GREENHOUSE EVALUATION OF GRAPEVINE FUNGAL ENDOPHYTES AND FUNGAL NATURAL PRODUCTS ANTAGONISTIC TO *XYLELLA FASTIDIOSA* FOR CONTROL OF PIERCE'S DISEASE

Principal Investigators
Philippe Rolshausen, Ph.D
Dept. of Botany and Plant Sciences
University of California
Riverside, 92521
Phone: (951) 827-6988
Fax: (951) 827-4294
philrols@ucr.edu

Co-Principal Investigators
Caroline Roper, Ph.D
Dept. of Plant Pathology and Microbiology
University of California
Riverside, 92521
Phone: (951) 827-3510
mcroper@ucr.edu

Katherine Maloney, Ph.D
Dept. of Chemistry
Point Loma Nazarene University
San Diego, CA 92106
(619) 849-3425
katherinemaloney@pointloma.edu

Cooperator
James B. Gloer, Ph.D
Dept. of Chemistry
University of Iowa
Iowa City, IA 52242
(319) 335-1361
james-gloer@uiowa.edu

Reporting Period: The results reported here are from work conducted on July 2012 to July 2016.

ABSTRACT.
The goal of this research was to identify fungal endophytes and fungal natural products antagonistic to *Xylella fastidiosa* (*Xf*) that could be implemented as preventative and curative treatment for Pierce's Disease (PD) management. We showed in *in vitro* bioassays that several fungal endophytes isolated from grapevine wood possess anti-*Xf* properties, due to the production of natural products. One of those fungus (*Cryptococcus*) inhibited PD symptoms development and *Xf* titer in *in planta* bioassays. In addition, we purified and characterized one natural product (radicinin) produced by *Cochliobolus* sp. as and effective inhibitor of *Xf*. Radicinin has a poor solubility in water and thus was not systemic and active against *Xf* when injected *in planta*. However, in collaboration with the private sector, we successfully developed an emulsion of radicinin and treated vines inoculated with *Xf*. Results will be obtained later on this year. In addition, we showed that the fractions from the crude extracts of three additional fungal endophytes (i.e., *Eurotium*, *Geomyces*, and *Ulocladium*) also possess activity against *Xf* in the *in vitro* bioassay. Active fractions from the crude extracts of these three fungal cultures are being examined using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) to identify their chemical structures and properties. These molecules and formulation are currently under review for patentability by the Executive Licensing Officer in the UC-Riverside Office of Research and, hence, their names cannot always be disclosed in this report.

LAYPERSON SUMMARY.

Several management strategies for Pierce's Disease (PD) are currently being deployed, but as of today successful management largely involve vector control through the use of insecticides. Here we propose to test an alternative control strategy to complement those currently in place or being developed. The goal was to identify fungi and natural products of fungi associated with grapevine that are antagonistic to *Xylella fastidiosa* (*Xf*) that could be implemented as preventative and curative treatment for Pierce's Disease (PD), either as a foliar spray, drench application or trunk injection. We have identified eight fungi naturally inhabiting grapevines that are antagonistic to *Xylella fastidiosa* (*Xf*) *in vitro*. One fungus (*Cryptococcus*) was able to inhibit PD symptoms development in greenhouse bioassays. In addition, we have identified one promising fungal molecule (radicinin) that is inhibitory to the bacterium in an *in vitro* bioassay. However, this molecule displayed poor water solubility and could not be used successfully as treatment for PD infected grapevines. We have developed an emulsion of radicinin in a concerted effort with the private sector and are currently testing the efficacy of this formulation in *in planta* bioassay. In addition, we recently showed that the fractions from the crude extracts of three additional fungal endophytes inhibited *Xf* in a disc bioassay. We are now in the process of characterizing the chemical structure and property of these molecules so they can be further tested in grapevine. These natural products and formulation of these products are currently under review for patentability by the Executive Licensing Officer in the UC-Riverside Office of Research and, hence, their names cannot always be disclosed in this report.

INTRODUCTION.

Xylella fastidiosa (*Xf*) is a Gram negative, xylem-limited, insect-vectored bacterium and is the causal agent of Pierce's Disease (PD) of grapevine (Hopkins and Purcell, 2002). PD is endemic to California but the recent introduction of a more effective vector, the Glassy-Winged SharpShooter (GWSS), *Homalodisca vitripennis*, to Southern California shifted the epidemiology of PD from a monocylic to a polycyclic disease. This led to a PD epidemic with severe economic consequences for the Southern California grape industry. The potential for the GWSS to become established throughout the state remains a severe threat to the other major grape-growing regions (Central and Northern California). Current PD management strategies largely involve vector management through the use of insecticides.

Control of PD with fungi or fungal metabolites is a largely unexplored research area. Fungi are receiving increasing attention from natural product chemists due to the diversity of structurally distinctive compounds they produce, together with the fact that many fungal species remain chemically unexplored. Fungi are excellent sources of interesting novel molecules that may be candidates with potential for control of bacterial diseases (Aldrich et al., 2015; Amna 2010; Proksch et al. 2010; Xu et al. 2008). We first characterized the microbial diversity in grapevines that escaped PD in natural vineyard settings, and compare this population to PD-infected grapevines with the goal of identifying fungi that are unique to PD-escaped vines. We identified eight fungal endophytes that possess anti-*Xf* properties, likely due to the production of natural products. Our objectives were to identify anti-*Xf* fungi and fungal natural products that we could use as preventative and curative treatment for PD.

OBJECTIVES

1- Evaluate grapevine fungal endophytes for *in planta* inhibition of *Xf* and PD development using our established greenhouse bioassay.

2- Purify and characterize additional natural products produced by the inhibitory fungi.

3- Evaluate radicinin and other natural products for their potential as curative treatments for vines already infected with PD.

RESULTS AND DISCUSSION

1- Evaluate grapevine fungal endophytes for *in planta* inhibition of *Xf* and PD development using our established greenhouse bioassay.

The goal of this objective was to provide increased tolerance to PD by inoculating grapes with natural fungal endophytes that possess anti-*Xf* properties. From 2010 to 2013 our research team collected plant tissue samples (sap, petioles, canes, spurs) from PD-escaped and PD-infected grapevines (**Fig.1**) and isolated fungi inhabiting these samples. Following this, we identified these specimens to the genus level by comparing the ribosomal DNA sequences to specimens deposited in the GenBank database. We tested the ability of all the organisms recovered to inhibit *Xf* growth using an *in vitro* bioassay. In brief, *Xf* liquid cultures are adjusted to OD_{600nm}=0.1 (approx. 10^7 CFU/ml); 300 µl of the *Xf* cell suspension are added to 3 ml of PD3 medium containing 0.8% agar and briefly vortexed. This mixture is then overlayed onto a petri plate containing PD3 medium. A sterile circle of agar is

drawn from the margin of an actively growing pure fungal culture and is placed onto the plates previously inoculated with *Xf*. Plates are incubated at 28ºC for seven days and then observed for an inhibition zone around the fungal colony (**Fig.2**; Rolshausen and Roper, 2011). This allowed us to select a total of nine biocontrol candidates (eight fungi and one bacterium). From those nine putative biocontrol agents, we narrowed the pool to 5 potential anti-*Xf* biological control agents (BCAs), based on preliminary data from our greenhouse bioassay and from fungal phenotypic traits (ability to grow rapidly and form spores). Those 5 BCAs (4 fungi and 1 bacterium) were re-introduced in grapevines cuttings prior to planting. To accomplish this, the organisms were grown on PDA medium for two weeks and spores were harvested in sterile water and the concentration was adjusted to 100 spores/µl in PBS buffer. Grape cuttings were vacuum infiltrated with spores, and planted in the greenhouse. Control plants were infiltrated with PBS buffer only. After a few weeks, the green shoots arising from these cuttings were inoculated with *Xf* (Temecula strain) by mechanical needle inoculation (Hill and Purcell, 1995). This experiment was repeated twice on grape cvs 'Merlot' and 'Cabernet Sauvignon' with 10 and 20 plants, respectively. In addition, a sub-sample of plants (n=10) were left un-inoculated with *Xf* to determine if the concentration of spores used is detrimental itself to the grape cuttings. Plant symptoms were rated after 14 weeks on a disease scale from 0 to 5 every two weeks (0= no symptoms; 5= Plant dead or dying) according to Guilhabert and Kirkpatrick (2005) (**Fig. 3**). Our results showed that in the buffer inoculated vines, no biocontrol treatments were lethal or caused PD-like symptoms to grapevines (data not shown). In the *Xf* inoculated grapevine cultivars, one fungus (CRY: *Cryptococcus*) significantly reduced disease severity and *Xf* titer (Rolshausen and Roper, 2013; **Fig.4**).

Figure 1: PD-symptomatic (red arrow) and PD-escaped (blue arrow) grapevines in a vineyard located close to a riparian area in the Napa valley, California.

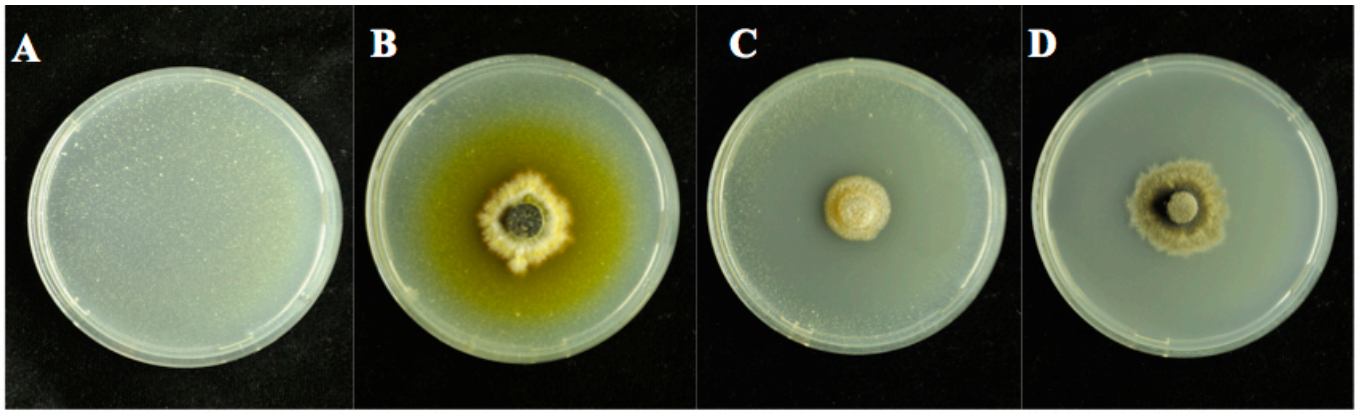


Figure 2: *In vitro* inhibition assay used to evaluate fungal activity towards *Xf*; *Xf* cells were plated in top agar and agar plugs containing fungi were placed on top. Inhibition was evaluated after 8 days of incubation at 28˚C. A) *Xf*-only control; B) No *Xf* inhibition; C) Mild *Xf* inhibition; D) Total *Xf* inhibition.

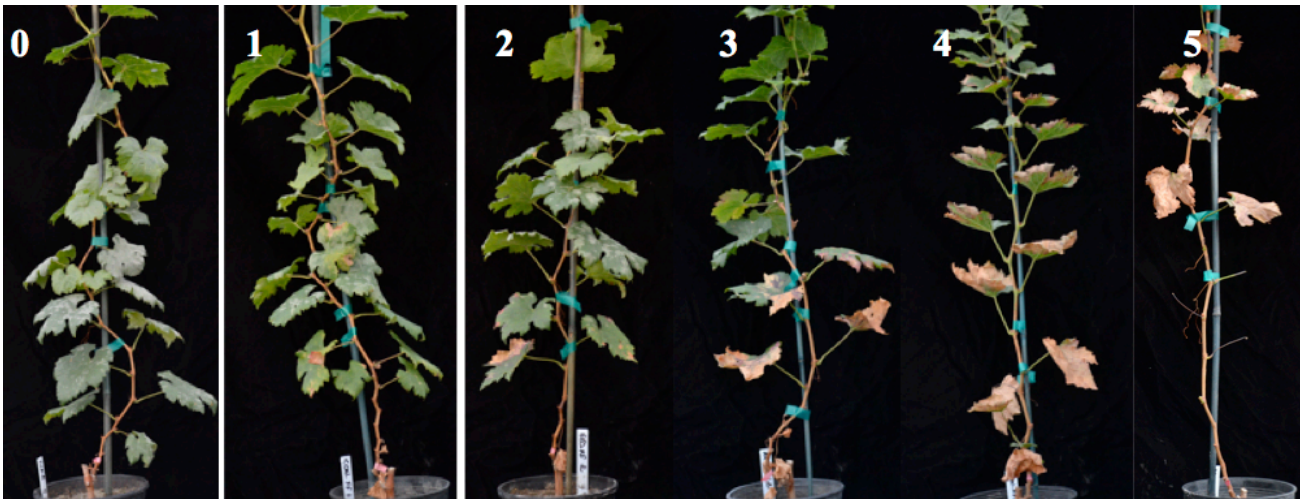


Figure 3: Pierce's Disease symptoms severity rating in grapevine cv. 'Merlot'; 0= no symptoms; 1 through 5= grapes infected with the wild type strain (Temecula) showing an increase in the disease severity.

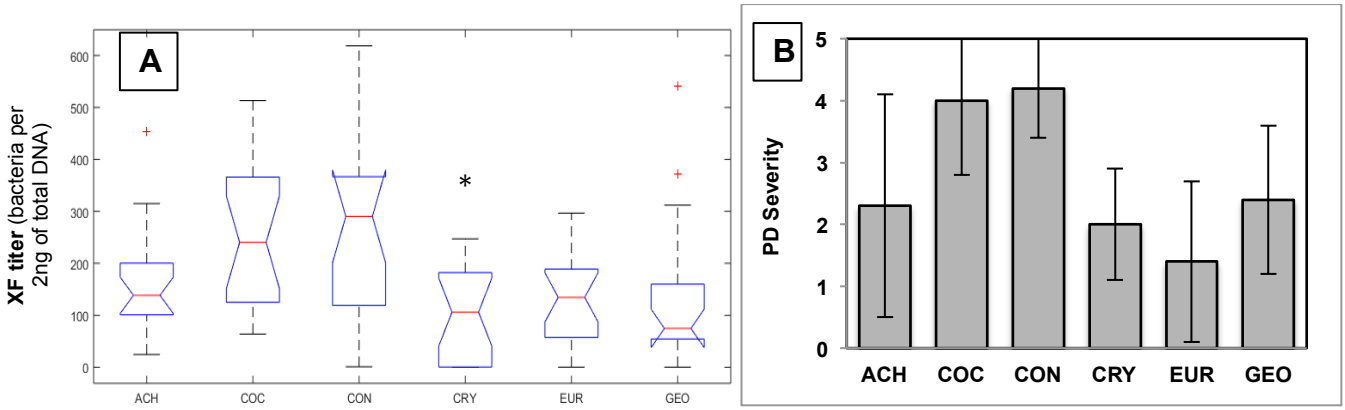


Figure 4: *Xf* titer and PD severity in grapevines (n=10) inoculated with 5 grapevine endophytes or 1X PBS alone (control) and challenged with *Xf* (ACH= *Achromobacter*; COC= *Cochliobolus*; CON= Control; CRY= *Cryptococcus*; EUR= *Eurotium*; GEO= *Geomyces*). **A**; Box plots illustrate the distribution of *Xf* titer in all 6 treatments. Asterisks * indicate significance at $P<0.05$. *Xf* titer was measured by qPCR. *Xf* titer was significantly decreased in vines that were pre-treated with *Cryptococcus* as compared to vines that were pre-treated with 1X PBS only. In addition, *Xf* titer was also decreased (just above statistical significance) in vines that were pre-treated with *Achromobacter* as compared to those inoculated with 1X PBS only. **B**; PD severity average as measured by our disease rating scale (0-5; **Fig.3**). Error bars represent standard deviation.

2- Purify and characterize additional natural products produced by the inhibitory fungi.

The goal of this objective was to identify fungal natural products produced by endophytes that can be used as curative treatments for control of PD. We previously identified eight fungal specimens inhabiting grapevine tissues (xylem sap, shoot, petioles and spur) that were able to inhibit *Xf* in a bioassay (See Objective 1). In addition, crude extracts collected from the fungal cultures showing inhibition towards *Xf* were collected for evaluation using a similar growth inhibition assay as described above. In brief, agar plugs of 0.5 cm diameter of each fungus were used to inoculate 250 mL liquid media, and the fungi were cultivated at room temperature on a shaker. After 10 days, each culture was filtered and further extracted with ethyl acetate, re-suspended in sterile methanol to an extract mass of 1mg, pipetted onto sterile paper discs and allowed to dry in a laminar flow hood. Once dry, the paper discs containing the crude extracts are placed onto the *Xf* cultures and incubated at 28ºC for 7 days. Following this, plates were observed for a halo of inhibition around the paper disc and compared to control *Xf*-only plates and plates with paper discs treated with methanol only. Crude extracts showing inhibition were further processed to purify and identify the inhibitory molecules. Thus far, we have purified and characterized the chemical structure of two molecules (radicinin and cytochalasin) that are active against *Xf* growth *in vitro*. Radicinin is produced by *Cochliobolus* sp. and cytochalasin is produced by *Dreschlera* sp. However, cytochalasin

showed to be toxic to mammals so we decided to discontinue this research axis. In addition, we pursued our efforts for the bioassay-guided isolation of natural products from the remaining fungi able to inhibit *Xf* in our lab bioassay (**Fig.1**), including *Cryptococcus* sp., *Ulocladium* sp., *Eurotium* sp. and *Geomyces* sp. We hope to identify additional anti-*Xf* natural products that could be tested as a curative treatment for PD in the greenhouse bioassay.

Cochliobolus:

Radicinin showed great potential *in vitro* (Aldrich et al., 2015). Hence, in an *in vitro* dose response assay, where *Xf* cells are submitted to an increasing concentration of a fungal molecule, radicinin was able to inhibit *Xf* growth (**Fig. 5**). We have been developed of a more efficient procedure for isolating radicinin from *Cochliobolus* sp. This is a critical step, as it will allow us to produce substantial amount of derivatives and further test them *in planta*. Radicinin is not commercially available, and we had been employing a multistep isolation procedure involving liquid-liquid extraction of *Cochliobolus* cultures followed by an expensive and time-consuming chromatography step to obtain pure radicinin for all our studies to date. Recently, we developed a procedure for purifying radicinin by recrystallization instead of chromatography. In this way, we were able to increase our yield of radicinin from 60.5 mg/liter of culture to 150 mg/liter of culture. This procedure also makes scaling up of the isolation for commercial use much more practical. In addition, the radicinin obtained by this new procedure is significantly more pure, as observed by NMR spectroscopy.

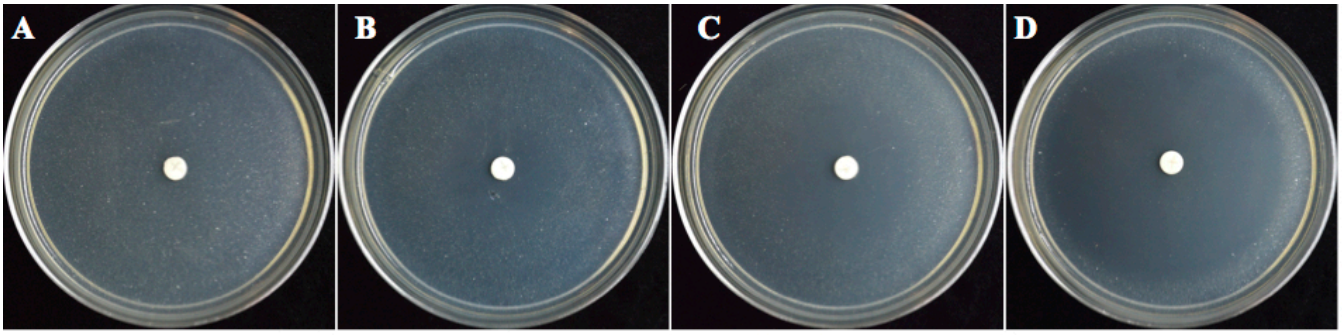
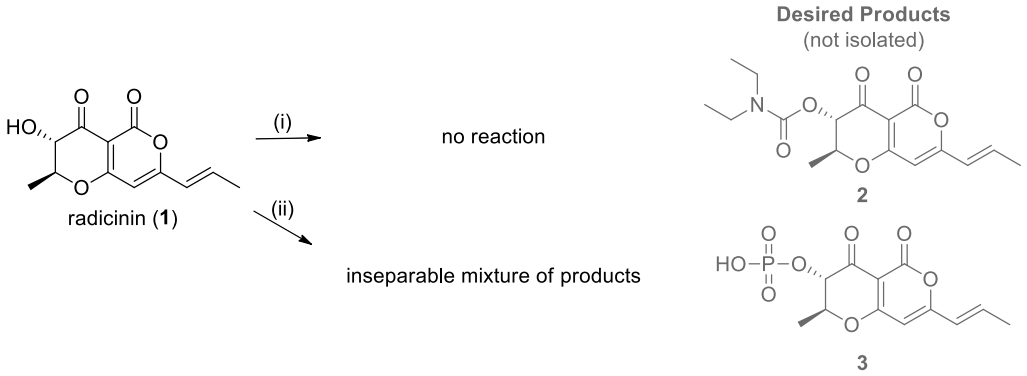


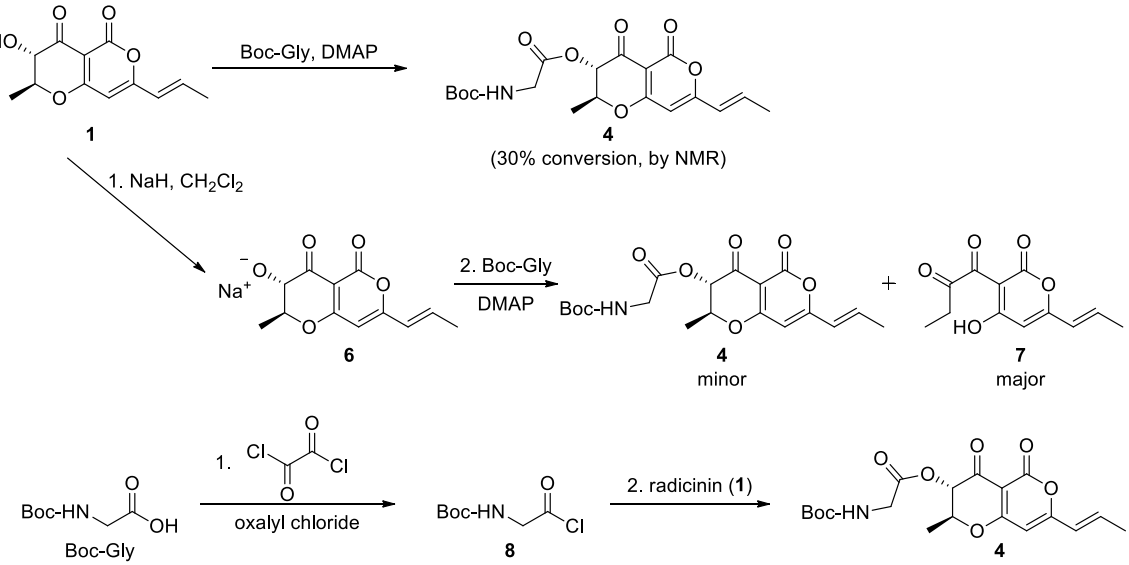
Figure 5: Dose response assay to evaluate *in vitro Xf* inhibition at increasing concentration of a fungal molecule. A) 0 µg molecule R1 (control); B) 50 µg molecule R1; C) 100 µg molecule R1; D) 250 µg molecule R1.

Now that we have figured out how to scale up radicinin production and purification, the next step was to prepare water-soluble semisynthetic derivatives of radicinin to facilitate testing *in planta*. We determined the solubility of radicinin in water to be 0.15 mg/mL, which is considered very slightly soluble. We have shown that acetylradicinin, which was modified at the hydroxyl group of radicinin, retains its anti-*Xf* activity (Aldrich et al., 2015). This result suggests that modification of this position may provide a viable strategy for increasing the water-solubility of radicinin without loss of activity. Adding ionizable groups is a commonly employed strategy for improving the water-solubility of bioactive molecules (Kumar and Singh, 2013), so we had proposed to add two such groups at the hydroxyl position of radicinin (**Scheme 1**). The carbamate (2) is weakly basic and should form a water-soluble salt in low pH solutions, while the phosphate (3) is acidic and should form a water-soluble salt at high pH. Both carbamates and organophosphates are commonly found in pesticides, so we had good reason to believe that one or both of these compounds would be able to move into the xylem of grapevines. However, attempts to prepare the weakly basic carbamate and the acidic phosphate were unsuccessful. Specifically, the reaction with diethylcarbamoyl chloride (i) did not go to completion, while the phosphate reaction (ii) gave a mixture of products that we were unable to purify.

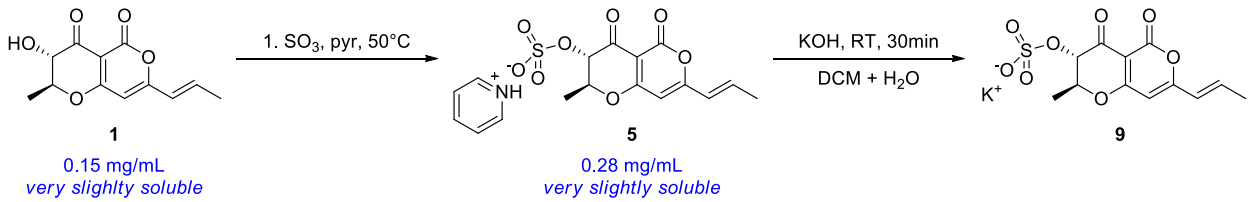


Scheme 1: *Xf*-inhibitory natural product radicinin (1), and semisynthetic derivatives (2-4). Reagents: (i) N,N-diethylcarbamoyl chloride, triethylamine (Vougogiannopoulou et al. 2008). (ii) 1. Cl3CCN, 2. (n-Bu)4NH2PO4, CH3CN, 3. DOWEX 50WX8, NH4HCO3.

We then attempted to make two alternate ionizable radicinin derivatives: a glycine-derivative (4, **Scheme 2**), and radicinin pyridinium sulfate (5, **Scheme 3**). The failure of reactions to form either 2 or 4 suggested that the alcohol group of radicinin is much less nucleophilic than we originally expected. We attempted to increase the nucleophilicity of this group by first deprotonating with sodium hydride to give an alkoxide (6, **Scheme 2**). We isolated 6 and found it to be more than a thousand-fold more water-soluble than radicinin, at 218 mg/mL, (which is considered freely soluble). However, the high pH of the alkoxide solution leads us to be concerned about possible nonspecific toxicity. We also doubt that this high water solubility would be maintained in a cellular environment, which is buffered at neutral pH. Despite the increased nucleophilicity of 6, we never observed any formation of carbamate 2, and observed only minimal formation of the boc-glycine derivative 4. Under the reaction conditions to form 4, radicinin appeared to undergo tautomerization and ring-opening to give isomer 7 (**Scheme 2**). We successfully prepared a sulfate of radicinin, as the pyridinium salt 5. Salt 5 maintained its activity against *Xf* in our disc assay (**Fig. 5**). This reaction proceeded to completion and the product proved easy to isolate. Unfortunately, the water solubility of 5 was only about twice that of radicinin: 0.28 mg/mL, lower than we had hoped. Recently, we were able to successfully replace the pyridinium counterion with potassium to give salt 9 (**Scheme 3**), which we hope will be more water soluble than 5, while retaining activity.



Scheme 2: Attempts to form the Boc-Gly derivative of radicinin using traditional peptide coupling methodology (top), or deprotonating first with sodium hydride (middle) gave the desired derivative as only a minor product, along with a ring-opened isomer of radicinin (7). We next plan to try activating Boc-glycine to the acid chloride (8) using oxalyl chloride, prior to reaction with radicinin (bottom).



Scheme 3: We prepared the pyridinium sulfate of radicinin (5), which was roughly twice as water-soluble as radicinin. Recently, we were able to exchange the pyridinium counterion for a more polar potassium ion in the potassium sulfate 9.

After a series of mostly-unsuccessful attempts at preparing water-soluble radicinin derivatives, we decided to explore another strategy for getting radicinin into grapevines, namely, using surfactants. We tested the solubility of radicinin in a variety of organic solvents that are compatible with agriculture, including o-xylene, canola oil, castor oil, mineral oil, and cyclohexanone. Radicinin was completely soluble in cyclohexanone, but was not soluble in any of the other solvents. We have been working with a private company (Evonik Corporation; http://www.break-thru.com/product/break-thru/en/Pages/default.aspx) to help us get the radicinin in the plant. Following their recommendation we dissolved radicinin in cyclohexanone plus one of Evonik's emulsifier, to prepare a water-cyclohexanone emulsion for application on grapevine leaves. These are currently being evaluated in greenhouse biossays (Objective 2).

Cryptococcus

Although live cultures of *Cryptococcus* sp. inhibited *Xf in vitro*, previous attempts to extract the active compound from liquid cultures failed to yield an active organic extract, either because the activity is not due to a small molecule natural product, or because the particular strain of *Cryptococcus* failed to produce the compound in liquid monoculture in Potato Dextrose Broth (PDB). We tried to stimulate the production of any active metabolite(s) by growing three *Cryptococcus* strains (the original strain CRY1, along with two more recently-isolated strains CRY3 and CRY4) in the following conditions:

- PDB control
- PDB with added *Vitis* sp. leaves (lyophilized and autoclaved with the media)
- PD3 medium (the medium used for the in vitro *Xf*-inhibition assay)

After 14 days of fermentation with shaking at room temperature, each culture was centrifuged to separate the cell pellet from the culture broth. The broths were extracted twice with ethyl acetate, and the pellets were lyophilized, ground in a mixture of 1:1 dichloromethane:methanol, and filtered to give a crude extract. Extracts were evaporated and submitted for the disc diffusion assay for activity against *Xf* (**Fig.5**). We are currently waiting for the results.

Ulocladium

We previously observed a compound in the ethyl acetate extract of *Ulocladium* sp. which high-resolution mass spectrometry revealed to have a molecular formula of $C_{10}H_8Cl_2O_4$; this compound has consistently been found in the active fractions from repeated fermentations and separations of *Ulocladium*. In an effort to produce enough of this compound, we fermented 5.5 L of *Ulocladium* sp. and fractionated the organic extract by silica gel chromatography. This yielded 23.4 mg of a semi-purified fraction containing the compound of interest. This was enough material to permit collection of two-dimensional NMR spectral data (including gdqCOSY, gHMBC, HSQC and NOESY experiments), which we are in the process of analyzing. All fractions have been submitted for bioassay against Xf.

Characteristic 1H NMR signals revealed the following features in the major compound from the active extracts:

- A phenol, indicated by a singlet at 12 ppm (and corroborated by the fact that the compound ionizes better in negative ionization mode on the LCMS, and by a phenol-specific ferric chloride TLC stain)
- One or more pair(s) of aromatic hydrogens in an ortho- relationship (indicated by doublets in the 7-8 ppm range, with J = 8-9 Hz), and
- A 1,2-disubstituted cis-alkene, indicated by coupled doublets at 6.3 and 7.7 ppm (J = 11.5 Hz).

We recently began a time-course study to observe the appearance of the active compound over time. The results of this study will be used to optimize production of the molecule of interest.

Geomyces

Previous active fractions from *Geomyces* sp. strain GEO1 revealed weak activity and no major small molecules. However, the active fraction of a more recently isolated Geomyces sp. strain (GEO3) showed strong activity in the *in vitro Xf*-inhibition assay. We fractionated this extract by silica gel chromatography and submitted the 6 fractions for bioassay. We are currently waiting for the results.

3- Evaluate radicinin and other natural products for their potential as curative treatments for vines already infected with PD.

The goal of this objective was to evaluate the anti-*Xf* efficacy of fungal natural products derivatives identified in Objective 2 *in planta*. Once this proof of concept is established in the greenhouse, the experiment will be carried over to the field. The deliverable for this objective is the development of a commercial product for the cure of PD. We have currently identified one fungal natural product (radicinin) as an active molecule inhibitory to *Xf* (see Objective 2). We had previsouly developed greenhouse assays to inject radicinin on PD-infected vines (**Fig.6**). However, we observe no reduction of PD symptoms development because we established that radicinin was not water-soluble and did not likely get in the plant systemically. Recently, we developed a water-cyclohexanone emulsion with radicinin (2g/L) and without for application on grapevine leaves. Potted grapevine cuttings (6-8 leaves) were inoculated with *Xf* as previously described (Hill and Purcell, 1995) and sprayed on leaves with our emulsions after 10 days of incubation. Three treatments with 7 plants per treatment consisted of; 1) *Xf* x blank emulsion; 2) *Xf* x radicinin emulsion; 3) buffer x radicinin emulsion. Plants will be rated for PD symptoms appearance and *Xf* titer as previously described (See Objective 1). Results will be obtained in October 2016.

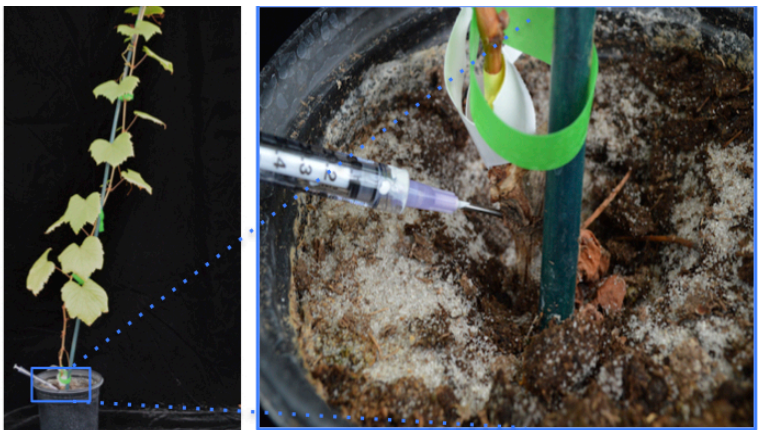


Figure 6: Needle-injection of an anti-*Xf* molecule in the xylem of PD-infected grapevine cuttings.

CONCLUSIONS

We aim to investigate preventative and curative measures for management of PD as part of a sustainable PD management program. Our strategy was to evaluate fungi inhabiting grapevine and the metabolites they produce as a mechanism to control PD. We have discovered that one fungus (*Cryptococcus*) inhibited PD development and Xf titer in *in planta* bioassay and that one active fungal natural products, radicinin (produced by *Cochliobolus*) inhibited *Xf* growth in *in vitro* bioassay. However, radicinin did not show efficacy in our greenhouse trials on PD-infected vines likely because it is not water-soluble. In a concerted effort with industry partners we successfully developed an emulsion of radicinin that was sprayed on PD-infected vines and currently awaiting for the results. In addition, we are also searching for additional active natural anti-*Xf* compounds. We have recently identified fractions from the crude extracts of three additional fungal endophytes that possess activity against *Xf* in the *in vitro* bioassay and we are in the process of identifying their chemical structure and properties. BCAs and fungal natural products that mitigated PD symptoms development and bacterial titer *in planta*, will be further tested in field trials.

REFERENCES CITED

Aldrich, T.J., Rolshausen, P.E., Roper, M.C., Reader, J.M., Steinhaus, M.J., Rapicavoli, J., Vosburg, D.A., Maloney, K.M. 2014. 2015. Radicinin from Cochliobolus sp. inhibits Xylella fastidiosa, the causal agent of Pierce's Disease of grapevine. Phytochemistry 116:130-137.

Amna, A., Khokhar, I, Mukhtar, I., and Mushtaq, S. 2010. Comparison of antibacterial properties of Penicillium species. International Journal of Biology and Biotechnology 7:393-396.

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

Hill, B. L., and A. H. Purcell. 1995. Multiplication and Movement of Xylella fastidiosa within Grapevine and Four Other Plants. Phytopathology, 85 (11):1368-1372.

Hopkins, D. L., and A. H. Purcell. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Disease, 86 (10):1056-1066.

Kumar, P. and Singh, C. 2013. A study on solubility enhancement methods for poorly water soluble drugs. American Journal of Pharmacological Sciences 1:67-73.

Proksch, P., Putz, A., Ortlepp, S., Kjer, J., and Bayer, M. 2010. Bioactive natural products from marine sponges and natural endophytes. Phytochemistry Reviews 9:475-489.

Rolshausen, P.E., and Roper, M.C. Control of Pierce's Disease with fungal endophytes of grapevines antagonistic to *Xylella fastidiosa*. In Proceedings, 2011 Pierce's Disease Research Symposium, pp. 166-172. California Department of Food and Agriculture, Sacramento, CA.

Rolshausen, P.E., and Roper, M.C. Greenhouse evaluation of grapevine fungal endophytes and fungal natural products antagonistic to *Xylella fastidiosa* for control of Pierce's Disease. In Proceedings, 2013 Pierce's Disease Research Symposium, pp. 161-168. California Department of Food and Agriculture, Sacramento, CA.

Xu, L., Zhou, L., Zhao, J., Li, J., Li, X., and Wang, J. 2008., Fungal endophytes from Dioscorea zingiberensis rhizomes and their antibacterial activity. Letters in Applied Microbiology 46:68-72.

FUNDING AGENCIES

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

ACKNOWLEDGEMENTS

The grapevine cuttings utilized in this study were graciously provided by Foundation Plant Services, University of California, Davis.