European Journal of Plant Pathology **105:** 927–932, 1999.

Short communication

Characterization of *Pseudomonas viridiflava* associated with a new symptom on tomato fruit

D.E. Goumas[1], N.E. Malathrakis[2] and A.K. Chatzaki[1]
[1]*National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 1802, 711 10 Heraklio, Crete, Greece (Fax: +81-245858; E-mail: dgoumas@her.forthnet.gr);* [2]*Technological Education Institute, P.O. Box 140, 711 10 Heraklio, Crete, Greece*

Accepted 25 August 1999

Key words: bacterial disease, identification

Abstract

A bacterial disease of tomato fruits was recorded during the winter in outdoor crops in Crete, causing damage of up to 30% in commercial production. Bacterial spots appeared on the upper surface of middle size immature fruit and the spots became larger with the increase of fruit size. Spots were round or elongated, superficial and lightly sunken, 1.5–2 cm in diameter. The centre of the spots dried out and became progressively gray, brown to black-brown and had intensely coloured margins. The infected area did not develop into soft rot. The bacterium *Pseudomonas viridiflava* (Burkholder) Dowson was identified as the causal agent on the basis of morphological, physiological, biochemical and pathological characters. This appears to be the first time that these symptoms have been described on tomato fruit.

Symptoms of an unknown bacterial disease have been frequently recorded throughout the island of Crete on tomato plants (*Lycopersicon esculentum Mill*.) grown in greenhouses and outdoors. The disease was first recorded during January 1994 in outdoor crops at Antiskari area of Heraklion Province and since then it has been observed on different cultivars, only during winter, in all tomato growing areas of Crete. The disease frequently caused damage of up to 30% in commercial production. The symptoms of the disease were restricted to fruits. Lesions develop on immature fruit, as water-soaked light brown spots 1–2 mm in diameter. On middle sized fruit, the spots, which appear on the upper surface, are round or elongated, superficial and lightly sunken, 1.5–2 cm in diameter, and become progressively larger as fruits develop and mature. The lesion centre dries out, becoming progressively gray, brown to black-brown and is surrounded by a narrow blackish margin (Figure 1). Under favourable conditions, the spots usually coalesce into scabby necrotic areas covering a large area of the fruit surface (Figure 2), but never results in necrosis of the whole fruit. Soft rot was never observed on those infections in the field. The symptoms were easily differentiated from those of other known bacterial infections of tomato fruit such as bacterial speck and bacterial spot. The disease reduces fruit quality making many unmarketable. A preliminary report on the first occurrence of the disease has been presented (Goumas and Chatzaki, 1997).

Initial streak-plate isolations, made on King's B medium (KB) (King et al., 1954), from the affected plants tissues, consistently resulted in essentially pure cultures of a fluorescent bacterium. In preliminary studies these isolates induced a hypersensitive reaction on tobacco leaves and rotted potato slices (Malathrakis and Goumas, 1987). To characterize the causal agent of this disease, tissues were removed aseptically from the margin of necrotic lesions of tomato fruits, and triturated in a few drops of sterile distilled water. Loopful of the suspensions were streaked onto plates of KB medium and Nutrient Dextrose Agar (NDA) and

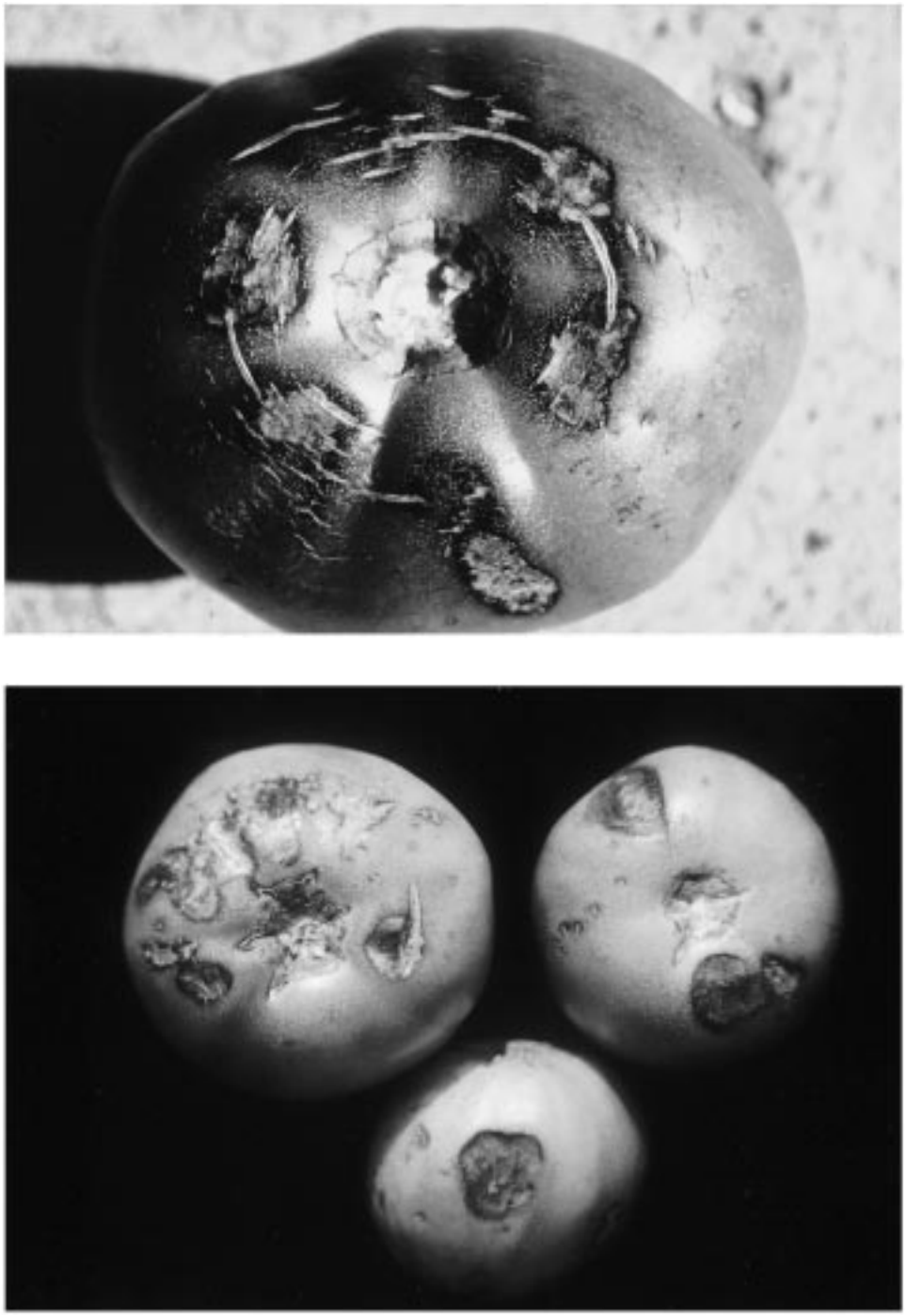

Figure 1. Symptoms on immature fruits. Superficial and lightly sunken, round or elongated spots, surrounded by a narrow blackish halo.

Figure 2. Spots coalescing to form scabby necrotic areas covering a large area of the fruit surface.

incubated for 48 h at 30 °C. Single colonies were subcultured, checked for purity and stored as slant cultures at 4 °C on NDA.

Isolates were initially tested according to the LOPAT tests (Lelliott et al., 1966) and fifteen representative isolates from all tomato growing areas of Crete (Ierapetra, Arvi, Messara, Goudouras), were selected for further characterization using the differential tests presented in Table 1. The methods for conducting the tests have been previously described (Malathrakis and Goumas, 1987; Goumas and Chatzaki, 1998). Nutritional tests were conducted on mineral salts medium (Ayers et al., 1919) at a final carbon source concentration of 0.1% (w/v). Production of pectinase was determined by the method of Hildebrand (1971) at three pH levels (4.5, 7, and 8.3). Liquefaction of gelatin and the presence of lipase were tested as described by Lelliott and Stead (1987). The differential capacity of *P. viridiflava*, *P. syringae* pv. *tomato* and *P. syringae* pv. *syringae* to fluoresce on iron-deficient medium containing sucrose, erythritol or DL-lactate as single carbon source was tested (Jones et al., 1986). Reactions of reference bacterial strains are also presented in Table 1 for comparison. Each test was repeated at least twice.

In preliminary inoculations on tomato fruits, many isolates were screened for their ability to reproduce disease symptoms. For the pathogenicity tests, three isolates of the bacterium (TKK542, TKK5a, and TKK615) were compared with a strain from tomato bacterial stem soft rot (PV442) and a strain from cucumber bacterial leaf necrosis (PV400), both previously characterized as *P. viridiflava* (Goumas and Chatzaki, 1998), and *P. syringae* pv. *tomato* (Pst18) and *P. syringae* pv. *syringae* (Pss5). Inoculations were made on immature detached and attached tomato fruits (cv. Menglo) (Lelliott and Stead, 1987). Fruits were swabbed with 70% ethanol and washed in sterile water and stabbed with a sterile needle at six sites. Inoculations were made by deposition of 20 µl of a bacterial suspension on the upper surface of tomato fruits. Four fruits were used for each isolate. Inocula were prepared from 48 h-old cultures on KB medium, suspended in sterile distilled water, and adjusted to approximately 10^8 cfu/ml by turbidity measurement (A_{600}). Serial tenfold dilutions within 10^6 and 10^4 cfu/ml were used. After inoculation, tomato fruits were kept in closed transparent boxes lined with moist blotting paper, either in incubators (10 °C and 28 °C) or at room temperature (12–28 °C)

Table 1. Comparison of isolates from tomato fruit with strains of *P. viridiflava* and *P. syringae* pathovars *syringae* and *tomato*

Tests	Isolates from: tomato fruits (15)[1]	*P. viridiflava* (3)	*P. syringae pathovar*	
			syringae (2)	*tomato* (2)
Levan	−[2]	−	+	+
Oxidase	−	−	−	−
Potato rot	+	+	−	−
Arginine dihydrolase	−	−	−	−
Hypersensitivity	+	+	+	+
Nitrate reduction	−	−	−	−
Fluorescent pigment	+	+	+	+
Gelatin hydrolysis	+	+	+	−
Pectate gel pitting	+[3]	+	−	NT
Lipases	+	+	+	NT
2-Ketogluconate	−	−	−	−
Utilization of				
D(−) Mannitol	+	+	+	+
D(+) Cellobiose	−	−	−	−
D(−) Sorbitol	+	+	+	+
D(+) Trehalose	−	−	−	−
i-Inositol	+	+	+	+
L(−) Rhamnose	−	−	−	−
D(−) Arabinose	−	−	−	−
Adonitol	−	−	−	−
Betaine	+	+	+	+
D(+) Sucrose	−ng[4]	−ng	+gf	+gf
Erythritol	+gf	+gf	+gf	−ng
DL-Lactate	+gf	+gf	+gf	−ng
L(−) Lactate	+	+	+	−
L(+) Tartrate	−	−	−	−
D(−) Tartrate	+	+	−	+
Malonate	+	+	+	+
Anthranilate	−	−	−	−
L-Valine	−	−	−	−
β-Alanine	−	−	−	−
Pathogenicity on				
Bean pod	+[5]	+	−	−
Lemon	−	−	+[6]	−
Pear	+[7]	+	+[8]	−

[1]( ): Number of isolates used. *P. viridiflava*: NCPPB 1249 (*Chrysanthemum morifolium*), Pv400 (*Cucumis sativus*), Pv442 (*Lycopersicon esculentum*). *P. syringae* pv. *tomato*: Pst18, Pst30 (*Lycopersicon esculentum*). *P. syringae* pv. *syringae*: Pss5 (*Citrus limon*), NCPPB 2778 (*Pyrus communis*). NCPPB: National Collection of Plant Pathogenic Bacteria, Harpenden, UK.

[2]−: Negative reaction, +: Positive reaction, NT: not tested.

[3]Pectate gel pitting occurs at pH 7 and 8.3 for *P. viridiflava*.

[4]ng: neither growth nor fluorescence; gf: growth and fluorescence when the pattern of Jones et al., (1986) was used for fluorescence on single carbon source media.

[5]+: Rust-coloured lesion.

[6]+: Deep black necrotic pit.

[7]+: Soft-rot.

[8]+: Dark water-soaked necrotic spot.

with a 16 h photoperiod. All fruits were assesed daily for ten days to record disease symptoms. In inoculations of immature fruit on tomato plants growing in a greenhouse during winter (9–28 °C), fruits were enclosed in clear polyethylene bags for three days and disease symptoms were recorded within 15 days. Controls were similarly treated with sterile water. Also, tomato plants (cv. Menglo) at the 4–5 true leaf stage were inoculated with an appropriate culture by stabbing the tip of a sterilized toothpick into a bacterial colony and then into the stem above the first true leaves. Inoculated sites were covered with parafilm and plants were placed in a mist chamber at 22 °C with a 16 h photoperiod for ten days during which disease symptoms were recorded. Controls were similarly treated with sterile toothpicks. Finally, inoculations were conducted on snap bean (*Phaseolus vulgaris* L. cv. Kentucky Wonder) pods (Cheng et al., 1989) on immature lemon and pear fruits (Lelliott and Stead, 1987).

On KB the colonies of isolates appeared opaque, convex, shiny, semifluid and produced a bright green-blue diffusible fluorescent pigment. On 5% sucrose nutrient agar the centre of the colonies became greenish (Lelliott and Stead, 1987). In LOPAT tests, all tested isolates gave a similar profile to reference stains of *P. viridiflava* from cucumber and tomato and were consistent with group II of Lelliott et al. (1966). Results of additional identification tests of isolates are presented in Table 1. These are similar to those obtained with the reference strains of *P. viridiflava*. Other reference strains used in this work gave results which were consistent with their designated classification (Goumas and Chatzaki, 1998). Quick identification of isolates as *P. viridiflava* was successful when the pattern of Jones et al. (1986) was used (Table 1).

The symptoms induced on inoculated fruits were similar to those due to natural infections on both detached fruits and those remaining on the plant, independently of the concentration used. The symptoms developed more rapidly when the concentration was higher but no other differences in symptom appearance were observed. On tomato fruits the disease started as water-soaked spots which developed in 3–4 days into small or large irregular lesions. The centre of the lesion later became dry and tan to black in colour. These symptoms differed from symptoms caused by the strains of *P. viridiflava* isolated from bacterial soft rot of tomato (PV442) and bacterial necrosis of cucumber (PV400). Although symptoms were initially similar to those produced by tomato fruit isolates, in most cases (about 80%) these infections resulted in a soft rot within 10 days at all incubation temperatures (461 soft-rotted sites per 576 infected sites). Soft rot symptoms were obtained in a few cases (about 15%) only at 10 °C incubation temperature with the isolates from tomato fruits (33 soft-rotted sites per 216 infected sites). Inoculations with Pst18 resulted in typical speck symptoms, whilst Pss5 produced necrotic deep-black lesions. All isolates of *P. viridiflava* produced bacterial soft rot on tomato plant after stab inoculation of the stem, (Malathrakis and Goumas, 1987). Finally, all isolates of *P. viridiflava* and reference strains of the bacterium, caused rust-coloured lesions within 48 h on excised snap bean pods. Strains Pst18 and Pss5 caused only a necrotic reaction on bean pods. Isolates of *P. viridiflava* induce soft rots on pear and did not produce any symptoms on detached lemon fruits. In contrast, strain Pss5 reproduced a black pit symptom on lemon and a water-soaked necrotic spot on pear fruits. Reisolations made from the artificially infected fruits and plants yielded, pure cultures of *P. viridiflava*. Their identification was confirmed by LOPAT tests. No symptoms developed on control plants or fruits.

On the basis of morphological, physiological biochemical and pathological characteristics, the fifteen representative isolates of *Pseudomonas* spp were identified as *P. viridiflava* (Burkholder) Dowson, according to the determinative schemes proposed by Lelliott et al. (1966), Sands et al. (1970) and Billing (1970). *P. viridiflava* has been characterized as an opportunistic pathogen on a wide variety of plants (Bradbury, 1986). In Greece *P. viridiflava* has often been found to cause severe disease problems on different plants grown in plastic greenhouses or outdoors (Goumas and Chatzaki, 1998). To our knowledge this is the first record of the bacterium causing these symptoms on tomato fruit (Bradbury, 1986). Further investigations are needed to determine the environmental factors predisposing the tomato fruits to bacterial infection and to explain why the isolates from tomato fruit do not cause soft rots. The disease symptoms were not associated with wounds or cracks in the epidermis of tomato fruits growing under unfavourable conditions. The disease appears during winter from the middle to the end of the cropping period (November–January). At this time the environmental conditions are favourable for bacterial infections and disease initiation and the conditions are adverse to the growth of tomato plants (cool and humid weather, low night temperature, reduced illumination, increased inoculum levels and stressed plants). The fact

that *P. viridiflava* is involved in diseases of different hosts only during the winter season in Crete (Goumas and Chatzaki, 1998) supports the suggestion that this bacterium is an opportunistic cool weather pathogen, which causes diseases of local economic importance.

References

Ayers SH, Rupp P and Johnson WT (1919) A study of the alkali-forming bacteria found in milk. United States Department Agriculture Bulletin pp 782

Billing E (1970) *Pseudomonas viridiflava* (Burkholder, 1930; Clara, 1934). Journal of Applied Bacteriology 33: 492–500

Bradbury JF (1986) Guide to Plant Pathogenic Bacteria. CAB, International Mycological Institute, Kew, UK.

Cheng GY, Legard DE, Hunter JE, and Burr TJ, (1989) Modified bean pod assay to detect strains of *Pseudomonas syringae* pv. *syringae* that cause bacterial brown spot on snap bean. Plant Disease 73: 419–423

Goumas DE and Chatzaki AK (1997) A bacterial disease of tomato fruits caused by *Pseudomonas viridiflava* in Crete. In: Albajes R and Carnero A (eds) Proceeding of Integrated Control in Protected Crops 'Mediterranean Climate', Espana, Tenerife. Vol 20: (pp 77–83) IOBC wprs Bulletin

Goumas DE and Chatzaki AK (1998) Characterization and host range evaluation of *Pseudomonas viridiflava* from melon, blite, tomato, chrysanthemum and eggplant. European Journal of Plant Pathology 104: 181–188

Hildebrand DC (1971) Pectate and pectin gels for differentiation of *Pseudomonas* sp. And otrer bacterial plant pathogens. Phytopathology 72: 1430–1436

Jones JB, Gitaitis RD and McCarter SM (1986) Fluorescence on single-carbon sources for separation of *Pseudomonas syringae* pv *syringae* , *P. syringae* pv *tomato* and *P. viridiflava* on tomato transplants. Plant Disease 70: 151

King EO, Ward MK and Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescein. Journal of Laboratory and Clinical Medicine 44: 301–307

Lelliott RA, Billing E and Hayward AC (1966) A determinative scheme for fluorescent plant pathogenic pseudomonads. Journal of Applied Bacteriology 29: 470–489

Lelliott RA and Stead DE (1987) Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications, Oxford

Malathrakis NE and Goumas DE (1987) Bacterial soft rot of tomato in plastic greenhouses in Crete. Annals of Applied Biology 111: 115–123

Sands DC, Schroth MN and Hildebrand DC (1970) Taxonomy of phytopathogenic pseudomonads. Journal of Bacteriology 101: 9–23