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Cucumber thick root - Report on a study visit to Holland June 1998

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PRACTICAL SECTION FOR GROWERS

Introduction

In Holland, a disorder of rockwool-grown cucumber plants know as 'thick root' or 'root thickening' ("wortelverdikking") has occurred with increasing frequency since 1993. The problem usually develops during plant propagation, sometimes from as early as 6 days after sowing, and affected plants can be very slow to root into slabs with consequent poor growth, wilting and reductions in yield. In England, the problem has occurred occasionally since February 1994 on imported Dutch plants, but in spring 1998 it occurred for the first time on UK raised cucumber plants, affecting both a propagation nursery and several production nurseries.

The **objectives** of the study visit reported here were to discuss cucumber thick root with Dutch researchers and plant raisers; to determine how the problem has been investigated in Holland; and to suggest possible areas for new work on the problem.

Summary of findings

Cucumber thick root has proved an intractable problem. The cause remains unknown despite many theories having been put forward and investigated. Until very recently, researchers have not been able reliably to reproduce symptoms. No pest or pathogen has been consistently isolated from affected roots. When examined microscopically, thick roots show breakdown of the epidermis and swelling and distortion of the cortical cells, with the vascular system apparently unaffected. No evidence of a toxic chemical in propagation cubes, vermiculite or feed solution has been found. Alterations in feed solution pH, conductivity and composition did not lead to symptom development. Ethylene induced root curling but not thick root. A high water content in propagation cubes appears to be important for thick root to develop. The causal agent can be transmitted in re-circulated water used for ebb-flood irrigation. Small volumes of re-circulation 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 re-circulation 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. These recent results suggest a thermolabile chemical, or chemicals, may be the cause; the origin of any such chemical is unknown. Further experiments at Naalkwijk on water filtration and heating are planned.

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 reported to be more susceptible to *Pythium* because of the damaged root epidermis and precautions should be taken against this disease.

A series of suggestions to investigate the cause and control of cumber thick root are made.

SCIENCE SECTION

1. Symptoms and occurrence of cucumber thick root

Plants with thick root are usually first recognised from their stunted growth and dark leaf colour. Examination of the roots at the side and/or base of the propagation cube reveals tightly curled fine roots (about 1 mm thick), markedly different in appearance from the straight roots of a normal plant. This root curling is believed to be the first stage in thick root development. Sections of lateral roots bearing these curly fine roots then develop a glassy, water-soaked appearance, due to abnormal cell enlargement in the root cortex. The vascular system appears unaffected. Finally, the areas of water-soaked roots swell and undergo secondary thickening, becoming corky in appearance. Lateral roots which are normally only 1-2 mm in diameter may become up to 10 mm in diameter. Swelling and thickening is often uneven along the length so that roots appear to have galls or nodules on them. Thickened roots are found within the cube near the stem base, on the outside of the cube or, occasionally, on roots within the growing slab. Photographs of cucumber thick root, alongside those of cucumber root mat for comparison, are shown in HDC Report PC 149.

Plants obviously affected by thick root when placed on slabs are usually very slow to root into the slabs and may wilt and die. Those which do root into the slabs appear, at least initially, unable to support a normal fruit load. Thick root becomes less of a problem if plants are able to get well established in a slab, although it is suggested that such plants are more susceptible to *Pythium* and other root diseases because of the damaged epidermis of the thickened roots.

Symptoms of thick root may develop from as early as 6 days after sowing seed, although sometimes they do not develop until around 20-22 days, just as the plants become ready for placing on growing slabs. It is possible that plants apparently healthy at dispatch may develop symptoms after placing them on slabs. It was stated that plants affected by thick root soon after sowing may perform adequately when planted out and, alternatively, plants first showing symptoms at around 20 days after sowing may perform poorly after planting out (i.e. there appears to be no progressive effect, as often occur with a disease).

It is reported (Verkerke & Kipp, 1998) that cucumber thick root may occur on plants in various growing media (e.g. rockwool, glasswool, foam, perlite). However, there appears to be less risk of thick root when plants are grown in peat. Growers in Holland report that if they use only peat propagation cubes no symptoms occur, even when there were problems previously on the nursery using rockwool cubes. Thick root has occasionally been observed on plants in peat cubes standing next to rockwool cubes with symptoms. Interestingly, it was recently reported that new roots allowed to grow through potting compost or coir placed around the stem base of affected plants (to encourage rooting), usually do not develop thick root (Anon, 1996).

It is reported that plants developing curly roots do not always develop water-soaked or thick roots. Possibly there may be more than one factor which can cause curly roots in cucumber, and only one factor which causes curly roots which subsequently progress to thick root. If so, factors which are found to induce curly root may be mistakenly identified as the cause of thick root. This may in part explain the numerous theories proposed for the cause of thick root.

Comments were made that a large proportion of plants in propagation can develop thick root symptoms very quickly. Also, that some plants in propagation remain unaffected even when surrounded by affected plants.

A survey of cucumber propagation nurseries in Holland in 1997 revealed most have now seen thick root on at least one occasion since 1993. The problem may affect just part, or all, of the production and may disappear for no apparent reason. It was reported that it may occur at any time of the year, although subsequent discussion suggested a greater risk in the winter/spring than in the summer. The problem has occurred on direct sown cubes, and where seedlings have been pricked out.

Early experimental work at Naaldwijk (1995) found that different depths of sowing, removal of the cube and slab wrappers, and using fertilisers from different sources did not result in thick root symptoms. In 1997, it was found that wounding roots at the cube base (by scratching on the floor), by picking up and putting down plants (e.g. when spacing plants), can result in curly roots but not thick roots.

Work by plant breeders was said to indicate that all commonly grown cucumber varieties are susceptible to thick root with faster growing varieties appearing more susceptible than others. In tomatoes, it has been noted that a weaker-rooted variety (Favorita) showed more symptoms than a stronger-rooted variety (Espero).

Trials conducted by Naalkwijk researchers on nurseries affected by thick root found no benefit from removal of cube wrappers, addition of *Trichoderma* to cubes or wetting cubes 1 day before sowing. It was stated that high water content and 'crowded plants' were necessary to maintain thick root development.

Cucumber thick root has been noted in Holland, England and Canada (Kipp, pers. comm.). There appears to be no published information on the problem other than that from Holland (see Section 10).

2. Factors associated with thick root development

2.1. Water content in the propagation cube

Development of thick root appears to be favoured by a high water content in the propagation cube. Root curling developed on plants grown in cubes with a constant high water content, but not in cubes held at a continuous low water content (Van Moolenbroek & Verkerke, 1996; Verkerke & Kipp, 1998). Measurement of variation in water content between and within propagation cubes revealed an association of thick root with a high water content both within and between cubes. On one propagation nursery, it was reported that thick root developed at the base of cubes on plants grown on concrete floors with ebb-flood re-circulation (where the base of cubes is liable to remain wet), but not on plants grown on aluminium ebb-flood benches with drainage holes. On another propagation nursery, thick root was reported to be more common on plants at the lower edges of profiled bays, where there was a greater chance of irrigation overlap and of cubes remaining wet.

2.2. Re-circulation of irrigation water

Many propagation nurseries in Holland use ebb-flood systems to irrigate cucumber plants. While small volumes of water collected from affected nurseries failed to cause thick root, 5 m^3 of recycled water transferred from an affected commercial nursery to an experimental ebb-flood system at Naaldwijk Research Station has resulted in development of thick root in test plants. No symptoms developed in an identical ebb-flood system in an adjacent glasshouse compartment which used the local water supply, also on a recycled system. These results suggest the cause of thick root can occur in recycled irrigation water.

However, thick root can also occur when irrigation water is not recycled. Thick root has occurred on propagation nurseries both in Holland and in the UK where irrigation water is not recycled. Although thick root appears to be more common on propagation nurseries where irrigation water is recycled, re-circulation is evidently not essential for occurrence of thick root.

It was stated in Holland that cucumber nurseries not re-circulating irrigation water were less at risk from thick root, but that it was no guarantee of freedom. It was suggested that large-scale re-circulation was needed for the problem to develop, but once present a single watering was sufficient to cause thick root. Possibly a high water content in propagation cubes is more common where plants are grown on flood-floor systems than with irrigation-to-waste systems. Additionally, spread of a water-borne causal agent between affected plants would be more likely in an ebb-flood system.

3. Recent Experimental work at Naaldwijk

An experimental system recently established at Naaldwijk (Figure 1), using 5 m^3 of water from an affected nursery in a small ebb-flood system, has proved a major advance. Thick root has been reproduced in test plants on a regular basis. Control plants in an adjacent compartment, using recycled mains water, remained unaffected. The following results were obtained from this system:

1. Water from the affected nursery has continued to lead to thick root development, even though it has now been diluted around 8 times, by topping-up with mains water.

2. Water taken from the collection tank of the experimental system and used to irrigate cucumber plants in an isolated (non-re-circulated) system resulted in thick root after just one or two waterings.

3. Cucumber plants grown in an isolated irrigation system in the same glasshouse compartment as the affected plants on the ebb-flood system remained healthy (i.e. there was no evidence for an aerial spread causal agent).

4. Water treated by heating to 45° for 30 minutes, or greater, no longer resulted in thick root when used to irrigate plants.

5. Water passed through a 0.22 µm filter resulted in thick root development. The residue taken from the filter surface also resulted in thick root development. Plating of the residue from the filter surface onto agar media revealed a mixture of micro-organisms. This experiment was repeated with the same results. A filter of this size would exclude both fungal spores and bacteria but may not exclude virus particles. [It should be noted that another research organisation in Holland (TNO) has reported that filtration does remove the cause of thick root.]

6. The experimental system has resulted in fewer thick root symptoms in recent tests. It was suggested that this may be due to high temperatures which occurred in the glasshouse in May.

7. Water transferred from another propagation nursery (not ebb-flood) affected by thick root, and used to establish an ebb-flood system at Naaldwijk, was ineffective in causing thick root symptoms.

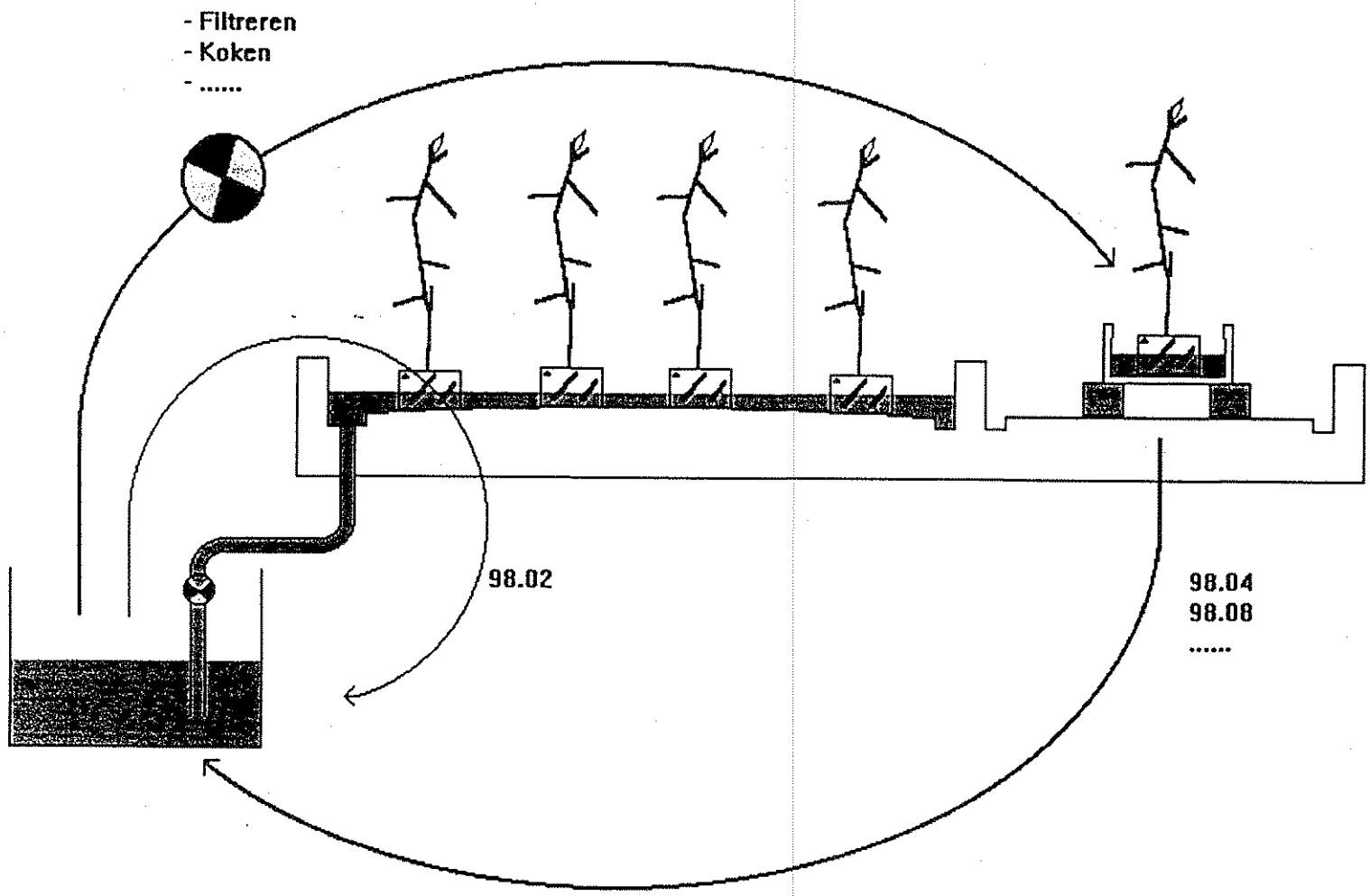

Fig 1. Design of experimental system for reproducing cucumber thick root, with water collected from affected nurseries (PBG Naaldwijk, Holland)

4. Possible causes of thick root

4.1. Fungi

Examination of and isolation from affected roots has failed to identify any fungus consistently associated with the problem. Usually, no fungus was isolated. *Olpidium* is sometimes observed within roots, but this fungus, the vector of melon necrotic spot virus and tobacco necrosis virus in cucumbers, is also found in unaffected roots.

4.2. Bacteria

No single bacterium has been consistently isolated from affected roots. Researchers at Wageningen isolated a number of bacteria, but none were able to infect test plants. They concluded that rhizogenic *Agrobacterium* bv. 1 (recently confirmed as the cause of cucumber root mat) was not involved.

Three samples of UK cucumber plants with thick root were examined for bacteria in early 1998 at CSL York. A mixture of bacteria were recovered, none consistently, but including rhizogenic *Agrobacterium*.

4.3. Viruses and viroids

Thick root symptoms are not consistent with any known virus or viroid disease. Tests for virus are currently being undertaken in Holland. An undescribed virus or viroid cannot be ruled out as the cause of thick root.

4.4. Pests

Nematodes are sometimes found in large numbers in roots affected by thick root. A sample from a UK cucumber crop was examined at CSL York this year and no plant parasitic species were found.

Sciarid and shore fly larvae are often found in and around thickened cucumber roots. These pests occur on dead organic matter and it is assumed that they occur as a consequence of thick root, rather than being the cause. The number of larvae in a plant with thick root can be 6 x greater than in a healthy plant.

4.5. Growing media

Chemical constituents of inert grown media used in plant propagation have been suggested as the cause of thick root. Chemical analysis of cubes from affected nurseries revealed no evidence of excess amounts of heavy metals, nitrate or nitrite. The effect of excess binding agent, excess wetting agent (50 x normal), and production of cubes at above and below the optimum polymerisation temperatures were investigated using Cultilene slabs. None of the treatments led to development of thick root symptoms (Verkerke & Kipp, 1998). Curly roots occurred in plants in all block types grown under constant wet conditions (80% moisture) and not in plants grown dry (50% moisture) or on an ebb flood system. Excess binder resulted in root tip browning.

The fact that propagation cubes are produced by a limited number of companies and sold in both Holland and the UK, but thick root, until 1998, was a problem only on Dutch propagation nurseries, also indicates that propagation cubes *per se* are unlikely to be the source of the problem.

4.6. Feed solution

Detailed monitoring of feed solution on affected and unaffected nurseries revealed no significant difference in solution pH, conductivity, redox value, heavy metals, nitrate or nitrite content. Experimental high, low and varying pH regimes failed to lead to symptom development. Grower observations in Holland suggest the problem is worse at a high pH.

High ammonium concentrations can lead to root curling, but not to thick root. Liquid fertiliser has been suggested as a possible source of thick root; but it was reported that only 8 propagators in Holland are using liquid fertiliser, and many more nurseries were affected by thick root.

4.7. Aluminium

Aluminium can be toxic to root growth. The usual symptom of aluminium toxicity in plants is root tips and laterals becoming thickened and brown, followed by development of dark green leaves and stunted growth as phosphate uptake and translocation is affected (Mengel & Kirkby, 1987). Cucumbers with thick root show dark green leaves and stunted growth, but not root tip browning. Pepper plants with thick root show root tip browning.

At normal pH values in cucumber production, aluminium binds with phosphate and is unavailable to plants. Aluminium becomes more readily available at pH 5.0 or less. The dominant aluminium ion species at low pH are Al^{3+} and $AlOH^{2+}$ with the latter much more toxic to plants. Aluminium toxicity in soil is reported to be greater around pH 4.5 because the $AlOH^{2+}$ concentration is high at this value (Mengel & Kirkby, 1987).

The role of aluminium in cucumber thick root was investigated at Naalkwijk. Cucumber seed were raised in vermiculite at pH 3.0 and in perlite at pH 5.5. No root swelling occurred at the low pH, where aluminium is likely to be available, or in the control plants in perlite. This result suggests aluminium is not involved in cucumber thick root.

4.8. Ethylene

High concentrations of ACC (1 - aminocyclo - propane - 1 - carboxylic acid), an ethylene precursor, were found in cubes of affected plants. Work at the University of Leiden has shown that ethylene can induce root curling but that symptoms do not progress into thick root. Interestingly, reported effects of ethylene on plant growth include cell expansion and tissue swelling as well as root curling (Lurssen, 1982)

4.9. Other gases

Research was started in 1997 to investigate the concentration of oxygen, nitrogen dioxide, carbon dioxide and other gases in and around roots. The Institute of Agrobiology and Soil Fertility Research at Wageningen is determining the oxygen requirement of cucumber roots. There is a theory at present that growers using hard water (high bicarbonate) are more at risk from thick root, [perhaps due to a resultant variable pH until all dissolved carbon dioxide has dissipated or to a low oxygen content when there is a high carbon dioxide content dissolved in water]. No results from this work were available. On one cucumber propagation nursery visited in Holland, the recycled solution was being oxygenated as a precaution against low oxygen content.

4.10. Water soluble organic chemicals

The recent demonstration that the causal agent can pass through a 0.22 μm filter but is inactivated by heat (30 minutes at 45°C) may indicate a water-soluble organic molecule. Such a chemical might originate, for example, from micro-organisms present in the recycled water or associated with cucumber roots, or be a chemical exuded from cucumber roots.

5. Measures to alleviate the effects of thick root

Various measures have been recommended in Holland:

In propagation

- Rinse the propagation cubes before sowing with twice their own volume of water. Discard this water.
- Space plants as soon as possible and preferably before roots emerge from the bottom of the cube [very difficult to achieve in practice]
- Only water when the average weight of the cube falls below 300g
- Water cubes up to around 450g. Do not saturate them. Check and adjust sprinklers if necessary to provide as even and as accurate watering as possible.
- Preferably water with overhead sprinklers, rather than ebb and flood irrigation
- Ensure there is good pH control
- Try to keep the cube and floor temperature below 25°C. (One nursery was adding mains or reservoir water to recycled water to prevent its temperature rising above 22°C.)

After placing on slabs until plants are well rooted

- Grow plants as dry as possible
- Maintain a low fruit load
- Control sciarid flies (as these may exacerbate root damage)
- Treat plants to control any *Pythium* infection

The suggestions on watering by weight and up to a specific weight during propagation would require a near-perfect watering system, because of the variability between cubes and the difficulty in re-wetting cubes which become very dry.

The practicality of achieving good pH control in propagation cubes is also likely to be difficult, with the pH varying as cubes dry out and are re-wetted.

An experiment is in progress at Naaldwijk evaluating different crop management practices on the effect of thick root on growth and yield of sweet pepper plants.

6. Observations on thick root in the UK in 1998

Thick root affected at least eight cucumber crops in England in 1998 and in two of them caused substantial yield reductions. In the other crops where the problem was slight, plants were slower than usual in rooting into the media but they grew away and there was no noticeable loss of yield. Some of the affected crops were grown from Dutch plants, others from UK raised plants; both first crops and re-planted crops were affected. One pepper crop was badly affected.

Symptoms were observed both in propagation cubes and on roots that developed within the rockwool slab. Cucumber plants supplied by one propagator with no history of thick root developed thick root when placed on used slabs which had previously grown affected plants.

On one nursery in Bedfordshire, the problem occurred in the second crop despite replacing old slabs with new slabs, disinfecting irrigation lines and equipment, and buying-in young plants from a nursery with no history of thick root (i.e. the problem appears to be persistent). Symptoms in the second crop did not appear until around 2 weeks after planting, by which time the plants had rooted well into the slabs, and yield appeared (at 4 weeks after planting) to be largely unaffected by the problem. This is consistent with reports from Holland that, for cucumber growers, the problem is greatest when plants visibly badly affected by thick root are planted.

7. Other protected vegetable crops affected by symptoms similar to thick root

There is evidence that aubergine, pepper and tomato plants can be affected by a problem similar to cucumber thick root. Although the gross symptoms are different in the different crop species (e.g. small, knobbly swellings in pepper roots) microscopically the affected roots appear similar with swollen cortical cells and collapse of the epidermis. The thick root problem is currently much more common in cucumber than in other crops.

8. Suggestions for possible research into the cause and control of thick root

Primary

1. Fresh samples of thick-root affected and unaffected cucumber plants should be tested for: a) the presence of rhizogenic and/or tumourigenic *Agrobacterium*, for *Rhizomonas* and for other bacteria, b) for fungi, by isolation and other appropriate tests, c) for possible virus and viroid infection by EM examination of affected roots and by host transmission tests.

2. Water samples shown to be capable of inducing thick root should be compared with unaffected water and with treated or sterilised affected water, by 1) chemical analysis 2) microbiological tests (total colony-forming units (cfu) and species composition)

3. A re-circulating system for reliably producing thick root symptoms should be established and used to investigate:

1. Effect of adding a wetter (e.g. Agral) on transmission of the problem
2. Effect of back filling the planting holes in rockwool cubes with peat and or coir
3. Effect of addition of selected micro-organisms on development of thick root (e.g. *Bacillus subtilis*, *Trichoderma harzianum*, root growth stimulants)
4. Infectivity of supernatant from centrifuged water
5. Dilution end point for infective water
6. Effect of chemical disinfectants (e.g. Jet 5, Reciclean, sodium hypochlorite) on transmission
7. Effect of using activated carbon in the production system

Secondary

4. Consider development of a propagation system to produce 'robust' young plant, for example by inoculating seed or the growing medium with benign or beneficial micro-organisms, by ensuring adequate oxygen content reaches the roots.

5. Investigate if rootstocks differ in their susceptibility to thick root.

6. Investigate the current seed treatment applied to cucumbers (heat and thiram dressing?) and evaluate alternative treatments.

7. Investigate the possibility of testing the efficacy of filters used in experimental work in Holland.

8. In Holland, the possible association of different water treatment systems (e.g. sand filter, UV, ozone, heat) used to treat re-cycled water on propagation nurseries, and occurrence of thick root could be investigated.

9. Acknowledgements

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Appendix. Organisations in Holland investigating or who have investigated cucumber thick root

1. Glasshouse Crops Research Station Naaldwijk (PBG)
2. Plant Pathology Service, Wageningen (PD)
3. Institute of Agrobiological and Soil Fertility Research, Wageningen (AB-DLO)
4. Dutch Organisation for Applied Scientific Research (TNO)
5. University of Leiden
6. University of Utrecht
7. Haskoning (independent research service employed by Grodan)
8. Barend Groen, Delft (independent consultant)