DEVELOPING A METHOD TO DETECT *XYLELLA FASTIDIOSA* IN GLASSY-WINGED SHARPSHOOTER

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INTRODUCTION

In spite of recent advances to reduce the spread and impact of Pierce's disease spread, few advances have been made in diagnostics of the pathogen, *Xylella fastidiosa*. This is in part due to a perceived lack of need for better diagnostics. Commercial and government laboratories routinely determine the presence of *X. fastidiosa* in plant tissue using serological assays (i.e. ELISA), polymerase chain reaction (PCR), and culturing techniques. Each of these techniques is valuable, the choice depending on the circumstances surrounding their use.

Unfortunately, none of these techniques have been developed to routinely detect *X. fastidiosa* in sharpshooter vectors that are known to spread *X. fastidiosa*. Several laboratories have detected the bacterium in *Homalodisca coagulata*, including laboratories under the supervision of D. Cook, D. Cooksey, H. Costa, T. Miller, A. Purcell, and R. Redak of the University of California. Thus far, the detection limit of *X. fastidiosa* in sharpshooter vectors is not established for any technique. Nor is the relationship between detection in *H. coagulata* and inoculation probability.

Why is it important to detect *X fastidiosa* in sharpshooter vectors? Precisely so we can define the window of time during which grapevines are most susceptible to an inoculation event leading to chronic/terminal infection. An awareness of this window of time will allow new and promising plant protection tactics to be deployed optimally. Currently, we know that systemic neonicotinoid insecticides not only induce GWSS mortality for several weeks after treatment, but also inhibit feeding for much longer (Blua and Redak 2001, Bethke et al 2001). This later characteristic may be more important to reducing disease spread than the former. If the sharpshooter does not feed, it cannot inoculate the pathogen into a non-infected plant or acquire it from an infected plant. In experiments that optimized *X. fastidiosa* transmission by the GWSS, inoculation efficiency jumped from 2% after 1 hour of inoculation access time to 23% after 6 hours (A. Purcell, personal communication). Substances that limit feeding to seconds or minutes could reduce transmission efficiency to the point of essentially blocking the spread of *X. fastidiosa*. Unfortunately, restraints on the use of neonicotinyl insecticides in grapevines do not allow adequate protection throughout the year. Thus, we need to determine when their use would have the greatest impact on infectious GWSS. These arguments can be applied to other plant-protection tactics including the use of substances that disrupt GWSS behavioral cues, and others that inhibit establishment of *X. fastidiosa* in grapevines.

OBJECTIVES

Our long-term goal is to identify the window of time during which grapevines are most susceptible to inoculation by glassy-winged sharpshooters (GWSS) carrying the Pierce's disease bacterium, *Xylella fastidiosa*. In support of this goal we propose first to generate a method of detecting *X. fastidiosa* in glassy-winged sharpshooter vectors that maximizes sensitivity, and is amenable to large sample sizes.

RESULTS AND CONCLUSIONS

Several commercially available PCR preparation kits are useful in detecting *X. fastidiosa*. These kits use different combinations of cell lysing agents and DNA capture methods. All kits we examined were relatively cheep, easy to manage, and amenable to a large number of samples (Table 1).

All of the kits and procedures detected *X. fastidiosa* from pure culture with the exception of Cell Lytic from Sigma (Table 2). A liquid nitrogen extract of *H. coagulata* heads interfered with Dneasy (Qiagen), FTA genecard (Whatman), and the Single Fly procedure, even though DNA was detected from all extracts. None of the kits detected *X. fastidiosa* from liquid nitrogen-extracted *H. coagulata* heads that were collected from citrus at U.C. Riverside. In a more extensive examination of the gDNA Blood Mini Kit (Eppendorf) our lower detection limit was 9×10^2 CFU with a sharpshooter head background.

Our preliminary attempts to detect *X. fastidiosa* in *H. coagulata* that were allowed to feed on infected grapevines have shown inconsistent results (Table 2). Several issues need to be explored. First, bacterial titer in *H. coagulata* that have acquired it frequently may be lower than our detection limits. Second, bacteria may be "trapped" in areas of *H. coagulata* mouthparts or foregut in ways that inhibit extraction. Third, interfering substances in the insect may inhibit extraction or PCR. Further studies will focus on these possibilities to optimize detection of *X. fastidiosa* in *H. coagulata*.

Table 1: Aspects of PCR kits examined for the detection of Xylella fastidiosa.

Kit/Procedure	Company	LYSIS	DNA Capture	Time (24 preps)	Cost /sample
GeneClean	Bio 101	SDS-Detergent	Silica Glassmilk	1.0 hr	$1.00
Dneasy tissue	Qiagen	Proteinase K	Silica Gel Membrane	1.0 hr	$2.00
DNAzol	MRC Inc.	guanidine-detergent	Phase Separation	1.5 hr	<$1.00
FTA genecard	Whatman	Chemical[2]	None	<20 min	<$1.00
gDNA Blood mini	Eppendorf	SDS/Proteinase K	Silica Gel Membrane	1.0 hr	$1.00
DNA extraction	Fermentus	Chemical[2]	Silica glass beads	1.5 hr	$1.00
Cell Lytic	Sigma	Lysozyme/SDS/Chemical	None	1.5 hr	<$1.00
Single Fly[1]	N/A	Proteinase K	None	1.0hr	<$1.00
Phenol Extract	N/A	Phenol	Phase Separation	Varies	<$1.00

[1]Procedure that uses proteinase K for lysis and directly to PCR
[2]Proprietary information

Table 2: Detection of DNA and Xylella fastidiosa (X.f.) by PCR kits examined.

Method	Company	Cultured *X. fastidiosa*		Cultured X.f. + *H. coagulata*		Field Collected *H. coagulata*		X.f. from *H. coagulata*	
		DNA	X.f.	DNA	X.f.	DNA	X.f.	DNA	X.f.
GeneClean	Bio 101	Yes	+	Yes	+	Yes	-	Yes	-
Dneasy tissue	Qiagen	Yes	+	Yes	-	Yes	-	Yes	-
DNAzol	MRC Inc.	Yes	+	Yes	+	Yes	-	Yes	-
FTA genecard	Whatman	Yes	+	Yes	-	Yes	-	Yes	-
gDNA Blood mini	Eppendorf	Yes	+	Yes	+	Yes	-	Yes	-
DNA extraction	Fermentus	Yes	+	Yes	+	Yes	-	Yes	+
Cell Lytic	Sigma	No	-	No	-	No	-	No	-
Single Fly	N/A	Yes	+	Yes	-	Yes	-	Yes	-
Phenol Extract	N/A	Yes	+	Yes	+	Yes	-	Yes	-

Samples were from cultured *X. fastidiosa* (n=4), cultured X. fastidiosa with Homalodisca coagulata adult heads extracted with liquid nitrogen (n=8), *H. coagulata* heads from adults collected in citrus and extracted as above (n=4), and heads extracted as above from *H. coagulata* adults allowed to feed for 4 days on grapevines infected with *X. fastidiosa* (n=4). PCR used primers set 31 and 33 (Minsavage et al. 1994).

REFERENCES

Bethke, J.A., M.J. Blua and R.A. Redak 2001. Effect of selected insecticides on *Homalodisca coagulata* (Homoptera: Cicadellidae) and transmission of oleander leaf scorch in a greenhouse study. J. Econ. Entomol. 94: 1031-1036.

Blua, M.J., R.A. Redak, D.J.W. Morgan and H.S. Costa. 2001. Seasonal flight activity of two *Homalodisca* species (Homoptera: Cicadellidae) that spread *Xylella fastidiosa* in southern California. J. Econ. Entomol. 94: 1506-1510.

Minsavage, G.V., C.M. Thompson, D.L. Hopkins, et al. (1994). Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathol. 84: 456-461.

Redak, R.A. and M.J. Blua. 2001. Impact of layering control tactics on the spread of Pierce's disease by the glassy-winged sharpshooter. Proceedings of the Pierce's Disease Research Symposium, M. Athar Tariq, Stacie Oswalt and Tom Esser (eds.), California Department of Food and Agriculture, Sacramento, CA, pp. 109-110.

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