CALIFORNIA CITRUS NURSERY BOARD
ANNUAL PROJECT REPORT

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This project is: Year 1 of two years.

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Project Title: Application of real time PCR for the detection of *Spiroplasma citri* in citrus nurseries and budwood trees.

Objectives: Optimize real-time PCR detection of *S. citri* and determine when infection can be detected following transmission.

1. Under greenhouse/screenhouse conditions, determine the number of days post inoculation when *S. citri* becomes detectable by real-time PCR.

Madam Vinous sweet orange plants were inoculated with 3-4 side grafts taken from *Spiroplasma citri*-infected sweet orange field trees. Inoculations were conducted in late winter (Feb. 22); spring (April 9); summer (July 25); and late summer (Aug. 15), 2008. No transmissions occurred in the late winter or spring inoculations; whereas 4 of 13 (31%) and 8 of 13 (62%) transmissions occurred with the summer and late summer inoculations, respectively. Real time PCR detected *S. citri* DNA after 63 days and 39 days post inoculation in the summer and late summer inoculations, respectively. This experiment is continuing.

2. Under field conditions using young and mature sweet orange trees with known *S. citri* infections, determine the seasonal within tree distribution and relative duration of *S. citri* detection by real-time PCR.

Twenty-year-old Thompson Improved (TI) Navel on Carrizo rootstock, infected with *S. citri* was selected to examine titer of *S. citri* within the tree canopy or with respect to symptom severity. The average *S. citri* titer in severely symptomatic trees ($7.1 \times 10^3$ cells/mg) was statistically higher than that in mildly symptomatic trees (1.2 cells/mg.) No statistical differences were related to tree canopy tier (base, middle or top). Furthermore, symptoms in severely symptomatic trees were more pronounced in the western or afternoon sunny side of the tree canopy compared to that of the eastern

canopy. Cycle threshold (Ct) values of *S. citri* DNA extracted from fruit columellae collected from the western tree canopy had a *S. citri* concentration of $11.1 \times 10^3$ cells/mg compared to those collected from the eastern side of 3.2 cells/mg. Therefore, samples for *S. citri* detection should be collected from the most symptomatic tissue and/or from the afternoon sunny side of the tree.

Other findings:

1. Comparison of fruit columellae vs leaf midrib as DNA source for PCR detection of *S. citri*.

Real time PCR detected *S. citri* in a total of 204 of 768 trees sampled from the Ducor field plot. One hundred forty seven of these positive trees were positive with both midrib and columellae tissue (72%); whereas 37 trees (18.1%) and 20 trees (9.8%) were positive with only leaf midrib or fruit columellae samples, respectively. Overall, midrib samples led to detection in 90.2% of the trees known to be *S. citri* positive; whereas the columellae samples yielded amplicons in only 81.9% of the known positives. Thus, leaf midrib tissue can be used for reliable detection of *S. citri* infection in field trees by PCR.

2. Is the incidence of *S. citri* increasing in some citrus orchards in the San Joaquin Valley?

A 40 A grove of Spring Navel on Carrizo rootstock, ~20 years of age, was selected for this study. This orchard is west of Ducor in Tulare Co. Samples were taken from three plots, each of 256 trees (16 rows x 16 trees). In 2007, the number of *S. citri* positive trees were 44, 53 and 74 (171 total) in plots 1, 2 and 3, respectively. In 2008, the number of *S. citri* positive trees were 46, 62 and 95 (203 total), in plots 1, 2 and 3, respectively. Therefore, 32 new trees were found in one year in these plots. The incidence in 2007 was 22.2%; whereas in 2008, it was 26.4%. This 4% increase could be explained by in field spread of *S. citri*; or by an increase in *S. citri* titer in infected trees from non-detectable (in 2007) to detectable levels (in 2008). This study is continuing.

Summary

- Graft transmission of *S. citri* from field sources was successful only when inoculum was obtained in summer. Optimum transmission occurred when inoculum was collected in August. This could be due to the possibility that spiroplasmas are believed to move into the root tissues in winter and redistribute in aerial plant parts when temperatures increase.
- Real time PCR detected transmission in as little as 39 days post inoculation.
- Higher distribution or titer of *S. citri* was found in severely symptomatic sectors of *S. citri*-infected trees. Therefore, samples from field trees should be collected from the most symptomatic canopy sector which is likely to be on the afternoon sunny side of the tree.
- Leaf midrib tissue was found to be slightly better than fruit columellae as DNA sources for *S. citri* detection in field trees.

- A 4% increase in *S. citri*-infected trees was found in a mature Navel orange plot near Ducor, CA and suggested some natural spread of citrus stubborn disease is occurring or that pathogen titer finally increased to detectable levels.