Integration of molecular diagnostics and air sampling to study plant pathogens

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INTRODUCTION The timing and dispersal of propagules of many plant pathogens has been studied by trapping airborne particles onto sticky surfaces (e.g. trap surfaces of Hirst-type or rotating-arm samplers) followed by detailed observation by light microscope. Visual identification and quantification can be a cheap and rapid technique particularly for large and easily identified spores. However it is sometimes difficult to identify spores to the species level and when target particles are present in very low numbers, visual identification under a microscope can be either time-consuming or insensitive because usually only a small proportion of the trap surface is examined in a series of microscope traverses.
RECENT DEVELOPMENTS A technique developed to detect ascospores of Sclerotinia sclerotiorum by PCR is more sensitive than traditional microscopy as it can detect the presence of only a few spores on a Hirst or rotating-arm trap surface, in a background of thousands of other spores and pollens. Other recently developed molecular techniques can now provide additional genotypic information such as presence of genes conferring fungicide resistance. Spore numbers can be estimated directly from the amount of pathogen DNA detected by quantitative PCR. Fraaije et al. (2005) quantified daily numbers of M. graminicola ascospores, trapped using traditional Hirst (Burkard) spore samplers, but in the same reaction, they also quantified the proportion of spores with alleles conferring resistance to strobilurin (QoI) fungicides. Each daily spore trap tape section was divided longitudinally to give a sub-section for microscopy and a sub-section for the real-time PCR assay. The advantage of this technique over using molecular diagnostics on samples collected directly in Eppendorf tubes is that the section available for microscopy can be used to confirm that the DNA detected was from ascospores and not conidia that may become airborne as an aerosol during rain events. The study showed that compared to an untreated wheat plot there was a very rapid increase in the proportion of spores that possessed alleles conferring fungicide resistance trapped above a fungicide-sprayed wheat plot following applications of the respective fungicide. Spore production decreased after harvest, due to removal of spore-producing debris and as a result the proportion of 'fungicide resistant' spores trapped above unsprayed or sprayed plots moderated to an intermediate level under the increased influence of the regional background spore load. The research suggests that integration of spore trapping with quantitative PCR has great potential for forecasting diseases of arable crops and as a method to manage fungicide resistance.

Preliminary results on pollen as indicator of heavy metal pollution in an alpine valley

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INTRODUCTION: Many authors have hypothesized the possibility of estimating effects caused by airborne pollutants analysing pollen grains. Aim of this work is to evaluate the use of pollen as a bioindicator of the presence of heavy metals in the particulate matter and moreover to assess possible stress symptoms under air pollution.
METHODS: The selection of sampling sites was based on the levels of anthropization in Turin urban area and in Susa Valley (North-west Italy). Analyses were carried out on Corylus avellana pollen collected from catkins at full bloom. The male anthesis in Hazelnuts occurs before the broad-leaved trees, living in the corresponding phytoclimatic area, come into leaf. Pollen samples were dried in an oven and then mineralised. The quantitative analyses of the extracted solutions were performed with atomic absorption spectroscopy for the measurement of Cd, Cu, Cr, Mn, Fe, Ni, Pb, Zn content. Viability was evaluated by FDA test and germinability was tested in vitro. Morphological observations were performed by Scanning Electron Microscopy and Light Microscopy.
RESULTS: Results are reported from localities representing three different environmental types: relatively unpolluted sites (U), polluted areas with prevalently road traffic emissions (P), and sites characterized by local source of pollutants (L). Pollen seems to be able to show presence on exine surface of heavy metals. The presence of a local source of emission is underlined by a significant increase in the values (e.g. Pb rate varies from 0.48 ppm in U to 2.03 ppm in L). A correlation between pollen germinability and environmental factors was verified: germinability increases from 11.3%, in L to 62.0 % in U; in the place with prevalently road traffic emissions there is moreover the highest percentage of anomalous grains (29.3% instead of 6.3% in unpolluted areas). A correlation is not evident between the presence of heavy metals on pollen surface and its viability (viability data always range from 71 to 89%). SEM analyses didn't show any morphological changes on the grains surface.
CONCLUSION: Short time contact between particulate matter and Corylus pollen seems to be enough to underline the presence of heavy metals in air when pollen is freshly collected from catkins at anthesis. So Corylus pollen can be an efficient environmental sensor in winter and in early spring, because male anthesis takes place before appearance of leaves, that can represent a bound in the accumulation process. In tested places heavy metal concentration seems to be able to have a negative influence on germinability, especially when a local pollutant source occurs. Viability and morphology are unchanged.