AHDB Horticulture

Project title:	Biocontrol as a key component to manage brown rot disease on cherry
Project number:	CTP_FCR_2017_3
Project leader:	Xiangming Xu, NIAB EMR and Michael Shaw, University of Reading
Report:	Annual report, October 2019
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Key staff:	Sophia Bellamy
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Industry Representative:	NA
Date project commenced:	October 2017

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Two microbial biocontrol agents (BCAs) (*Aureobasidium pullulans* and *Bacillus subtilis*) have been identified and show biocontrol promise against brown rot disease of stone fruits.

Background

Brown Rot, caused by *Monilinia* spp., is one of the most important diseases in stone fruits worldwide. Brown rot can cause blossom wilts and fruit rots in the orchard as well as latent fruit infections leading to post-harvest rot. Current control methods rely on scheduled spraying of fungicides. However, new pathogen strains resistant to fungicides and the continuing pressure to reduce fungicide use have led to an increase in research into alternative management methods, such as biological control. NIAB EMR recently identified two microbes that significantly reduced sporulation of *M. laxa* under laboratory conditions. These two isolates were a bacterial species *Bacillus subtilis* (B91) and yeast-like fungus *Aureobasidium pullulans* (Y126), and currently being formulated into commercial products. We aim to investigate the potential to use these two novel biocontrol microbes to reduce the latent infection of cherry fruit by *M. laxa*.

Summary

Y126 and B91 are being studied for their efficacy against *M. laxa* in terms of reducing sporulation on mummified fruits, blossom wilt and latent fruit infections in cherry. In year 2, we specifically investigated the use of these two microbes for reducing latent infection of cherry fruit, hence reducing post-harvest rot development.

Financial Benefits

Further research is needed to fully assess the direct effects of these two biocontrol microbes on commercial fruit production. However promising results in a latent infection trial showed the two biocontrol agents reduce the disease incidence post-harvest by nearly 30% when applied two weeks before harvest. However this was a small trial and not conducted under commercial conditions. A follow up exeriment in year three will be done in commercial conditions to beter quantify this.

Action Points

There are no grower action points at this early stage of the project.

SCIENCE SECTION

Introduction

Latent infections are characterised by the penetration of *M. laxa* of young fruits but remaining symptomless (latent) until fruit riping near harvest. The latent infection resumes as the fruit matures, often manifesting as post-harvest rots. It is understood that infections of *M. laxa* via wounds result in visible symptoms in the orchard pre-harvest, and latent infections occur on intact fruits usually manifesting as fruit rot at or following harvest. A study looking at *M. fructicola,* another dominate brown rot pathogen, on prune showed a positive correlation between blossom blight incidence, latent infections on immature fruits and post-harvest rots (Luo *et al.*, 2005).

Infection of *M. laxa* on stone fruit is closely related to environmental factors. The optimum temperature for *M.laxa* sporulation is around 10 ^{o}C. It can produce conidia at even lower temperatures. Germination of *M. laxa* conidia may occur at temperatures as low as -4 ^{o}C (Tamm and Fluckiger, 1993; Tian and Bertolini, 1999). However, infection rarely occurs at temperatures lower than 10˚C (Casals *et al.*, 2010).

Infection of fruit by *M. laxa* is affected by fruit maturity: fruit tends to be increasingly susceptible as it matures (Gell *et al.*, 2008). As the fruit matures, there are changes in its biochemical and physiological composition that result in changes in its susceptibility to infection (Biggs, 1988; Wade and Cruickshank, 1992). In the first stage of fruit formation, the green fruitlet is photosynthetically active and is susceptible to infection. This susceptibility is thought to be due to the active stomata forming, an easy entry point for the fungi. The following stage, pit hardening, is the least susceptible due to the increase in production of secondary compounds such as catechin, epicatechin, and phenolic compounds. Once the pericarp forms and hardens, the concentration of chlorogenic andneochlorogenic acid reduces. This leads to increased susceptibility of fruit to *M. laxa* as chlorogenic andneochlorogenic acid can affect melanin production in *M. laxa* that is essential for penetration (Oliveira Lino *et al.*, 2016).

Many of the current studies on latent infection are carried out on different *Prunus* species, *Monilina* species and using different techniques. These variations may account for inconsistencies in latent infection at different stages of fruit maturity (Oliveira Lino *et al.*, 2016). It is, however, generally understood that the susceptibility of stone fruits to the pathogen increases with fruit maturity (Oliveira Lino *et al.*, 2016). Latent infections can quickly develop into visual rots and easily spread via contact within cold storage (Fourie and Holz, 2003). The ability of *M laxa* being able to develop rapidly at 5-10 ^{o}C can lead to the rapid spread of rot post-harvest. With the restriction of fungicide application post-harvest the spread of rot can lead to significant post-harvest crop loss (Martini and Mari, 2014).

Pre-harvest applications of microbial antagonists could be an effective control measure for reducing post-harvest decay of fruits. Theoretically, a pre-harvest application would allow beneficial microbes to colonise the fruit surface before harvest. Therefore if wounds were sustained during harvest, these would be colonised by the antagonist suppressing the spread of the pathogen (Sharma *et al.*, 2009).

In 2013, two microbial strains of *M. laxa* were identified from indigenous populations within the UK (Rungjindamai, Xu and Jeffries, 2013): *Aureobasidium pullulans* (Y126), a yeast-like fungus, and *Bacillus subtilis* (B91), a bacteria. These two strains showed promise in suppressing pathogen sporulation as well as being able to survive over a range of temperatures under lab conditions.

This study is to access the efficacy of these two strains (B91 and Y126) in terms of reducing latent infection of *Monilinia laxa* on cherry fruit. We expect that applying these two strains close to harvest would lead to reduced latent infection, hence post-harvest rot on cherry.

Materials and Methods

Treatments

There were three treatments: fruit treated with the two biocontrol microbes in addition to fruit that did not receive biocontrol microbes but were treated with sterile distilled water, as a control. All fruits of three treatments were inoculated with *M. laxa* 24 hours after biocontrol application.

Inoculum production

Single colonies of B91 and Y126 were grown in liquid media (liquid broth and potato dextrose broth, respectively) for 24 hours on a shaking incubator (180 rpm, 25 °C). Propagule concentration was estimated with a spectrophotometer and adjusted to OD600 0.2 (B91) and 0.01 (Y126) to achieve a propagule concentration of 1×10^8 CFU ml^{-1}. The biocontrol suspensions and the control of sterile distilled water were transferred to handheld sprayers.

M. laxa inoculum was grown on ripe plums, spores were harvested and suspended in sterile distilled water and adjusted to $1x10^5$ spores ml^{-1} with a haemocytometer.

Application

Three trees of cultivar Kordia in an open-air orchard at NIAB EMR were selected, with three branches from each tree selected at random, one for each treatment. Two weeks before harvest fruits on each branch were sprayed with an appropriate biocontrol strain until runoff. Twenty-four hours later, all fruits, including the control treatment, were sprayed with *M.laxa* spore suspension.

Disease assessment

Two weeks after treatment, visibly healthy ripe fruits were harvested and stored at 20 °C on sterile trays. Visible rot was assessed on days 1, 2 and 4; on each assessment, diseased fruits removed to prevent secondary contact spread.

Results

The data was logit transformed, and an ANOVA performed in R to assess the efficacy of the treatments to reduce latent infection.There appears to be treatment effect on all three assessments (Fig. 1). The ANOVA (Table 1) shows there is a statistical significance between treatment and disease incidence ($p < 0.0001$). A Post-Hoc Tukey analysis on treatment revealed a significant difference between the control treatment and the two biocontrol agents, Y126 and B91, and the control treatment of SDW ($p < 0.0001$ and $p < 0.001$ respectively). There is no significant difference between the biocontrol agents ($p = 0.94$).

Table 1: ANOVA table to show whether the disease incidence is affected by the treatment or day of storage.

	Degrees of Freedom	Sum of Squares	Mean Squared	F value	Pr(>F)
Treatment	2	1.665	0.832	17.83	**0.0000298**
Day	1	4.013	4.013	85.968	**0.00000000716**
Treatment: Day	2	0.002	0.001	0.026	0.974
Residuals	21	0.98	0.047		

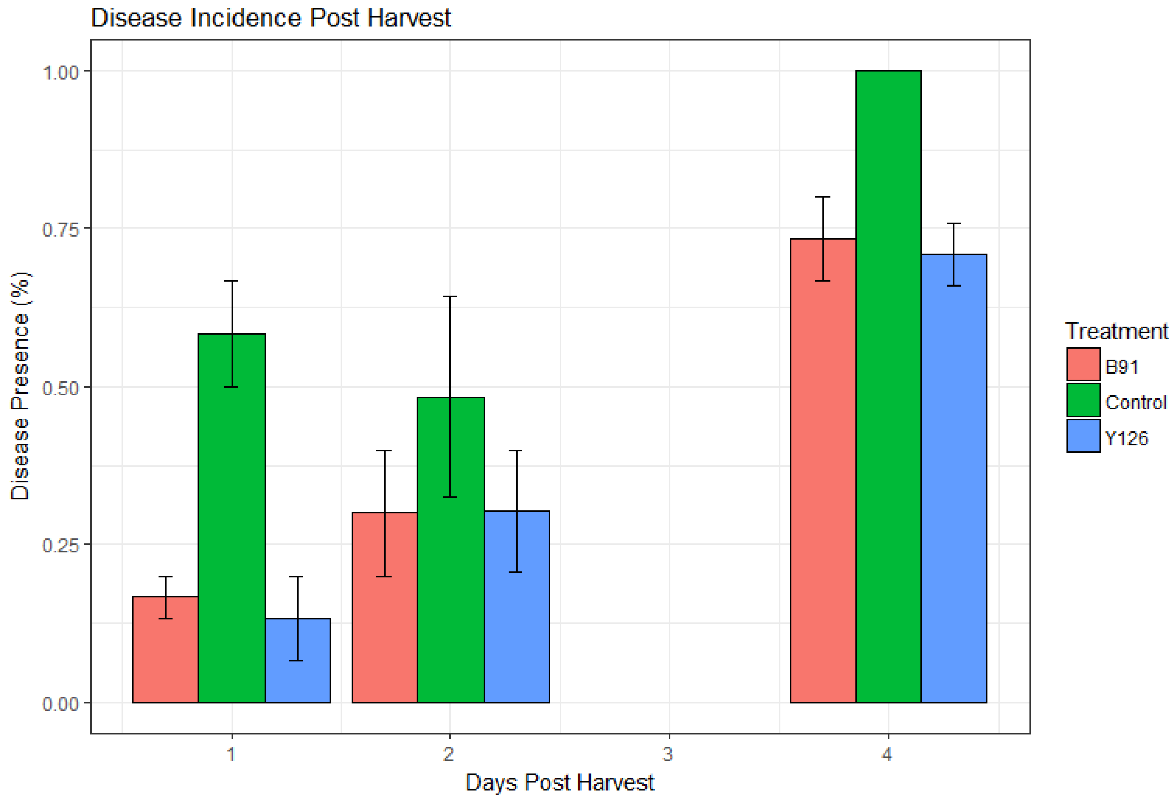

Figure 1: The mean percentage of fruits with visible rot by treatment and across three time points (Days post-harvest) with standard error bars.

Discussion and Conclusions

The results of the present experiment showed that application of the two biocontrol agents can significantly reduce the disease incidence post-harvest when applied two weeks before harvest with a reduction of nearly 30% on day four compared to the control in which all fruit were rotted. However, it is not clear whether this is achieved through delayed pathogen development or the prevention of infection. To determine this a follow up experiment will be conducted in year three, trees will be treated with *M. laxa* early in the season to mimic latent infection as well as trees inoculated late in the season for 'new infection' after a biocontrol treatment fruit will be harvested and the rot profile assessed over time. The 100% rate of disease incidence in the control group would suggest that infection had occurred on most fruits in the field. However, further investigation would be needed to confirm this, particularly under low temperatures, which replicate commercial post-harvest cold storage.

Knowledge and Technology Transfer

- BSPP PhD Conference – Presentation at the BSPP PhD conference 2019
- AHDB Tree fruit day – Presentation at the AHDB tree fruit day 2019
- Biotechnology YES competition 2018
- BSPP Grand Challenges in Plant Pathology 2018
- XV Meeting of the IOBC-WPRS - Poster presentation at the International organisation for biological and integrated control conference in Lleida, Spain. Secured 500 euro travel grant.
- NIAB Poster day 2018 - Poster presentation at NIAB poster day 2018 in Cambridge, with the CTP.

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