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Field Evaluation of DSF-producing grape for control of Pierce's disease

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Abstract:
A cell density-dependent gene expression system in *X. fastidiosa* (*Xf*) mediated by a small signal molecule called diffusible signal factor (DSF) which we have now characterized as 2-Z-tetradecenoic acid (hereafter called C14-cis) controls the behavior of *Xf*. The accumulation of DSF attenuates the virulence of *Xf* by stimulating the expression of cell surface adhesins such as HxfA, HxfB, Xada, and fimA (that make cells sticky and hence suppress its movement in the plant) while down-regulating the production of secreted enzymes such as polygalacturonase and endogluconase which are required for digestion of pits and thus for movement through the plant. Artificially increasing DSF levels in plants in various ways increases the resistance of these plants to Pierce's disease. Disease control in the greenhouse can be conferred by production of DSF in transgenic plants expressing the gene for the DSF synthase from Xf; such plants exhibit high levels of disease resistance when used as scions and confer at least partial control of disease when used as rootstocks. This project is designed to test the robustness of disease control by pathogen confusion under field conditions where plants will be exposed to realistic conditions in the field and especially under conditions of natural inoculation with insect vectors. We are testing two different lineages of DSF-producing plants both as own-rooted plants as well as rootstocks for susceptible grape varieties in two field sites. Plants were established in one field site in Solano County on August 2, 2010. Plants were planted at a Riverside County site on April 26, 2011. The plants established in the Solano County site have grown well and were in general in excess of 2 to 3 meters in length by July, 2011. All plants at the Solano County experimental site were needle-inoculated with a suspension of *X. fastidiosa* on July 22, 2011. From one to 4 vines per plant were inoculated, each at a given site with a 20 ul droplet of *X. fastidiosa* containing about 20,000 cells of *X. fastidiosa*. Disease severity and population size of the pathogen will be assessed in the plants as a means of determining their susceptibility to Pierce's disease.

Layperson Summary:
Xf coordinates its behavior in plants in a cell density-dependent fashion using a diffusible signal molecule (DSF) which acts to suppress its virulence in plants. Artificially increasing DSF levels in grape by introducing the *rpfF* gene which encodes a DSF synthase reduces disease severity in greenhouse trials. We are testing two different lineages of DSF-producing plants both as own-rooted plants as well as rootstocks for susceptible grape varieties. Disease severity and population size of the pathogen will be assessed in the plants as a means of determining their susceptibility to Pierce's disease.

Introduction:
Our work has shown that *Xf* uses DSF perception as a key trigger to change its behavior within plants. Under most conditions DSF levels in plants are low since cells are found in relatively small clusters, and hence cells do not express adhesins that would hinder their movement through the plant (but which are required for vector acquisition) but actively express extracellular enzymes and retractile pili needed for movement through the plant. Disease control can be conferred by elevating DSF levels in grape to "trick" the pathogen into transitioning into the non-mobile form that is normally found only in highly colonized vessels. While we have demonstrated the principles of disease control by so-called "pathogen confusion" in the greenhouse, more work is needed to understand how well this will translate into disease control under field conditions. That is, the methods of inoculation of plants in the greenhouse may be considered quite aggressive compared to the low levels of inoculum that might be delivered by insect vectors.

Likewise, plants in the greenhouse have undetermined levels of stress that might contribute to Pierce's disease symptoms compared to that in the field. Thus we need to test the relative susceptibility of DSF-producing plants in the field both under conditions where they will be inoculated with the pathogen as well as received "natural" inoculation with infested sharpshooter vectors. We also have recently developed several new sensitive biosensors that enable us to measure *Xf* DSF both in culture and within plants. We could gain considerable insight into the process of disease control by assessing the levels of DSF produced by transgenic *rpfF*-transformed grape under field conditions.

Objectives:

1) Determine the susceptibility of DSF-producing grape as own-rooted plants as well as rootstocks for susceptible grape varieties for Pierce's disease.
2) Determine population size of the pathogen in DSF-producing plants under field conditions.
3) Determine the levels of DSF in transgenic *rpfF*-expressing grape under field conditions as a means of determining their susceptibility to Pierce's disease.

Results and Discussion:

Disease susceptibility of transgenic DSF-producing grape in field trials.

Field tests are being performed with two different genetic constructs of the *rpfF* gene in grape and assessed in two different plant contexts. The *rpfF* has been introduced into Freedom (a rootstock variety) in a way that does not cause it to be directed to any subcellular location (non-targeted). The *rpfF* gene has also been modified to harbor a 5' sequence encoding the leader peptide introduced into grape (Thompson seedless) as a translational fusion protein with a small peptide sequence from RUBISCO that presumably causes this RpfF fusion gene product to be directed to the chloroplast where it presumably has more access to the fatty acid substrates that are required for DSF synthesis (chloroplast-targeted). These two transgenic grape varieties are thus being tested as both own-rooted plants as well as rootstocks to which susceptible grape varieties will be grafted. The following treatments are thus being examined in field trials:

Treatment 1	Non-targeted RpfF Freedom
Treatment 2	Chloroplast-targeted RpfF Thompson
Treatment 3	Non-targeted RpfF Freedom as rootstock with normal Thompson scion
Treatment 4	Chloroplast-targeted RpfF Thompson as rootstock with normal Thompson scion
Treatment 5	Normal Freedom rootstock with normal Thompson scion
Treatment 6	Normal Thompson rootstock with normal Thompson scion
Treatment 7	Normal Freedom
Treatment 8	Normal Thompson

Treatments 5-8 serve as appropriate control to allow direct assessment of the effect of DSF expression on disease in own rooted plants as well as to account for the effects of grafting per se on disease susceptibility of the scions grafted onto DSF-producing rootstocks.

One field trial was established in Solano County on August 2, 2010. Twelve plants of each treatment were established in randomized complete block design. Self-rooted plants were produced by rooting of cuttings (about 3 cm long) from mature vines of plants grown in the greenhouse at UC Berkeley. Cuttings were placed in a sand/perlite/peatmoss mixture and subjected to frequent misting for about 4 weeks, after which point roots of about 10 appeared. Plants were then be transferred to 1 gallon pots and propagated to a height of about 1 m before transplanting into the field. Grafted plants were produced in a similar manner. 20 cm stem segments from a susceptible grape variety were grafted onto 20 cm segments of an appropriate rootstock variety and the graft union wrapped with grafting tape. The distal end of the rootstock variety (harboring the grafted scion) was then be placed in rooting soil mix and tooted as described above. After emergence of roots, the grafted plant were then transplanted and grown to a size of about 1 m as above before transplanting into the field site.

The plants have all survived and are growing well (Figure 1). The plants were too small to inoculate in the 2010 growing season and hence were inoculated on July 26, 2011 (no natural inoculum of *X. fastidiosa* occurs in this plot area and so manual inoculation of the vines with the pathogen will be performed. The plants established in the Solano County site have grown well and were, in general, in excess of 2 to 3 meters in length by July, 2011 (Figure 2). All plants at the Solano County experimental site were needle-inoculated with a suspension of *X. fastidiosa*. From one to 4 vines per plant were inoculated, depending on the size and number of vines per plant. Each inoculation site received a 20 ul droplet of *X. fastidiosa* containing about 20,000 cells of *X. fastidiosa* (Figure 3). Because researchers from both UC-Berkeley and UC-Davis will be contributing treatment to each plot, and since the

Figure 1. Overview of research plot in which DSF-producing plants are established (top). Close-up of transgenic Freedom vines in mid-September (bottom).

controls for some researchers will be the same, some control plants are being shared between research groups. All plants at UC-Davis were inoculated by needle puncture through drops of a common inoculum source of *X. fastidisoa* of about 10^6 cells/ml as in previous studies.

Some of the pants needed to establish the trial at Riverside county were damaged in the greenhouse at UC Berkeley in 2010 due to pesticide applications, and since plants from other researchers at UC Davis were also not ready for transplanting to the field in 2010, a decision was made to establish all of the plants from the UCB and UCD research groups together in early 2011. The plants for the Riverside County have now been generated and were transferred to a lath house at UC Davis on March 23, 2011 to harden off for about 3 weeks. The plants were then transported to Riverside County for establishment in the field experiment together with plants from researchers at UC Davis and were planted on April 26, 2011 (Figure 4). The plants at the Riverside County trial will not be artificially inoculated, but instead will be subjected to natural infection from infested sharpshooter vectors having access to *X. fastidiosa* from surrounding infected grape vines. Disease symptoms will be measured bi-weekly starting at 8 weeks after inoculation at the Solano County trial, or about 10 weeks after transplanting into the field site at Riverside County. Leaves exhibiting scorching symptoms characteristic of Pierce's disease will be counted on each occasion, and the number of infected leaves for each vine noted. ANOVA will be employed to determine differences in severity of disease (quantified as the number of infected leaves per vine) that are associated with treatment.

Conclusions:

The transgenic plants have been successfully established at two field sites in California. The first disease assessments will be made in late-summer, 2011. Since substantial disease control has been observed in these plants in the greenhouse, these tests should provide a direct assessment of the utility of such transgenic plants for disease control in the field.

Funding Agency:

Funding for the project described here was provided by the Pierce's Disease and Glassy-winged Sharpshooter Board.

Figure 2. Images of Thompson Seedless grape (left) and Freedom grape (right) at the Solano County field trial in July 2011.

Figure 3. Process of inoculation of grape at the Solano County field trial in July, 2011. A needle was inserted through a vine and a droplet of inoculum applied to the needle tip. After withdrawal of the needle, the bacterial inoculum is drawn into the vine due to the tension of the water in the xylem vessels.

Figure 4. Establishment of grape trial in Riverside County.