>3</sup>, Gabriele TONIDANDEL<sup>4</sup> Luca PEDROTTI<sup>5</sup>

<sup>1</sup> *Research and Innovation Centre, Fondazione Edmund Mach, elena.gottardini@fmach.it*

<sup>2</sup> *Research and Innovation Centre, Fondazione Edmund Mach, antonella.cristofori@fmach.it*

<sup>3</sup> *Research and Innovation Centre, Fondazione Edmund Mach, fabiana.cristofolini@fmach.it*

<sup>4</sup> *Agenzia Provinciale per la Protezione dell'Ambiente, Trento, gabriele.tonidandel@provincia.tn.it*

<sup>5</sup> *Parco Nazionale dello Stelvio, Servizio Sviluppo Sostenibile e Aree Protette, luca.pedrotti@libero.it*

### Introduction

Airborne pollen is usually monitored at urban centers, where most people live, to provide information on types and amount of allergenic pollen. Thus, very few aerobiological data are available for remote, mountainous sites. This study aims to characterize the biological quality of the air in an alpine valley in Trentino (North Italy), a region highly interested by the presence of tourists (e.g., 9.3 million overnight stays registered during June - September 2021).

### Materials and Methods

An aerobiological study was carried out during late spring and summer in 2020 and 2021 in Val di Rabbi, an alpine valley located in the Trentino sector of Stelvio National Park. Gravitational Sigma-2 samplers were selected for the survey, for their reliability and efficiency (VDI\_2119 2013) and easy handling at remote sites (Gottardini et al. 2021), and installed at 2 m above ground level in 5 sites located at altitudes ranging from 700 to 2000 m a.s.l. Sampling surfaces, treated with an adhesive medium, were analyzed by optical microscopy (OM; 400×; Leitz Diaplan) for pollen identification and count (weekly samples); the average daily sedimentation rate ( $P \text{ cm}^{-2} \text{ d}^{-1}$ ) was calculated for the June-August period. In 2021, an active Hirst-type sampler was concurrently installed in one of the five sites (daily samples). Samples were processed and analyzed following conventional techniques and standardized protocols (UNI EN 16868:2019).

### Results and Discussion

In the two study periods, the number of identified pollen taxa ranged from 25 at the highest elevation site (2000 m a.s.l.) to 39 at the lowest elevation (700 m a.s.l.). The proportion of arboreal pollen slightly decreased with the altitude, from 51% to 42%. The three most abundant pollen taxa

were pine (*Pinus*), grasses (*Poaceae*) and nettle (*Urticaceae*), even if in different proportion at each site. The only grass and nettle allergenic pollen represented on average the 52% of the total pollen. The pollen season peak for grasses showed a delay of about 4 days every 100 m of elevation (Figure 1).

47 pollen taxa were identified, by active sampling, 10 more than by the passive one. The most abundant pollen taxa detected by active sampling were pine, grasses, and nettle, as for passive sampling, with the last two representing even the 71% of the total. The pollen season peak for grasses was on 22<sup>nd</sup> June, coherently with the passive sampling results at the same site.

## Conclusions

Our mountain aerobiological study reveals the presence of allergenic pollen even at high altitude, with noteworthy shifts in the pollen season. The surveyed data can be further exploited for studying the plant biodiversity in remote areas, as well as to provide useful information for allergic tourist and hikers. Moreover, passive sampling proved to be a feasible solution for aerobiological studies in remote or orographically complex areas, typically excluded in routine air quality monitoring.

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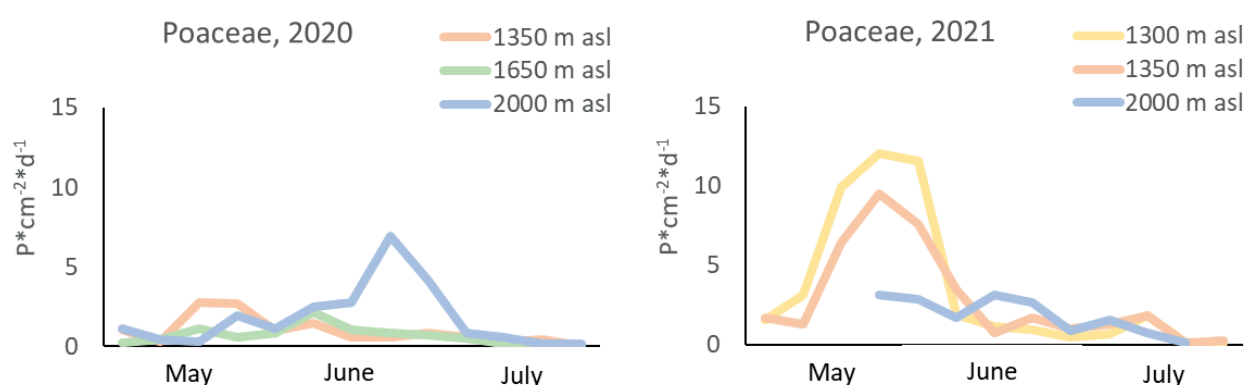
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## Acknowledgements

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Figure 1







**KEY WORDS: CAMBIAMENTO CLIMATICO VEGETAZIONE,  
RISCHIO AMBIENTALE E RISCHIO ALLERGOLOGICO**

## THE DISTRIBUTION OF *AMBROSIA SPP.* POLLEN GRAINS THROUGH NORTH EAST OF ITALY, SLOVENIA AND CARINTHIA (AUSTRIA) IN THE YEAR 2021

Francesca TASSAN-MAZZOCCO<sup>1</sup>, Pierluigi VERARDO<sup>2</sup>, Irene GALLAI<sup>3</sup>, Damaris SELLE<sup>4</sup>, Stefania LAZZARIN<sup>5</sup>, Elena GOTTARDINI<sup>6</sup>, Veronica KOFFLER<sup>7</sup>, Edith BUCHER<sup>8</sup>, Helmut ZWANDER<sup>9</sup>, Andreja KOFOL SELIGER<sup>10</sup>, Anja SIMČIČ<sup>11</sup>, Tanja CEGNAR<sup>12</sup>

<sup>1</sup> Regional Agency for Environmental Protection Friuli Venezia Giulia, Italy. e-mail: [francesca.tassan.@arpa.fvg.it](mailto:francesca.tassan.@arpa.fvg.it)

<sup>2</sup> Regional Agency for Environmental Protection Friuli Venezia Giulia, Italy. e-mail: [pierluigi.verardo@arpa.fvg.it](mailto:pierluigi.verardo@arpa.fvg.it)

<sup>3</sup> Regional Agency for Environmental Protection Friuli Venezia Giulia, Italy. e-mail: [irene.gallai@arpa.fvg.it](mailto:irene.gallai@arpa.fvg.it)

<sup>4</sup> Regional Agency for Environmental Protection Veneto, Italy. e-mail: [damaris.selle@arpa.veneto.it](mailto:damaris.selle@arpa.veneto.it)

<sup>5</sup> Regional Agency for Environmental Protection Veneto, Italy. e-mail: [stefania.lazzarin@arpa.veneto.it](mailto:stefania.lazzarin@arpa.veneto.it)

<sup>6</sup> Fondazione Edmund Mach (TN), Italy. E-mail: [elena.gottardini@fmach.it](mailto:elena.gottardini@fmach.it)

<sup>7</sup> Biologisches Labor Bozen, Italy, e-mail: [Veronika.Kofler@provinz.bz.it](mailto:Veronika.Kofler@provinz.bz.it)

<sup>8</sup> Biologisches Labor Bozen, Italy, e-mail: [Edith.Bucher@provinz.bz.it](mailto:Edith.Bucher@provinz.bz.it)

<sup>9</sup> Kärntner Landesregierung, Austria, e-mail: [hzwander@aon.at](mailto:hzwander@aon.at)

<sup>10</sup> National Laboratory of Health, Environment and Food, Slovenia. e-mail: [andreja.kofol.seliger@nlzoh.si](mailto:andreja.kofol.seliger@nlzoh.si)

<sup>11</sup> National Laboratory of Health, Environment and Food. e-mail: [anja.simcic@nlzoh.si](mailto:anja.simcic@nlzoh.si)

<sup>12</sup> Slovenian Environment Agency, Slovenia. e-mail: [tanja.cegnar@gov.si](mailto:tanja.cegnar@gov.si)

### Introduction

The data sharing of *Ambrosia spp.* pollen particles detection among the Environmental Agencies of North East Italy, Carinthia (Austria) and Slovenia has allowed the development of a geographical map showing where ragweed was mostly concentrated in 2021.

### Materials and Methods

*Ambrosia spp.* pollen grains were identified during the routine microscope analysis of the samples collected in 2021 from n.24 stations belonging to the monitoring networks of the Environmental Agencies involved in the study. Specific training in the recognition of *Ambrosia spp.* pollen was previously carried out to distinguish it from similar genera. Pollen concentration data collected from each station were reported as annual pollen index, i.e. the sum of daily pollen concentration over the year (fig.1). Kriging model was applied to spatialize the punctual annual data and visualize the areas of greatest presence of the pollen.

### Results and Discussion

The annual pollen indexes of *Ambrosia spp.* from North East of Italy, Carinthia (Austria) and Slovenia, spatialized with the Kriging method are represented in figure 2.

On this map, the sampling stations are also reported and since each station represents the main phytoclimatic areas of each territory, it was possible to validate the result obtained. It is evident a higher concentration of Ambrosia pollen in the eastern-south part of the study area (Slovenia, Carinthia, and the Italian Veneto region) that tends to decrease toward North-West (Italian region of Friuli Venezia Giulia and Trentino).

## Conclusions

Many studies on the presence of Ambrosia in Italy and in Europe show that this genus is abundant in North West Italy as well as in Hungary and Serbia. The sharing of annual monitoring data made it possible to verify that in 2021 ambrosia was very abundant on the border between Slovenia and Hungary, along the lower valley of the Drava and Sava rivers, both tributaries of the Danube. The Venetian plain seemed not to be very interested by the presence of this invasive plant, except for the Vicenza's area, while mountain areas were almost free of this pollen.

CITY	X	Y	AMBROSIA gr/m3
Belluno	12.22360568	46.16098718	111.85
Bolzano	11.342099	46.499332	85.32
Brunico	11.936946	46.800067	16.62
Feltre	11.91321206	46.01443649	131.98
Lignano Sabbiadoro	13.1162	45.6764	720
Padova	11.8875172	45.40276071	148.2
Pordenone	12.68305616	45.95712675	235.25
Rovigo	11.81650464	45.07303337	181.97
San Michele all'Adige	11.135	46.194	139
Silandro	10.778849	46.62891	32.41
Tolmezzo	13.01031	46.40588	83.12
Treviso	12.24902283	45.66567596	137.78
Trieste	13.77430339	45.64705821	604
Val Canali	11.86851005	46.1992681	47
Venezia	12.25309923	45.47796371	755.8
Verona	10.99065954	45.43779327	297.43
Vicenza	11.54652049	45.54779178	1094.22
Izola	13.66	45.54	1143
Lendava	16.45	46.55	7961
Ljubljana	14.51	46.06	1080
Maribor	15.63	46.55	1770
Klagenfurt	14.3	46.62	516
Villach	13.83	46.6	446
Weizelsdorf Rosental	14.172	46.5225	1630

Fig.1 quantity of Ambrosia pollen in the year 2021 in the study locations.

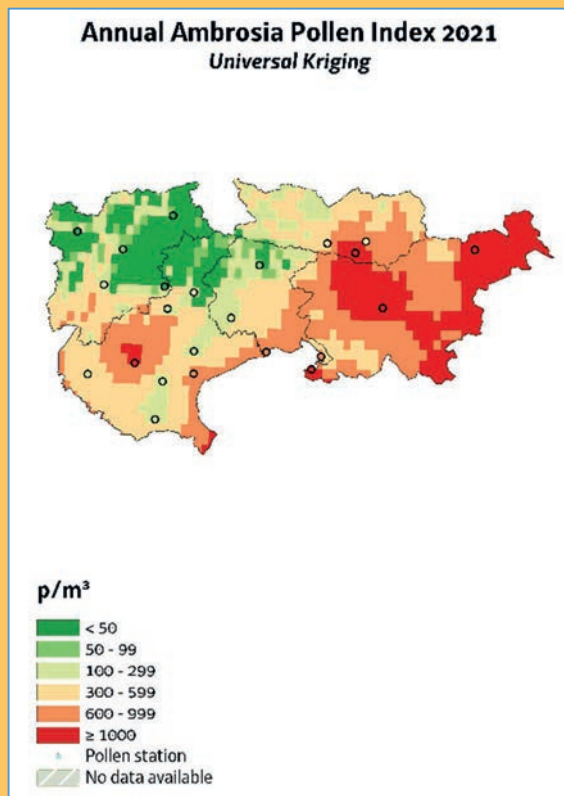


fig.2 Distribution of *Ambrosia spp.* through the North east of Italy (Trentino, Veneto, Friuli Venezia Giulia regions), Slovenia and Carinthia (Austria).



## LE RETI DI MONITORAGGIO AEROBIOLOGICO E AEROBIOLOGIA 4.0

### Pollen Monitoring by Different Techniques: a Comparative Study between Optical Microscopy and DNA Metabarcoding

Mattia FRAGOLA <sup>1</sup>, Sabrina DATTOLI <sup>2</sup>, Augusto ARSIENI <sup>3</sup>, Nicola CARELLI <sup>2</sup>, Sante MAIELLARO <sup>3</sup>,  
Maria Rita PERRONE <sup>1</sup>, Salvatore ROMANO <sup>1</sup>

<sup>1</sup> Department of Mathematics and Physics, University of Salento, [mattia.fragola@unisalento.it](mailto:mattia.fragola@unisalento.it);  
[mariarita.perrone@unisalento.it](mailto:mariarita.perrone@unisalento.it); [salvatore.romano@unisalento.it](mailto:salvatore.romano@unisalento.it)

<sup>2</sup> Agenzia Regionale per la Prevenzione e la Protezione dell'Ambiente (ARPA) Puglia, [s.dattoli@arpa.puglia.it](mailto:s.dattoli@arpa.puglia.it);  
[n.carelli@arpa.puglia.it](mailto:n.carelli@arpa.puglia.it)

<sup>3</sup> Azienda Sanitaria Locale (ASL) Brindisi, [arsienia@tin.it](mailto:arsienia@tin.it); [sante.maiellaro@asl.brindisi.it](mailto:sante.maiellaro@asl.brindisi.it)

#### Introduction

Pollen is extensively studied worldwide to investigate human sensitivity to this aeroallergen and the severity of related allergic symptoms. Indeed, allergies are amongst the most common chronic disorders, so the knowledge of airborne pollen concentrations is mandatory to achieve a better management of the associated diseases (Clot et al. 2020).

In this exploratory study, the contributions of pollen families collected at two different sites, Brindisi and Lecce (South-eastern Italy), by the Hirst-type trap/optical-microscopy system and DNA metabarcoding approach, respectively, were compared, with the main goal of discussing benefits and limits of the two monitoring techniques. The relationships between pollen concentrations, meteorological parameters, and long-range transported air masses were also investigated at both sites.

#### Materials and Methods

Environmental samples were simultaneously collected by means of a volumetric and a gravimetric air sampler in Brindisi and Lecce, respectively, from July 2018 to June 2019. In Brindisi pollen measurements are based on the reference method, i.e. a morphological analysis by optical microscopy according to EN 16868:2019 protocol. In more detail, a volumetric Hirst-type sampler was used to continuously sample airborne pollen grains in ambient air: atmospheric particles were captured on a rotating drum with an adhesive tape, which was analysed under an optical microscope. In Lecce gravimetric samplings were performed on the roof of the Mathematics and Physics Department of University of Salento, by a low volume HYDRA-FAI dual-sampler equipped with a PM10



sampling head, which allows collecting bioaerosol with an aerodynamic diameter  $\leq 10 \mu\text{m}$  on 47-mm-diameter polytetrafluoroethylene filters. Then, DNA metabarcoding approach, based on the 18S rRNA-gene high-throughput sequencing, was applied to DNA extracts from the collected PM<sub>10</sub> samples to identify pollen at family level (Fragola et al. 2022).

## Results and Discussion

Twenty-one pollen families were detected in Brindisi throughout the sampling period by morphological analysis with optical microscopy and pollen grain concentrations were mainly correlated with temperature, while no significant correlation with wind speed (WS) was observed, likely for the small number of samples collected during the one-year sampling. On the contrary, five out of the twenty-four pollen families detected in Lecce by DNA metabarcoding were positively correlated with WS. Few Urticaceae pollen grains were detected in Brindisi, only during heavy rainy days, also characterised by high relative humidity (RH) values; indeed, pollen grains may absorb moisture from the air and swell. Consequently, especially after heavy rain, swelling may cause the grains to rupture, preventing their identification by optical microscopy. No impact of heavy rain and/or high RH on the number of detected families and sequence reads was instead observed in Lecce. Only 9 out of the 24 pollen families detected in Lecce were in common with the ones detected in Brindisi; particularly, the nine families were identified in all samples in Lecce, whereas in Brindisi they were detected mainly in spring samples, which was likely due to the different pollen sampling/detection systems used at the two sites. The four-day analytical back-trajectory analysis has proved that both sites were similarly affected by airflows, but their impact on the 9 shared pollen family contributions varied differently with advection patterns at both sites.

## Conclusions

Whole pollen grains (10–100  $\mu\text{m}$  diameter) are required for the pollen morphological detection by optical microscopy in Brindisi. Conversely, the PM<sub>10</sub> sampler used in Lecce allows the collection of only airborne pollen grains and/or fragments with an aerodynamic diameter  $< 10 \mu\text{m}$ . The deposition of whole pollen grains markedly decreases with increasing distance from the source because of their large size and, consequently, the long-range transport of pollen fragments  $< 10 \mu\text{m}$  is likely favoured over that of pollen grains. Hence, pollen families were detected in Brindisi mainly during their blooming period, while they were on average detected throughout the sampling time in Lecce.

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### Long-term trends of annual pollen concentrations in the Veneto region, Italy (2008-2020)

Sofia TAGLIAFERRO<sup>1</sup>, Pierpaolo MARCHETTI<sup>1</sup>, Barbara DALL'ARA<sup>2</sup>, Francesco DOMENICHINI<sup>2</sup>, Stefania LAZZARIN<sup>2</sup>, Morena NICOLIS<sup>3</sup>, Damaris SELLE<sup>2</sup>, Camillo SILIBELLO<sup>4</sup>, Alessandro MARCON<sup>1</sup>

<sup>1</sup> *Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona. Email: sofia.tagliaferro@univr.it, pierpaolo.marchetti@univr.it, alessandro.marcon@univr.it*

<sup>2</sup> *Regional Agency for Environmental Protection Veneto, Italy. E-mail: barbara.dallara@arpa.veneto.it, francesco.domenichini@arpa.veneto.it, stefania.lazzarin@arpa.veneto.it, damaris.selle@arpa.veneto.it*

<sup>3</sup> *Section of Hygiene and Preventive, Environmental and Occupational Medicine, Department of Diagnostics and Public Health, University of Verona. E-mail: morena.nicolis@univr.it*

<sup>4</sup> *ARIANET, Milan, Italy. E-mail: c.silibello@aria-net.it*

**Introduction:** pollen-induced allergies affect approximately 20% of the world's population (D'Amato et al. 2016). Climate change plays a role in altering pollen allergenicity, seasonality, distribution and load (Beggs 2021). The rising temperature has been correlated to increasing pollen concentrations, as well as longer and earlier pollen seasons over time (Beggs 2021; Choi et al. 2021). This evidence suggests a possible future increase in human exposure to pollen and a consequent increase in the burden of allergic diseases. This study aimed to evaluate long-term trends in pollen load and possible climatological differences within the Veneto region, northern Italy.

**Materials and methods:** for the period 2008-2020, daily concentration data of 10 allergenic pollen families/genera (Betulaceae, Betula, Alnus, Cupressaceae, Oleaceae, Graminaceae, Urticaceae, Corylaceae, Ambrosia, Artemisia) for 12 monitoring centres was provided by the Regional Agency for Environmental Protection Veneto (ARPAV). The centres were classified into two different climatic zones (Barbi et al. 2011): 3 alpine centres (Belluno, Feltre, Agordo) and 9 sub-continental centres (Treviso, Verona, Legnago, Vicenza, Padova, Teolo, Venezia, Jesolo, Rovigo). The pollen dataset was checked using the quality control function of the R AeRobiology package (Rojo et al. 2019) and the moving mean method was applied for the imputation of missing data. The 95-percentage method was applied to identify the start/end dates of pollen seasons during each year (Nilsson and Persson 1981; Andersen 1991), and the seasonal cumulative pollen concentration (Seasonal Pollen Integral, SPI<sub>n</sub>) was calculated as well. Temporal trends of SPI<sub>n</sub> (dependent variable) were analysed with the statistic software STATA using two-level linear regression models with year and climatic zone as independent variables, and a random intercept term for the centre. Climatic heterogeneity in trends was investigated by testing the interaction between year and climatic zone using likelihood ratio tests. When the p-value was <0.10, separate analyses were conducted for alpine and sub-continental centres.



Results and discussion: a general increase in concentrations emerged for all pollens except for Urticaceae, Betula, Ambrosia and Artemisia, which did not show a significant trend over time. An annual average increase of 78.8 (95%CI: 25.5, 132.2), 135.1 (95%CI: 84.9, 185.3), and 407.5 (95%CI: 266.0, 549.1) pollen/cubic meter (p/m<sup>3</sup>) was observed for Betulaceae, Oleaceae, and Corylaceae, respectively. A significant climatic heterogeneity resulted in the temporal trends of Cupressaceae (Alpine vs. Sub-continental: 28.3, 95%CI: -146.7, 203.4, vs. 251.1, 95%CI: 141.4, 360.7 p/m<sup>3</sup>), Graminaceae (45.4, 95%CI: -41.7, 132.6, vs. 205.6, 95%CI: 150.2, 261.1 p/m<sup>3</sup>) and Alnus (13.8, 95%CI: -46.1, 73.6, vs. 78.4, 95%CI: 41.7, 115.0 p/m<sup>3</sup>).

Conclusions: these results suggest a general increase in seasonal pollen load for most pollens, which was more marked in the sub-continental centres for Cupressaceae, Graminaceae and Alnus, compared to the alpine centres.

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## LE RETI DI MONITORAGGIO AEROBIOLOGICO E AEROBIOLOGIA 4.0

### Aerobiology in spatial knowledge: focus on Veneto.

Damaris SELLE<sup>1</sup>, Silvano DE MAS<sup>1</sup>, Barbara DALL'ARA<sup>1</sup>, Stefania LAZZARIN<sup>1</sup> Anna BORDIN<sup>2</sup>, Morena NICOLIS<sup>3</sup>, Rodolfo MUZZOLON<sup>4</sup>

<sup>1</sup>Veneto Regional Agency for Environmental Protection : damaris.selle@arpa.veneto.it, silvano.demas@arpa.veneto.it, barbara.dallara@arpa.veneto.it, stefania.lazzarin@arpa.veneto.it

<sup>2</sup>University of Padua: anna.bordin@unipd.it ; <sup>3</sup>University of Verona<sup>4</sup>: morena.nicolis@univr.it; Azienda ULSS n. 1 Dolomiti Belluno: muzzolon.ulss1@gmail.com

#### Introduction

A 10-year study, from 2012 to 2021, of the Total Pollen Index (TPI) in the Veneto region has made it possible to identify the aerobiological specificities of the region and the pollinoses present in the territory.

#### Materials and methods

The analysis was performed by processing the data obtained from the monitoring carried out at the eight sampling sites distributed throughout the region (fig. 1), using a VPPS-Lanzoni sampler. For each station, the Total Pollen Index (TPI) was calculated, i.e. the sum of the average daily concentrations (expressed in granules/mc air) of all the pollens detected during the year in question (fig.2), the TPI over the 10 years of some tree families (fig. 3) and of some herbaceous families (fig.4). The main pollinoses were then considered (%).

#### Results and discussion

2020 was the year with the highest pollen concentration in all sites and 2013 the one with the lowest. Aceraceae, Betulaceae and Cupressaceae are present above all in the lowland and coastal areas, especially in Treviso, Verona, Vicenza and Venice, and Platanaceae in the lowlands and in the west of the region (Padua, Rovigo, Verona). Salicaceae are especially found in the Rovigo lowland site, while in the mountain area (Belluno and Feltre), Corylaceae and Oleaceae (genus *Fraxinus*) are the most representative family. The *Olea* genus is particular to the Verona area. Herbaceous plants have shown trends of higher concentrations in the plains and towards the coast than in the mountainous areas, in particular the Amaranthaceae in the Rovigo site and the Composite plants in the west of the region. The prevalence of pollen allergy is estimated at between 30 and 40% of the population, and the prevalence of pollinosis reflects the distribution of pollen taxa in the different vegetative areas. Grass pollen is the main cause of pollinosis, accounting for more than 70% of allergic sensitisation in lowland areas; in mountainous areas, sensitisation to Corylaceae-Betulaceae plays an important role, affecting up to 40% of pollinosis



patients. Sensitisation to Cupressaceae affects no more than 10 % of allergic patients; *Parietaria* sp. plays a more significant clinical role in coastal areas, affecting about 30 % of allergic patients. Sensitisation to Oleaceae (Olea tree in the Veronese and lowland areas, *Fraxinus* and *Ligustrum* in hilly-mountainous areas) affects between 10 and 30 % of patients. Sensitisation to Composites (mainly *Artemisia* sp.) affects a percentage of patients of around 10-20 %. It should also be noted that patients are often sensitised to more than one pollen taxa at the same time; multisensitisation mainly concerns Corylaceae-Betulaceae, Graminaceae and Urticaceae (*Parietaria* sp).

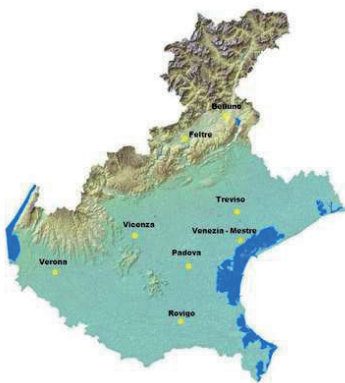


Figure 1 - The monitoring network

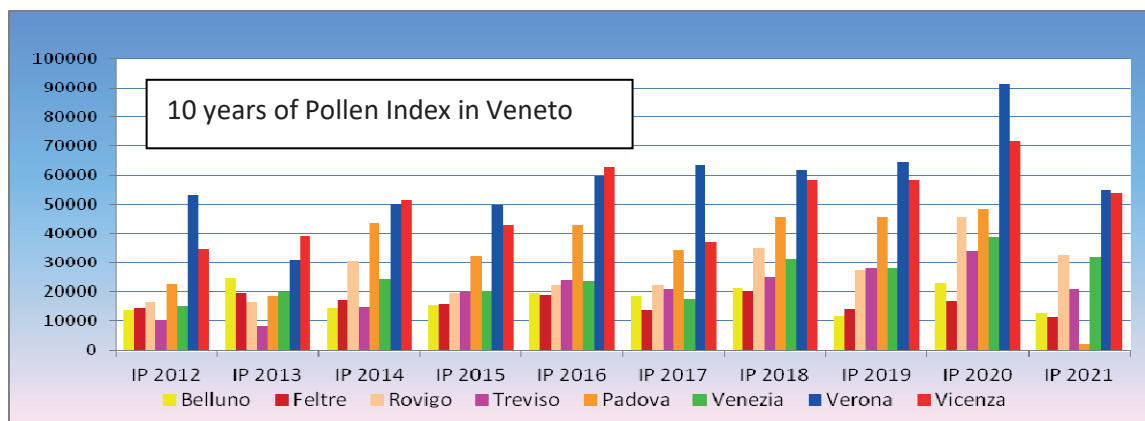


Figure 2- Pollen Index from 2012 to 2021 for each monitoring site.

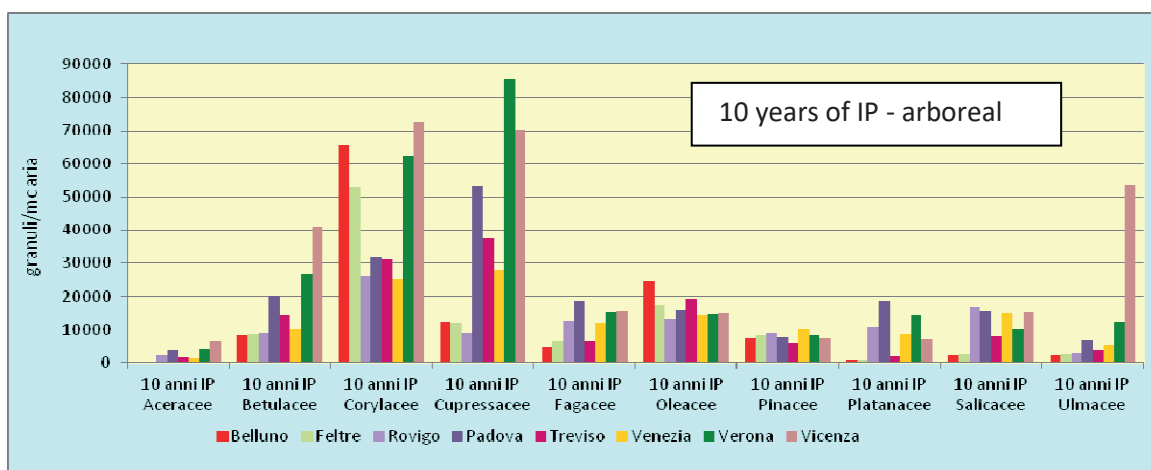


Figure 3- 10 years of tree IPs per monitoring site

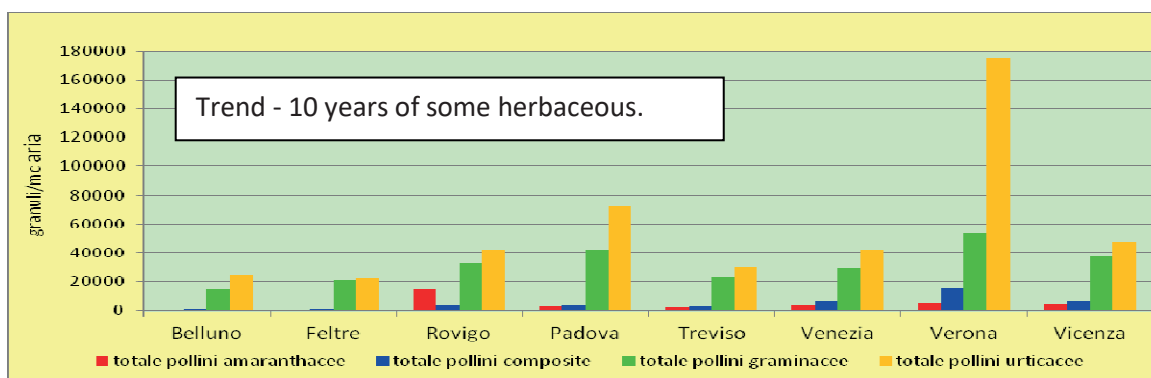


Figure 4- 10 year IP of some herbaceous plants per monitoring site





## LE RETI DI MONITORAGGIO AEROBIOLOGICO E AEROBIOLOGIA 4.0

### ALLERGENIC POLLENS OF WEED PLANTS IN NORTH EAST ITALY

Pierluigi VERARDO<sup>1</sup>, Francesca TASSAN MAZZOCCO<sup>2</sup>, Damaris SELLE<sup>3</sup>, Barbara DALL'ARA<sup>4</sup>, Stefania LAZZARIN<sup>5</sup>

<sup>1</sup> Regional Agency for Environmental Protection Friuli Venezia Giulia, Italy. e-mail: [pierluigi.verardo@arpa.fvg.it](mailto:pierluigi.verardo@arpa.fvg.it)

<sup>2</sup> Regional Agency for Environmental Protection Friuli Venezia Giulia, Italy. e-mail: [francesca.tassan.@arpa.fvg.it](mailto:francesca.tassan.@arpa.fvg.it)

<sup>3</sup> Regional Agency for Environmental Protection Veneto, Italy. e-mail: [damaris.selle@arpa.veneto.it](mailto:damaris.selle@arpa.veneto.it)

<sup>4</sup> Regional Agency for Environmental Protection Veneto, Italy. e-mail: [barbara.dallara@arpa.veneto.it](mailto:barbara.dallara@arpa.veneto.it)

<sup>5</sup> Regional Agency for Environmental Protection Veneto, Italy. e-mail: [stefania.lazzarin@arpa.veneto.it](mailto:stefania.lazzarin@arpa.veneto.it)

#### Introduction

In North East Italy, in addition to the allochthonous plants that arrived in the past, new plant species that produce allergenic pollens have recently arrived and are spreading. Their spread and consistency are not always well known. An analysis of their pollen can indicate their presence, quantity and danger to health and the environment.

#### Materials and Methods

The sampling slides of the pollen monitoring network are used to identify pollen from alien plants. In some cases, specific training is required on the recognition of some rare or little known taxa. Kriegering programs are used to spatialize the sampling data to visualize the places of greatest presence.

#### Results and Discussion

The pollens of some species of weeds such as Ambrosia and Amaranthus are frequently detected by the palynologists of the Italian POLLnet network.

Ambrosia has been studied throughout Italy for years and its distribution shows that it is mainly present in the North (fig. 1).

Amaranthus has shown a significant increase in recent years, due to the pesticide resistance of this plant, a typical crop pest (fig. 2).

In recent years specific courses and tests have been carried out to improve the competence of operators in the recognition of some taxa, such as Ailanthus, Xanthium, Broussonetia, Maclura. The reports of botanists have been useful for the search for new plants such as Baccharis (fig. 3), Impatiens, Senecium. Still others are not part of the Italian flora, but sometimes pollens are found in the air due to transboundary transport phenomena from not far away territories, where plants are present: this is the case of the VAT genus. In the routine monitoring of allergenic pollen, these pollen types are often not considered individually, but the counts refer to generic classes "Other pollen grains" or "Other Asteraceae".

In order to investigate the phenomenon of the diffusion of many allctone weeds, both quantitative sampling data and reports of their presence on the territory were collected; in some cases the information was reworked to obtain geographic distribution maps; in others, the possible impacts on human health and the ecosystem were assessed. In some cases, measures have been taken to contain the spread and consequent damage.

## Conclusions

The study of airborne pollen can be of great help in knowing the presence, consistency and danger of some plant species that have arrived in north-eastern Italy. If the pollen is identifiable and detected in several stations, it is possible to create a distribution map that identifies the areas at greatest risk. The distribution maps of the plant over the years help to follow the phenomenon over time.

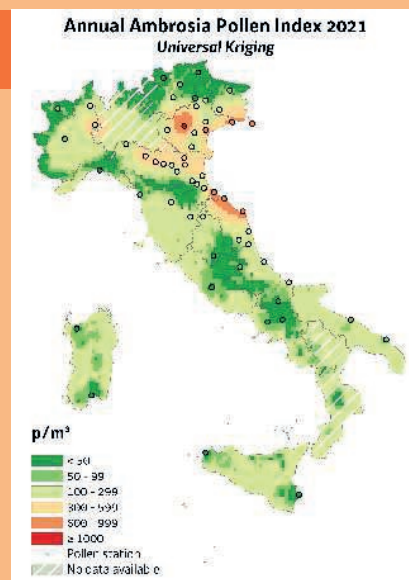


fig.1. Distribution of Ambrosia in Italy in 2021.

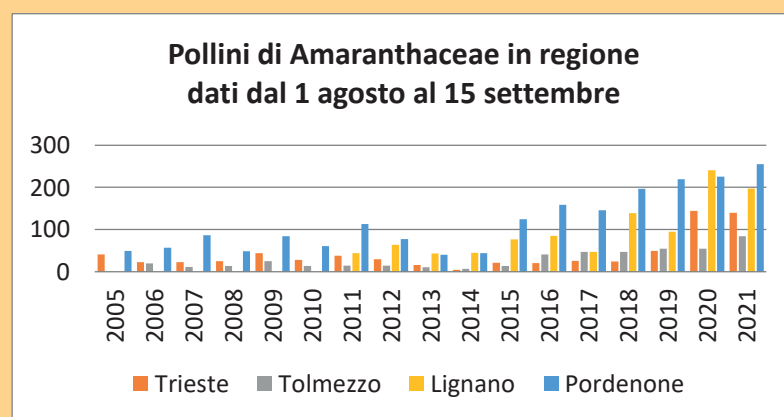


fig.2. total of *Amaranthus* pollen in some locations in North East Italy.



fig.3. *Baccaris halimifolia* in the Venice lagoon.

## ONE HEALTH AND AIR QUALITY MONITORING

### Focus *Alternaria sp.*: 22 years of monitoring in Padua

Maria Angiola CRIVELLARO<sup>1</sup>, Anna BORDIN<sup>1</sup>, Stefania LAZZARIN<sup>2</sup>, Barbara DALL'ARA<sup>2</sup>, Damaris SELLE<sup>2</sup>

<sup>1</sup>University of Padua: anna.bordin@unipd.it

<sup>1</sup>University of Padua: mariangiola.crivellaro@aopd.veneto.it

<sup>2</sup>Regional Agency for Environmental Prevention and Protection of the Veneto Region:  
stefania.lazzarin@arpa.veneto.it; barbara.dallara@arpa.veneto.it ; damaris.selle@arpa.veneto.it

#### Introduction:

*Alternaria sp.* is a fungus belonging to the Ascomycota division as it produces spores in a characteristic type of sporangium called asco. This genus, as well as being of interest in the agronomic field because it can damage plants and their products (cereals, flax, sunflowers, potatoes, tobacco, etc.), is also the cause of allergic pathologies.

#### Materials and methods

Monitoring was carried out weekly using a VPPS- Lanzoni sampler. The period considered was from 2000 to 2021. In addition to the monitoring data, data on allergic diseases caused by this fungus were considered.

#### Results and discussion

Figure 1 shows the trend over 22 years of monitoring. Analysis of the data shows an increase in the quantity of spores from 2015 to 2020. Figure no. 2, on the other hand, shows the trend in the years of greatest sporulation (2015 to 2020); it can be seen that the period of greatest spore emission is concentrated mainly in the summer, late summer and autumn months.

Due to its great ability to spread and the small size of its spores, *Alternaria sp.* is easily inhaled and often causes respiratory difficulties.

The greatest *Alternaria* spore emission, has been connected to respiratory allergy; the exposure to spores *Alternaria sp.* is considered significant increase in the prevalence of allergic respiratory diseases observed in the summer and autumn months



[12-15] and an important contributor to the development and exacerbation of asthma. It has been suggested that *Alternaria* spore can also interact with airborne PM and acts as adjuvants by altering the immunogenicity of allergenic proteins.

As a contributing factor, climate change (in particular temperature, relative humidity and ultraviolet radiations) is known to affect abundance and properties of bioaerosols as carriers of aeroallergens.

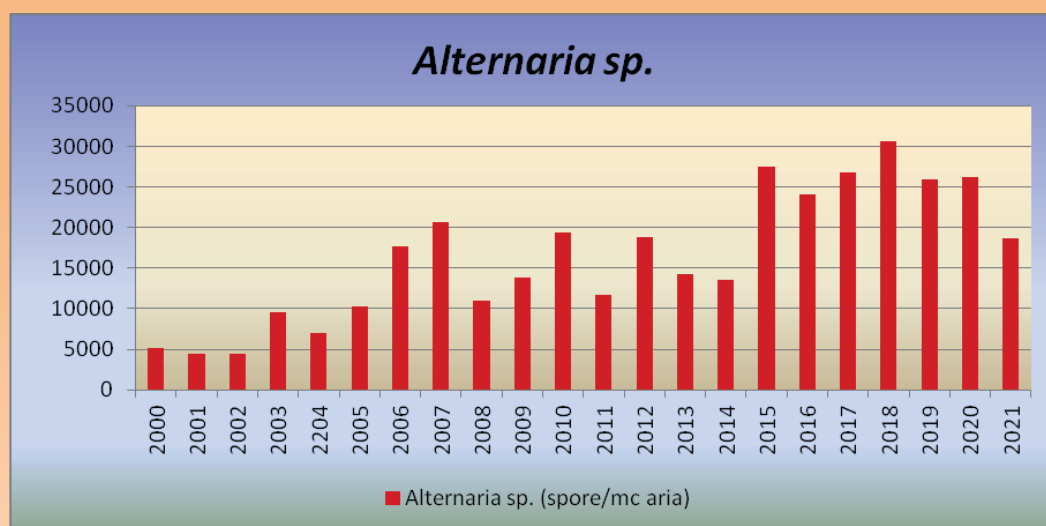


Figure no. 1 - *Alternaria* sp. in Padua from 2000 to 2021

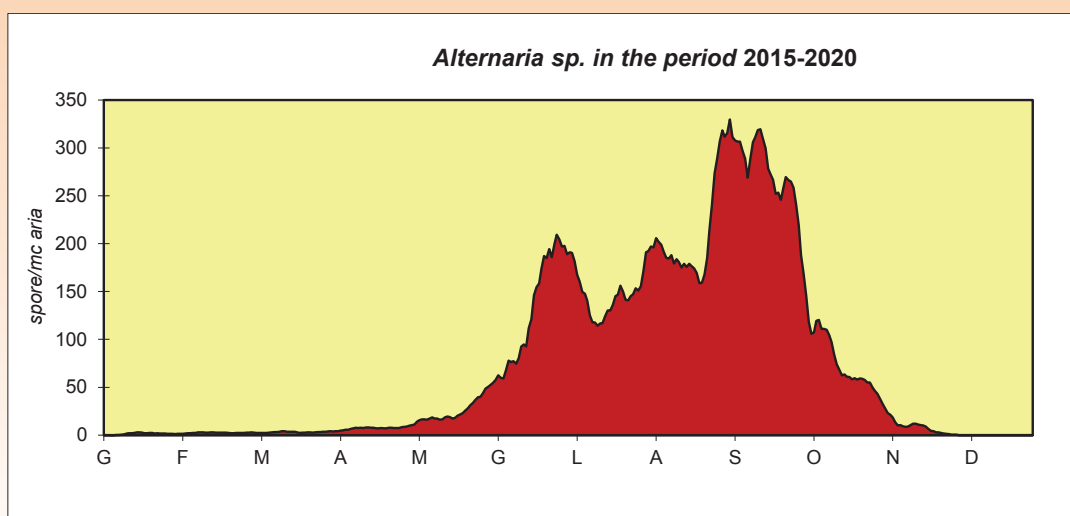


Figure no. 2 - Evolution of *Alternaria* sp. spores in the period 2015-2020



## SCHOOL-UNIVERSITY COLLABORATION, A CASE STUDY: AIRBORNE FUNGAL SPORES MONITORING IN CROPS FOR FARM ANIMALS

Elena RUGGERI<sup>1</sup>, Sergio BENEDETTI<sup>2</sup>, Massimo ZERANI<sup>1</sup>, Emma TEDESCHINI<sup>3</sup>

<sup>1</sup> Department of Veterinary Medicine, University of Perugia, Italy  
[elena.ruggeri@studenti.unipg.it](mailto:elena.ruggeri@studenti.unipg.it), [massimo.zerani@unipg.it](mailto:massimo.zerani@unipg.it)

<sup>2</sup> Institute of Higher Education "G. Garibaldi", Agricultural Institute of Macerata, Italy  
[sergio.benedetti@agrariamacerata.org](mailto:sergio.benedetti@agrariamacerata.org)

<sup>3</sup> Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy  
[emma.tedeschini@unipg.it](mailto:emma.tedeschini@unipg.it)

**Introduction** School-university collaboration is important for the greater practical technical skills available to students. Agricultural Institute of Macerata (founded in 1868) has an agricultural farm that plays a key role in the student education. In this institute, a student of the degree course "Animal production" carried out her degree thesis, thus making the skills learned in the university course available to the students of the institute.

Fungal spores have been a cause of great concern in the food farming sector for years. Fusariosis of the ear, present on both wheat and barley, is a disease caused by several species of the genus *Fusarium* (*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*). Among the species causing fusariosis, *F. graminearum* is the prevalent species. This complex disease is feared for its resurgence and for the health implications associated. In favorable environmental conditions it can lead to production losses of up to 45%. In addition, the *Fusarium* species produce mycotoxins, substances harmful to attacked plants that resist during processing and are therefore present in food derivatives for humans and animals. Following this they were regulated by the legislature with maximum permissible limits in grain binding for the production and marketing of derivatives (food and feed products). The presence of airborne spores is an indication of the risk of developing phytopathology so of the possible release of mycotoxins that can spread into food. According to the recent One Health concept, declared by Superior Institute of Health, it's important to pay attention to any interference from these toxins, which could destabilize the balance that connects human, animal and ecosystem health. The aim of this research is to monitor the presence of airborne fungal spores pathogenic of the cereals used as food for animals and humans.



**Materials and Methods** The aerobiological monitoring is carried out at Agricultural Institute of Macerata (Central Italy) from May to July 2022, the period corresponding to the maturation of the inflorescences until harvest. The cereal crops present in the monitored area are: *Triticum durum* cv Senatore Cappelli, *Triticum aestivum* cv Bologna, Marco Polo, Pioneer PR22R58, Bandera, *Hordeum vulgare* cv Digersano, *Vicia faba* cv Chiaro di Torre Lama, *Medicago sativa* cv Bella campagnola, *Lolium multiflorum* cv Mowestra. The aerobiological monitoring was carried out by a volumetric trap of Lanzoni VPPS 2000 spores placed on a terrace at about 10 m.

**Results and Discussion** Considering the unfavorable weather conditions of the year for the development of fusariosis, the research involved the monitoring of the *Alternaria* genus (Figure 1), that like *Fusarium* is a saprophytic spore, phytopathogenic and pathogen for respiratory system of domestic animals and humans (Budd, 1986). Moreover, in 2011 a report of European Food Safety Authority (EFSA) indicates the presence of toxins in feed and food attributed to *Alternaria* genus. *Alternaria* species are characterized by a great environmental adaptation, inducing severe epidemics in both humid and semi-arid regions. Some *Alternaria* species can infect and induce symptoms on plants during their growing stages, while others only cause damage after harvest in storage, trade and processing (Agrios, 2005). *Alternaria* conidia have a typical morphology that allows recognition in monitoring samples: are multicellular, light brown and claviform shape with size 15-20 x 30-40 µm. EFSA specifies that exposure to mycotoxins can occur through the consumption of contaminated food or animals fed with contaminated feed. This evidence stimulates scientific interest by directing research towards an in-depth study of the behavior of this spore and its actual toxicity regarding the risk of developing plant diseases and consequent development of carcinogenic toxins in animals and humans. In central Italy regions (Umbria and Marche), the *Alternaria* spores can produce about 70 phyto-toxins, of which only a limited number have been chemically characterized. The phyto-toxins produced by the *Alternaria* species are classified as emerging toxins, or rather, toxins of certain toxicological interest, which are not yet subject to European legislation (Pecorelli 2018).

**Conclusions** The attention of the European Commission in relation to the toxicity of *Alternaria* species stops at foodstuffs for human use, underestimating the repercussions that zootechnical nutrition has on human health. Until now, European Commission has only issued a recommendation on monitoring the presence of *Alternaria* toxins in food (European Commission, 2022). This research comes from the need to constitute another possible starting point for the launch of further studies on the toxicity of *Alternaria*. The results showed that *Alternaria* parasitised organically grown *Triticum aestivum* at a low level and chemically treated *Triticum durum* at a high level. (Figure 2). According to the researches and recommendations mentioned, the study of *Alternaria* is crucial in preventing the domino effect that would arise at the time of a fungal attack by developing phytotoxicity with direct and indirect consequences on animal and human health.

In the context of the multidisciplinary that characterizes training in Italian higher education, the design of this research aims to create a bridge between school and university specialization and in this case to introduce knowledge of the airborne microbiome.

**Contributions** E.R.: sampling and result analysis; S.B. institute tutor; M.Z.: university tutor, idealization and result analysis; E.T.: idealization, study design and result analysis.

**Acknowledgements** The Authors thank the Dean of the Institute Prof. Maria Antonella Angerilli for her willingness to school-university collaboration and the Professors Ludovica Carducci and Etaferahu Sime for their support in making the collaboration successful.

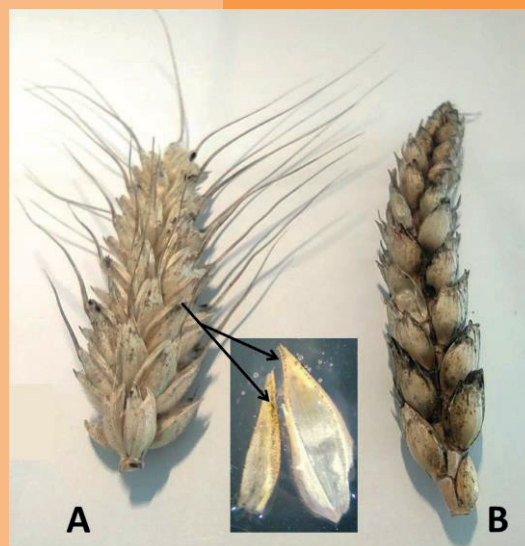
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#### Figures



**Figure 1.** *Alternaria* genus



**Figure 2.** A: *Triticum aestivum*, cv Bologna. B: *Triticum durum*, var. Platone C3S. Insert: glume with alternariosis

# AIRBORNE POLLEN: A POTENTIAL WARNING ALERT FOR TICK-BORNE ENCEPHALITIS RISK

Antonella CRISTOFORI <sup>1</sup>, Fabiana CRISTOFOLINI <sup>2</sup>, Giovanni MARINI <sup>3</sup>, Francesca DAGOSTIN <sup>4</sup>,  
Valentina TAGLIAPIETRA <sup>5</sup>, Annapaola RIZZOLI <sup>6</sup>, Elena GOTTARDINI <sup>7</sup>

<sup>1</sup> Research and Innovation Centre, Fondazione Edmund Mach, [antonella.cristofori@fmach.it](mailto:antonella.cristofori@fmach.it)

<sup>2</sup> Research and Innovation Centre, Fondazione Edmund Mach, [fabiana.cristofolini@fmach.it](mailto:fabiana.cristofolini@fmach.it)

<sup>3</sup> Research and Innovation Centre, Fondazione Edmund Mach, [giovanni.marini@fmach.it](mailto:giovanni.marini@fmach.it)

<sup>3</sup> Research and Innovation Centre, Fondazione Edmund Mach, [francesca.dagostin@fmach.it](mailto:francesca.dagostin@fmach.it)

<sup>3</sup> Research and Innovation Centre, Fondazione Edmund Mach, [valentina.tagliapietra@fmach.it](mailto:valentina.tagliapietra@fmach.it)

<sup>3</sup> Research and Innovation Centre, Fondazione Edmund Mach, [annapaola.rizzoli@fmach.it](mailto:annapaola.rizzoli@fmach.it)

<sup>3</sup> Research and Innovation Centre, Fondazione Edmund Mach, [elena.gottardini@fmach.it](mailto:elena.gottardini@fmach.it)

## Introduction

The circulation of tick-borne encephalitis virus (TBEv) depends on population dynamics of host tick and rodents, which in turn depend on nutrient resources. Tree seeds are the main food for rodents, and their fluctuating production is strongly correlated to pollen abundance. Our study aims to fill the gap and investigate whether airborne pollen is directly associated to recorded TBEv human cases in the Alpine biogeographical region.

## Materials and Methods

We focused our study within the province of Trento (northern Italy, 6,000km<sup>2</sup>, 500,000 inhabitants). The territory is included in the Alpine biogeographical region (EEA Report No 1/2002) and the main forest tree species growing within a 5-km radius from the pollen sampler are represented by hop-hornbeam (*Ostrya carpinifolia* Scop.), beech (*Fagus sylvatica* L.), spruce (*Picea abies* L.), pine (*Pinus sylvestris* L. and *P. nigra* J. F. Arnold), downy oak (*Quercus pubescens* Willd.), manna ash (*Fraxinus ornus* L.), and hazel (*Corylus avellana* L.). Airborne pollen concentration has been monitored since 1989 at Fondazione Edmund Mach, in San Michele all'Adige (Latitude 46.19 N, Longitude 11.13 E, 220 m a.s.l.), while TBEv human cases have been recorded since 1992 by the local Public Health Agency. Airborne pollen was sampled by a Hirst-type trap, processed, and analyzed following conventional techniques and standardized protocols (UNI EN 16868:2019). First, we statistically investigated the association between the annual total pollen concentration of the dominant arboreal plant taxa and the annual number of TBEv human cases (1989-2020) with

different time lags by univariate linear models. Consequently, we built a full model by considering all significant covariates, we computed all possible sub-models and finally we selected the best (the one with the lowest Akaike's Information Criterion score).

## Results and Discussion

We found a significant positive association between pollen abundances for beech ( $p=0.04$ ), oak  $p=0.012$ ), hop hornbeam ( $p=0.013$ ) and TBEv human cases with a two-year lag (Figure 1). All other lags and taxa resulted in non-significant relationships. Subsequently, we identified the best model, which considered only hop-hornbeam and oak pollen quantities, both with positive coefficients, consistently with the univariate analysis.

## Conclusions

To the best of our knowledge, this is the first attempt at quantifying the potential relationship between airborne pollen abundances of tree species and TBEv infections, based on a three-decade time series of data. If validated at a larger spatial scale, pollen data might therefore be used to realize an early warning system for the risk of TBEv transmission, two years in advance. Moreover, as pollen monitoring is routinely performed worldwide at multiple sites and provides quantitative measures, the association between pollen abundances and TBEv infections could be replicated in different biogeographical regions.

## References

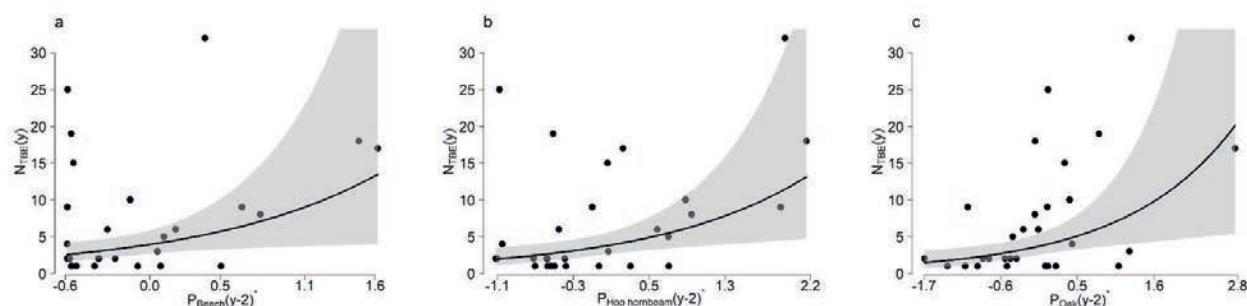
EEA Report No 1/2002 [https://www.eea.europa.eu/publications/report\\_2002\\_0524\\_154909](https://www.eea.europa.eu/publications/report_2002_0524_154909)

UNI EN 16868:2019 Ambient air - Sampling and analysis of airborne pollen grains and fungal spores for allergy networks - Volumetric Hirst method

## Acknowledgements (Funds)

This study was partially funded by EU grant 874850 MOOD.

Figure 1







## VALORE ECONOMICO DEL POLLINE E COSTO SOCIALE DELLE POLLINOSI

# MEASURING THE CLINICAL IMPACT OF NATURAL EXPOSURE TO POLLEN AND MOLDS THROUGH E-DIARIES. A PILOT STUDY IN 100 PATIENTS WITH SEASONAL ALLERGIC RHINITIS.

Luigi PALMIERI<sup>1,2</sup>, Stephanie DRAMBURG<sup>1</sup>, Salvatore TRIPODI<sup>3</sup>, Alessandro TRAVAGLINI<sup>4</sup>, Paolo Maria MATRICARDI<sup>1</sup>

<sup>1</sup> Dept. of Pediatric Pulmonology, Immunology and Critical Care Medicine, Charité -Universitätsmedizin Berlin, Berlin, Germany, [luigipalmieri88@gmail.com](mailto:luigipalmieri88@gmail.com)

<sup>2</sup> Department of Pediatric, Aldo Moro University of Bari, Bari, Italy

<sup>3</sup> Allergology Service, Policlinico Casilino, Rome, Italy

<sup>4</sup> Department of Biology, University of Rome Tor Vergata, Rome, Italy

### Introduction

E-diaries are increasingly used in the diagnosis and monitoring of seasonal allergic rhinitis. Sequences of daily data recorded produce trajectories of disease severity scores over time, which can be matched against simultaneous trajectories of pollen counts (Bianchi A. et al. 2016). The graphs produced by these two types of trajectories can be analyzed either by direct observation or by means of mathematical parameters (Tripodi S. et al. 2020) (Dramburg et al. 2022). However, guidelines helping the interpretation of these graphs for diagnostic purposes have never been developed.

### Materials and Methods

We examined the trajectories of daily symptoms scores (dSS) collected through e-Diary (AllergyMonitor™, TPS, Rome - Italy) by 100 polysensitized patients affected by seasonal allergic rhinitis during 2018 in Rome (Italy) and matched against those of the concentration of pollen grains (Cupressaceae, Fagales, Oleaceae, Graminaceae, Urticaceae) and *Alternaria* spores in the air. By watching each patient's graphs (Fig. 1), and following internal guidelines, three independent experts graded, with a score-class ranging from 0 (null) to 4 (max), 5 different parameters (Tab. 1) referred in each patient to each allergen. Consensus was considered achieved when the scores attributed by different experts to the same item fell into two contiguous classes.

## Results and Discussion

Full consensus on data completeness by the three experts was achieved in 531/600 (88.5%) evaluations (Tab. 2), of which 495 fulfilled the criteria for further judgement. Among these, full consensus was found in 439 (90.1%) for average symptoms severity, 467 (94.3%) for global symptoms burden, 381 (77.0%) for allergen priority, and 381 (77.0%) for allergen clinical relevance. At population level, average scores ranged 2.9-3.2 for data completeness, 1.5-2.0 for symptom severity, 1.5-2.3 for global burden of allergen exposure, 1.5-2.3 for allergen priority and 0.9-1.4 for allergen overall clinical relevance.

## Conclusions

The visual analysis and interpretation of graphs generated by e-Diaries of patients with seasonal allergic rhinitis can be well supported by guidelines. However, a certain degree of heterogeneity in experts' subjective interpretation cannot be eliminated. This visual analysis, which is demanding and time consuming, might be substituted by mathematical algorithms, making e-Diaries' reading and interpretation reliable, standardized, fast and user-friendly.

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## Tables

E-diary basic parameters (by pollen season)
1) <b>data completeness</b>
2) average <b>severity</b> of symptoms
3) <b>overall burden</b> of symptoms over the year
4) <b>priority</b> (hierarchical position between allergens)
5) <b>clinical relevance</b> (AIT?)

**Table 1** - The parameters asked to be judged by the three experts

**Table 2** - Effectiveness of guidelines in generating concordance of judgement among experts. Comparison of the aetiological diagnostics (identification of the allergen responsible for the symptoms) by comparing the trajectories of seasonal allergic rhinoconjunctivitis symptoms of 100 Roman patients (January-September 2018) and simultaneous pollen trajectories of cupressaceae, fagales, oleaceae, gramineae, urticaceae and alternaria.

Difference between scores	expert comparisons			global	
	1v2	1v3	2v3	n	%
<b>evaluuable comparisons*</b>	2609	2616	2652	7877	100,00
<b>difference (score)</b>					
-4	0	1	0	1	0,01
-3	9	14	4	27	0,34
-2	63	126	36	225	2,86
-1	433	628	312	1373	17,43
0	1372	1422	1350	4144	52,61
+1	644	371	830	1845	23,42
+2	81	48	117	246	3,12
+3	7	5	2	14	0,18
+4	0	1	1	2	0,03
<b>concordants (0±1)**</b>	<b>2449</b>	<b>2421</b>	<b>2492</b>	<b>7362</b>	<b>93,46</b>
<b>discordants (&gt;1, &lt;-1)</b>	<b>160</b>	<b>195</b>	<b>160</b>	<b>515</b>	<b>6,54</b>

\* the theoretical number of comparisons that can be made is 9000 (100 patients\*6 allergens\*5judgments). The actual number is lower, since when the expert judged the adherence to the compilation as insufficient (score <1), the remaining 4 judgements for that patient/allergen combination were not made.

\*\* two judgements were considered to be in agreement with each other if the score expressed by the two experts coincided or differed by only one unit

## Figures

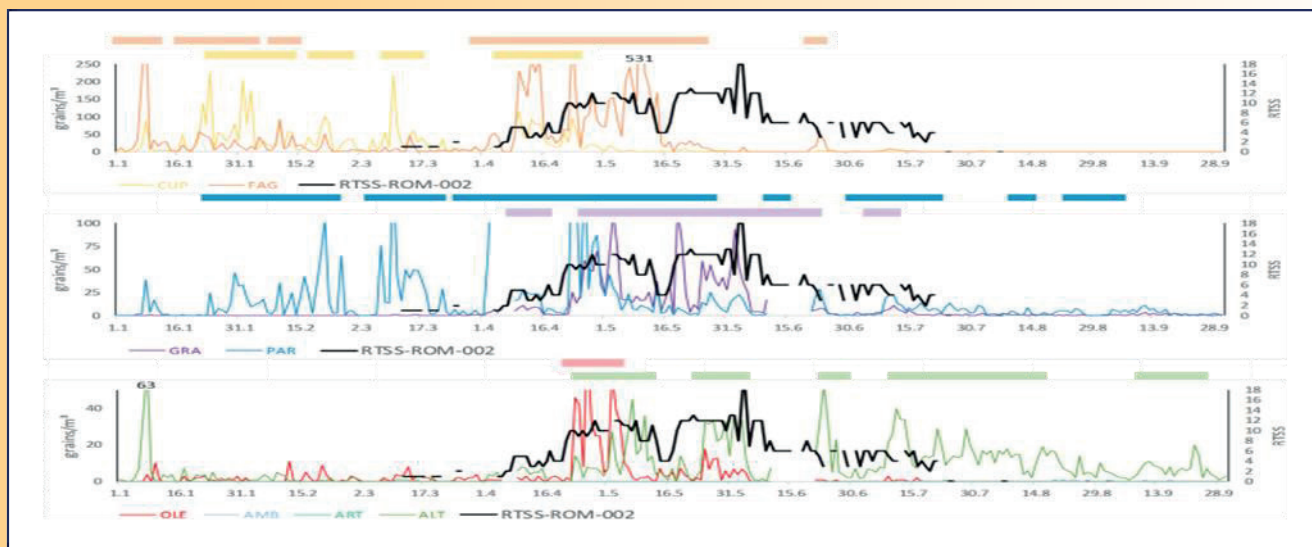


Figure 1 - Example of a graph generated by AllergyMonitor and examined by the three experts.





## VALORE ECONOMICO DEL POLLINE E COSTO SOCIALE DELLE POLLINOSI

### BIOLOGICALS DECREASE PSYCHOLOGICAL DISTRESS, ANXIETY AND DEPRESSION IN SEVERE ASTHMA, DESPITE COVID-19 PANDEMIC

Roberta ZUNNO<sup>1</sup>, Girolamo PELAIA<sup>3</sup>, Corrado PELAIA<sup>4</sup>, Vincenzo PATELLA<sup>1, 2</sup>

<sup>1</sup> U.O.C. of Internal Medicine - G.O.I. of Allergology. Company Center for Allergic and Immunological Diseases and Company Center

PSOCARE/Atopic Dermatitis. ASL Salerno. Hospital "Santa Maria della Speranza" of Battipaglia (SA)  
patella@allergiasalerno3.it

<sup>2</sup> School of Specialization in Allergology and Clinical Immunology. Faculty of Medicine and Surgery. University of Naples  
patella@allergiasalerno3.it

<sup>3</sup>Respiratory Medicine Unit, University "Magna Græcia" of Catanzaro, Catanzaro, Italy

pelaia@unicz.it

<sup>4</sup>Department of Health Sciences, University "Magna Græcia" of Catanzaro, Catanzaro, Italy  
pelaia.corrado@gmail.com

#### Introduction

Patients with respiratory diseases suffer more from problems of severe psychiatric comorbidity than the general population. Asthma might cause psychiatric disorders and affect patients' quality of life negatively. Previous studies reported that mental disorders prevail in asthmatic patients, causing anxiety, depression, and suicidal risk (Baumeister et al. 2005; Katon et al. 2007; Zhang et al. 2019). The aim of this study is to evaluate in real life the prevalence of psychological comorbidities in asthmatics with severe asthma treated by biologicals (Benralizumab, Mepolizumab, Omalizumab).

#### Materials and Methods

This study starts with the hypothesis that psychological distress, anxiety, depression and suicidal risk in severe asthma patients decreases if treated by biologicals. This study involves a sample of 90 patients (32 males, 58 females and aged  $53.92 \pm 15.92$ ) suffering from severe asthma and treated



with the biological drugs of Benralizumab, Mepolizumab, Omalizumab during Covid-19 pandemic (Table 1). At the beginning of the treatment (T0) and after 16 weeks (T1), there have been reported results from both clinical disease control, assessed using the ACT, and psychological disorders, assessed with the PSS (Cohen et al. 1983), HADS (Zigmond et al. 1983) and C-SSRS (Posner et al. 2008).

## Results and Discussion

The main results highlighted a significant improvement between T0 (baseline) and T1 (16 weeks of treatment with biologics) in terms of asthma control, perceived stress, anxiety and depressive symptoms, independent of the type of biologic drugs used during the pandemic, despite Covid-19 pandemic (Fig. 1). The regression model shows that the ACT is a good predictor of stress, anxiety and depression. The ACT and psychological parameters are negatively related: an increase in the control of the disease, measured by the ACT, is associated with a decrease in stress, anxiety and depression scores (Table 2).

## Conclusions

Further results have confirmed the hypothesis that, despite the high impact of the pandemic on asthma described in literature, all patients who had started therapy before Covid continued their treatment with biologics during the Covid-19 pandemic. It was shown that there was a significant improvement in disease management, perceived stress, anxiety and depressive symptoms, following treatment of 16 weeks with biological therapy during the pandemic, independent from the type of biologics used (Fig. 1). Our results confirm that biologics might additionally contribute to a decrease in psychological distress, anxiety and depression.

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## Tables

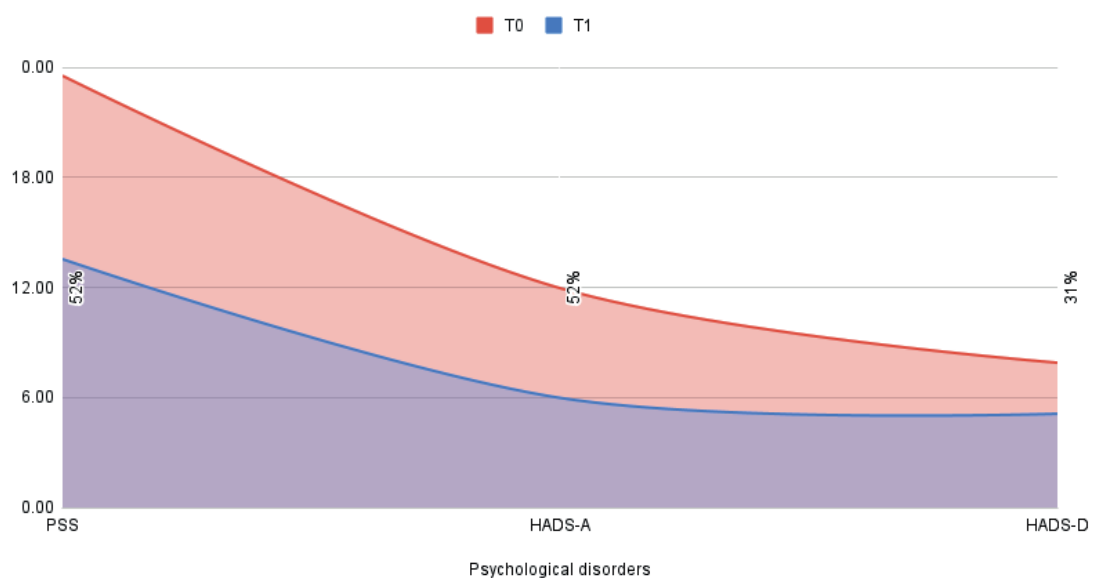
Table 1. Baseline Demographic and Disease Characteristics of Patients			
Characteristic	Patients, No (%)		
	Benralizumab (n= 30)	Mepolizumab (n= 27)	Omalizumab (n= 33)
Sex			
Male	12 (40)	8 (29.63)	12 (36.36)
Female	18 (60)	19 (70.37)	21 (63.63)
Age, mean (SD)	55.67 (11.53)	54.04 (14.73)	52.24 (20.05)
Disease duration since diagnosis, mean (SD)	18.25 (9.64)	21.59 (16.10)	24.64 (14.79)
IgE, mean (SD)	614.30 (737.48)	1071.75 (1524.02)	1430.67 (1061.45)
EOS, mean (SD)	1815.71 (960.62)	1185.70 (1035.43)	1247.74 (1292.96)
FEV1, mean (SD)	970.76 (928.18)	1128.64 (704.60)	1054.77 (803.78)

Table 2. Correlation between psychological tests and asthma control test

ACT				
	r Correlation	95% CI	R squared	P value
T0				
PSS	-0.082	-0.284 to 0.128	0.007	0.444
HADS-A	0.017	-0.191 to 0.223	0.000	0.874
HADS-D	-0.122	-0.321 to 0.009	0.015	0.252
T1				
PSS	-0.340	-0.511 to 0.143	0.116	0.001
HADS-A	-0.233	-0.42 to 0.027	0.054	0.027
HADS-D	-0.481	-0.626 to -0.304	0.32	<0.0001

## Figure

Fig. 1. Summary of statistical data of psychological disorders





### LENTIGO MALIGNA AND ATYPICAL FIBROXANTHOMA OF THE HEAD: A CASE REPORT.

Rosalba BUQUICCHIO<sup>1</sup>, Carlo FUSO<sup>1</sup>, Vincenzo ALTAMURA<sup>1</sup>

<sup>1</sup>*U.O. of Dermatology, Melanoma Unit, Perrino Hospital, Brindisi (ITALY), [rosalba.buquicchio77@gmail.com](mailto:rosalba.buquicchio77@gmail.com)*

#### Introduction

Lentigomaligna (LM) is a melanocytic neoplasm found on chronically sun-exposed areas of the body, particularly in the head and neck areas. It occurs commonly in middle-aged and elderly individuals, and as such, it is referred to as "senile freckle" (Franke JD et al., 2021). LM is a type of in situ melanoma.

Atypical fibroxanthoma (AFX) is an unusual dermal mesenchymal tumour, characterised as a dermal spindle-cell sarcoma, considered a superficial and clinically benign presentation of pleomorphic dermal sarcoma, malignant fibrous histiocytoma, and undifferentiated pleomorphic sarcoma (Soleymani T et al., 2019). Clinically, it appears as a red or pink nodule or papule, located on the head and neck skin areas (Iglesias-Pena N et al., 2020), with visual characteristics akin to basal cell carcinoma, squamous cell carcinoma and pyogenic granuloma. Histology shows a variable mix of histiocytoid, spindle, epithelioid, and/or giant multinucleated cells with pleomorphic nuclei.

#### Case presentation

We report the case of 90 years old male with a history of sun exposure, presenting with an erythematous nodule in the vertex area of the scalp. At dermatoscopy, the lesion presented an atypical vascular pattern with arboriform telangiectasias; the lesion was surgically excised and the initial diagnosis of LM was confirmed by histology. Concurrently, we observed an ulcerated, irregularly pigmented skin lesion in the frontal region, suspect for basal or squamous cell carcinoma; the lesion removed via excisional biopsy and skin grafting, revealed on histology as an atypical ulcerated fibroxanthoma. Immunohistochemical assays were positive for CD68, S-100+, vimentin+, Melan A-CKAE1/AE3-.

#### Discussion

Lentigomaligna occurs as a pigmented, asymmetric macule commonly located in the head and neck skin domains, and slowly spreading. The diagnostic standard for LM is excisional biopsy. In our case, histology shows proliferation of atypical melanocytes at the epidermal-dermal junction, grouped in

small nests or as single cells. Early diagnosis and treatment is crucial to achieve complete remission when possible and to prevent progression to invasive melanoma and the correlated risk of metastases. As such, surgery is the treatment of choice for lentigomaligna.

Based on the histological findings of atypical fibroxanthoma, we performed a review of available literature data. Current opinions suggest surgical margin clearance is the strongest predictor of clinical recurrence; improved recommendations for appropriate surgical margins help delineate standardized safe excision radiuses when intraoperative lesion margin assessment is not available (Jibbe A et al., 2021). In our case, the patient was proposed two treatment options for the purpose of radicalization: either radiotherapy or surgical revision with increased margins of exeresis (specifically,  $\varnothing$  2 cm).

## Conclusions

In consideration of current literature data, surgery remains the gold standard in AFX management, with radical cancer extirpation being the preferable treatment choice compared to radiotherapy, with complete margin control being critical to avoid recurrences.

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## VALORE ECONOMICO DEL POLLINE E COSTO SOCIALE DELLE POLLINOSI

### ASSOCIATION OF OBESITY WITH TOTAL SERUM IGE LEVELS AND ALLERGY SYMPTOMS IN CHILDREN AND ADOLESCENTS

Salvatore GUERCIO NUZIO <sup>1</sup>, Eleonora ARDIA <sup>1</sup>, Giancarlo CIMINO <sup>1</sup>, Paola COCCORULLO <sup>1</sup>,  
Manuela GRIMALDI <sup>1</sup>, Livio D'ISANTO <sup>1</sup>

<sup>1</sup> Division of Pediatrics, Santa Maria della Speranza Hospital of Battipaglia, ASL Salerno,  
[pediatria.pobattipaglia@gmail.com](mailto:pediatria.pobattipaglia@gmail.com)

#### Introduction

The adiposity of the southern Italian pediatric population has been growing steadily. Allergic disease has also been on the rise in recent decades (Hay and Henrickson 2021). Some researchers (Visness CM et al. 2009; Vehapoglu A et al. 2021) have shown pediatric obesity to be related to allergy symptoms or to higher total serum IgE levels (a marker for atopy), while others have not (Lucas JA et al. 2017). Differences in the ages of the study populations, the specific outcomes examined, and the methods used for categorizing obesity may account for the disparate findings. This analysis explores the complex relationships between total serum IgE, allergy symptoms, clinical and laboratory data of a group of obese Italian children living in the province of Salerno, Campania.

#### Materials and Methods

A retrospective study was conducted on 48 children (20M, 28F, age 5-16 years) with obesity (BMI z-score  $2.86 \pm 0.27$ ) assessed at the pediatric endocrinology service of the Pediatric Unit – Hospital of Battipaglia - ASL Salerno from January 2016 to January 2020. All of them practiced: weight, height, BMI z-score, waist circumference (WC) and blood pressure measurement; glucose, insulin (for HOMA index calculation), c-peptide, ALT, AST, gamma GT, uric acid, total cholesterol (TC), HDL, LDL, triglycerides, ESR, PCR blood dosages. They also followed the oral glucose tolerance test. The correlation coefficient was calculated between total serum IgE and clinical/laboratory values identified.

## Results and Discussion

Total IgE levels significantly correlate with fasting blood glucose levels ( $r=0,36756$ ,  $p=0,02319$ , Figure 1) but not with HOMA index ( $r=0,09141$   $p=0,58522$ ); moreover, total IgE values significantly increase with TC levels ( $r=0,34183$   $p=0,03838$ ) of our pediatric population (Figure 2). Although total IgE levels are not significantly correlated with weight ( $r=0,23715$   $p=0,28794$ ) and BMI ( $r=0,09924$   $p=0,68604$ ) the highest IgE values were found in children with higher BMI. By selecting children with known allergic oculo-rhinitis and mild-moderate asthma in course of daily therapy with inhaled cortisone drugs associated with antihistamines and/or montelukast ( $n=20$ ), a positive correlation between total IgE and waist circumference is evident ( $r=0,49380$   $p=0,02691$ , Figure 3). The analysis of our pediatric obese population supports the concept that increased weight is associated with larger allergic predisposition. In particular, the raise in WC, baseline blood glucose and TC concomitant with total IgE levels also suggests a possible increased risk of metabolic complications in the subgroup of atopic patients (Papoutsakis C et al. 2015).

## Conclusions

Childhood obesity may be the most important health issue facing southern Italian children today. While an increase in allergy may not be the most consequential health risk faced by overweight children, it does provide additional motivation for undertaking the difficult challenge to reduce childhood obesity.

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## Figures

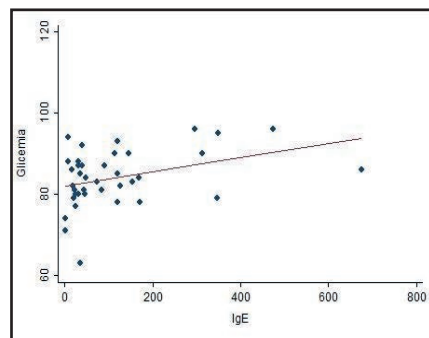


Figure 1

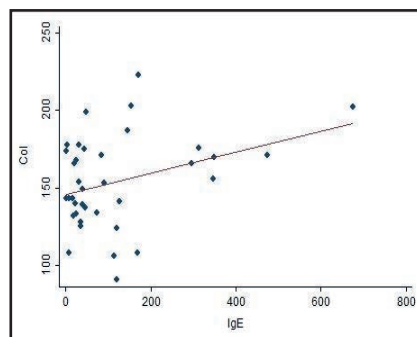


Figure 2

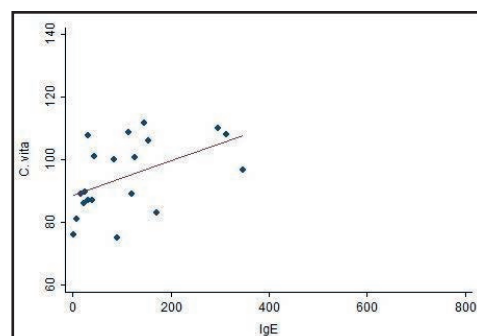


Figure 3

## QUERCUS IN ROME CITY: FENOLOGY AND POLLEN

Maria Antonia BRIGHETTI<sup>1</sup>, Denise DE FRANCO<sup>1</sup>, Alessandro DI MENNO di BUCCHIANICO<sup>2,3</sup>,  
Annarosa MIRAGLIA<sup>1,2</sup>, Alessandro TRAVAGLINI<sup>1</sup>

<sup>1</sup> Department of Biology, University of Rome Tor Vergata  
[maria.antonina.brighetti@uniroma2.it](mailto:maria.antonina.brighetti@uniroma2.it); [denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com);  
[annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com); [travagli@uniroma2.it](mailto:travagli@uniroma2.it)

<sup>2</sup> PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata

<sup>3</sup> Italian National Institute for Environmental Protection and Research (ISPRA), Rome, Italy  
[alessandro.dimenno@isprambiente.it](mailto:alessandro.dimenno@isprambiente.it)

**Introduction:** *Quercus* genus in Rome includes 9 species (*Q. xcrenata* Lam., *Q. cerris* L., *Q. dalechampii* Ten., *Q. frainetto* Ten., *Q. ilex* L. subsp. *ilex*, *Q. pubescens* Willd. subsp. *pubescens*, *Q. robur* L. subsp. *robur*, *Q. suber* L., *Q. virgiliana* (Ten.) Ten.) and belongs to the order Fagales together with the *Alnus*, *Betula*, *Carpinus*, *Castanea*, *Corylus*, *Fagus*, genera, all characterized from allergenic pollen. In particular, *Quercus* pollen despite having a low allergenicity, shows cross-reactivity with *Betula* pollen allergens, responsible for about 30% of pollen sensitization in northern Europe.

**Material and Methods:** In this work the following were considered:

- meteorological data, temperatures and precipitation, from the Monte Mario and Tor Vergata stations of the Hydrometeorological Network of the Regional Functional Center of the Regional Civil Protection Agency (1997-2020);
- pollen data from the monitoring stations RM5 - Tor Vergata (1997-2020) and RM6 - San Pietro (2000-2020) relating to the Tor Vergata Monitoring Center in Rome;
- phenological data (2008-2020) according BBCH guidelines (Meier, 2001) of the park of Caffarella (south Rome area) and Villa Pamphili (north Rome area) sites, .

The data was processed to calculate phenological and production indicators, cumulative temperatures and precipitation, and Pearson correlations using IBM's SPSS software.

**Results and Discussion:** *Quercus* pollen in Rome represents over the years, more or less, 10% of the total airborne pollen, with an average APIn of 4975. Flowering on average lasts 52 days starting on 10 April and ending on 31 May, and begins in the north of the city until you reach the east quadrant.



The influence that weather conditions have on the phenology of plants is now known, in particular, its responsible to determining the parameters of the flowering season. In the case of the *Quercus* genus, flowering and consequent pollen release are influenced by the average minimum Temperature of the winter months, by the millimeters of rain accumulated in the summer months of the previous year and by the number of rainy days in the autumn months of the previous year.

**Conclusions:** Furthermore, a high correlation emerges from the comparison between the data on phenological observations and the presence of airborne pollen; in particular, the phenophase R02 (swollen catkins, close to hatching) is recorded 7 days before the presence of the first airborne pollen.

These data demonstrate the importance of pollen monitoring and phenological observations as a tool for the prevention of allergic symptoms and improvement of the health conditions of the allergic population.

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## COMPARISON BETWEEN DAILY AND HOURLY READINGS OF AIRBORNE POLLEN AND SPORES IN THE CITY OF ROME: A CASE STUDY CIPRO AND TOR VERGATA

Denise DE FRANCO<sup>1</sup>, Maria Antonia BRIGHETTI<sup>1</sup>, Alessandro DI MENNO di BUCCHIANICO<sup>2,3</sup>, Annarosa MIRAGLIA<sup>1,2</sup>, Alessandro TRAVAGLINI<sup>1</sup>

<sup>1</sup> Department of Biology, University of Rome Tor Vergata,  
[denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com);

[maria.antonia.brighetti@uniroma2.it](mailto:maria.antonia.brighetti@uniroma2.it); [annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com); [travagli@uniroma2.it](mailto:travagli@uniroma2.it)

<sup>2</sup> PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata

<sup>3</sup> Italian National Institute for Environmental Protection and Research (ISPRA), Rome, Italy,  
[alessandro.dimenno@isprambiente.it](mailto:alessandro.dimenno@isprambiente.it)

**Introduction:** The World Allergy Organization recognized the pollinosis as a global epidemic (WAO 2013) considering it one of the most common diseases. The data of the World Health Organization indicate that in 2030 the 50% of the European will be allergic.

Therefore, to know and investigate the daily and hourly trend of a different pollen *taxa* in different areas is the assumption to realize of an effective tools of prevention the onset of allergic symptoms.

**Material and Methods:** In this work have been considered two aerobiological monitoring stations localized in the city of Rome, Tor Vergata (TV-RM5) and Cipro (CP): the area investigated by RM5 is a suburban area, mostly surrounded by countryside and wild vegetation. The second station CP is located in the centre of Rome, a densely inhabited area, characterized by the presence of several buildings, road trees and public gardens.

The daily reading, for a total of 2185 sampling slides, was carried out during three-year period 2018-2020. 68 pollen *taxa* have been recognized, this results a photograph of the most representative *taxa* in the two different areas and their relative distribution.

Furthermore, the hourly reading has been carried out in relation to 75 days of the same three-year period and of the same sampling areas. The data were processed through the software SPSS's IBM.

**Results and Discussion:** The daily reading shows a greater presence of the arboreal *taxa* (Cupressaceae-Taxaceae and Betulaceae, especially *Corylus avellana* L.) in the CP sampler area, on the contrary a greater presence of herbaceous *taxa* characterizes the TV-RM5 sampler area.



Moreover, the statistical elaboration allowed to calculate the phenological (beginning, end, duration of the pollen season) and productive (API - Annual Pollen Integral, peak day and maximum concentration) indicators.

In particular about the phenological indicators in 2019 the beginning of the Poaceae pollen season at CP is very early (about 80 days) compared to TV. This result can probably be explained by the microclimatic phenomenon of the island of urban heat, which involves a rise in the temperature of 4-5 °C, both in the summer and winter, than to the periphery (Miraglia et al. 2021). From the comparison of the length of the pollen seasons of the three-year period 2018-2020 it is clear that the pollen season for TV is longer than CP, probably because the herbaceous plants, most represented on TV, have a spring-summer flowering period, much more extended than the arboreal species present at CP with flowering limited to the winter period. The hourly readings confirm the distribution of the various *taxa*, but in particular allow to identify the peak hours: from the analysis of the hourly trends of the main allergenic pollen *taxa*, it is clear that in both sampling sites the hourly range of greatest pollen emission is from about 10 to 14.

**Conclusions:** In conclusion, the daily readings contribute to elaborate of pollen bulletins and calendars for allergic users; in addition, in terms of prophylaxis, knowing the hourly trend of the concentration of the each allergenic *taxa* is advantageous. This allows to reduce the exposure to pollen sources during the times of maximum pollen concentration cause for pollinosis (Suanno et al. 2021).

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## POSTER

# DIFFUSION OF INFORMATION REGARDING AIRBORNE POLLEN AND SPORES IN THE CITY OF ROME

Denise DE FRANCO<sup>1</sup>, Maria Antonia BRIGHETTI<sup>1</sup>, Alessandro DI MENNO di BUCCHIANICO<sup>2,3</sup>,  
Annarosa MIRAGLIA<sup>1,2</sup>, Alessandro TRAVAGLINI<sup>1</sup>

<sup>1</sup> *Department of Biology, University of Rome Tor Vergata,*  
[denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com);

[maria.antonia.brighetti@uniroma2.it](mailto:maria.antonia.brighetti@uniroma2.it); [annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com); [travagli@uniroma2.it](mailto:travagli@uniroma2.it)

<sup>2</sup> *PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata*

<sup>3</sup> *Italian National Institute for Environmental Protection and Research (ISPRA), Rome, Italy,*  
[alessandro.dimenno@isprambiente.it](mailto:alessandro.dimenno@isprambiente.it)

**Introduction:** Aerobiological monitoring is an important tool for prevention of pollinosis. The World Allergy Organization indicate the pollinosis as a global epidemic (WAO 2013). It is one of the most common diseases, and available data of the World Health Organization indicate that in 2030 the 50% of the European will be allergic. A correct information of the start and end of allergenic pollen seasons is important for a focused diagnostic.

So, all strategy to improve life quality of allergic people is welcome. Diffusion of palynological data is important and all way can be used. Weekly bulletin can be carried out in different way, internet, newspaper, SMS, app that it is possible downloaded by store. And of course, mail.

**Material and Methods:** In this work have been considered how Monitoring Centre of Rome Tor Vergata distributes its pollen bulletin. Actually, there are available a web page ([polline.uniroma2.it](http://polline.uniroma2.it)), a Facebook profile ([facebook.com/Polline.uniroma2](https://facebook.com/Polline.uniroma2)), on account Instagram (<https://www.instagram.com/ilpollinediroma>), and a mailing list.

Moreover, many followers downloaded the app Allergy monitor, by TPS production. Every week both on Facebook than on Instagram, we receive question on the pollen season and pollen presence. Furthermore, we send mail to whom that request the bulletin. Every week we send about mail to 350 different mail address.

**Results and Discussion:** Sending the e-mails made it possible to establish an exchange of information between allergy sufferers and the center. Many of them have begun to provide



information on the area of residence and the state of flowering, mainly for Cupressaceae, *Platanus* and *Parietaria*. This information helped us to calibrate the Cupressaceae bloom predictions. For this family the center also carries out phenological monitoring. Another important feedback was the sharing with users of the evaluation of some apps dedicated to air quality. Many users have reported the difference in the presence and concentration of pollen between what is reported in the bulletin and what is reported by the apps (Travaglini et al. 2022).

Last important aspect, through the comment of the bulletin, environmental education was also carried out: the criticalities of urban green management, the choice of species present and / or introduced by the competent authorities or by private citizens were pointed out. Periodically prepared botanical pills have helped raise the awareness of allergy sufferers who have learned to recognize the plants responsible for their allergy.

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## ACCURACY AND REPRESENTATIVENESS OF POLLEN SEASON WITH DIFFERENT CALCULATION CRITERIA

Alessandro Di Menno di Bucchianico<sup>1,2</sup>, Raffaella GADDI<sup>1</sup>, Stefano MARCHESI<sup>3</sup>, Cecilia SCARINZI<sup>4</sup>, Maria Antonia BRIGHETTI<sup>5</sup>, Denise DE FRANCO<sup>5</sup>, Annarosa MIRAGLIA<sup>2,5</sup>, Alessandro TRAVAGLINI<sup>5</sup>

<sup>1</sup> *Italian Institute for Environmental Protection and Research (ISPRA), Rome, Italy;*  
[alessandro.dimenno@isprambiente.it](mailto:alessandro.dimenno@isprambiente.it); [raffaella-gaddi@isprambiente.it](mailto:raffaella-gaddi@isprambiente.it)

<sup>2</sup> *PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata*

<sup>3</sup> *Emilia-Romagna Environmental Protection Agency (ARPAE), Modena, Italy;*  
[smarchesi@arpae.it](mailto:smarchesi@arpae.it)

<sup>4</sup> *Piedmont Environmental Protection Agency (ARPA Piedmont), Turin, Italy;*  
[ceciscar@arpa.piemonte.it](mailto:ceciscar@arpa.piemonte.it)

<sup>5</sup> *Department of Biology, University of Rome Tor Vergata, Rome, Italy*  
[maria.antonia.brighetti@uniroma2.it](mailto:maria.antonia.brighetti@uniroma2.it); [denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com); [annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com);  
[travagli@uniroma2.it](mailto:travagli@uniroma2.it)

### Introduction

Phenological indicators such as the beginning, end and duration of the pollen season are among the main indicators used for reporting and communicating to the public the presence of allergenic pollens in the air. In Italy, pollen concentration data are disseminated, among others, by the weekly bulletins of the POLLnet network of the Italian National System for Environmental Protection, SNPA ([www.pollnet.it](http://www.pollnet.it)) and by regional and national reports, which describe the main allergenic pollen taxa present in the area.

In particular, the national reports published by the Italian Institute for Environmental Protection and Research give the pollen seasons of seven of the most important allergenic pollen emitting families or genera monitored on the Italian territory: (Betulaceae, Coryloideae, Oleaceae, Cupressaceae-Taxaceae, Poaceae, Asteraceae and Urticaceae).

### Materials and Methods

The duration of the pollen season is the period of time in which significant quantities of anemophilic pollen are dispersed into the atmosphere. If we consider the seven families that represent almost all of the allergenic pollens monitored on the Italian territory (Betulaceae, Coryloideae, Oleaceae,





Cupressaceae-Taxaceae, Poaceae, Asteraceae and Urticaceae) we will have seven different pollen seasons that follow one another and overlap the one to the other without interruption.

There are several methods of calculating the pollen season, the POLLnet SNPA network follows the definition of Jäger and colleagues (Jäger *et al.*, 1996) in which the start pollen season is defined as the first day, with a pollen amount exceeding 1% of the annual total, providing this was not followed by more than six consecutive days with zero values, and the end of the season was defined as the number of the day upon which 95% of the annual total was reached. But many other criteria have been proposed and applied, during the year, by various studies that can return different and, *a priori* acceptable, start and end dates of pollen season (Jato *et al.*, 2006).

## Results and Discussion

In this study, the pollen season was determined using the *R* project for statistical computing software by means of various alternative to Jäger calculation criteria, for some representative stations in Northern, Central and Southern Italy for a period of time ranging from 2003 to 2021. We considered data of the seven allergenic pollen taxa above mentioned measured by a selection of POLLnet SNPA monitoring station and, for Lazio region, by the Aerobiological Monitoring Center of the University of Rome Tor Vergata. The results were compared with the duration obtained with the more common Jäger method and the representativeness of different results was assessed by calculating the average deviation and significance level over time and space.

## Conclusions

The comparison, for representative sites of the Italian territory, among pollen trends outlined by different definitions of pollen season, based on different percentage thresholds and different eligibility criteria, leads to the conclusion that the choice of the methodological criterion can lead to highly variable outputs for the temporal trends (Glick *et al.* 2021).

It is therefore important that, at national and EU level, representative studies are launched as soon as possible, bringing a comparison for the conscious and shared choice of the most accurate calculation methodology for this important aerobiological indicator.

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## POSTER

# POLLEN AND LIPID BIOMOLECULES FROM SEDIMENTARY ARCHIVES: A CASE STUDY FROM THE NEAR-SITE CORES OF THE TERRAMARA S. ROSA DI POVIGLIO (PO PLAIN, N ITALY)

Assunta FLORENZANO <sup>1</sup>, Eleonora CLÒ <sup>1</sup>, Jérémy JACOB <sup>2</sup>

<sup>1</sup> Laboratorio di Palinologia e Paleobotanica, Dipartimento di Scienze della Vita, Università degli Studi di Modena e Reggio Emilia, Via G. Campi 287, 41125 Modena, Italy; [assunta.florenzano@unimore.it](mailto:assunta.florenzano@unimore.it), [eleonora.clo@unimore.it](mailto:eleonora.clo@unimore.it)

<sup>2</sup> LSCE (UMR CEA-CNRS-UVSQ 8212), Orme des Merisiers, CEA/Saclay Bât. 714, 91191 Gif-sur-Yvette Cedex, France; [jeremy.jacob@lsce.ipsl.fr](mailto:jeremy.jacob@lsce.ipsl.fr)

## Introduction

Molecular biomarkers detected in sedimentary archives are increasingly being utilized as a proxy for examining past environmental conditions. Among them, plant-derived biomolecules are particularly informative on the local presence of a plant when coupled with pollen and other palynomorphs, in absence of any plant macroremains in the stratigraphic deposit.

In sedimentary deposits, the detection of miliacin – a pentacyclic triterpene methyl ether specific of *Panicum* and other few Poaceae, is being regarded as trace evidence of past presence of millets. Combined with the evidence from pollen analysis, the presence of miliacin in sediments may be considered the molecular evidence of millet cultivation.

This paper relies on a project (2020 FAR-DSV UNIMORE, in collaboration with CNRS-LSCE) dealing with the integration of biomolecular techniques and pollen analysis to improve information on the integration of broomcorn millet (*Panicum miliaceum* L.) into subsistence strategies of the Terramare culture (1650–1150 B.C.) in the central Po Plain.

## Materials and Methods

The correspondence between miliacin content and *Panicum* pollen has been verified in three continuous near-site cores (Near-S3, Central-S1, Far-S2) collected at different distances (150 m, 320 m, 525 m) from the Terramara S. Rosa di Poviglio (Clò 2022). A subsampling step was performed for cores with a selection of 31 samples related to Holocene chronology. The extraction of miliacin from sediments was carried out according to the method for lipid extraction and separation developed by



the CNRS-LSCE, and the extracted compounds were investigated using gas chromatography–mass spectrometry (GC–MS) (Jacob et al. 2005).

## Results and Discussion

Concentration in miliacin was very low (few nanograms per gram) in all samples. Interestingly, three Bronze Age samples in the core closest to the site (N-S3; up to 14.4 ng/g) and a stratigraphically related sample in the central core (C-S1; up to 18.1 ng/g) displayed clear peaks on the chromatogram. In the same level of C-S1, traces of *Panicum* pollen (0.3%) are index of increased crop diversity (mainly wheat and barley until then), and the combined evidence of miliacin is a further support of millet cultivation during the dry phase in Recent Bronze Age.

## Conclusions

This research shows that pollen and plant biomarkers are complementary tool to improve knowledge on past presence/use of specific plants. In the near-site cores of the Terramara of S. Rosa di Poviglio, the combined evidence of *Panicum* pollen and sedimentary miliacin testify the local presence of the plant even in the lack/scarcity of millet grains in the archaeocarpological record, providing empirical evidence of the introduction of millet crops.

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## POSTER

### ANAPHYLAXIS, QoL AND PSYCHOLOGICAL DISORDERS

Giovanni FLORIO<sup>1</sup>, Roberta ZUNNO<sup>1</sup>, Luciana PIERRO<sup>1</sup>, Vincenzo PATELLA<sup>1,2</sup>

<sup>1</sup> U.O.C. of Internal Medicine - G.O.I. of Allergology. Company Center for Allergic and Immunological Diseases and Company Center

PSOCARE/Atopic Dermatitis. ASL Salerno. Hospital "Santa Maria della Speranza" of Battipaglia (SA)  
patella@allergiasalerno3.it

<sup>2</sup> School of Specialization in Allergology and Clinical Immunology. Faculty of Medicine and Surgery. University of Naples  
patella@allergiasalerno3.it

#### Introduction

Anaphylaxis is a severe allergic reaction triggered by hymenoptera, medications or foods, which can lead to death if not treated quickly. Adrenaline (epinephrine) is the first-line treatment for anaphylaxis and its prompt administration is vital to reducing mortality. Individuals with a history of anaphylaxis are prescribed adrenaline autoinjectors (AAI), designed to administer adrenaline intramuscularly, for use in emergency situations in severe anaphylactic reactions until medical help arrives (Moss et al. 2021; Chime et al. 2017; Liberman et al. 2015). During anaphylactic shock, people may experience strong emotional stress and have the feeling of being about to die, an experience that can be experienced by the person as traumatic and associated psychological distresses, including anxiety and depression (Lee Y et al., 2020; Man Cheung Chung et al. 2011). These emotional experiences can be exacerbated by respiratory and/or skin physical symptoms and intense states of anxiety and hyperalertation, linked to the fear of not being able to breathe and the continuous vigilance necessary to prevent exposure to the trigger. This condition can have a significant impact on patients' quality of life (QoL), greatly affecting their physical and mental health (Oude Elberink 2006). The aim of this observational study is to evaluate, retrospectively, in real life, the effect of adrenaline treatment on QoL and psychological parameters in patients with a history of anaphylaxis to hymenoptera, drugs or foods, in patients treated with ready-to-use adrenaline 300 mg.

#### Materials and Methods

The study involves 90 patients with a history of anaphylaxis (age 18-80, mean age  $41 \pm 16.34$ ; 45 males, 45 females) who were prescribed self-injectable adrenaline in the period between 2020 and 2022, at the G.O.I. of Allergology, Business Centre for Allergic and Immunological Diseases and



Psocare/Atopic Dermatitis Business Centre, of the U.O.C. of Internal Medicine of the Hospital of Battipaglia, ASL Salerno. Patients were interviewed through psychological counseling online at the baseline (T0) and after (T1) the self-injectable adrenaline prescription. Overall physical and mental health was assessed through SF-12 (Ware et al. 1992). Perceived stress was assessed using the PSS (Cohen et al. 1983), anxiety and depression were assessed using HADS (Zigmond et al. 1983). In addition, all patients were given a qualitative interview, based on 4 questions related to the concern about the anaphylaxis management and the awareness of disease, including if they feel safer with a 500 mg vial or the 300 mg vial they usually use.

### Results and Discussion

Our data show an improvement in perceived stress and symptoms of anxiety and depression, and in general in physical and mental health, after prescribing self-injectable adrenaline (T1) compared to baseline (T0). The information obtained from the qualitative interview provided useful advice to improve the management of disease and the QoL of patients with a history of anaphylaxis.

### Conclusions

It is also important to assess the quality of life in patients with a history of anaphylaxis. Prescribing adrenaline reduces stress, anxiety and depression, and improves the physical and mental health of patients. Through psychological counseling it is possible to better understand the symptoms, difficulties and concerns of patients and improving anaphylaxis management and QoL.

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## AEROBIOLOGICAL MONITORING IN AN OCCUPATIONAL ENVIRONMENT USING A REAL-TIME BIOAEROSOL SAMPLER

Andrea LANCIA <sup>1,2</sup>, Maria Concetta D'OVIDIO <sup>1</sup>, Angela GIOFFRÈ <sup>3</sup>, Donatella MAGRI <sup>2</sup>

<sup>1</sup> *Dipartimento di Medicina, Epidemiologia, Igiene del Lavoro e Ambientale (DiMEILA), Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro (INAIL), Monte Porzio Catone, Roma. Emails: [a.lancia-sg@inail.it](mailto:a.lancia-sg@inail.it), [m.dovidio@inail.it](mailto:m.dovidio@inail.it)*

<sup>2</sup> *Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Roma. Emails: [andrea.lancia@uniroma1.it](mailto:andrea.lancia@uniroma1.it), [donatella.magri@uniroma1.it](mailto:donatella.magri@uniroma1.it)*

<sup>3</sup> *Dipartimento di Medicina, Epidemiologia, Igiene del Lavoro e Ambientale (DiMEILA), Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro (INAIL), Lamezia Terme, Catanzaro. Emails: [an.gioffre@inail.it](mailto:an.gioffre@inail.it)*

### Introduction

Aerobiological monitoring is usually realized using traditional volumetric samplers, which operate collecting bioaerosol particles on sampling surfaces or containers, that have to be later observed through microscopy to identify pollen and fungal spores or analyzed for an evaluation of bacterial charge and diversity. These methods are usually quite reliable, but they are also time consuming, as they require specialized operators and are limited in the type of information they provide. Recently, new types of automatic real-time bioaerosol samplers have been developed (Huffman et al. 2020) and are becoming increasingly widespread around the world. The use of these samplers lightens the work load for operators and provides a wealth of information on the single analyzed particle. Their use in occupational contexts may have a great potential. Here, we evaluated the effectiveness of a real-time sampler in an occupational environment.

### Materials and Methods

In December 2021, a real-time sampler was used to perform the sampling in an indoor occupational environment. The sampler operated continuously for two entire weeks, through both working and non-working hours. The instrument used was the WIBS-NEO, a sampler that operates through fluorescence spectroscopy to distinguish different types of particles based on the absorption and emission of light of different wave lengths. The sampled particles have been identified by associating them to different channels (A, B, C) based on their fluorescence parameters (Hernandez et al. 2016). The values for each particle in each channel were considered significant if equal to or higher than

the average channel values obtained operating the sampler in forced trigger mode, plus three times the standard deviation (Li et al. 2020). Additionally recorded was the temporal distribution of the various particles during the hours of each day.

## Results and Discussion

Here we show the results from one week of sampling as a representative. During the course of the week, a total number of 6,030,099 particles were collected, 17.06% of which were fluorescent in at least one channel, while the remaining 82.94% were considered non fluorescent since their value was below the chosen threshold. The majority of these particles (99.91%) have a diameter  $<10\ \mu\text{m}$ . A total of 729,971 particles were fluorescent in the C, BC, or ABC channels, these combinations being usually associated with pollen (Hernandez et al 2016). A total of 839 particles had a diameter  $>10\ \mu\text{m}$ , reasonably referred to intact pollen grains. Among these, 137 had an asphericity factor  $<10$ , indicating a round shape, typical of non-deformed pollen grains. In addition, 46,320 particles were possible bacteria (A channel and diameter  $<2\ \mu\text{m}$ ) and 862 fungal spores (A or AB and diameter between 2 and  $10\ \mu\text{m}$ ). The daily distribution of excited particles showed a difference between working and non-working days, and between hours of the day when employees were and were not in the room.

## Conclusions

The real time sampler resulted to be a very powerful instrument for aerobiological monitoring in occupational environments. It is able to grant very detailed information about bioaerosol particles. For example, by registering the exact moment in which every particle was sampled, it enables accurate assessments of the temporal variations of bioaerosol concentrations. Its ability to recognize different types of pollen, fungi or bacteria is still limited though, compared to other techniques. Thus, at the moment it could be considered a very useful tool to associate to traditional monitoring techniques in occupational environments.

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## POSTER

### Variability between Hirst-type pollen traps is reduced by resistance-free flow adjustment.

Marina MUÑOZ-TRIVIÑO<sup>1</sup>, José M. MAYA-MANZANO<sup>1\*</sup>, Fiona TUMMON<sup>2</sup>, Bernard CLOT<sup>2</sup>,  
Lukasz GREWLING<sup>3</sup>, Carsten SCHMIDT-WEBER<sup>1</sup>, Jeroen BUTERS<sup>1</sup>

1. Center of Allergy & Environment (ZAUM), Member of the German Center for Lung Research (DZL), Technical University and Helmholtz Center Munich, Munich, Germany.
2. Federal Office of Meteorology and Climatology MeteoSwiss, Chemin de l'Aérologie, Payerne, Switzerland.
3. Laboratory of Aerobiology, Department of Systematic and Environmental Botany, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

\*Email: jose.manzano@tum.de

## Introduction

Pollen measurements are routinely performed at more than 600 stations worldwide according to the pollen monitoring map of the world ([www.zaum-online.de/pollen](http://www.zaum-online.de/pollen)) (Buters et al. 2018), typically using manual Hirst-type pollen traps. But manual instruments have been shown to suffer from systematic errors that are not always sufficiently compensated for, such as the error produced when calibrating the trap with flow meters that have an internal airflow resistance (Oteros et al. 2017). Here we provide an easy way of providing most accurate results by correcting for erroneous airflow measurements, taking as example four hirst traps running in parallel during the framework of the EUMETNET AutoPollen Programme and the ADOPT COST Action (Intercomparison campaign 2021 held in Munich, Germany).

## Materials and Methods

Different flowmeters were tested, including the hand-held rotameters from Burkard Scientific Ltd, Burkard Manufacturing Co Ltd, and Lanzoni Srl, as well as the anemometer-based easyFlux® and a self-designed RFF (resistance-free flow meter). The easyFlux® and RFF were calibrated externally by an external laboratory. All 4 Hirst-type pollen traps were set to measure 10 L/min using the standard hand-held rotameter and monitored thereafter on a weekly basis for the entire campaign using the same rotameter. Finally, the resulting data were then corrected using the easyFlux® airflow values and differences between the corrected and uncorrected pollen concentrations were evaluated.

## Results and Discussion

Uncorrected pollen concentrations were 26.5% (hourly data) and 21.0% (daily data) higher than those obtained after correction with the easyFlux®. The differences between traps before and after flow correction were tested for the eight main pollen types by comparing the median standard deviations for concentrations at various temporal resolutions. For hourly values, the median standard deviation across the traps for the eight pollen types was reduced by 28.2% ( $p < 0.001$ ) compared to the uncorrected data. For daily values, a significant decrease in the median standard deviation (21.6%) between traps was observed for 7 out of 8 of the pollen types, ( $p < 0.05$  or lower).

## Conclusions

We therefore recommend continuing to calibrate Hirst-type pollen traps with standard hand-held rotameters, but also that resistance-free flow meters are used occasionally so as to be able to apply flow corrections to the final pollen concentrations reported. This method thus guarantees there is no change in flow that may impact the measurement characteristics of the traps and simultaneously ensures improved accuracy of the final results.





### Acknowledgements (Funds)

This article contributes to the EUMETNET AutoPollen Programme, which is developing a prototype of European automatic pollen monitoring network. The intercomparison campaign was funded by the Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL) and MeteoSwiss. We also thank financial support to the COST ADOPT action CA18226 – *New approaches in detection of pathogens and aeroallergens*.

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## POSTER

# A NEW PALAEOENVIRONMENTAL RECORD OF THE LAST 10 KA IN THE LESSER CAUCASUS: FIRST DATA FROM LAKE TETRI TBA (SAMSARI-JAVAKHETI, GEORGIA)

Erwan MESSAGER<sup>1</sup>, Andréa JULIEN<sup>1</sup>, David ETIENNE<sup>2</sup>, Anne-Lise DEVELLE<sup>1</sup>, Emmanuel MALET<sup>1</sup>

<sup>1</sup> UMR 5204 EDYTEM Environnement Dynamique et Territoire de la Montagne, CNRS/Université Savoie Mont Blanc, Campus Technolac, FR-73376 Le Bourget du Lac, [erwan.messenger@univ-smb.fr](mailto:erwan.messenger@univ-smb.fr)

<sup>2</sup> UMR 042 CARTEL Centre Alpin de Recherche sur les Réseaux Trophiques et Ecosystèmes Limniques Université de Savoie/INRA, CISM, FR-73376 Le Bourget du Lac

## Introduction

The volcanic plateaux of Georgia and Armenia are covered by steppic grasslands. The origin of these herbaceous ecosystems is still questioned, because little is known regarding the influence of agro-pastoralism in shaping these mountain ecosystems. In this area, previous palaeoecological works have been carried out on lakes and peatbogs this last decade (Messenger et al. 2013, 2017, 2021; Joannin et al. 2014, Cromartie et al. 2020). They have provided the complete Holocene vegetation history of the region. These studies do not record significant human impact before the last two thousand years, while a lot of protohistoric sites (especially Bronze Age) have been discovered in the area.

## Materials and Methods

In 2016, we started to work on a new target: Lake Tetri Tba (2610 m a.s.l, 41,443-43,703, Samsari volcanic range), a small lake located 6km far from the larger lake of Paravani (in which our team has already conducted several works). The Tetri Tba watershed is composed of moraine deposits and meadows, still exploited by nomadic shepherds. Several cores have been retrieved from this lake. The sequence TET 16-02 covers the last ten thousand years. It was sampled for sediment analysis and for pollen and fungal spore analysis.



## Results and Discussion

The pollen analysis reveals a grass-dominated vegetation throughout the last ten millennia. The main shift in the vegetation history is an increase of tree taxa from 9000 cal. BP, at the same period than recorded in Paravani Lake (expansion of forests at lower altitude). While there are regular occurrences of ruderal taxa, the vegetation dynamic does not reflect a significant impacts of human activities. However, the analysis of coprophilous fungal spores reveals a long pastoral history in the Tetri Tba catchment area. Indeed, this history seems to have started as early as the Neolithic period and to continue throughout the Holocene, with marked phases of exploitation (Early and Middle Bronze Age, beginning of the Iron Age or the Medieval period), and phases of decline (Chalcolithic period or at the end of the Middle Bronze Age). These new results make it possible to discuss the phases of exploitation of the mountain in relation to the archaeological results.

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## POLLEN RISK INDEX IN GREEN AREAS: AN INSTRUMENT FOR THE PREVENTION OF POLLINOSIS IN AN URBAN ENVIRONMENT – A CASE-STUDY IN ROME

Annarosa MIRAGLIA<sup>1,2</sup>, Maria Antonia BRIGHETTI<sup>1</sup>, Denise DE FRANCO<sup>1</sup>, Alessandro TRAVAGLINI<sup>1</sup>

<sup>1</sup> *Department of Biology, University of Rome Tor Vergata,*  
[annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com);

[maria.antonia.brighetti@uniroma2.it](mailto:maria.antonia.brighetti@uniroma2.it); [denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com); [travagli@uniroma2.it](mailto:travagli@uniroma2.it)

<sup>2</sup> *PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata*

**Introduction:** Nowadays urban green spaces play a key role in socially, economically and environmentally sustainable urban development, producing a series of long-term benefits. But pollen released by urban flora is one of the main ecosystems disservices. So, knowing the allergenic risk in densely populated areas is an important tool for prevention practices for allergy people. The most important method to evaluate the allergenicity of the urban green areas is I<sub>UGZA</sub> of Cariñanos *et al.* (2014), so the purpose of this work is to determine a methodological variant which considers seasonal variations in terms of pollen concentrations.

**Material and Methods:** To achieve this objective, the I<sub>UGZA</sub> have been modified, specifically introducing aerobiological parameters and modifying mode to expression some intrinsic parameters (surface occupied, length of pollination period).

The intent is not to generically label an area as allergenic or non-allergenic, but to produce an index that calculates the allergenic risk of the area weekly in relation to the flowering of one or more *taxa* in a specific period. The index results in a value between 0 (null allergenicity) and 1 (maximum allergenicity). This index was applied to Passeggiata del Pincio in Villa Borghese of Rome.

**Results and Discussion:** The results show the area has a pollen risk of 0 throughout the second half of the year. While for 15 weeks in 2020 and for 13 weeks in 2021 (February- May) the allergenic risk is greater than 0.3 (threshold value). 2 weeks in 2020 and 1 week in 2021 have an allergen risk higher than even 0.5. Observing the values of the index, the pollen risk trend reflects the trend of pollen concentrations of the most allergenic and most representative species of the place (*Cupressus*



*sempervirens* L., *Platanus x hispanica* Mill., *Quercus ilex* L., *Pinus pinea* L., *Cedrus libani* A.Rich., *Olea europaea* L.).

Therefore, we can affirm that Passeggiata del Pincio isn't a healthy space for all citizens. Specifically, for people sensitive to *Cupressus sempervirens* and/or *Platanus x hispanica* and/or *Quercus ilex* pollen: pollen emissions in their respective flowering periods constitute a disservice to health.

**Conclusions:** This index is a prevention tool, able to consider that the allergenic risk of a green areas varies with the seasons and to attribute to one or more *taxa* the exceeding of the risk threshold value. So it's also an opportunity to reiterate to the administrations that the planning of mowing, pruning and care of the greenery is not only a question of urban decor, but also a health issue.

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## COMPARISON BETWEEN TWO PORTABLE SAMPLERS: PARTRAP FA52 AND LANZONI VPPS® 1000

Annarosa MIRAGLIA<sup>1,2</sup>, Maria Antonia BRIGHETTI<sup>1</sup>, Denise DE FRANCO<sup>1</sup>,  
Alessandro TRAVAGLINI<sup>1</sup>

<sup>1</sup> Department of Biology, University of Rome Tor Vergata,

[annarosa.miraglia@gmail.com](mailto:annarosa.miraglia@gmail.com);

[maria.antonia.brighetti@uniroma2.it](mailto:maria.antonia.brighetti@uniroma2.it); [denise.defranco29@gmail.com](mailto:denise.defranco29@gmail.com); [travagli@uniroma2.it](mailto:travagli@uniroma2.it)

<sup>2</sup> PhD Program in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata

**Introduction:** The aerobiological samplers Hirst type (Burkard, Lanzoni) are the base of the Italian and European aerobiological monitoring (Hirst 1952). The fixed samplers have a sampling range of 10-15 km, so they determine pollen concentration of large area, but in some cases using the aerobiological portable samplers is useful to determine pollen spectrum of smaller area. Thus, in the field of larger project, we wanted to test two different portable samplers: Lanzoni VPPS® 1000 and Partrap FA52 (Coppa, Biella, Italy). Both devices have an identical aspiration flow of 10 l/min, but different operation mode.

**Material and methods:** At first to ensure their comparability it's important to test the functioning of the samplers. The Lanzoni VPPS® 2000 is placed, according to the protocol, on the rooftop of Biology Department of the University of Rome Tor Vergata at 15 m above the ground. So the Partrap FA52 and the Lanzoni VPPS® 1000 was placed on the upper part of the Lanzoni VPPS® 2000, to which they were fixed. In this way, the opening of aspiration of three samplers were aligned vertically. Therefore, the samplers rotate like a single block to the wind direction. The aerobiological sampling was carried out in 10 non-consecutive days of springtime, from h. 8.00 to h 18.00 (Fiorina et al. 1997).

Thereafter, we have chosen to use two mobile samplers for sampling campaign in 9 green areas of Rome. The campaign included sampling of 2 hours on a weekly basis over a period of 12 months (May 2020 - October 2020 and January 2021-April 2021). The samplings were always scheduled at different times, in a time slot between sunrise and sunset, to also consider the physiological variability of pollen concentrations during the day. A total of 135 samplings were carried out. The samples obtained from the two samplers were analysed under the microscope according to the continuous strip reading method (Miraglia et al. 2020)



**Results and discussions:** The results of the comparison test with the fixed sampler Lanzoni VPPS® 2000 highlighted the complete overlap of the data of the Lanzoni VPPS® 1000 portable sampler with the fixed sampler (96%), while an underestimation is highlighted in the data obtained with the Partrap FA52 portable sampler (65%). While, about the monitoring campaign *in situ* in the green areas of Rome at eye level: Lanzoni VPPS® 1000 detects on average about 200% more granules than the Lanzoni VPPS® 2000, but still up to 10000% more, as expected. Partrap FA52 shows fluctuating results, probably the performance of this sampler is more conditioned by some external variables, still to be explored. In same case it detects the same percentage of Lanzoni VPPS® 1000, but approximately half of the times it records a concentration inconsistent with others phenological evidences.

**Conclusions:** In conclusion the Lanzoni VPPS® 1000 is more reliable to detect the pollen spectrum of small area than Partrap FA52. However, the small size of the Partrap FA52 allows to wear on the belt the sampler and therefore to investigate the exposure of the individual allergic patient.

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## EVALUATION OF THE EFFICACY OF THE PREDICTIVE RESPONSE TO IMMUNOTHERAPY SCORE (PRIS) IN IDENTIFYING ELIGIBLE PATIENTS FOR ALLERGEN IMMUNOTHERAPY

Ilaria Mormile<sup>1</sup>; Felicia De Rosa<sup>1</sup>; Francesca Wanda Rossi<sup>1,2</sup>; Francescopaolo Granata<sup>1</sup>, Amato de Paulis<sup>1,2</sup>

<sup>1</sup>*Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy*

<sup>2</sup>*Center for Basic and Clinical Immunology Research (CISI), WAO Center of Excellence, University of Naples Federico II, Naples, Italy*

**Introduction:** Allergen immunotherapy (AIT) is the only disease-modifier treatment for allergic diseases as it could prevent both the onset of new allergic sensitizations and disease progression (Dhamiet al. 2017). A specific predictive factor of AIT outcome has not been identified yet. This study aims to evaluate the efficacy of a disease score referred to as Predictive Response to Immunotherapy Score (PRIS) we developed to predict the response to AIT and identify eligible patients.

**Materials and Methods:** 110 patients (68 males and 42 females) diagnosed with allergic rhinitis with or without concomitant asthma were enrolled in this study. Before beginning single-allergen, or two-allergen sublingual immunotherapy (SLIT), patients were fully evaluated by analyzing clinical and laboratory parameters. A specific rating was assigned to each parameter to be combined in a total score named PRIS. The parameters were chosen based on literature review and previous works evaluating AIT responsiveness and possibly predictive factors. The parameters included age, clinical features, disease onset, number of positive allergens (assessed with Skin Prick Test and/or specific IgE), presence of symptoms following exposure to the allergen(s) to which the patient is sensitized, specific IgE/total IgE ratio, allergen dominance, and component resolved diagnosis. At baseline (T0) and follow-up [after 24 months (T24) of SLIT treatment] rhinitis and asthma symptoms, as well as the use of on-demand therapy, were measured using the Visual Analogue Scale (VAS) to calculate a mean symptom score (MSS). Finally, the variation between the MSS at T0 and T24 ( $\Delta$ MSS-24) was measured.

**Results and Discussion:** We observed a significant improvement of symptoms at T24 as compared to T0 in all groups undergoing SLIT. Overall, PRIS significantly predicted  $\Delta$ MSS-24 by regression analysis ( $R=0.622$ ;  $F(1,97) = 60.810$ ;  $p<0.001$ ; Figure 1). PRIS was effective in predicting  $\Delta$ MSS-24 in patients treated with single-allergen SLIT ( $R=0.708$ ;  $F(1,65)=64.453$ ;  $p<0.001$ ) as well as with two-allergen SLIT ( $R=0.599$ ;  $F(1,31)=16.833$ ;  $p<0.001$ ). In addition, PRIS was effective in predicting



$\Delta$ MSS-24 in both patients with only rhinitis ( $R=0.660$ ;  $F(1,48)=36.313$ ;  $p<0.001$ ) and with concomitant asthma ( $R=0.674$ ;  $F(1,48)=39.207$ ;  $p<0.001$ ). As shown in Table 1, all PRIS parameters were significant predictors for our outcome, and the parameters' score categories (assumed in the model on an ordinal scale) adequately reflect the different progression in comparison with the references.

**Conclusion:** PRIS assessment before SLIT prescription might represent a useful tool to individuate potential responders.

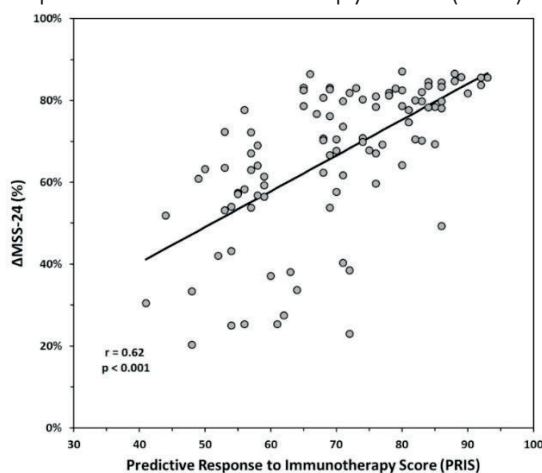
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**Table 1.** Predictive Response to Immunotherapy Score (PRIS).

Linear regression models using as predictors all PRIS parameters	
PRIS Parameter	<i>p</i> -Value
Age (years)	<0.001
Clinical features	<0.001
Disease onset (years)	0.006
Number of allergen sensitizations	0.003
Symptoms when exposed to allergen(s) to which the patient is sensitized	<0.001
Specific IgE/Total IgE (s/t) ratio	<0.001
Component-Resolved Diagnosis for major allergens	<0.001
Allergen Dominance	0.002

**Figure 1.** Correlation analysis revealed a significant direct correlation between the predictive response to immunotherapy score (PRIS) and  $\Delta$ MSS-24 ( $p<0.001$ ).



## MICROSCALE APPROACH FOR THE STUDY OF PREHISTORIC SOCIO-ENVIRONMENTAL INTERACTIONS IN BARCELONA CITY. THE CASE STUDY OF THE CAGALELL AREA

Ana PENA PÉREZ <sup>1</sup>, Santiago RIERA MORA <sup>2</sup>, Ramón JULIÀ BRUGUÉS <sup>3</sup>

<sup>1</sup> SERP, IAUB, Departament d'Història i Arqueologia, Universitat de Barcelona, [anapena@ub.edu](mailto:anapena@ub.edu)

<sup>2</sup> SERP, IAUB, Departament d'Història i Arqueologia, Universitat de Barcelona, [rieram@ub.edu](mailto:rieram@ub.edu)

<sup>3</sup> SERP, IAUB, Departament d'Història i Arqueologia, Universitat de Barcelona, [nomar.ailuj@gmail.com](mailto:nomar.ailuj@gmail.com)

### Introduction

In the littoral plain of Barcelona, several paleoenvironmental studies are being conducted in order to reconstruct socio-ecological dynamics between human communities and their environment. Barcelona is a narrow plain that stretches between the Besòs and Llobregat rivers (NE and SW), closed inland by the Collserola mountain range (NW) and open to the Mediterranean Sea. The evolution of this plain was deeply affected by regressive and transgressive marine processes during the Holocene. Thus, environmental evaluations deal with complex coastal dynamics (e.g. high sensitivity to climate and sea level changes).

In addition, the study faces the contemporary urban development, which covers ancient geomorphological and hydrological features, and favours the fragmentary recovery of archaeological and paleoenvironmental information. To overcome these difficulties, a targeted survey methodology has been articulated, requiring the collaboration of public administrations and private companies in order to access to paleoenvironmental and archaeological records.

### Materials and Methods

Despite the dense urban fabric, several paleowetland areas have been surveyed, allowing the execution of high-resolution paleoenvironmental studies at spatial microscale.

The Cagalell is one of these littoral paleowetlands. Located between two littoral promontories called *Mons Taber* (16 m.a.s.l.) and Montjuïc (185 m.a.s.l.), these lacunar deposits date back





to 8260-7070 cal. yr BC and their maximum expansion is set around 20-25 ha (Julià and Riera, 2012). Coring revealed a 15 m deep sequence, suitable for high-resolution studies.

In recent years, urban development has temporarily opened up new plots in the area. These were accessed to extract new sedimentary sequences and complete a 400 m SW-NE transect of the outermost area of the Cagalell. Paleoecological analyses (pollen and non-pollen palynomorphs) were performed in selected cores chosen by their organic content.

## Results and Discussion

The Cagalell Holocene sequence starts on top of Pliocene deposits with transgressive marine sediments associated to Early Holocene sea level rise. From -10 m.a.s.l., the records reveal a sedimentary unit 8 to 12 m thick formed by silts and organic clays, with peat levels in between. The upper levels correspond to floodplain silts and stream deposits. In this area, deposit formation predominantly occurred throughout the Neolithic period, according to the chronological framework defined by 16 new radiocarbon dates. During this period, infilling of the topographic depression occurred, which partially covered wetland levels. Archaeological remains indicate occupation on those levels, both in Late Neolithic times (-0,15 m.a.s.l) and Late Bronze Age (1,30 m.a.s.l).

The first farming communities of the Early Neolithic settled in a forested landscape (AP=80%) dominated by submediterranean and humid trees such as deciduous oaks and common hazel, and pinewoods. This woodland was disturbed by phases of forest clearance, allowing the mastic expansion and shrubby vegetation growth. It is worth noting the low presence of thermo- or mesomediterranean vegetation during EN times, and the presence of taxa inclined to humid and cool environments (e.g. *Tilia*, *Corylus*, *Betula*), including riverine forest (e.g. *Salix*, *Fraxinus*, *Alnus*). Otherwise, weeds and apophyte expansion, occurrences of *Cerealia*-t pollen and increase in coprophilous fungi coincide with the earliest dates of Neolithic occupations (ca. 5600 cal. yr BC), showing a farming productive system based on agriculture and livestock.

## Conclusions

Microlocal studies, combined with high temporal resolution and the integration of archaeological and paleoenvironmental data, enables the reconstruction of human-environment interactions during prehistoric times in a highly altered urban area. The current analyses show that distribution of Neolithic settlements and land uses occurred near freshwater wetland environments in the Barcelona Plain

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## HOLOCENE PALEOENVIRONMENTAL RECONSTRUCTIONS IN THE BAY OF BREST

C. Valero<sup>1</sup>, C. Lambert<sup>2</sup>, M. Vidal<sup>1</sup>, P. Stéphan<sup>3</sup>, Y. Pailler<sup>4</sup>, A. Ehrold<sup>5</sup>, A. Penaud<sup>1</sup>

<sup>1</sup> *Univ Brest (UBO), UMR 6538 Laboratoire Geo-Ocean, 29280 Plouzané, France*

<sup>2</sup> *Univ Vannes (UBS), UMR 6538 Laboratoire Geo-Ocean, 56000 Vannes, France*

<sup>3</sup> *Univ Brest, CNRS, UMR 6554 Littoral Environnement Télédétection Géomatique (LETG) Brest, 29280 Plouzané, France*

<sup>4</sup> *Inrap, UMR 8215 Trajectoires, IUEM, 29280 Plouzané, France (??)*

<sup>5</sup> *Ifremer, UMR 6538 Laboratoire Geo-Ocean, 29280 Plouzané, France*

### Introduction

The Bay of Brest is a semi-enclosed basin of 180 km<sup>2</sup> subject to tidal dynamics and to the fluvial influences of both Aulne and Elorn Rivers, which drain watersheds representing 2600 km<sup>2</sup> around the Bay, the major one being the Aulne River catchment. This coastal environment, at the land-sea interface, is subject to natural climate oscillations, typical of the North Atlantic Basin (Penaud et al., 2020), with the superimposed paleoenvironmental transformations inherited from the post-glacial sea level rise (Gregoire et al., 2017) and the anthropogenic forcing increasing from the Neolithic (6,950 ka BP) and especially from the Bronze Age (4,200 ka BP). Therefore, it is an ideal site for the reconstruction of the interactions and retroactions between climate, environment and human dynamics at the western tip of Brittany. To that end, a Holocene paleoenvironmental study was conducted thanks to eight cores collected around the Bay of Brest in four different subtidal areas (i.e., the Roscanvel Bay, the Brest Harbour, the South of the Plougastel peninsula and the mouth of the Aulne River) more or less impacted by oceanic vs. fluvial influences (Lambert et al., 2019, 2020).

### Materials and Methods

First, a spatial comparison of pollen and dinoflagellate cyst assemblages (terrestrial and aquatic bioindicators, respectively) was conducted thanks to cores retrieved at the mouth of the Aulne River (twin cores “F” and “KS22”; fluvial influence) and close to the Brest Harbour (twin cores “KS06” and “KS05”; oceanic influence), on same time intervals encompassing 200 years: i) first around 4 ka BP and ii) second around 1 ka BP. Second, a stack of 8 Bay of Brest study cores was established to discuss the paleo-landscape evolution over the last 9 kyrs BP in this macro-tidal estuarine environment.

### Results and Discussion

Spatial comparisons, between the mouth of the Aulne River and the Brest Harbour, allowed recognizing fluvial-palynological tracers among palynological assemblages (*Alnus* and *Corylus* for pollen taxa; *Lingulodinium machaerophorum* for dinocyst ones). This spatio-temporal investigation was then discussed in parallel with palynological data acquired on the southern Brittany shelf over

the last 7 kyrs BP (core CBT-CS11; Penaud et al., 2020). This allowed discussing the synchronous character of environmental observations considered as regional for western France (i.e., the general decline of the forest cover and related increase in Poaceae testifying of the landscape opening) and also local characteristics specific of Bay of Brest watersheds and linked to paleo-regimes of winter precipitation regimes above northern Europe. In addition, we reconstructed the paleo-landscape evolution of Bay of Brest watersheds. Since the Neolithic period, the forest cover decreased in favour of open landscapes, including cultivated and grazed areas, in connection with the development of agriculture, or wastelands in the case of the metal industry. Nevertheless, the rate of opening of the landscape did not follow the trend of herbaceous taxa: the forest cover slowly declined around 4 ka BP, then strongly decreased at the end of the Iron Age, before experiencing a revival of about 5 centuries at the end of the Gallo-Roman period (Lambert et al., 2020). This latter period is discussed at the light of archaeological evidences of withdrawal of coastal societies in Brittany potentially echoing a climate deterioration combined to an unfavourable socio-economic context (Lambert et al. 2020). Finally, a drastic fall is recorded at the start of the Middle Ages and is related to the population dynamics around the Brest Harbour and in particular to intensive agro-pastoral practices.

## Conclusions

This study therefore represents the first long-term Holocene trend of Bay of Brest paleo-landscapes allowing discussing both climatic and anthropogenic forcing thanks to the permanent change of scale performed when interpreting local signals (Bay of Brest) face to macro-regional evidences of climate and environmental changes thanks to north-eastern Atlantic marine records.

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## MeSCAL project: mobility & cultural contact in the shaping of southern California landscapes: Palaeoecological approach.

Reyes LUELMO-LAUTENSCHLAEGER<sup>1</sup>, R. Scott ANDERSON<sup>2</sup>, Nathalie COMBOURIEU-NEBOUT<sup>3</sup>, Yannick MIRAS<sup>3</sup>, Alexander SIMMS<sup>4</sup>, Aude BEAUGER<sup>5</sup>, Elisabeth ALLAIN<sup>5</sup>, Ana EJARQUE<sup>1</sup>

<sup>1</sup> ISEM, CNRS, [reyes.luelmo@umontpellier.fr](mailto:reyes.luelmo@umontpellier.fr); [ana.ejarque@umontpellier.fr](mailto:ana.ejarque@umontpellier.fr)

<sup>2</sup> Northern Arizona University, [Scott.Anderson@nau.edu](mailto:Scott.Anderson@nau.edu)

<sup>3</sup> HNHP, CNRS, [yannick.miras@mnhn.fr](mailto:yannick.miras@mnhn.fr); [nneboutcombourieu@mnhn.fr](mailto:nneboutcombourieu@mnhn.fr)

<sup>4</sup> University California Santa Bárbara, [asimms@geol.ucsb.edu](mailto:asimms@geol.ucsb.edu)

<sup>5</sup> Geolab, CNRS, [Aude.beauger@uca.fr](mailto:Aude.beauger@uca.fr); [Elisabeth.allain@uca.fr](mailto:Elisabeth.allain@uca.fr)

### Introduction: Mediterranean landscapes across the ocean

Mediterranean cultural landscapes have been built through millennia as a consequence of natural-anthropogenic dynamics, but also by trading, migration and colonial processes. During the latter, settlers adapt the new territories to their own socio-economic and cultural patterns, introducing new plants and land-uses (Anderson 1952) with serious consequences on local ecosystems (Ejarque et al. 2015). However, palaeoenvironmental research addressing the role of human mobility and cultural interactions in the shaping of past landscapes is still scarce (Sluyter, 2002). The MeSCAL Project has been designed to fill this gap in the Mediterranean landscapes of southern California (SoCal). The Californian coast is a biodiversity hotspot attesting to a rich history of human settlement over the last ~13ky. MeSCAL will analyze the spatial distribution of land-uses and plants following Late Holocene (~4ky) migratory and colonial processes, assessing their impact on native SoCal native flora, wetlands and soilscares.

### Materials and Methods: an integrated paleoenvironmental and archeo-historical approach

The MeSCAL project proposes an interdisciplinary approach based on the coupling of 1) multi-proxy palaeoenvironmental analyses –i.e. pollen, non-pollen palynomorphs (NPP), fire history analysis, diatoms, sedimentology, geochemistry- in continental wetlands and marine records, respectively providing local and regional information on vegetation and land-use changes; 2) calibration of fossil palaeoenvironmental datasets with modern pollen and NPPs analogues of vegetation and land-uses; 3) archaeobotanical analyses furnishing direct information on past consumption and use of plants; and 4) coupling of paleoenvironmental results with archaeo-historical and ethnographic datasets. MeSCAL includes a micro-regional approach, with case-study locations distributed along a coastal-inland transect. This will allow the tracking of differences in landscape changes following colonial settling between coastal areas under direct colonial control and hinterland areas with a smaller colonial influence that may have served as refuge for native populations and landscapes.



## Results and Discussion: preliminary palaeoecological results.

Initial fieldwork and research have focused on obtaining and radiocarbon dating sediment records suitable for palaeoecological analysis in the study areas. Two continental wetlands -one in Kern County and another in San Diego County have provided organic-rich sedimentary records covering the last ~4ky in the inland and coastal locations, respectively. Forthcoming coring is planned to obtain more sedimentary records in the Santa-Barbara coastal region. In this latter location on-going palynological analyses are being conducted in the Late Holocene section of a previously cored and dated Santa Barbara Basin marine record (MD02-2503) located ~ 20 km offshore of the Santa Barbara coast (Beaufort & Grelaud, 2017). When combining this information with that obtained from the palynological study of small continental wetlands in the project area we will be able to assess the local or regional extent of vegetation and land-use changes following human mobility in coastal SoCal.

In addition to on-going paleoenvironmental research, the study of modern pollen and NPP assemblages of vegetation and land-uses in SoCal has been launched to calibrate fossil palynological datasets. Surface samples have been collected in 37 locations along an altitudinal gradient from 40 to 2525 m.a.s.l. from the Santa Barbara-Ventura coast across the Transverse Ranges in Kern County. The reference transect covers the main coastal and mountain vegetation communities and landscape settings of our study areas. Samples were also taken from restored historic gardens and orchards of the Santa Barbara Mission as a reference of introduced colonial landscapes.

## Conclusions

The MeSCAL Project will help to better understand how human mobility and settling has contributed shaping the current landscapes of southern California, measuring the long-term impact of transported plants and practices over the cultural landscape in this territory marked by human migrations and colonial processes. These kinds of studies contribute to engaging citizens with their own history and identity that is reflected in the landscapes they inhabit. In addition, results obtained by the project will inform SoCal native tribes about their ancestral landscapes and land-uses, helping to protect their cultural identities, landscape heritages and traditional ways of life.

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## Dinoflagellate Cysts Response to Climate Change During the MIS 12/11 Transition

Saeed MALEKI<sup>1</sup>, Dael SASSOON<sup>1</sup>, Nathalie COMBOURIEU-NEBOUT<sup>1</sup>, Vincent LEBRETON<sup>1</sup>,  
Laurent LONDEIX<sup>2</sup>, Marie-Hélène MONCEL<sup>1</sup>

<sup>1</sup>*Muséum national d'Histoire naturelle, UMR 7194 Histoire Naturelle de l'Homme Préhistorique*

<sup>2</sup>*Université de Bordeaux, UMR CNRS 5805 EPOC (Environnements et Paléoenvironnements Océaniques et Continentaux), Allée Geoffroy St Hilaire, 33615 Pessac, Cedex, France*

Organic-walled dinoflagellate cysts (dinocysts) are biostratigraphical markers and paleoceanographical sea surface indicators (e.g., Traverse, 1988). They provide detailed information on climate, productivity and salinity changes in marine environments (Sluijs et al., 2005). Different time periods recorded in ODP site 976 (Alboran Sea) have been previously studied at various resolutions using calcareous nanoplanktons, foraminifera, dinocysts and pollen grains (e.g., Combourieu-Nebout *et al.*, 1999, von Grafenstein *et al.*, 1999; González-Donoso *et al.*, 2000; Joannin *et al.*, 2011; Bazzicalupo *et al.*, 2018). This work aims to investigate climate and environmental changes during the MIS 12/11 transition using dinocysts with comparison to synchronous pollen data.

Palynological samples are treated following a standard method (Faegri and Iversen, 1989), avoiding acetolysis to preserve dinocysts. Selected samples from MIS 12 and 11, with a high resolution on the transition V (MIS 12/11), illustrate the changes in dinocyst assemblages. Warm/cold water dinoflagellate ratios are used to show changes in sea surface temperatures. Results confirm that climate changes during the MIS 12/11 transition had a strong effect on dinocyst assemblages. We observed a significant shift characterised by a decrease in abundance of species belonging to a cool climate and an increase in warm taxa at the onset of MIS 11. High concentrations of *Nematosphaeropsis labyrinthus*, *Operculodinium centrocarpum*, and *Bitectatodinium tepikiense* in MIS 12 samples support the idea of a cool climate with more nutrients and a productive condition compared to MIS 11. *Pentapharsodinium dalei*, associated with cold waters in polar to equatorial regions (Zonneveld et al., 2013), is only reported in MIS 12.

Synchronous pollen data from ODP Site 976 during the MIS 12/11 transition also record such a climatic shift from cool to warm conditions interpreted based on the transition from predominantly herbaceous and steppic taxa and a high abundance of *Pinus* during MIS 12, towards a greater abundance of temperate and Mediterranean taxa in MIS 11.

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## DID HUMAN ACTIVITIES DRIVE THE MID TO LATE HOLOCENE WIDESPREAD EXPANSION OF *CEDRUS ATLANTICA* RANGE?

Daniel ABEL-SCHAAD<sup>1</sup>, Francisca ALBA-SÁNCHEZ<sup>1</sup>, Antonio GONZÁLEZ-HERNÁNDEZ<sup>1</sup>, José Antonio LÓPEZ-SÁEZ<sup>2</sup>

<sup>1</sup> Dpto de Botánica, Facultad de Ciencias, Universidad de Granada, [dabels@ugr.es](mailto:dabels@ugr.es), [falba@ugr.es](mailto:falba@ugr.es), [aglehdez@gmail.com](mailto:aglehdez@gmail.com)

<sup>2</sup> Centro de Ciencias Humanas y Sociales, Consejo Superior de Investigaciones Científicas, [joseantonio.lopez@cchs.csic.es](mailto:joseantonio.lopez@cchs.csic.es)

### Introduction

*Cedrus atlantica* is an endemic and relict species whose populations are highly fragmented along the mountains of North Africa (Thomas, 2013). These forests have undergone an outstanding migration since the Last Glacial Maximum as a response to climate and anthropogenic changes. Palaeoecological studies, mainly based on pollen analyses, have reported their dynamics, showing a high resilience both to climatic and human-induced disturbances, which have allowed their persistence in current interglacial refugia (Abel-Schaad et al, in prep).

Human influence in the area has been evidenced since at least 6500 cal yr BP, although it seems to have been quite low until the last 2500 years, when grazing by livestock, together with an increasing aridity, led to their progressive decline (Cheddadi et al., 2019). On the other hand, human exploitation of cedars began 10,000 years ago in the Near East and then spread to Greece and to the western Mediterranean around 5500 to 4500 years BC (M'hirit & Benzyane, 2006). Recent studies have shown the likelihood of ancient plantations of cedar since before 4000 cal BP in the eastern Mediterranean (Özcan & Çiçek, 2022). Indeed, north African cedar forests show a wide expansion during the transition from Mid to Late Holocene, especially in the Middle Atlas, coinciding with the first traces of human activities (Abel-Schaad et al, 2022). An explanation pointing to a human-induced driving of this process becomes a tempting hypothesis.

### Materials and Methods

We have analyzed two cores located in the Middle Atlas (Morocco) spanning the last 13000 years and also made a revision of all available pollen studies. We have focused on pollen and non-pollen types related to human activities and their relationship with the curves of tree taxa.

### Results and Discussion

While the site of N'harcha shows emerging human traces at this time (Fig. 1), a multivariate analysis in Tigalmamine (Figs. 2 & 3) provides a strong relationship between *Cedrus* and human-related indicators, like coprophilous fungi and Cerealia. This linking seems to be indirect, as human impact would have been more pronounced on oak forests, where both cereal cultivation and pasturelands were easily accessible. This disturbance could have promoted a subsequent spread of cedar forests, without ruling out some kind of protection or even the plantation of this latter species.

The striking widespread expansion of *Cedrus* detected by palaeoecological studies in the transition from Mid to Late Holocene, especially in the Middle Atlas, could not only have been a consequence of reported climate changes, as human traces became more apparent. Although they did not reach high levels, it seems that human activities could have promoted the cited spread of cedar forests in this area. Further and complementary studies, especially those focusing on philogeography, are needed to broaden this new discussion on the dynamics of this emblematic species.

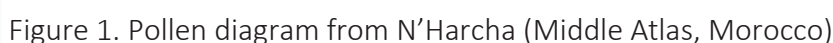
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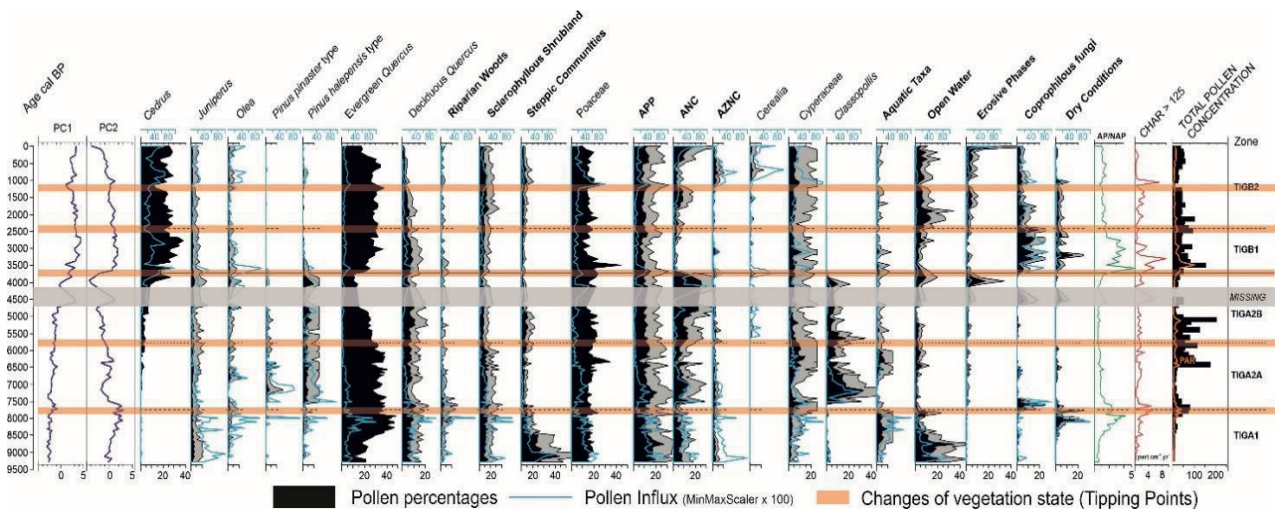


Figure 2. Pollen diagram from Tigalmamine3 (Middle Atlas, Morocco)

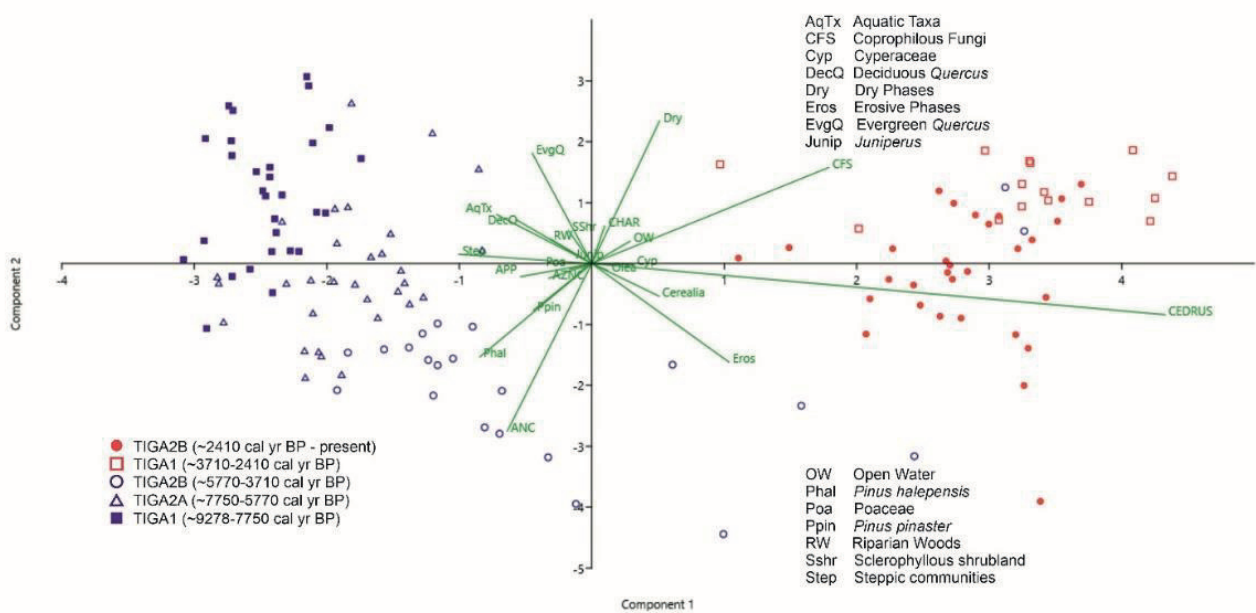


Figure 3. Biplot for the PCA from Tigalmamine3 (Middle Atlas, Morocco)

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