

Progress Report APP# 91941

Title: Etiology and Diagnosis of Eutypa Dieback in Grapes in the Midwest

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Project Objectives:

- 1.) Characterize and determine the pathogenicity of *Eutypella vitis*
- 2.) Compare symptoms produced by *El. vitis* and *E. lata* in major grape cultivars
- 3.) Optimize field sampling protocol using PCR diagnostic method

Results to date

1) Characterize and determine the pathogenicity of *El. vitis*

Forty-one isolates of *Eutypella vitis* from Michigan, and 12 isolates of *E. lata* (11 from Michigan and 1 from California) were screened for pathogenicity on potted 'Concord' canes. Healthy, 1-year-old canes with two intact nodes were collected from 'Concord' vineyards in December 2005 and January 2006. For inoculation, a cordless drill was used to make a 3-mm-diameter hole approximately 2 cm below the upper node of each cane. A plug of agar with mycelium was inserted into the hole and sealed with parafilm®. The bottom node was cut and dipped in rooting powder and the cane was planted in a mixture of 2 parts sand and 1 part potting mix in an 8-inch pot. Four canes inoculated with the same isolate were planted in each pot with a total of 4 pots (16 canes) for each isolate. The negative control consisted of canes inoculated with a sterile plug of PDA, and an untreated control consisted of canes that were kept intact and not drilled. The pots were placed in a randomized complete block design and maintained under greenhouse conditions for 6 months. After disease severity evaluation, the bark was removed from the cane, and the length of tissue necrosis around the site of inoculation was measured. Tissue was removed approximately 5 mm above and below the edge of the site of inoculation, surface sterilized, and plated on ampicillin-amended PDA. The presence or absence of *E. lata* or *El. vitis* was determined after 1 week of incubation based on morphological characteristics.

Pathogenicity screening of *El. vitis* isolates resulted in a wide range of lesion sizes (5.1 to 10.3 mm) with xylem necrosis characteristic of Eutypa dieback. Among *E. lata* isolates, E30, an isolate from California was the most virulent, causing a lesion of 16.8 mm in length. Michigan isolates of *E. lata* and *El. vitis* fell within the same range with respect to lesion size. When viewed in cross section, canes infected by isolates of *El. vitis* displayed wedge-shaped areas of necrosis, characteristic of the cankers formed by *E. lata*. We were also able to recover nearly all of the isolates from necrotic tissue on agar plates with the exception of two isolates (EV74 and EV344).

In the spring of 2006, we inoculated mature and 2-year-old 'Concord' vines in vineyards with ascospores obtained from stromata of both *El. vitis* and *E. lata*. This was done to more closely mimic the natural infection process. These vines will be analyzed for xylem necrosis and canker development in the summer of 2007.

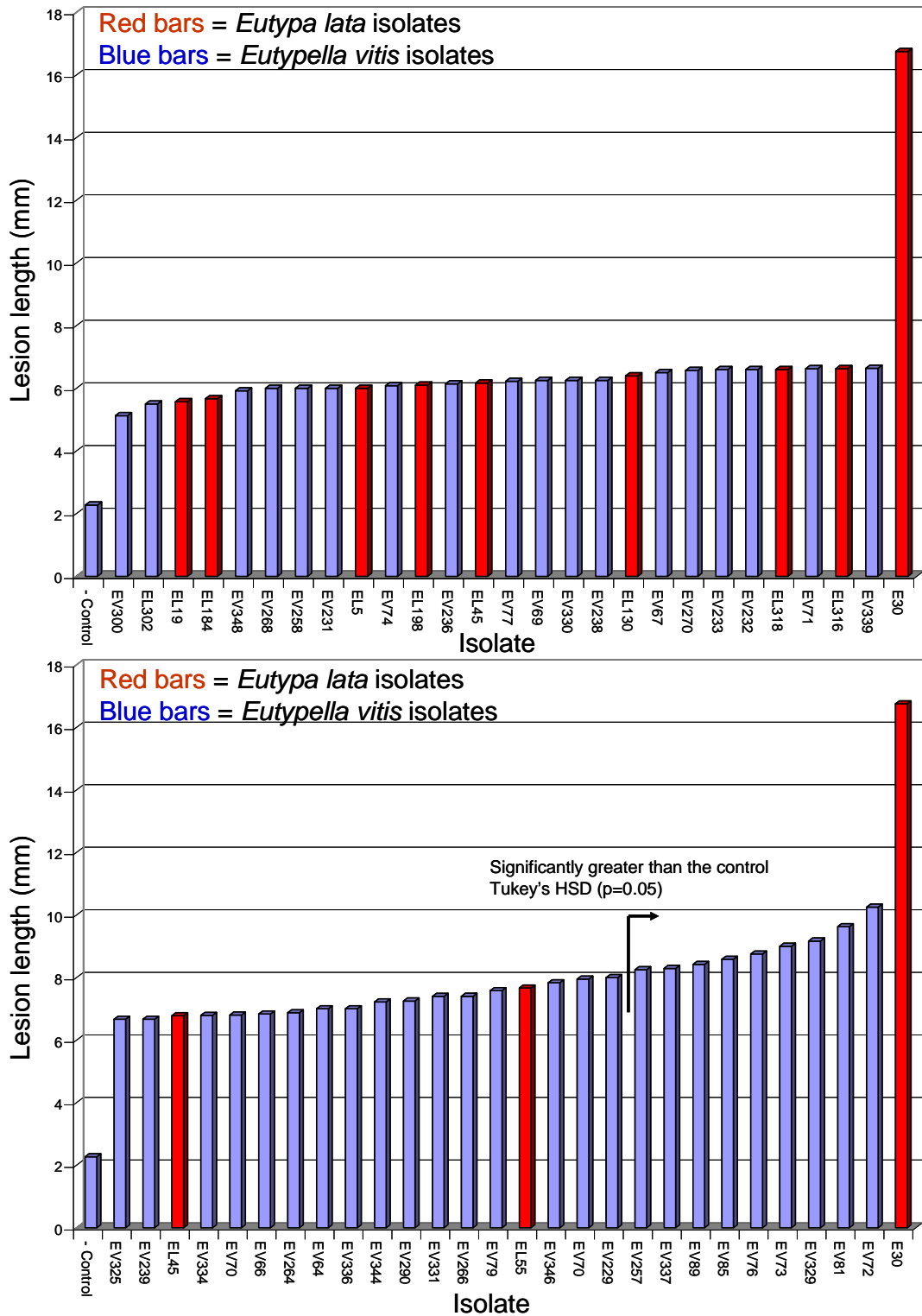


Figure 1. Lesion development on potted ‘Concord’ canes six months after inoculation with isolates of *Eutypella vitis* and *Eutypa lata*. Average length (mm) of necrotic lesions with the size of the inoculation hole (3-mm diameter) removed. Red bars represent isolates of *E. lata* and blue bars represent isolates of *El. vitis*. Isolates significantly greater than the control are to the right of the bar and arrow (Tukey’s HSD, p=0.05)

2) Compare symptoms produced by *El. vitis* and *E. lata* in commonly grown grape cultivars

The isolates EV73, EV257, EV339, EV70, EL45, EL55, and E30 were selected for their greater virulence based on the amount of necrosis and disease severity shown on ‘Concord’ potted cuttings inoculated in the winter of 2004-2005 (see previous progress report, January 6th, 2006) These isolates were used to inoculate dormant potted canes of the cultivars, ‘Concord’, ‘Niagara’, ‘Seyval’, ‘Vignoles’, Chardonel’, ‘Chardonnay’, and ‘White Riesling with the same experimental design and technique as described previously. After six months, no foliar symptoms were visible on any cultivar. Vines were harvested to see if there lesions and if these differed in size among cultivars. ‘Niagara’ appeared to be the most resistant to infection by both *E. lata* and *El. vitis*, whereas ‘Concord’ was relatively the most susceptible to infection by both species (Table 1). The other cultivars were intermediate in their susceptibility, but generally showed longer lesion lengths in response to *E. lata* (primarily due to E30, which appeared more virulent than Michigan isolates of *E. lata*).

Table 1. Lesion development on potted grape canes six months after inoculation with isolates of *Eutypella vitis* and *Eutypa lata*. (Lesion length measured in mm)

Isolate ^Z	Cultivar ^{xy}						
	N	S	Cl	R	V	Cy	Cn
Control	1.1 a	1.7 a	1.6 ab	2.0 a	1.7 ab	1.3 a	1.7 a
EV70	2.2 ab	3.3 bc	1.5 a	4.7 c	2.3 ab	2.2 ab	8.8 bcd
EV73	2.1. ab	4.7 e	2.3 ab	3.8 bc	1.0 a	2.9 ab	7.4 bdc
EV257	3.3 b	2.0 a	2.4 ab	2.9 ab	4.8 cd	4.5 b	7.8 cd
EV339	1.1. a	3.5 cd	3.8 ab	4.8 cd	5.0 cd	3.0 ab	6.3 bcd
EL45	1.3 a	2.1 ab	2.5 ab	4.8 cd	7.3 d	2.9 ab	6.8 bcd
EL55	1.9 ab	3.4 bdc	2.9 b	6.1 e	3.2 bc	3.1 ab	5.6 bc
E30	3.0 b	4.5 cde	10.5 c	11.5 f	12.0 e	12.4 c	16.5 e
Average							
<i>El. vitis</i>	2.0	3.3	2.5	3.1	4.1	3.3	7.5
<i>E. lata</i>	2.1	3.2	5.5	5.9	7.3	7.5	9.4

^x Cultivars; N=Niagara, S=Seyval, R=White Riesling, Cy=Chardonnay, Cl=Chardonel, V=Vignoles, Cn=Concord

^y Significance assigned using Tukey’s HSD (p=0.05)

^z Isolates with an EV designation are *Eutypella vitis*, isolates with an EL or E designation are *Eutypa lata*

3) Optimize field sampling protocol using PCR diagnostic method

The classical method for diagnosing *E. lata* is culturing the fungus from wood taken directly from a canker, but this method often takes between several weeks to a month before the cultures are morphologically distinguishable enough to identify. Also, once the cultures are mature, it is impossible to distinguish *E. lata* from *El. vitis*. This method can also be complicated by the presence of saprophytic fungi in infected wood that outgrows and overtakes *E. lata* in culture. By using a PCR technique to detect the presence of *E. lata* and *El. vitis*, we are able to reliably distinguish between both *E. lata* and *El. vitis* and other organisms in less than 2 days. An example of the PCR results from sampled vines is shown in Figure 2.

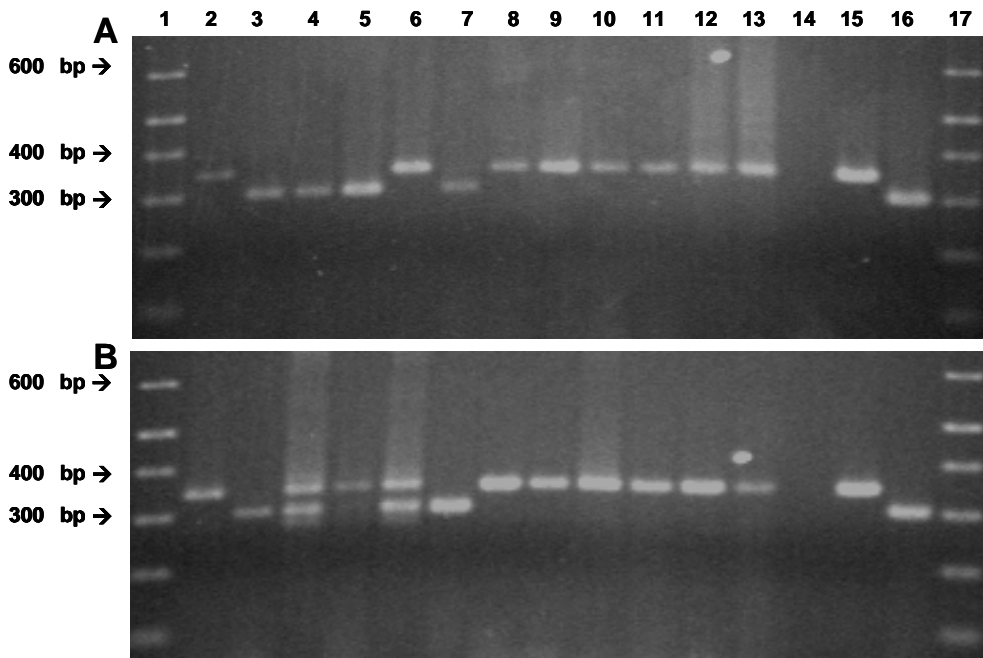


Figure 2. Agarose gel showing the detection of *Eutypa lata* and *Eutypella vitis* in cankers without visible stromata from naturally infected grapevine using nested multiplex PCR, A) PCR products from the centers of the cankers and B) PCR products from the margins of the cankers. Lane 1, 17: 1KB+ DNA ladder; lanes 2-13: wood samples from grapevine cankers without visible stromata; lane 14: wood sample from apparently healthy vine (negative control); lane 15: *E. lata* DNA (positive control); lane 16: *El. vitis* DNA (positive control). The presence of *E. lata* is indicated by a 350 bp band. The presence of *El. vitis* is indicated by a 300-bp band.

One hundred and nine symptomatic vines from 2 vineyards in Southwest Michigan were sampled and tested for the presence of *E. lata* and *El. vitis* using our nested multiplex PCR technique. Forty seven of the vines were sampled directly from a region of the canker with fruiting bodies in stromata. Fifty two of the vines were sampled twice from cankers with no apparent fruiting bodies, once in the margin of the canker and once in the center. Results are shown in Table 2. Approximately half (26 of 47) of the cankers with stromata were positive for the presence *El. vitis*, more than twice that of *E. lata* (12 of 27). Five of the 47 cankers contained both fungi. Cankers of without fruiting bodies tested positive primarily for *E. lata* (48 of 52 cankers) with very little *El. vitis* (13 of 52). When *El. vitis* was present, it was likely found with *E. lata* (11 of 52 cankers). There was little difference between the margins and the centers of the cankers when it came to detecting either fungus.

Previous sampling with wood chips from cankers plated in agar was successful about 25% of the time. Our PCR technique has reliably giving a positive identification for 90% of the samples. This research has demonstrated that the PCR technique is a valuable tool in detecting and diagnosing *Eutypa* dieback. We have sampled several other vineyards and are in the process of PCR analysis. We hope to continue to show that this method is both faster and more reliable than previous detection methods

Table 2. Detection of *Eutypa lata* and *Eutypella vitis* in naturally infected, symptomatic grapevines (*Vitis labrusca* ‘Concord’) using multiplex nested PCR.

	Isolation Success Ratio	Detection of fungal species by PCR ^a		
		<i>Eutypa lata</i>	<i>Eutypella vitis</i>	both
Cankers with stromata^b	43/47	12/47	26/47	5/47
Cankers without stromata				
<i>Vineyard A</i>				
PCR of wood from margin of canker	30/32	20/32	2/32	7/32
PCR of wood from center of canker	29/32	21/32	2/32	6/32
<i>Vineyard B</i>				
PCR of wood from margin of canker	17/20	16/20	0/20	1/20
PCR of wood from center of canker	17/20	13/20	0/20	4/20

^a Positive detection based on presence of PCR amplification product of the expected size for *E. lata* and *El. vitis* (350 bp and 300 bp, respectively)

^b Cankers sampled exclusively from Vineyard A

APPENDIX

Impact Statement:

This research so far has contributed to a better understanding of the potential role of *Eutypella vitis* in Eutypa dieback, a chronic and serious disease of grapes, though further research is needed. This also included field evaluation and optimization of a newly developed PCR (polymerase chain reaction) method for timely and accurate diagnosis of Eutypa dieback. This research is aimed at helping growers increase the longevity of their vineyards and productivity of the vines, contributing to the economic viability of grape production in the Great Lakes Region.

Publications/presentations

A poster entitled “*Eutypella vitis*, a potential pathogen of grapevines in Michigan” was developed from information gained in this study and displayed at the national American Phytopathological Society meeting in 2005 in Austin, Texas (Jordan, S., and Schilder, A. 2005. *Eutypella vitis*, a potential pathogen of grapevines in Michigan. Phytopathology 95:S51). The poster was also presented at the 2005 Great Lakes Fruit, Vegetable and Farm Market EXPO, in Dec. 2005 in Grand Rapids, Michigan.

An oral presentation entitled “Characterization of *Eutypella vitis*, a potential pathogen of grapevines” was presented at the 5th International Workshop on Grapevine Trunk Diseases on the campus of the University of California at Davis on September 11th, 2006.

Once the project is completed, we plan to submit a manuscript entitled: “Morphological, genetic and pathogenic characterization of *Eutypella vitis*, a pathogen of grapevines” to Plant Disease.