Fungi and CHIPS

Prepared by: Natalie van Zuydam (MSc student working on the project entitled: "Molecular characteriztion of Leptographium species using a high-density, oligonucleoride microarray chip"

'Who done it?'…Identification of fungal pathogens causing diseases on plants is a complicated process and this is even true for professional mycologists. Accurate species identification is typically entrusted to taxonomists who are experienced in dealing with particular groups of fungi. We are at an exciting point in the history of molecular biology where increasing volumes of sequence data are available for fungi. It has consequently now become standard practice, when describing fungal species, to use morphological as well as molecular characters. The sequence data may be used in a number of molecular applications. One of these is to DNA CHIPS or microarrays that are capable of discerning species based on their molecular characters.

A DNA CHIP is made up of a microscope slide that has DNA sequences printed on its surface at defined locations. These sequences are derived from sequence data that have been analysed to locate a unique DNA signature that identifies a particular species. Samples are prepared from a number of species through PCR. These samples are then labelled and hybridised to the DNA CHIP. Only complementary sequences will pair and they then emit a fluorescent signal at a particular position on the array (Figure 1). The position and intensity of the signal will indicate the identity of the organism from which the sample was prepared.

DNA CHIPS have been used for the identification of many species of bacteria as well as fungi in the genera *Phytophthora, Fusarium* and *Pythium.* One of the groups of fungi that we are interested in are those belonging to the so-called *Leptographium* complex. These fungi are insect associates and they include important tree pathogens and agents of sap stain. They are notoriously difficult to identify and we have thus applied DNA CHIP technology for this purpose. This is a very exciting development as it is one of the first diagnostic chips for fungi that rely on sequence data rather than expression data as the identifying character.

DNA CHIP technology has well defined applications for *Leptographium* spp. as well as other fungal genera. It will allow for multiple species to be accurately identified in a single assay whereas traditional methods would involve multiple sequencing reactions and the development of complex phylogenies. Our aim is also to use it to analyse environmental samples in which we wish to know more about the populations of different organisms that are present. This is particularly important for organisations that are of quarantine importance. It will thus be possible to identify potential bio-security threats, such as the black stain root disease pathogen *Leptographium wageneri,* in a shorter time frame than was needed using time intensive traditional methods that rely on pure cultures and variable morphological characters (Figure 2).

Conference attended: **Van Zuydam NR, Wingfield BD, Jacobs K, Wingfield MJ.** 2007. Microarrays: A novel diagnostic technology for *Leptographium*. 45th Congress of the Southern African Society for Plant Pathology, Benoni, South Africa.

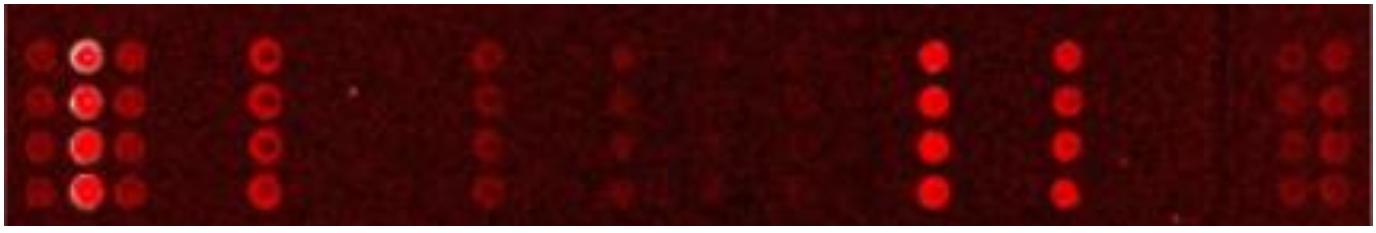

Figure 1 The intensity of the fluorescent areas and the position indicates labelled target binding and identifies specific species of *Leptographium*

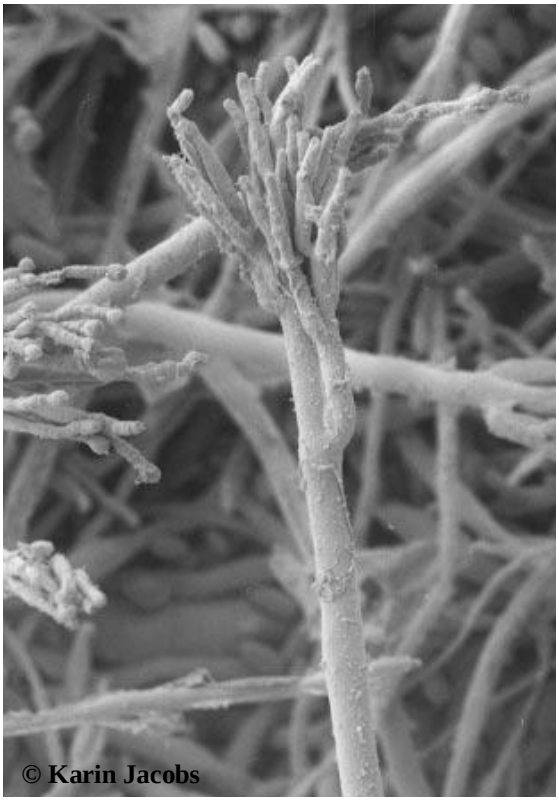

Figure 2 The conidiogenous apparatus of *Leptographium sibiricum.* The morphology of this apparatus is often used to discern species.