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Spread of viruses by recirculated nutrient solutions in soilless cultures

Spredning af virus i recirkuleret næringsstofopløsning i jordløse kulturer

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Summary

The risk of spreading viruses through contaminated water has become greater in recent years due to the development of new growing systems, such as soilless culture and recirculated nutrient solutions.

As the biological balance which earlier could be found in the soil no longer exists and recirculated nutrient solutions are used, the conditions of virus infection and vector survival have changed.

In order to investigate this new situation, infection trials were carried out with different viruses which were spread with and without vectors to plants grown in a system with a recirculated nutrient solution.

Cucumber green mosaic virus (CGMV), tomato mosaic virus (ToMV), tobacco necrosis virus (TNV) and the lettuce big vein agent (LBVA) were all spread by the watering system.

The spread of the CGMV and the ToMV – both without any known vectors – increased during the continuation of the culture. Root infections occurred to a very high degree after 1–2 months of culture, and systemic top infections to a lower degree after 2–5 months.

The spread of the TNV and the LBVA, both transmitted by the vector *Olpidium brassicae (O. b.)*, on the other hand, decreased during the continuation of the culture, but the infection rate became very high after only 1 day of exposure to contamination.

The *O. b.* zoospores were only active for the first 8 days of the culture.

The addition of the surfactant 'Teepol' to the recirculating water decreased the infection rate of LBVA, but did not eliminate infection.

Key words: Virus spread, cucumber green mosaic virus, lettuce big vein agent, tobacco necrosis virus, tomato mosaic virus, soilless culture, recirculated nutrient solution.

Resumé

Risikoen for virusspredning igennem forurenet vand er øget i de senere år på grund af udvikling af nye dyrkningssystemer, som f.eks. jordløs kultur og recirkuleret næringsstofopløsning.

Faktorer, som har ændret forholdene for både virusspredning og vektorer, er, at den biologiske balance, som tidligere eksisterede i jord, mangler, og at recirkuleret næringsstofopløsning nu anvendes.

For nærmere at undersøge disse nye forhold er der udført infektionsforsøg med forskellige virus, som spredes med eller uden vektorer til planter dyrket i et anlæg med en recirkuleret næringsstofopløsning.

Agurkgrønmosaikvirus (AGMV), tomatmosaikvirus (ToMV), tobaknekrosevirus (TNV) og salatnervebåndsklorose-agent (SNBA) er alle blevet spredt i vandingsanlægget.

Spredningen af AGMV og ToMV, begge uden kendte vektorer, øgedes med kulturtiden. Rodinfektioner forekom i stort antal efter 1–2 måneders kultur, og systemisk topinfektion i mindre omfang efter 2–5 måneder.

Spredningen af TNV og SNBA, som begge overføres af svampen *Olpidium brassicae,* mindskedes derimod med kulturtiden, men infektionsprocenten var allerede høj, efter at planterne havde været udsat for smitte gennem kun 1 dag.

Zoosporerne af *Olpidium brassicae* har kun været aktive gennem de første 8 dage af kulturperioden.

Tilsætning af afspændingsmidlet 'Teepol' til den recirkulerende næringsstofopløsning reducerede infektionen af SNBA, men eliminerede ikke angreb.

Nøgleord: Virusspredning, agurkgrønmosaikvirus, salatnervebåndsklorose-agent, tobaknekrosevirus, tomatmosaikvirus, jordløs kultur, recirkulerende næringsstofopløsning.

Introduction

During the last decade, new soilless growing systems have been introduced in the nurseries as an alternative way of controlling soilborne pathogens.

As rooting media peat and inorganic fibreglass or rockwool products are used, either in polythene bags or placed on polythene sheets as a barrier against soil-borne pathogens.

A nutrient solution is supplied either periodically, for instance in dripping and ebb-flood systems, or constantly by recirculating the nutrient solution using the so-called nutrient film technique (2).

Soil-borne viruses have been described earlier (3, 4, 13), and further investigations have shown virus spread through contaminated drainage water. The virus spread occurs either without known vectors, as is the case of cucumber green mosaic virus (CGMV), tomato mosaic virus (ToMV) and tomato bushy stunt virus or through vectors like *Olpidium brassicae (O. b.),* as is the case of big vein agent in lettuce (LBVA) and tobacco necrosis virus (TNV) (5, 6, 7, 9, 15, 16, 17, 20). Melon necrotic spot virus is also transmitted by *Olpidium* (1).

In soilless growing systems LBVA has already caused enormous problems, especially in England. However, very effective control has been obtained by adding surfactant substances such as »Agral« (alkyl phenol ethylene oxide) (18, 19).

Virus infection in cucumber plants grown in rockwool has also been described (1, 17).

Preliminary Danish investigations concerning the spread of LBVA by the fungus *O. brassicae* from infected to healthy plants in a recirculating watering system showed that the infection already occurred during the first hour after the plants had been exposed and that the infection percentage was independent of the time of exposure.

However, no infection occurred in other healthy indicator plants placed in the system using the same infector plants (10, 11).

The present paper deals with experiments concerning virus spread by a recirculated nutrient solution. The experiments were carried out at the Institute of Plant Pathology in Lyngby from 1978 to 1982, and some of the results have been published (8, 11, 12).

Method

Common to all experiments:

The set-up consisted of a container with 60 litres of a nutrient solution from which the solution was pumped round by means of an EHEIM air-cooled membrane pump No. 381 with a dosage of max. 228 litres/hour. The solution was fed, via tubes, into 7 channels on a slightly sloped table top from which the solution was returned to the container (Fig. 1). The tubes and the con-

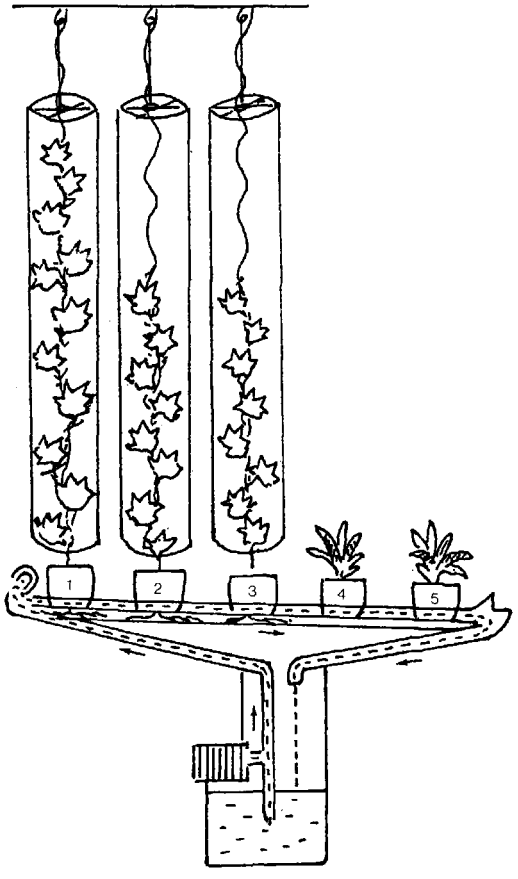

Fig. 1. The experimental set-up showing the recirculating nutrient solution and the position of plants.
1. Infector plant in foil tube used for long cultivation time.
2, 3. Younger indicator plants in foil tubes used for long cultivation time
4, 5. Indicator plantlets without foil tubes used for short cultivation time.

Jens Begtrup del.

Forsøgsanlæggets opstilling med den recirkulerende næringsstofopløsning og planternes placering:
1. *Infektorplante i plasticfolierør for en længere kulturperiode.*
2, 3. *Yngre indikatorplanter i plasticfolierør for en længere kulturperiode.*
4, 5. *Små indikatorplanter uden beskyttelse for en kort kulturperiode.*

tainer were dark and the area of cultivation was kept dark in order to prevent formation of algae. The nutrient solution consisted of 1 ‰ of the mixture NH_4NO_3 and a fertilizer mixture 'Superba' in the proportion 1:2 with addition of 0.1 ‰ 'Substral' micronutrient. The pH value was controlled weekly and kept at about 6 while the electrical conductivity was kept at about 16 mS/cm. The solution consumed was replaced by deionized water with or without nutrients depending on the conductivity figure. The nutrient solution itself was replaced before every new experiment.

All plants were raised either in blocks of inorganic rockwool or in »Einheitserde« according to Professor *Anton Frühstorfer*. »Einheitserde« developed especially for small-plant breeding and with a content of 60% peat and 40% clay. Later the plants were transferred to larger rockwool blocks or potted in rockwool granulate before the plants were placed in the watering channels.

An infector plant was placed in each channel at the upper end and followed by 4 to 8 healthy plants (Fig. 2). The nutrient solution flowed past the infector plant down to the healthy ones.

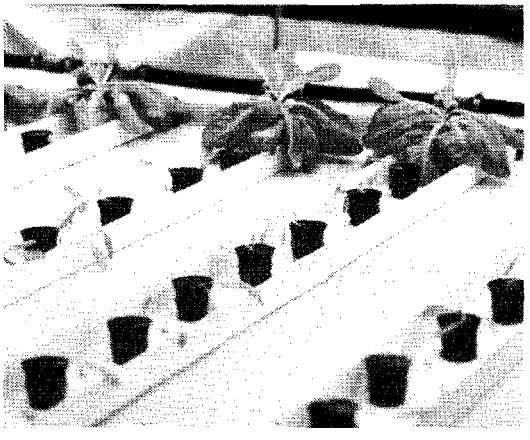

Fig. 2. Infection trial comprising lettuce big vein agent and *Olpidium brassicae* carried out in the system with recirculating nutrient solution. Healthy lettuce plantlets exposed to contamination from older infector plants.

Photo: Jens Begtrup.

Infektionsforsøg med salatnervebåndsklorose-agent og Olpidium brassicae *i anlægget med recirkulerende næringsstofopløsning. Sunde salatplanter udsat for smitte fra ældre infektorplanter.*

All plants grown for longer periods were isolated from each other in a 175 cm transparent plastic foil tube with a diameter of 28 cm (Fig. 3). In this way no leaf contact occurred.

On the other hand the roots of the infector and the healthy plants were allowed to grow freely together in the single channel (Fig. 4).

Leaf and root samples were tested for virus infection on suitable indicator plants at certain intervals and leaf symptoms were assessed. The leaf samples consisted of younger leaves and the root samples either of roots developed in the growing medium without risk of contamination or of roots developed in the channels. The latter root samples were placed in small gauze bags and rinsed in running tap water for half an hour in order to wash off contaminating virus particles.

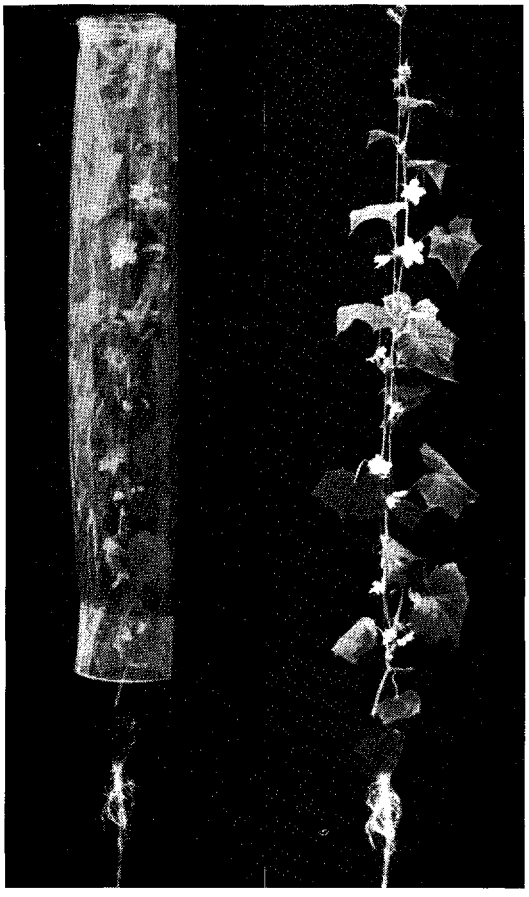

Fig. 3. Infection trials with cucumber green mosaic virus carried out in the system with recirculating nutrient solution. Cucumber plants after 1 month of culture with and without the isolating plastic foil tube.
Photo: Jens Begtrup.
Infektionsforsøg med agurkgrønmosaikvirus i anlægget med recirkulerende næringsstofopløsning. Agurkplanter efter 1 måneds kultur med og uden plasticfolierør.

Fig. 4. Tomato root development in the channel after 1 month of culture.
Photo: Jens Begtrup.
Udvikling af tomatrødder i vandingsrende efter 1 måneds kultur.

The surfactant 'Teepol', ('Teepol' GD53 15 per cent, alkyl benzene sulphonate, alcohol ethoxysulphate, alcohol ethoxylate, Shell), was used in the experiments in order to elimate the zoospores of the vector *Olpidium brassicae*.

Virus spread without vectors

Cucumber green mosaic virus

Infection trials were carried out with the gherkin variety 'Ideal Nova' used both as infector and indicator plants (Fig. 5). All the plants were raised in rockwool blocks, and the infector plants were sap-inoculated with CGMV.

At the time when the infector plants showed virus symptoms and the indicator plants had developed the cotyledons or the first true leaf, the plants were placed simultaneously in the water channels. At the end of the experiments leaf and root samples were tested on gherkin plants by sap-inoculation and the developed leaf symptoms were assessed.

Tomato mosaic virus

Tomato plants of the variety 'Revermun' were used both as infectors and indicators (Fig. 6). A yellow mosaic type of ToMV was used as virus source. All other conditions were similar to those mentioned for CGMV. Leaf and root samples were tested on detached leaves of *Nicotiana tabacum* 'Xanthi'.

Virus spread with vectors

Tobacco necrosis virus

The stipple streak strain of TNV was kindly supplied by Dr. *L. Bos*, Holland, for infection trials in combination with the fungus *Olpidium brassicae*.

Bean plants (*Phaseolus vulgaris* 'Bonita') were used both as infector and indicator plants. The infector plants were sown in rockwool granulate mixed with *O. b.*-infected lettuce root debris. A fortnight later the developed infector roots were assessed for *O. b.* zoosporangia and the plants were sap-inoculated with the TNV strain.

O. b. zoosporangia and TNV infection were seen in the roots of all the infectors and the TNV was spread systemically in the plants. The infectors were placed in the recirculating watering system with the indicator plants which were exposed to contamination for 24 hours at 3 intervals:

When infector plants were transferred to the channels, 3 days later and 8 days later.

Leaf and root samples were tested for TNV on detached leaves of *Nicotiana t.* 'Xanthi' one month after the exposure.

Lettuce big vein agent (LBVA)

Lettuce plants were used in 2 infection trials as infectors and as indicators (Fig. 2). The varieties 'Hjerter Es' and 'Baccarat' were used in the first and the second experiment respectively.

Fig. 5. Cucumber plants in plastic foil tubes grown in the system with recirculating nutrient solution.
Photo: Jens Begtrup.
Agurkplanter i plasticfolierør dyrket i anlægget med recirkulerende næringsstofopløsning.

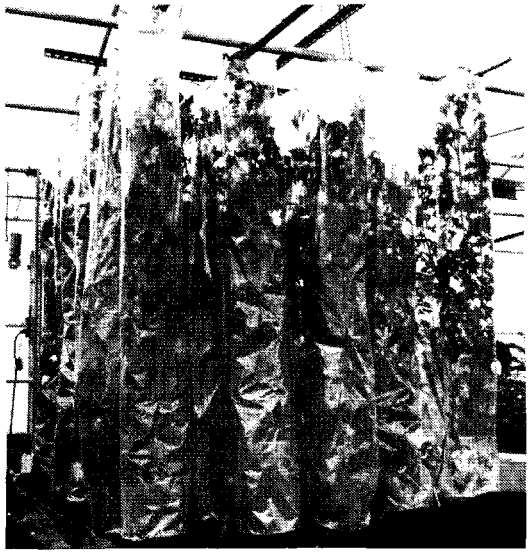

Fig. 6. Tomato mosaic virus infection trial carried out in the system with recirculating nutrient solution. Tomato plants after 1 month of culture.
Photo: Jens Begtrup.
Infektionsforsøg med tomatmosaikvirus i anlægget med recirkulerende næringsstofopløsning. Tomatplanter efter 1 måneds kultur.

The infector plants showed all the typical LBVA symptoms and were also infested with *O. b.* zoosporangia. The indicator plants were exposed to contamination for 8 to 24 hours for varying numbers of days from the start of the experiment.

The surfactant 'Teepol' was added to the nutrient solution in a concentration of 20 ppm one hour before start of the experiment and then every four days.

The treated plants were grown under isolated conditions, for 2 months, to avoid further root contact. The results were based on the LBVA symptoms found at the end of the experiment.

Results

Cucumber green mosaic virus

The following experiments with gherkins were carried out:

1. 14 days of culture where the plants only showed a limited growth with 1–2 permanent leaves and without any root contact between the plants.
2. 14 days of culture where the plants developed a moderate growth with 4 permanent leaves and partial root contact.
3. 30 days of culture where the plants grew strongly to a height of 150 cm and with 30 cm roots mixing freely with other roots. The results are shown in Table 1.

Table 1. Spread of cucumber green mosaic virus in gherkins by the recirculating nutrient solution.
Spredning af agurkgrønmosaikvirus i drueagurker med den recirkulerende næringsstofopløsning.

Culture time in days *Dyrknings-tid i dage*	Root contact *Rod-kontakt*	Samples: total number *Antal i alt*	per cent with virus: leaves *% med virus blade*	roots *rødder*
14[1]	none	106	0	17
14[2]	partial	56	4	77
30[2]	full	56	68	93

[1] Average of 4 experiments
[2] Average of 2 experiments

Virus contamination was further found in the nutrient solution after 9, 12, 14 and 30 days of culture, but not after 3 and 4 days.

Tomato mosaic virus

The experiments consisted of 2 infection trials where root contact occurred between all the tomato plants. Leaf and root samples were tested at weekly intervals. The results are shown in Table 2.

Table 2. Spread of tomato mosaic virus in tomatoes by the recirculating nutrient solution[1].
Spredning af tomatmosaikvirus i tomater med den recirkulerende næringsstofopløsning.

Culture time in weeks *Kultur-tid i uger*	Samples: total number *antal i alt*	per cent with virus: leaves *% med virus blade*	roots *rødder*	Per cent of plants showing virus symptoms at end of culture *% planter med virussymptomer ved afslutning af kulturen*
2	56	0	0	0
3	56	0	4	0
5	56	4	21	0
7	56	7	29	0
10	56	7	68	2

[1] Average of 2 experiments

Tobacco necrosis virus

Bean plants were used in an infection trial as both infectors and indicators. 3 sets of indicator plants were exposed to contamination 3 times for 24 hours without any leaf and root contact. The result is shown in Table 3.

Table 3. Spread of tobacco necrosis virus stipple streak in beans by the recirculating nutrient solution.
Spredning af tobaknekrosevirus stipple streak i bønner med den recirkulerende næringsstofopløsning.

3 sets of indicators exposed for 24 hours at various times after start *3 hold indikatorer udsat for smitte i 24 timer på forskellige tidspunkter*	Samples: total number *antal i alt*	per cent with virus: leaves *% med virus blade*	roots *rødder*	Per cent of plants showing virus symptoms at end of culture *% planter med virussymptomer ved afslutning af kulturen*
Set 1 after 0 days[1]	25	84	100	28
Set 2 after 3 days	25	64	100	12
Set 3 after 8 days	25	8	72	0

[1] Average of 2 experiments

Lettuce big vein agent

Set of lettuce plants were exposed to contamination for 8 or 24 hours at various intervals without any leaf and root contact, and the effect of the detergent 'Teepol' was investigated.

The results are shown in Tables 4 and 5.

Table 4. Spread of lettuce big vein agent in lettuce plants by the recirculating nutrient solution.
Spredning af salatnervebåndsklorose-agent i salat med den recirkulerende næringsstofopløsning.

5 sets of indicators exposed for 24 hours at various times after start *5 hold indikatorer udsat for smitte i 24 timer på forskellige tidspunkter*	Without 'Teepol'[1] total number of plants *Uden 'Teepol'[1]* *antal planter i alt*	 per cent with symptoms *% med symptomer*	'Teepol' added[2] total number of plants *'Teepol' tilsat[2]* *antal planter i alt*	 per cent with symptoms *% med symptomer*
Set 1 after 1 day	15	80	9	33
Set 2 after 3 days	17	100	8	50
Set 3 after 8 days	18	89	8	0
Set 4 after 16 days	17	0	7	0
Set 5 after 21 days	18	0	8	0

[1] Average of 2 experiments
[2] 20 ppm added 1 hour before start and every fourth day
20 ppm tilsat 1 time før start og hver 4. dag

Table 5. Spread of lettuce big vein agent in lettuce plants by the recirculating nutrient solution.
Spredning af salatnervebåndsklorose-agent i salat med den recirkulerende næringsstofopløsning.

3 sets of indicators exposed for 8 hours at various times after start *3 hold indikatorer udsat for smitte i 8 timer på forskellige tidspunkter*	'Teepol' added ppm *'Teepol' tilsat*	Total number of plants *Antal planter i alt*	Per cent with symptoms *% med symptomer*
Set 1 after 1–2[1] days	0	173	31
Set 2 after 3 days	20	89	15
Set 3 after 4 days	40[2]	90	10

[1] Average of 2 experiments
[2] 20 ppm added after 2 and 3 days of culture
20 ppm tilsat efter 2 og 3 dages dyrkning

Conclusion and discussion

The viruses cucumber green mosaic virus (CGMV), tomato mosaic virus (ToMV), tobacco necrosis virus (TNV) and the lettuce big vein agent (LBVA) were all spread by the recirculating watering system.

CGMV and ToMV, where no vectors are known, both spread increasingly during the continuation of the culture (Table 1, 2). Root infections already occurred after 2 to 3 weeks even without root contact, and reached a very high level after 1 to 2 months of culture.

Systemic top infection also occurred after 2 to 5 weeks but at a lower level. It is known that generally virus moves easily from top to root but only with difficulty from root to top. The result corresponds with the experiments of *Rast* (14) showing that the ToMV only moves to the top after a long growth period of up to 10 weeks.

TNV and LBVA, which are spread by the vector *Olpidium brassicae,* were both spread in the watering system. However, the spread decreased as the culture was continued (Table 3, 4).

The *O. b.* zoospores were only active during the first 8 days of the culture, which indicates that the living conditions for the zoospores were not suitable even with the total absence of metal ions, which are known to be toxic to the zoospores (19).

The addition of the surfactant »Teepol« decreased the LBVA infection rate to some extent, in contrast to English experiments, where infection was effectively avoided (18).

The results show that plants cultivated in systems with recirculating nutrient solution are in danger of epidemic virus attacks when first introduced into the system.

Viruses which are able to infect plants through the roots, without any vector, will, under normal growing conditions, be spread constantly causing an increasing rate of infection.

The possibility of inactivation of those viruses in the watering system has not yet been investigated in contrast to the fungus and virus vector *Olpidium brassicae* (18, 19).

When both fungus and virus are introduced

into the system, fungus-borne viruses will instantly be spread and cause a very high rate of infection.

It has been demonstrated quite clearly that inactivation of the vector for these fungus-borne viruses is possible by means of surfactants as for instance »Agral« (18), but in the present experiment, where the surfactant »Teepol« was used, this one was not effective enough.

In order to avoid virus attacks in watering systems virus resistant plant material may be used or sensitive plants may be grown under highly hygienic conditions in combination with an effective surfactant. This may be done by the use of controlled healthy plantlets grown in clean inorganic material and with an effective isolation against soil pathogens.

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