Come out, come out, wherever you are!

New findings reveal that early detection and quantification of *Botrytis cinerea* in plums and weeds in the orchard may be key to optimise decay control.

SPECIES OF THE genus *Botrytis* are plant pathogens of numerous food and ornamental crops. *Botrytis cinerea*, specifically, is a generalist fungus that infects more than 220 host plants worldwide, among them numerous fruit crops, and also stone fruit such as plums. The fungus is viewed as an aggressive necrotrophic pathogen. This means it survives on dead plant tissue, and it infects fruit by entering through wounds, and then causes decay by killing off cells. However, new discoveries in various crop types have revealed that the life cycle of the fungus includes a period where it lives inside healthy plants, like a welcome visitor, without causing harm.

Due to the commercial importance of this fungus, comprehensive studies have been conducted on the nature of on *B. cinerea* over the past few decades. However, it now seems that the interaction between the plant and the fungus is not fully understood, and that there are more pieces to the puzzle than previously accepted. This brings into question what is known about the biology, as well as the life and disease cycle of *B. cinerea*.

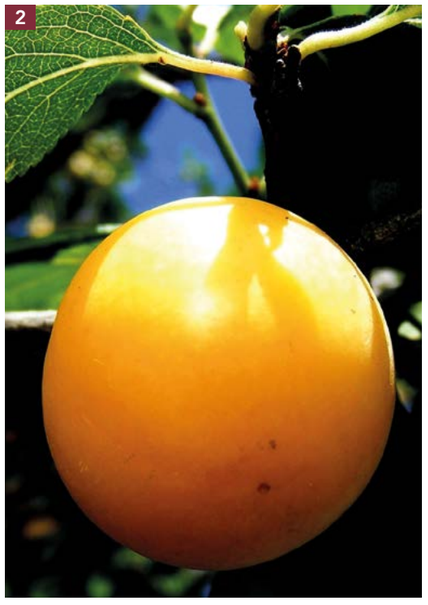
Interestingly, the harmless presence of *B. cinerea* has not only been reported in cultivated crops; it has also been found in weeds. This raises further questions with regards to the epidemiology of *B. cinerea*. For example: could inoculum from weeds infect blossoms and fruit? If so, the continuous presence of the fungal inoculum from weeds could mean that once fungicide applied to trees wears off, the infection could continue indefinitely.

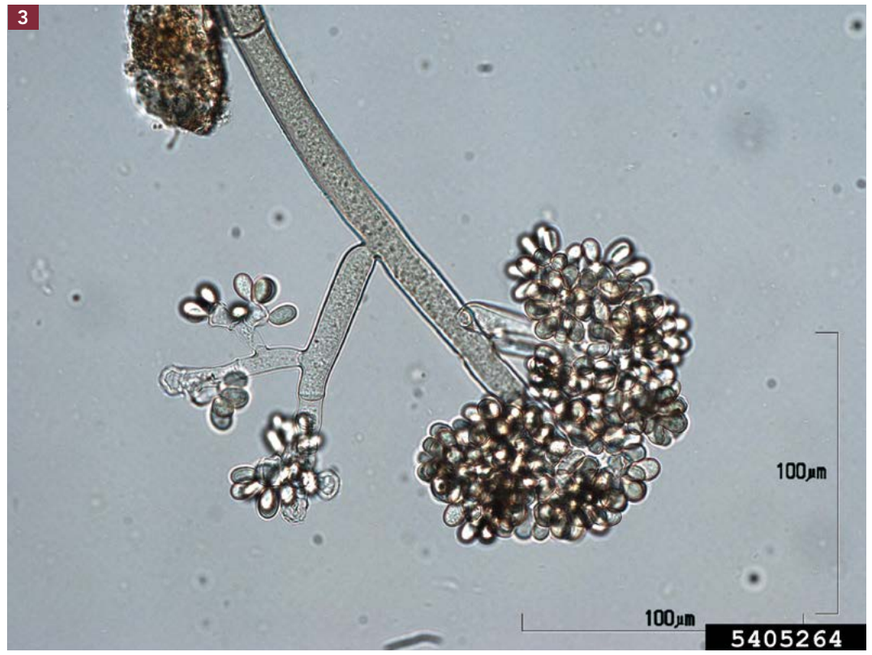
Answering new questions

Under the leadership of Dr Ida Wilson, a crop protection specialist at ExperiCo, and Dr Stephan Ferreira, research manager at WestCape Biotech in Stellenbosch, a study was launched to clarify whether or not *B. cinerea* could live harmlessly in plum tissue, and if it is present in weeds in a South African plum orchard.

Execution started with identifying an orchard with a known high incidence of *Botrytis* grey mould and high weed infestation. Twenty-five trees were chosen and samples of blossoms, small fruit, mature fruit at harvest, and fruit after storage were evaluated. Using quantitative real-time PCR (qRT-PCR), the researchers wanted to determine the presence of *B. cinerea* DNA, as well as the quantity. The same technique was used to detect the presence and quantity of DNA in weeds. Data were gathered for a two-year period.

B. cinerea was found in all plum tissue types.





Courtesy: Paul Bachi, University of Kentucky Research and Education Center, Bugwood

Botrytis presence in blossoms were particularly high, with fungus DNA present in at least 95% of the blossoms. Traces of the fungus were also found in more than 14 species of weeds, including both broad-leaved and grass weeds.

First year results revealed that greater weed infestation per tree coincided with a higher presence of fungus DNA in blossoms. "We don't know yet if there is a causal relationship," says Dr Wilson. "Our recommendation is that further studies into how weed infection may influence blossom infection are necessary, given that *B. cinerea* is vectored by insects, which makes the transferral of *B. cinerea* from weed flowers to blossoms a possibility."

Notably, the study found that, given the presence of *B. cinerea* DNA in fruit of different maturities, standard control measures for grey mould control had no influence the presence of the fungal DNA in plums. The amount of *B. cinerea* DNA increased in a linear relationship with the maturation of the fruit. Thus, despite the application of fungicide to the surface of fruit, *B. cinerea* remained present inside fruit tissue and the amount of DNA progressed as fruit matured. "Our finding suggests that fungicide cannot abolish *B. cinerea* present inside plums," says Dr Ferreira.

During the first year of the study, fruit were stored for up to four weeks. All fruit remained healthy, hence the relation between the presence of *B. cinerea* DNA and the occurrence of fruit decay could not be ascertained. In the second year of the study, however, fruit were stored for the whole cooling period it would have been exposed to in commercial industry. From cold-storage, fruit were transferred to higher storage temperatures to simulate shelf life, but also "extended shelf life", which presented a point in time where fruit decay was inevitable.

Results indicated a direct correlation between the average amounts of *B. cinerea* DNA detected in fruit with the average percentage rot that developed during storage. Although the sample size was small (analyses over six points in time represented 3000 fruit in total), a potential relationship between the presence of *B. cinerea* DNA and the development of post-harvest decay in plums is evident.

Technical support in the study was rendered by four matriculants who were partaking in the Premier of the Western Cape's Advancement of Youth Programme, an initiative to give youth workplace experience, in order to assist them

1 Dr Ida Wilson.
2 The study was done on a yellow plum variety.
3 *Botrytis* as it appears under a microscope.

PROJECT TITLE
The prevalence of *Botrytis* in plums and other hosts in the orchard – a preliminary investigation using molecular technology

PRINCIPAL INVESTIGATORS
Dr Ida Wilson and Dr Stephan Ferreira

CONTACT DETAILS
+27 (0)21 887 1134
ida@experico.co.za
+27 (0)21 865 2531
stephan@westcapebiotech.co.za

DURATION
Two years

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R283 577 & R273 577

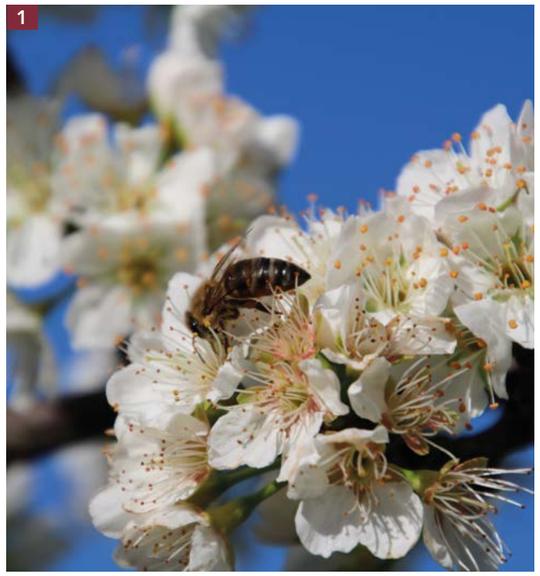
LEAD INSTITUTIONS
ExperiCo (Pty) Ltd and WestCape Biotech (Pty) Ltd

BENEFICIARY
South African Stone Fruit Producers' Association

HUMAN CAPITAL DEVELOPMENT
Four interns of Western Cape Premier's Advancement of Youth Programme and an intern of Cape Peninsula University of Technology's Work Integrated Learning Programme participated in the project.

PUBLICATIONS
One

PRESENTATIONS AND PAPERS
One



1 Although grey mould decay caused by *Botrytis cinerea* is a disease that manifests post-harvest, plums may be first infected in the orchard during the pollination stage when blossoms are most vulnerable.
2 At the time of bloom, plum orchards should be kept free of weeds, grasses and other hosts of *Botrytis cinerea* to keep early infection at bay.
3 A stray non-stone fruit tree in the bottom left corner of the plum orchard had symptoms of die-back and a high incidence of *B. cinerea* in its blossoms.

to be taken up in workplaces. This industry and PHI Programme-funded project, also formed part of one national diploma student's thesis.

Recommendations

"Based on our findings, we recommend that disease control efforts for *B. cinerea* should be revisited," says Ida. A new strategy could focus on protecting blossoms from primary infection early in the growing season, and eradicating weeds when trees are blooming.

Moreover, results support the development of a decay risk prediction tool. Detection of a high *B. cinerea* DNA in blossoms may predict a high risk for decay in fruit post-harvest. This theory will be tested in follow-up trials in the 2017/2018 season.

Arguably, the study's most significant contribution was pointing out that the biology of pathogens in the field is not necessarily fully understood. Without a clear picture of where the fungus lives, and what it is doing there, control efforts cannot be targeted optimally. Results obtained from this study encourage the further exploration of the lifestyles of fruit pathogens in general, emphasise the importance of questioning the status quo and support the urgent conceptualisation of new, more accurately directed decay control strategies, which could be highly beneficial to the fruit industry.

WHAT IS THE VALUE OF QUANTITATIVE REAL-TIME PCR (QRT-PCR)?

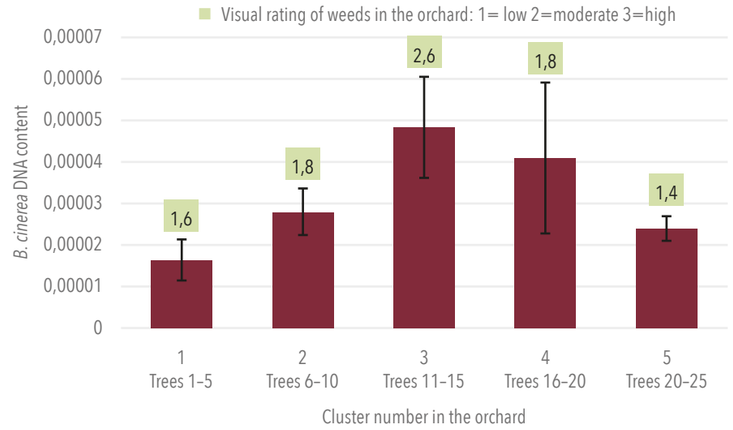
Techniques that are able to measure the amount of DNA from a specific pathogen have become the gold standard in disease diagnostics in all life science fields, including medicine, veterinary science and agricultural sciences.

To detect and quantify DNA, qRT-PCR has been established as a cost-effective, accurate and sensitive method to routinely detect and quantify pathogens in their hosts. Several studies have shown the validity of using this technique to determine the levels of *Botrytis cinerea* present in crops.

Moreover, several studies have found a linear correlation between *B. cinerea* DNA detected by qRT-PCR after artificial inoculation, and observed grey mould symptoms in fruit. Studies conducted at WestCape Biotech also accurately and sensitively quantified *B. cinerea* in fruit with natural grey mould infection.

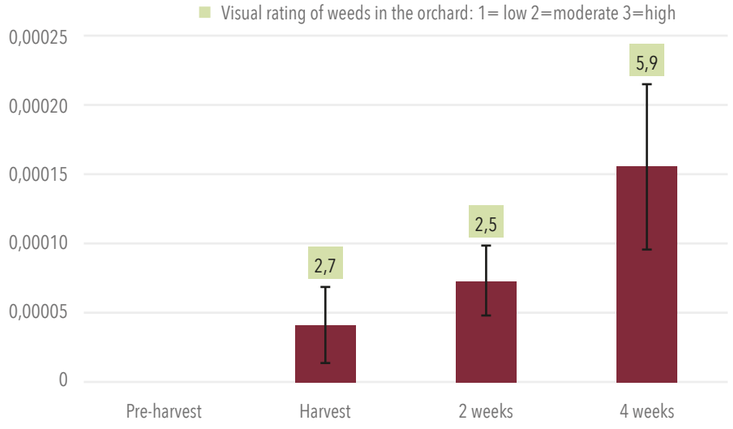
Sources: Dr Stephan Ferreira, Spotts et al. (2008), Sanzani et al. (2012), Cadle-Davidson (2008), Suarez et al. (2005)

Figure 1: *B. cinerea* DNA levels in blossoms per single cluster of trees indicating the visual presence of weeds per cluster

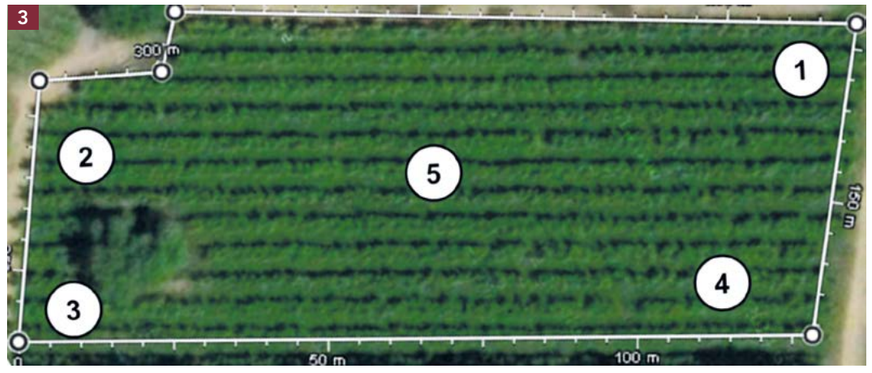


The severity of weed infestation and presence of *Botrytis* DNA (the greater the visual weed incidence, the higher the *B. cinerea* DNA level from blossoms of that cluster of trees). (Error bars indicate standard error (n=5).)

Figure 2: Quantity of *B. cinerea* DNA per time point of analyses measured over 25 trees



Average level of *B. cinerea* DNA, for 250 fruit for each sampling point. (Error bars indicate standard error (n=5–7).)



> "Results obtained from this study should encourage the further exploration of the life cycles of fruit pathogens in general. The results emphasise the importance of questioning the status quo and it supports the urgent conceptualisation of new, more accurately directed decay control strategies.
>
> **Dr Ida Wilson**