CUCUMBER SYSTEMIC NECROSIS CAUSED BY A STRAIN OF TOBACCO NECROSIS VIRUS

BY WAYNE THOMAS* AND P. R. FRY*

(Received 3 July 1972)

ABSTRACT

Cucumber systemic necrosis virus (CSNV), a strain of tobacco necrosis virus (TNV), was found occurring naturally on glasshouse cucumbers planted during the winter, and was transferred experimentally to produce symptoms in cucumber, french bean, cowpea, and tobacco.

The virus was transmitted from cucumber to seedlings of cucumber and lettuce using isolates of Olpidium brassicae (Wor.) Dang. from lettuce as the vector. Light microscope studies revealed high concentrations of Olpidium sporangia and resting spores within roots of naturally infected cucumber plants.

The host range, physical properties, serology, and electron microscopy of the virus identify it as cucumber systemic necrosis virus, a strain of tobacco necrosis virus. This is the first record of cucumber systemic necrosis virus in New Zealand.

INTRODUCTION

In October 1970 a virus was isolated from cucumber plants showing a severe systemic necrosis of stems and leaves in glasshouses at Whangarei, Auckland, and Hastings. Identical symptoms have since been found in Kaikohe and Otaki glasshouses. Loss of plants and production has ranged from 20–50%, depending on planting date. Symptom expression was most severe during winter and early spring, because of low light intensity and low temperature.

The symptoms produced on cucumber, bean, and a range of host plants resembled those caused by tobacco necrosis virus (TNV) (Smith 1937a; Price 1940), and indicated that the disease might be caused by cucumber necrosis virus (CNV), which is considered to be a strain of TNV (van der Want 1948; van Koot and van Dorst 1955).

As no disease of this nature had previously been recorded on cucumbers in New Zealand, the host range, physical properties, transmission, and serological relationships of the virus were investigated.

SYMPTOMS

The virus produces yellow to green spots on young leaves, vein clearing, and general chlorosis. At a later stage of infection, necrotic...
spots develop inside the chlorotic ones, producing a brown centre. Gradual death of surrounding leaf tissue follows. Often a distinctive stem and leaf necrosis occurs (Fig. 1), which is typical of the less susceptible varieties such as Princess and Triumph. Early infection can cause very stunted plants, with rosetting of the growing point (Fig. 2), as a result of restricted internodal elongation. Plants may die, especially when there is severe basal necrosis. (Fig. 3).

On severely infected plants which set fruit, the fruit may be covered with small, round, sunken, light-coloured spots with dark green water-soaked edges. Infected fruit is usually small, badly mis-shapen, and unmarketable.

Of all the cucumber varieties grown commercially in New Zealand, Marketer appears to be especially susceptible to the disease, and the F₁ hybrid variety Triumph is the least susceptible.

Symptoms are most severe during winter and early spring, probably because of low temperature and low light intensity. Virus symptoms may be visible 1 month after planting out, especially on unthrifty plants. Mildly infected plants recover during late spring and summer, when growing conditions improve. Virus is still found in the stems and roots, but causes little damage.

**Incidence**

The disease has been found on glasshouse cucumbers in Auckland, Hastings, Kaikohe, Otaki, and Whangarei. The disease has probably been in New Zealand since at least 1962, when samples were first sent from Whangarei.

Loss of plants and production has ranged from 20–50%, being especially severe on crops planted from the end of June until the end of July, when the soil tends to be wet and cold, and light intensity low.

**MATERIALS AND METHODS**

Unless otherwise stated, plants were grown in steam-disinfected potting soil in a greenhouse with temperatures controlled at 18–21°C. Leaves of test plants were dusted with grade 3F carborundum powder before mechanical inoculation. Inoculum was prepared by homogenising leaf, stem, or root tissue with an equal weight of 0.1 M phosphate buffer, pH 7.6.

**Virus purification**

Four to 6 days after inoculation 120–140 g of infected cucumber cotyledons were harvested and homogenised in 240–280 ml of 0.1M phosphate buffer, pH 7.6, containing 0.1% mercaptoethanol. The homogenate was strained through cheesecloth and the extract clarified by low-speed centrifugation. The supernatant liquid was frozen for 24 hr, thawed, and subjected to another low-speed clarification. Freezing and thawing were repeated until the preparation was clear (usually three cycles). After two cycles of high- and low-speed centrifugation.
the pellet was re-suspended in 0.1M phosphate buffer, pH 7.6, and layered on a sucrose gradient. Gradient columns were prepared by layering 6 ml of solutions containing 10, 20, 30, and 40% sucrose dissolved in 0.1M phosphate buffer, pH 7.6. One-and-a-half ml of partially purified virus preparation was floated on the top of each gradient and centrifuged for 120 min at 24,000 rpm in an S.W.25 Spinco rotor. Purified virus was recovered, using an Isco density-gradient fractionator.
**Electron microscopy**

Purified virus preparations were negatively stained with 2% potassium phosphotungstate, pH 6.5, ratio 1:1, and placed on collodion-coated 200-mesh copper grids. The preparations were examined in a 'Philips EM-200' electron microscope.

**Serology**

Antiserum to the virus was produced in a rabbit injected intravenously with 2.0 mg of purified virus, at 7-day intervals for 3 weeks, then, after 7 days, with 2.0 mg of virus emulsified in Freund's incomplete adjuvant, injected intramuscularly at 7-day intervals for a further 3 weeks. The rabbit was bled 10 days after the last injection. Serological relationships were determined by the agar gel diffusion method (Ouchterlony 1958). Tests were carried out in 10 cm disposable plastic Petri dishes containing 12 ml of 8.5% agar (Davis, N.Z.) in 0.85% NaCl. Sodium azide was added as a preservative. Plates were incubated at room temperature, and precipitin patterns were recorded after 24–48 hr.

**Physical properties**

Physical properties of the virus were determined in extracted cucumber sap, using cucumber as assay host. The thermal inactivation point was determined by exposing 2 ml aliquots of infected sap to each temperature for 10 min. Other properties examined were the dilution end-point on infectious sap and longevity of the virus in sap at room temperature.

**Sedimentation coefficient**

The sedimentation coefficient (S_{20W}) was determined, using Markham's (1962) protractor on data from a Model E Spinco set to run at 35 600 rpm.

**Transmission by Olpidium brassicae**

A strain of *Olpidium brassicae* (Wor.) Dang. obtained from lettuce roots was used in fungal transmission tests. Host plants were newly germinated cucumber or lettuce grown in steam-disinfected (87°C for 90 min) sand or potting soil.

A suspension of zoospores was obtained from lettuce roots washed in 0.05M glycine, pH 7.6, and mixed with an equal volume of partially purified virus (2 mg), and poured on the roots of young cucumber and lettuce plants. Appropriate controls were inoculated with CSNV alone, zoospores alone, or buffer alone. After 14–21 days inoculated roots were assayed for the presence of CSNV by rubbing extracted sap on cucumber cotyledons or leaves of *Chenopodium quinoa*. The roots of both cucumber and lettuce were also examined for the presence of *Olpidium*. 
Electron microscopy

Electron micrographs of negatively stained virus, purified from cucumber, revealed high concentrations of isometric particles, about 26 nm in diameter, similar to measurements made on strains of TNV by Kassanis and Nixon (1961). Turnip yellow mosaic virus (TYMV) particles photographed at the same magnification were used as a size reference, the majority having an average diameter of 28 nm (Matthews 1970).

A cucumber necrosis virus (CNV) recorded in Canada by McKeen (1959) was obtained from Dr H. F. Dias of the Research Station, Canada Department of Agriculture, Vineland Station, Ontario, for comparative purposes in this study (Canadian cucumber necrosis virus is designated CCNV throughout the paper). Electron micrographs of negatively stained CCNV revealed isometric particles of 30 nm in diameter, confirming the measurements made by Dias and Doane (1968) on CCNV particles. This suggests that the Canadian and New Zealand necrosis diseases of cucumber are unrelated.

Serology

Antisera were prepared to virus isolates from Auckland, Hastings and Whangarei, and all were found to have a titre in excess of 1 in 500 with the homologous virus in the Ouchterlony agar double-diffusion test. There was no reaction to normal plant proteins in either purified preparations or cucumber sap.

The serological reactions of the three New Zealand virus isolates and an isolate of New Zealand tobacco necrosis virus (NZTNV) with their corresponding antisera showed that the four isolates are related but not identical, spur formation being observed between the homologous and heterologous lines of precipitation. Thus CSNV symptoms can be caused by several strains of TNV.

An antiserum against the CCNY was also obtained from Dr H. F. Dias, with which purified CCNV formed a specific precipitin pattern. However, there was no reaction between the local CSNV and the antiserum for CCNV, or between CCNV and antiserum for CSNV. Serological tests thus indicate that CCNV and CSNV are unrelated, even though they have many properties in common. These results confirm the work of Babos and Kassanis (1963a) and Dias and Doane (1968), who found CCNV and TNV to be unrelated.

In gel-diffusion tests with local virus and antisera to both the A and D serotypes of TNV, CSNV was found to react strongly with NZTNV anti-serum, which is a member of the D serotype group, but only a weak reaction occurred between local virus and antiserum for Rothamsted TNV, which is serotype group A. No reaction was obtained against satellite virus antisera.

Purified CSNV's were found to react with antisera for TNV obtained from Dr H. F. Dias and R. G. Grogan of the University of California, Davis, U.S.A.
Physical properties

The thermal inactivation point for the virus in cucumber sap occurred between 90-95°C, which agrees with that found by Price (1938) and Babos and Kassanis (1963b). The dilution end-point lay between $10^{-5}$ and $10^{-6}$, as reported by Behncken (1968). When infected cucumber sap was stored at room temperature, the virus was found to be still active after 90 days, but much of its infectivity had then been lost. This agrees with the findings of Bawden (1941) and Behncken (1968).

Sedimentation coefficient

The sedimentation coefficients ($S_{20w}$) determined for both CSNV and CCNV were 112 and 126 respectively, a further indication that the two viruses are unrelated. Dias and Doane (1968) have recorded similar sedimentation coefficient differences between TNV (116) and CCNV (133), using the same method.

Host range studies

Tobacco necrosis virus has an extremely wide natural host range, but causes few diseases. Natural infections usually occur in symptomless roots of many weeds and cultivated plants. In these studies the root systems of tomatoes (*Lycopersicum esculentum* Mill.) grown as an autumn crop in the CSNV-infected glasshouses were heavily infected with CSNV and *Olpidium*. Temmink (1970) also found the tomato root system to be an excellent host for virus multiplication and *Olpidium*. Thus tomatoes may play an important role in the epidemiology of CSNV in glasshouse cucumbers.

Experimental inoculations with TNV have infected 88 species in 37 families, including monocotyledons (Price 1940), the virus usually causing necrotic lesions restricted mainly to inoculated leaves.

Local CSNV was mechanically inoculated to a number of species including members of Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Leguminosae, and Solanaceae. Species to which CSNV was transmitted experimentally and the symptoms produced on these hosts are as follows:

Local lesions only: *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste and Reyn.; *C. quinoa* Wild; *Zinnia elegans* L.; *Cucurbita maxima* Duch. (Buttercup squash); *Glycine max* (L) Merr. (Soybean); *Lathyrus odoratus* L. (sweetpea); *Datura stramonium* L. (devils thorn-apple); *Lycopersicum esculentum* Mill. (tomato); *Nicotiana glutinosa* L.; *Nicotiana tabacum* L. (tobacco) cv. Samsun and White Burley: *Petunia hybrida* Vilm.

Local lesions followed by systemic infection: *Cucumis sativus* L. (cucumber) cv. short green prickly. Local, necrotic lesions developed in inoculated cotyledons, followed in some instances by systemic necrotic spotting and vein necrosis of leaves. No steam necrosis was observed: *Phaseolus vulgaris* L. (French bean) cv. Prince. Local necrotic lesions, and some spreading vein necrosis of primary leaves. Necrotic streaks then appeared on the stem, followed by systemic vein necrosis: *Vigna senensis* (L.) Savi (cowpea). Large necrotic lesions with chocolate surrounds occurred on inoculated leaves, followed by wilting and eventual death.
Transmission by Olpidium brassicae

Successful transmission of CSNV by zoospores of Olpidium brassicae was detected by the appearance of local lesions on cucumber cotyledons or leaves of C. quinoa 3 days after inoculation with root sap extracted from infected cucumber seedlings. No virus was detected when CSNV alone, Olpidium zoospores alone, or buffer alone had been added to pots of young cucumber plants. These results indicate that the cucumber virus being investigated is readily transmitted by Olpidium zoospores, as was found for TNV by Teakle (1962); Teakle and Gold (1963); and Kassanis and Macfarlane (1964).

On examination of both cucumber and lettuce roots from plants inoculated with a suspension of Olpidium zoospores + virus and Olpidium zoospores alone, Olpidium sporangia and resting spores were found mainly in the region of elongation, 5–15 mm from the root tip. These bodies were present in large numbers, especially in lettuce. No Olpidium was detected in those plants inoculated with virus alone or buffer alone.

Roots of naturally infected cucumber plants, showing typical systemic symptoms of the disease, were found to have high concentrations of Olpidium sporangia (Fig. 4) and resting spores.

IDENTIFICATION

Symptoms in cucumber from commercial plantings and those produced by experimental inoculation of cucumbers, beans, and tobacco are identical with those caused by CNV in Holland. The reactions of purified virus with antisera prepared locally to TNV, and with that obtained from Dr H. F. Dias and Dr R. G. Grogan, provide strong evidence that the local virus is closely related to CNV as known in Holland, but not to CCNV as known in Canada. Spur formation on gel-diffusion plates suggests that the local isolates of CSNV are different strains of the same virus.

Physical properties such as aging, thermal inactivation and dilution end-point agreed with those found for TNV by Price (1938), Bawden (1941), Babos and Kassanis (1963b), and Behncken (1968). Measurement of particle size and morphology agreed with those found by Kassanis and Nixon (1961).
Thus the virus isolate causing a necrotic disease of cucumbers in New Zealand is identified as cucumber necrosis virus, a strain of tobacco necrosis virus. This is the first record of a strain of TNV causing a systemic disease in New Zealand.

**DISCUSSION**

TNV was first described by Smith and Bald (1935) as a virus disease of tobacco and *N. glutinosa* seedlings. They also found it present in the roots of certain normal-looking plants.

Although TNV is extremely widespread, it causes few diseases of economic importance, the main ones being:

1. Tulip necrosis or ‘Augusta’ disease, which was first recorded in Holland and since reported in the U.S. and Germany (de Bruyn Ouboter and van Slogteren 1949; Kassanis 1949; Fulton 1950; Uschlaweit 1952).  
2. Bean stipple streak, which has been recorded in Holland, Germany, the U.S., and Australia (van der Want 1948; Bawden and van der Want 1949; Quantz 1956; Natti 1959; and Behncken 1968).  
3. One form of cucumber necrosis virus recorded in Holland (van Koot and van Dorst 1955).  
4. ABC disease of potato tubers in California (Noordam 1957).

More recently TNV has been obtained from pear trees showing ring pattern and bark split symptoms (Kegler *et al.* 1969), from grapevines (Cesati and van Regenmortel 1969), and from citrus (Kassanis 1970).

*Olpidium brassicae* and TNV have been found in a number of New Zealand commercial tomato glasshouses, and therefore, CSNV could become a serious problem for growers who plant cucumbers for the winter and early spring market after tomato crops.

Man may have been instrumental in the spread of the disease, either by transporting wet soil containing *Olpidium* and virus on working boots and implements or by handling plants when pruning (van Koot and van Dorst 1955). This may have occurred on three properties on which the virus disease has been recorded in the Auckland district, as the owners have had close contact with each other in the past, often visiting each other’s glasshouses.

The problem has been accentuated by some growers who plant the young, 3- to 5-week-old (1-2 leaf stage) cucumbers between the existing tomato plants to provide them with a better microclimate and thus encourage good growth before the tomatoes are pulled out. However, under this scheme much of the tomato root system is left behind to avoid disturbing the cucumber plant. Since a tomato root system is an excellent host, especially for virus multiplication and for *Olpidium*, the ideal situation for spread of the disease is created, with a heavy inoculum of virus and the *Olpidium* vector located close to the young cucumber plant.

If control measures are to be successful, they should be applied before planting the tomato crop. This would prevent rapid build-up of both virus and vector for the subsequent cucumber crop.
Acknowledgments

Dr H. F. Dias, Research Station, Canada Department of Agriculture, Vine-
land Station, Ontario, for CCNV virus, and CCNV and TNV antisera; Dr R. G.
Grogan, Davis University, California, for TNV antiserum; Dr S. Bullivant, Cell
Biology Department, University of Auckland, for electron micrographs; and
officers of the Department of Agriculture, who assisted in field studies.

REFERENCES

Babos, P.; Kassanis, B. 1963a: Serological relationships and some pro-
properties of tobacco necrosis virus strains. Journal of General
Microbiology 32: 135.

1963b: The behaviour of some tobacco necrosis
virus strains in plants. Virology 20: 490.

Bawden, F. C. 1941: The serological reaction of viruses causing tobacco

Bawden, F. C.; van der Want, J. P. H. 1949: Bean stipple streak caused
by a tobacco necrosis virus. Tijdschrift over Plantenziekten 55:
142.

Behnken, G. M. 1968: Stipple streak disease of French bean caused
by a tobacco necrosis virus in Queensland. Australian Journal of
Agricultural Research 19: 731.

Bruyn Ouboter, M. P. de; van Slogteren, E. 1949: Het Augusta-ziek de
tulpen een virusziekte von het tabakonecrose type. Tijdschrift
over Plantenziekten 55: 262.

Cesati, R. R.; van Regenmortel, M. H. V. 1969: Serological detection
of a strain of tobacco necrosis virus in grapevine leaves. Phyto-
pathologische Zeitschrift 64: 363.

Dias, H. F.; Doane, F. W. 1968: Evidence for lack of relationship
between Canadian cucumber necrosis and tobacco necrosis virus.

Phytopathology 40: 298.

Kassanis, B. 1949: A necrotic disease of forced tulips caused by tobacco

1970: Tobacco necrosis virus. C.M.I./A.A.B. Descriptions
of Plant Viruses No. 14.

Kassanis, B.; Macfarlane, I. 1964: Transmission of tobacco necrosis
virus by zoospores of Olpidium brassicae. Journal of General
Microbiology 36: 79.

Kassanis, B., Nixon, H. L. 1961: Activation of one tobacco necrosis
virus by another. Ibid. 25: 459.

Tabaknekrosevirus (tobacco necrosis virus) in Obstgehölzen.

van Koot, Y.; van Dorst, J. H. M. 1955: Een nieuwe virusziekte bij
komkommers. Tijdschrift over Plantenziekten 61: 163.

Markham, R. 1962: The analytical ultracentrifuge as a tool for the

Matthews, R. E. F. 1970: Turnip yellow mosaic virus. C.M.I./A.A.B.
Descriptions of Plant Viruses No. 2.
Cucumber systemic necrosis


——— 1940: Comparative host ranges of six plant viruses. Ibid. 27: 530.


Smith, K. M. 1937a: Studies on a virus found in the roots of certain normal looking plants. Parasitology 29: 70.


van der Want, J. P. H. 1948: Het stippelstreep van de boon (Phaseolus vulgaris) een ziekte veroorzaakt door een virus, dat in de grand overblijft. Tijschrift over Plantenziekten 54: 85.