Biocontrol as a key component to manage brown rot diseases on cherry

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Brown rot on cherry:

Monilinia spp. cause brown rot disease on cherry and other *Prunus* species., causing blossom blight, cankers and fruit rot (Fig. 1). The current control relies on scheduled application of fungicides. There is a growing need for new management strategies less reliant on fungicides.

Figure 1. (left) Mummified fruit on tree, the primary source of inoculum; and (right) brown rot on cherry.

Potential for biocontrol:

NIAB EMR identified two microbes that reduce sporulation of *M. laxa* as well as fruit infection in small-scale studies. These two isolates are a bacterium *Bacillis subtilis* (B91) and yeast-like fungus *Aureobasidium pullulans* (Y126).

Aims:

The overall aim is to understand the ecology of these two potential biocontrol agents (BCAs) to improve biocontrol efficacy. Specifically, we studied the survival of these two BCAs under natural conditions and their effect on overwintering of *M. laxa* on mummified fruits.

Method:

A propidium monoazide (PMA) based qPCR method was developed to estimate the size of viable microbial population. PMA is a dye that binds to and modifies DNA so that it can not be amplified in PCR. However PMA can not permeate cell membrane but bind DNA only in dead cells. Thus PMA-based qPCR only quantifies DNA from viable cells (Fig. 2).

A field experiment was conducted where mummified fruit was treated with the two BCAs. Treated samples were taken over time and subjected to the PMA-qPCR method for estimation of the viable population size.

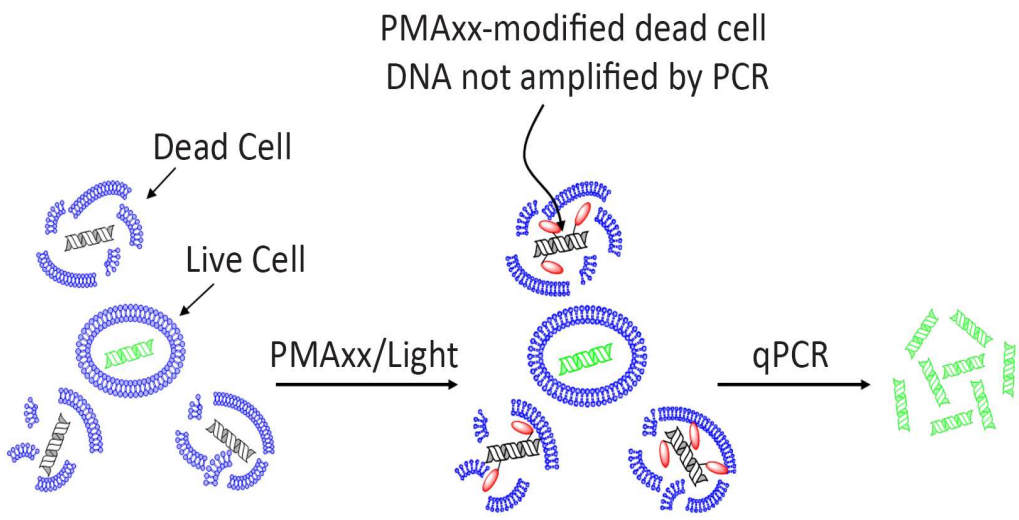

Figure 2. The principal of selective detection of live cells using PMA dye (Nocker *et al.,* 2006) .

Results and discussion:

Viable population of Y126 declined over time from the initial 1x 10^8 to 0 five weeks after application. A simple linear model accounted for nearly 84% of the total variability in the viable population (Fig. 3).

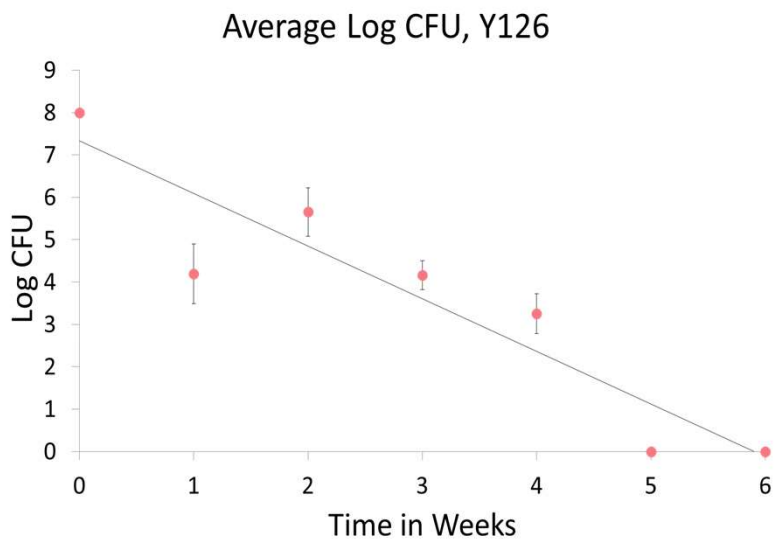

Figure 3. Estimated viable population size (Log CFU) for Y126 over time; the line is the fitted linear model with $R^2 = 0.84$.

Viable population of B91 initially declined before levelling out; even at six weeks after application, the viable population was still as high as 10^6 CFU (Fig. 4). There is an apparent cyclic pattern in the viable population size, which is difficult to explain.

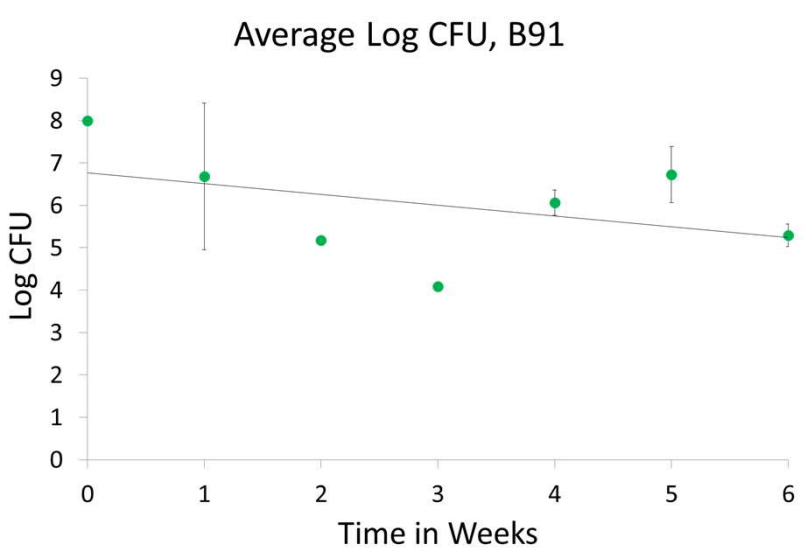

Figure 4. Estimated viable population (Log CFU) of B91 over time with a linear model only accounting for 19% of the observed variability.

References:

Nocker A., Cheung C., Camper A. (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. *Journal of Microbiological Methods*, 67:310-320

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