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WINTER-TYPE CALIFORNIA TOBACCO RATTLE VIRUS IN ITS RELATION TO THE INFECTED CELL[1]

Het winter-type van het Californische ratelvirus van tabak in zijn relatie tot de geïnfecteerde cel

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Particles of California tobacco rattle virus (CTRV) were observed in thin sections of vascular elements of leaves showing oak leaf symptoms. At no time during this study were particles observed in mesophyl cells of these leaves. A peculiar chloroplast breakdown is described.

INTRODUCTION

In a recent study of California tobacco rattle virus (CTRV) (DE ZOETEN & SHALLA, 1966) it was established that this virus isolate conforms to the pattern of symptom variability generally found in rattle virus isolates. This is particularly well illustrated by the occurrence of chlorotic line patterns and oak leaf patterns (Fig. 1A), apart from other symptoms in about 3 % of all tobacco plants six weeks after inoculation with CTRV.

It has been suggested elsewhere (DE ZOETEN & SHALLA, 1966) that in CTRV the occurrence of these oak leaf patterns is comparable with the observed "Winter-type" (KÖHLER, 1956) or "NM" type (CADMAN, 1962) symptoms reported for other strains of TRV. CADMAN (1962) has indicated that the virus in leaves showing oak leaf patterns was in an RNA-ase sensitive, unstable form.

Since CTRV particles (Fig. 1B) and the effect of CTRV infection on the ultrastructural appearance of mesophyl cells have been studied earlier (DE ZOETEN, 1966) it seemed interesting to study the winter-type CTVR in its relation to the infected leaf tissues.

MATERIALS AND METHODS

The virus used in this investigation was first described by GOLD *et al.* (1963). Glurk tobacco *(Nicotiana tabacum* var. *Xanthi*-nc) was used for propagating the virus. Growth conditions, assay host, transmission and other properties of this rattle isolate have already been described elsewhere (DE ZOETEN & SHALLA, 1966). The leaf tissue used for electron microscopy was killed and fixed in Dalton's chrome osmium solution (DALTON, 1955) at about 4 °C for 24 hours. The fixed tissue was dehydrated in ethanol, then transferred into two changes of propylene oxide for one hour each. The tissue pieces were then embedded in Araldite 6005 (CIBA) expoxy resin. Serial sections were cut on a Porter-Blum Ultramicrotome. The sections were stained for ten minutes on one side with 1 % strontium permanganate in water, and subsequently viewed in an RCA EMU-3G electron microscope.

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RESULTS

An interesting pattern of cytological breakdown was observed in leaves showing the oak leaf pattern symptoms, differing in most aspects from the earlier (DE ZOETEN, 1966) described symptoms of CTRV-infected mesophyl cells (Fig. 2A, B).

A striking degeneration was noted in chloroplasts (Fig. 2C). The lamellar organization of the grana was reduced to a few stacked membranes, which were not differentiated into grana and intergrana lamellae. The intergrana lamellae and most of the grana lamellae appeared as vesicular material occupying the lumen of chloroplasts (Fig. 2C, D, E). The number and size of osmiophyllic bodies in the chloroplasts increased with progressive degeneration. During this time a progressive decrease in lamellae was observed. Degradation appeared to start at one end of the chloroplast, since in some cases one end seemed to be degenerated while the other parts looked apparently normal. Cytoplasmic inclusions could be resolved in the chloroplasts while the cytoplasm itself often had a foamy appearance.

The ribosomes in the cytoplasm clumped together to produce a coarsely granular appearance of the cytoplasm.

Mitochondrial changes occurred resulting in higher electron densities of these organelles, while loss of cristae was sometimes observed.

Virus particles were at no time observed in the mesophyl cells showing the described breakdown syndrome. However, particle aggregates were seen in vascular elements believed to be sieve tube elements (Fig. 1C).

CONCLUSION AND DISCUSSION

Although at this time it seems premature to draw any far-reaching conclusions from the observed cytological changes it is interesting to note the difference between CTRV and winter-type CTRV in this respect.

The chloroplast breakdown in leaves with oak leaf symptoms resembles that occurring in phosphorous-deficient plants as reported by THOMPSON *et al.* (1964). A gradual breakdown or possible hydrolysis of the proteinaceous skeleton of the lamellae seems to occur. This may explain the progressive formation of osmiophyllic globuli (Fig. 2E, $O_1 O_2 O_3$) these possibly being residual lipid materials.

The failure to see CTRV in its particulate form in the mesophyl cells on infected leaves showing oak leaf symptoms might be due to the low concentration of virus particles in these cells. An alternative explanation might be found in the fact that winter-type CTRV is an RNA form of CTRV and thus not resolvable with the techniques employed.

Repeated single lesion isolation failed to yield a stable winter-type CTRV isolate (DE ZOETEN & SHALLA, 1966). The reported observation of particles in the sieve tube elements could mean that more than one strain is involved. This then would indicate that two closely related strains of the same virus, one in an RNA form, can exist in the same tissue in very close proximity to each other. However, the occurrence in leaf tissue of one strain of CTRV in two physically different forms might render an alternative explanation.

SAMENVATTING

Deeltjes van het Californisch ratelvirus waren te zien in ultradunne coupes van vaatweefsel in bladeren van enkele tabaksplanten, die eikebladsymptomen vertoonden. Virusdeeltjes werden nooit in de mesofylcellen van deze bladeren gevonden. Een opvallende degeneratie der chloroplasten wordt beschreven.

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FIG. 2. A. A young chloroplast in a healthy cell. × 34,000.
B. A young chloroplast in a CTRV-infected mesophyl cell 15 days after inoculation. A cytoplasmic inclusion (CI) is seen while a prolamellar body (P) is present. The lamellar structures are swollen (G1, I1). × 44,000.
C. A chloroplast in a mesophyl cell of a leaf showing oak leaf patterns. Vesiculation (Ves) is seen next to the stacked lamellae (Sl). × 42,000.
D. Breakdown of a chloroplast in a chlorotic region of a leaf showing oak leaf symptoms. Stacks of lamellae (L) are seen without pronounced differentiation in grana and intergrana lamellae being observed. Invagination of the limiting membrane (arrow) may give rise to cytoplasmic inclusions (CI). The lamellar stacks seem to lose structure during progression of the disease. × 40,000.
E. Breakdown of a chloroplast in a chlorotic region of a leaf showing oak leaf symptoms. Invaginations resulting in a vesiculate chloroplast stroma are numerous (arrows). Loss of structure of lamellar stacks is further developed and it is seen that this will result via stages O_1, O_2 in O_3, a structureless osmiophyllic globule. × 45,000.

A. Een jonge chloroplast in een gezonde cel. 34.000 ×.
B. Een jonge chloroplast in een met CTRV geïnfecteerde cel, 15 dagen na inoculatie. De lamellaire onderdelen van de chloroplast zijn gezwollen. 40.000 ×.
C. Een chloroplast in een mesofylcel van een blad met eikebladsymptomen 42.000 ×.
D. Een gedegenereerde chloroplast in een chlorotisch gedeelte van een blad met eikebladsymptomen. Opeenhopingen van lamellen zijn te zien zonder een schijnbare differentiatie in grana- en intergrana-lamellen. Cytoplasmatische insluitsels ontstaan door instulping der buitenste membranen (pijl). Gedurende de voortschrijdende afbraak der chloroplasten verliezen de lamellaire eenheden hun structuur. 40.000 ×.
E. Afbraak van een chloroplast in een chlorotisch gedeelte van het blad. O_1, O_2 en O_3. zijn stadia in de afbraak der lamellaire structuren, eindigend met de vorming van zgn osmiofiele druppels. 45.000 ×.

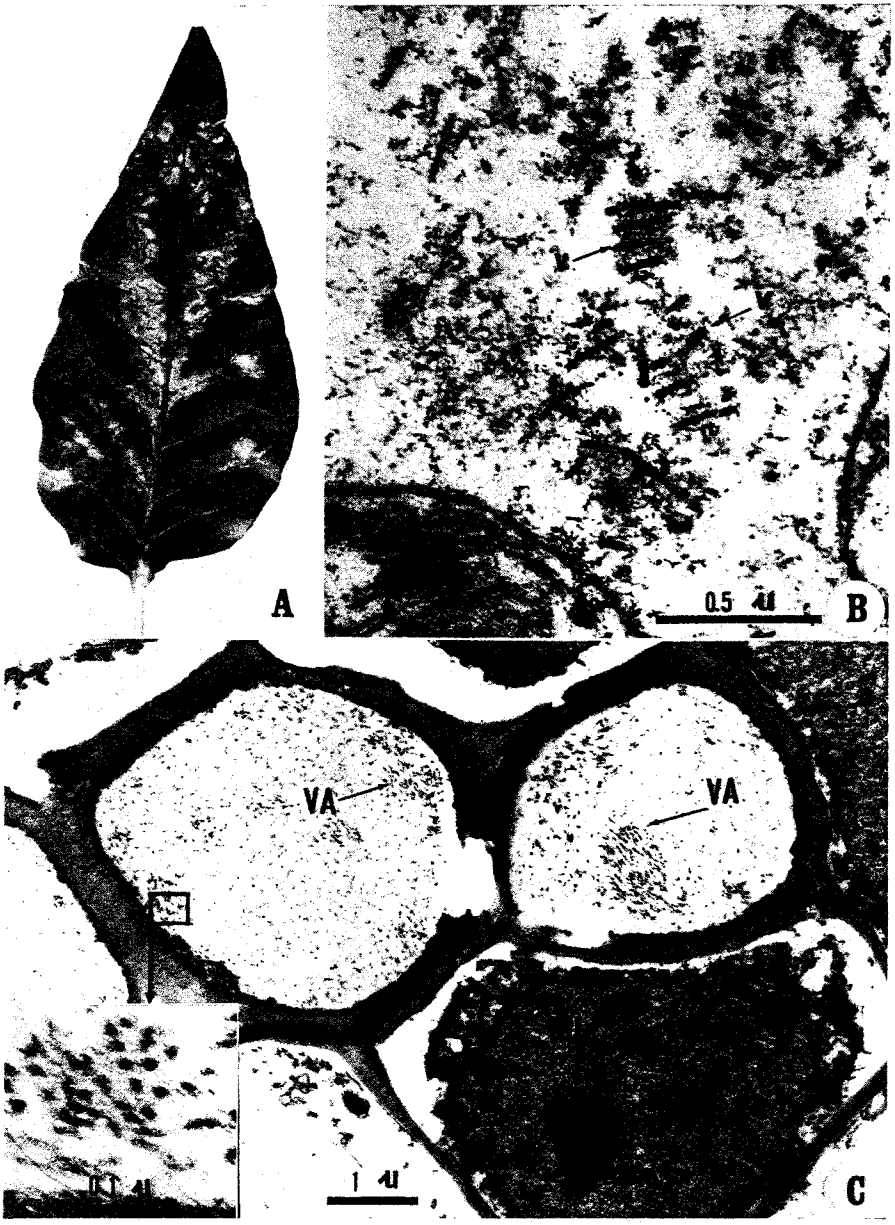
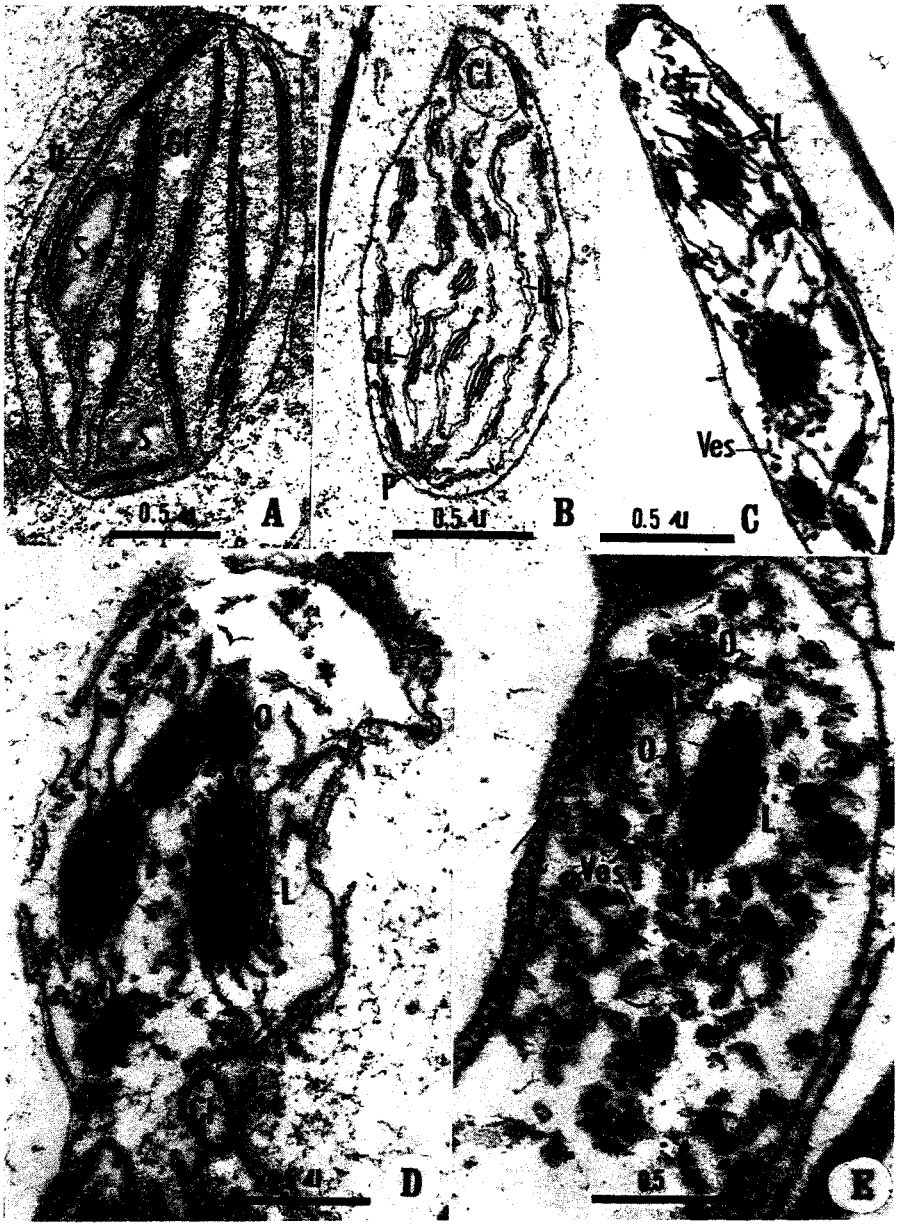


FIG. 1. A. Oak leaf symptom on a tobacco leaf systemically infected with CTRV.
B. CTRV particles are shown in the cytoplasm as they usually occur in older infections. Chrome osmium fixed. × 50,000.
C. Phloem elements of veinlets in a leaf showing oak leaf pattern, containing viral aggregates (VA) (particles 20–25 mμ wide). The darkly stained bordering cell is believed to be a companion cell. × 14,000.

A. Eikebladsymptomen in systemisch geïnfecteerde tabaksbladeren.
B. Deeltjes van CTRV, zoals ze gevonden worden in het cytoplasma van mesofylcellen in oudere infecties. 50.000 ×.
C. Zeefvaatelementen van een blad met eikebladsymptomen. Virusaggregaten (VA) zijn te zien. De dikte der waargenomen deeltjes is 20–25 mμ. De donkergekleurde aangrenzende cellen zijn begeleidende cellen. 14.000 ×.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