J. Crop Prot. 2015, 4 (1):131-137

Research Article

First Report of *Xylella fastidiosa* associated with Oleander Leaf Scorch in Lebanon

Mirvat Temsah[1], Lucia Hanna[2] and Adib Saad[2]

1. Lebanese University, Beirut, Lebanon.
2. American University of Beirut, Beirut, Lebanon.

Abstract: Symptoms of the leaf scorch disease on the leaves of oleander infected by *Xylella fastidiosa* appearing for the first time in Lebanon were confirmed by ELISA tests and anatomical observations of sections of petioles by a scanning electron microscope. *Xylella fastidiosa* was detected in the xylem forming bacterial aggregates in the lumen of tracheary elements. The bacterium colonizes and invades the conducting tissues degrading pit membranes (PMs) of tracheary elements. Dense networks of fibrillar material and tyloses, originating from the plant, as a defense mechanism form around the bacterial aggregates to limit the movement of the pathogen. The bacterial aggregates that plug the tracheary elements and the tyloses produced by the infected oleander plants cause water stress and consequently leaf scorch symptoms. To the best of our knowledge, this is the first report of *Xylella fastidiosa* in Lebanon.

Keywords: oleander, Elisa, SEM, *Xylella*

Introduction

Xylella fastidiosa Wells *et al*., 1987 is a xylem-limited gram negative bacterium transmitted from one plant to another by xylem-sap feeding insects. The bacterium is mainly distributed in the USA where it was first discovered associated with Pierce's disease (PD) of grapevine in 1973. The bacterium causes water stress symptoms on a very wide host range including in addition to grapes, citrus (citrus variegated chlorosis (CVC) or citrus X disease), oleander (Oleander Leaf Scorch (OLS), alfalfa (alfalfa dwarf), peach (phony peach disease (PPD), plum (plum leaf scald or PLS), almond (almond leaf scorch or ALS), elm, coffee, sycamore, oak, maple, and pear. The pathogen can also be detected in hundreds of asymptomatic plant species (Chatterjee *et al*., 2008). Several pathogenic strains of the bacterium have been described, that are often host specific. For example, strains that cause Pierce's disease (PD) of grapevine do not infect peach, and peach strains do not cause disease in grapevines, but PD strains do cause disease in almond and alfalfa. This pathogen multiplies in the xylem forming bacterial aggregates that degrade the pit membranes (PMs). Bacterial aggregates restrict water movement in the xylem.

Nerium oleander L. plants at the American University (AUB) of Beirut Campus showed symptoms of leaf chlorosis, scorching and stunting similar to those expressed by the leaf

Handling Editor: Dr. Masoud Shams-Bakhsh

* **Corresponding author**, e-mail: asaad@aub.edu.lb
Received: 5 June 2014, Accepted: 24 November 2014
Published online: 10 December 2014

scorch disease and typical to those expressed by *Xylella fastidiosa* infections (Fig. 1).

In this study, the presence of *Xylella fastidiosa* in the petioles of affected leaves was confirmed by the ELISA tests and by in situ observations made in a scanning electron microscope (SEM). This is the first report of *Xylella fastidiosa* in Lebanon. So far, no research has been conducted in Lebanon to determine the principal insect vector(s) of this pathogen, however, leafhoppers were observed to be common on plants on the AUB campus.

Figure 1 Leaf scorch symptoms on oleander.

Materials and Methods

Petiole tissues (1g) were ground in the ELISA extraction buffer (Agdia) in a 1:10 ratio of plant material to buffer. Extraction buffer was used as a negative control for ELISA testing. Agdia positive control and serial dilutions of *Xylella fastidiosa* cells from culture were both used as positive controls. A sample was considered positive with ELISA if its absorbance reading was twice as great as the mean. Plates were read after 15 min at 490 nm on a BioTek™ ELx800™ Absorbance Microplate Reader (Bio-Tek Instruments). For SEM observations, cross and longitudinal petiole sections, less than 1mm in thickness, were cut with a razor blade and immediately fixed in pure methanol for 5 min. Sections were then washed in pure ethanol once and stored in pure ethanol until use (Schwab and Hulskamp, 2010). For observations with SEM, sections were mounted onto stubs with an adhesive copper tape on top. The edges of the tape were bordered with colloidal graphite. Finally, the samples were sputtered with gold-palladium and observed in a TESCAN VEGA3 SEM.

Results

Fifteen symptomatic samples of one gram petiole tissues were tested by ELISA for the presence of *Xylella fastidiosa*, four of these samples showed a positive reaction. Observations of cross sections of a healthy petiole of oleander leaf under SEM microscopy revealed the following tissues: an epidermis, a collenchyma, a parenchyma and the primary conductive tissues (xylem and phloem). The tissues of the petiole were comparable to those of the primary tissues of the stem. The vascular tissues form a continuous arc that opens towards the adaxial side of the petiole (Fig. 2). Vascular tissues of the petiole have one collateral bundle; a phloem on the abaxial side and xylem on the adaxial side (Esau, 1965). The primary xylem consists of tracheary elements, tracheids or protoxylem and a metaxylem. The walls of the tracheary elements are impregnated with secondary walls and have porous pit membranes (PMs). Pit membranes are composed of the middle lamella and adjacent primary cell walls that limit pits externally (Evert and Eichhorn, 2006). The water moves through the tiny openings of the PMs of a tracheary element to adjacent tracheary elements with minimal restrictions. Tracheids are narrow imperforated cells through which sap flows radially through the pit membranes of one cell to another. The metaxylem vessels are large in size with perforated plates forming open tubes. Water enters the ducts of the metaxylem and out by diffusion through the pit membranes (Siau, 1984; Tyree and Zimmermann, 2002).

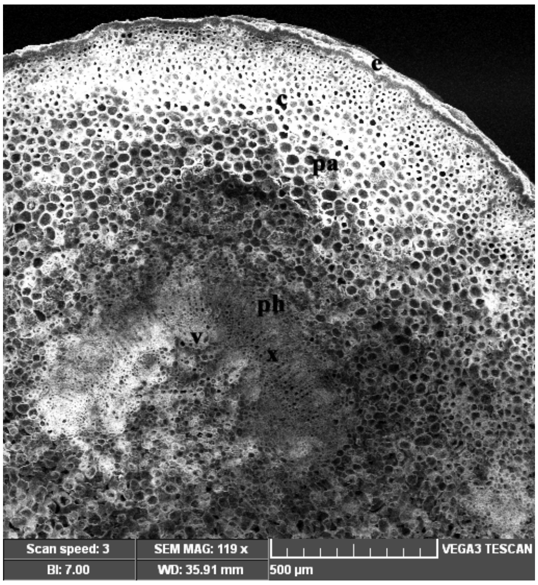

Figure 2 Cross section of a petiole showing vascular bundle tissues as observed by a scanning electron microscope, SEM. **e**, epidermis; **c,** collenchyma; **pa**, parenchyma; **v**; vascular bundle; **x**, xylem; **ph**, phloem.

Bacteria were observed in the lumen of tracheary elements grouped in clusters or aggregates (Fig. 3A and B). They were particularly abundant in the tracheids (Fig. 3B). PMs are perforated by openings in the infected xylem, while they are intact in the petioles of healthy plants. Further degradation was marked by expansion of openings or a partial or total loss of PMs where bacterial aggregates occupied several adjacent tracheary elements (Fig. 4). Bacterial aggregates were found to be surrounded by dense networks of fibrillar material connected to walls of the tracheary elements (Fig. 5A and B). In some tracheary elements, tyloses were observed to add to the dense networks of fibrillar material and block the spread of bacterial aggregates (Fig. 6).

No previous studies were carried out on the characteristics of the growth of *Xylella fastidiosa*, in situ in oleander using Scanning Electron Microscopy (SEM).

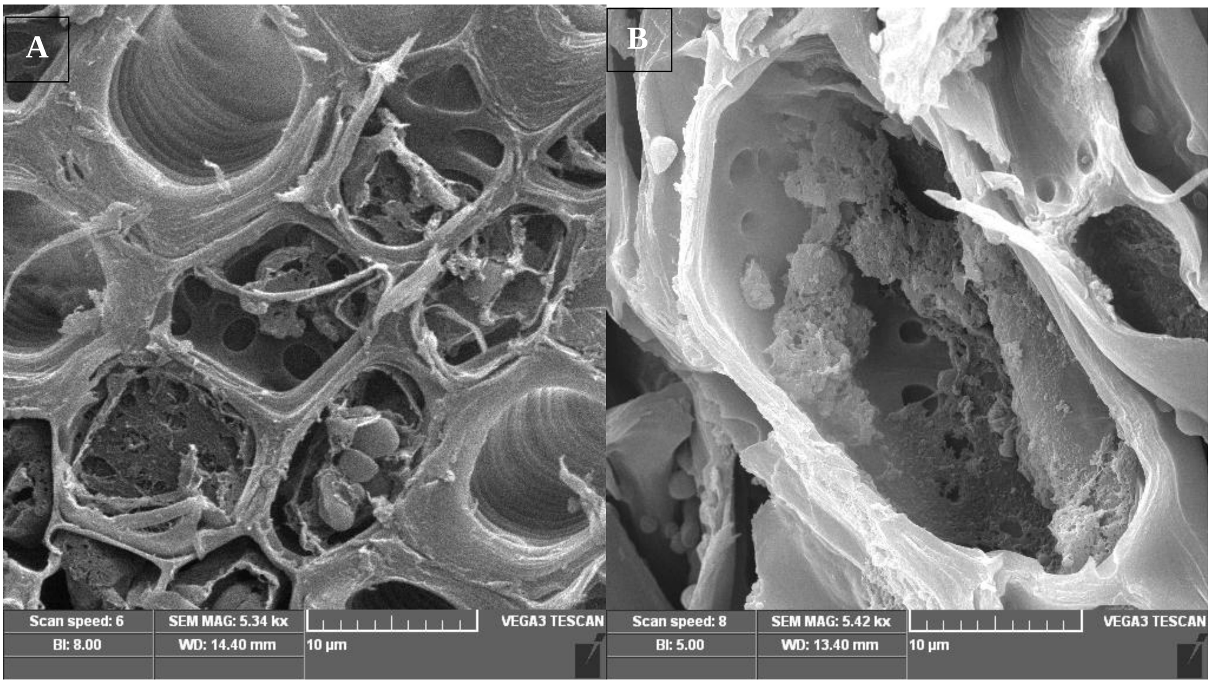

Figure 3 Compact bacterial aggregates in the lumen of tracheary elements of a petiole of oleander in a cross section (A) and in a longitudinal section of a tracheid (B). Some lumens of tracheary elements are partially or totally plugged.

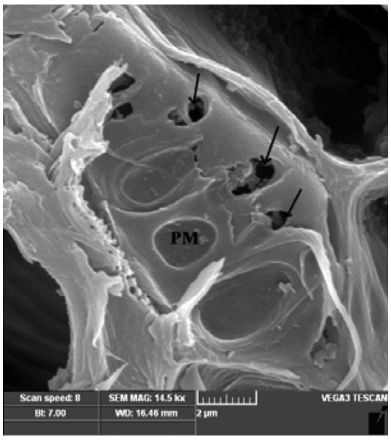

Figure 4 Longitudinal section of an infected tracheid with partially or completely degraded PMs (at arrows).

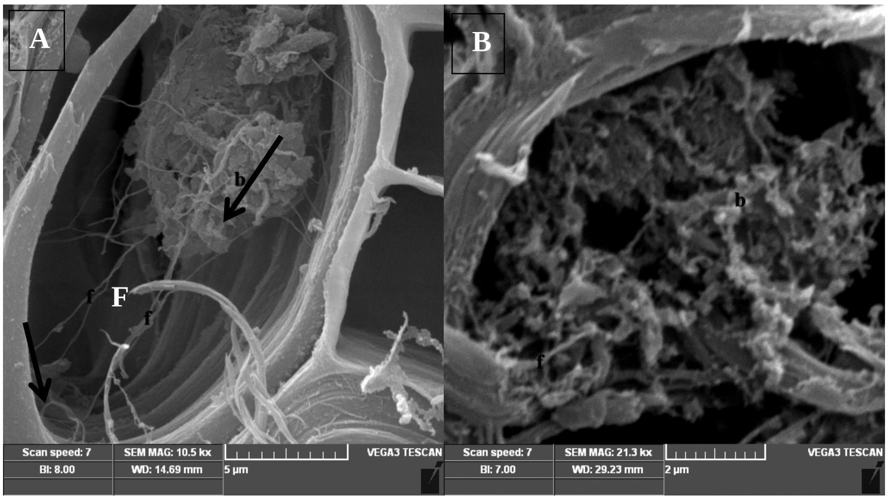

Figure 5 (A and B).Cross sections of lumens of tracheary elements showing bacterial aggregates and fibrillar material in close contact with the wall of a tracheary element (arrows). PM, pit membrane; b, bacteria; f; fibrillar material.

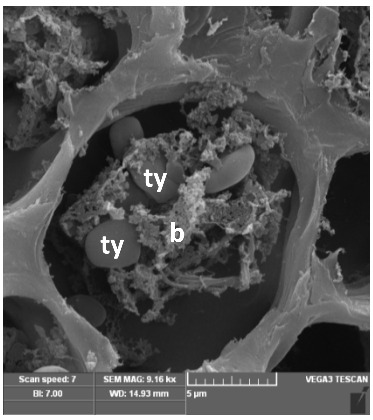

Figure 6 Tyloses, a dense network of fibrillar material, and bacterial clusters in the lumen of a tracheary element in a cross section of an infected petiole of oleander; ty, tyloses; b, bacteria.

Discussion

Xylella fastidiosa is a vascular pathogen in the tracheary elements of petioles of oleander leaves. Newman *et al*., 2003, indicated that for bacterial populations to become systemic, bacterial cells must move from a tracheary element to another through the PMs of two adjacent tracheary elements. The small pore size in PMs is a security mechanism to prevent the spread of bacterial pathogens from one tracheary element to another as the water moves throughout the vascular system (Sperry and Tyree, 1988; Tyree and Zimmermann, 2002). However, the rod shaped *X. fastidiosa* has a size of about 1000 nm, which makes it too large to pass freely through the small pores of PMs (size 5 to 20 nm) as indicated by Choat *et al*., 2003.

Oleanders are dicot plants where the primary walls of PMs are composed of cellulose microfibrils embedded in a polysaccharide matrix of homogalacturonans, xyloglucans and pectins (Carpita and Gibeaut, 1993). Degradation of PMs suggests the involvement of various extracellular enzymes produced by *X. fastidiosa*, such as the polygalacturonases, endo-1, 4-β-glucanases, xylanases, and xylosidases (Chatterjee *et al*., 2008). These enzymes degrade PMs thus expanding and increasing the size of the pores allowing *X. fastidiosa* to move freely from one tracheary element to another and systemically invade the xylem. This movement results from the inter vascular capacity of *X. fastidiosa* to digest PMs by enzymes and was also observed in the xylem of the stems of vines (Aguero *et al*., 2005). Roper *et al*., (2007) have shown that mutants of *X. fastidiosa* lacking the gene encoding polygalacturonases lose their pathogenicity. Accordingly, they indicated and concluded of the importance of polygalacturonases in the degradation of PMs and in the colonization and dissemination of *X. fastidiosa* in grape vines. This explains how a small population of virulent strains of *X. fastidiosa* introduced into oleander leaves by insect vectors can multiply and spread within oleander and cause the leaf scorch disease. Hopkins (1989) showed that the development of symptoms of Pierce's disease in the veins of leaves, petioles and stems of grapevines was in correlation with the concentration of *X. fastidiosa* or its products in the xylem of the host.

The water flow in the tracheids is slower than that of the metaxylem vessels, since a tracheid diameter is small (about 10 microns). This may explain the abundance of *X. fastidiosa* in the cells of tracheids where nutrient media move at a constant flow rate.

The dense network of fibrillar material of the exopolysaccharide protective layer, synthesized by the walls of the tracheary elements and the tyloses formed by contact cells (parenchyma cells giving rise to tracheary elements) immobilize bacterial populations and limit their spread. These structures produced by the plant, therefore, act as a physical barrier to invasions by systemic vascular pathogens.

It has been shown that the plugging of tracheary elements of diseased plants is due to the production of tyloses and gums (Mollenhauer and Hopkins, 1976). Moreover other materials such as globular material and networks of fibrillar material (Greta *et al*., 1984), and other products are secreted by the host to restrict the movement of pathogens. Such a phenomenon contributes to the appearance of leaf scorch symptoms of infected plants.

References

Aguero, C. B., Uratsu, S. L., Greve, C., Powell, A. L. T., Labavitch, J. M., Meredith, C. P. and Dandekar, A. M., 2005. Evaluation of tolerance to Pierce's disease and Botrytis in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. Molecular Plant Pathology, 6: 43-51.

Carpita, N. C. and Gibeaut, D. M., 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant Journal, 3: 1-30.

Chatterjee, S., Almeida, R. P. and Lindow, S., 2008. Living in two Worlds: the Plant and Insect Lifestyles of *Xylella fastidiosa*. Annual Review of Phytopathology, 46: 243-71.

Choat, B., Ball, M., Luly, J. and Holtum, J., 2003. Pit membrane porosity and water stress-induced cavitation in four co-existing dry rainforest tree species. Plant Physiology, 131: 41-48.

Esau, K., 1965. Plant Anatomy. John Wiley and Sons, New York.

Evert, R. F. and Eichhorn, S. E., 2006. Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. John Wiley and Sons, New York.

Greta, E. T., Stojanovic, B. J., Kuklinski, R. F., DiVittorio, T. J. and Sullivan, M. L., 1984. Scanning electron microscopy of Pierce's disease bacterium in petiolar xylem of Grape leaves. Phytopathology, 75: 264-269.

Hopkins, D. L., 1989. *Xylella fastidiosa*: Xylem limited bacterial pathogen of plants. Annual Review of Phytopathology, 27: 271-290.

Mollenhauer, H. H. and Hopkins, D. L., 1976. Xylem morphology of Pierce's disease infected grapevines with different levels of tolerance. Physiological Plant Pathology, 9: 95-100.

Newman, K. L., Almeida, R. P. P., Purcell, A. H. and Lindow, S.E., 2003. Use of a green fluorescent strain for analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. Applied and Environmental Microbiology, 69: 7319-7327.

Roper, M. C., Greve, L. C., Warren, J. G., Labavitch, J. M. and Kirkpatrick, B. C., 2007. *Xyllela fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. Molecular Plant Microbe Interactions, 20: 411-419.

Schwab, B., and Hulskamp, M., 2010. Quick and easy fixation of plant tissues for scanning electron microscopy. Cold Spring Harbor Protocols 2010, 8: 1. *doi:10.1101/pdb.prot4934.*

Siau, J. F., 1984. Transport Processes in Wood. Springer-Verlag, New York.

Sperry, J. S. and Tyree, M., 1988. Mechanism of water stress-induced xylem embolism. Plant Physiology, 88: 581-587.

Tyree, M. T. and Zimmermann, M. H., 2002. Xylem Structure and the Ascent of Sap, 2nd ed. New York: Springer-Verlag.

اولین گزارش از ارتباط *Xylella fastidiosa* با سوختگی برگ درختچه‌های خرزهره در لبنان

میروات تمساه[1]، لوسیا حنا[2] و ادیب سعد[2]*

۱- دانشگاه لبنان، بیروت لبنان.
۲- دانشگاه امریکایی بیروت، بیروت، لبنان.
* پست الکترونیکی نویسنده مسئول مکاتبه: asaad@aub.edu.lb
دریافت: ۱۵ خرداد ۱۳۹۳؛ پذیرش: ۵ آذر ۱۳۹۳

چکیده: ظهور علائم سوختگی برگ خرزهره ناشی از آلودگی باکتری *Xylella fastidose* با آزمون‌های الایزا و بررسی‌های آناتومیکی مقاطع دمبرگ گیاه به‌وسیله میکروسکوپ الکترونی نگاره برای اولین بار در لبنان به اثبات رسید. *X. fastidiosa* به‌شکل توده‌های باکتریایی در مجرای عناصر تراکئیدی گیاه مشاهده شد این باکتری به لوله‌های آوند چوبی گیاه رخنه کرده غشاء گودک‌های عناصر تراکئیدی را تخریب می‌کند. در اثر مکانیسم دفاعی گیاه شبکه‌های متراکمی از الیاف فیبری و تیلوز توسط گیاه در اطراف توده باکتریایی تشکیل می‌شود تا انتشار باکتری بیمارگر محدود شود. توده‌های باکتریایی که عناصر تراکئیدی را مسدود می‌کنند و نیز تولید تیلوز توسط گیاه میزبان آلوده سبب تنش کم آبی و در نتیجه بروز علائم سوختگی برگ می‌شوند. براساس اطلاعات نگارندگان این اولین گزارش از وجود *X.fastidiosa* در کشور لبنان است.

واژگان کلیدی: خرزهره، الایزا، SEM، باکتری سخت‌کشت