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Conduciveness of different soilless growing media to *Pythium* root and crown rot of cucumber under near-commercial conditions

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Abstract

Substrates made from rockwool, coir dust, pumice and perlite were compared for conduciveness to *Pythium* root and crown rot in cucumber under near-commercial conditions. Rockwool slabs of 7 cm height were more conducive to the *Pythium* disease than coir dust slabs, pumice or perlite under these conditions. Temperature, oxygen concentration and water content were determined in the substrates to explain differences in conduciveness between the inorganic substrates rockwool, pumice and perlite by differences in the physical conditions. Temperature and oxygen concentration could not explain the differences but the higher disease level on rockwool was associated with a much higher water content of this substrate as compared to coir dust, pumice and perlite. Increasing the height of the substrate from 7 to 14 cm greatly decreased the percentage of diseased plants due to the *Pythium* disease on rockwool but had no effect on the level of disease on perlite when the substrate had been infested 4 cm below the planting hole. This difference in response in substrate height between rockwool and perlite could be explained by a much larger decrease in water content with substrate height in the rockwool than in the perlite substrate. Temperature in the substrates were above 30 °C for more than 6 h on sunny days in June and reached maximum values of 35 °C or more. These temperatures are highly favourable for the pathogen *P. aphanidermatum* but will have adverse effects on most biocontrol strains.

Introduction

In the Netherlands, most glasshouse growers have changed from growing plants in soil to systems in which plants are grown on artificial substrates between 1980 and 1990 (Wever, 1999). The main reason for this change was generally higher yield on artificial substrates than when plants were grown in soil. Due to Dutch legislation cultivation of most glasshouse crops is only allowed in closed systems. In these systems, the nutrient solution is reused to prevent leaching of nutrients into the environment. Another important reason was the avoidance of root diseases such as black root rot of cucumber caused by *Phomopsis sclerotioides* and diseases caused by root knot nematodes. Problems with *Phomopsis sclerotioides* or root knot nematodes rarely occur in artificial substrates. However, *Pythium* root and crown rot which is not a serious threat in soil-grown cucumber plants, appeared to be a very serious disease of substrate-grown cucumber and a few other crops. Apparently, the conditions in the most commonly used substrate, rockwool, are very conducive for *Pythium* diseases as compared to those occurring in natural soils. A presumably low microbial activity in the inorganic rockwool as compared to soil may be a reason for its

conduciveness for *Pythium* rot since high microbial activities have often been associated with suppressiveness against *Pythium* diseases (Chen et al., 1988; Craft and Nelson, 1996). Other reasons for the conduciveness of rockwool slabs for *Pythium* diseases may be the high substrate temperature and water content during cultivation.

Rockwool is the most popular artificial substrate in the Netherlands. However, other substrates like pumice, perlite and substrates made from coir dust or fibres are now also being used in glasshouse horticulture and perlite especially is increasing in popularity. These substrates may differ in suppressiveness against *Pythium* diseases due to differences in biological and/or physico-chemical characteristics. Coir is an organic product and the microbial activity in the substrate is relatively high during use (Kipp et al., 2000). Due to its higher microbial activity, coir substrates may be less conducive for *Pythium* rot than substrates made from rockwool. Pumice and perlite are inorganic like rockwool and presumably do not support high microbial populations. However, these substrates are relatively dry substrates compared to rockwool slabs under common horticultural practices and these substrates may, therefore, be less favourable for *Pythium* spp., which are favoured by high water content. The height of the substrate may also affect its conduciveness to *Pythium* disease since the water content will decrease with increasing height.

Nowadays, *Pythium* rot is generally controlled by the preventive use of chemical pesticides. However, the Dutch government and other European countries wish to restrict the use of chemical pesticides in agriculture and the preventive use of chemical pesticides may be forbidden or restricted by law in the future. Alternative methods or strategies are, therefore, needed to decrease the risk of yield losses by *Pythium* diseases. Much research has been done on the biocontrol of *Pythium* root and crown rot in artificial substrates or hydroponic systems (Rankin and Paulitz, 1994; Buysens et al., 1995; Ongena et al., 1999, 2000; Paulitz and Belanger, 2001; Folman et al. 2003). However, no commercial biocontrol products are presently on the market which consistently suppress *Pythium* rot in rockwool. Biocontrol especially fails under warm conditions in spring or summer time during which plant losses by *Pythium* rot can be most serious. A strategy which may be much more promising to prevent plant losses due to *Pythium* rot may be the choice of the substrate. However, little information is available on differences in conduciveness for root diseases among artificial substrates and how disease may be affected by substrate height. Therefore, the goal of the present study was to compare the conduciveness of four commonly used substrates, rockwool, coir, pumice and perlite for *Pythium* rot of cucumber under practical conditions and to determine which abiotic substrate conditions are related to conduciveness.

Materials and methods

Plant material and substrates

Long English cucumber plants (cv. Odessa) were raised in rockwool blocks (Grodan, $10 \times 10 \times 6$ cm). No pesticides were used. Plants were placed on the substrate at the second or third leaf stage (second or third leaf fully unfolded). Four substrates were used: rockwool slabs (Cultilène; one-year, size: $100 \times 15 \times 7$ cm), compressed coir dust growing slabs (Profit, Forteco B.V., Kwintsheul, the Netherlands; size: $100 \times 15 \times 7$ cm after wetting), pumice (Bol Peat Import B.V., Schiedam, the Netherlands; size: 2–6 mm) and perlite no. 2 (Brinkman Agro B.V., Gravenzande, the Netherlands; size: 0.6–2.5 mm). The bulk density, porosity and water retention curves were determined for each substrate (Table 1). Perlite and pumice were analysed according to the EN standard (Anon, 1999). Rockwool and coir were analysed according to Wever (2003) and Kipp et al. (2000), respectively. Rockwool slabs and the coir slabs were placed in containers specially made for them (Librabak, Brinkman, Agro B.V., 's Gravenzande, the Netherlands) unless stated otherwise. Drainage holes were cut in the plastic covering the rockwool and coir slabs at the bottom at both ends of each slab. Pumice and perlite were placed in 12-l buckets unless stated otherwise. A bucket was filled with about 10 l of substrate reaching a substrate height of 18.5 cm. A drainage hole (2 cm width, 1 cm high) was made 2 cm above the bottom of the bucket.

Greenhouse site and watering frequency

All experiments were performed in the same greenhouse (size: 9.6×14.5 m). This greenhouse

Table 1. Physical characteristics of the tested growing media at leak out and at different pressure heads

Characteristic	Perlite	Pumice	Rockwool	Coir
Bulk density (kg m^{-3})	60	329	53	96
Water content (% volume)				
Leak out[a]	55	44	90	85
−10 cm (−1 kPa)	31	32	43	75
−20 cm (−2 kPa)	29	32	4	60
−50 cm (−5 kPa)	25	32	2	51
−100 cm (−10 kPa)	19	31	2	49

[a]For perlite and pumice the pressure head at leak out is −2.5 cm (−0.25 kPa), for rockwool −3.5 cm (−0.35 kPa) and for coir −3.0 cm (−0.30 kPa).

had 36 gutters not connected to each other on which the substrates were placed. Each plant obtained nutrient solution through a dripper (water release: 4 l h^{-1}) which was placed in each propagation block. The nutrient solution was automatically supplied using a transpiration model (De Graaf, 1988). This model calculates the amount of transpiration based on the initial plant length, global radiation and glasshouse temperature. The nutrient solution contained 1.3 NH_4, 8.0 K, 5.2 Ca, 1.6 Mg, 18.2 NO_3, 1.6 SO_4 and 1.4 P in mM and 25.0 B, 0.8 Cu, 0.5 Mo, 3.0 Zn, 10.0 Mn and 15.0 Fe in μM (EC 2.5 mS cm^{-1}, pH 5.5). The same nutrient solution was used for each substrate but the watering regime was adjusted to the height and the physical properties of the different substrates. For example, the 'setpoint gift', the calculated amount of transpiration after which the plants are irrigated, was highest for coir and lowest for pumice and perlite (Table 2). The nutrient solution was not recirculated to avoid spread of inoculum with the nutrient solution since no equipment was available to disinfest the nutrient solution and approximately 30% of the nutrient solution supplied was drained off. In the Netherlands, disinfestation of the drain water is common practice on commercial cucumber nurseries.

Pythium *inoculum and disease assessment*

An isolate of *P. aphanidermatum*, originally isolated from a diseased long English cucumber plant in Naaldwijk, was grown on V8-agar for three weeks (Expt. I) or on PDA (BioTrading Benelux B.V., Mijdrecht, the Netherlands) for two weeks (Exps II and III) at 24 °C. V8-agar was prepared as follows: 1 l of tomato-vegetable juice (Tomatientje, Albert Heijn, Naaldwijk, the Netherlands) was mixed with 10 g $CaCO_3$ for 20 min, the mixture was centrifuged at 10,000 × *g* for 5 min and the supernatant filtered through cheese cloth. The filtrate was diluted five times with demineralized water (V8-liquid medium). V8-agar was made by dissolving 15 g agar (Agar Technical, Difco, Brunschwig Chemie, Amsterdam, the Netherlands) in 1 l V8-liquid medium followed by autoclaving at 121 °C for 20 min. The agar colonized by the *P. aphanidermatum* isolate was cut into pieces of 1 × 1 cm and one piece of agar with the pathogen was placed in the substrate 4 cm below the surface in the middle of a planting site prior to planting. In rockwool and coir dust the agar piece was positioned by making a small crack from the side with a knife. In pumice or perlite the agar piece was placed in a hole made from the surface after which it was covered with pumice or perlite,

Table 2. Setpoint irrigation and irrigation time for each substrate (height) used in the experiments

Substrate		Setpoint irrigation (calculated transpiration in ml m^{-2})[a]	Irrigation time (s)
Material	Total height (cm)		
Rockwool, perlite	7	110	180
Coir	7	165	270
Pumice	18.5	55	110
Rockwool	14	55	110
Perlite	14/18.5	55	110

[a]The transpiration was calculated using the initial plant length, global radiation and temperature (De Graaf, 1988).

respectively. One piece of agar was used per plant unless stated otherwise. Plants were placed on the substrate within 2 h after infestation. Plants were observed for disease symptoms twice a week from 1 week after planting. A plant was recorded as diseased when it showed stem base rot and/or was wilted. It was also recorded when a diseased plant had died.

Experimental design and treatments

Three experiments were performed, one in the summer of 2001 (Expt. I) and two in the spring of 2002 (Exps II and III). In the first two experiments, different substrates were compared for suppressiveness against *Pythium* rot with substrate heights commonly used in practice. In the third experiment, different substrate heights were chosen for rockwool and perlite in order to explain differences in suppressiveness against *P. aphanidermatum* by differences in the physical properties of the substrates

Expt. I. Three substrates were compared: rockwool slabs, coir dust slabs and pumice. Each substrate had a non-infested control which resulted in a total of six treatments. Each treatment had six replicate gutters. On a gully, three rockwool slabs, three coir dust slabs (each 1 m in length) or six buckets filled with pumice were placed. In each bucket was placed one plant and on each rockwool slab or coir slab were placed two plants. The distance between plants was 56 cm.

Expt. II. This experiment consisted of six different treatments: four substrates (rockwool slabs, coir slabs, pumice and perlite) with two different amounts of inoculum in pumice and perlite; 50 cm rockwool and coir slabs were made from 1 m slabs. Five 50 cm rockwool or coir slabs were placed on each gully with two plants per slab and a planting distance of 30 cm. Pumice or perlite were placed in buckets as described above and two plants were placed on each bucket. Thus, each treatment had six replicate gutters with 10 plants each. The first slab or bucket on each gully was not infested with *P. aphanidermatum* and was used to measure different physical conditions in the substrate (see below). The total substrate volume per plant was about twice as high in pumice and perlite (5 l $plant^{-1}$) than in rockwool or coir (2.5 l/plant). Therefore, the experiment included extra treatments with pumice and perlite with two agar pieces with *P. aphanidermatum* per plant to obtain the same amount of inoculum per volume unit substrate as in the rockwool and coir. The two agar pieces were placed 4 and 8 cm below the planting site. At time of planting, 20 extra plants were placed on trays in the same greenhouse compartment, watered by hand, and observed for two weeks in order to determine if young plants had been infected or contaminated with pathogenic *Pythium* spp. prior to planting.

Expt. III. The experiment had six different treatments: two substrates (rockwool and perlite) at two different heights (7 and 14 cm) with two different infestation sites in the higher substrate. In the higher (14 cm) substrate an agar piece with *P. aphanidermatum* was either placed 4 or 11 cm below the substrate surface. 50 cm rockwool slabs were made as described for Expt. II. Plastic covers which are normally used to cover the rockwool slabs were filled with perlite to create similar substrate sizes for the two substrates. In this way 50 cm long slabs filled with perlite were created ('perlite slab', height: 7 cm). A 14 cm substrate height was obtained by placing a plastic tube ($12.5 \times 12.5 \times 7$ cm) on a rockwool slab or perlite slab and filling this tube with a piece of rockwool (cut out from a slab) or perlite, respectively. Two tubes were placed 30 cm apart on each 50 cm slab. Five 50 cm slabs were placed on each gulley. The first slab on each gully was not infested and was used to measure the substrate water content and temperature (see below). Twenty extra plants were raised on trays as described for Expt. II.

Substrate water content

In Expts II and III the water content of the substrate was determined using a FD-sensor calibrated for each substrate (Baas and Straver, 2001). The FD-sensor has three pins, each 6 cm in length. At each measuring time, the pins were pressed horizontally in the substrate and the water content recorded. The holes made in the plastic covers of the rockwool slabs and coir dust slabs using the pins were covered by plastic tape after each measurement. An opening had been made in the buckets filled with pumice or perlite prior to planting 8.5 cm above the drainage hole in order to measure the water content and this hole was covered with tape between measuring times. The water content in each substrate was measured on

different days after planting and always between 12:30 h and 13:30 h. The water content in the rockwool slabs and coir dust slabs was determined at 5 cm above the drainage hole. The water content was measured at 8.5 cm above the draining hole in buckets filled with pumice or perlite. The water content in the pumice and perlite substrates was not measured at the same height as in the rockwool slab because these substrates were higher and relatively dry at the top. It was decided to measure the water content in about the middle of these substrates. In Expt. III, the water content was measured at the 3 and 10 cm substrate height in both the perlite and rockwool substrates since in this experiment the rockwool and perlite substrates had the same heights. The water content in the rockwool blocks was determined 1.5 cm above the substrate surface 21 days (at 12:30 h) and 22 days (at 8:30 h) after planting in Expt II and 16 days after planting (12:30 h) in Expt. III.

Oxygen concentration

In Expt. II, gas sampling tubes were placed in the substrate as described by Wever et al. (2000). Briefly, tubes with an inner diameter of 0.7 cm and an inner volume of 3.8 ml were inserted horizontally into the substrate and fixed through holes made in the containers. Each tube had five small openings (0.4 cm diam) for gas exchange in the substrate. The tube was made air-tight at one end and at the other end the tube was closed with a septum. Gas sampling tubes were made at two different heights in the substrate: at 2 and 5 cm in rockwool and coir dust and at 3 and 14.5 cm above the bottom of the drainage hole in pumice or perlite. A gas sample was taken by pressing a needle through the septum of a cell. Oxygen concentration in the air sample was determined using a fiber optic oxygen sensor (Blok and Wever, 2001; Gérard and Blok, 2001.). The oxygen concentration was determined in four replicate rockwool slabs, coir slabs or buckets filled with pumice or perlite. Air samples were taken and oxygen concentration determined 1, 3, 7, 10, 14, 17, 21 and 24 days after planting.

Temperature

In Expt. II, the temperature in the substrate was measured together with the water content using the FD-sensor as described above two and eight days after planting. In Expt. III, temperature was recorded using temperature sensors connected to a data logger (DTL 1232, Ludo Herman, Belgium). In the 7 cm high substrates one sensor was placed 4 cm below the planting hole. In the 14 cm high substrate sensors were positioned 4 and 11 cm below the planting hole. Sensors were placed in the non-infested slab on each of three replicated gutters. Temperature was recorded every hour during the first 62 h after planting. Data on air temperature and global radiation were obtained from a local weather station in Naaldwijk.

Data analysis

The area under the disease progress curve (AUDPC), the curve describing the time course of the percentage diseased plants, was computed for each replicate. Data were tested for homogeneity of variance by observation of the residual plots and when no trends were found in these plots data were subjected to ANOVA (Gomez and Gomez, 1984). Data on the AUDPC and the percentage diseased plants on the final assessment day and the oxygen concentration and water content on each assessment day were analysed using analysis of variance (ANOVA). Mean values of the different treatments were compared using Fisher's protected LSD ($P < 0.05$).

Results

Expt. I

Disease development. Plants showed typical *Pythium* rot symptoms at the stem base both in the non-infested control as in the infested treatments. On an average 14 and 25% of the plants were diseased in the non-infested treatments and infested treatments, respectively, at the end of the experiment. A filamentous micro-organism was isolated from plants with stem base necrosis in the non-infested control at the end of the experiment on water agar (15 g l^{-1} Difco Agar Technical) and on *Pythium* selective medium (Difco Cornmeal agar amended with pimaricin (10 μg ml^{-1}), vancomycin (200 μg ml^{-1}), penicillin (100 μg ml^{-1}) and PCNB (100 μg ml^{-1})). The isolate was identified as a *Pythium* sp. based on its morphology

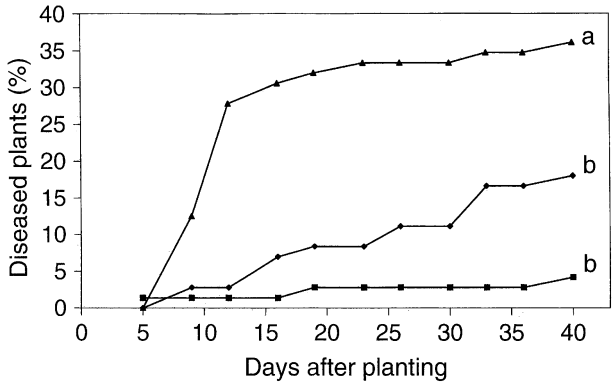

Figure 1. Expt. I: Percentages cucumber plants with *Pythium* stem base rot. Plants were grown on coir (♦), pumice (■) or rockwool (▲). Data points on the final observation day followed by the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.05$). The AUDPC of the rockwool treatment was significantly larger that those of the coir and pumice treatments (Fisher's Protected LSD, $P < 0.05$). The treatments coir and pumice were not significantly different according to this analysis.

and its ability to grow on *Pythium* selective medium. No significant interaction was present between the experimental factors substrate and infestation either for the AUDPC's nor for the final percentages of diseased plants (F-test, $P > 0.05$). The percentage of diseased plants was not significantly higher on artificially infested substrate than on non-infested substrate (F-test, $P > 0.05$). The mean percentage of diseased plants was significantly higher on rockwool than on pumice or coir (LSD, $P < 0.05$) (Figure 1); differences between coir and pumice were not significant (LSD, $P > 0.05$). On rockwool 23.6% of the plants were dead on the final observation day (day 40) and significantly lower percentages of plants had died on coir (5.6) and pumice (1.4) (LSD, $P < 0.05$).

Expt. II

Disease development. None of the plants placed on non-infested substrate nor any of the 20 control plants placed in trays showed any disease symptoms. Seven days after planting more than 20% of the plants on rockwool slabs infested with *P. aphanidermatum* showed stem base necrosis. On the other substrates none or less than 3% of the plants showed disease symptoms at that time (Figure 2). On the final observation day, 100% of the plants grown on rockwool showed disease symptoms of which 50% were dead. Significantly lower percentages of plants were dead on coir dust (4%), pumice (4%) and perlite (8%) (LSD, $P < 0.05$). Doubling of the amount of *Pythium* inoculum in pumice or perlite at the start of the experiment did not have a significant effect on the percentages diseased plants (LSD, $P > 0.05$; Figure 2).

Water content. The water content was highest in the rockwool slabs and lowest in perlite and pumice (Table 3). The water content in the rockwool blocks was similar for blocks placed on rockwool slabs, coir dust or pumice and was on an average 75% (v/v). Significantly lower water contents (61%) were measured in the rockwool blocks which had been placed on perlite (LSD, $P < 0.05$).

Temperature. The temperatures measured 2 cm below the substrate surface in the rockwool slabs and coir dust slabs and 8 cm below the substrate surface in pumice and perlite did not differ significantly and were on average 27.3 and 24.2 °C (at 13:00 h) 2 and 8 days after planting, respectively.

Oxygen concentration. Air samples could not be taken on some occasions when the gas sampling tube contained water. In that case the septum was removed and the tube emptied after which a new septum was placed. During the first three days after planting the oxygen concentration of the air samples was similar to that of the air in the greenhouse. Between three and seven days the oxygen density in the air decreased in the lower part of the rockwool slabs and became significantly lower than in the other substrates or in the upper part of the rockwool slabs (Table 4). From

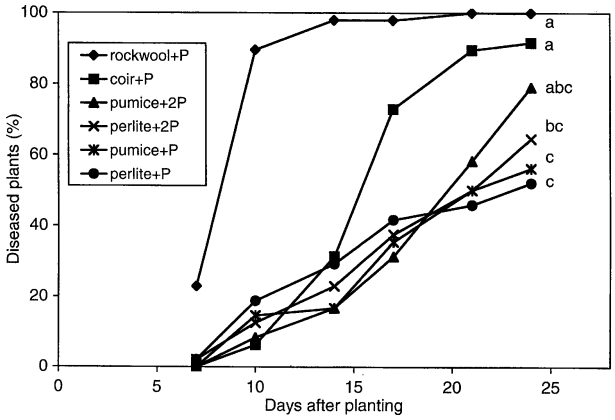

Figure 2. Expt. II: Percentages diseased cucumber plants on coir, perlite, pumice and rockwool. The substrates had been artificially infested with one agar piece colonized with *Pythium aphanidermatum* prior to planting. Perlite and pumice had either been infested with one (P) or two pieces of agar (2P). Data points on the final observation day followed by the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.05$). The AUDPC of the rockwool treatment was significantly larger than those of the other curves (Fisher's Protected LSD, $P < 0.05$). The other treatments were not significantly different according to this analysis.

10 days on, the oxygen concentration of the air in the lower part of both the rockwool slabs and the coir dust slabs were generally lower than in the upper parts or in the other substrates (Table 4).

Expt. III

Disease development. None of the plants placed on non-infested substrate nor any of the 20 control plants placed in trays showed any disease symptoms. All plants grown on 7 cm high rockwool slabs were diseased 23 days after planting and significantly lower percentages of plants were diseased on perlite or the 14 cm high rockwool substrate (Figure 3; LSD, $P < 0.05$). Total percentages of plants showing above ground disease symptoms were significantly higher on perlite than on 14 cm high rockwool except when *Pythium* inoculum had been placed 11 cm below the substrate surface in the 14 cm high perlite substrate (Figure 3; LSD, $P < 0.05$). On the final observation day, 75% of the plants grown on 7 cm high rockwool were dead and significantly lower percentages of plants (0–16.7) were dead on the other substrates (LSD, $P < 0.05$).

Water content. The water content decreased more with increasing substrate height in rockwool than in perlite. The water content of the rockwool was higher at 3 cm but of similar value at 10 cm substrate height compared to perlite (Table 5). The water content in the rockwool blocks on the different substrates was highest on the 7 cm high substrates and was similar for blocks placed on rockwool slabs and perlite with an average of 84%.

Table 3. Expt. II: volume water content (%) in rockwool slabs, coir dust slabs, pumice and perlite different days after planting and growing Long English cucumber plants

Substrate	Substrate height[a]	Measuring height	Days after planting				
			2	6	8	14	21
Rockwool	7	5	82a[b]	77a	76a	76a	69a
Coir dust	7	5	67b	60b	63b	62b	59b
Pumice	16.5	8.5	40d	37c	37c	36c	41c
Perlite	16.5	8.5	52c	43c	43c	43c	42c

[a]Centimetres above draining hole.
[b]Values followed by the same letter in each column are not significantly different according to Fisher's protected LSD ($P > 0.05$).

Table 4. Expt. II: oxygen concentration in air samples taken from four different substrates at different heights and different days after young long English cucumber plants had been planted

Substrate	Sampling height[b]	Days after planting[a]							
		1	3	7	10	14	17	21	24
Rockwool	2	19.9a	19.9a	17.7b	18.5c	17.2c	15.4c	16.2c	18.9bc
Rockwool	5	20.6a	20.3a	20.4a	20.2ab	20.2ab	19.5ab	19.7ab	20.4ab
Coir dust	2	20.1a	20.0a	20.2a	19.5bc	19.3b	18.1b	17.9bc	18.8c
Coir dust	5	21.6a	20.3a	20.6a	20.7a	20.8a	20.5a	20.8a	21.1a
Pumice	3	21.3a	20.4a	20.8a	20.9a	21.0a	20.9a	20.9a	21.3a
Pumice	14.5	21.3a	20.4a	20.8a	21.0a	21.1a	21.0a	21.1a	21.4a
Perlite	3	19.8a	19.0[c]	20.8a	21.0a	20.5ab	20.4ab	20.2a	20.8a
Perlite	14.5	20.9a	20.3a	20.8a	21.0a	20.8a	21.0a	21.1a	21.5a
Greenhouse air		21.5	20.4	20.6	21.3	21.2	21.4	21.5	22.0

[a]Values followed by the same letter in each column are not significantly different according to Fisher's protected LSD ($P = 0.05$).
[b]Centimetres above the draining hole.
[c]Value based on 1 replicate, not used in statistical analyses.

On the 14 cm high rockwool substrate the water content was 48% and significantly lower than on the 14 cm high perlite substrate (66%).

Temperature. During the first three days of the experiment the maximum air temperature outside the glasshouse was 20.3 °C. The weather was sunny at day of planting (day 0) and at day 2 with mean radiation between 10:00 h and 22:00 h of 490.9 and 529.4 W m^{-2}, respectively. The weather was cloudy on day 1 with a mean radiation of 121.6 W m^{-2}. No significant differences were found in the mean temperature between the substrates perlite and rockwool at 3 cm height nor at different heights (3 and 11 cm) in the same substrate. At 11 cm in the 14 cm high substrates, the average temperature was significant higher in rockwool (26.2 °C) than in perlite (25.3 °C) (LSD, $P < 0.05$). On sunny days the temperature in the substrates was above 30 °C for more than 6 h and maximum temperatures were about 35 °C. In the 14 cm high rockwool substrate the maximum temperature was about 38 °C.

Discussion

In the present experiments, rockwool slabs were much more conducive to *Pythium* rot than coir dust slabs, pumice and perlite under the applied

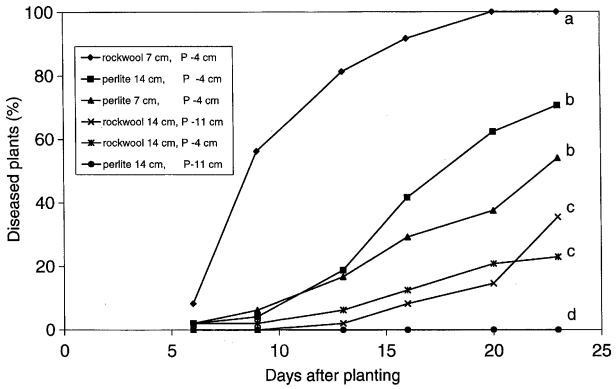

Figure 3. Expt. III: Percentages of diseased cucumber plants on perlite and rockwool substrates artificially infested with *Pythium aphanidermatum*. Two substrate heights, 7 or 14 cm were used and in the higher substrate *Pythium* inoculum was placed either 4 or 11 cm below the substrate surface prior to planting. Data points on the final observation day followed by the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.05$). The AUDPC of the treatment with 7 cm high rockwool was significantly larger that those of all other treatments; the perlite treatments with inoculum placed 4 cm below the surface were different from all other treatments according to this analysis (Fisher's Protected LSD, $P < 0.05$).

Table 5. Expt. III: Volume water content (%) in rockwool and perlite different days after planting long English cucumber plants

Substrate	Height (cm)	Measuring height (cm)	Days after planting							
			1	2	6	9	13	16	20	22
Rockwool	7	3	96a[a]	89a	86a	84a	83a	83a	86a	84a
	14	3	96a	89a	85a	86a	84a	83a	87a	85a
		10	59c	54c	54c	52c	53d	56c	56c	55c
Perlite	7	3	69b	68b	68b	66b	68b	66b	69b	69b
	14	3	71b	71b	72b	70b	70b	71b	72b	72b
		10	58c	54c	54c	54c	58c	57c	60c	59c

[a]Values followed by the same letter in each column are not significantly different according to Fisher's protected LSD ($P > 0.05$).

water regime resembling commercial growing conditions. Physical factors were assumed to play a major role in conduciveness of artificial substrates and, therefore, the present study focused on the differences in oxygen concentration, temperature and water content between the substrates.

At low oxygen concentrations plants may be more susceptible for *Pythium* root rot than at high oxygen concentrations (Chérif et al., 1997). In the present study, the oxygen concentration in the lower part of the rockwool slabs was significantly lower than in the pumice or perlite at day 7 and later on but it seems unlikely that this can explain the higher number of plants seriously affected by *P. aphanidermatum* for several reasons. Firstly the lower oxygen levels found in the rockwool substrate are probably still too high to expect oxygen deficiency (Baas et al., 2001; Wever et al., 2001). Secondly, lower oxygen concentrations were also measured in the lower part of the coir dust slabs and relatively few plants died on this substrate due to the *Pythium* disease. The oxygen concentration in the upper part of the rockwool slabs was similar to that in pumice and perlite and in pilot experiments it was found that cucumber plants were more heavily affected by *P. aphanidermatum* when inoculum had been placed higher in the substrate. Moreover, plants showed severe disease symptoms in the upper part of the rockwool slab (root rot directly below the rockwool block) and higher (stem base necrosis) where oxygen levels were similar to those in pumice and perlite.

Temperature could not explain the differences in *Pythium* disease and the high water content in the rockwool slabs was probably the main reason for the high disease level on this substrate as compared to pumice and perlite. High water contents favour the rapid formation of zoosporangia and zoospores (Duniway, 1979), and *P. aphanidermatum* may produce more zoospores in the rockwool slabs than in the other substrates. At higher water contents, zoospores may also have a better opportunity to swim through the substrate in the free available water and reach more locations where they can infect the roots. It was shown that doubling of the substrate height of the rockwool significantly decreased the risk of plant losses due to the *Pythium* disease whereas no significant difference in disease levels was found between the 7 and 14 cm high perlite substrate when the inoculum had been placed 4 cm below the substrate surface (Figure 3). The water content in the upper part of the 14 cm high rockwool substrate was much lower than in the lower part of this substrate whereas this difference in water content was much lower for the 14 cm high perlite substrate (Table 5). The water content was measured at 3 and 10 cm height. With increasing height above 10 cm the water content in rockwool will drop much more strongly than in perlite (Table 1; Kipp et al., 2000). This difference in water retention between rockwool and perlite may explain why the percentages of diseased plants decreased much more with the increased substrate height in rockwool than in perlite. Probably, the water content and not the pressure head is the main factor influencing the disease development since the water pressure head at 3 cm height in rockwool and perlite will be of similar value as also indicated by the water content close or even above the water content at leak out (Tables 1, 5).

The higher water content in the perlite substrate (Table 5) as compared to the water content at leak out (Table 1) was not unexpected since the water content in perlite was measured 2 cm above the draining hole whereas the water content according to the EN Standards is measured at a pressure

head of 2.5 cm (Anon., 1999). Moreover, roots present in the substrate will contribute to higher water content measurements, and perlite takes up more water over time (the EN standard measures the water content after 72 h of saturation) (Wever, 1995; Wever et al., 2004).

The lowest level of disease was found in perlite when the inoculum had been placed 11 cm below the substrate surface. Lower disease levels may be expected when the pathogen occurs at a larger distance from the planting hole: younger cucumber plants are more susceptible to the *Pythium* disease. If the plant is infected at an early stage, the chance that the stem base will be infected by the pathogen will increase and the risk that the plant will die also increases. The position of the inoculum may be less important when the conditions in the upper part of the substrate (close to the planting hole) are unfavourable (e.g. relatively dry) for the disease. For example, no effect of the position of the inoculum was found in the 14 cm high rockwool substrate.

A lower water content in the rockwool substrate may also be obtained by decreasing the water supply instead of increasing the substrate height. However at the start of the culture this is rather difficult because there is only a minor water uptake by the plant. The substrate will, therefore, remain relatively wet in this period. Also the lower centimetres of rockwool slabs are generally saturated with water under commercial growing conditions and rather low amounts of water will need to be given to obtain water contents unfavourable for *Pythium* spp. also in that part of the substrate. A low water supply will result in a small water buffer in the substrate and a high risk of water shortage for the plant and thereby lower yields. On the other hand a large water supply in perlite will generally not lead to a large increase in the water content of the substrate as water is easily drained off in this substrate making it less conducive for *Pythium* diseases than rockwool.

In Expt. I, control plants were diseased and the greenhouse where the plants had been raised had probably been contaminated with the pathogen since more plants raised in this greenhouse showed typical symptoms of *Pythium* root and crown rot. This unplanned contamination indicated that the kind of substrate on which the plants are planted may still have an effect on disease development even when the plants or propagation blocks and not the substrate have been infected or contaminated. In Expt. II, the water content of the rockwool blocks placed on rockwool slabs, coir dust slabs or pumice was not significantly different. No water content measurements had been done in Expt. I but there is no reason to believe that significant differences in water content of the rockwool blocks occurred in Expt. I since the water regime in Expt. I was similar to that in Expt. II. The results, therefore, suggest that different conditions in the substrates below the propagation blocks had caused the differences in the disease levels. Possibly, the lower water content in the pumice and coir dust may have been responsible for the lower disease levels on these substrates than on the rockwool slabs.

The temperature in the substrate was above 30 °C for more than 6 h and reached values of 35 °C or even more on sunny days in June when the crop was still small and not fully shading the substrate (Figure 4). The air temperature outside the glasshouse was 20 °C at maximum and higher substrate temperatures may have been expected when the outdoor temperature would have been higher than 20 °C, which is not unusual for June. Such high temperatures favour the pathogen, *Pythium aphanidermatum*, as this *Pythium* species has an optimum temperature of 35–40 °C (Van der Plaats – Niterink, 1981). The temperature did not reach such high values when the crop was shading the substrate (data not shown). However, the first weeks after planting is a critical period for plant losses due to the *Pythium* disease and many young plants may be lost during the first weeks when the substrate becomes infested with the pathogen. A second criticial period for plant losses is the period after fruit set. In that period, the plants are shading the substrate and substrate temperatures will increase less than during the first weeks after planting due to sunlight. Various researchers have been working on the control of *Pythium* rot in hydroponic-grown cucumber using antagonistic micro-organisms like *Pseudomonas*, *Streptomyces* and *Trichoderma* strains (Rankin and Paulitz, 1994; Buysens et al., 1995; Postma et al., 1995, 2001). Most of these strains will have maximum growth temperatures between 30 and 35 °C and an optimum below 30 °C. The efficacy of these biocontrol strains may therefore be low during sunny days in spring and summer time shortly after planting when the conditions are highly favourable for the pathogen and are causing most damage to the crop. Therefore, this temperature effect should

be much more addressed in studies on biocontrol of *Pythium* disease in hydroponics since effective control strategies are especially needed during warm and sunny weather during the first weeks after planting of the crop.

In the present experiments, high percentages of cucumber plants died on rockwool slabs due to *Pythium* root and crown rot, whereas relatively low percentages of plants died on perlite or on a double layer of rockwool even at temperatures highly favourable for the *Pythium* pathogen. The results indicate that the water content plays a major role in the development of root and stem rot caused by *P. aphanidermatum* in hydroponics and that the type and height of the substrate are important tools for decreasing the water content in the root zone and for decreasing the chance of yield losses due to the disease.

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