Pseudomonas corrugata **and** *Pseudomonas marginalis* Associated with the Collapse of Tomato Plants in Rockwool Slab Hydroponic Culture

Václav KŮDELA, Václav KREJZAR and Iveta PÁNKOVÁ

Division of Plant Heath, Crop Research Institute, Prague-Ruzyně, Czech Republic

Abstract

Kůdela V., Krejzar V., Pánková I. (2010): *Pseudomonas corrugata* **and** *Pseudomonas marginalis* **associated with the collapse of tomato plants in rockwool slab hydroponic culture.** Plant Protect. Sci**., 46**: 1-11**.

Plant pathogenic species *Pseudomonas corrugata* and *P. marginalis* were detected and determined in collapsed tomato plants in rockwool slab hydroponic culture in southern Moravia, Czech Republic. Surprisingly, *P. marginalis* was also determined before planting in apparently healthy grafted tomato transplants grown in hydroponic culture. Moreover, non-pathogenic *P. fluorescens, P. putida, P. synxantha*, and *Stenotrophomonas malthophilia* were identified. The Biolog Identification GN2 MicroPlate™ System (Biolog, Inc., Hayward, USA) was used for identification of bacterial isolates. Cultures of *P. corrugata* and *P. marginalis* were used in a greenhouse pathogenicity experiment. Seven weeks old tomato plants of cv. Moneymaker grown in sterilised perlite were inoculated into the stem with a hypodermic needle at one point above the cotyledon node. In inoculated tomato plants, disease symptoms were observed that included external and internal dark brown lesions around the inoculation site, watering and collapse of pith and sometimes also vascular browning and wilting of leaves. In comparison with *P. marginalis, P. corrugata* appeared to be a much stronger pathogen. Both tested *Pseudomonas* species were recovered from inoculated tomato plants. *P. corrugata* was found to move both upwards to the apex of the stem and downwards from the site of the inoculated stem into roots. When inoculated into potato tuber slices, some tomato strains of *P. marginalis, P. fluorescens, P. synxantha*, and *Pseudomonas* sp. produced soft rot. However, other strains of the same species were not able to macerate the potato tissue. It is concluded that *P. corrugata* and *P. marginalis* can be associated with the collapse of tomato crop in soilless culture grown in a greenhouse. This is the first report on *P. corrugata* in tomato plants in the Czech Republic. The role of plant pathogenic bacteria, fungal root rot and vascular pathogens and *Pepino mosaic virus* in the collapse of tomato plants is discussed.

Keywords: *Pseudomonas corrugata*; *Pseudomonas marginalis*; *Pseudomonas fluorescens*; *Pseudomonas synxantha*; *Lycopersicon esculentum*; bacterial diseases; wilting; root rot; open hydroponic system

Over traditional outdoor field systems, greenhouse production provides the option of off-season crop production and expansion of markets. Most greenhouse horticultural crops are grown in soil. Recently, however, there has been an increasing interest in the use of hydroponics or soilless techniques for producing greenhouse horticultural crops. The most popular are so called aggregate hydroponic or soilless technologies for growing plants in nutrient solution with the use of an artificial inert medium (substratum), such as rockwool (mineral wool), to provide mechanical support for roots.

Production of greenhouse tomatoes in hydroponics (water culture) or soilless culture has increased in popularity in Europe and North America in the last decades. However, in the Czech Republic, no more than 10 ha of hydroponics growing system with rockwool are in use mainly for the production of tomatoes and cucumbers and no further increase is expected in the years to come.

One of the advantages of hydroponic systems is that they provide a relatively sterile pathogen- and pest-free growing medium (Staunton & Cormican 1978). As distinct from soil, soilless culture is supposed to be a relatively ideal pathogen-free environment for plants.

Nevertheless, microbiological biocoenosis in the phyllosphere and rhizosphere of plants grown in rockwool hydroponic systems and other hydroponic production techniques is still imperfectly known concerning both plant growth promotional and plant heath effects.

In 2008, the collapse of mature tomato plants causing severe losses was recorded in rockwool hydroponic culture in a commercial glasshouse in Moravia, the Czech Republic, and we were asked to participate in solving this problem. The objectives of this study were: (1) to find out if the relatively sudden collapse of tomato plants observed in a period between the first fruit set and the first fruit maturation was associated with the presence of plant pathogenic bacteria; (2) to search for plant pathogenic bacteria in tomato transplants for planting; (3) to verify the pathogenicity of isolated bacteria for tomato plants by artificial inoculations.

MATERIALS AND METHODS

During the 2008 growing season and from January to May 2009, more than 60 mature collapsed tomato plants were taken in rockwool slab hydroponic culture in order to examine histological symptoms of diseased and apparently healthy tomato plants and to perform bacteriological analysis with the aim to search for plant pathogenic bacteria. Besides, more than 50 apparently healthy grafted tomato transplants grown in small rockwool cubes saturated with nutrient solution were analysed.

Isolations. Bacteria were isolated both from diseased tomato plants and apparently healthy transplants as described by Schaad *et al.* (2001). Stem and root segments about 1.5–2.0 cm long were surface sterilized for 3 min in a 1:10 dilution of a household bleach (5.25% active sodium hypochlorite). After rinsing in sterile water, stem or root segments were superficially dried, longwise cut, and parts of the discoloured vascular strands or pith were excised using a sterile scalpel. The excised small pieces of tissue were placed in a droplet of sterilised water in a flamed watch glass and mechanically crushed. Fifteen to twenty minutes after mechanical crushing, a loopful of macerate was streaked onto King's B medium and nutrient agar (NA) in Petri dishes and incubated at 26°C. Single bacterial colonies with cultural characteristics resembling that of pectobacteria or pseudomonads (both fluorescent and nonfluorescent) were re-streaked to obtain pure cultures of representative strains.

Identification. Each bacterial isolate was screened for Gram reaction using the simple KOH technique (Schaad *et al.* 2001). The Biolog Identification GN2 MicroPlate[TM] System (Biolog, Inc., Hayward, USA) was used for the identification of bacterial isolates obtained from tomato plants according to standard protocols. The microplates were incubated for 16 and 24 hours. Biolog's MicroLog 2 4.2 software was used to identify the bacterium from its carbon substrate oxidation pattern. Calculations for the identification of bacteria as to the genus, species and other taxonomic units are based on similarity indices. A similarity index of ≥ 0.500 was considered to indicate a good species match (Table 1).

Pseudomonas corrugata (ex Scarlett *et al.* 1978) Roberts and Scarlett 1981 was used as a positive control for all identification and pathogenicity tests.

Pathogenicity test. Cultures of bacterial isolates obtained from tomato plants, 8K/1 (isolated from root), 8S/3 (from stem) and Corr 1 (from pith), were used in a greenhouse pathogenicity experiment (Table 2). Seven weeks old tomato plants of cv. Moneymaker, grown in sterilised perlite and watered with Knop's nutrient solution, were inoculated. A suspension of each tested bacterial isolate (1×10^8 CFU/ml) in sterile distilled water from 24-h cultures was used as the inoculum. In one experimental variant, 23–25 plants in one experimental variant were inoculated by injection of approximately 0.2 ml of inoculum into stem internodes with a hypodermic needle at one point above the cotyledon node. Five plants injected with sterile water were used as controls.

After inoculation, the plants were placed into clear plastic bags and maintained in a moisture saturated atmosphere for four days and then they

Table 1. Identification of bacterial isolates from tomato plants based on the carbon substrate oxidation pattern (BIOLOG)

Isolate or strain number	BIOLOG identification	Similarity index[1]	Probability[2]	Pectolytic activity[3]
Ra Pc Corr1; 8S/3; 3; 4; 5; 6; 7; 8; 8K/1	*Pseudomonas corrugata*	0.9; 0.7; 0.9; 0.82; 0.93; 0.92; 0.93; 0.91	100; 89; 100; 100; 100; 100; 100; 100; 100	NT
DSM 7228[T] (=ATCC 29736[T] = NCPPB 2445[T])	*Pseudomonas corrugata*	0.94	100	NT
Ra G 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11	*Pseudomonas fluorescens* biotype G	0.62; 0.62; 0.78; 0.57; 0.86; 0.87; 0.79; 0.67; 0.71; 0.87	100; 100; 100; 100; 100; 100; 100; 100; 100; 100; 100	NT
Ra Sm 1; 2; 3; 4	*Stenotrophomonas maltophilia*	0.59; 0.57; 0.51; 0.69	100; 100; 100; 100	NT
Ra Pf 1	*Pseudomonas fluorescens*	0.51	97	+++
Ra Pf 2	*Pseudomonas fluorescens*	0.64	85	–
Ra Pf 3	*Pseudomonas fluorescens* biotype A	0.54	81	–
Ra Pm 1; 3; 6; 8; 9; 10	*Pseudomonas marginalis*	0.67; 0.55; 0.6; 0.59; 0.57	100; 100; 99; 99; 91	–
Ra Pm 2; 4; 5; 7	*Pseudomonas marginalis*	0.54; 0.82; 0.63; 0.65	98; 100; 98; 99	+++
Ra Psyn 1; 3; 4	*Pseudomonas synxantha*	0.57; 0.54; 0.52	83; 94; 100	–
Ra Psyn 2	*Pseudomonas synxantha*	0.5	90	+++
Ra Ppu 1	*Pseudomonas putida*	0.54	93	–
Ra Ppu 2	*Pseudomonas putida* biotype A	0.68	100	NT
Ra P 1	*Pseudomonas* sp.	–	–	+++

[1]At 16–24 h of incubation, the similarity index must be at least 0.50 to be considered an acceptable species identification
[2]% probability – allows to compare identifications with other systems that use this type of calculation
[3]+++ high pectolytic activity (tissue maceration has spread from the site of inoculation through the entire potato disk); + weak pectolytic activity (tissue maceration has spread 5–20 mm from the site of inoculation); – no pectolytic activity; NT not tested

were unbagged. The plants were grown in a greenhouse at 20°C to 25°C and 60–70% relative humidity. Eleven weeks after inoculation, external and internal symptoms were evaluated. The length of external and internal stem lesions around the inoculation points was measured (Table 2).

To determine if *P. corrugata* and *P. marginalis* could move from the inoculated site on the stem upwards to the apex of the stem and downwards into the roots and cause disease symptoms, the stems and roots were examined for vascular browning, discoloration or disintegration of the pith and rot of roots. Isolations were made from different parts of stems and roots.

Pectolytic ability. Potato tubers were surface sterilised with alcohol, peeled aseptically and sliced into disks about 7 mm thick. These were placed in sterile Petri dishes with sterile moistened filter paper. A very heavy cell suspension was applied into shallow pits made in the centre of potato disks of cv. Dita, and the dishes were incubated at 27°C. The inoculated disks were examined in 24, 48 and 72 h for soft rot probing the tissue surrounding the inoculum site with a loop to assess the ability to macerate the potato tuber tissue (Bradbury 1970; Schaad *et al.* 2001).

RESULTS

Symptoms on collapsed tomato plants

External symptoms

The first symptoms of suddenly collapsed tomato plants were observed in a period between the first fruit set and the first fruit maturation (Figure 1).

Table 2. Pathogenicity test with five isolates of *Pseudomonas corrugata* (Pc) and one isolate of *P. marginalis* (Pm) from tomato plants

Variant	Isolate/strain	Number of plants	Wilting plants		Mean length of lesions (mm)			
			number	percentage	external lesions	internal lesions	external + internal lesions	variant 1 = 100
1	Pc NCPPB	25	4	16	109	309	418	100
2	Pc (from pith) 8SK	23	14	60	183	145	328	78
3	Pc (from stem) 8S4	25	13	52	120	160	280	67
5	Pc (from roots) 8K1	25	14	56	70	77	147	35
4	P m ŠB2	24	13	54	83	67	150	36
6	Control	5	0		0	0	0	0

Stems and petioles of symptomless plants exhibited a conspicuous fragility.

Initially, affected plants showed daytime wilting near the top of the stem, then merging into progressive leaf wilting. Finally, severely affected plants displayed sudden drying and dying, with or without yellowing (Figure 2). As the disease advanced, the whole plants collapsed.

In some cases, external elongated dark brown to blackish lesions appeared on the stems at the beginning of picking (Figure 3). The cortex sloughed easily from the stele (Figure 4).

Stems cut lengthwise showed watery browning of the cortex, light to dark browning of vascular tissues, discoloration or disintegration of the pith of stems. The pith was watery, soft and rotted, sometimes with cavities near the base of the stem (Figure 5). The appearance of numerous protuberances and short adventitious roots was observed both at the base and in the middle of affected stems (Figure 6). In most cases, the root system was severely destroyed. Browning of the root cortex and survived central cylinders was a characteristic symptom observed.

Histological symptoms

In order to evaluate the grade of colonisation of xylem vessels, i.e. the relative proportion of affected

Figure 1. Collapsed mature tomato plants in rockwool hydroponic culture in a commercial glasshouse (Photo V. Krejzar)
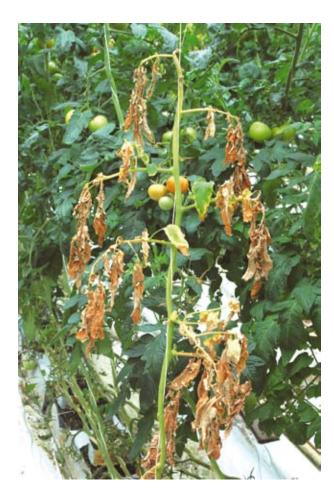
Figure 2. Wilting, drying and dying of tomato plant. Some basal leaflets of leaves show marginal yellowing – natural infection (Photo V. Krejzar)

Figure 3. Brown to black streak along the length of the stem of tomato plant with symptoms of wilting and drying of leaves – natural infection (Photo V. Krejzar)

xylem tissues, we evaluated histological symptoms on a cross-section of the main roots and the base of stems of more than 60 diseased tomato plants. We found out that on average only 25% to 30% of outer xylem vessels in the stems were occluded in the analysed collapsed plants.

Identification of bacterial pathogens and non-pathogens

Pseudomonas corrugata and *P. marginalis* were detected and determined in collapsed tomato plants in rockwool slab hydroponic culture. Besides, non-pathogenic *P. fluorescens*, *P. putida*, *P. synxantha*, and *Stenotrophomonas malthophilia* were identified. *P. marginalis* was also determined in apparently healthy grafted tomato transplants. Attempts to isolate *Clavibacter michiganense* subsp. *michiganense* from collapsed tomato plant failed.
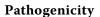

Figure 4. Disintegration of the cortical tissue and its sloughing off at the base of tomato plant with symptoms of wilting and drying of leaves – natural infection (Photo V. Krejzar)

Figure 5. Laddering of pith in the longitudinal section of tomato plant with symptoms of wilting and drying of leaves – natural infection (Photo V. Krejzar)

Figure 6. Numerous short adventitious roots grouped in longwise splits on the stems – natural infection. (Photo V. Krejzar)

Pathogenicity

In tomato plants inoculated with a hypodermic needle at one point above the cotyledon node, external and internal symptoms were observed which included external dark brown lesions around the place of inoculation, water-soaked pith and not infrequently vascular browning and wilting of leaves. Initial adventitious roots vertically arranged above the necrotic lesion appeared on the stems of infected plants (Figures 7–9).

In comparison with *P. marginalis*, *P. corrugata* appeared to be a much stronger pathogen. Both tested *Pseudomonas* species were recovered from

Figure 7. Reactions of cv. Moneymaker tomato plants to the inoculation with *P. corrugata* (on the right) compared to the inoculation with the fungus *Pyrenochaeta lycopersici* (on the left) (Photo V. Krejzar)

Figure 8. An external necrotic lesion on the tomato stem spreading upwards from the point of inoculation with *Pseudomonas corrugata*, isolate 8S/3. Note the development of initial adventitious roots vertically arranged above the necrotic lesion (Photo V. Krejzar)

Figure 9. The tomato stem split longitudinally to show internal water-soaked pith. Note also light brown lesions on secondary roots at the site of their attachment to the main root (see arrows) – artificially infected with *Pseudomonas corrugata*, isolate 8S/3 (Photo V. Krejzar)

inoculated tomato plants. In some cases the re-isolation of *Pseudomonas corrugata* from tissue segments cut out from various places of inoculated tomato stems yielded nearly the clear culture of the originally inoculated bacterial isolate. *P. corrugata*

Figure 10. Reisolation of *Pseudomonas corrugata* from tissue segments cut out from various places of the inoculated tomato stem. With increasing distance from the point of inoculation the number of recovered bacterial colonies on an agar plate decreased (Photo V. Krejzar)

was found to move both upwards to the apex of the stem and downwards from the site of the inoculated stem into roots. The number of recovered bacterial colonies on an agar plate decreased with increasing distance from the point of inoculation (Figure 10). Bacteria were recovered from the external light brown lesions on secondary roots at the site of their attachment to the main root (Figure 9).

Pectolytic activity. When inoculated into potato tuber slices, some strains of *P. marginalis* (i.e. 4 out of 10 isolates), *P. fluorescens* (1 out of 3 isolates), *P. synxantha* (1 out of 4 isolates) and *Pseudomonas* sp. (1 out of 1 isolate) produced soft rot (Table 1).

DISCUSSION

The identification of specific factor(s) that cause the collapse of tomato plants in the open field or protected crops manifesting itself by mass and relative rapid and complete wilting, drying and dying is an uneasy task in the majority of the cases. Generally, the collapse of tomato plants can have two main causes, i.e. water deficit (water stress) or diseases or pests which destroy roots or damage the principal water-conducting tissues. Discoloration of tomato stem vessels caused by various bacterial and fungal pathogens is difficult to distinguish (Blancard 1992).

The browning of the vascular system and wilting of tomato plants are characteristic of fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* and also verticillium wilt caused by *Verticillium albo-atrum* or *V. dahliae*. Stem rot of tomato caused by *Fusarium merismoides* was described by Fletcher and Lord (1985) in the United Kingdom.

In the surveyed greenhouse hydroponic tomato culture in South Moravia, we found out that on average only 25% to 30% of outer xylem vessels in the stems and main roots of collapsed plants were occluded. Therefore, although vascular fungal pathogens of *Verticillium* sp. and *Fusarium* spp. were isolated from diseased plants, we concluded that these potential fungal vascular pathogens were not the main or the only cause of collapse of surveyed tomato crop. For that reason, on the one hand we focused on searching for pathogenic fungi that destruct cortical tissues of roots (Kůdela *et al.* 2009) and on the other hand, in this study, we were looking for vascular and root rot pathogens of bacterial origin.

Clavibacter michiganensis subsp. *michiganensis*, causal agent of tomato canker or vascular wilt, is regarded as one of the most destructive tomato pathogens. In Europe, it is an EPPO quarantine organism. Bacterial tomato canker has been known in Bohemia since the 1950s (Staněk, Ujevic' personal communication) and in southern Moravia since the 1960s (Pokorný & Nováková-Pfeiferová 1961). In recent years, we have seen several outbreaks of canker disease in glasshouse and plastic greenhouse tomato crops in the Czech Republic. It is worthy of note that in the samples of diseased tomato plants collected in a plastic greenhouse at Otaslavice, Prostějov district, Moravia in 2004, we isolated and determined *C. m.* subsp. *michiganesis* together with *Pseudomonas corrugata* (Kůdela & Krejzar, unpublished). In spite of our effort, we did not confirm the association of *C. m.* subsp. *michiganensis* with the collapse of tomato plants in rockwool hydroponic culture in a commercial glasshouse in Moravia in 2008.

According to the hypothesis of Soler-Aleixandre *et al.* (2005), tomato collapse would be associated with necrosis of the vascular system caused by *Pepino mosaic virus* (PepMV) accumulation. Nearly 90% of the non-collapsed plants showed systemic infection by PepMV and necrosis on the vascular system was observed among all the collapsed plants analysed. According to Soler *et al.* (2005), PepMV is an agent necessary, but not sufficient, for the development of collapse. However, it does not accord with our previous experiences obtained during solving the problem of causes of collapsed tomato plants (Kůdela & Krejzar, unpublished). The role of PepMV in the development of collapse of tomato plants seems to be overrated by Soler-Aleixandre *et al.* (2005).

Several bacteria can cause external browning of the tomato stem or stem soft rot, pith and xylem browning, leaf yellowing, wilting and premature dying of the whole plants. A list of potential pathogens includes such bacteria as *P. cichorii* (Wilkie & Dye 1974), *P. corrugata* (Scarlett *et al.* 1978), *Erwinia carotovora* subsp. *carotovora* (Dhanvantari & Dirks 1987), *P. fluorescens* biotype A, *P. viridiflava* and *E. c.* subsp. *atroseptica* (Malathrakis & Goumas 1987), fluorescent *Pseudomonas* sp. (Dhanvantari 1990), *P. mediterranea* (Catara *et al.* 2002), *Erwinia chrysanthemi* (Asyan *et al.* 2003) and *Ralstonia solanacearum*, biovar 2, race 3 (Loreti *et al.* 2007). The tomato diseases caused by the above-mentioned bacteria are called vari-

ous common names in countries where they occur such as tomato pith necrosis, pit necrosis of tomato, bacterial stem rot of tomato, tomato stem necrosis or bacterial wilt of tomato.

Of the plant pathogenic bacterial species which are capable to induce the wilt and finally collapse of the whole tomato plants, *Pseudomonas corrugata* and *P. marginalis* were detected and determined in collapsed tomato plants in rockwool slab hydroponic culture in southern Moravia, Czech Republic. Besides, *P. fluorescens, P. putida, P. synxantha* and *Stenotrophomonas malthophilia* were identified.

Many fluorescent pseudomonads are involved in several interactions with plants. They are typical inhabitants of the phyllosphere and rhizosphere. Some species have a strict commensal relationship with their host plants. Furthermore, potentially phytopathogenic pseudomonads can be found on their specific hosts, as well as on plant species that are not susceptible to these pathogens. Pseudomonads belong to the most versatile organisms known to man. According to Berkelmann and Wohanka (1994), members of the genus *Pseudomonas*, accounting for 40%, represented the major group of 160 bacterial strains isolated from the recycled nutrient solution of a hydroponic system in rockwool. Taking into account the extraordinary biochemical versatility, adaptability, optimal growth temperature and strictly aerobic metabolism of bacteria of *Pseudomonas* genera, this environment offers optimal conditions for extensive propagation of pseudomonads.

P. marginalis is regarded as one of many oxidase positive, soft rot fluorescent pseudomonads (Lelliott *et al.* 1966; Janse *et al.* 1992). Recently, based on 16S rRNA analysis, *P. marginalis* has been placed in the *P. fluorescens* group (Anzai *et al.* 2000). According to Schaad *et al.* (2001), most of the fluorescent plant-associated saprophytic strains belong to the *P. fluorescens-P. putida* complex of organisms. Clear distinction and identification of most strains, unless their properties happen to fit almost exactly the description of either *P. fluorescens* or *P. putida* or one of their subgroups, is difficult. *P. synxantha* is another fluorescent rhizosphere bacterium which has been recently placed in the *P. flurescent* group (Anzai *et al.* 2000). *Stenotrophomonas malthophilia*, previously classified as *Pseudomonas maltophilia*, is ubiquitous in water, soil and rhizosphere of several cultured plants: cabbage, rape, mustard, maize,

beet (Debette & Blondeau 1980) and potato (Messiha *et al.* 2007). *S. maltophilia* is regarded as the emerging pathogen in human medicine. It can cause some nosocomial respiratory, bloodstream, and urinary infections.

In our study, some isolates of *P. marginalis*, *P. fluorescens*, *P. synxantha* and *Pseudomonas* sp. produced soft rot when inoculated into potato tuber slices and other strains of the same species were not able to macerate the potato tissue (Table 1). With the exception of *P. marginalis*, the role of pectolytic pseudomonads in the collapse of tomato plants in surveyed rockwool slab hydroponic culture was not determined in greater detail.

Generally, the ability of a bacterium to macerate the plant tissue confirms its pectolytic nature and provides an indication of pathogenicity. The maceration ability, however, does not prove the pathogenicity of the bacterium in a natural environment. Various plant tissues such as potato tubers, peppers or celery stalks can be used to test the maceration ability (Schaad *et al.* 2001). As for *P. corrugata*, it cannot be looked over that this species is not regarded as pectolytic. Scarlett *et al.* (1978) reported that *P. corrugata* did not rot onion slices. On the other hand, in the test of Lukezic (1979) this bacterium was capable of rotting onion sections if inoculated onto a cut surface. As regards *P. marginalis* (*sensu stricto*), this fluorescent pseudomonad serves as a typical species indicating the positive pectolytic activity. Potato-macerating isolates among the fluorescent pseudomonads represented a continuum of phenotypes from *P. fluorescent* to *Pseudomonas putida* (Sands & Hankin 1975; Janse *et al.* 1992).

Little is also known about interactions between bacterial and fungal vascular pathogens inside the affected plants at combined activities of various types of vascular pathogens. Noteworthy is the fact that *P. corrugata* has a great potential as a biological control agent against plant pathogenic fungi and bacteria, as well as a phytotoxin indicator. It is used as a biological control agent against pre- and post-harvest plant pathogens (Catara 2007). Similarly, *S. maltophilia* may be useful for the control of *Ralstonia solanacearum*, causal agent of potato brown rot (Messiha *et al.* 2007).

Dispersal of root, vascular and foliar pathogens by recycled nutrient solution is a major concern in *closed* hydroponic cultures. To limit such dispersal, ultraviolet (UV) disinfection technology has

been used to remove pathogens from hydroponic nutrient solution. On the other hand, main steps to control root pathogens in *open* hydroponic crops is to use pathogen-free planting material and/or resistant cultivars.

A crucial problem regarding the sources of *P. corrugata* and *P. marginalis* in the surveyed collapsed tomato crop remains unsolved. It is known that water from ponds and rivers used for irrigation can contain *P. marginalis* (Clark & Graham 1962). Nevertheless, in the surveyed rockwool slab hydroponic tomato crop, water from a well was used for irrigation. We did not conduct any thorough studies to determine the source of either pathogen. However, when more than 50 apparently healthy grafted tomato transplants grown in hydroponic culture were analysed before planting, surprisingly, *P. marginalis* was determined.

Summing up, the association of *P. corrugata* and some pectolytic bacteria in the collapse of tomato plants in rockwool slab hydroponic culture was proved in this study. In practice, however, the collapse of tomato, similarly like of other plants, results most frequently from combined effects of more than one harmful abiotic and/or biotic factor. It can also be assumed that the mass rapid attack of tomato plants both by root rot and vascular pathogens in the surveyed greenhouse hydroponic culture was initiated by some (until undefined) abiotic stress(es).

References

Anzai Y., Kim H., Park J.Y., Wakabayashi H., Oyaizu H. (2000): Phylogenic affiliation of the pseudomonads based on 16S r.RNA sequence. International Journal of Systematic and Evolutionary Microbiology, **59**: 1563–1589.

Asyan Y., Karatas A., Cinar O. (2003): Biological control of bacterial stem rot caused by *Erwinia chrysanthemi* on tomato. Crop Protection, **22**: 807–811.

Berkelmann B., Wohanka W. (1994): Characterization of the bacterial flora in circulating solutions of a hydroponic system with rockwool. Acta Horticulturae, **361:** 372–381.

Blancard D. (1992): A Colour Atlas of Tomato Diseases. Observe, Identify, Control. Wolf Publishing Ltd., London.

Bradbury J.E. (1970): Isolation and preliminary study of bacteria from plants. Review of Plant Pathology, **49**: 213–218.

Catara V. (2007): *Pseudomonas corrugata*: plant pathogen and/or biological resource? Molecular Plant Pathology, **8**: 233–234.

Catara V., Sutra L., Morineau A., Achouak W., Christen R., Gardan L. (2002): Phenotypic and genomic evidence for the revision of *Pseudomonas corrugata* and proposal of *Pseudomonas mediterranea* sp. nov. International Journal of Systematic and Evolutionary Microbiology, **52**: 1749–1758.

Clark M.R., Graham D.C. (1962): A disease of forced rhubarb associated with *Pseudomonas marginalis.* Plant Pathology, **11**: 31–33.

Debette J., Blondeau R. (1980): Présence de *Pseudomonas maltophilia* dans la rhizosphère de quelques plantes cultivées. Canadian Journal of Microbiology, **26**: 460–463.

Dhanvantari B.N. (1990): Stem necrosis of greenhouse tomato caused by a novel *Pseudomonas* sp. Plant Disease, **74**: 124–127.

Dhanvantari B.N., Dirks V.A. (1987): Bacterial stem rot of greenhouse tomato: Etiology, spatial distribution, and the effect of high humidity. Phytopathology, **77**: 1457–1463.

Fletcher J.T., Lord K.J. (1985): A stem rot of tomato caused by *Fusarium merismoides.* Plant Pathology, **34**: 443–445

Janse J. D., Derks H.J., Spit B.E., Tuin van der W.R. (1992): Classification of fluorescent soft rot *Pseudomonas* bacteria, including *P. marginalis* strains, using whole cell fatty acid analysis. Systematic and Applied Microbiology, **15**: 538–553.

Kůdela V., Krejzar V., Pánková I. (2009): *Pyrenochaeta lycopersici* causing destruction of tomato root system in soilless culture. In: Book of Abstracts. XVIII. Czech and Slovak Plant Protection Conference, Brno, September, 2–4, 2009.

Lelliott R.A., Billing E., Hayward A.C. (1966): A determinative scheme for the fluorescent plant pathogenic pseudomonads. Journal of Applied Bacteriology, **29**: 470–489.

Loreti S., Fiori M., De Simone D,. Falchi G., Gallelli A, Schiaffino A., Ena S. (2007): Bacterial wilt, caused by *Ralstonia solanacearum*, on tomato in Italy. New Disease Reports, **15**: 1.

Lukezic F.L. (1979): *Pseudomonas corrugata,* a pathogen of tomato, isolated from symptomless alfalfa roots. Phytopathology, **69**: 27–31.

Malathrakis N.S., Goumas D.E. (1987): Bacterial soft rot tomato in plastic greenhouses in Crete. Annals of Applied Biology, **111**: 115–123.

Messiha N.A.S., Diepeningen van A.D., Farag N.S., Abdallah S.A., Janse J.D., Bruggen van A.H.C.

(2007): *Stenotrophomonas malthofillia:* a new potential biocontrol agent of *Ralstonia solanacearum*, causal agent of potato brown rot. European Journal of Plant Pathology, **118**: 211–225.

Pokorný K , Nováková-Pfeiferová J. (1961): K výskytu bakteriálního vadnutí rajčat v r. 1960 v jihomoravské zelinářské oblasti. Ovocnářství a zelinářství: 182–183,

Sands D.S., Hankin L. (1975): Ecology and physiology of fluorescent pectolytic pseudomonads. Phytopathology, **65**: 921–924.

Scarlett C.M., Fletcher J.T., Roberts P., Lelliot R.A. (1978): Tomato pith necrosis caused by *Pseudomonas corrugata* n. sp. Annals of Applied Biology, **88**: 105–114.

Schaad N.W., Jones J,B., Chun W. (eds) (2001): Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd Ed. APS Press, St. Paul.

Soler-Aleixandre S., Lopez C., Diez M.J., Pérez de Castro A., Nuez F. (2007): Association of *Pepino mosaic virus* with tomato collapse. Journal of Phytopathology, **153**: 464–469.

Staunton W.P., Cormican T.P. (1978): The behaviour of tomato pathogens in a hydroponic system. Acta Horticulturae, **82**: 133–135.

Wilkie J.P., Dye D.W. (1974): *Pseudomonas cichorii* causing tomato and celery diseases in New Zealand. New Zealand Journal of Agriculture Research, **17**: 123–130.

Corresponding author:

Prof. Ing. Václav Kůdela, DrSc., Výzkumný ústav rostlinné výroby, v.v.i., odbor rostlinolékařství, oddělení bakteriologie, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 427, fax: + 420 233 106 636, e-mail: kudela@vurv.cz