Project Title: Diagnostic system for apple diseases using a DNA array
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Quarterly report (4th)

Note: ***Due to the actual project start date (late March early April 2004) we are presenting this as a 4th quarter report and propose to continue the research and give a final report and presentation at the next meeting in May, 2005.***

Objectives:
1. Compare the sensitivity, specificity and reproducibility of DNA array, and Real-time PCR detection techniques for Powdery mildew & Fire blight.
2. Evaluate the usefulness of the techniques for monitoring and predictive purposes using fire blight and powdery mildew predictive models as a reference in comparison to traditional monitoring.
3. Evaluate relative cost effectiveness of the various techniques for commercial development including potential applications to micro-array and microchip technologies.

Real time PCR assay: Fire blight and Powdery mildew. Primers for the real time PCR test have been designed from the pEA29 plasmid (pEA29-F1 and pEA29-F3), and tested with the reverse primer AJ76 (McManus and Jones) in both a single amplification and a nested amplification protocol. With a single amplification protocol approximately 66% of all isolates were detected and with the nested amplification protocol, detection of Fire bight isolates increased to 90%. The nested real time PCR protocol requires an additional 2.5 hours to complete but would still allow for the detection and quantification analysis to be complete within one working day.

The primer pair PM-ITS / Un28S22 has been tested in a real time PCR assay and has detected *P. leucotricha* the apple mildew pathogen. Similar to the fire blight assay, environmental sampling of apple mildew with I-rod spore samplers required a nested PCR for detection with the real time PCR analysis.

The nested real time PCR allows for specific detection of both pathogens from environmental samples, but due to assay parameters, which cause software conflicts, sample quantification is not generated. Several changes to assay parameters are being investigated to clear up this problem.

DNA array hybridization: Fire blight and Powdery mildew. The DNA array membrane includes ribosomal DNA and pEA29 plasmid DNA probes which are specific for the Fire blight pathogen. The membrane also includes ribosomal probes for apple powdery mildew. The PCR intended for DNA array analysis, for both fire blight and apple mildew are nested reaction combining universally conserved outer primers, with internally nested species-selective primers. The apple mildew PCR targets the ribosomal DNA which corresponds to the rDNA probes on the array. The fire blight PCR is a multiplex reaction simultaneously amplifying ribosomal DNA and pEA29 plasmid DNA thereby targeting both DNA regions with specific probes incorporated on the array.

Field samples: Low levels of fire blight were detected throughout the growing season by both real time PCR and DNA array hybridization (Table 1). As mentioned above quantification was

not obtained with the (nested) real time PCR and therefore cell numbers for fire blight were given as being equal or grater than 2 x 10^0, or values were obtained from standard real time PCR runs conducted earlier. There was a general agreement between the real time PCR detection and the hybridization results. However on May 18th and 21st , real time PCR detected the pathogen while the array did not.

Results for the detection and quantification of apple powdery mildew using the DNA array are shown in Figure 1. Daily spore counts in the orchard ranged from zero to 2 x 10^4. Results for the real time PCR were not quantitative but detected an almost constant presence of the pathogen in the orchard (results not shown). Work will continue to overcome the problems surrounding the quantification issues with the real time assay.

Table 1. Summary of results comparing real time PCR vs. DNA array Hybridization for the detection of *E. amylovora* (fire blight). Shown are sample dates with positive reactions that indicate the presence of the pathogen. Also shown are results of a predictive model that indicates the pathogen potential for the corresponding dates.

	Model	Positive Nested Q-PCR				Positive Nested PCR/Hybridization			
Date	**Pathogen potential**	**Sample #**	**Sample type**	**Location**	**Estimated # of cells**	**Sample #**	**Sample type**	**Location**	**Estimated # of cells**
Apr 20	V. low	1	DM*	F9	2.8 x 10*1	1	DM*	-	2 x 10*1
Apr 23	V. low	3	DM*	F9	2.3 x 10*1	3	DM*	-	2 x 10*0
Apr 29	V. low	6	DM*	F9	≥ 10*0	6	DM*	-	2 x 10*1
May 6	V. low	9	DM*	F9	≥ 10*0	9	DM*	-	2 x 10*0
May 18	V. low	44	BL**	F20	≥ 10*0	44	BL**	-	0
May 20	V. low	12	DM*	F16	≥ 10*0	12	DM*	-	2 x 10*0
May 21	High	45	BL**	F20	≥ 10*0	45	BL**	-	0
June 5	High	-	-	-	No samples	-	-	-	No samples
June 7	High	-	-	-	No samples	-	-	-	No samples
June 24	High	62	L**	F20	≥ 10*0	62	L**	-	2 x 10*1

** DM (door mat) = siliconized plastic tape placed at the entrance to beehives and sampled daily for DNA extraction.*

*** BL (blooms); and L (leaves), randomly sampled from the orchard on a daily basis and used in DNA extractions.*

Figure 1. Apple mildew detection and quantification from an I-rod spore samplers using DNA array hybridization.