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Some biological observations on pale fruit, a viroid-incited disease of cucumber

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Abstract

A viroid-incited disease characterized by pale fruits, crumpled flowers, and rugosity and chlorosis on the leaves of cucumber, occurs occasionally in cucumber crops grown in glasshouses in the Netherlands. The disease is found primarily in crops planted in spring, rarely in those planted in summer but not in those planted in late summer. The pathogen can be transmitted with sap, during pruning, by grafting and with dodder to cucumber and a number of other cucurbitaceous species, but not with *M. persicae*. There is no evidence for seed or nematode transmission. The incubation period is 21 days at high temperatures (30 °C) but shorter after inoculation by razorblade slashing.

The number of glasshouses with the disease has increased since 1965, but the number of diseased plants is usually low. The initial distribution of diseased plants in the glasshouses suggests that the pathogen is introduced by an insect.

Introduction

In 1963 a disease in cucumber especially attracting attention by a light green colour on the fruits, but also with affected flowers and young leaves, occurred in two glasshouses in the western part of the Netherlands. The disease, now called pale fruit disease, has since been observed in different places over the whole country. The number of affected plants in a glasshouse is mostly less than 0.1 %. The disease spreads slowly; diseased plants found later usually occur in the same row near those first observed attacked.

The disease soon proved to be infectious, but no agent could be detected by electron microscopy. The cause has now (paper in preparation) been found to resemble the pathogens causing potato spindle tuber (Diener, 1971, 1972; Singh and Clark, 1971), citrus exocortis (Semancik and Weathers, 1972; Sänger, 1972) and chrysanthemum stunt (Diener and Lawson, 1973; Hollings and Stone, 1973). These pathogens are often called viroids (Diener, 1971).

This paper deals with biological features of the disease, such as symptomatology, transmission and host range within the Cucurbitaceae. Its occurrence in glasshouses and distribution over the Netherlands are also described.

Materials and methods

Cucumber plants 'Sporu', 2 to 3 weeks old, were used as test plants. They were grown in a mixture of bog-peat, moor-peat and dune sand (10:7:1). After adjusting the pH to 5.8 with lime, to every m^3 of this mixture was added: 240 g N, 350 g P_2O_5, 300 g K_2O and 250 g of Sporumix PG, containing 25% MgO, 0.3% Cu, 0.1% B, 0.6% Mo and 0.5% Mn. The plants were fertilized once or twice per experiment with a nutrient mixture containing N, P_2O_5, K_2O and MgO in the ratio of 15:5:16:6. Approximately 0.7 g of this mixture was added to each pot containing 0.75 l of the soil mixture. The plants were kept in a glasshouse at 20–25°C and a rel. humidity of 60–80%. In later experiments the plants were kept at temperatures of 30–32°C by day and 25–27°C by night. Supplementary illumination of 600 ft candles was given in winter.

The inocula were prepared by grinding leaves with pestle and mortar. Inoculations were made by rubbing the first two true leaves with a plug of cotton wool using carborundum 500 mesh as an abrasive. After inoculation, the plants were rinsed with tap water. In later experiments inoculations were also made by razorblade slashings. Slashes were made at 15 places in the hypocotyledon with sharply angled pieces broken from a razorblade. These pieces were placed in a holder and dipped in the inocula at every 5 slashes. This technique (Semancik and Weathers 1972) was brought to our attention by Dr Bové.

Observations and results

Symptoms on cucurbitaceous plants. In preliminary experiments sap inoculations were made on *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana clevelandii*, *N. glauca*,

Fig. 1. Fruits of *C. sativus* 'Sporu' affected by pale fruit disease. Bottom: fruit of a healthy plant.

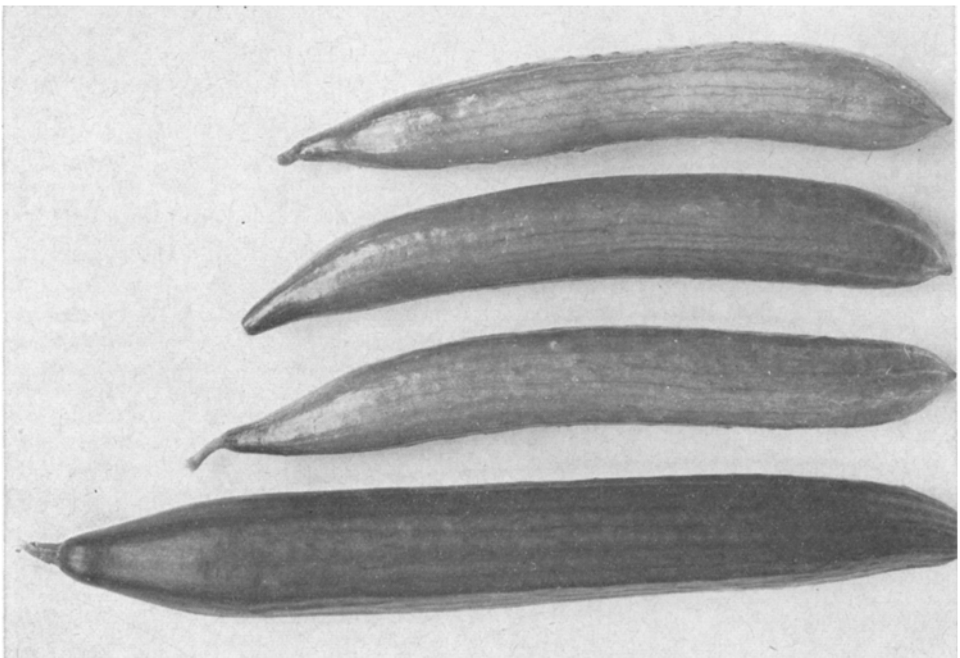

Fig. 1. Vruchten van C. sativus 'Sporu', door bleke-vruchtenziekte aangetast. Onder: vrucht van een gezonde plant.

Fig. 2. Flowers of a healthy (left) and an affected (centre and right) *C. sativus* 'Sporu' plant.

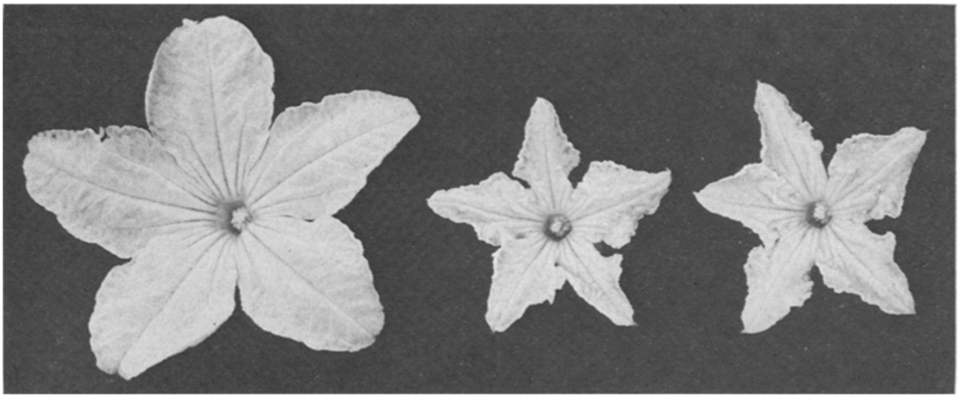

Fig. 2. Bloemen van een gezonde (links) en een aangetaste (midden en rechts) komkommerplant 'Sporu'.

N. glutinosa, N. rustica, N. tabacum 'White Burley', 'Samsun' and 'Xanthi', *Phaseolus vulgaris* 'Verschoor', and *Datura stramonium*. None of these plants showed symptoms after 4 weeks. However, a number of Cucurbitaceae appeared to be susceptible. Therefore a study on the host range was mainly restricted to species of this family in order to find one which might be a more suitable test plant than cucumber.

In *Cucumis sativus* symptom severity strongly depends on the temperature. The most distinctive symptom is found on the fruits. They are pale green in colour, retarded in growth and most are slightly pear-shaped (Fig. 1). The flowers may also be abnormal. Both male and female ones are then stunted and crumpled (Fig. 2). The edge of the petals is slightly notched. Developing leaves may be smaller, bluegreen and rugose i.e. the leaf surface between the veins is raised, thus decreasing the smoothness and flatness of the leaves. The leaf blades are undulated, their edges turned downward, and the tips bent downwards or even turned backwards (Fig. 3). On ageing the leaf symptoms fade and a chlorosis appears. The internodes of the younger parts of affected plants are shorter than those of healthy plants. Consquenetly, the plant may be somewhat stunted. When plants are grown at 30°C leaf symptoms appear before flowering and development of the fruit. The symptoms are then more intense. Necrosis has never been observed in cucumber.

Although the cucumber and gherkin cultivars of *C. sativus* are botanically closely related, gherkins react quite differently to infection. At 30°C the first symptom is a chlorotic spotting on the developing leaves. Later the leaves show a conspicuous chlorotic vein banding, reduction in size and a blue tinge, but no edge curling (Fig. 4). Internode shortening leads to plant stunting. At low temperatures (about 20°C) the leaves are chlorotic and growth retardation is less. At low as well as at high temperatures the flowers remain smaller and are crumpled. The fruits are small and pale, locally almost white in colour. The disease has not yet been found in gherkin crops.

Cucumis melo, Citrullis vulgaris and *C. colocynthis* show vein chlorosis which extends gradually into the mesophyll and develops into necrosis. The internodes and leaves are strongly reduced in size (Fig. 5), giving the plants a bushy appearance. Many flower buds, mainly pistillates, may be formed in the leaf axils (Fig. 6), but few

Fig. 3. Symptoms of pale fruit disease at the tip of an affected cucumber plant (left). Right: tip of a healthy plant.

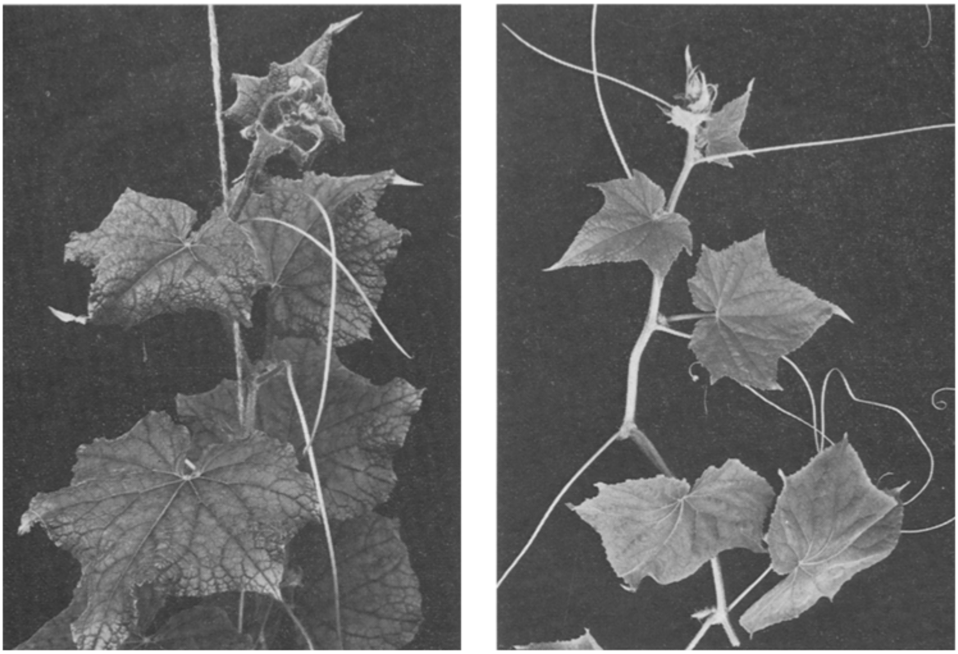

Fig. 3. Symptomen van de bleke-vruchtenziekte in de top van een aangetaste komkommerplant (links). Rechts: top van een gezonde plant.

Fig. 4. Vein chlorosis in the leaves (centre and left) of *C. sativus* 'Levo', a gherkin producing cultivar. Healthy leaf on the right.

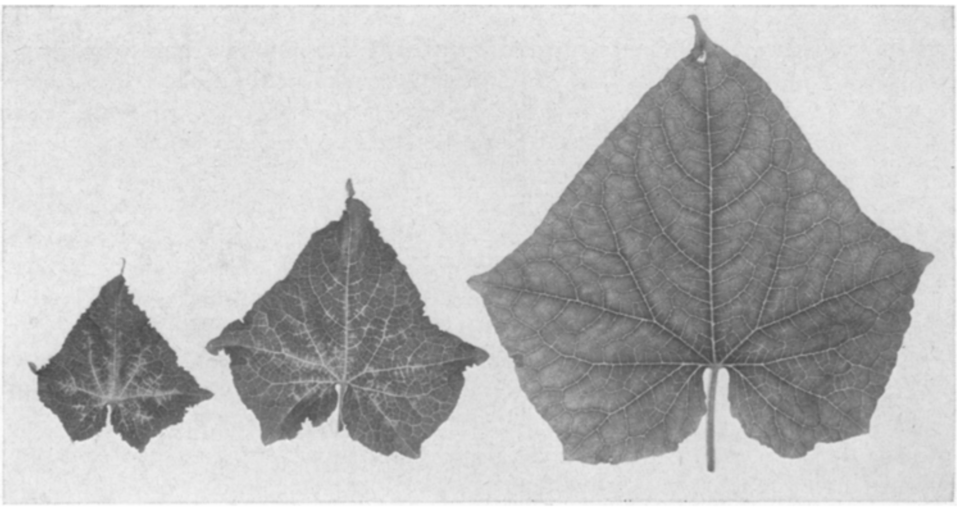

Fig. 4. Nerfchlorose in de bladeren (midden en links) van C. sativus 'Levo', een augurk. Het rechtse blad is van een gezonde plant.

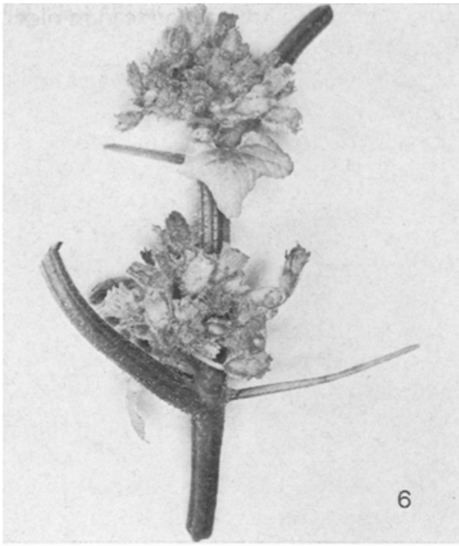

Fig. 5, 6 and 7. Symptoms on *C. melo* 'Witte Suiker' caused by the pathogen of cucumber pale-fruit disease.
Fig. 5. Tip of a healthy (right) and an affected plant (left).
Fig. 6. Leaf axils with numerous flowerbuds on an affected plant.
Fig. 7. Fruit of an affected plant, grown at 20–24 °C.

Fig. 5, 6 en 7. Symptomen op C. melo 'Witte Suiker', die geïnfecteerd zijn met het pathogeen van de bleke-vruchtenziekte van komkommer.
Fig. 5: Top van een gezonde (rechts) en een zieke plant (links).
Fig. 6: Bladoksels van een geïnfecteerde C. melo waarin talrijke bloemknoppen zijn aangelegd.
Fig. 7: Vrucht van een zieke plant, die werd opgekweekt bij 20–24 °C.

or none will flower. The affected plants of *Cucumis melo* eventually die. Wide and deep brown-grey cracks are formed on the fruits of *C. melo* 'Witte Suiker' (Fig. 7).

The growth of *Cucumis anguria*, *C. dipsaceus* and *C. myriocorpus* is strongly inhibited, the internodes remain extremely short and the leaves are small. The flowers are crumpled and fruits set but do not develop. *C. anguria* is the least sensitive.

Luffa acutangula, *L. cylindrica* and *L. operculata* show a slight chlorosis in leaf veins, which is more pronounced in older leaves than in younger ones. The leaf edges of *L. acutangula* curl.

The leaves of *Melothria japonica* become slightly chlorotic and the infected plants are later severely stunted.

In *Cucumeropsis edulis*, *Cucumis metuliferus*, *Lagenaria vulgaris* and *Trichosanthes anguina* the agent produces identical symptoms. Chlorosis starts in the small veins of the youngest leaves and extends gradually into the adjacent mesophyll then becoming necrotic. The leaf edge curls downwards. Growth is retarded. *C. edulis*, *C. metuliferus* and *L. vulgaris* usually die soon after inoculation (Table 1).

The leaves of *Bryonopsis laciniosa* become chlorotic, curl, and finally die. Developing side-branches may die later.

In *Benincasa cerifera* and *B. hispida* growth is severely reduced. The veins of developing leaves yellow .This may be followed by a slight chlorosis and a vaulting of these leaves (Fig. 8). At a later stage the plant tip may become necrotic, followed by death of the plant. When plants become infected late, the growth is severely reduced (Fig. 9). The symptoms then resemble those caused by growth substances and finally develop into necrosis. Numerous flower buds are formed which do not develop. In some cases the plant dies. *B. hispida* is slightly more sensitive than *B. cerifera*.

In addition to the species listed, others were also tested for their susceptibility and sensitivity by looking for symptoms and by back inoculation on to cucumber. The results obtained are given in Table 1. Most of the species tested were susceptible; some

Fig. 8. Symtoms on *B. hispida*.

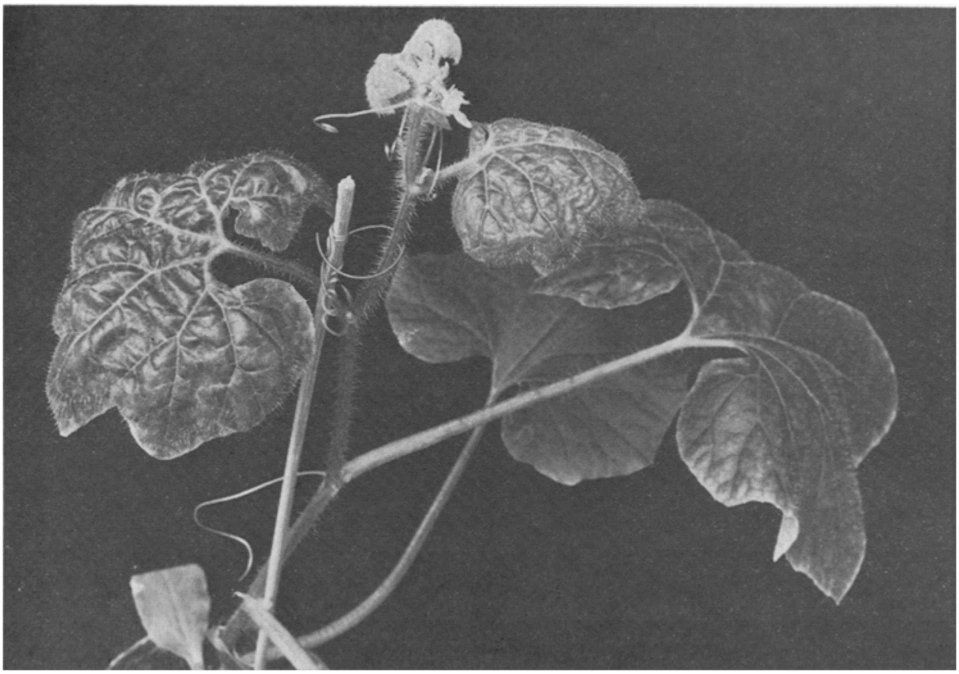

Fig. 8. Symptomen op B. hispida.

Fig. 9. Tip shoots of *B. cerifera*. Right: healthy. Left: shoot of an infected plant.

Fig. 9. Toppen van planten van B. cerifera. Rechts: gezond. Links: top van een geïnfecteerde plant.

even died. From a few species, although producing symptoms, the pathogen could not be recovered.

Transmission. The pathogen was mechanically transmitted with crude sap expressed from infected cucumber plants to several Cucurbitaceae. Infectious inocula were prepared from leaves, flowers, fruits and stems. Sap inoculation on cucumber and gherkin resulted in 100% infection whereas inoculation to other Cucurbitaceae was less efficient. When sap inoculations were made on to *C. melo* 'Witte Suiker' 25% of the plants became infected and other species were even less susceptible. See also Table 2.

Grafting was a more successful way of transmission. When cucumber shoots were grafted on to cucumber, *C. melo*, or *L. vulgaris*, all plants became infected.

Slashing of the stems with a razor blade also appeared to be a successful method of infecting plants. The susceptibility appeared to be increased by this method (Table 1, see footnote 2) and the incubation period is shorter (see '*Incubation period*').

The disease can also be transmitted during pruning as was demonstrated in one experiment. Two plants grown from infected scions were placed at the beginning of each of 8 rows of 20 cucumber plants. Pruning of the plants and harvesting of the fruits started in every row with the infected plants, and was done once or twice a week for a period of two months. The plants adjacent to the infected plants showed symptoms about 42 days, and those at the end of the rows 60–70 days, after the onset of pruning and harvesting. All plants became infected and the mean period in which the first symptoms were noticed, was 60 days after pruning was started.

The pathogen could also be transmitted by dodder. In one experiment 3 out of 4 cucumber plants used, were infected via *Cuscuta subinclusa*. Contact with the diseased

Table 1. The susceptibility and sensitivity of Cucurbitaceae.

Species tested	Susceptibility	Symptoms	Plant death	Results of back inoculation on *C. sativus* 'Sporu'
Acanthosicyos horrida	–			0/5[1]
Benincasa cerifera	+	+	+	2/4[2]
B. hispida	+	+	+	1/5[2]
Bryonia dioica	–			0/5
Bryonopsis laciniosa	+	+	+	3/4
Cayaponia africana	+	–		5/5
Citrullus colocynthis	+	+		0/5
C. vulgaris	+	+		0/5
Coccinia sessilifolia	+	–		1/5
Cucumeropsis edulis	+	+	+	3/3
Cucumis africanus	+	–		4/5
C. anguria	+	+		5/5
C. dipsaceus	+	+		5/5
C. melo	+	+	+	5/5
C. metuliferus	+	+	+	5/5
C. myriocarpus	+	+		2/5
C. sativus	+	+		5/5
Cucurbita andreana	+	–		4/4
C. ficifolia	+	–		2/5
C. maxima	+	–		2/5
C. mixta	+	–		2/5
C. moschata	+	–		2/5
C. pepo	+	–		2/5
Cyclanthera pedata	–			0/5
Ecballium elaterium	–			0/5
Kedrostis africana	+	–		1/5
Langenaria vulgaris	+	+	+	5/5
Luffa acutangula	+	+		0/5
L. cylindrica	+	+		0/5
L. operculata	+	+		1/5
Melothria japonica	+	+		3/5
M. pendula	+	–		2/5
M. scabra	+	–		4/4
Momordica balsamina	–			0/5
M. charantia	–			0/5
M. foetida	–			0/5
Trichosanthes anguina	+	+		5/5

[1] Number of infected plants over number of inoculated plants. The back inoculations were made (with inocula prepared from a leaf sample of the inoculated plants) by razor blade slashings.
[2] A higher number of infected plants was obtained if the razor blade was contaminated by cutting the infected plants first and then slashing the plants to be inoculated.

Tabel 1. De vatbaarheid en gevoeligheid van komkommerachtingen.

and healthy plants lasted for 2 weeks. *Cuscuta campestris* did not transmit the pathogen from cucumber to cucumber. No symptoms could be produced on *Nicotiana clevelandii*, *N. rustica*, *N. tabacum* 'Xanthi nc', *Sonchus oleraceus* or *Vinca rosea* after a dodder contact with cucumber for 2 weeks. No back inoculations were made on to cucumber.

Attempts to transmit the pathogen with aphids were unsuccessful. In these trials aphids (*Myzus persicae*) were placed either for a short period after 3 h fasting or for a week on infected plants. After these acquisition feeding periods the aphids were tested for infectivity on cucumber plants for one week.

To study whether transmission through soil could be obtained soil samples were collected around the first diseased plants found in a glasshouse. They were taken from a depth of 0–25 cm and 25–50 cm. A group of 30 cucumber plants was kept in each of the two samples for a period of 6 weeks. The plants were then replanted in steam-sterilized soil, and kept under observation for 4 months. As none of these plants developed symptoms it is concluded that the disease is not soil-borne.

In preliminary experiments, seeds obtained from infected plants proved to be free of the pathogen. In addition, there was no evidence of seed transmission from studies on the origin of seeds from which the affected crops were grown.

Incubation period. The length of the incubation depends greatly upon the temperature at which the plants are grown. The results of one experiment are given in Table 2.

Table 2. The influence of temperature on the length of the incubation period after mechanical inoculation of the leaves.

Growth temperature (°C) day	night	Infectivity[1]	Mean incubation period[2]
20	20	7/10	76
25	20	9/10	49
30	25	10/10	35
30	30	10/10	21

[1] Number of infected plants over number of inoculated plants.
[2] In this experiment the plants were placed in buckets of 10 l.

Tabel 2. De invloed van de temperatuur op de incubatieperiode na mechanische inoculatie van de bladeren.

When plants were grown at day and night temperatures of approximately 30°C the incubation period was 21 days. However, it was considerably longer (76 days) when the plants were grown at temperatures of 20°C. The development of the symptoms and their ultimate severity were also positively correlated with the temperature. Leaf symptoms developed clearly at 30°C, but were less pronounced at lower temperatures.

In the earlier experiments the plants were inoculated by rubbing plant sap over the leaves with carborundum. Recent experiments showed that the incubation period on cucumber could be drastically reduced if the plants were inoculated with razorblade slashings into stems. At 30°C, the incubation period could then be further reduced to 12 days.

With grafting the incubation period was usually shorter than with sap transmission; in *C. melo* and *L. vulgaris* 2–3 weeks as compared to 4–5 weeks.

Table 3. The number of glasshouses with cucumber plants affected by pale fruit disease.

Year	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972
Number of glasshouses with affected plants	2	0	2	1	3	5	11	13	19	17
Number of infested glasshouses where the disease had previously been observed			1[a]		1[a]	1[a]	3	6	7	8

[a] These refer to the same case.

Tabel 3. Het aantal warenhuizen met komkommers aangetast door de bleke-vruchtenziekte.

The occurrence and distribution of the disease. The disease was first observed in the Westland, a vegetable growing district with extended glasshouse holdings in the western part of the Netherlands. Since 1965 diseased plants have been observed in an increasing number of glasshouses (Table 3). In 1968 a few diseased plants were found in a glasshouse in the south-eastern part of the country near Venlo and the disease has occurred in the same glasshouse every year since. In 1971 it was also found in some other glasshouses in that area and there have been isolated reports from all other cucumber growing areas in the country.

The disease has so far been reported to occur in 46 different glasshouses of which 16 were affected more than once. There is a higher chance of finding the disease in a glasshouse, where it has occurred in previous years, than in one in which the disease has not been encountered before.

The sites at which the disease is found in a glasshouse usually differs from year to year. This further suggests that the agent is not soil-borne.

The number of infected plants is mostly small, viz. less than 20 in a crop of 2,500 to 25,000 plants. However, some higher incidences have been found, perhaps due to spreading by pruning and other cultivation measures. The first diseased plants are often found near the outer walls of the glasshouse or close to the main walk. In other cases the infection was distributed at random over the glasshouse. Plants developing symptoms after those first found diseased, mostly occur near these in the same row, probably as a consequence of pruning.

Most diseased plants are found from the beginning of April through May. Some are found earlier in crops planted at the end of the preceding year and grown at about 24 °C during the day. Under such conditions the incubation period is about 2 months. Thus the infection may have taken place in February or March or even the last week of January. Occasionally in June some diseased plants can be found in crops planted in May. No infection has ever been found in cucumbers planted in July or August.

Discussion

Several Cucurbitaceae became infected and showed easily discernible symptoms. Of these, *B. hispida* may be the most useful test plant as its incubation period is short and the symptoms are the most pronounced. A local lesion host has not been found.

The agent could not be recovered by sap inoculation or razor blade slashing from some of the species which showed symptoms. One possible explanation for this anomaly is that the agent may be inactivated during the inoculation. A second possiblility is that the concentration in the infected plant is not sufficiently high to infect cucumber.

The observation that the first diseased plants are found near the sides and then often near fissures and near the main walk of glasshouses suggests that the disease may be introduced by a vector, supposedly an insect. This vector may enter the glasshouse along the main walk with carts, material transported, or on workers' clothes. The small number of diseased plants may indicate that the vector does not live on cucumber but merely probes on some plants.

With respect to the supposed transmission of this disease by a vector it should be noted that most of the diseased plants are found during April and May; and we may safely assume that the plants concerned have been infected approximately two months earlier. Thus the vector may infect the plants either after its diapause in the glasshouse or after entering the glasshouse when there are hardly any insects encountered outdoors, although some may be active alongside the outer walls of the glasshouse.

In summer and autumn infected plants are found only rarely. The vector might then have sufficient host plants outside the glasshouse to live on. A consequence of this idea is the assumption that the pathogen occurs in plants and in vectors in the field.

High temperature favours symptom development by drastically shortening the incubation period and increasing symptom severity. It has been reported that high levels of nitrogen and manganese have a positive influence on symptom expression of citrus exocortis (Weathers, 1964) and potato spindle tuber (Lee and Singh, 1972). Although we made no studies on the effect of nutrients on symptom development it was evident in our experiments that conditions less optimal for cucumber growth diminished the symptom severity, lengthened the incubation period and decreased plant sensitivity.

The increase in number of infected cucumber crops does not necessarily mean that the disease is spreading. It may reflect the growing number of farmers who are able to recognize this disease.

Samenvatting

Enige biologische waarnemingen over de bleke-vruchtenziekte van komkommer die door een viroïd wordt veroorzaakt

Een door een viroïd veroorzaakte ziekte die bleke vruchten, verfrommelde bloemen, diepnervigheid en later bladchlorose veroorzaakt, wordt zo nu en dan op komkommers, die in warenhuizen worden geteeld, aangetroffen (Fig. 1–3). De ziekte wordt voornamelijk gevonden op die gewassen die in de winter en het voorjaar worden geplant en niet op die, welke 's-zomers of in de herfst worden geplant.

De veroorzaker van deze ziekte kan mechanisch van komkommer naar komkommer en andere komkommerachtigen, zoals augurk, watermeloen en suikermeloen, worden overgebracht (Fig. 4–9; Tabel 1). Het pathogeen is ook met *Cuscuta* en door enting over te brengen en kan met snoeigereedschap worden verspreid. Overdracht met zaad, *Myzus persicae* of nematoden kon niet worden vastgesteld.

De incubatietijd, die onder praktijkomstandigheden ongeveer 6–8 weken is, kon tot ruim 3 weken worden teruggebracht door de planten bij 30 °C te kweken (Tabel 2) en kon worden gereduceerd tot 12 dagen door het pathogeen over te brengen aan een scheermesje, waarmee kleine sneetjes worden aangebracht.

Het aantal bedrijven waarop de ziekte wordt gevonden neemt sinds 1965 toe (Tabel 3). Mogelijk is dit toe te schrijven aan het aantal telers dat de ziekte kan herkennen. Het aantal besmette planten in een warenhuis is gewoonlijk laag. Het vóórkomen en de verspreiding van de zieke planten in een warenhuis suggereert dat de ziekte door een insekt in het warenhuis wordt binnengebracht.

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References

Diener, T. O., 1971. Potato spindle tuber 'virus'. IV. A replicating low molecular weight RNA. Virology 45: 411–428.

Diener, T. O., 1972. Potato spindle tuber viroid. VIII. Correlation of infectivity with a UV-absorbing component and thermal denaturation properties of the RNA. Virology 50: 606–609.

Diener, T. O. & Lawson, R. H., 1973. Chrysanthemum stunt: a viroid disease. Virology 51: 94–101.

Hollings, M. & Stone, O. M., 1973. Some properties of chrysanthemum stunt, a virus with the characteristics of an uncoated ribonucleic acid. Ann. appl. Biol. 74: 333–348.

Lee, C. R. & Singh, R. P., 1972. Enhancement of diagnostic symptoms of potato spindle tuber virus by manganese. Phytopathology 62: 516–520.

Sänger, H. L., 1972. An infectious and replicating RNA of low molecular weight: the agent of the exocortis disease of citrus. Adv. Biosci. 8: 104–116.

Semancik, J. S. & Weathers, L. G., 1972. Exocortis disease: evidence for a new species of 'infectious' low molecular weight RNA in plants. Nature New Biology 237: 242–244.

Singh, R. P. & Clark, M. C., 1971. Infectious low molecular weight ribonucleic acid from tomato. Biochem. Biophys. Res. Comm. 44: 1077–1083.

Weathers, L. G., 1964. Nitrogen as a factor in the development of exocortis of citrus. Phytopathology 54: 968–969.

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