Project title:	**Cucumber: investigation of the cause of thick root**
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The results and conclusions in this report are based on a series of experiments and surveys. The conditions under which the work was carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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PRACTICAL SECTION FOR GROWERS

1. Introduction and objectives

In Holland, a disorder of rockwool-grown vegetables, particularly cucumber plants, known as 'thick root' (wortelverdekking), has occurred with increasing frequency since 1993. The problem develops during plant propagation, sometimes from as early as 6 days after sowing, and plants with affected roots are very slow to root into slabs with consequent reductions in yield. In England, thick root has been seen occasionally on imported Dutch cucumber plants (e.g. March 1994) but in spring 1998 it occurred for the first time on UK raised plants. The problem principally affected cucumber plants though abnormal growth was also seen on roots of occasional sweet pepper and tomato crops.

A study visit to Holland in June 1998 (PC 153) found that cucumber thick root was proving an intractable problem. Until very recently, researchers had not been able reliably to reproduce symptoms. No pest or pathogen has been consistently isolated from affected roots. When examined microscopically, these roots show breakdown of the epidermis and swelling and distortion of cortical cells, with the stele apparently unaffected. No evidence of a toxic chemical in rockwool cubes, vermiculite or feed solution has been found (Verkerke & Kipp, 1999). Alterations in feed solution pH, conductivity and composition did not lead to symptom development. A high water content in propagation cubes appears to be important for thick root to develop. Ethylene induces root curling but not glassiness or root swelling. The causal agent can occur in recirculated water used for ebb-flood irrigation. Small volumes of recirculation water transferred from affected nurseries to Naaldwijk Research Station did not lead to thick root but a 5 m^3 batch transferred and used in a recirculation system was effective. The causal agent passed through a 0.22 μm filter, but activity was lost on heating water to 45°C for 30 minutes.

Avoiding a high water content in propagation cubes appears to reduce the risk of thick root. Reducing fruit load on young plants appears to allow more rapid production of normal roots from affected plants into the slabs. Plants with thick root are probably more susceptible to *Pythium* because of the damaged root epidermis and precautions should be taken against this disease.

The objectives of the current project are:

1. To continue liaison with Dutch researchers investigating the cause and control of thick root.

2. To collect samples of cucumber and other protected crops affected by thick root in the UK and to test them for bacteria, particularly *Agrobacterium.*

3. To develop an experimental system in which thick root can be reliably reproduced (as a lead-in to studies on control of the problem).

4. To conduct isolations of candidate causal micro-organisms from such a system, for further testing.

2. Summary of findings

The cause of cucumber thick root remains unknown, although there is increasing evidence that bacteria, or bacterial products, are involved. This hypothesis is based on the loss of activity when hydroponic solution from an affected crop is filtered, heated or treated with antibiotics; and from the maintenance of activity with dilution. In Holland, some bacteria isolated from affected solution caused thick root in some tests, but the results were not reproducible.

Cucumber thick root was reported at two propagation nurseries in Holland in 1999, and none in England. The problem did not recur at UK production nurseries badly affected by thick root in 1998. Propagation nurseries appear to be using strict hygiene and disinfection procedures to reduce the risk of bacterial infection. In Holland, both propagation and production nurseries are introducing novel growing practices so as to create a drier root environment (thick root is known to be favoured by wet roots). These include: i) no wrapper on the propagation cube; ii) loosely wrapped rockwool slab stood in a drained trough; iii) overhead irrigation in preference to flood-drain during propagation; iv) new cube placed on top of the old cube at replanting v) production in tall containers (12 litre buckets) of pumice.

Experimental systems to maintain thick root were established at HRI Stockbridge House. Thick root symptoms were produced most reliably in an NFT system inoculated with affected solution from one of the 1998 UK outbreaks. Bacterial isolations were made from hydroponic solutions and from roots of affected plants from England and Holland. A wide range of bacteria were isolated including species of *Aeromonas, Alteromonas*, *Arthrobacter, Flavobacterium, Bacillus, Micrococcus*, *Pseudomonas* and *Rhodococcus.* After surface sterilisation, the quantity of bacteria isolated from healthy and affected roots differed, with more bacteria isolated from affected roots. This held true for both English and Dutch plants. From affected roots, potentially plant pathogenic bacteria isolated were *Pseudomonas* sp. from UK plants and *Curtobacterium* and a probable *Xanthomonas* species from Dutch plants. No rhizogenic *Agrobacterium* sp. were isolated from any samples, although non-rhizogenic *Agrobacterium* sp. were found in some samples. Numerous bacterial isolates have been placed in storage in preparation for testing to determine if any are capable of causing thick root.

3. Action points for growers

1. Be aware of the symptoms of thick root and their difference from root mat (see photographs of both diseases in HDC report PC 149, and *HDC News* **52** , 8-9).

2. Consider trying methods of growing cucumber with a drier root environment, which is considered to reduce the risk of thick root.

4. Recommendations for future work

It is recommended that work should continue to investigate the cause of thick root in hydroponic crops. Suggested areas of study include:

(i) to continue liaison with the research group at PBG Naaldwijk and to establish what research work will be undertaken at Naaldwijk in the near future.

(ii) to investigate, monitor and test for bacteria any outbreaks of thick root on cucumbers, peppers or tomatoes occurring in the UK.

(iii) to conduct Koch's postulates on up to 20 bacteria, isolated from affected plants and nutrient solutions in 1999.

(iv) once Koch's postulates are proven, use molecular methods to identify the causal organism.

SCIENCE SECTION

1. Progress on thick root in Holland

The following information was gained from discussions with growers and others in Holland, from observations made on nursery visits in Holland and from studying reports published in Groenten en Fruit and Naaldwijk publications.

1.1 Occurrence in 1999

Thick root was reported to be less of a problem this year than in 1998, with affected cucumber plants produced by just two propagators. In August 1999, during a visit to four cucumber nurseries in south Holland, symptoms were observed in a crop of cv. Korinda grown from plants produced by one of the known affected propagators. The crop had established well and produced an acceptable yield of fruit, but affected plants wilted in the hot weather of late July. Roots at the base of cubes showed gross irregular swelling, identical to that observed in some affected crops in the UK in 1998.

1.2 Recent experimental work at PBG Naaldwijk

1.2.1 Drainage water collected from a nursery with thick root was still able to cause thick root symptoms after dilution many times (Anon., 1998). This result suggests that the cause is either a micro-organism, or a chemical active at very low concentrations.

1.2.2 Using affected solution which has been diluted 100 times generally results in more thick root than using undiluted solution (Pittens van der Heijden, 1999).

1.2.3 Water containing the cause of thick root was still able to cause the problem after passage through a 0.22 μm filter (Anon., 1998). This result suggests that the immediate cause of thick root may be a chemical rather than a bacterium. Single cells of many bacteria are unlikely to pass through a 0.22 μm filter. Single molecules of a large protein such as haemoglobin (0.0068 μm) would easily pass through.

1.2.4 Some bacteria isolated from thick root-affected plants caused thick root symptoms when used to inoculate test plants but results were not reproducible (Anon, 1999; Pittens van der Heijden,1999).

1.2.5 The effect of various irrigation levels and fruit loads on the ability of sweet pepper plants to tolerate thick root was investigated (Anon., 1999). Results have not been publicly reported.

1.2.6 DNA techniques will be used to help investigate the involvement of micro-organisms in thick root (Pittens van der Heijden, 1999).

1.2.7 A disinfectant based on hydrogen peroxide and organic acids did not cause a decrease in thick root (Pittens van der Heijden, 1999).

1.2.8 Feed solution which had been in contact with affected roots for 1 week did not cause thick root. But if the feed solution was diluted it could result in thick root (Pittens van der Heijden,1999).

1.3 Recent experimental work at TNO, Leiden

1.3.1 Water containing the cause of thick root was no longer able to cause the problem after passage through an ultra-membrane filter which excludes particles greater than 100 KDa in size. The residue which collected on the filter did cause thick root (Anon., 1998). [The Dalton is a unit of mass used in connection with molecular and cell dimensions and is equal to the mass of one hydrogen atom (1.67×10^{-24} g). For comparison, haemoglobin, a globular protein, has a mass of 65 KDa and is 6.8 nm in width. If the filtration results of 1.2.3 and 1.3.1 are both correct, the cause of thick root appears to be larger than a haemoglobin molecule and smaller than an average bacterial cell].

1.4 Recent experimental work by Groen Agro Control (GAC), Delft

1.4.1 As at PBG Naaldwijk, Groen Agro Control are able to maintain thick root in a recirculating system, producing symptoms as required by placing young plants in affected water. An NFT system is used by GAC (Gastel, 1999).

1.4.2 Groen reports that ultrafiltration results show the cause of thick root to be 400 - 800 nm in size (Gastel, 1999). If correct, this result indicates that the causal agent is larger than an organic chemical, smaller than a fungal or algal cell and most probably a bacterium. [NB This result and 1.3.1 appear to be in conflict].

1.4.3 Electron micrographs of filters retaining the cause of thick root revealed several types of bacteria. Residue on filters caused thick root when tested by inoculation of plants in NFT (Gastel, 1999).

1.4.4 Water containing the cause of thick root lost its ability to cause symptoms when treated with an antibiotic (effective against a certain [unspecified]group of bacteria), but not when treated with a fungicide or algaecide (Gastel, 1999).

1.4.5 Water from one nursery with thick root only caused thick root when it was diluted. Groen hypothesised that this is consistent with a bacterium inhibited by other organisms, but able to multiply more quickly than other organisms under certain conditions.

1.4.6 Acetyl salicylic acid (aspirin) applied in nutrient solution to young cucumber plants was reported to prevent thick root in laboratory tests (van Gastel, 1998b). In a glasshouse test it reduced the incidence of root curling and glassiness by up to 80% in a test recirculation system. The concentration used was not reported, though it was stated that at concentrations greater then 10 µm (1.8g ASA/1000 litres) acetyl salicylic acid was harmful to plants. ASA is a signalling molecule which reduces a plant's sensitivity to ethylene, a known cause of root curling. There are many reports of it being used experimentally, to induce resistance to various foliar disease. The observed effect on early thick root symptom is not unexpected. **Commercial use of acetyl salicylic acid to control plant diseases is not permitted in either the UK or Holland.**

1.5 Examination by the Plant Protection Service (PD), Wageningen

1.5.1 It was reported that none of the micro-organisms known to PD is the direct cause of thick root (Gastel, 1998a).

1.6 Observations on grower practices to minimise the risk of thick root

1.6.1 It is now generally accepted in Holland that the cause of thick root is probably a bacterium or a bacterial product. Propagation nurseries are therefore adopting strict hygiene precautions to reduce the risk of site contamination. Disinfectants commonly used included sodium hypochlorite and Horticlean (a hydrogen peroxide product, ex Belgium). Slab surfaces may be drenched with Horticlean before replanting.

1.6.2 Earlier work in Holland showed a clear association between high water content and increased risk of thick root (see PC 153). Some growers are therefore adopting measures to grow plants in a drier medium including: (i) using a propagation cube without a plastic sleeve; (ii) overhead watering rather than ebb-flood irrigation; (iii) placing rockwool slabs in plastic troughs with good drainage and a loose plastic top cover; (iv) replanting on top of the propagation cube of a previous crop (providing no *Pythium* or thick root is present) (v) growing plants in 12 litre buckets of granular (4-8 mm) pumice or, less commonly, perlite.

1.6.3 'Grow Group' plant propagators at Naaldwijk are attempting to promote microbial activity in rockwool cubes, in an attempt to inhibit multiplication of any bacterium involved in the cause of thick root (Cooke, 1999). Two types of powder (lava and Humax) are suspended in water and drenched onto cubes; Humax is reported to be a host to *Trichoderma*, a possible beneficial fungus. It may be relevant that thick root rarely occurs in plants raised in peat blocks. However, it should also be noted that earlier work by Pim Paternotte at Naaldwijk indicated that test biocontrol agents (identity not revealed) did not control thick root.

1.6.4 Growers in Holland with whom I discussed the thick root problem, reported that all cucumber varieties they had tried, including grafted plants, were susceptible to the problem.

2. Progress on thick root in the UK

2.1 Occurrence in 1999

During the 1999 season there were no reported outbreaks of thick root on commercial nurseries (cucumber, tomato or pepper) in the UK where plants had been raised by UK propagators. No thick root was observed on the cucumber nurseries in Bedfordshire and Essex, where crops were severely affected by thick root in 1998. Thick root was observed, however, on some batches of young cucumber plants imported from Holland. Symptoms were slight and plants grew away from the problem.

2.2 An experimental system to maintain thick root

Introduction

A series of small scale re-circulating hydroponic systems were established at HRI Stockbridge House in a Venlo glasshouse unit (House M17) and inoculated with solution and roots taken from affected slabs collected from a commercial nursery in the UK that had suffered a thick root outbreak during 1998 (Fig 1). Uninoculated control systems were set up alongside to ensure the symptoms were not the result of crop production systems.

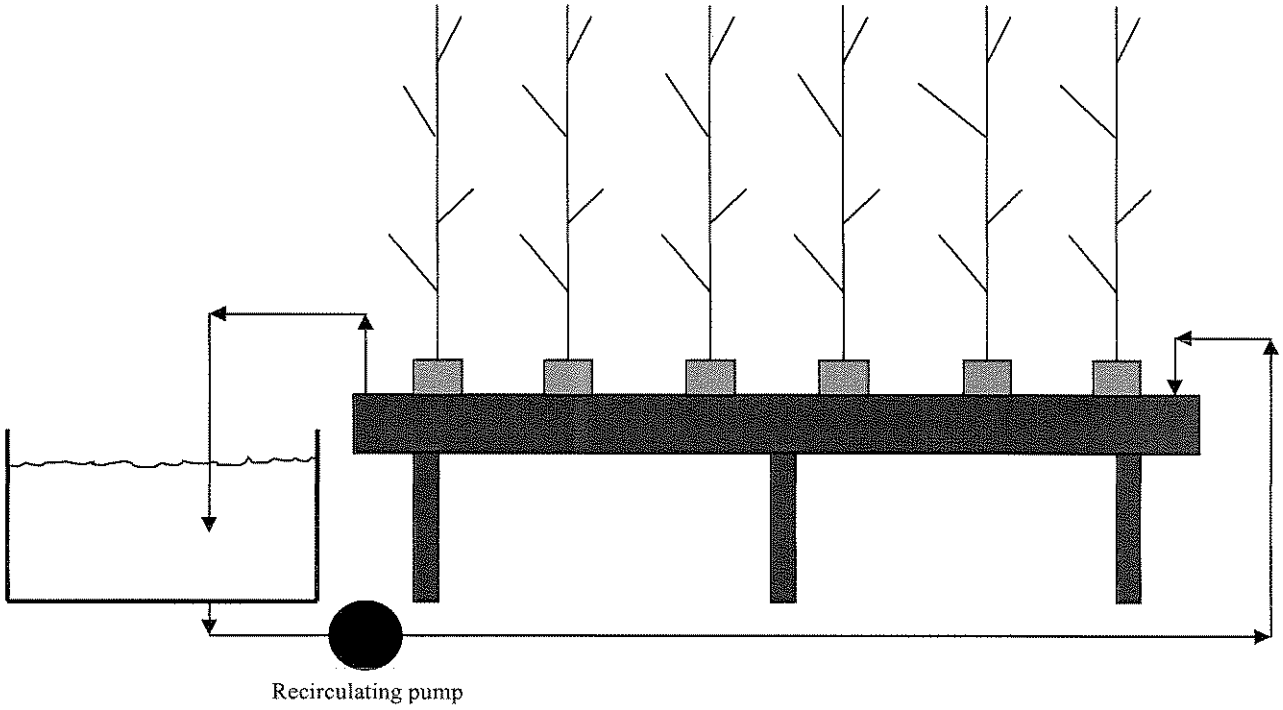
Methods

Four treatments were set up to examine symptom development:

1. Uninoculated control. Fresh nutrient solution prepared at Stockbridge House.
2. Inoculated. Affected solution collected by capillary action into fresh nutrient solution over a 24 hour period.
3. Inoculated. Affected slabs soaked in fresh nutrient solution for 1 week.
4. Inoculated. Affected slabs placed in NFT channel and young plants placed on top.

Two separate re-circulating NFT units were set up for each treatment, with 8 cucumber plants/unit. The water flow rate and environmental conditions followed an HRI blueprint growing regime. The plants in each treatment were monitored weekly for development of symptoms and root growth. The plants were removed and re-planted periodically with healthy young cucumber plants raised in a separate glasshouse.

During maintenance of the thick root culture, it was essential to ensure a high standard of hygiene in the vicinity of the re-circulating systems to avoid cross contamination between individual channels and to other protected salad crops on site. A Plant Health Policy outlining a Code of Practice was compiled detailing the precautions to be taken by all staff in contact with the trial. While crop working, staff wore shoes or shoe covers that were within the containment area, as well as gloves and laboratory coats. These were disposed of or cleaned as appropriate. Foot-dips were maintained at all entrances. Sciarid and shore flies were controlled to avoid potential spread. All excess nutrient solution from this area was pasteurised prior to disposal.



——— Flow of recirculating nutrient solution

Figure 1. Schematic diagram of the experimental recirculation system at HRI Stockbridge House set up for the thick root cultures.

Results and discussion

All methods of inoculating plant material resulted in thick root. The first symptoms noticed was tightly curled root growth, which developed from as soon as 5-7 days after plants were placed in the solution. This was followed by extensive root swelling with roots becoming water soaked or glassy in appearance. As the plants matured, the roots became corky or galled. Transferring around 2 litres of affected solution into 200 litres (dilution 1:100) of fresh nutrient solution resulted in symptoms developing on the young plants.

Throughout the duration of the work, a number of *Pythium* species occurred in the recirculation systems, leading to root discoloration, rotting and plant collapse. Despite regular fungicide application it proved difficult to eliminate. No *Pythium* spp. were isolated from the controls, suggesting that the *Pythium* isolates were associated with the original source of plant material affected by thick root. A number of isolates of *P. sylvaticum* and *P.* Group F were identified; other isolates are currently awaiting confirmation. It is likely that the nature of the cortical cell development with thick root could dispose roots more liable to infection, especially as the species of *Pythium* identified are considered only weakly pathogenic.

A number of *in vitro* fungicide resistance tests were conducted to assess the effectiveness of the fungicides to the *Pythium* species isolated. Some displayed resistance towards Filex and Fongarid in these tests, at 2-20 and 2 ppm active ingredient, respectively.

Over time, plants growing in the affected channels developed root symptoms similar to those caused by rhizogenic *Agrobacterium* biovar 1 strain, with a mass of root growth in the propagation block. However, only one strain isolated was found to be an *Agrobacterium* species and it proved negative when tested for the presence of Ri plasmid.

2.3 Analysis of root growth and structure

Roots from plants displaying thick root symptoms were examined microscopically to determine if any cortical cell swelling and cell disruption was present. Variation in cell size similar to that described previously by Dutch researchers was found. Sectioning the root tissue proved difficult due to the breakdown of the epidermis, the distortion and swelling of the cortical cells and infection by *Pythium* spp.

2.4 Isolation and identification of bacteria from hydroponic solution and cucumber roots

Isolation were made from (i) solution from the experimental recirculating system at HRI Stockbridge House; (ii) affected cucumber plants from this system; iii) affected plants from the experimental system at PBG Naaldwijk; (iv) affected plants collected from a commercial crop in Holland in August 1999. Over 100 isolates were identified to genus level by fatty acid profiling (FAP).

Samples of the re-circulating hydroponic solution from unaffected (control) and affected systems were taken to isolate potential casual agents of thick root. Water samples were filtered through a 0.45 μm membrane filter, re-suspending the filter contents in water in a dilution series. Small samples (0.1 ml) from each dilution were plated onto a range of culture media (SPA, King's B, NA, PDA and selective *Pythium* and *Phytophthora* agars) and incubated at 25°C for 8-72 hours. The range of organisms isolated on each media from the control and affected systems were monitored. In addition, root samples from all treatments were forwarded to CSL to test for the presence of *Agrobacterium* and other bacteria.

Unless stated all roots examined at CSL were surface sterilised (5 mins - 10% bleach solution) and then ground up in sterile phosphate buffer. 100μl of the resulting suspension was then spread onto a variety of media (to maximise the chance of culturing strains less able to compete on common media such as NA) and incubated for 48-72 hrs at 28°C.

Media Used:

NA - Nutrient Agar	Bergerson's rhizobium medium
Schroth's (for *Agrobacterium* bv. 1)	DSMZ rhizobium medium
Brisbane and Kerr's (for *Ag.* bv.2)	YGM - Yeast Glucose Mineral Agar
Rot and Sasser's (for *Ag.* bv. 3)	SPA - Sucrose Peptone Agar

2.4.1 Hydroponic solution

A wide range of bacteria were isolated (Table 1). Affected solution differed from control solution with a number of *Pythium* isolates recovered from the affected solution but not the control solution. *Phytophthora* was not isolated from any system.

Table 1. Bacteria isolated from hydroponic solution at HRI Stockbridge House.

Plate	Isolation media and code	FAP Identification	Source
1	NA 20	*Pseudomonas* sp.	Affected
2	NA 30	*Arthrobacter* sp.	Affected
3	NA 31	*Alteromonas* sp.	Control
4	SPA 4	*Pseudomonas* sp.	Control
5	SPA 14	*Rhodococcus* sp.	Affected
6	SPA 23	*Flavobacterium* sp.	Control
7	SPA 34	*Pseudomonas* sp.	Affected
8	KB 2	*Pseudomonas* sp.	Affected
9	KB 3	*Alteromonas* sp.	Control
10	KB 5	*Cytophaga* sp.	Control
11	KB 9	*Bacillus* sp.	Control
12	KB 15	*Pseudomonas* sp.	Control
13	KB 16	*Aeromonas* sp.	Affected
14	KB 22	*Micrococcus* sp.	Control
15	KB 24	*Flavobacterium* sp.	Affected
16	KB 33	*Acidovorax* sp.	Control
17	KB 36	*Arthrobacter* sp.	Affected
18	KB 37	*Rhodococcus* sp.	Control
19	KB 38	*Pseudomonas* sp.	Affected

There were differences in the range and number of bacterium isolated from the treatments exhibiting thick root compared to the control treatments. Colonies showing similar, or common, morphologies were forwarded to CSL for identification. In the control solution there was a higher number of some bacterial cultures including *Bacillus* spp., *Flavobacterium* sp., and *Alteromonas* sp. whereas *Pseudomonas* spp. and *Micrococcus* sp. were more prevalent in the infected solution. *Arthrobacter* sp. and *Rhodococcus* sp. were present in both systems at similar levels. In addition, *Cytophagus* sp., *Aeromonas* sp. and *Acidovorax* sp. were isolated and a few isolates were unidentified.

2.4.2 HRI Stockbridge House plants

Plants from five separate treatments were received at CSL. Roots were taken from each and surface sterilised before being ground up and plated out onto NA, Schroth's medium, SPA and YGM. Isolations were made from thick root affected roots. As with other thick root samples, a large number of bacterial colonies appeared on plates from infected material, with little or no bacterial growth from the healthy control tissue.

Table 2. Bacteria isolated from roots of cucumber plants from HRI Stockbridge House thick root experiment

No.	Sample From:	FAP Identification
1	Slabs in NFT Channel.	*Pseudomonas* sp.
2	Slabs in NFT Channel.	*Pseudomonas* sp.
3	Slabs in NFT Channel.	*Pseudomonas* sp.
4	Slabs in NFT Channel.	*Pseudomonas* sp.
5	Slabs in NFT Channel.	*Pseudomonas* sp.
6	Slabs in NFT Channel.	*Pseudomonas* sp.
7	Slabs in NFT Channel.	*Pseudomonas* sp.
8	Slabs in NFT Channel.	*Pseudomonas* sp.
9	Slabs in NFT Channel.	*Pseudomonas* sp.
10	Slabs in NFT Channel.	*Pseudomonas* sp.
11	Slabs in NFT Channel.	*Agrobacterium* sp.
12	Slabs in NFT Channel.	*Pseudomonas* sp.
13	Slabs in NFT Channel.	*Pseudomonas* sp.
14	Slabs in NFT Channel.	*Pseudomonas* sp.
15	Healthy Control	*Bacillus* sp.
16	Soaked Slabs.	*Sphingomonas* sp.
17	Soaked Slabs.	*Sphingomonas* sp.
18	Soaked Slabs.	*Sphingomonas* sp.
19	Soaked Slabs.	*Sphingomonas* sp.
20	Soaked Slabs.	*Sphingomonas* sp.
21	Capillary.	*Pseudomonas* sp.
22	Capillary.	*Pseudomonas* sp.
23	Capillary.	*Pseudomonas* sp.
24	Capillary.	*Pseudomonas* sp.
25	Capillary.	*Pseudomonas* sp.
26	Capillary.	*Pseudomonas* sp.
27	Capillary.	*Pseudomonas* sp.
28	Capillary.	*Pseudomonas* sp.
29	Capillary.	*Pseudomonas* sp.
30	Capillary.	*Pseudomonas* sp.
31	Capillary.	*Pseudomonas* sp.
32	Capillary.	*Pseudomonas* sp.
33	Capillary.	*Pseudomonas* sp.
34	Capillary.	*Pseudomonas* sp.
35	Capillary.	*Pseudomonas* sp.
36	Capillary.	*Pseudomonas* sp.
37	Capillary.	*Pseudomonas* sp.
38	Capillary.	*Pseudomonas* sp.
39	Capillary.	No Match
40	Capillary.	*Pseudomonas* sp.

Unless stated otherwise, all samples were surface sterilised and from affected tissue.

2.4.3 Dutch plants

Seven infected cucumber samples (cv. Odessa) were received from PBG Naaldwijk in Holland together with a healthy sample from a commercial Dutch nursery (variety unknown). These samples were processed in two ways, firstly individual samples were ground up in phosphate buffer and spread onto 8 different media without surface sterilisation. Secondly, the affected samples were bulked, surface sterilised and spread onto the same media together with a surface sterilised healthy sample. There was little in the way of typical thick root symptoms on the affected plants.

Initial observations showed no clear differences in bacterial growth between non-surface sterilised samples on any of the media. However, clear differences were observed on NA, *Rhizobium*, YGM, and SPA media between the affected and healthy surface sterilised samples with more growth being produced from the affected samples. Little or no growth resulted on plates from unaffected tissue. The differences on the SPA medium were especially marked. 40 colonies from all the plates were isolated and subjected to Fatty Acid Profiling (Table 3).

Table 3. Bacteria isolated from roots of healthy and affected cucumber plants from Holland, April 1999.

Plate	Sample from:	FAP Identification
1	Infected.	*Curtobacterium* sp.
2	Infected.	*Curtobacterium* sp.
3	Infected.	*Curtobacterium* sp.
4	Infected.	*Curtobacterium* sp.
5	Infected.	*Agrobacterium* sp.[1]
6	Non-SS. Infected.	*Agrobacterium* sp.[1]
7	Non-SS. Infected.	*Agrobacterium* sp.[1]
8	Non-SS. Healthy.	*Agrobacterium* sp.[1]
9	Non-SS. Healthy.	*Xanthomonas* sp.
10	Non-SS. Healthy.	*Agrobacterium* sp.[1]
11	Non-SS. Healthy.	*Agrobacterium* sp.[1]
12	Non-SS. Infected.	*Xanthomonas* sp.
13	Non-SS. Infected.	*Stenotrophomas maltophilia*
14	Infected.	*Curtobacterium* sp.
15	Infected.	*Curtobacterium* sp.
16	Infected.	*Curtobacterium* sp.
17	Infected.	*Curtobacterium* sp.
18	Infected.	*Curtobacterium* sp.
19	Infected.	*Curtobacterium* sp.
20	Infected.	*Curtobacterium* sp.
21	Infected.	*Curtobacterium* sp.
22	Infected.	*Curtobacterium* sp.
23	Infected.	*Curtobacterium* sp.
24	Infected.	*Curtobacterium* sp.
25	Infected.	*Curtobacterium* sp.
26	Infected.	*Curtobacterium* sp.
27	Infected.	*Curtobacterium* sp.
28	Infected.	*Curtobacterium* sp.

29	Infected.	*Curtobacterium* sp.
30	Infected.	*Curtobacterium* sp.
31	Infected.	*Curtobacterium* sp.
32	Infected.	*Curtobacterium* sp.
33	Infected.	*Curtobacterium* sp.
34	Infected.	*Curtobacterium* sp.
35	Infected.	*Pseudomonas corrugata*
36	Infected.	No Match
37	Infected.	*Stenotrophomonas maltophilia*
38	Infected.	*Stenotrophomonas maltophilia*
39	Infected.	*Stenotrophomonas maltophilia*
40	Infected.	*Stenotrophomonas maltophilia*

[i] Ri-plasmid negative.
Plates 8-10 were isolated from healthy control plants. Unless stated, all samples were surface sterilised

Further cucumber samples, cv. Korinda, showing obvious symptoms of thick root were received in August 1999. Isolations were made from the roots before and after surface sterilisation (Table 4).

Table 4. Bacteria isolated from roots of affected cucumber plants, from Holland, August 1999.

No.	FAP Identification
1	*Pseudomonas putida - fluorescens* complex
2	*Enterobacteriaceae - Serratia* sp. complex
3	*Enterobacteriaceae* sp.
4	*Pseudomonas putida - flourscens* complex
5	*Stenotrophomonas maltophilia*
6	*Agrobacterium* bv 1 sp.
7	*Agrobacterium* bv 1 sp.
8	*Agrobacterium* bv 1 sp.
9	*Agrobacterium* bv 1 sp.
10	*Agrobacterium* bv 1 sp.
11	*Enterobacteriaceae* sp.
12	*Enterobacteriaceae* sp.
13	*Cytophagaceae* (Genus Unknown)
14	*Enterobacteriaceae* sp.
15	*Pseudomonas* sp.
16	*Pseudomonas* sp.
17	*Cytophagaceae* (Genus Unknown)
18	No Match
19	*Pseudomonas putida - flourscens* complex
20	*Enterobacteriaceae - Serratia* sp. complex
21	Unknown Gram +ve Bacterium (*Bacillus* sp.?)
22	Unknown Gram +ve Bacterium (*Bacillus* sp.?)
23	Unknown Gram +ve Bacterium (*Arthrobacter* sp.?)
24	Unknown Gram +ve Bacterium (*Bacillus* sp.?)

25	Unknown Gram +ve Bacterium (*Bacillus* sp.?)
26	Unknown Gram +ve Bacterium (*Bacillus* sp.?)
27	*Xanthomonas* sp.
28	*Xanthomonas* sp.
29	*Xanthomonas* sp.
30	No Match
31	No Match
32	Unknown Gram +ve Bacterium (*Bacillus* sp.?)
33	Unknown Gram +ve Bacterium (*Bacillus* sp.?)
34	*Bacillus* sp.
35	Unknown Gram +ve Bacterium (*Bacillus* sp.?)

All samples were from surface sterilised thick root affected material

2.4.4 Dendrogram of fatty acid profiles from isolated strains

Fatty Acid Profiles are automatically stored by the computer controlling the Gas Chromatography machine. A dendrogram, based on unweighted pair match grouping analysis (UPGMA) of all the strains isolated from the thick root samples was constructed. Only one major cluster contained strains from all three sites. Two strains were selected from this cluster and inoculated into the leaves of a tobacco plant at ~10^9 cells ml^{-1}. No hypersensitive reaction resulted on the inoculated areas of this plant. Such a reaction can lead to the organism being considered a plant pathogen (i.e. a hrp gene cluster), though the absence of a reaction does not rule this out.

Comments

There were clear differences in the number of bacteria isolated from surface - sterilised roots from thick root affected and healthy plants, with far greater bacterial growth from affected roots. This held true for both Dutch and English plant samples. From non-surface sterilised roots, there were no clear differences in the bacterial flora isolated from healthy and affected samples. There were also clear differences in the range of bacteria isolated from the recirculating nutrient solution of control and inoculated experimental systems established at HRI Stockbridge House (see 2.4.1)

The bacteria isolated from Dutch and English plants differed. Potentially plant pathogenic bacteria isolated were *Curtobacterium* and a probable *Xanthomonas* from Dutch plants, and a *Pseudomonas* sp. from English plants.

It should be noted that not all bacteria are culturable and it is possible that the cause of thick root, assuming it to be a bacterium, may not grow in culture.

3. Recommendations for work in 2000

3.1 To maintain liaison with the research group at PBG Naaldwijk and cucumber consultants in Holland. To establish what work on thick root will be undertaken at Naaldwijk in 2000.

3.2 To investigate, test for bacteria, and monitor any outbreaks of thick root on cucumbers, peppers or tomatoes occurring in the UK.

3.3 To test the ability of up to 20 bacteria, isolated from affected plants and nutrient solutions in 1999, for their ability to cause cucumber thick root symptoms, and to attempt re-isolation of the introduced organisms from symptomatic plants (Koch's postulates).

3.4 Once Koch's postulate are proven, to isolate bacteria retrieved from filtering solutions obtained from affected crops. A PCR will then be performed on these strains using 16S rRNA primers. Resulting DNA fragments can then be sequenced and compared with known databases in an attempt to identify the casual organism.

3.5 To analyse filtrates obtained from infected crops by MALDI-TOF Mass Spectrometry (chemical 'fingerprint') and by DGGE profiling (microbiological 'fingerprint')

3.6 To report on the above to UK growers by means of an annual report, an article in HDC News and presentation at an appropriate grower group meeting or conference.

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