Introduction

Angular leaf spot is an important disease of cultivated strawberry. The disease is transmitted primarily through systemically infected nursery stock. This creates problems for nurseries wishing to export plants to Europe because of quarantine restrictions. Currently, field inspections for symptoms are used to certify plants free of X. fragariae, but visual inspection is not useful for detecting plants infected systemically. To detect systemic infections, PCR is the desired tool because of its sensitivity, specificity, and ease of use.

In previous work, we developed three sets of real-time PCR primers and probes (q241, q245, q295) and determined optimal reaction conditions for use of these primers for the detection of X. fragariae in strawberry crown tissue. The objectives of this study were to: 1) Evaluate the performance of the three primer pairs with Receiver Operating Characteristic (ROC) curve analysis; and 2) Provide information for the selection of optimal cutoffs for each primer set.

Materials and Methods

Primer sensitivity and specificity: The sensitivities of the qPCR primer pairs were determined individually from 10-fold serial dilutions of 1) genomic DNA extracted from pure cultures of X. fragariae, 2) whole cell bacteria, and 3) DNA extracts from mixtures of strawberry crown tissue extract with whole cell bacteria. qPCR was performed on all dilution series to determine the sensitivity and specificity of the qPCR primer pairs. X. fragariae strains X1, X6, and X128 were used in all experiments. All reactions were performed in triplicate for each primer pair, and were run with a X. fragariae X13 positive control and a master-mix-negative control.

ROC curve analysis: The data were partitioned into 2 groups based on whether their AUC was less than an arbitrary cutpoint called the cutpoint, $T_{cp}$. By convention, the cutpoint is often set to 35.

The number of data sets classified as cases and controls, the Mann-Whitney U-statistic, the AUROC, its standard error and associated $z$-value, was dependent upon the primer pair and the tolerance threshold ($D_{thresh}$) selected. Ct values less than 35 are to be expected. These shortcomings may be resolved by selecting a cutpoint other than 35 and this can be done through ROC curve analysis.

Results & Conclusions

The introduction sets up the background for the study, explaining the importance of detecting strawberry diseases and the current methods used. The materials and methods section details the experimental procedures, including primer sensitivity and specificity tests and ROC curve analysis. The results section presents the data and analysis, and the conclusions summarize the findings, emphasizing the need for optimized cutpoints for qPCR primer sets.