

RISK ASSESSMENT RESEARCH FOR THE PD PROJECT

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Pierce's disease (PD) is described by Dr. Lampe in an earlier session. It is a disease of grapevines caused by a specific strain of the bacterium, *Xylella fastidiosa*, a xylem-limited bacterium that is acquired by xylem-feeding leafhoppers called sharpshooters carried in the pre-cibarium of the mouthparts/foregut and is transmitted readily as the sharpshooters move and feed. The PD strain of *X. fastidiosa* has no effect on any other plant so far as we know. PD is endemic in the southeastern USA and northeastern Mexico where the pathogen appears to have co-evolved with the glassy-winged sharpshooter, *Homalodisca coagulata* (GWSS). Although the pathogen has been present in California for many years, native sharpshooters are not capable of supporting epidemics. This changed with the arrival of GWSS. We do not know when it arrived, but I noticed large numbers of GWSS feeding on yucca in my yard in Riverside in 1988.

The first hint of trouble from GWSS occurred when the insect began transmitting a strain of *X. fastidiosa* to oleanders causing an epidemic of oleander leaf scorch in southern California. This was followed by an outbreak of PD in vineyards in the winegrowing area of Temecula at the end of the 1990s. Soon growers and their bankers in Temecula generated emergency industry, federal and state funding for control and a cure.

If there was a cure for PD, there could be a thriving winegrowing industry in Florida and Texas. In fact, Texas has a struggling wine industry north of San Antonio on the fringe of the pathogen and GWSS distributions and Florida has a very modest effort.

The PD in California is now controlled by an expensive chemical treatment program and an equally expensive and inconvenient quarantine program aimed at the large local nursery industry with active inspection and chemical treatment protocols for nursery stock that is shipped from southern California. Both measures target GWSS, which has so far been contained south of Fresno. We have learned that citrus near vineyards is the highest risk condition for infesting vineyards and spreading the pathogen causing PD in the spring; therefore, the control program treats citrus to protect vineyards. The citrus and grape industries have to get along to make this work and the treatments have to be compatible with the outstanding biological control programs in place in California, but one can't see this going on for a protracted period of time. Eradication of GWSS is not considered feasible in the foreseeable future. On top of this, we do not know exactly what causes PD in the grapevine, nor the exact method of transmission other than physical. Knowing the entire genome of *Xylella* hasn't provided a control method.

Since a solution is demanded, we proposed symbiotic control. This requires new technology never tried before, which is why we require interaction with regulatory officials. Symbiotic control targets the act of transmission of the pathogen by the sharpshooter in a form of competitive displacement. We identified a preliminary symbiont to test, *Alcaligenes xylosoxidans denitrificans*, (*Axd*) which has some of the necessary properties. It was isolated from the pre-cibarium of GWSS, where it attaches alongside and has access to the pathogen. It is a xylem-limited bacterium, again has access to the pathogen. It is smaller than the pathogen, fully 1/10 the physical size of PD-*Xylella*. This attracted enough USDA-APHIS funding to start a 4-member team.

Dave Lampe provided the first genetically transformed *Axd* with *egfp* and then *DsRed* marker genes. Dave also found an antibody that seemed to recognize the coat protein of the pathogen, S1. We tested a phage-antibody version of this antibody and found that it disrupted the transmission of the pathogen by GWSS. At this time I applied for a permit to do field trials with support from the California grape and wine industry; past experience suggested the sooner the better. It took a while to learn that EPA was the regulatory body; it took EPA a while to figure out which work group was responsible, but they eventually read that *Axd* was found in nosocomial infections in lungs of patients. After a meeting of the EPA SAPs, severe restrictions

were placed on field tests (we had to burn the grapevines at the end of the test). This restriction was not compatible with the BL-1 level of laboratory handling approved by the UCR BioSafety committee. It was also non-negotiable (there was no mechanism to explain the ruling nor to appeal). Bill Schneider warned that *Axd* could well suffer the same fate as *Burkholderia cepacia*, which was dropped from developed due to similar SAP concerns.

This ruling affected what research we did from then on. I asked graduate student Jennifer Parker to do comparative nucleotide identification (a logical step anyway). That cost about 2 man-years. One third of our entire research effort went into identifying the fate of *RAxd* in grapevine, soil and the entire team was distracted by questions of making *Axd* safe rather than making it work to prevent PD. Dave Lampe was distracted from making an *Axd* version that had both the marker gene and his S1 antibody (so-called S1-*RAxd*). Ravi Durvasula then asked Lampe and I to join him in a CSREES-BRAG grant proposal to address risk assessment issues. We obtained a high rating and then the agency asked us to cut the request in half so they could fund 20% of the applications in that round. This allowed an abbreviated look at horizontal gene movement and design of *Axd* versions that would self-destruct (and presumably be more acceptable to EPA) and took us further still away from PD.

Our consultant, Frank Richards, inventor of the symbiotic control strategy for Chagas disease, then devoted considerable time trying to get workers in the cystic fibrosis field to consider the use of *Axd* as a delivery vehicle to clear lung infections of pathogens. His calls to the CF Foundation were not returned and he had to abandon the effort. This proposed new use of symbiotic control still hangs in the air.

In the end we learned that *RAxd* disappeared quickly from commercial grapevines and would not colonize soil unless the soil was first sterilized (in other words our *RAxd* was not competitive). We suspected that our *Axd* was not the same as the one found in fluids in hospitals because of the extreme differences in niches occupied. We will almost certainly pick another symbiont to deliver the anti-PD strategy for ecological reasons, which means the entire interaction with EPA was necessary only for the experience. One thing was clear, no hazard from *Axd* was every identified, which resonates with UCR BioSafety BL-1 rating. And EPA has a better idea how to deal with symbiotic control, at least this application.

References: Miller, T.A., D.J. Lampe and C.R. Lauzon. 2007. Transgenic and paratransgenic insects in crop protection. In: *Insecticide Design Using Advanced Technologies*, edited by: I. Ishaaya, R. Nauen and R. Horowitz, Springer-Verlag, Heidelberg, Germany (in press). Miller, T.A., C.R. Lauzon, D.J. Lampe, R. Durvasula and C. Matthews. 2006. Paratransgenesis applied to control insect-transmitter plant pathogens: The Pierce's disease case. In: *Insect Symbiosis 2*, edited by: K. Bourtzis and T.A. Miller. Taylor and Francis, London/CRC Press Boca Raton, FL. Bextine, B., D. Lampe, C. Lauzon, B. Jackson and T.A. Miller. 2005. Establishment of a genetically marked insect-derived symbiont in multiple host plants. *Current Microbiol.* 50: 1-7. Miller, T.A. 2004. Rachel Carson and the adaptation of biotechnology to crop protection. *Amer. Entomol.* 50: 194-198. Bextine, B., C. Lauzon, S. Potter, D. Lampe and T.A. Miller. 2004. Delivery of a genetically marked *Alcaligenes* sp. to the glassy-winged sharpshooter for use in a paratransgenic control strategy. *Current Microbiol.* 48: 327-331.