The hunt for *Botrytis cinerea*

By developing a method to detect *Botrytis* infection before the rot shows itself, the South African pear export industry hopes to boost its reputation and profitability.

CONFIDENCE IN THE quality of produce is a cornerstone of international trade in fresh fruit. Post-harvest diseases undermine this confidence, especially when they manifest only after fruit consignments have been delivered to their foreign destinations. The consequent financial losses can be ruinous to a producer, while the reputational damage can be impossible to repair.

Botrytis rot, caused by *Botrytis cinerea*, is a major issue in the pear industry. *B. cinerea* can infect pears at the calyx end, the stem-end, and (or through) puncture wounds. Although infection can occur at any time during the pear's lifecycle, calyx-end infection occurs mainly during the blossom period when pistils and stamens are infected and the inoculum is retained within the floral tube of the fruit. The infection remains latent until visual symptoms finally appear at some point during post-harvest storage.

According to Dr Stephan Ferreira, manager of biotechnology at WestCape Biotech, the unpredictability of especially calyx-end rot is hugely problematic for the local industry. "It would therefore be of great value to have a way to detect and quantify *Botrytis cinerea* levels within a batch of fruit before visual symptoms appear," he says. "This will allow producers to make informed decisions about corrective action, and help them to plan logistics that will reduce the risk of delivering fruit that is likely to develop *Botrytis* rot to the market."

At the moment, however, no economically viable method exists to monitor the presence of the *Botrytis cinerea* inoculum at different stages of the production and supply chain.

1 Merle Wells-Lee, laboratory assistant (front), and Dr Stephan Ferreira, project leader and biotechnology manager, WestCape Biotech.

The DNA solution

Techniques based on measuring the amount of DNA from a specific pathogen have become the gold standard in disease diagnostics in all life science fields, including agriculture.

Quantitative real-time polymerase chain reaction (qRT-PCR) has been established as a cost-effective, accurate and sensitive method to routinely detect and quantify pathogens in their hosts by detecting and quantifying DNA.

Importantly, several studies have already confirmed the validity of this technique to determine the levels of *Botrytis cinerea* in crops. A 2008 study showed that a strong linear correlation existed between the amount of *Botrytis cinerea* DNA on pear fruit after artificial inoculation and observed stem-end grey mould symptoms. Studies conducted at WestCape Biotech also accurately and sensitively quantified *Botrytis cinerea* in a variety of crop plants from artificial and natural inoculation experiments.

> "We found that *B. cinerea* DNA was detectable in the blossoms at full bloom and in the calyx end of fruit harvested at optimal maturity."
>
> Dr Stephan Ferreira

Building on this existing body of work, WestCape Biotech and its collaboration partners proposed a project to develop a system based on the qRT-PCR technique to accurately, sensitively and rapidly determine the level of *Botrytis cinerea* present at the calyx end of 'Packham's Truimph' and 'Abate Fetel' flowers and fruit.

"By sampling specific stages of the production and supply chain, we hoped to generate information that would introduce changes in agricultural practices, pesticide application and post-harvest management to address *Botrytis cinerea* infection levels and financial losses associated with the problem," says Stephan.

He furthermore foresees that the outcome of the team's work could lead to a predictive model capable of assessing the likelihood of disease development during the storage and distribution of pears. Such a tool would make a significant contribution in reducing financial and reputational damages to the South African fruit industry.

The proposal received PHI Programme and industry funding and in August 2014 the team, consisting of Dr Stephan Ferreira and Dr Ida Paul, set to work.

Objective, methodology and results

The objective of the project was to develop a method to continuously detect and quantify *B. cinerea* levels in orchards, packhouses and packed fruit.

To achieve this, pear blossoms were collected from orchards at full bloom, and fruit at optimal harvest maturity. These samples were either artificially inoculated with known amounts of *B. cinerea* spores or left uninoculated. Genomic DNA was extracted from the samples and used in a qRT-PCR reaction to determine the relative *B. cinerea* DNA content present

2&3 *B. cinerea* DNA was detectable in the blossoms at full bloom (**2**) and in the calyx end of fruit (**3**).

PROJECT TITLE
Detection and quantification of *Botrytis cinerea* incidence and severity in harvested pears for the development of a monitoring system to support commercial exports

PRINCIPAL INVESTIGATORS
Dr Lucienne Mansvelt, Dr Stephan Ferreira and Dr Ida Wilson

CONTACT DETAILS
+27 (0)21 865 2532
stephan@westcapebiotech.com
+27 (0)21 887 1134
ida@experico.co.za

DURATION
One year and six months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R181 000 & R171 000

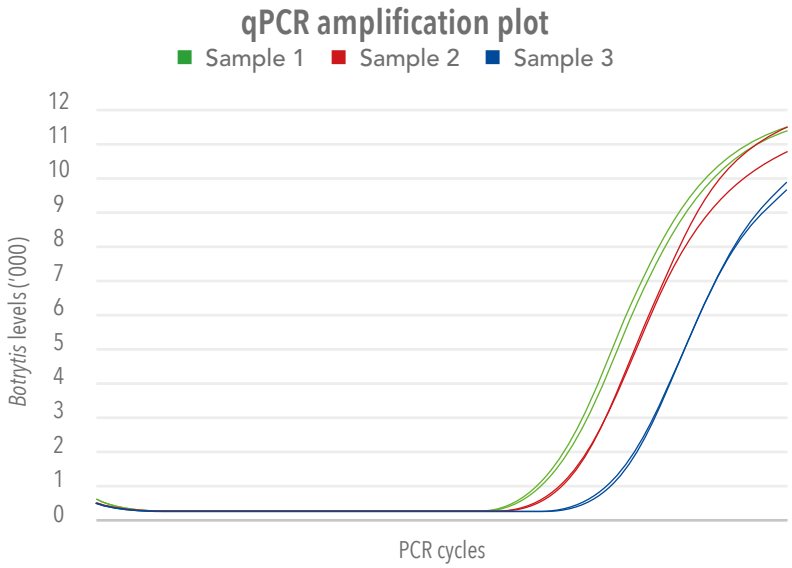
LEAD INSTITUTIONS
WestCape Biotech (Pty) Ltd and ExperiCo (Pty) Ltd

BENEFICIARY
The South African pome industry

FOCUS AREA
Post-harvest disease control

PUBLICATIONS
Pending

PRESENTATIONS
Two



in the blossoms and in the calyx end of the mature pears at specific time points.

The research team found that:

- *B. cinerea* DNA was detectable in the blossoms at full bloom and in the calyx end of fruit harvested at optimal maturity.
- There was a strong correlation between the *B. cinerea* DNA content and the number of spores artificially inoculated in both blossoms and the calyx end of fruit.
- The method that WestCape Biotech had developed previously as a research tool could be used to monitor the increase in *B. cinerea* DNA in the calyx end of pear fruit in storage.
- There was no significant reduction in the *B. cinerea* DNA content in the calyx end after chlorine flume treatment.
- Infections early in the production period, most probably at bloom, are crucial in determining the inoculum level present throughout the production and storage period.

The results showed that WestCape Biotech's method detected and quantified inoculum levels sensitively and accurately on pear blossoms and at the calyx end of mature pears. They also confirmed its application as a way to monitor *B. cinerea* inoculum levels in stored pear fruit.

Consequently, the method can be used to inform packhouse and logistics decisions in the interest of reducing financial losses incurred due to infected fruit.

THE BANE OF *BOTRYTIS*

Botrytis cinerea, the causal agent of *Botrytis* rot, is a filamentous fungus that infects more than 200 host species, leading to significant economic losses worldwide in various commercial crops.

The impact of *Botrytis* rot in commercial crops is devastating. One study has estimated that around 20% of all crop losses can be attributed to this fungus; another claimed it is responsible for more than 50% of post-harvest rot.

The cost of fungicide used to fight the disease is estimated at around $540 million annually, which represents around 10% of the global fungicide market.

1 Calyx-end decay caused by *B. cinerea* in an advanced stage. The decayed area appears light brown to dark brown and is spongy.
2 There was no significant reduction in the *B. cinerea* DNA content in the calyx end after chlorine flume treatment.

1

2