2007

Annual Report

IASMA RESEARCH CENTRE

FONDAZIONE EDMUND MACH

Allergenic pollens: new techniques for identification and quantification

Elena Gottardini
Sara Longhi
Antonella Cristofori
Fabiana Cristofolini

The analysis of airborne pollen is time-consuming and requires considerable effort in terms of scientific skills. The methodology currently used (Norma UNI 11108) is based on morphologically microscopic identification of sampled particles (Faegri, Knut and Iversen, 1989). The difficulties associated with this method make it hard to establish a sampling network able to guarantee spatially-detailed pollen information. It is essential to know the actual pollen concentrations in order to more efficiently communicate the potential risk to the allergic population. In Trentino, aerobiological monitoring has been carried out since 1989 at the Istituto Agrario di San Michele all'Adige - Fondazione Edmund Mach (IASMA). Our research is aimed at finding a more rapid and semi-automatic method for aerobiological sample analysis and increasing the number of sampling sites, in order to take into account the wide variability in the vegetation of this region.

A new methodological approach

The IASMA aerobiology research unit has, since 2006, been involved in co-ordinating a research project entitled CARPOL, which was established with the aim of finding suitable innovative techniques to solve the above-mentioned problems. The project is divided into two independent lines of research: bio-molecular and spectroscopic. In recent years, bio-molecular tests based on amplification of DNA regions, associated to real-time PCR and array hybridisation, have been used as diagnostic tools in several fields (Gachon *et al.* 2004). In fact, many DNA Barcode projects have been developed, aimed at rapid identification of different animal and vegetal species and to determine their phylogenetic links utilising short DNA sequences as bar codes (Kress *et al.* 2005; Chase *et al.* 2005). Bio-molecular research within the current project is aimed at exploiting these new technologies, detecting and amplifying specific DNA tracts by PCR reaction for each pollen taxon, and associating genetic material quantification by specific probes (real-time PCR). The objective is to apply real-time PCR to pollen specimens, determine sampled taxa and quantify their DNA content. At the same time we plan to develop a sys-

Fig. 1 - Amplification of extracted DNA - primer designed on *Corylus*

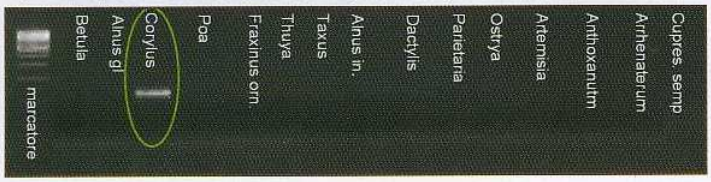
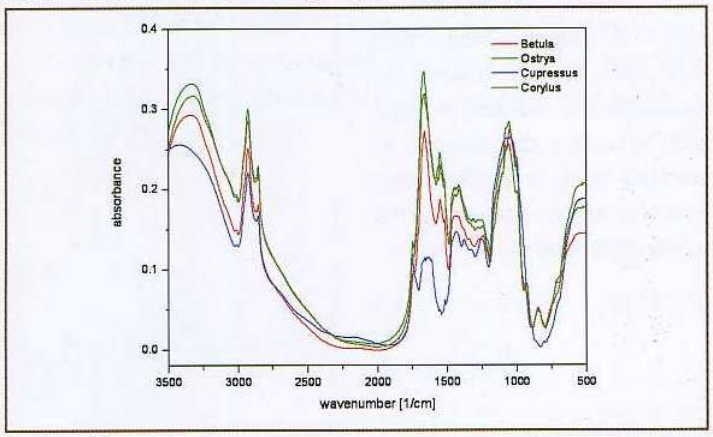


Fig. 2 - Example of FT-IR spectroscopy on pure samples in KBr



was recently applied to the study of pollen by Pappas et al. (2003); it allows several cellular components, such as lipids, peptides, proteins, nucleic acids, sugars and many metabolites, to be surveyed contemporaneously, thus acquiring information on each compound and on its respective rates. The main objective of spectroscopic research is to characterise FT-IR spectra for known pollen samples and to build a reference spectra library. FT-IR analysis on unknown samples and comparison with spectra library should allow pollen samples to be identified.

The CARPOL project, financed by the Fondazione Caritro, involves several research units: the Aerobiology and Molecular Genetics Unit of the IASMA Research Centre, the Microsystems Division of Fondazione Bruno Kessler - in association with the University of Verona - and the Materials Engineering and Industrial Technologies Unit of the University of Trento.

Vegetal species selection

Selection of the group of plants to study within the project was made on the basis of their presence in local flora and their allergic relevance. Each species was collected from three different localities, with the aim of assuring that any genetic differences which may exist among different populations were properly represented.

Pollen samples were collected between January and September 2007, following single species flowering times; leaf samples were collected at the same time for bio-molecular analysis.

Protocol setting for pollen DNA extraction and quantification of allergenic species

The first step in the bio-molecular approach was in-silico research for taxa-specific DNA regions, developed by analysing sequences stored in on-line data banks (NCBI). Primer couples and probes were then designed on selected sequences with the purpose of applying real-time PCR. In the laboratory, DNA from the leaves was extracted to preliminary test primers and probes. The successive pollen DNA extraction required an ad hoc protocol to be set up. Amplifying specific DNA sequences allowed some allergenic species to be identified (Fig. 1) and quantification of some of them was verified quite rapidly. In addition, the possibility of applying these analyses to airborne pollen samples was tested and proved positive.

Concluding remarks

The evaluation carried out at the end of the first year of the project signals the potential of the bio-molecular approach for developing a rapid and reliable method for characterising and quantifying aero-dispersed