THE GENETICS OF RESISTANCE TO PIERCE'S DISEASE

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INTRODUCTION

This project is part of the American Vineyard Foundation Long Term Project on Pierce's Disease. My component of this project focuses on understanding the genetics of resistance to *Xylella fastidiosa*, the causal agent of Pierce's disease (PD).

OBJECTIVES

1. Complete analysis of a series of crosses (Design II mating scheme) allowing the quantitative inheritance of *X. fastidiosa* resistance to be evaluated.
2. Complete a genetic map of a *Vitis rupestris* x *Muscadinia rotundifolia* seedling population using AFLP (amplified fragment length polymorphism) markers to allow the identification of DNA markers to *X. fastidiosa* resistance and eventual identification of *X. fastidiosa* resistance genes and their genetic engineering into vinifera cultivars.
3. Utilize genetic markers to *X. fastidiosa* resistance to accelerate the introgression of *X. fastidiosa* resistance into table, raisin and wine grapes.
4. Develop DNA markers from resistance sources other than *M. rotundifolia* for use in breeding table, raisin and wine grapes through the development of additional mapping populations and the bulk segregant analysis of DNA markers.

RESULTS AND CONCLUSIONS

Accomplishments to date have occurred in the following areas:

A. ***Inheritance studies of Xylella fastidiosa resistance in a Muscadinia rotundifolia background.*** Alan Krivanek, a PhD student, is studying the inheritance of *X. fastidiosa* resistance in *M. rotundifolia*. He has completed a broad series of crosses among 12 siblings from an F1 population of *Vitis rupestris* x *M. rotundifolia*, with 6 males crossed to each of 6 females. He will test a 4 x 4 mating scheme and evaluate a portion of the seedlings from each of the 16 possible seedling populations for resistance to *X. fastidiosa* by using needle inoculation followed by symptom evaluation and ELISA to determine the extent of *X. fastidiosa* movement. The results of this research will give estimates for the number of genes involved in resistance. Evaluation of the seedling populations has begun and will be completed over the coming year.

B. ***Defining Xylella fastidiosa resistance.*** We are defining *X. fastidiosa* resistance as the ability of a genotype to limit the movement of *X. fastidiosa*, particularly in a downward direction. A set of resistant and susceptible individuals to determine whether lack of movement is a valid resistance indicator has been tested. These tests used known susceptible, potentially resistant and known resistant genotypes. The known susceptible genotypes were *V. rupestris* 'A. de Serres' (the female parent of the 89 population), Chardonnay and the *V. rupestris* x *M rotundifolia* genotype 8909-19. The potentially resistant genotypes were 8909-04 and 8909-11 and the resistant genotypes were 8909-15 and 8909-17. A time course study with 4 replicates of these genotypes was sampled over 4, 8 and 16 weeks using greenhouse grown potted vines. The presence of *X. fastidiosa* was determined with optimized ELISA (see below) at the point of inoculation (poi), 10 cm above and below, and 20 cm above and below the poi. To allow quantification of the ELISA reading a three-fold dilution of *X. fastidiosa* in healthy plant sap – 1.8×10^6 cfu/ml; 1.8×10^5 cfu/ml and 1.8×10^4 cfu/ml was used. The ELISA reading threshold for a positive reaction was 0.100 at 450 OD, which corresponds to about 10,000 cfu/ml.

After 4 weeks, *X. fastidiosa* was easily detectable in the three susceptible genotypes. There was limited upward movement at this time, although movement seems greatest in 8909-19. At 8 weeks after inoculation downward movement in the susceptible genotypes was easily detected at 10 cm below the point of inoculation, and *X. fastidiosa* seemed to move most readily in *V. rupestris* 'A. de Serres'. By 16 weeks the differences among resistant and susceptible genotypes are very clear in terms of both symptom expression in leaves and unevenly lignified stems and ELISA readings. All three susceptible genotypes had mean ELISA values well above the OD 0.1 or 10,000 cfu/ml threshold at positions both above and below the point of inoculation. We are evaluating resistance after 16 weeks.

C. ***Optimizing detection of X. fastidiosa to better define resistance.*** We have optimized the sensitivity and reproducibility of ELISA for *X. fastidiosa* detection and can now reliably detect 10,000 cfu/ml of ground plant sap in 150 samples with duplicate readings, over the course of one day and at a cost of $0.28 per sample. Our definition of resistance depends upon a very accurate measure of the extent to which *X. fastidiosa* moves in the stem. We are using various PCR techniques (standard, IC-, and quantitative) to best evaluate movement. These efforts are being spearheaded by Nihal Buzkan, (post-doc). We are working towards a blot hybridization technique that will allow sensitive, rapid and inexpensive evaluation of movement in the hundreds of seedlings we must process. Nested IC-PCR techniques are able to detect 10 –20 cfu/ml of plant sap. However, the cost is 10X higher than ELISA and the throughput is 10X lower.

D. ***Developing a genetic map and markers for X. fastidiosa resistance.*** We are now finalizing a genetic map created from a cross of two *V. rupestris* x *M. rotundifolia* siblings (8909-15 x 8909-17). This map was initiated about 2 years ago and is now being completed with the addition of about 200 AFLP, SSR and ISSR markers, bring the total number of markers to about 500. This map was constructed to develop markers for resistance to the dagger nematode (*Xiphinema index*) and to enable the identification of the gene responsible for this resistance. Alan Krivanek tested about 50 individuals from the 8909 and 8913 populations and found strong resistance to *X. fastidiosa* in several including 8909-08, 89-15 and 8909-17. He was also able to document a wide range of responses from very susceptible to very resistant. Although the 8909-15 x 8909-17 seedling population is a cross of two resistant individuals, its progeny segregate widely for *X. fastidiosa* resistance. About 120 members of this sibling generated F2 have been evaluated for *X. fastidiosa* resistance and the character will be soon be mapped. Previous examinations of X. fastidiosa resistance in southeastern US grape species have found that at lest three genes are involved, and preliminary results from Alan also suggest that more than one gene is involved in *X. fastidiosa* resistance from *M. rotundifolia*. However, it may be possible to find markers very tightly associated with *X. fastidiosa* resistance, and preliminary analysis found that *X. fastidiosa* resistance does map in the 8909-15 x 809-17 population.

We are also examining several other populations that contain *X. fastidiosa* resistance from *V. rupestris* x *M. rotundifolia* selections. Alan Krivanek will be testing a population from 8909-15 x B90-116 (a larger berried seedless *V. vinifera* table grape from David Ramming) and then use Bulked Segregant Analysis (BSA) and AFLP markers to develop DNA markers for resistance. These will be compared to resistance markers developed with the mapping population. Resistance markers from the 8909-15 x B90-116 population may be easier to develop because of the much greater genetic difference between the two parents than in the mapping population and therefore greater segregation and genetic polymorphism. Shannon Reis, MS student, will also be using BSA to examine 8909-08 (another *V. rupestris* x *M. rotundifolia X. fastidiosa* resistant selection) x three Ramming *V. vinifera* seedless grapes. Her study will verify the existence and utility of the markers for *X. fastidiosa* resistance.