

## TAXONOMY OF FRUIT-ROTTING FUNGAL PATHOGENS: WHAT'S REALLY OUT THERE?

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### ABSTRACT

This paper discusses the role of taxonomy in understanding the epidemiology of fruit rotting diseases and in determining the biosecurity status of the fungi associated with those diseases. The taxonomy of most highly specialised fruit-rotting pathogens is well understood, but some degree of uncertainty or confusion applies to the taxonomy of many less specialised pathogens. The taxonomic lumping of morphologically similar but genetically and biologically distinct taxa often confounds our ability to understand diseases, frustrates the interpretation of research data, and can result in misinformation about biosecurity status. Such unspecialised pathogens are widespread in New Zealand, but whether they cause a problem in a specific orchard depends on environmental conditions, management of the orchard, and host plant susceptibility. As management practices and preferred cultivars change, so does the spectrum of these low-specificity taxa that cause disease problems. Five examples illustrate the need for unambiguous taxonomy to facilitate more effective control and management strategies of plant pathogens. Accurate identification allows a clear understanding of the biology of the pathogens and subsequently the epidemiology of their associated diseases.

**Keywords:** *Botryosphaeria*, *Cadophora*, *Diaporthe*, *Glomerella*, *Neofabraea*, *Pezicula*.

### INTRODUCTION

This paper discusses the difficulties that different levels of taxonomic knowledge impose on our understanding of five major fungal pathogens attacking fruit in New Zealand and how this influences their biosecurity status and the nature and control of the diseases they cause. Most of the important diseases of flowers and immature fruit are caused by species with few taxonomic controversies, e.g. *Venturia inaequalis* (on apple), *Monilinia fructigena* (on stone fruit) and *Botrytis cinerea* (on grape, kiwifruit, etc.). Such straightforward taxonomy allows isolates to be unambiguously identified, which has enabled the epidemiology of these diseases to be clearly understood and sophisticated control methods to be developed. Examples include the restriction of apple black spot fungicide applications to periods of high infection risk (Tate et al. 1996), and kiwifruit canopy thinning to control *Botrytis* inoculum build-up (Manning & Pak 1993).

In contrast, understanding epidemiology and developing effective control methods has proved difficult for many fungi associated with post-harvest fruit rots, including genera such as *Glomerella*, *Diaporthe*, *Botryosphaeria*, *Neofabraea*/*Pezicula*, and *Cadophora* (= *Phialophora* sensu lato). Difficulties experienced in distinguishing meaningful taxa of these fungi are due in part to a combination of scarcity of morphological characters for species differentiation (e.g. all with small, unornamented, unicellular sexual and asexual spores) and a lack of taxonomic understanding of the genetic complexity of these organisms. Many of their species names encompass poorly defined sets of genetically

distinct taxa, which may differ in pathogenicity towards different host species or cultivars, susceptibility to fungicides, behaviour under CA storage, etc. This taxonomic lumping of genetically and biologically distinct taxa under a single name only confounds our understanding of diseases and frustrates the interpretation of research.

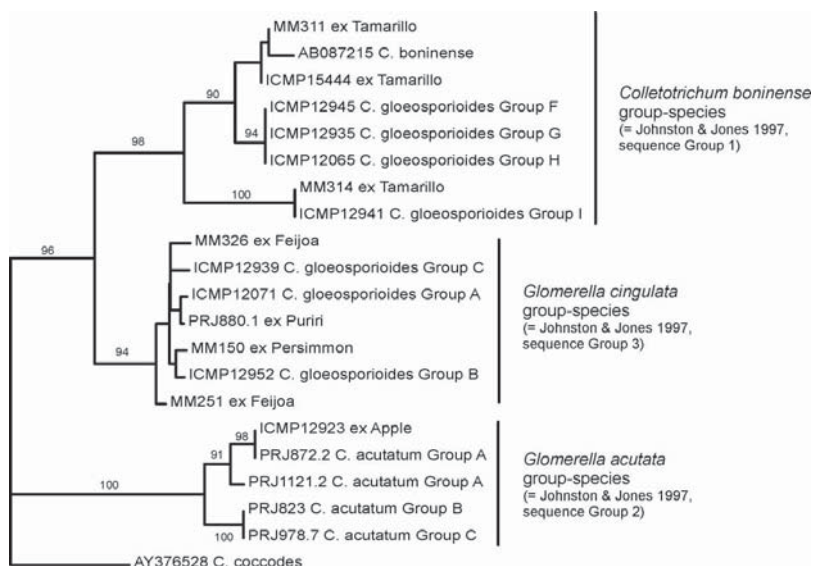
Most species within these genera are relatively unspecialised in their host range and disease symptoms (e.g. the diseases apple bitter rot and avocado anthracnose are caused by both *Glomerella acutata* and *G. cingulata*). This multiplicity of fungi means that experiments aiming to understand the epidemiology of such diseases without accurate identification of the pathogens involved are unlikely to succeed. Many of these fungi appear to occur at low levels throughout the country. Whether or not a particular species causes a problem in a specific orchard depends on climate and weather, management of the orchard, and the susceptibility of the crop species and cultivar being grown. As management practices and preferred cultivars change, so does the spectrum of the taxa within these low-specificity fungi that cause disease problems. The following examples illustrate how valuable the unambiguous taxonomy of five important plant pathogens can be in determining biosecurity status, understanding the epidemiology of disease and implementing successful control measures.

## THE PATHOGENS

### *Glomerella* (asexual state *Colletotrichum*)

Before the mid 20<sup>th</sup> century many *Glomerella* species were described solely on the basis of their host association. Arx (1957) revised the genus, placing more than 450 described species in synonymy with *G. cingulata* (*C. gloeosporioides*). For the next 40 years, *G. cingulata* was considered a species highly variable in morphology and biology. In recent years, several research groups have been using DNA to clarify relationships within the genus. No consensus has yet been reached, but there appear to be a number of biologically specialised forms within several 'group-species' that can be clearly defined using DNA sequences. New Zealand isolates previously referred to *G. cingulata* or its anamorph are distributed across two broad taxa, *G. cingulata* and *C. boninense* (a species recently described from Japan). Also common on fruits in New Zealand is *G. acutata* (*C. acutatum*). Figure 1 shows a phylogenetic tree based on DNA sequences illustrating these relationships. In apple orchards, changes in preferred cultivars and preferred fungicides (Freeman et al. 1998), have shifted the *Glomerella* populations associated with bitter rot from a dominance by *G. cingulata* (Brook 1977) to a dominance in recent years by *G. acutata* (M.A. Manning, unpubl. data).

Although *G. acutata* and *G. cingulata* are generally recognised as distinct in surveys of fungi associated with fruit rots, *C. boninense* is seldom separated from *G. cingulata*. Just as *G. acutata* and *G. cingulata* differ in fungicide susceptibility, *C. boninense* and *G. cingulata* are likely to differ in some of their biological characteristics. Each of the *Glomerella* group-species contains several distinct taxa. Some of these are distributed across several crops, while others are host-specialised and associated with a particular disease. An intensive survey of any one plant species will record several of these taxa; some may be pathogenic and some saprobic on the target plant (Johnston 2000). Distinguishing these genetically distinct taxa can be difficult, but failure to do so would mask the true aggressive nature of the pathogenic isolates, and prevent a clear understanding of the epidemiology of associated diseases and susceptibility to fungicides.



**FIGURE 1:** Neighbour-joining tree from PAUP\* using ITS sequences from selected New Zealand *Glomerella* isolates, together with *C. boninense* and *C. coccodes* sequences from Genbank. *C. gloeosporioides* Group A to Group I annotations refer to morphological groups defined by Johnston & Jones (1997). *C. acutatum* Group A to Group C annotations refer to groups defined by Lardner et al. (1999) on the basis of RAPD banding patterns. Isolates from ICMP culture collection (Landcare Research), as well as the collections of M.A. Manning (MM numbers) and P.R. Johnston (PRJ numbers). Numbers on branches are bootstrap values (% of 1000 replicates). Tree rooted using *C. coccodes* as an outgroup.

### *Botryosphaeria* (asexual state *Diplodia* or *Fusicoccum*)

This genus provides an example of a taxonomically difficult group of fungi for which genetic relationships are rapidly being resolved. Dingley (1969) listed only two *Botryosphaeria* species from New Zealand, including all those with a *Diplodia* asexual stage under a broad concept of *B. quercuum*, and all those with a *Fusicoccum* asexual stage under a broad concept of *B. dothidea*. The taxa from New Zealand previously grouped under *B. quercuum* are now distinguished as *B. obtusa* and *B. stevensii* (Laundon 1973), whereas *B. quercuum* sensu stricto has never been recorded here. Similarly, following morphological/cultural studies by Pennycook & Samuels (1985) and recent molecular studies (e.g. Slippers et al. 2004), it is now known that taxa previously grouped under *B. dothidea* for New Zealand include *B. dothidea* (sensu stricto), *B. lutea*, *B. parva*, and *B. ribis*. Biologically these taxa differ in their sensitivity to benzimidazole fungicides, their host preferences and the kinds of fruit rots they cause.

### *Cadophora*

Fungi forming *Phialophora*-like conidiophores are commonly isolated from ripe fruit rots. Molecular studies have shown that the genus *Phialophora* sensu lato is polyphyletic. All of the New Zealand fruit-rotting isolates investigated are genetically related to the discomycete order Helotiales, and these are now referred to the genus *Cadophora*. Several genetically diverse *Cadophora* species are known from kiwifruit and apple in New Zealand. The biological significance of these species is yet unknown.

***Diaporthe* (asexual state *Phomopsis*)**

*Diaporthe* species are commonly isolated from a wide range of fruit throughout New Zealand. Although the names placed on some suggest there may be host-specialisation (e.g. *Diaporthe actinidiae*), the morphological variation between isolates is poorly understood, with similar ranges of variation in sets of isolates from a wide range of crops and localities. Preliminary genetic data (Kandula et al. 2003) suggested that apparent morphological and cultural variation may not correlate well with genetic variation. The relationship between isolates from different hosts remains a 'black box'. Resolution of the *Diaporthe* taxonomy will require intensive molecular studies, such as the recent Niekerk et al. (2005) paper clarifying relationships amongst the *Diaporthe* species associated with grapes in Australia and South Africa.

***Pezicula* and *Neofabraea* (asexual state *Cryptosporiopsis*)**

Species of these genera are commonly isolated from a wide range of fruits. Recent molecular studies (e.g. Verkley 1999) have started to resolve the relationships amongst species referred to these genera, but questions remain about the taxa present in New Zealand. DNA sequence data has confirmed the occurrence of four species in New Zealand: *P. cinnanomea*, an undescribed *Pezicula* sp. (also known from conifer bark from Europe), *N. alba* and *C. actinidiae* (P.R. Johnston, unpubl. data). Other related species are also likely to occur in New Zealand. We have been unable to confirm that either *N. malicorticis* or *N. perennans* occurs in New Zealand. Although both have been reported (Brien 1932, 1934; Brook 1957; Dingley 1969), the nomenclature in these earlier publications is confused and confusing, and no vouchered cultures support the records. During a recent study describing *Cryptosporiopsis actinidiae* (Johnston et al. 2004), an attempt was made to include isolates of *N. malicorticis*, but the single available New Zealand culture under this name proved to be *C. actinidiae*. Given the morphological similarities between *C. actinidiae*, *N. malicorticis* and *N. perennans*, it remains uncertain whether *N. malicorticis* and *N. perennans* were ever in New Zealand. These taxa are of biosecurity significance, making resolution of their status in New Zealand important.

Like *Glomerella*, the *Neofabraea* population associated with apple appears to have changed over the last 50 years. Historically, ripe spot (caused by both *N. alba* and "*N. perennans*") was a major disease problem for commercial orchards in New Zealand (Brook 1957), but after the advent of the first broad spectrum organic fungicides (dithiocarbamates) it became relegated to a disease of the home garden or neglected apple trees. Recently, with the introduction of more advanced fungicides (e.g. demethylation inhibitors) the disease has made something of a comeback, but now the pathogen causing damage is exclusively *N. alba* (M.A. Manning, unpubl. data).

*C. actinidiae* was first described from kiwifruit and at present causes an economic problem only on the kiwifruit cultivar Hort 16A. However, it has been isolated at low levels from other fruits including apple and persimmon, and also from native forest. It is likely to be widely distributed in New Zealand. Isolates of the fungus belong to multiple vegetative compatibility groups (R.E. Beever, pers. comm.), implying that sexual recombination is occurring somewhere, in an as-yet-undiscovered *Neofabraea* stage. As new fruit cultivars are developed and orchard management systems change, *C. actinidiae* has potential to cause a problem on other crops as well. Similarly, other *Pezicula*, *Neofabraea* and *Cryptosporiopsis* species that currently appear benign, may become economically important in the future.

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