THE CONTRIBUTION OF THE PECTIN-DEGRADING ENZYME POLYGALACTURONASE (PG) IN TRANSMISSION OF *XYLELLA FASTIDIOSA* TO GRAPE AND USE OF PG-INHIBITOR PROTEINS FOR TRANSGENIC RESISTANCE TO PIERCE'S DISEASE

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INTRODUCTION

Pierce's disease (PD) develops because (1) inoculative glassy-winged sharpshooters (GWSS) feeding on grapevines transfer *Xylella fastidiosa* (*Xf*) bacteria into the vine, and (2) the *Xf* population in the vine's water-conducting cells increases and spreads throughout the vine, triggering a set of responses that result in vine collapse and death. Our work on PD development thus far has focused on the spread of the bacteria once they have been introduced into the vine. The cell wall polysaccharide "fabric" of the pit membranes that separate xylem vessels from one another has interpolymer gaps (referred to as cell wall "porosity") that are substantially smaller than *Xf* cells. Thus, the systemic spread of *Xf* is likely to be facilitated by the action of enzymes that digest some of the pit membrane's constituent polysaccharides. Plant cell wall digestion is a common aspect of the biochemistry of most plant interactions with fungal and bacterial pathogens (Powell et al., 2000). In the report describing our continuing work to test a hypothetical model of PD development (Labavitch et al., 2001 & 2002,; Labavitch and Matthews, 2003 and Labavitch et al. in these Proceedings), we have described studies to determine whether *Xf* genes presumed to encode cell wall-degrading enzymes actually do encode the polygalacturonase (PG) and β-1,4-glucanase that their sequences predict. Apparently they do. The work of Dr. Cecilia Aguero (Meredith and Dandekar, 2002 & 2003; Aguero et al., 2004) shows that transgenic grapevines that express the pear fruit gene that encodes a PG-Inhibiting Protein (PGIP) show slower and reduced symptom development, following needle inoculation, than do untransformed grapes. We presume that this is a consequence of the PGIP's inhibition of an *Xf* PG that is crucial for bacterial spread through the vine

As a follow up to work we are doing on plant-insect interactions, we have identified glucanase and PG activity in protein extracts of homogenized GWSS heads. We presume that the enzymes were located in the insect's salivary apparatus and represent some of the proteins in GWSS salivary secretions. If GWSS penetrates grapevine tissues and inserts its stylets in the water-conducting cells of the vine using only mechanical force, why should the saliva of the insect contain PG and other cell wall-degrading enzymes? Dr. Elaine Backus, co-PI on this proposal suggests that the salivary enzymes are important contributors to the insect's feeding success, both in penetration and in correct stylet placement. If this is correct, and if the pear PGIP that has been introduced into transgenic grapevines inhibits the GWSS PG, then the transgenics should also be less susceptible to *Xf* transfer from the insect than untransformed vines.

The Objectives of our work in this proposal are to obtain PG enzyme from both GWSS and *Xf*, and determine the extent to which PGIP inhibits the PGs from the bacteria and insect. Several PGIPs with differing abilities to inhibit PGs from various fungal plant pathogen sources are known (Stotz et al., 2000). If we find that pear PG inhibits either the *Xf* or GWSS PG, or both, continuing research will screen PGIPs from other sources with the intent of identifying an inhibitor with maximal ability to slow infection and disease development in grapevines.

OBJECTIVES

1. To determine whether the pectin-degrading enzyme of *X. fastidiosa* contributes to the systemic spread of the bacterial population in inoculated grapevines (1st priority)
2. To determine whether the pectin-degrading enzyme(s) in the salivary secretions of GWSS contributes to inoculation success of *X. fastidiosa* into grapevines (2nd priority)

RESULTS

This is a new project and funding was only recently received to begin work on specific Objectives. However, because the project is an extension of other PD research (that of others as well as our own) we have some relevant results to present in this progress report.

Grapevines for Testing.

Dr. Cecilia Aguero has teamed with Profs. Meredith and Dandekar to generate transgenic *V. vinifera* (cultivars 'Thomson Seedless' and 'Chardonnay') expressing the pear fruit PGIP gene. These vines accumulate PGIP protein in tissues and in the xylem sap and show decreased susceptibility to infection by *X. fastidiosa* (Aguero et al., 2004). These vines will be the key biological material for testing in the work of this proposal. Dr. Aguero has expanded the populations of these vines to provide the plant material that we will need.

GWSS Cell Wall-digesting Enzymes.

David Morgan has provided to the Labavitch lab several samples of killed GWSS for biochemical analysis. The best samples to examine for their enzyme complement will be excised insect salivary glands and a large-scale collection/dissection "party" is planned for later in the year. In the meantime, we have isolated insect heads, homogenized them in a protein extraction buffer (1M NaCl in 0.1M NaAcetate, pH 5.5), stirred the homogenate at room temperature for 3 h in the presence of protease inhibitors (2% v/v Sigma inhibitor mix) and collected the soluble protein-enriched supernatant following centrifugation at 15,000 x g for 15 min. The extracts are then assayed for PG and β-1,4-glucanase activities using standard radial diffusion assays.

The PG content of the GWSS head protein extracts we have prepared thus far has been quite variable, often rather low. We will wait until we have obtained a substantial number of isolated GWSS salivary glands to attempt the PG purification. However, because the β-1,4-glucanase (BGase) activity has been substantial in all extracts, we have tested many of our insect enzyme purification approaches with the glucanase and made excellent progress.

Protein isolated (as above) from excised heads of 40 GWSS was chromatographed on Concanavalin A Sepaharose. While the protein did not bind "absolutely" to the lectin column, its passage was retarded somewhat. Over 65% of the protein in the extract eluted rapidly from the column, while 90% of the BGase activity was delayed, thus giving a useful first purification step. The active fractions from this step were pooled and subjected to size-exclusion chromatography (SEC) on a Sephacryl S-200 column, This step removed an additional 20% of the protein while allowing us to recover a peak of BGase representing 35% of the initial activity. The final purification involved passage of the pooled, SEC-purified BGase through a Q-Sepharose anion exchange column, eluting first with 5 column volumes of 0.05M Tris-HCl (pH 7.0) and then a linear gradient (0 to 1M NaCl in the Tris-HCl). The elution of the BGase activity was retarded on this column, emerging as a clean peak of activity corresponding to a protein peak. The fractions with BGase activity were pooled , concentrated and run on an SDS-PAGE gel to determine its protein species distribution. A single protein was seen when 40mg of protein was subjected to electrophoreseis, suggesting that a BGase protein had been substantially (or, perhaps, absolutely) purified. The protocols that we have developed for the GWSS BGase should prove useful when we have substantial GWSS to work with.

Work for the Coming Year.

Our plan is to obtain PG-active proteins from GWSS and *Xf*, purify them and test for inhibition by PGIP. In addition, we will monitor the relative infection of control and pear PGIP-expressing transgenic grapevines by GWSS carrying *Xf,* to assess PGIP's contribution to resistance to bacterial transmission from the insect.

REFERENCES

Aguero CB, Uratsu SL, Greve LC, Powell ALT, Labavitch JM, Meredith CP and Dandekar AM (2004) Evaluation of tolerance to Pierce's Disease and *Botrytis* in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. Molecular Plant Pathology (in press).

Backus E, Yan F, Habibi J and Bennett W (2003) Sharpshooter feeding behavior in relation to transmission of the Pierce's Disease bacterium. 2003 Pierce's Disease Research Symposium. p. 127-131.

Labavitch JM, Matthews MA and Greve LC (2002) The development of Pierce's Disease in xylem: the roles of vessel cavitation, cell wall metabolism and vessel occlusion. 2002 Pierce's Disease Research Symposium. p. 61-64.

Labavitch JM and Matthews MA (2003) The development of Pierce's Disease in xylem: the roles of vessel cavitation, cell wall metabolism and vessel occlusion. 2003 Pierce's Disease Research Symposium. p. 150-153.
Meredith C and Dandekar AM (2002) Directing potential anti-*Xylella* gene products to the xylem of transgenic grapevines. Symposium Proceedings: 2002 Pierce's Disease Research Symposium. p. 37-38**.**
Meredith C and Dandekar AM (2003) Directing potential anti-*Xylella* gene products to the xylem of transgenic grapevines. Symposium Proceedings: 2003 Pierce's Disease Research Symposium. p. 23-25.
Powell ALT, van Kan J, ten Have A, Visser J, Greve LC, Bennett AB, Labavitch JM (2000) Transgenic expression of pear PGIP in tomato limits fungal colonization. MPMI 13:942-950.
Stotz HU, Bishop JG, Bergmann CW, Koch M, Albersheim P, Darvill AG and Labavitch JM (2000) Identification of target amino acids that affect interactions of fungal polygalacturonases and their plant inhibitors. Physiol. and Mol. Plant Pathol. 56:117-130.

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