Genetic Studies of Pathogenicity in *Botrytis cinerea* (*Botryotinia fuckeliana*)

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Abstract

Botrytis cinerea is a common pleomorphic fungus causing 'grey mould' disease on a wide range of crops resulting in serious losses both pre- and post-harvest. Traditional control measures rely heavily on frequent fungicide applications. A greater understanding of the infection process and more information on the factors determining successful and unsuccessful host/pathogen interactions is important for the development of new control strategies. Although widely studied, relatively little is known about its genetics and the factors that determine its pathogenic ability.

This study examines the genetics and pathogenicity of *B. cinerea* through mutation and selection. Two new genetic markers were developed based on resistance to the toxic analogues sodium selenate and potassium chlorate. These markers were then utilised in sexual crosses and competition studies *in planta*.

Selenate resistant (SelR) mutants of *Botrytis cinerea* were selected by plating conidia or mycelial plugs onto minimal medium amended with selenate and taurine. Mutants could be divided into three classes based on growth in the presence of selenate or chromate and on improved growth in response to taurine in minimal media. Some mutants grew poorly on minimal media but were responsive to taurine, indicating they were defective in sulphate reduction. Strains showing the SelR phenotype may result from mutations in different genes; the genetic symbol *Sel1* was allocated to one.

Nitrate non-utilising (Nit) mutants, generated as spontaneous sectors on minimal media amended with chlorate, behaved as *nit1* mutants in growth tests (putatively defective in nitrate reductase apoenzyme) and the genetic symbol *nit1* was allocated to one of these mutants. When *nit1* mutants were paired on medium with nitrate as sole nitrogen source, some pairings complemented, behaviour attributed to intragenic complementation.

Selected crosses of SelR and *nit1* mutants with wild type strains gave 1:1 segregation of both phenotypes and no evidence of linkage to either *Mbc1* (benzimidazole resistance) or

Daf1 (dicarboximide resistance) markers; loose linkage was confirmed between *Mbc1* and *Daf1*. Both *Sel1*R and *nit1* mutants were stable following subculture and retained pathogenicity in a French bean leaf assay. Complementation was demonstrated between a taurine responsive SelR mutant and a *nit1* mutant selected from the same parent.

Non-aggressive mutants were isolated from a single-ascospore strain of *B. cinerea* following mutagenic treatment (ultraviolet and 4-nitroquinoline-1-oxide) and screening on French bean leaves. Crosses with reference strains SAS56 or SAS405 revealed one u.v. mutant (Mp97) in which the non-aggressive phenotype segregated 1:1; indicating a single gene of major effect on pathogenicity to which the genotypic symbol *Pat1* was allocated. No evidence of linkage was found between *Pat1* and either *Mbc1*, *Daf1*, *nit1* or *Sel1*.

Further characterisation of this gene in studies involving *Pat1* and wild-type strains revealed various host and temperature responses. *Pat1* strains produced small, restricted lesions on French bean and soybean leaves and slowly spreading lesions on rose flowers. On tomato stems at 20 and 25°C the mutant was essentially non-pathogenic, although a reduced number of invasive infections were produced at 10 and 15°C. *Pat1* strains grow relatively normally, are indistinguishable from wild-type in gross morphology, and grow well on minimal medium indicating no unusual nutrient requirements, and it was concluded from comparison of physiological characteristics that the non-aggressive character is unlikely to be due to gross unfitness.

No difference was found between *Pat1* and wild-type strains in total polygalacturonase activity, and differences in polygalacturonase isozyme profiles were not correlated with the presence of the *Pat1* gene. *Pat1* was found to correlate with low acid production indicating a role for organic acid in pathogenesis of *B. cinerea*. Microscopic examination of 4-day-old lesions showed a distinctly stained ring of mesophyll cells surrounding lesions of Mp97 but not its parent (A4), suggesting a difference in host response. Differences in phytoalexin induction in soybean were not found. It is possible that *Pat1* strains may be deficient in the ability to tolerate or metabolise defence compounds.

Two hypotheses are presented for further investigation. The first that *Pat1* strains may have reduced toxicity due to low production of organic acids, and the second that these strains are non-aggressive due to a reduced ability to metabolise defence compounds.

In competition experiments aggressive and non-aggressive strains were found to co-exist in the same lesion when inoculated at the same time but when challenge inoculations were delayed 6 hours or more the initial inoculation was found to dominate, suggesting non-aggressive strains may be useful as biocontrol agents.

Acknowledgments

This thesis is dedicated to the memory of my brother Ian (Loopy) Weeds whose busy productive life and too early death (●ctober 1987) inspired me to get on with life: this work being one result.

I thank my supervisors Ross Beever and Peter Long for their help and patient encouragement. I am also very grateful to the following people for their important contribution; Keith Sharrock for advice and assistance with the polygalacturonase activity and phytoalexin induction work and his contribution of the PG isozyme studies, Shiva Ganeshanandam for advice and assistance with data analysis, Daryl Cook for help with the monoclonal antibody work, Lorraine Davis & Hugh Nielson for trouble shooting and technical stuff at Massey, Martin Heffer for the photography and for always being there when the fungus was photogenic, Ian Hallett and Paul Sutherland for help with the microscopy, Henry Pak for translating all those German texts, Patrick Connolly & Rod Ball for the logit analysis, Michael Eden for the tomato plants, pots and timely advice, Christoph Janisch & Willie Cornett, for all that computing help, Ken Young for the donation of copious glasshouse roses, Bob Fullerton for supplying pre-conditioned *Sclerotinia sclerotiorum* sclerotia and culturing advice, Molly Dewey for the gift of the BC-KH4 monoclonal antibody, the staff at the Mt Albert library for finding all those urgent references so fast, HortResearch for the use of facilities, Crop and Food for the use of a glass house, AGMARDT for the funds, and Landcare Research Mt Albert for a friendly and supportive working environment, my air conditioned office and the generous use of facilities.

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Victory to the truth. Triumph to the invincible sun.

Contents

Tables

Figures

Preface

This thesis consists of an introduction and literature review (Chapter 1), a description of the techniques developed and general methodology (Chapter 2), followed by the main experimental work grouped into three subject areas (Chapters 3 – 5) and written as discrete units to facilitate publication, and ending with a general discussion and outline of possible future work (Chapter 6). To keep the text uncluttered some information has been placed in the appendices; a list of abbreviations (Appendix 1), components, instructions, and references for media and solutions (Appendix 2), source and genotype of B. *cinerea* strains (Appendix 3), a glossary of selected terms (Appendix 4), a summary of genetic nomenclature (Appendix 5), a summary of genetic symbols (Appendix 6), a summary of enzymes related to pathogenicity with relevant references (Appendix 7), and experimental details of the polygalacturonase (PG) cup-plate-assay (Appendix 8)

Chapter 3 has been accepted for publication in Mycological Research co-authored with supervisors Drs Beever (Landcare Research, Mt Albert) and Long (Massey University). Preliminary results and conclusions from the work presented in Chapter 3 were presented at the X1th International Botrytis Symposium held in Wageningen (the Netherlands) in June 1996.

> **Weeds PL, Beever RE, Long PG**. 1996. New genetic markers for *Botrytis cinerea*. In *XIth International Botrytis Symposium Programme and book of abstracts*. Wageningen, the Netherlands. Pp12.
>
> **Weeds PL, Beever RE, Long PG**. 1997. New genetic markers for *Botrytis cinerea* (*Botryotinia fuckeliana*). *Mycological Research*. In press.

Chapter 5 is being prepared for submission to Physiological and Molecular Plant Pathology, co-authored with supervisors Drs Beever and Long, and Dr Sharrock (Hort Research, Ruakura) who advised on PG enzyme and phytoalexin assays and carried out isoelectric focusing experiments on mutant and wild-type strains. Results and conclusions from the PG isoelectric focusing experiments are included within the text (boxed) for

completeness. An extension of these studies, showing segregation patterns of PG isozymes, was presented as a poster at the Australasian Plant Pathology Society 11th Biennial Conference held in Perth (Australia) in September 1997 (Appendix 9).

> **Weeds PL, Beever RE, Sharrock KR, Long PG**. 1997. A major gene controlling pathogenicity in *Botrytis cinerea* (*Botryotinia fuckeliana*). *Physiological and Molecular Plant Pathology*. In preparation.
>
> **Sharrock KR, Weeds PL, Beever RE**. 1997. Segregation of polygalacturonase isozymes in sexual progeny of *B. cinerea* (*Botryotinia fuckeliana*). Proceedings of the 11th Biennial Conference of the Australasian Plant Pathology Society, 29 Sept. - 2 October, Perth, Australia. p. 142.

Clarification of terms

Some terms are used inconsistently through this thesis. Selenate resistant (SelR and Sel1R) mutants are sometimes referred to as selenate mutants. NaSeO4 and SeO4 and selenate are used interchangeably.

The term 'parent' refers to both the wild-type progenitor of mutants and both the sclerotial and fertilising strain in sexual crosses.