ULTRASTRUCTURAL CHANGES
IN ASTER YELLOWS PHYTOPLASMA
AFFECTED *LIMONIUM SINUATUM* MILL. PLANTS.
I. PATHOLOGY OF CONDUCTING TISSUES

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ABSTRACT

Changes in anatomy and cytology of conducting tissues of *Limonium sinuatum* Mill. plants affected by aster yellows phytoplasma were investigated. In the phloem tissues of affected plants stem necrosis takes place. In necrotic regions no sieve tubes were observed only necrotic cells and parenchyma cells. The sieve tubes present on the border of necrosis showed collapsed walls and were rich in vesicles.

Phytoplasma cells were observed in sieve tubes present in nonnecrotic regions of the phloem. Various structural changes in sieve elements were investigated. The endoplasmic reticulum cisternae were often localised in the lumen of the sieve element without contact with the walls. Such localisation of endoplasmic reticulum was never observed in healthy plants. Vesicles of different size, fuzzy material and clumping of p-proteins were characteristic for sieve elements from nonnecrotic part of phloem. No correlation with the sieve tube structure and the appearance of phytoplasma in a single sieve element was found.

In control plants of *L. sinuatum* phloem observed were phloem parenchyma cells with spiny vesicles (SV). In infected plants there were a remarkable increase in cells with SV. Also the SV itself had not only a vesicular but also a tubular or extended cistern shape.

KEY WORDS: phytoplasma, aster yellows, phloem structure.

INTRODUCTION

Phytoplasmas are procaryotes belonging to the class Mollicutes. They cause serious diseases in a wide variety of dicotyledonous and monocotyledonous plants (McCoy et al. 1989). Alto phytoplasmas generally are confined to the sieve tube elements of infected plants (Esau 1977), there are little observations about influence of the pathogen to the sieve tubes structure. General changes observed in numerous plant species infected by phytoplasma are phloem necrosis (Dyer and Sinclair 1991; Braun and Sinclair 1976; Braun and Sinclair 1979; Dijkstra and Van der Vant 1970; Hiruki and Dijkstra 1973; McGovern et al. 1989a, b). The cells from that region collapsed and necrotised. The phytoplasmas were also observed in sieve tubes in nonnecrotic phloem (Pisti and Bellardi 1990; Bertaccini and Marani 1980; Chang et al. 1992; Hwang et al. 1997). These sieve tubes had little or no structural changes in comparison with to healthy plants. Parthasarathy (1974) has not observed changes in sieve tubes structure of palm phloem infected by phytoplasma. He mentioned only vesi-

cles of unknown origin present in sieve tubes lumen. Schlag and Gal (1996) found no influence of phytoplasma on nacreous layer of sieve – element walls in apple trees. Only Esau (1977) observed changes in sieve tube plastid structure.

Our examination of *Limonium sinuatum* Mill. with proliferation and virescence symptoms ultra thin sections revealed phytoplasma resembling those found in other countries (Muller et al. 1973; Baker et al. 1983; Chang et al. 1996). The PCR investigations suggest that the disease in Poland was caused by aster yellows phytoplasma (Kamińska et al. 1996, 1999). The ultrastructure of infected plants phloem compared to phytoplasma presence was the subject of examination presented in these paper.

MATERIAL AND METHODS

Diseased and asymptotic plants of annual statice *Limonium sinuatum* Mill. were collected from field plantations, potted and maintained in an insect-proof greenhouse.

The diseased plants show leaves and flowers discolouration and malformation, shoots dieback, proliferation and flowers virescence.

Specimens for electronmicroscopical examination were fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer pH = 7.2, for 4 h. Postfixation in 1% osmium tetroxide for 2 h at 4°C was applied. The material was dehydrated in concentration gradients of ethanol (10-70%), acetone (70-100%) and embedded in Epon. The ultrathin sections on copper grids were stained with uranyl acetate and lead citrate. Observations were taken on a JEM 1220 electron microscope in the Laboratory of Electron Microscopy Warsaw Academy of Agriculture.

RESULTS

Anatomy of control and infected Limonium sinuatum Mill. plants stem

Healthy *Limonium sinuatum* Mill. plants in a cross section of the flowering stem showed a regular ring of collateral beams situated in the stem stele, and little beams without cambium in the cortex. Under the epidermis a very distinct region of assimilating parenchyma was seen.

In infected plants in the part showing witch's broom symptoms the beams were not separated one from the other by parenchyma cells. The vascular tissues of the stem were changed and the fascicles had not a regular shape. In the phloem part there were numerous necrosis situated generally in the outer part of the phloem on the region of the protophloem. Signs of irregular divisions were observed. The regions of divided cells extend even from the phloem of main bundles to bundles situated in the cortex.

Changes in sieve tube structure

No sieve tubes were observed in the necrotic part of the phloem (Figs 1, 2). Only sometimes parenchyma cells could be distinguished in this region although in most cases all cells were necrotised. The sieve tubes present in the border of necrosis showed collapsed walls and were rich in vesicles and with dark osmophilic inclusions present in their protoplast.

In nonnecrotic parts of the phloem phytoplasmas were seen in almost all sieve tubes (Figs 3, 4, 5). Most sieve tubes had only a few phytoplasma cells seen in the cross section situated near the cell wall of the sieve element and especially near the sieve plate (Fig. 3). Most phytoplasma cells were oval in shape, although some elongated and branched structures were seen. All of them had a very distinct middle part of a cell with nucleoid and a narrow outer one with ribosomes. The phytoplasma cells were surrounded with three-layered external membrane (Fig. 4). Sieve tubes filled with tightly packed phytoplasma were much less numerous (Fig. 5) and the phytoplasmas from these sieve elements were oval or round, never elongated.

The structure of the observed sieve tubes varies. Some of them from the nonnecrotic part of the phloem do not show much difference in structure from those of control plants (Fig. 3). Although they possess phytoplasmas in their protoplast, the p-proteins structure and distribution in the sieve tube seems not changed in comparison with healthy plants. Other sieve elements had multivesicular structures and big

vesicles were observed in the lumen of sieve tubes (Figs 6, 7, 8). These vesicles vary in number and size. Sometimes the vesicles were so big, that they filled the whole lumen of the sieve tube. Other sieve tubes were filled with fine fibrillar material (Figs 9, 10). These fuzzy deposits were distributed randomly in the sieve element lumen changing its structure. Only the presence of endoplasmic reticulum cisternae typical for sieve tubes permit us to state that it was in fact a sieve tube. In those sieve tubes no p-protein was seen, neither vesicles mentioned before (Fig. 10).

Sieve tube endoplasmic reticulum from *L. sinuatum* Mill. control plants was situated near the longitudinal walls of the sieve tube with a parallel arrangement of the cisterns to the sieve tube wall. It was composed of 3-5 cisternae. Sieve tube endoplasmic reticulum from infected plants had often changed the orientation to the sieve wall (Fig. 11) and sometimes was situated in the lumen of the sieve tube without contact with the sieve tube walls (Fig. 12). Such a localisation of endoplasmic reticulum was never observed in control plants. The endoplasmic reticulum cisternae were sometimes in close contact with the phytoplasma (Fig. 12).

L. sinuatum Mill. sieve tube plastids were of the s-plastid type. In diseased plants very few of them seem to be intact. Others show breakages of outer membranes, and sometimes only starch granules appeared in the sieve element lumen (Fig. 13).

Most of the sieve tubes did not show changes in the structure of the sieve tube plate. Beside the phytoplasma present in the neighbourhood of the sieve pores and even in their lumen the sieve tube plate pores were not plugged, but had only thin deposits of callose around the pores. But near the necrotising phloem zone the plugging of sieve plate pores with callose was observed (Fig. 14). Also the outgrowths of the vertical sieve tube wall, plugging the sieve plate pores, took place (Fig. 15).

No correlation between the sieve tube structure and the appearance of phytoplasma cells and the number of them present in a single sieve element was observed.

The phloem parenchyma cells with spiny vesicles

In control plants of *L. sinuatum* phloem observed were parenchyma cells with spiny vesicles (SV). These structures were a type of small vesicles surrounded by spines, with one end bound in the membrane of the vesicle (Fig. 16). The tubular nature of these spines could be seen in some sections. The cells with SV had small vacuoles and endoplasmic reticulum cisternae, as well as dictyosomes. Their plastids were small, with a few tylacoids and sometimes starch granules. In SV cells there were sporadically p-protein present, but more often SV were not accompanied by p-proteins. The cells with SV were situated in the protophloem.

In infected plants there was a remarkable increase of cells with SV (Figs 17, 18). They appear in different parts of the phloem, in the outer part of the bundle, as well as in the phloem situated near the xylem. In these cells there were large regions of cytoplasm filled with SV (Fig. 18). Also the SV itself had not only vesicular but also a tubular or extended cistern shape (Figs 18, 19). The structure of spines and the way they were joined with the membrane was the same in infected and control plants. No phytoplasma was seen in the cells with SV.

Fig. 1. The necrosis of phloem composed of collapsed cells with strongly curved cell walls and dark, homogenous protoplast. Phytoplasma infected *L. sinuatum* plant. Magn. 20 000 $\times$, bar represents 1 μ m.

Fig. 2. A fragment of phloem tissue. Sieve element (SE) with the phytoplasma on the left and the necrotic cells on the right (star). Phytoplasma infected *L. sinuatum* plant. Magn. 10 000 $\times$, bar represents 1 μ m.

Fig. 3. Sieve elements with phytoplasma (stars) situated mostly near the sieve plate (SP). Phytoplasma infected *L. sinuatum* plant. Magn. 7 000 $\times$, bar represents 2 μ m.

Fig. 4. A group of phytoplasma cells from a sieve tube. In the middle part of phytoplasma seen nucleoid (star) and a narrow outer part with ribosomes. The phytoplasma cells were surrounded with three-layered external membrane (arrow). Phytoplasma infected *L. sinuatum* plant. Magn. 90 000 $\times$, bar represents 100 nm.

Fig. 5. Sieve tubes filled with tightly packed phytoplasma (P). Phytoplasma infected *L. sinuatum* plant. Magn. 17 700x, bar represents 1 μ m.

Fig. 6. Sieve tube with multivesicular structures (star) and endoplasmic reticulum (arrow) accompanied with lipid droplets and moved from the sieve tube wall. Phytoplasma infected *L. sinuatum* plant. Magn. 31 000x, bar represents 500 nm.

Fig. 7. Sieve tube with vesicles (stars) filling the lumen of the sieve tube. Phytoplasma (P) present in sieve element. Phytoplasma infected *L. sinuatum* plant. Magn. 23 000x, bar represents 500 nm.

Fig. 8. Sieve tube with a vesicle (star) in the middle of the sieve tube lumen. Phytoplasma (P) present in sieve element. Phytoplasma infected *L. sinuatum* plant. Magn. 15 000x, bar represents 1 μ m.

DISCUSSION

Phytoplasmas are widespread and important plant pathogens that live exclusively in sieve tubes (Esau 1977). Although the phytoplasma infection affected the phloem on the histological (necrosis), as well as cytological level (structure of sieve tubes and the spiny vesicles presence in

phloem parenchyma cells), there was no correlation between the phytoplasma presence in the sieve elements and sieve tubes structure. The necrosis, so typical for phytoplasma infections in *L. sinuatum* plants, occurs in the outer part of the phloem more often than in the part near the cambial zone, although in this region there were numerous phytoplasmas in the sieve tubes observed. Also the

Fig. 9. Sieve tube with phytoplasmas (P) and fine fibrillar material (star). Phytoplasma infected *L. sinuatum* plant. Magn. 17 000 $\times$, bar represents 1 μ m.

Fig. 10. The fuzzy deposits (stars) and phytoplasmas (P) in sieve elements. Endoplasmic reticulum (ER) present. Phytoplasma infected *L. sinuatum* plant. Magn. 33 000 $\times$, bar represents 500 nm.

Fig. 11. Sieve tube endoplasmic reticulum (ER) from infected plant phloem showing changed orientation to the sieve wall (CW). Phytoplasma infected *L. sinuatum* plant. Magn. 47 000 $\times$, bar represents 500 nm.

Fig. 12. Sieve tube with endoplasmic reticulum (ER) situated in the lumen of the sieve tube without contact with the sieve tube walls, but with close contact with phytoplasmas (P). Phytoplasma infected *L. sinuatum* plant. Magn. 48 000 $\times$, bar represents 500 nm.

process of sieve tube plates plugging was rather correlated with necrotic spots than with the presence of phytoplasma in the sieve elements protoplasts itself. No special correlation was noted between the structural changes in sieve tubes like the position of endoplasmic reticulum cisternae and the presence of vesicles and fuzzy material in sieve element protoplast and the number of phytoplasma present in

the lumen of the sieve tubes. Thus, rather the physiological changes on the plant level due to phytoplasma infection seems to be the reason of structural changes in sieve tubes than the presence of phytoplasma cells in a particular single sieve tube, and its interaction with cell components.

Spiny vesicles were observed in other species, not related to *Limonium* (Esau and Gill 1970; Newcomb 1967; Ster

Fig. 13. A part of sieve element with starch granules (s). Phytoplasma infected *L. sinuatum* plant. Magn. 24 000 $\times$, bar represents 500 nm.

Fig. 14. Sieve tube plate with callose (star) in the sieve tube with phytoplasmas. Phytoplasma infected *L. sinuatum* plant. Magn. 9 000 $\times$, bar represents 1 μ m.

Fig. 15. Outgrowing (star) of the vertical sieve tube wall plugging the sieve plate pores in sieve tube with phytoplasmas (P). Phytoplasma infected *L. sinuatum* plant. Magn. 18 000 $\times$, bar represents 500 nm.

and Newcomb 1969; Cronshaw and Esau 1968), which suggests that those structures are a normal component of plant phloem. In some of these cases spiny vesicles were found to be accompanied by p-protein (Esau and Gill 1970; Ster and Newcomb 1969; Cranshaw and Esau 1968), which suggests that there was a functional relation between the two-cell components. Spiny vesicles present in *Limonium* phloem parenchyma cells were not closely connected with p-protein neither in infected nor control plants. Phytoplasma infection increased their number in the

phloem but did not increase the number of p-protein regions present in phloem parenchyma cells.

The phytoplasma infection had a strong influence on spiny vesicles. Not only there were more cells with SV, but also the SV number increased. Similarly TMV infection of tobacco increased the number of spiny vesicles containing cells and the number of vesicles present in those cells (Esau and Gill 1970). Thus the completely different pathogens have a similar effect on SV in different plant species. Infection with phytoplasma not only increases the

Fig. 16. A part of a phloem parenchyma cell with spiny vesicles (arrow head) from control, noninfected plant. Magn. 56 000 $\times$, bar represents 200 nm.

Fig. 17. A phloem parenchyma cell with spiny vesicles from phytoplasma infected plant. Magn. 17 000 $\times$, bar represents 1 μ m.

Fig. 18. A group of spiny vesicles and spiny cistern in phloem parenchyma cell from phytoplasma infected plant. Magn. 53 000 $\times$, bar represents 200 nm.

Fig. 19. Spiny vesicles and spiny cysternae in the cell from phytoplasma infected plant. Magn. 73 000 $\times$, bar represents 200 nm.

appearance of spiny vesicles in *L. sinuatum* plants phloem, but is also responsible for changes in their shape to elongated tubules or cisterns. Spiny tubules were observed by Esau and Gill 1970 in *Tetragonia*, but those two structures – tubules and vesicles – do not occur in the same plant species. No phytoplasma was observed in phloem parenchyma cells with spiny vesicles, thus not phytoplasma alone changes the number and pattern of these structures.

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ZMIANY CYTOPATOLOGICZNE
ZACHODZĄCE W ROŚLINACH *LIMONIUM SINUATUM* MILL.
PORAZONEGO ŻÓŁTACZKĄ ASTRĄ.
I. ZMIANY PATOLOGICZNE W TKANKACH PRZEWODZĄCYCH

STRESZCZENIE

Zmiany anatomiczne i cytologiczne łyka roślin *Limonium sinuatum* Mill. porażonych żółtaczka astrów były przedmiotem badań. W wiązkach przewodzących łodyg roślin porażonych obserwowano zmiany nekrotyczne występujące na terenie łyka tych wiązek. W obrębie nekrozy, oprócz komórek znekrotyzowanych obserwowano wyłączone komórki miękiszowe. W rurek sitowych w nieznokrotyzowanych częściach floemu roślin porażonych obserwowano fitoplazmy. Elementy sitowe wykazywały szereg zmian cytologicznych takich jak zmianę położenia retikulum endoplazmatycznego, którego cysterny odsuwały się od ściany komórkowej. W świetle rurek sitowych występowały różnej wielkości pęcherzyki. Obserwowano również pojawianie się kłaczkowatych wtrąć oraz zbijanie się p-protein rurki w skupiska. Nie obserwowano jednak korelacji tych zmian patologicznych z ilością występujących fitoplazm w danej rurce.

W roślinach kontrolnych *L. sinuatum* na terenie łyka obserwowano komórki miękiszu łykowego zawierające pęcherzyki o specyficznej strukturze (spiny vesicles – SV). W roślinach porażonych fitoplazmami komórki zawierające wspomniane pęcherzyki występowały znacznie częściej. Ponadto oprócz pęcherzyków obserwowano również wydłużone cysterny, których budowa odpowiadała SV. W komórkach miękiszu łykowego ze SV nie obserwowano obecności fitoplazm.

SŁOWA KLUCZOWE: fitoplazma, żółtaczka astrów, struktura łyka.