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Ethylene and Structure-Function Relations of *Xylella fastidiosa* in *Vitis vinifera* in Pierce's Disease in Plants

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*Xylella fastidiosa*a, a xylem-limited rickettsia-like microorganism, is responsible for Pierce's disease in grape vines and citrus chlorosis in citrus trees. The disease results in a slow, but significant water loss due to plugging of the xylem by tyloses and gums. Previously, it had been thought that the pathology is simply a result of physical plugging of the xylem by the bacteria. However, ethylene, a gaseous plant hormone, has been implicated in the etiology of Pierce's disease and other plant pathologies [1, 2]. We examined the ultrastructural pathology of field grown, naturally infected and Ethiphon®, precursor of ethylene, treated uninfected grape vines, *Vitis vinifera*, to verify a role for ethylene stimulation of tyloses and gums in the pathology of Pierce's disease by immunocytochemical localization of 1-aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase), a biomarker for ethylene production.

Petioles of naturally infected *V. vinifera* were collected in the Texas A&M vineyard and fixed for transmission electron microscopy (TEM) as previously described [3, 4]. Uninfected plants raised in the greenhouse were treated with Ethiphon®, a precursor of ethylene, and then fixed for TEM. Thin sections were examined and the pathology was compared in both naturally infected and Ethiphon® treated plants. Sites of ACC oxidase were localized with goat anti-ACC oxidase (Santa Cruz Biotechnology, Santa Cruz, CA) followed by donkey anti-goat IgG secondary antibody labeled with 12 nm collodial gold (Jackson ImmunoResearch, West Grove, PA). Semi-quantitative data from colloidal gold counts were compared between the naturally infected, Ethiphon® treated uninfected and uninfected field grown plants.

ACC oxidase localized at low levels in uninfected petioles. There were tyloses in both naturally infected and Ethiphon® treated specimens and there was extensive localization of ACC oxidase as documented by colloidal gold immunocytochemistry (Figs. 1 and 2). Semi-quantitative analysis (Table 1) of the colloidal gold distribution indicated high levels of ethylene in the xylem of both naturally infected and in Ethiphon® treated uninfected petioles. Ethiphon® treated uninfected xylem had the highest levels of ACC oxidase (56.5 ± 3.28 gold particles/μm^2) that were higher than the naturally infected xylem (42.0 ± 2.57, 34.1 ± 1.27 gold particles/μm^2) while uninfected xylem had much lower levels (19.5 ± 9.76 gold particles/μm^2).

The immunocytochemical localization of ACC oxidase demonstrated the production of ethylene by the pathogen, *X. fastidiosa*, as well as by the host plant. Elevated levels of ethylene have been reported in a number of plant pathologies [2] and in the pathogens. In addition, ethylene is auto-catalytic and the production of ethylene by the pathogens can stimulate additional production of ethylene by the host plant as well. The evidence presented here indicates a role for increased production of tyloses and gums in response to increased ethylene production by both the pathogen, *X. fastidiosa*, and the host, *V. vinifera*.

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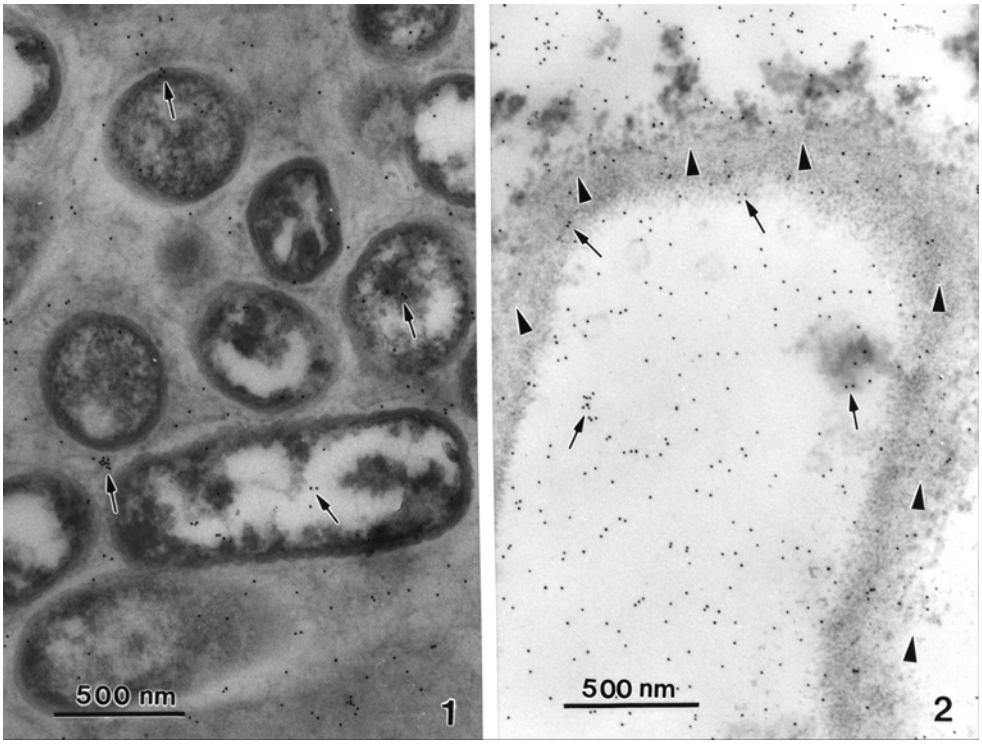

Fig. 1 Localization of ACC oxidase (arrows) by colloidal gold immunocytochemistry in xylem of naturally infected petiole.
Fig. 2 Localization of ACC oxidase (arrows) by colloidal gold immunocytochemistry in xylem of an uninfected, Ethiphon® treated petiole. Note the large tylose (arrowheads) in the uninfected xylem.

TREATMENT	Colloidal Gold Particles/μm^2
Ethiphon® Treated Uninfected (Chardonnay)	56.5 ± 3.28
Xylella Infected (LaRouge)	34.1 ± 1.27
Xylella Infected (Sangiovese)	42.0 ± 2.57
Uninfected, Untreated	19.5 ± 9.76

Table 1. Localization of ACC Oxidase in Xylem

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