

BREEDING PIERCE'S DISEASE RESISTANT TABLE AND RAISIN GRAPES

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INTRODUCTION

This project is jointly funded through the California Table Grape Commission and the California Raisin Marketing Board and the CDFA/APHIS Pierce's disease program. It is a collaborative effort between UC Davis and the USDA/ARS-Fresno, and is focused on breeding new PD resistant cultivars of table and raisin grapes. This project also builds off the Walker lab portion of the AVF Long Range Project on PD, which focuses on identification of DNA markers for *Xylella fastidiosa* resistance and the mapping of *X. fastidiosa* resistance leading to identification of resistance genes. Success in the AVF PD project will expedite progress in PD resistant table/raisin grape breeding by accelerating the selection process, fine-tuning parent selection, and allowing future resistance gene transformation efforts.

OBJECTIVE

Develop Pierce's disease resistant table and raisin grapes by crossing a variety of *Xylella fastidiosa* resistance sources with large berried and seedless *V. vinifera* table and raisin grapes.

RESULTS AND CONCLUSIONS

In 2000, we began a two-pronged approach for introducing PD resistance into our crosses. First resistance derived from *Muscadinia rotundifolia* via *V. rupestris* x *M. rotundifolia* selections that greatly suppress *X. fastidiosa* development. The second approach utilizes resistance derived from field resistant *Vitis* cultivars from various historic breeding programs in the Southeastern United States (SEUS). Fruit quality is better in these resistant sources, but still needs improving to meet commercially acceptable quality in California. We now have about 50 PD resistant cultivars at UCD and 13 at USDA-Fresno for use as breeding parents. There are also additional selections with high powdery mildew resistance. Fruit quality is coming from advanced seedless tablegrape genotypes at the USDA-Fresno.

Last year, about 180 embryo rescue seedlings resulted from crosses of a male *V. rupestris* x *M. rotundifolia* resistance source (8909-08) with advanced tablegrape genotypes; these were done at the USDA-Fresno. The seedlings were planted in Fresno and copies were given to UCD for *X. fastidiosa* resistance screening. To date, more than 100 of these have been screened for *X. fastidiosa* resistance. Genotypes that do not allow appreciable *X. fastidiosa* movement have been identified. Currently the Bulk Segregant Analysis (BSA) technique is being used to develop a genetic marker for this source of resistance. This marker will dramatically speed our work in selecting resistant seedlings.

Similarly, in mid-summer of this year, copies were made from about 130 seedlings in the UCD vineyard resulting from the 2000 cross of an advanced table grape cultivar onto a female *V. rupestris* x *M. rotundifolia* resistance source (8909-15). This population is also being screened and the BSA technique utilized to develop a genetic marker for resistance. Seedlings from both the male and female resistance source are expected to fruit in 2002 and crosses will be made onto them.

In the past year, over 35 of the SEUS field PD - resistant *Vitis* cultivars and accessions were subjected to the greenhouse PD screen. All were found to harbor ELISA – detectable levels of *X. fastidiosa* after two months of plant growth. A wide range of PD levels in this field resistant material were observed: from barely detectable, to levels equal to susceptible *vinifera* cultivars. Until a better definition of resistance is developed, we will use parents that allow the lowest levels of *X. fastidiosa* development. We have also established a field trial in a PD hotspot in northern California using 13 different SEUS genotypes that allow various *X. fastidiosa* titers in their xylem. Nine resistant and seven susceptible selections from the *V. rupestris* x *M. rotundifolia* population were also included.

In Spring 2000, 1,667 seedlings from PD crosses made using our two strategies were planted at UCD. Plants were derived from both embryo rescue and from seed. From the *V. rupestris* x *M. rotundifolia* resistance source strategy, 5 advanced seedless tablegrape selections were crossed onto a resistant female and a resistant male was crossed unto one seeded and one seedless tablegrape cultivar with 606 seedlings planted to the field. Within the *Vitis* source resistance strategy, 16 different female sources of resistance were used and 7 different male sources resulting in 43 different cross combinations and 1061 seedlings planted to the field. This wide diversity was chosen to allow for multiple trait selection as well as likely alternative PD defense mechanisms, and insure a broad base to the breeding program. Vineyard establishment of these plants has been excellent and most are large enough to fruit in 2002. Seedlings were also field established at the USDA-Fresno. These include the above mentioned embryo rescued seedlings (about 180), and 439 seedlings (pre-screened for powdery mildew resistance) from crosses of two *Vitis* resistance sources and one *V. rupestris* x *M. rotundifolia* selection.

2001 Crosses

At UCD, 6 different resistant females were crossed with 9 different advanced tablegrape selections creating 24 different cross combinations and yielding more than 6,100 seeds. Seven clusters of crosses of an advanced tablegrape female by resistant SEUS males and 3 shipments of resistant pollen from a number of the SEUS resistant vines were sent to USDA-Fresno for their crosses.

At USDA-Fresno, 16 resistant males were crossed to 8 seedless tablegrape selections to continue the embryo rescue Xf resistant program. These efforts produced 1,186 embryos. Ten PD resistant males onto 3 seeded tablegrape females to incorporate large berry size with PD resistance, and produced about 500 seeds.

VIRULENCE ANALYSIS OF THE PIERCE'S DISEASE AGENT *XYLELLA FASTIDIOSA*

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INTRODUCTION

The glassy-winged sharpshooter, *Homolodisca coagulata*, spreads the causative agent of Pierce's disease, the bacterium *Xylella fastidiosa* (*Xf*). Depending on whether the glassy-winged sharpshooter can establish itself in Northern California, Pierce's disease may represent a multi-billion dollar threat to the grape and wine industry and the associated tourist trade. The symptoms of Pierce's disease include a yellowing and gradual necrosis (scorching) of grapevine leaf edges, stunting of cane growth, and, particularly in the spring for vines infected for two or more seasons, inter-veinal chlorosis of leaves (Hewitt, 1970). (Lee et al., 1982) reported that detached grape leaves from grape cultivars that are particularly sensitive to Pierce's disease, showed typical marginal yellowing and scorching in less than 12 hr after petiole uptake of cell-free washing from *Xf* cells grown on agar plates. The activity did not survive autoclaving or multiple freezing and thawing and was not inactivated by incubation with proteinase K. No activity was observed for washings of uninoculated agar plates.

Applying the conditions of (Lee et al., 1982), (Goodwin et al., 1988) also observed phytotoxicity after petiole uptake of cell-free washing, but from both *Xf*-populated and uninoculated agar plates. Further, (Goodwin et al., 1988) found a correlation between increased midday stomatal resistance and symptom development on leaf margins and an approximately six-fold increase in leaf proline content (fresh-weight basis) associated with Pierce's disease. They discounted phytotoxins as significant contributors to Pierce's disease symptoms and state that "The biophysical and biochemical changes observed for diseased vines indicate that marginal leaf necrosis occurs when water stress develops. Diseased leaves are apparently water stressed because of vascular dysfunction which, when prolonged, may result in accelerated leaf senescence." (Goodwin et al., 1988) also found that higher stomatal resistance was associated with spring inter-veinal chlorosis but was not as pronounced as the stomatal resistance increase observed for leaves showing symptoms first at the margin.

OBJECTIVE

The objective of this project is to identify gene product(s) and gene(s) of *Xf* that contribute to its virulence.

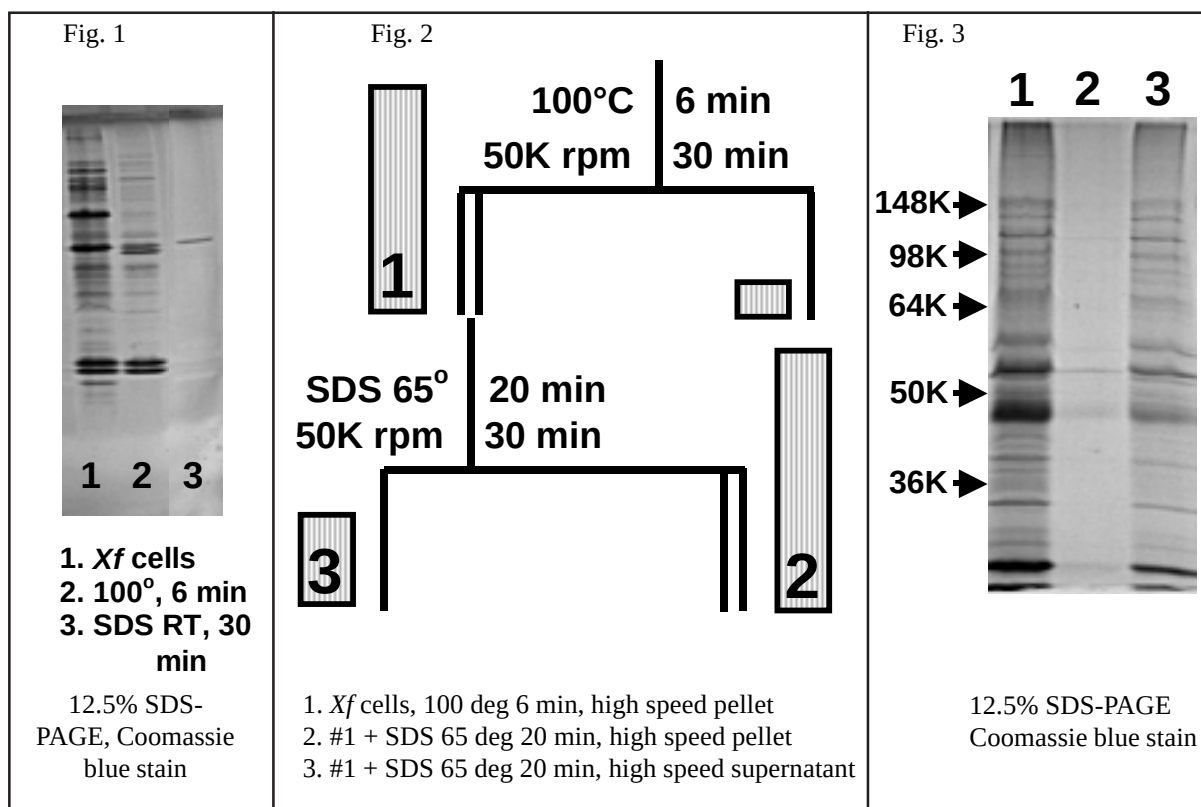
RESULTS AND CONCLUSIONS

Experimental infection of grape to induce disease generally requires weeks or months to the appearance of symptoms. We wondered whether other plants might exhibit more rapid symptom development upon exposure to *Xf*, particularly to *Xf* infiltrated directly into the leaf lamella. A survey of plant species and lines commonly used as experimental hosts for plant pathogens revealed that *Chenopodium quinoa* (Cq) developed chlorosis corresponding to the infiltrated area within 48 hr of infiltration of 10^8 to 10^6 cfu/ml suspensions of *Xf* cells. Light microscope cytological studies carried out by Prof. Judy Jernstedt, UC Davis Agronomy and Range Sciences Department, revealed that the observed chlorosis of Cq leaves infiltrated with *Xf* is the result of chlorophyll loss from chloroplasts in all photosynthetic cell types without other observable effect. Xanthomonads are considered to be closely related to *Xylella* spp., and *Xanthomonas campestris* pv. *Vesicatoria* and some other, but not all, *Xanthomonas* spp. induce a similar chlorosis in Cq leaves. *Xanthomonas campestris* pv. *campestris* induced necrosis 3-4 days after infiltration. Currently we are testing the effects of petiole-feeding of grape leaves with *Xf* and fractions (see below) derived from *Xf*.

Chlorosis development varied significantly from leaf to leaf on a Cq plant and from plant to plant. However, comparisons of infiltrated opposite leaf halves appear to provide valid measures of the relative potency of *Xf*-derived preparations and forms the basis for a semi-quantitative assay used here. *Xf* cells from liquid culture had a slightly greater specific chlorosis-inducing (CI) activity than *Xf* cells from agar plates. However, the yield of *Xf* cells from plates was greater, and results

reported here are from plate-derived *Xf* cells. The CI activity was associated with cells, not washings of cells. Heating *Xf* cells at 100°C for 6 min slightly enhanced the CI activity. Treatment with sodium dodecyl sulfate (SDS) at 25°C or 65°C did not destroy the CI activity. Most proteins will not survive in active form after treatment at high temperatures or with SDS, but the CI activity was greatly reduced or destroyed by incubation with protease K, trypsin or chymotrypsin. Treatment with acetic acid or chloroform also was inactivating, but incubation with periodate, lysozyme, or ethanol did not reduce CI activity.

The results described above are consistent with an essential role for an unusually stable protein in whatever constitutes CI activity. Fig. 1 presents an analysis of proteins collected as a precipitate after treatment of *Xf* cells at 100°C or with SDS at 25°C and centrifugation at 50,000 rpm for 30 min. Fig. 2 is a flow chart showing treatment at 100°C and collection of insoluble material, which then was treated with SDS at 65°C. A single vertical line represents supernatant; a double vertical line represents precipitate. Bars show the relative CI activity of four fractions. Even after treatment with SDS at elevated temperature, most of the material remained insoluble. However, exposure to SDS and mercaptoethanol solubilized proteins for gel electrophoresis. As indicated by Fig. 1 and Fig. 3, elevated temperature and SDS removed the bulk of the protein without destruction of CI activity. Lane numbers in Fig. 3 correspond to sample numbers in Fig. 2. Current effort is aimed at correlating CI activity with a specific protein band(s), identification of the corresponding protein, with aid of *Xf* genome sequences (Simpson et al., 2000), and testing of *Xf*-derived, CI fractions for their effects on grape.



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