Smith, Brett and Frank N. Martin* USDA-ARS, 1636 East Alisal St., Salinas, CA 93905. **A real-time PCR and digital droplet PCR assay for quantification of *Polymyxa betae* in sugar beet roots.**

Polymyxa betae is an obligate pathogen capable of vectoring several viruses of sugar beet that can cause large losses in production. In the past, quantifying *P. betae* infection levels required time-consuming staining and visual examination using light microscopy. A new species-specific quantitative real-time PCR assay that allows for rapid quantification of *P. betae* infection levels in sugar beet roots has been developed. The assay has been designed to target the *P. betae* internal transcribed spacer (ITS) region of the genomic rDNA using sequence data from forty isolates. To achieve species-specificity, the assay primers and probe were designed against nineteen *Polymyxa graminis* ITS sequences from GenBank. In practice, the assay has demonstrated species-specificity in all fifteen *P. betae* isolates tested and has not amplified *P. graminis* isolates. Three internal plant controls, two targeting single copy nuclear genes and one targeting the ITS region of the rDNA, have been multiplexed with the pathogen assay to standardize quantification results across samples. Further, the assay has also been optimized for digital droplet PCR, which allows for more precise quantification of minor differences in infection levels than conventional real time PCR. These tools will allow for more efficient quantification of *P. betae* infection levels in experimentation evaluating host resistance, variation in aggressiveness among isolates and virus transmission studies.