GRAPE RECOGNITION OF *XYLELLA* SURFACE PROTEINS AND THEIR RELATIONSHIP TO PIERCE'S DISEASE SYMPTOM DEVELOPMENT

Principal Investigator:
Paul Feldstein
Department of Plant Pathology
University of California
Davis, CA 95616
pafeldstein@ucdavis.edu

Cooperator:
George Bruening
Department of Plant Pathology
University of California
Davis, CA 95616
gebruening@ucdavis.edu

Cooperator:
Edwin Civerolo
San Joaquin Valley Agric. Sci. Center
USDA ARS
Parlier, CA 93648
eciverolo@fresno.ars.usda.gov

Reporting Period: The results reported here were derived from work conducted October 2010, through October 2011.

ABSTRACT
The aim of this project is to understand the role of *Xylella fastidiosa* (*Xf*) surface proteins, especially the translation elongation factor "temperature unstable" (EF-Tu) in disease development induced by this bacterium, the causitive agent of Pierce's disease (PD) of grapevine, and to use this understanding to interfere with disease development. Our earlier work showed that the infiltration of preparations enriched in *Xf* EF-Tu protein into *Chenopodium quinoa* leaves induced a chlorosis reaction, suggesting that EF-Tu may contribute to symptom development in *Xf* infections. EF-Tu of certain eubacteria has been recognized as a MAMP, a microbe-associated molecular pattern, i.e., a macromolecule that is characteristic of a class of microorganisms and thus well suited to signaling the invasion of a member of that class. Unlike the EF-Tu of most eubacteria, *Xf* EF-Tu is associated with an insoluble fraction, which makes its purification by conventional methods difficult. Previously, we demonstrated that using *Agrobacterium tumefaciens* to program plant cells to produce apoplast-targeted *Xf* Ef-Tu resulted in the development of scorching symptoms characteristic of PD in *Nicotiana tabacum* cultivar SR-1 and Thompson seedless grapevines. The application of transgenic Agrobacterium obviated the need to purify EF-Tu. We report here that the use of *A. tumefaciens* carrying new binary constructs with intron inserts in *Xf* EF-Tu gene, both with and without apoplastic targeting sequences, also induced PD-like symptoms in SR-1 tobacco. Infiltrations of these same *Agrobacterium* strains into *Nicotiana benthamiana* did not lead to symptom development, consistent with the known lack of an EF-Tu receptor in *N. benthamiana*. We expected that apoplastic targeting would be required for symptom induction because, in the infected plant, *Xf* resides in the xylem rather than interacting with living cells. Interpreting the similar response of SR-1 tobacco to EF-Tu targeted and not targeted to the apoplast is confounded by the much greater accumulation of EF-Tu for the non-apoplast-targeted, compared to the untargeted, construct. We employed transposon mutagenesis to map regions of EF-Tu needed for symptom induction. Our results suggest that the symptom-inducing region lies between amino acids 91 and 291. Previously, we found that digestion of *Xf* cells with lysozyme in a detergent solution generated a membrane- and EF-Tu-containing insoluble fraction in which EF-Tu is accessible to antibody, whereas the EF-Tu of intact *Xf* cells is not accessible. Here we demonstrate that infiltration of the insoluble, EF-Tu-containing fraction into Thompson seedless grapevine leaves stimulated expression from a grapevine promoter known to be specifically activated in *Xf* infection.

LAYPERSON SUMMARY
The elongation factor "temperature unstable", EF-Tu, is one of the most abundant proteins in most bacteria. In *Xylella fastidiosa (Xf)* it is found at or just below the cell surface and is associated with an insoluble fraction which makes purification difficult. To avoid the need to purify the *Xf* EF-Tu, we have used *Agrobacterium*, a bacterium that can program plant cells to produce exogenous proteins, to produce *Xf* EF-Tu either with or without targeting EF-Tu to the outside of the plant cell. Production of either targeted or untargeted EF-Tu in *Nicotiana tabacum* cv SR-1 led to development of Pierce's disease(PD)-like symptoms. We have shown that only part of the *Xf* EF-Tu protein is necessary to produce these symptoms. Also, the insoluble fraction, which has much more accessible EF-Tu protein than intact cells, is capable of stimulating grapevine responses in the same way that *Xf* infection can. The apparent ability of *Xf* EF-Tu protein to stimulate grape and the production PD-like symptoms in the absence of intact *Xf* cells or other *Xf* proteins supports a role for the *Xf* EF-Tu protein recognition in PD and could lead to novel methods for reducing PD symptom development.

INTRODUCTION
Effective long term control of Pierce's disease(PD) will likely require the development of resistant or tolerant grapevine cultivars. Resistance or tolerance could be achieved by interfering with symptom development, which presumably requires at least partial interference with the functioning of *Xylella fastidiosa (Xf)* virulence factors. While the mechanism of *Xf* virulence factor function has not been established, *Xf* surface proteins, such as the major outer membrane protein, MopB (Bruening and Civerolo 2004), the hemagglutinin-like minor outer membrane proteins H*Xf*A and H*Xf*B (Guilhabert and Kirkpatrick 2005) and a form of the translation elongation factor "temperature unstable" (EF-Tu), are possible candidates. We reported earlier that partially purified MopB was capable of inducing chlorosis in *Chenopodium quinoa* (Bruening et al. 2007); however, *Xf* MopB produced in and purified from *E. coli* did not induce this chlorosis. Eluted protein from a minor trailing band from electrophoresis of this partially purified MopB preparation through a sodium dodecyl sulfate-permeated polyacrylamide gel induced chlorosis in *C. quinoa*. The major component of this trailing band was EF-Tu suggesting that it, and not MopB, is the chlorosis inducing factor.

EF-Tu is one of several highly conserved eubacterial macromolecules known as "microbe-associated molecular patterns" (MAMPs) because of their ability to induce innate immunity in both plants (Jones and Dangl 2006) and animals. Flagellin, chitin and certain lipopolysaccharides are other examples of MAMPs. EF-Tu is the most abundant protein (up to 700,000 molecules per cell) in most bacterial cells making it a reasonable signal for the presence of bacterial infection. The MAMP activity of *E. coli* EF-Tu is illustrated by its ability to induce alkalization of the medium of cultured *Arabidopsis thaliana* cells at subnanomolar concentrations. Pressure infiltration of *E. coli* EF-Tu at 1μM into *Arabidopsis* leaves leads to both resistance to *Pseudomonas syringae* and the accumulation of defense gene mRNAs (Kunze et al. 2004). The *E. coli* EF-Tu and *Xf* EF-Tu are highly similar (77% identity and 88% similarity in their amino acid sequence), and they both induce chlorosis when pressure infiltrated into *C. quinoa* leaves. The regions that show identity between the *E. coli* and *Xf* EF-Tu protein sequence also show >90% identity with >100 eubacterial Ef-Tu sequences (Kunze et al. 2004). In some bacteria, the EF-Tu protein has at least one additional activity--that of an adhesin. *Mycoplasma pneumoniae* and *Lactobacillus johnsonii* appear to use EF-Tu for the binding of these bacteria to human cells, and, in the case of *M. pneumoniae*, antibody to EF-Tu was demonstrated to interfere with attachment to human cells (Dallo et al. 2002, Granato et al. 2004). Therefore, it would not be surprising that the apparently surface associated *Xf* Ef-Tu would be capable of inducing reactions in grapevine, including those that lead to symptom development. This work is an extension of our previous project entitled "Exploiting Xylella Fastidiosa Proteins For Pierce's Disease Control"**.** The objectives for the current project are given below.

OBJECTIVES

1. Test *Xf* EF-Tu for its ability to induce scorching in grapevine
2. Identify a grapevine receptor for *Xf* EF-Tu
3. Interfere with *Xf* EF-Tu-induction of scorching using RNAi or by expression of alternative receptor
4. Characterization of *Xf* EF-Tu and its immobilization and localization

RESULTS AND DISCUSSION

Transient expression of Xf EF-Tu in* Nicotiana tabacum *induces Pierce's disease-like symptoms

Our earlier agro-infiltration constructs were designed to express *Xf* EF-Tu for accumulation in the plant cell cytoplasm or in the intercellular spaces (apoplast). A protease-encoding sequence was incorporated to generate a near-wildtype amino-end to EF-Tu. These constructs apparently slowed the growth of *Agrobacterium tumefaciens*, and the protease provoked a reaction when it was transiently expressed in plants. **Figure 1** diagrams new constructs from which the protease was eliminated and into which an intron was inserted to prevent expression in *A. tumefaciens*. Extracellular targeting was through the P14 apoplastic targeting sequence (Vera et al. 1989), which has been shown by Jim Lincoln of the David Gilchrist laboratory to effectively cause secretion of the green fluorescent protein (GFP) into the apoplast. For convenience, in this report apoplast-targeted construct is designated P, and the cytoplasm-targeted construct is designated I. The positioning of the intron after codon 3 should prevent synthesis of EF-Tu in *A. tumefaciens* and thus any toxic effects from premature expression. Due to the uncertainty in the site of signal protease cleavage to release P14, the amino end produced by expression from these new constructs may not be the same as the authentic EF-Tu amino end.

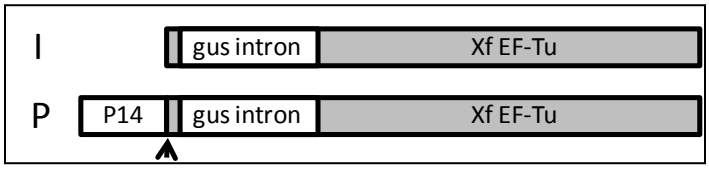

Figure 1. Coding sequence for I and P constructs. The I construct is intended to generate EF-Tu in the cytoplasm of agro-infiltrated leaves. The P construct is intended to direct intercellular accumulation of EF-Tu. In the P diagram, the arrow represents the site of cleavage that releases the P14 peptide. The grey boxes represent *Xf* EF-Tu coding regions. The gus intron is preceded in the construct by a sequence encoding only the first three amino acids of *Xf* EF-Tu. The intron was derived from the intron in the gus-encoding sequence found in the pCAMBIA vectors and was originally derived from the castor bean catalase gene. These constructs were cloned into pEAQ-HT, an expression vector that gives high levels of translation (Sainsbury and Lomonosoff, 2008).

The two strains of *A. tumefaciens* used here, in order of increasing aggressiveness, are LBA4404 and GV2260. Each was transformed with the P and with the I constructs, which were compared with untransformed *A. tumefaciens*. In later experiments, with each of the three *A. tumefaciens* strains, the untransformed strain and the same strain bearing pEAQ-HT vector without insert, induced very similar reactions on SR-1 tobacco (not shown), supporting the appropriateness of the untransformed *A. tumefaciens* control. LBA4404, bearing either the I or the P construct, induced mild scorching symptoms in agro-infiltrated SR-1 tobacco (*Nicotiana tabacum*), whereas untransformed *A. tumefaciens* induced, at most, only a mild chlorosis (compare the three panels on the left half of **Figure 2**). As expected, agro-infiltration with strain GV2260

containing the I and P constructs induced stronger scorching symptoms compared to LBA4404 (compare the left and right halves of **Figure 2**).

Figure 2. Comparison of scorch symptoms produced on SR-1 tobacco by infiltration with *Agrobacterium* alone (C) and *Agrobacterium* containing the I and P constructs. Two *Agrobacterium* strains were tested, LBA4404 and GV2260. Cultures were grown overnight at 28°C then diluted 1/10 and growth was allowed to continue for several hours until the cultures reached mid-log phase (A_{600} = ~0.6). The cells were washed once with water and their A_{600} were adjusted to 0.5 A_{600} before infiltration. Pictures were taken at five days post infiltration (dpi), although symptoms were usually easily seen between two and three dpi.

The I construct lacks the apoplast-targeting P14 sequence and therefore should direct synthesis of cell-confined EF-Tu. The observed strong scorching symptoms is inconsistent with recognition by an EFR homolog because the ligand-binding portion of the EFR protein is known to be extracellular (Albert et al. 2010) and *Xf* is not expected to be capable of delivering *Xf* proteins to the plant cell interior (Meidanis et al. 2002). The observed scorching symptoms after intracellular accumulation of *Xf* EF-Tu could be explained if there is (i) a functional intracellular receptor of EF-Tu, (ii) escape of some EF-Tu from the cell or (iii) toxicity of the EF-Tu protein not related to its MAMP activity. To address this last possibility, we infiltrated *N. benthamiana*, which is known not to have an EFR homologue, with GV2260 containing the I and P constructs. **Figure 3** shows *N. benthamiana* leaves infiltrated at the same time as the SR-1 tobacco leaves shown in **Figure 2**. No symptoms were present in the *N. benthamiana* compared with the strong scorching symptoms seen in the SR-1 tobacco at 5 dpi. By nine dpi, a mild yellow chlorosis was visible in the I construct infiltrated *N. benthamiana* leaves compared to the C and P construct infiltrated leaves. This lack of symptoms is consistent with the absence of an EFR homologue in *N. benthamiana*. It is also consistent with the EF-Tu not being generally toxic to plant cells, at least for *N. benthamiana*. This non-toxicity in *N. benthamiana* and is also supported by studies of GFP expression from the pEAQ-HT vector in GV2260, which show much greater accumulation of GFP in *N. benthamiana* than in SR-1 tobacco (results not shown).

Protein was extracted from the *N. benthamiana* leaves at nine dpi, subjected to denaturing gel electrophoresis and immunoblotted using anti-*Xf* EF-Tu antibodies. As shown in **Figure 4**, a small amount of protein, visible between the 37.5KDa and 50KDa standards, was detected only in the extract from the I sample while no signal was observed for the lane receiving the P sample, which is consistent with previous experiments where P14-containing, apoplast targeted GFP produced far less GFP fluorescence and GFP protein than was observed for the GFP construct lacking P14. GFP expressed from the pEAQ-HT vector represents a significant fraction of the *N. benthamiana* cell protein (Sainsbury and Lomonosoff, 2008). Therefore, the small amount of *Xf* EF-Tu produced in the otherwise superior protein-producing *N. benthamiana* supports the notion that a specific reaction with a receptor, rather than an inherent toxicity, is responsible for the scorching symptoms seen in SR-1 tobacco. The low level of protein produced may be due to codon bias differences between the plants used and the *Xf* bacteria, for example the preference for CGC codons for arginine in prokaryotes while it is rarely used in plants, or may be due to poor splicing. In either case, the amount of EF-Tu produced is sufficient to elicit a strong response.

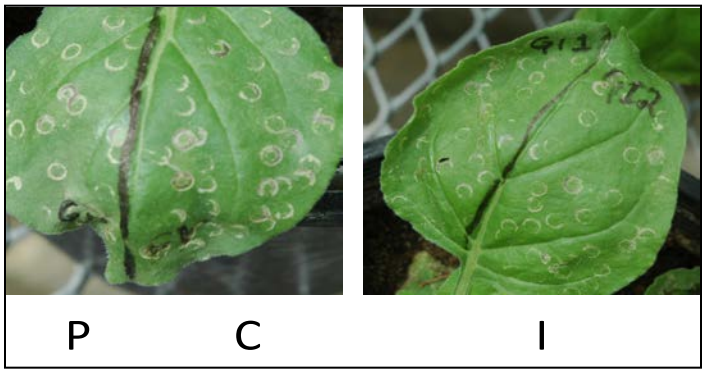

Figure 3. Test of symptom development in N. Benthamiana after infiltration with GV2260 containing I and P constructs. Left panel, opposite half leaves were infiltrated with GV2260 alone (C) or containing the P construct (P). Right panel, leaf infiltrated with GV2260 containing the I construct. The cultures used with the same as used in the experiment shown in **Figure 2** and were infiltrated at the same time. Images taken at five dpi.

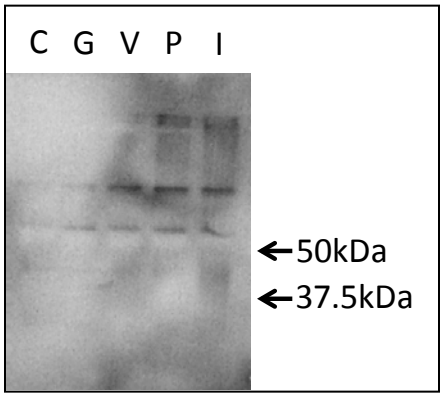

Figure 4. Immunoblot with anti-*Xf* EF-Tu antibody of protein extracts of *N. benthamiana* leaves extracted nine dpi. Extracts are from uninfiltrated leaves (C), leaves infiltrated with GV2260 only (G), GV2260 containing empty pEAQ-HT (V), GV2260 containing the P construct (P) and GV2260 containing the I construct (I).

Since the I construct produced scorching symptoms in SR-1 tobacco, we undertook experiments aimed at defining the portion of the EF-Tu protein that is recognized to initiate development of these symptoms. A EZ-Tn5 <Tet-1> transposon from Epicentre Technologies was inserted randomly into the I construct plasmid DNA in vitro. Transformation into *E. coli* followed by selection on kanamycin and tetracycline plates allowed only those plasmids that had at least one inserted transposon to be recovered. The promoter that transcribes the tetracycline resistance gene could lead to production of *Xf* EF-Tu protein fragments through internal initiation if the transposon is inserted in the appropriate orientation. Because this could lead to toxic effects during cloning, only those plasmids where the transposon is in the opposite orientation to the *Xf* EF-Tu were chosen. These clones were selected by PCR using a primer outside the *Xf* EF-Tu coding sequence and a primer within the transposon such that only the desired plasmids would give a PCR product. The size of this PCR product was used to roughly map the insertion sites and appropriate clones were sequenced to determine the exact insertion site. Two clones were transformed into *A. tumefaciens* strain GV2260 and infiltrated into SR-1 tobacco. **Figure 5** shows the locations of these two clones and shows that in clone I-10, the deletion of the C-terminal 105 amino acids did not destroy the ability of the produced *Xf* EF-Tu protein fragment to induce scorching. However, in clone I-12, leaving only the 91 N-terminal amino acids did destroy said ability. A peptide representing the 18 N-terminal amino acids is sufficient to bind and activate the *Arabidopsis* EFR protein (Kunze et al. 2004). When this peptide was infiltrated into *Chenopodium quinoa* leaves, the chlorosis that characteristically develops after infiltration of preparations enriched in *Xf* EF-Tu (Bruening et al. 2007) was not seen. These results suggest that the receptors for EF-Tu in *Chenopodium* and SR-1 tobacco may be recognizing different epitopes than the epitope recognized by the *Arabidopsis* EFR protein. Nevertheless, the receptor proteins in *Chenopodium* and SR-1 tobacco could be EFR homologs.

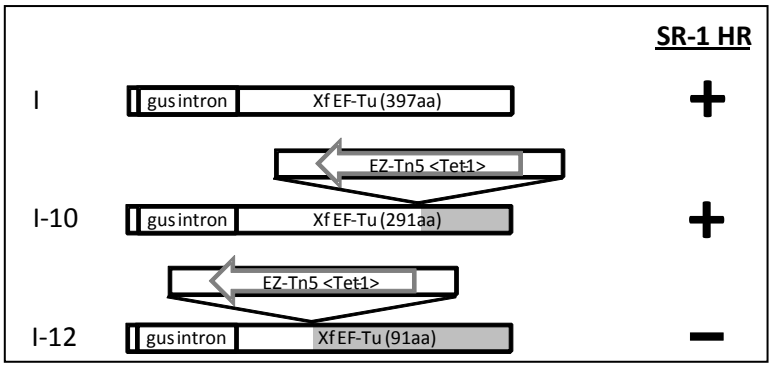

Figure 5. Transposon tagged construct I in *A. tumefaciens* strain GV2260 and tested in SR-1 tobacco. I-10 and I-12 are the designation for the tested clones. The number of amino acids of *Xf* EF-Tu before the insertion site for each clone tested is shown in parenthesis. The grey colored area corresponds to sequence 3' of the first stop codon resulting from the transposon insert.

The EF-Tu protein is considered to consist of three domains. Clone I-10 has enough of the carboxyl end sequence of EF-Tu deleted to completely remove domain 3 (Song et al. 1999). The spontaneous polymerization of E. coli EF-Tu maps to domain 3. Polymerized EF-Tu is speculated to be a component of the primative prokaryotic cytoskeleton (Soufo et al. 2010). One possible mechanism by which *Xf* EF-Tu could be inherently toxic is by polymerizing. The fact that SR-1 tobacco recognition of *Xf* EF-Tu does not require the polymerization domain suggests that *Xf* EF-Tu is not inherently toxic, as we have already tentatively concluded from other evidence presented and interpreted above.

Stimulation of an EF-Tu responsive grape promoter by a Xf cell-derived sub-cellular fraction

Previously, we described material that is released from *Xf* cells after incubation with Bugbuster, a commercial bacterial lysis solution, and lysozyme. A fraction, designated fraction P, was recovered by centrifugation and gradient centrifugation of the partially digested *Xf* cell material (Bruening et al. 2008). Electron microscopy of fraction P revealed spheres of approximately the same surface area of the original *Xf* cells, suggesting that components of fraction P are at or very near the surface of the intact cells (Feldstein et al. 2010). Immunogold electron microscopy with anti-*Xf* EF-Tu antibody showed that fraction P retained *Xf* EF-Tu (Feldstein et al. 2010). Separate incubations of fraction P and intact *Xf* cells with Alexa 488-labeled anti-*Xf* EF-Tu antibody, followed by washing and centrifugation, resulted in labeling of fraction P but not of the intact cells (**Figure 6A**). These results suggest that the *Xf* EF-Tu, like the EF-Tu proteins of a few other bacteria, is associated with the outer surface of the bacterial cell but is not accessible in the intact cell. For certain pneumococci and meningococci bacteria, the surface associated EF-Tu is not accessible to antibody and is made accessible only after treatment of the cells with heat or ethanol (Kolberg et al. 2008).

Viable or heat-inactivated *Xf* cells induce a chlorotic reaction when pressure infiltrated into *Chenopodium quinoa* leaves. This activity was traced to the EF-Tu of *Xf* cells (Bruening et al. 2007). Not surprisingly, fraction P stimulated a similar reaction after pressure infiltration into *C. quinoa* leaves. Transcription from a grapevine promoter, 9353, which was discovered in the laboratory of Douglas Cook and exploited in the laboratory of David Gilchrist, is activated by *Xf* infection of grapevine (Gilchrist and Lincoln 2008). To test the ability of fraction P to activate, in grapevine leaves, transcription from promoter 9353, *A. tumefaciens* bearing a 9353-driven GFP construct was co-infiltrated with fraction P. At five days after infiltration, leaf lamella were examined under a laser confocal microscope. **Figure 6B** shows that only when the fraction P material was infiltrated was a GFP signal seen in the infiltrated areas, suggesting that a component of fraction P, presumably EF-Tu, is sufficient to activate *Xf*-infection-specific promoter 9353.

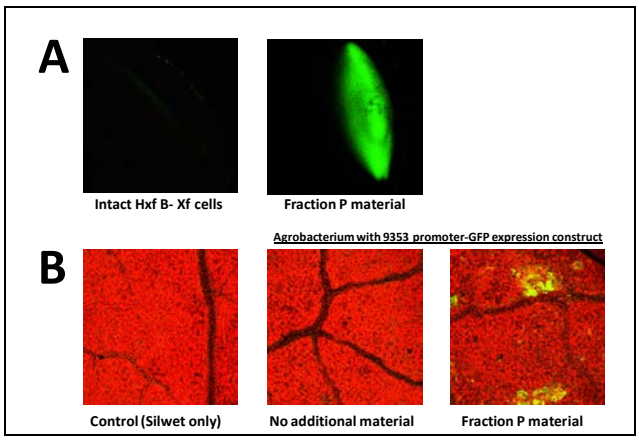

Figure 6. *Xf* cell-derived fraction P has antibody-accessible EF-Tu and activates transcription from a *Xf*-infection-specific grapevine promoter. (**A)** Recovery of fluorescent anti-*Xf* EF-Tu antibody in the insoluble fraction after incubation with intact *Xf*-H*Xf*B cells, **left panel,** and with fraction P material, **right panel**. Fraction P material or cells were incubated with Alexa 488-labeled anti-*Xf* EF-Tu and then washed with buffer and collected by centrifugation. The centrifugation pellet, collected in the bottom of an Eppendorf tube, was viewed under a fluorescent microscope. (**B**) Laser confocal microscope images of Thompson Seedless grape leaves infiltrated with Silwet only, **left panel**, with Silwet and *A. tumefaciens* strain bearing the *Xf* infection-specific 9353 promoter-GFP expression construct, **middle panel,** or with Silwet, the 9353 promoter-GFP expression construct and fraction P material, **right panel**. The Silwet wetting agent increases the entry of the *A. tumefaciens* cells in the infiltration method used.

CONCLUSIONS

Xf EF-Tu has been shown to be associated with or near the outer surface of the *Xf* cell. This peripheral location could allow recognition by the plant of the *Xf* EF-Tu protein. Due to the difficulties of purifying the *Xf* EF-Tu protein itself, we have used *Agrobacterium* to induce plant cells to produce the *Xf* EF-Tu protein themselves. This production leads to PD-like symptoms. While targeting of this protein to the intercellular spaces of the leaf does not appear necessary in these experiments, the difference in the amount of protein produced may allow some of the more highly produced intracellular protein to reach the presumably extracellular receptor. We have also found that removal of the C-terminal domain 3 of the EF-Tu protein does not interfere with symptom development, but that larger deletions did, suggesting that the recognized region of EF-Tu is between amino acids 91 and 291. This is inconsistent with the EF-Tu region known to bind to the *Arabidopsis* EFR receptor, which consists of the amino-end 18 amino acids. This suggests that the *Arabidopsis* receptor EFR and the presumed grapevine and SR-1 tobacco receptors may be recognizing different regions of the EF-Tu protein.

REFERENCES CITED

Albert M, Jehle AK, Mueller K, Eisele C, Lipschis M, Felix G. 2010 *Arabidopsis thaliana* pattern recognition receptors for bacterial elongation factor Tu and flagellin can be combined to form functional chimeric receptors. Journal of Biological Chemistry 285:19035–19042

Bruening, G., and E. L. Civerolo. 2004. Exploiting *Xylella fastidiosa* proteins for Pierce's disease control, pp. 286-289. *In* M. A. Tariq, S. Oswalt, P. Blincoe, A. Ba, T. Lorick and T. Esser [eds.], Pierce's Disease Control Program Symposium. California Department of Food and Agriculture, San Diego, CA.

Bruening, G., E. L. Civerolo, and P. A. Feldstein. 2007. Exploiting *Xylella fastidiosa* proteins for Pierce's disease control, pp. 173-176, Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA, T. Esser, editor, San Diego, CA.

Bruening, G., P. A. Feldstein, and E. L. Civerolo. 2008. Exploiting *Xylella fastidiosa* proteins for Pierce's disease control, pp. 142-148, Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA, T. Esser, editor, San Diego, CA.

Caldas, T. D., A. El Yaagoubi, M. Kohiyama, and G. Richarme. 1998. Purification of elongation factors EF-Tu and EF-G from *Escherichia coli* by covalent chromatography on thiol-sepharose. Protein Expression and Purification 14: 65-70.

Dallo, S. F., T. R. Kannan, M. W. Blaylock, and J. B. Baseman. 2002. Elongation factor Tu and E1 beta subunit of pyruvate dehydrogenase complex act as fibronectin binding proteins in *Mycoplasma pneumoniae*. Molecular Microbiology 46: 1041-1051.

Feldstein, P.A. 2010. Grape recognition of *Xylella* surface proteins and their relationship to Pierce's disease symptoms, Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA, T. Esser, editor, San Diego, CA.

Francis, M., E. L. Civerolo, and G. Bruening 2008. Improved Bioassay of *Xylella fastidiosa* Using *Nicotiana tabacum* Cultivar SR-1 Plant Disease 92: 14-20.

Gallie, D. R., Sleat, D. E., Watts, J. W., Turner, P. C., and T. M. A. Wilson. 1987. The 5' leader sequence of tobacco mosaic virus RNA enhances the expression of foreign genes *in vitro* and *in vivo*. Nucleic Acid Research 15: 3257-3273.

Gilchrist, D. and J. Lincoln. 2008. Functional testing and characterization of Pierce's disease induced promoters from Grape, pp. 204-207, Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA, T. Esser, editor, San Diego, CA.

Granato, D., G. E. Bergonzelli, R. D. Pridmore, L. Marvin, M. Rouvet, and I. E. Corthesy-Theulaz. 2004. Cell surface-associated elongation factor Tu mediates the attachment of *Lactobacillus johnsonii* NCC533 (La1) to human intestinal cells and mucins. Infection and Immunity 72: 2160-2169.

Guilhabert, M. R., and B. C. Kirkpatrick. 2005. Identification of *Xylella fastidiosa* antivirulence genes: Hemagglutinin adhesins contribute to X-fastidiosa biofilm maturation and colonization and attenuate virulence. Molecular Plant-Microbe Interactions 18: 856-868.

Jones, J. D. G., and J. L. Dangl. 2006. The plant immune system. Nature 444: 323-329.

Kolberg, J., Hammerschmidt, S., Frank, R., Jonak, J., Sanderov, H. and A. Aase. 2008. The surface-associated elongation factor Tu is concealed for antibody binding on viable pneumococci and meningococci. FEMS Immunology and Medical Microbiology, 53, 222-230.

Kunze, G., C. Zipfel, S. Robatzek, K. Niehaus, T. Boller, and G. Felix. 2004. The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. Plant Cell 16: 3496-3507.

Meidanis, J., Braga, M.D.V., and S. Verjovski-Almeida, 2002. Whole-Genome Analysis of Transporters in the Plant Pathogen *Xylella fastidiosa* Microbiology and Molecular Biology Reviews. 66: 272–299.

Sainsbury, F. and G. P. Lomonossoff. 2008. Extremely High-level and Rapid Transient Protein Production in Plants without the Use of Viral Replication. Plant Physiology 148: 1212-1218.

Song, H. Parsons, M.R., Roswell, S., Leonard, G. and S.E.V. Phillips. 1999. Crystal Structure of Intact Elongation Factor EF-Tu from *Escherichia coli* in GDP Conformation at 2.05 Å Resolution. Journal of Molecular Biology, 285, 1245- 1256.

Soufo, H.J.D., Reimolda, C., Linneb, U., Knusta, T., Geschera, J. and P.L. Graumanna. 2010. Bacterial translation elongation factor EF-Tu interacts and colocalizes with actin-like MreB protein Proceedings of the National Academy USA, 107, 3163-3168.

Vera P., Hernandez-Yago J. and V. Conejero 1989. Pathogenesis-related P1(p14) protein. Vacuolar and apoplastic localization in leaf tissue from tomato plants infected with citrus exocortis viroid, *in vitro* synthesis and processing. Journal of General Virology 70:1933-1942

Zipfel, C., G. Kunze, D. Chinchilla, A. Caniard, J. D. G. Jones, T. Boller, and G. Felix. 2006. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell 125: 749-760.

FUNDING AGENCIES

Funding for this project was provided by the USDA-funded University of California Pierce's Disease Research Grants Program.

ACKNOWLEDGMENTS

We are grateful to James Lincoln and David Gilchrist for providing grapevines, to James Lincoln for constructs and photomicroscopy, to Darleen Hoffman for immune-gold electron microscopy, and to George Lomonossoff for an expression vector. Steven Daubert provided a useful suggestion on agroinfiltration.