CYTOPATHOLOGICAL CHANGES IN *SCHEFFLERA ACTINOPHYLLA* HARMS. NATURALLY INFECTED WITH IMPATIENS NECROTIC SPOT VIRUS (INSV)

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ABSTRACT

Plants of *Schefflera actinophylla* Harms. with stunted growth, chlorotic and necrotic spots and patterns, leaf epinasty and distortion are infected with impatiens necrotic spot virus classified as a member of genus *Tospovirus*. Studies with electron microscope revealed that in cells of *S. actinophylla* leaves there were no virus particle inclusions typical for *tospovirus* infection, but only single particles were present. The isolate of INSV was defective and the amount of INSV particles was strongly reduced in *Schefflera* plants. Some inclusions (I type) were composed of short branched cisterns of endoplasmic reticulum and a dark substance forming characteristic bands. The II type represented inclusions that were usually ballshaped and composed of a dark substance resembling that of the I type, but not accompanied by endoplasmic reticulum cisterns. The III type of inclusions was composed of dark, osmophilic masses. The inclusions present in the cells differ from those typical for tospovirus infection.

KEY WORDS: *tospovirus*, impatiens necrotic spot virus, cytoplasmic inclusions.

INTRODUCTION

Impatiens necrotic spot virus (INSV) has been classified as a member of genus *Tospovirus* within the family Buniaviridae. Serologically it is very distinct from tomato spotted wilt virus (TSWV) and sequence analysis revealed that the N protein exhibits only 55% sequence homology to that of TSWV (Law and Moyer 1990). INSV has a limited but characteristic host range of which ornamental plants are the most important.

INSV has been first reported in *Impatiens* sp. in the USA in 1987 (Law and Moyer 1990). In Europe it was first described in the Netherlands in 1989 (Avila et al. 1992) and in Poland in 1996 (Kamińska and Rudzińska-Langwald 1996). Cytological changes in plants infected with the foreign isolates were described by Urban et al. (1991). In this paper we describe the results of cytopathological studies on a Polish isolate.

MATERIAL AND METHODS

Plants of *Schefflera actinophylla* Harms. from a commercial greenhouse, with stunted growth, chlorotic and necrotic spots and patterns, leaf epinasty and distortion, were found to be naturally infected with impatiens necrotic spot virus (Kamińska and Rudzińska-Langwald 1996)

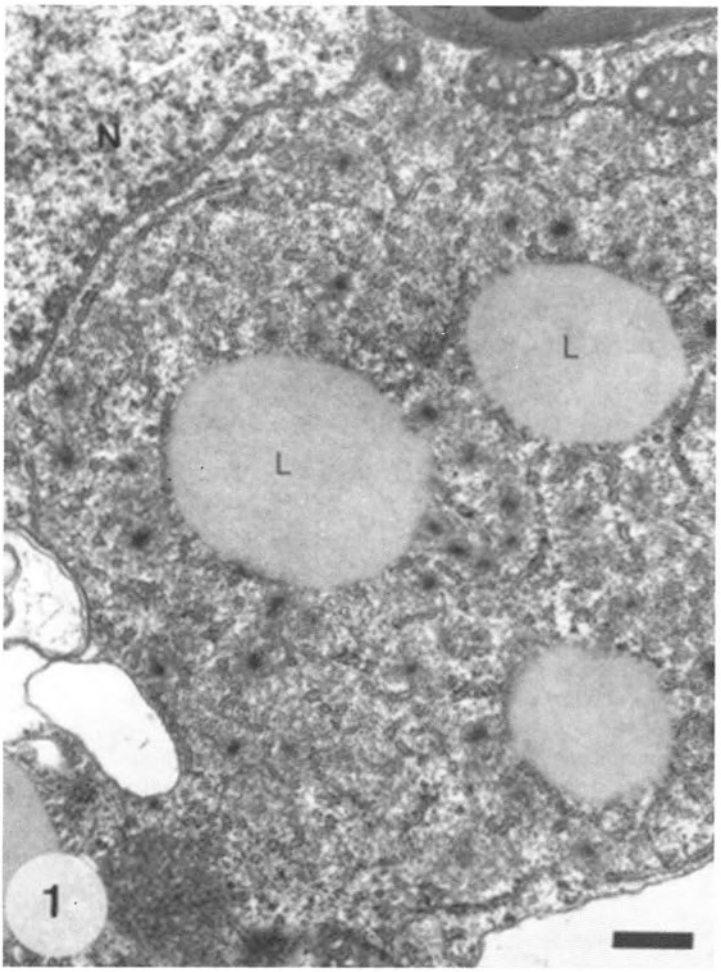
Leaf specimens were fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer pH = 7.2, for 4 h. Postfixation in 1% osmium tetroxide for 2h. at 4°C was applied. The material was dehydrated in concentration gradients of ethanol (10-70%), acetone (70-100%) and embedded in Epon 812. The ultrathin sections on copper grids were stained with uranyl acetate and lead citrate. Observations were taken on a JEM 100C electron microscope in the Laboratory of Electron Microscopy, Warsaw Academy of Agriculture.

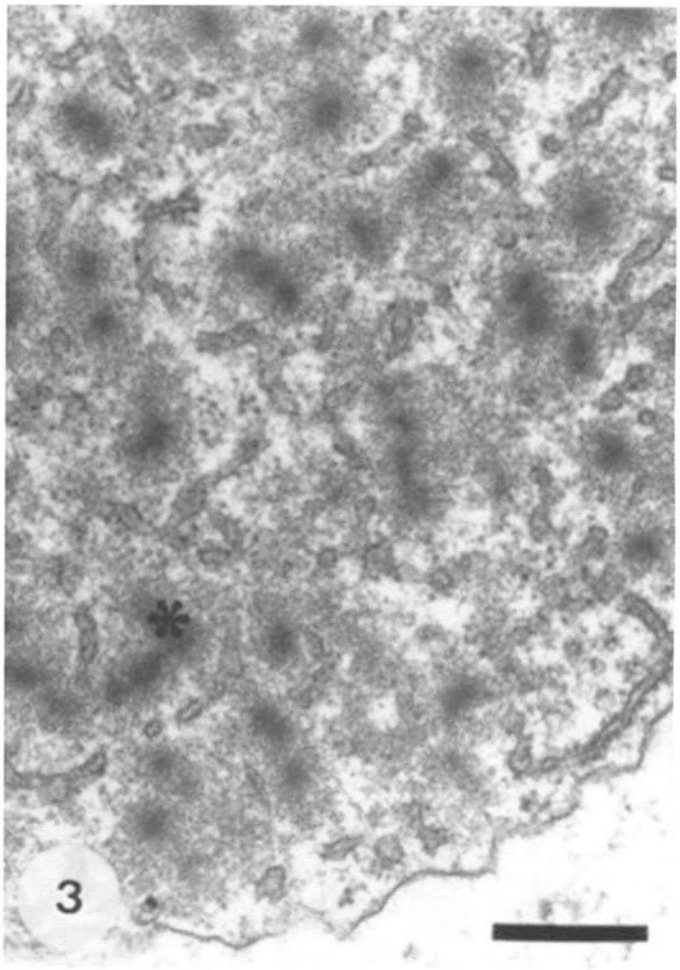
RESULTS

Composed leaves of *S. actinophylla* have a multiple epidermis on the upper surface of the leaf blade. This is a water storage tissue. The mesophyl parenchyma situated under the multiple epidermis is divided into palisade and sponge parenchyma. The inclusions connected with virus infection were present in mesophyl parenchyma, and in parenchyma of conducting tissues of the leaf phloem and xylem. There were no virus inclusions observed in water storage tissue, the cells of which have a big central vacuole and rather limited amount of cytoplasm.

The electron microscopy revealed that in cells of *S. actinophylla* leaves only single particles of INSV were present (Fig. 12). Usually they were situated in the cytoplasm on the borders of inclusions produced by INSV. The virus particles were surrounded by an extra membrane. Sporadically sacs were seen composed of a few virus particles surrounded by joint membrane. The sacs never formed virus particle inclusions typical for *tospoviruses*.

In the cytoplasm of infected plants some inclusions (I type) were composed of short branched cisterns of endoplasmic reticulum and a dark substance forming characteristically striped bands (Figs 1-3). Within lipid droplets were often seen. This type of inclusions usually occured in the neigh-





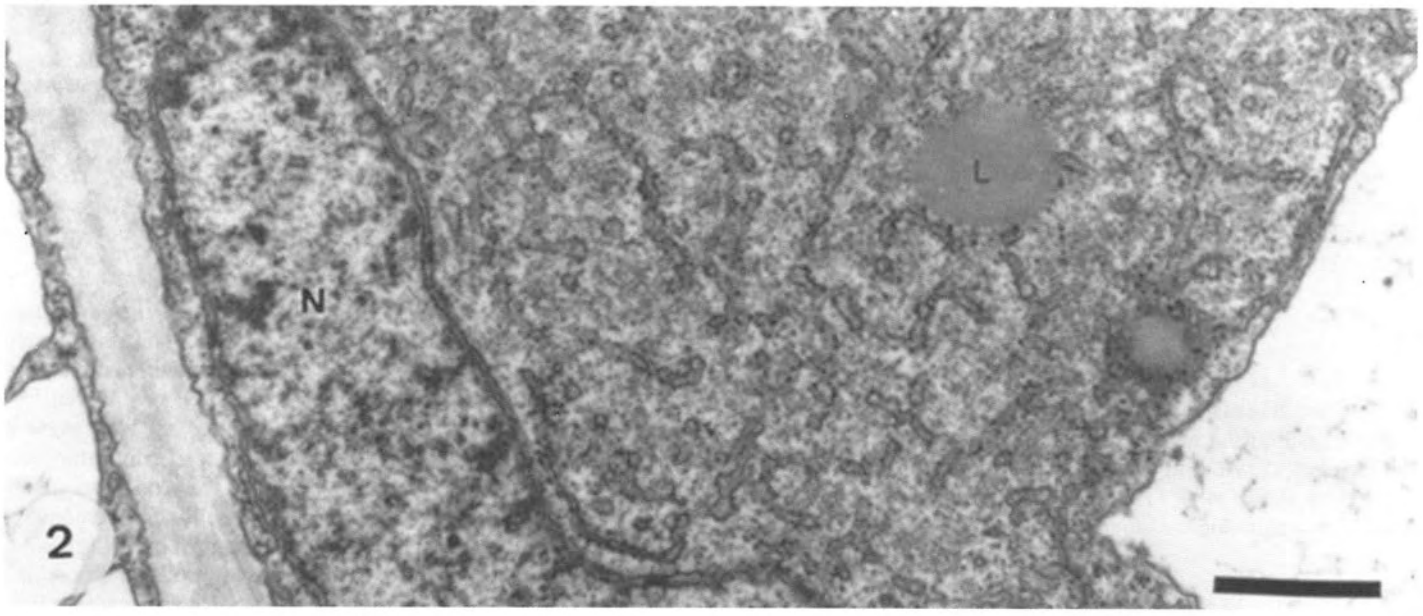
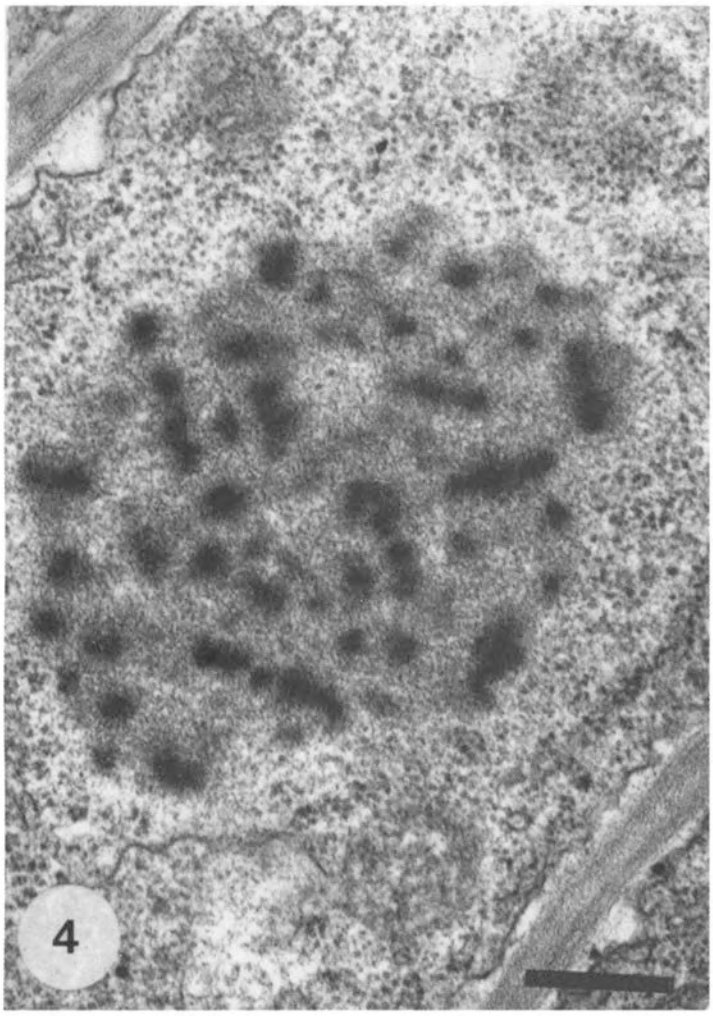
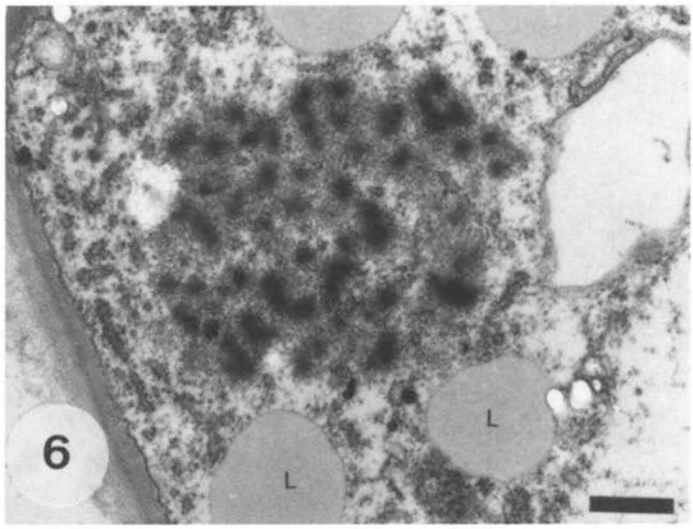


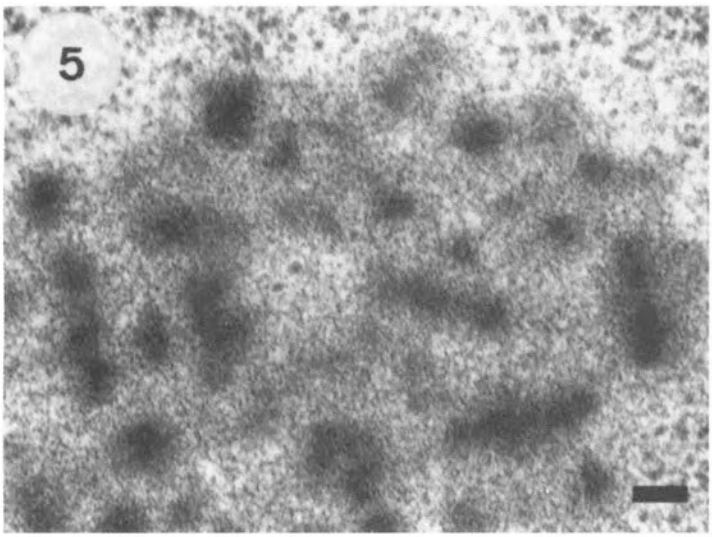
Fig. 1. The region of I type inclusion composed of endoplasmic reticulum cisterns and dark substances. In the inclusion lipid droplets (L) are seen. The inclusion is situated near a nucleus of the cell (N). Magn. 19 000 x. Bar length here and in subsequent figures is 500 nm.
Fig. 2. Fragment of the inclusion containing endoplasmic reticulum cisterns. Magn. 40 000 x.
Fig. 3. Fragment of the inclusion containing endoplasmic reticulum cisterns and dark substances (star). Magn. 40 000 x.

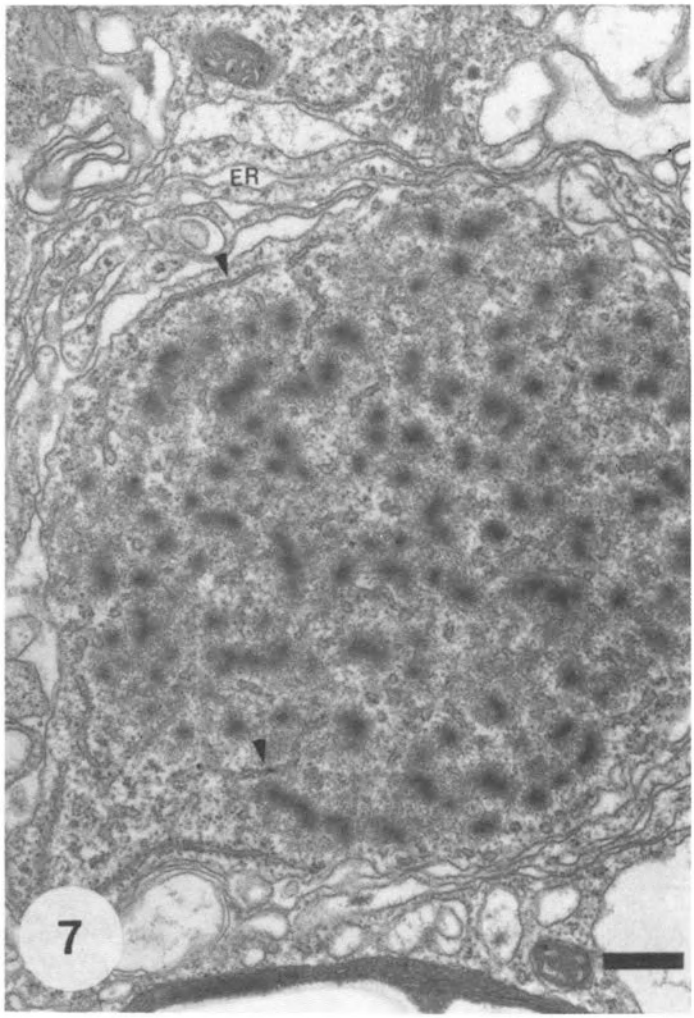
bourhood of the cell nucleus. Sometimes concavities of a nuclear envelope partly surrounded the inclusions (Figs 1, 2).

The inclusions of the II type were usually ballshaped and composed of a dark substance resembling that from the I type. There were no endoplasmic reticulum cisterns in the inclusion (Figs 4-6). No contact was seen between the inclusions of the II type and the cell nucleus. In the I type occured the inclusions with endoplasmic reticulum cisterns but with dark material reduced (Fig. 2). There were also inclusions with much dark material and a number of endoplasmic reticulum cisterns reduced (Fig. 7). Their presence suggests the sequence of changes between the different types of inclusions.







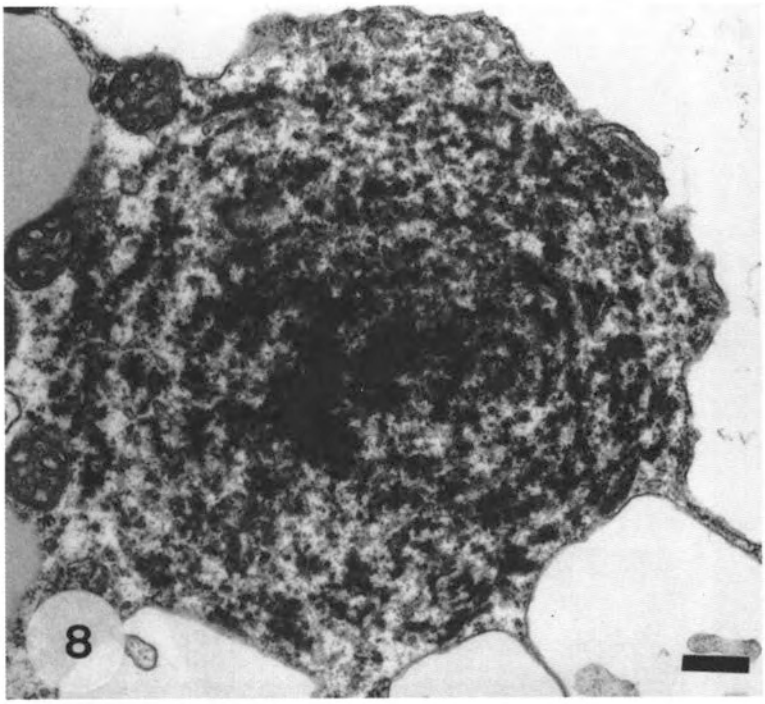


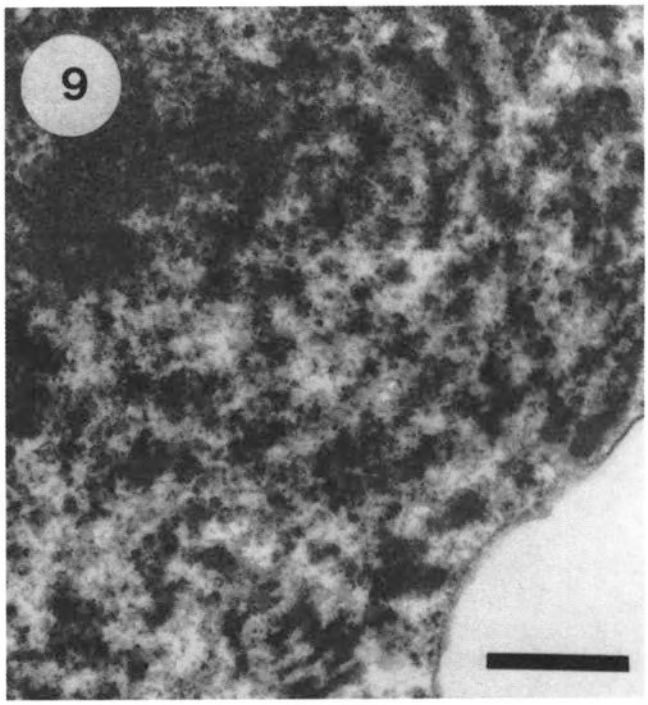
Figs 4-6. The II type of inclusions. Fig. 4. Magn. 39 000 x. Fig. 5. Magn. 67 000 x. Fig. 6. Magn. 20 000 x.
Fig. 7. Inclusion with reduced number of endoplasmic reticulum cisterns (arrow). Magn. 19 700 x.

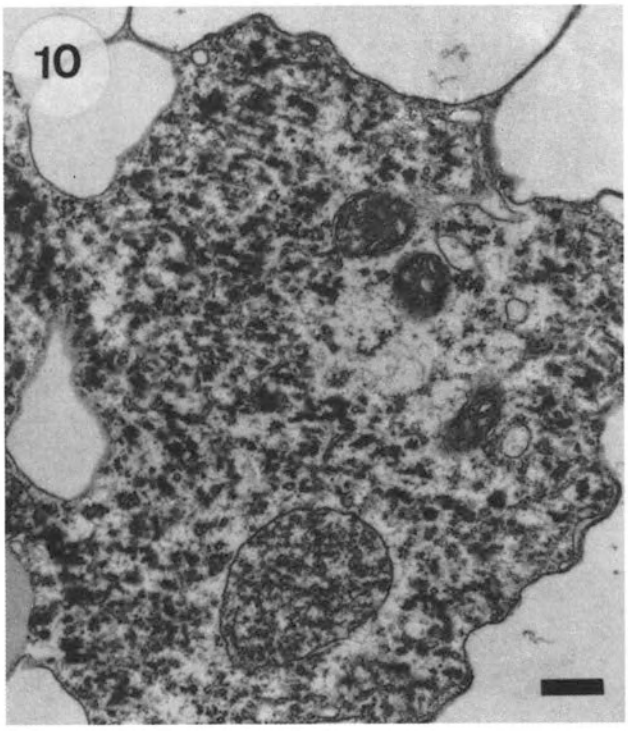
Suggested sequence of changes between the inclusions of type I (1, 2) and type II (4).

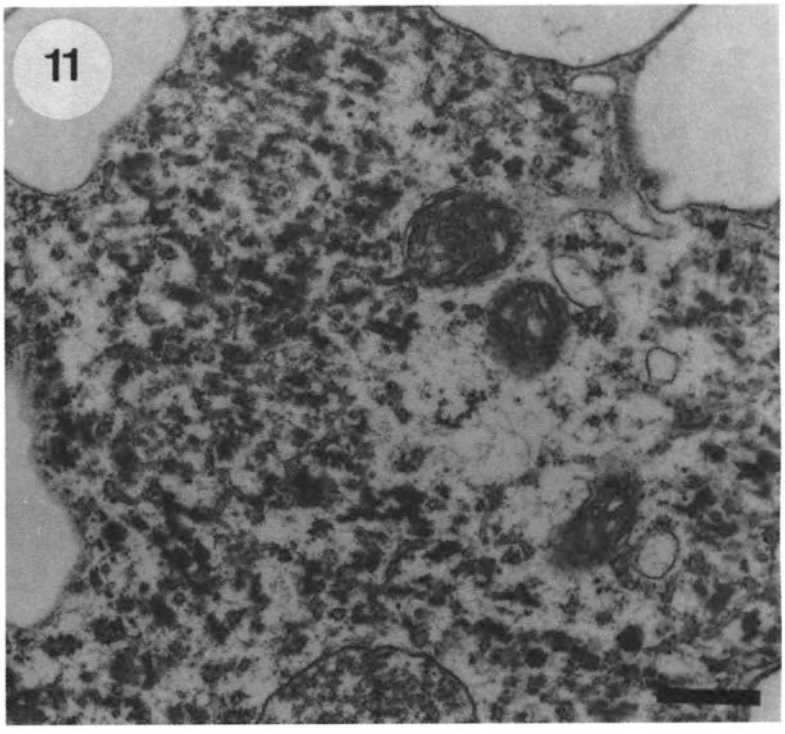
The III type of inclusions was composed of dark, osmophilic masses (Figs 8-11), different in shape from the substances present in other types and less numerous.

In mesophyl cells there were changes in the chloroplast structure such as numerous lipid globules in stroma and accumulation of large starch grains (Fig. 13). In the cells cell wall outgrowths were seen, composed of mosaic dark, osmophilic substances and regions not contrasted (Figs 14, 15).





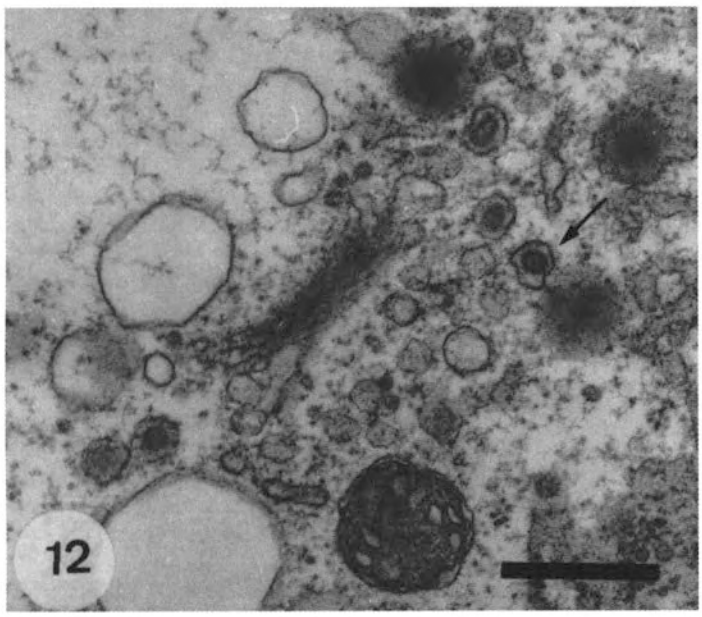


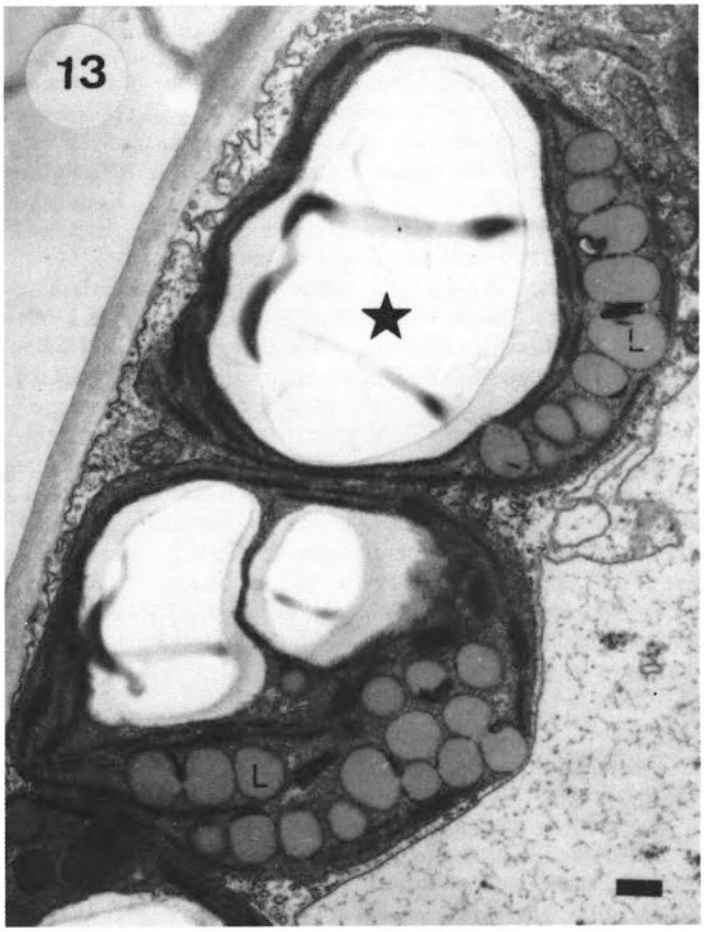


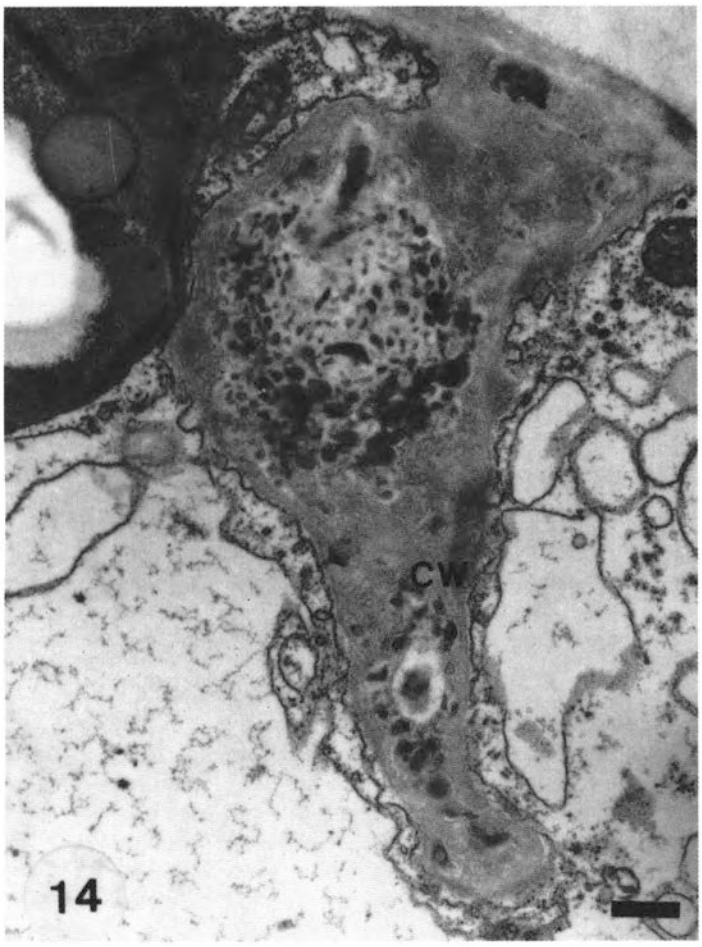
Figs 8-11. III type of inclusion composed of dark, osmophilic masses. Fig. 8. Magn. 18 000 x, bar here and in subsequent figures represents 500 nm. Fig. 9. Magn. 36 000 x. Fig. 10. Magn. 17 000 x. Fig. 11. Magn. 25 000 x.

DISCUSSION

Cytopathology of TSWV infections demonstrates that virus particles accumulate in sacs surrounded by membrane (Ie 1971, Kitajima et al. 1992, Milne 1970, Urban et al. 1991, Zielińska and Miciński 1987). Also several other types of cytoplasmic inclusions have been identified in TSWV infected plant cells. The inclusions called viroplasm are composed of chains of dense masses of (N) protein (Kitajima et al. 1992, Milne 1970, Verkley et al. 1983, Zielińska et al. 1987). The fibrous paracrystaline arrays of various shapes are associated with (Nss) protein. They form fibrous inclusions (Ie 1971, Kitajima et al. 1992, Milne 1970, Urban et al. 1992). The isolates of INSV described by Urban (1991) show similar cytopathological changes as TSWV does. In the case of defective isolates which do not produce virus particles the amorphous inclusions appeared in the cells of infected plants. Amorphous inclusions were characteristic of TSWV infection with defective isolates. Ie (1971) reported "dark diffused masses" in some plant species cells infected with TSWV. Kitajima et al. (1992) described such structures in plants infected with defective isolates of TSWV. Ullman et al. (1995) described amorphous inclusions in trips cells infected with TSWV. Amorphous inclusions have been reported in plant cells infected







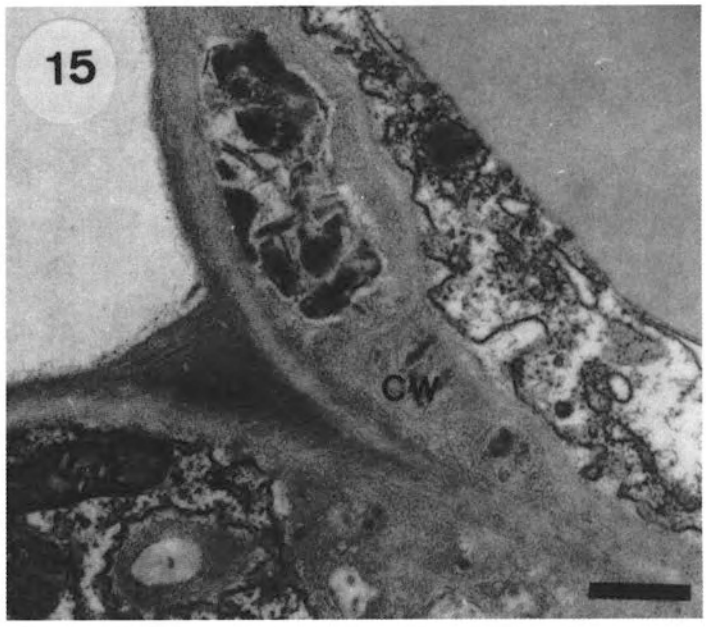


Fig. 12. Single particles of INSV surrounded by extra membrane (arrow) in the cytoplasm of mesophyl cells. Magn. 40 000 x.
Fig. 13. Chloroplasts with numerous lipid globules (L) in stroma and accumulated large grains of starch (star). Magn. 11 000 x.
Figs 14, 15. Cell wall outgrowths (CW) with a mosaic of dark, osmophilic substances and regions not contrasted. Fig. 14. Magn. 19 000 x. Fig. 15. Magn. 25 000 x.

with INSV (Urban et al. 1991). In contrast, the isolate of INSV from *S. actinophylla* does not form inclusions composed of virus particles so it is the defective one, but it doesn't form amorphous inclusions either. Therefore, the changes taking place in the cells of *S. actinophylla* infected with the described isolate of INSV differ from those characteristic for *tospoviruses*.

The virus inclusions are characteristic for the type of virus and its strain. They are similar in the different hosts of the virus (Martelli and Russo 1984). In the case of INSV it seems also that cytoplasmic inclusions are due more to the virus itself than to the host of this virus.

The inclusions of I and II type resemble viroplasm described for TSWV infection (Kitajima et al. 1992, Milne

1970, Verkley et al. 1983, Zielińska and Miciński 1987), but those were never accompanied by such characteristic arrangement of endoplasmic reticulum as it was in I type of inclusion produced by the described isolate of INSV. The inclusions of TSWV particles are usually placed in the vicinity of the cell nucleus, but it is not characteristic for viroplasm (Zielińska and Miciński 1987, Rudzińska-Langwald and Kamińska 1996b). The I type of inclusions produced by the examined isolate of INSV were situated near the nucleus.

In cells of *S. actinophylla* plants there is no increase in number of dictyosomes, which was characteristic for the TSWV infection, because those are connected with TSWV particle maturation (Rudzińska-Langwald and Kamińska 1996a). On the other hand, the particles formed in infection by defective strains of TSWV appear in the amorphous inclusions. In contrast virus particles of the examined INSV isolate which appeared in the cytoplasm, were surrounded by an additional membrane and resembled those from early stages of infection by nondefective isolates of TSWV (Rudzińska-Langwald and Kamińska 1996). The amount of INSV particles was strongly reduced in *Scheflera* plants but microscopical examination indicate a different mechanism of that reduction as compared with TSWV.

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ZMIANY CYTOLOGICZNE ZACHODZĄCE W ROŚLINACH *SCHEFFLERA ACTINOPHYLLA* HARMS. NATURALNIE PORAŻONYCH WIRUSEM NEKROTYCZNEJ PLAMISTOŚCI NIECIERPKA (INSV)

STRESZCZENIE

Rośliny *Schefflera actinophylla* Harms. porażone wirusem nekrotycznej plamistości niecierpka (INSV) wykazywały karłowaty wzrost, chlorotyczne i nekrotyczne plamy i wzory na liściach, oraz deformacje liści. W trakcie obserwacji przy użyciu mikroskopu elektronowego nie zaobserwowano typowych dla infekcji tospowirusami inkluzji cząstek wirusa, jedynie występowały w cytoplazmie pojedyncze cząstki. Tak więc opisywany izolat INSV był defektywny, a ilość cząstek występująca w komórkach liści *S. actinophylla* mała. W cytoplazmie komórek porażonych obserwowano inkluzje składające się z krótkich cystern retikulum endoplazmatycznego i ciemnych kłaczkowatych substancji tworzących charakterystyczne pasma (inkluzje I typu). Drugi typ inkluzji tworzyły kuliste masy ciemnych kłaczkowatych substancji przypominających te z inkluzji I typu lecz bez towarzyszących cystern retikulum endoplazmatycznego. III typ inkluzji wywoływanych przez INSV tworzyły ciemne osmofilne masy. Zmiany cytopatologiczne powstające pod wpływem porażenia roślin badanym izolatem INSV różniły się od typowych opisywanych w literaturze dla infekcji tospowirusami.

SŁOWA KLUCZOWE: tospowirus, wirus nekrotycznej plamistości niecierpka, inkluzje cytoplazmatyczne.