



## DISEASE NOTE

**FIRST REPORT OF CUCUMBER  
LEAF SPOT VIRUS IN BULGARIA**D. Kostova<sup>1</sup>, R.G. Milne<sup>2</sup>, G. Dellavalle<sup>2</sup> and V. Lisa<sup>2</sup><sup>1</sup> Maritza Institute for Vegetable Crops, 32,  
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Cucumber leaf spot virus (CLSV), family *Tombusviridae*, genus *Aureusvirus* (Martelli *et al.*, 1998) was detected in glasshouse cucumber plants in two areas of southern Bulgaria in 1999. Samples from 18 plants showing symptoms were collected, dried under vacuum in Bulgaria, brought to Torino and tested by ELISA for Cucumber mosaic virus (CMV) and for common cucurbit potyviruses. Three samples, positive for CMV, gave an unusual necrotic systemic disease when sap inoculated to *Nicotiana benthamiana* and *N. clevelandii*. In such plants, electron microscopy indicated the presence of CMV and tombusvirus-like particles. The CMV was inactivated by heating the infected sap for 10 min at 75°C. The surviving virus was increased in *N. benthamiana* and tested by agar gel diffusion with antisera to the cucumber fruit streak strain of CLSV (from D. Gallitelli, Bari), closely serologically related to type CLSV (Gallitelli *et al.*, 1986; Weber, 1986), Cucumber necrosis virus, genus *Tombusvirus* (from S. Winter, Braunschweig), Cucumber soil-borne virus, genus *Carmovirus* (from R. Koenig, Braunschweig) and Tobacco necrosis virus, genus *Necrovirus*, from the Torino Institute collection. The virus reacted only with the serum to CLSV, up to a serum dilution 1:32. The reaction of indicator plants sap-inoculated with our isolate what is reported for CLSV (Gallitelli *et al.*, 1983; Weber, 1986). Cucumber cv. 'Marketer' was systemically infected by our isolate, without showing symptoms. CLSV had previously been reported in Germany, Greece, U.K. and Jordan. This is the first report CLSV in Bulgaria.

Gallitelli D., Vovlas C., Avgelis A., 1986. Some properties of cucumber fruit streak virus. *Phytopathologische Zeitschrift* **106**: 149-162.

Martelli G.P., Russo M., Rubino L., Sabanadzovic S., 1998. *Aureusvirus*, a novel genus in the family *Tombusviridae*. *Archives of Virology* **143**: 1847-1851.

Weber I., 1986. Cucumber leaf spot virus. AAB Descriptions of plant viruses no. 319.

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## DISEASE NOTE

**LEAF BLIGHT OF IMPATIENS  
CAUSED BY PSEUDOMONAS  
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Plants of pot-grown *Impatiens* (*Impatiens wallerana* Hook. x *I. sultanii* Ferg.) cultivar 'New Guinea Hybrid' showing necrotic lesions on leaves were observed in spring 1998 in a commercial glasshouse located in Abruzzo (central Italy). Leaves showed either brownish marginal necrosis or irregular necrotic spots. In many cases, leaves appeared wrinkled. Leaf fall was also observed. Incidence of the disease was around 10%. Leaf tissue was taken from the margin of lesions and crushed in sterile physiological saline. From the suspensions, 0.1 ml aliquots from serial ten-fold dilutions were spread on medium B of King *et al.* and incubated at 25-27°C for 48 h. Fluorescent colonies were recovered in large numbers. Biochemical, physiological and pathogenicity tests were performed with some representative isolates. All isolates were levan-positive, oxidase-negative, potato soft rot-negative, arginine dihydrolase-negative, and tobacco hypersensitivity-positive (LOPAT test, group 1A). In addition, all isolates hydrolyzed esculin and arbutin, liquefied gelatin and were negative in nitrate reduction and tyrosinase activity. The mode of utilization of glucose was oxidative. They reproduced the symptoms when inoculated, at  $1-2 \times 10^7$  cfu ml<sup>-1</sup>, by pricking *Impatiens* leaves (Cooksey and Koike, 1990). Moreover, they caused necrotic lesions on lemon and orange fruits as well as on leaves of lilac (*Syringa vulgaris* L.). Comparison with *Pseudomonas syringae* pv. *syringae* van Hall type and reference strains using fatty acid analysis, matched the *Impatiens* isolates at a value higher than 90%. We conclude that the causative agent of the disease was *P. syringae* pv. *syringae* van Hall. To our knowledge, this is the first record of the pathogen identified at pathovar level, on *Impatiens* cultivar 'New Guinea Hybrid'.

Cooksey D.A., Koike S.T., 1990. A new foliar blight of *Impatiens* caused by *Pseudomonas syringae*. *Plant Disease* **74**: 180-182.

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## DISEASE NOTE

**FIRST RECORD  
OF *BURKHOLDERIA ANDROPOGONIS*  
ON BOUGANVILLEA IN ITALY**

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Pot-grown plants of Bouganvillea (*Bouganvillea spectabilis* Willd.) cultivar 'Temple fire' with leaf spot symptoms were observed in September 2000 in a commercial greenhouse in the province of Latina (central Italy). The leaf spots were irregular necrotic lesions with tan centers and reddish brown margins, sometimes, with a chlorotic halo. In many cases, spots coalesced and the leaf became twisted and fell (Moffett *et al.*, 1986). Infection could be present on all leaves of a twig. Disease incidence was around 10-15%. Leaf tissue taken from the margin of the lesions was crushed in mortars containing sterile physiological saline. From the suspensions, 0.1 ml aliquots of the serial ten-fold dilutions were spread on medium B of King *et al.* (KB) and incubated at 25-27°C for 5 days. The resulting greyish-white colonies were used in biochemical and pathogenicity tests as well as for comparison by SDS-PAGE of whole-cell protein extracts. All the isolates were negative in fluorescent pigment production on KB, levan production, presence of oxidase and arginine dehydrolase, aesculin and starch hydrolysis, gelatin liquefaction, nitrate reduction and tween 80 lypolysis. In addition, they showed the same protein profile as *Burkholderia andropogonis* Gillis *et al.* [Synonym: *Pseudomonas andropogonis* (Smith) Stapp] NCPPB 451, NCPPB 2386 and NCPPB 3195. When inoculated at  $1-2 \times 10^7$  cfu ml<sup>-1</sup> by puncturing leaves of Bouganvillea cultivar 'Temple fire', they reproduced the symptoms observed in the glasshouse within 15 days. Re-isolation on KB yielded the same colony type as observed in primary isolations. We conclude that the causative agent of the disease was *B. andropogonis* Gillis *et al.* To our knowledge, this is the first record of this disease of Bouganvillea in Italy and Europe.

Moffett M.L., Hayward A.C., Fahy P.C., 1986. Five new hosts of *Pseudomonas andropogonis* occurring in eastern Australia: host range and characterization of isolates. *Plant Pathology* 35: 34-43.

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## DISEASE NOTE

**FIRST REPORT OF *TOMATO YELLOW  
LEAF CURL VIRUS*  
IN APULIA (SOUTHERN ITALY)**

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In autumn 2000, symptoms of stunting, curling and yellowing of leaf margins, and marked reduction in the number of fruits were observed in some greenhouse-grown tomato plants of cv. 'Naxos' in the province of Bari (Apulia, southern Italy). The symptoms closely resembled those elicited in tomato by *Tomato yellow leaf curl virus* (TYLCV), (family *Geminiviridae*, genus *Begomovirus*), and nuclear fibrillar rings characteristic of geminivirus infection were detected in thin sections of such plants. Presence of TYLCV was confirmed by molecular hybridisation with a TYLCV riboprobe. For typing this TYLCV isolate, total nucleic acid preparations, extracted from three distinct naturally infected tomato plants were primed each with the primers TY1(+) and TY2(-) (Accotto *et al.*, 2000) and subjected to RT-PCR RFLP. In all cases, a 580 bp amplicon was obtained which, when exposed to the endonuclease *Ava*II, yielded a restriction pattern consisting of 360, 150 and 68 bp fragments. These results are consistent with the presence of an isolate of species TYLCV-Sar in all plants tested. The 580 bp amplicon was cloned into a pGEM-T vector (Promega, Madison, WI) and sequenced. This fragment shared 99.5% homology with the equivalent sequences of a clone from a Sicilian isolate (Acc. no. Z28390) and 97.5% with a clone from a Sardinian isolate (Acc. no. X61153) of TYLCV-Sar.

Accotto G.P., Navas-Castillo J., Noris E., Moriones E., Louro D., 2000. Typing tomato yellow leaf curl viruses in Europe. *European Journal of Plant Pathology* 106: 179-186.

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