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Development of Methods allowing Correlation of *Dothistroma* and Dothistromin *In Planta*

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**Abstract**

*Dothistroma septosporum* is a fungal pathogen of pines with a worldwide distribution. It is responsible for the disease red band needle blight, in which necrotic lesions appear on infected needles. The red colour of the disease is due to the presence of the mycotoxin dothistromin. This toxin is structurally related to the better characterised mycotoxins aflatoxin and sterigmatocystin. The function of these toxins is unknown, but dothistromin is hypothesised to act as a competition factor. While much work has been done on *D. septosporum* and dothistromin in broth culture, *in planta* work has been limited by the methods available.

This work focused first on the development of a method for the reliable and high yield extraction of DNA from infected lesions, as previously used methods were found to be inadequate. It was found that the addition of an enzyme lysis step to the Qiagen DNeasy protocol and the replacement of its column purification with chloroform purification gave a greatly increased yield of DNA with an acceptable loss of purity.

To allow quantification of dothistromin from the same lesion samples, previously used assay systems were optimised and compared in their accuracy and sensitivity. An HPLC-fluorescence method was found most effective, and was able to accurately quantify dothistromin at single lesion quantities.

The developed methods were used to give a correlation between *Dothistroma* biomass and dothistromin in lesions at various stages of development. While this correlation was not found to be statistically significant, continuation of this work should allow valid conclusions to be drawn.

To give insight into the evolution of dothistromin biosynthesis, the genomes of other dothideomycetes were examined for the presence of dothistromin biosynthesis gene homologs. While no homologs were conclusively identified, a number of genes were shown to have similarity to known toxin biosynthesis genes.

In summary, while not all research hypotheses were able to be proven or disproven, this work sets a firm basis for future investigation in these areas using the methods developed, and strongly suggests the direction continued study should take.
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Table of Contents

CHAPTER 1: INTRODUCTION .............................................................. 1
1.1 HISTORY, HOSTS, AND GEOGRAPHIC RANGE .............................. 1
1.2 IDENTIFICATION, DIFFERENTIATION, AND DNA EXTRACTION .......... 2
1.3 THE CHEMISTRY AND BIOSYNTHESIS OF DOTHISTROMIN .................. 3
1.4 DOTHISTROMIN AS A MYCOTOXIN .............................................. 5
1.5 THE BIOLOGICAL ROLE OF DOTHISTROMIN .................................... 6
1.6 PURIFICATION AND QUANTIFICATION OF DOTHISTROMIN ............. 8
1.7 RESEARCH HYPOTHESES .............................................................. 12
1.8 AIMS AND OBJECTIVES ............................................................... 12

CHAPTER 2: MATERIALS AND METHODS .................................... 14
2.1 DNA EXTRACTION AND PURIFICATION ........................................... 14
  2.1.1 DNA extraction; needles and fungal mycelia ................................. 14
  2.1.2 Sampling and freeze drying ......................................................... 14
  2.1.3 Grinding .................................................................................. 14
  2.1.4 Qiagen DNeasy DNA extraction ..................................................... 15
  2.1.5 Nucleon Phytopure DNA extraction ................................................. 16
  2.1.6 Combined DNA extraction method ............................................... 16
  2.1.7 Glucanex enzyme incubation ....................................................... 16
  2.1.8 Fastprep bead beater .................................................................... 17
  2.1.9 DNA quantification ..................................................................... 17
  2.1.10 PCR conditions .......................................................................... 17
  2.1.11 Surface sterilisation .................................................................... 18
  2.1.12 Nested PCR ................................................................................ 18
  2.1.13 DNA gels .................................................................................. 19
2.2 DOTHISTROMIN EXTRACTION AND QUANTIFICATION ................... 19
  2.2.1 Broth cultures ............................................................................... 19
  2.2.2 Solvent extraction ....................................................................... 19
  2.2.3 Dothistromin ............................................................................... 20
  2.2.4 ELISA ........................................................................................ 20
  2.2.5 Thin Layer Chromatography ....................................................... 21
  2.2.6 HPLC ........................................................................................ 22
  2.2.7 Mass Spectrometry ..................................................................... 23
2.3 DOTHISTROMA LESION SAMPLING ........................................... 23
  2.3.1 Seedling inoculation .................................................................... 23
  2.3.2 Labelling ..................................................................................... 23
  2.3.3 Quantification ............................................................................. 24
2.4 BIOINFORMATICS: ........................................................................ 25
  2.4.1 Cluster location: ......................................................................... 25
  2.4.2 Alignment: .................................................................................. 25

CHAPTER 3: DEVELOPMENT OF DNA EXTRACTION METHODS ................... 27
3.1 INTRODUCTION ........................................................................ 27
3.2 RESULTS ......................................................................................................................... 28
  3.2.1 DNA extraction optimization ....................................................................................... 28
  3.2.2 Extraction from small pine needle samples ................................................................. 32
  3.2.3 Extraction from needles infected with D. septosporum ............................................. 32
  3.2.4 Extraction from herbarium specimens ....................................................................... 33
  3.2.5 Fungal DNA extractions ............................................................................................ 33
3.3 DISCUSSION .................................................................................................................... 34

CHAPTER 4: DEVELOPMENT OF DOTHISTROMIN EXTRATION AND QUANTIFICATION METHODS .......... 37
4.1 INTRODUCTION ............................................................................................................. 37
4.2 RESULTS ....................................................................................................................... 38
  4.2.1 Extraction .................................................................................................................... 38
  4.2.2 TLC ............................................................................................................................. 39
  4.2.3 HPLC .......................................................................................................................... 42
  4.2.4 Internal standard ......................................................................................................... 47
  4.2.5 ELISA .......................................................................................................................... 48
  4.2.6 Summary of tested methods ....................................................................................... 49
4.3 DISCUSSION .................................................................................................................. 50

CHAPTER 5: DOTHISTROMIN IN PLANTA ............................................................ 54
5.1 INTRODUCTION ............................................................................................................ 54
5.2 RESULTS ....................................................................................................................... 54
  5.2.1 Lesion development and categorization ..................................................................... 54
  5.2.2 PCR biomass quantification ....................................................................................... 57
  5.2.3 Dothistromin-biomass relationship ......................................................................... 58
5.3 DISCUSSION .................................................................................................................. 61

CHAPTER 6: BIOINFORMATIC INVESTIGATION OF RELATED ORGANISMS ......................................................... 65
6.1 INTRODUCTION ............................................................................................................ 65
6.2 RESULTS ....................................................................................................................... 65
  6.2.1 Bioinformatics .......................................................................................................... 65
  6.2.2 Toxin assay .............................................................................................................. 78
6.3 DISCUSSION .................................................................................................................. 79

CHAPTER 7: SUMMARY ................................................................................................. 82

CHAPTER 8: APPENDIX .................................................................................................... 83
8.1 GEL ELECTROPHORESIS ............................................................................................. 83
8.2 ELISA BUFFERS .......................................................................................................... 83
8.3 LIST OF COMPARED GENES ..................................................................................... 84
8.4 ALTERNATIVE BUFFERS TESTED ............................................................................. 89
8.5 TLC BACKGROUND ..................................................................................................... 90
8.6 CAFFEIC ACID HPLC .............................................................................................. 91
8.7 LESION COMPONENT VARIATION ............................................................................ 92
8.8 SAMPLE GROUP DISTRIBUTION .............................................................................. 93

CHAPTER 9: BIBLIOGRAPHY ......................................................................................... 94
List of figures

FIG. 1.1: DOHISTROMIN AND RELATED SECONDARY METABOLITES ......................... 3
FIG. 1.2 PRODUCTION OF OXYGEN RADICALS BY DOHISTROMIN ......................... 5
FIG. 1.3: EARLY PRODUCTION OF DOHISTROMIN .................................................... 7
FIG. 3.1: YIELDS OBTAINED FROM DIFFERENT DNA EXTRACTION PROTOCOLS ......... 30
FIG 4.1: EFFECT OF INCREASED ACIDIFICATION ON DOHISTROMIN IONISATION .......... 39
FIG 4.2: SEPARATION OF TLC SOLVENT SYSTEMS .................................................. 40
FIG 4.3: TLC DILUTION SERIES .............................................................................. 41
FIG 4.4: SCANNING UV-VIS ABSORBANCE HPLC ................................................... 43
FIG 4.5: HPLC DETECTION ..................................................................................... 44
FIG 4.6: FLUORESCENCE DETECTION DOHISTROMIN DILUTION SERIES ............... 45
FIG 4.7: MASS SPECTRUM OF DOHISTROMIN PEAK .............................................. 47
FIG 4.8: ELISA DOHISTROMIN DILUTION SERIES .................................................. 48
FIG 5.1: VARIATION IN MICROSCOPIC APPEARANCE OF SAMPLES ....................... 56
FIG. 5.2: AMPLIFICATION OF SAMPLE SETS ............................................................. 58
FIG. 5.3: DOHISTROMIN CONTENT OF SAMPLES BY GROUP .................................. 59
FIG. 5.4: DNA VS DOHISTROMIN .......................................................................... 60
FIG. 6.1: M. GRAMINICOLA PKS7 CLUSTER ............................................................... 68
FIG. 6.2: C. HETEROSTROPHUS PKS19 CLUSTER .................................................... 70
FIG. 6.3: M. FIJIENSIS MYCF11.E_GW1.7.973.1 CLUSTER ......................................... 72
FIG. 6.4: A. BRASSICOLA AB06180.1 GENE CLUSTER ........................................... 74
FIG. 6.5: S. NODORUM JAM_SNOG_06672/ JAM_SNOG_06682 CLUSTER ............... 76
FIG. 6.6: M. GRAMINICOLA DOHISTROMIN HPLC ............................................... 78
FIG. 8.1: CHLOROFORM:METHANOL TLC BACKGROUND ........................................ 90
FIG. 8.2: CAFFEIC ACID HPLC CHROMATOGRAM ............................................... 91
FIG. 8.3: VARIATION IN HPLC FLUORESCENCE PEAKS ......................................... 92
FIG. 8.4: DNA VS DOHISTROMIN, BY SAMPLE GROUP .......................................... 93
List of tables

**TABLE 2.1**: PCR PRIMERS ................................................................. 18
**TABLE 2.2**: REALTIME PCR PRIMERS AND PROBES ................................................. 25
**TABLE 3.1**: SUMMARY OF METHOD MODIFICATIONS ................................................. 31
**TABLE 3.2**: EXTRACTION YIELD FOR SMALL SAMPLES .............................................. 32
**TABLE 3.3**: EXTRACTION YIELD FOR *DOTHISTROMA LESIONS* ................................ 33
**TABLE 3.4**: EXTRACTION YIELD FROM FUNGAL MYCELIUM ....................................... 34
**TABLE 4.1**: TLC DILUTION SERIES ............................................................................ 42
**TABLE 4.2**: FLUORESCENCE PEAK AREA OF DOTHISTROMIN HPLC STANDARDS .......... 46
**TABLE 4.3**: ABSORBANCE OF ELISA STANDARDS ..................................................... 48
**TABLE 4.4**: DOTHISTROMIN QUANTIFICATION METHODS SUMMARY ....................... 49
**TABLE 6.1**: BIOSYNTHESIS GENE MATCHES SURROUNDING POTENTIAL PKS HOMOLOGS ................................................................................................................... 66
**TABLE 6.2**: *M. GRAMINICOLA* PKS7 CLUSTER GENE MODELS ....................................... 69
**TABLE 6.3**: *C. HETEROSTROPHUS* PKS19 CLUSTER GENE MODELS ............................... 71
**TABLE 6.4**: *M. FIJIENSIS* MYCF1.E_GW1.7.973.1 CLUSTER GENE MODELS ................ 73
**TABLE 6.5**: *A. BRASSICOLA* AB06180.1 GENE CLUSTER MODELS ............................. 75
**TABLE 6.6**: *S. NODORUM* JAM_SNOG_06672/JAM_SNOG_06682 CLUSTER GENE MODELS .............................................................................................................................. 77
**TABLE 8.1**: *ALTERNARIA BRASSICOLA* GENE COMPARISONS ................................. 84
**TABLE 8.2**: *COCHLIOBOLUS HETEROSTROPHUS* GENE COMPARISONS ..................... 85
**TABLE 8.3**: *MYCOSPHAERELLA FIJIENSIS* GENE COMPARISONS ............................... 86
**TABLE 8.4**: *MYCOSPHAERELLA GRAMINICOLA* GENE COMPARISONS ....................... 87
**TABLE 8.5**: *STAGONOSPORA NODORUM* GENE COMPARISONS ................................. 88