British Society for Plant Pathology

Presidential meeting 2005
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University of Nottingham, UK
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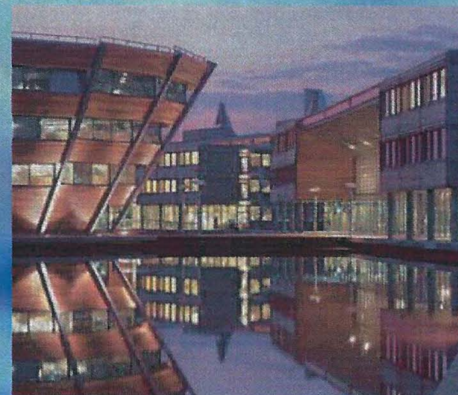
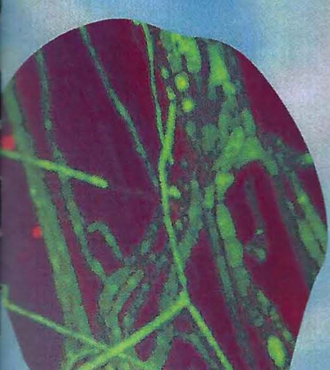


President: Prof. Phil Russell

Plant Pathology with a Purpose



Programme and Abstracts

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IN VITRO CULTURE CONDITIONS OF *COLLETOTRICHUM GLOEOSPORIOIDES* FOR SPORE PRODUCTION: b) EFFECT OF TEMPERATURE SHOCK ON SPORE PRODUCTION

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Hypericum perfuratum is an important plant species used for the production of hypericin, a metabolite of major application in the pharmaceutical industry. Its production is, however, strongly limited by *Colletotrichum gloeosporioides*, the causative agent of anthracnose. As *H. perforatum* tolerance to *C. gloeosporioides* is dependent on plant variety, we intend to select tolerant varieties under *in vitro* conditions for metabolite production without fungicide application. Selective pressure on plants is induced by inoculation with the fungus spores, but spores are difficult to obtain. The establishment of *in* vitro conditions for sporé production was the main goal of the trials here presented. The influence of changing temperature on spore production of *C. gloeosporioides* was tested. Four different temperature conditions were tested, using the environment temperature as reference. We tested the influence of an initial cold and heat shock, as well as a cold and heat shock after growth under environment conditions. Preliminary results showed that an initial heat shock induces spore production in *C. gloeosporioides*. Complementary trials on spore production and *in vitro* plant inoculation are being developed.

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In vitro fungicidal activity of *Artemisia scoparia* against *Macrophomina phaseolina*

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The biological activity of *Artemisia scoparia* was evaluated against root rot pathogen *Macrophomina phaseolina*. It was observed that aqueous leaf and stem extracts as well as the hexane fractions of *A. scoparia* severely inhibited the growth and development of pathogen *M. phaseolina*. Results indicate that leaf and stem of *A. scoparia* contain fungitoxic chemicals.

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Activity and expression of transposable elements in *Magnaporthe*

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Species of *Magnaporthe* are known to possess numerous distinct populations of transposable elements (TEs). Repeat regions in genomes arise through the proliferation of TEs, and these regions have been used to differentiate among apparent pandemic clones of *Magnaporthe*. Previous work has identified a long terminal repeat (LTR) retrotransposon called MAGGY (for MAGnaporthe GYpsy like element) that is specific to rice-infecting isolates of *Magnaporthe*. MAGGY has high variable copy number in all isolates surveyed to date. The recent acquisition of the genome sequence of a laboratory *Magnaporthe* rice-infecting strain and subsequent global transcriptional analyses presented the opportunity to study the relationship between TE genomic demography and expression patterns. The latter microarray studies suggested that MAGGY constituent ORFs, *gag* and *pol,* were highly expressed in conidia, relative to mycelial and appressorial stages of development. Reverse transcription polymerase chain reaction (RT PCR)-based analyses confirmed the pattern of expression observed in microarray experiments. Other TEs also present in high copy number in the *Magnaporthe* genome appear to have a similar pattern of expression. Here, we present microarray and RT PCR evidence in support of the conclusion that both type1 and 2 TEs, including MAGGY, MGL, Pot 2, Pot 3, Pot 4, Pyret and Occan are substantially more highly expressed in conidia than in other developmental stages in *Magnaporthe*. These observations and conclusions parallel similar findings in diverse eukaryotes, where TEs are active in germ and progenitor cells. Experiments in progress will determine whether high levels of TE expression truly signal active transposition and resulting observable genomic rearrangement in rice-infecting lineages of *Magnaporthe*. These, and related studies promise to offer profound insight into genome structure, function and evolution, as well as complex biological phenomena, such morphological development, pathogenesis and speciation.

In Vitro Culture Conditions of *Colletotrichum gloeosporioides* for Spore Production:

(b) Effect of temperature shock on spore production

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Introduction

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As *H. perforatum* tolerance to *C. gloeosporioides* is dependent on plant variety, we intend to select tolerant varieties under *in vitro* conditions for metabolite production without fungicide application. Selective pressure on plants is induced by inoculation with the fungus spores, but spores are difficult to obtain. The establishment of *in* vitro conditions for spore production was the main goal of the trials here presented.

The influence of changing temperature on spore production of *C. gloeosporioides* was tested. Four different temperature conditions were tested, using the room temperature as reference. We tested the influence of an initial cold and heat shock, as well as a cold and heat shock after growth under environment conditions.

Materials and Methods

1 - Inoculation of the different cultures in PDA and MMN medium.

2 - Growth in the different conditions as shown:

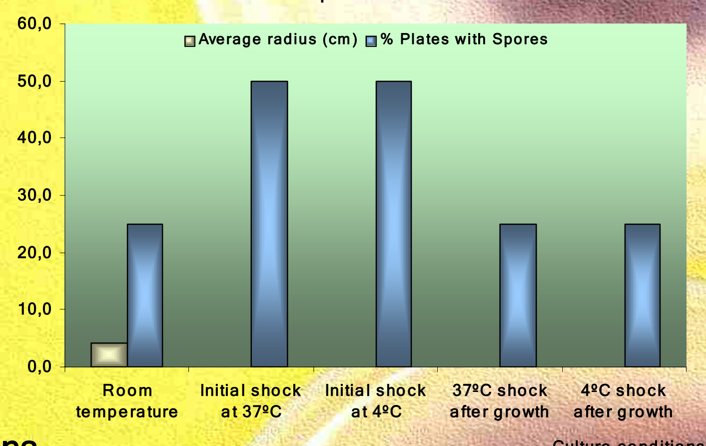
3 - The growth in radius was measured weekly.

4 - The percentage of plates with spores and average growth in radius were calculated.

Culture	PDA-PDA				PDA-MMN				MMN-PDA				MMN-MMN			
Day 1 to 10	37ºC	4ºC	room	room	37ºC	4ºC	room	room	37ºC	4ºC	room	room	37ºC	4ºC	room	room
Day 10 to 20	room	room	37ºC	4ºC	room	room	37ºC	4ºC	room	room	37ºC	4ºC	room	room	37ºC	4ºC
After 20th day	room	room	room	room	room	room	room	room	room	room	room	room	room	room	room	room

Results

Figure 1 - Influence of temperature shocks on the spore production



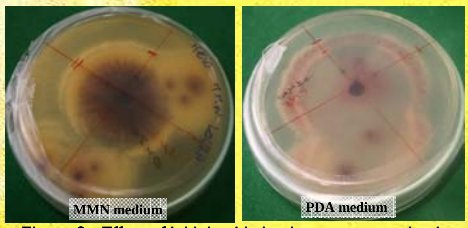


Figure 2 - Effect of initial cold shock on spore production

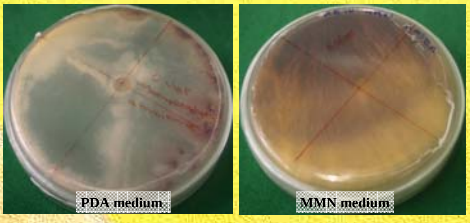


Figure 3 - Effect of initial heat shock on spore production

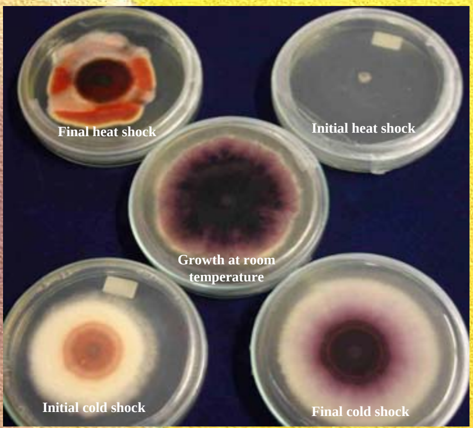


Figure 4 - Different aspects of *C. gloeosporioides* growth.

Conclusions

1 - Preliminary results on the effect of temperature shock on spore production of *C. gloeosporioides* showed that:

- an initial heat shock induces spore production (Fig. 1 & 3).
- an initial cold shock also induces spore production (Fig. 1 & 2).
- temperature shock after room temperature growth doesn't increase spore production (Fig. 1).

2 - During temperature shock the fungus doesn't grow (Fig. 1).

3 - Temperature shocks also have influence on growth pattern and on fungus mycelia colour (Figs. 3 & 4).

Complementary trials on spore production and *in vitro* plant inoculation are being developed.

Work financed by POCTI - AGR-40283-2001 "Study of defence mechanisms of *Hypericum perforatum* against *Colletotrichum gloeosporioides* infection (anthracnose)"