

DISEASE NOTE

**FIRST REPORT OF BIPARTITE
BEGOMOVIRUS ASSOCIATED
WITH LEAF CURL DISEASE
OF *DURANTA REPENS* IN PAKISTAN**

**M. Tahir, M.S. Haider, A.H. Shah, N. Rashid
and F. Saleem**

*School of Biological Sciences, University of the Punjab,
Quaid-i-Azam Campus, Lahore, Pakistan*

Pigeon Berry (*Duranta repens*) is a common ornamental plant of the family *Verbenaceae* that is often grown as a garden hedge in Pakistan. In an attempt to identify alternate hosts of *Cotton leaf curl virus* (CLCuV), leaf samples from *D. repens* showing leaf curl symptoms and apparently healthy (symptomless) plants were collected from Multan, Pakistan. Total DNA was isolated from both types of leaf samples. The presence of a begomovirus was confirmed by PCR amplification using a degenerate primer pair, designed from conserved sequences of genes for replication associated protein and coat protein of begomoviruses (Mansoor *et al.*, 1998). An amplification product of the expected size (i.e. approx. 1.5 kb) was produced from symptomatic samples whereas there was no amplification from the symptomless leaf samples. The PCR product was cloned and sequenced. The DNA sequences obtained from *D. repens* showed the highest levels of sequence identity to croton yellow vein mosaic virus segment A, C1 gene (91% over a stretch of 548 nucleotides in the virion-sense). A second genomic component DNA B (approx. 2.8 kb) was identified by PCR using a set of primers designed from published sequences. The DNA B sequences obtained from *D. repens* showed the highest level of identity with *Tomato Leaf Curl New Dehli Virus* segment B, movement protein (94% over a stretch of 400 nucleotides in the complementary-strand). This indicates that the leaf curl disease of *D. repens* is associated with a bipartite begomovirus.

Mansoor S., Hussain M., Khan S.H., Bashir A., Leghari A.B., Panwar G.A., Siddiqui W.A., Zafar Y., Malik K.A., 1998. Polymerase Chain Reaction-based Detection of Cotton Leaf Curl and Other Whitefly-transmitted Geminiviruses from Sindh. *Pakistan Journal of Biological Sciences* 1: 39-43.

Corresponding author: M. Tahir
Fax: +92.42.9230980
E-mail: tahirbs@yahoo.com

Received February 26, 2006
Accepted May 25, 2006

DISEASE NOTE

**FIRST RECORD OF *PEACH LATENT
MOSAIC VIROID* AND *HOP STUNT
VIROID* IN KOSOVA**

**L.R. Susuri¹, L. Dida², S. Matic³, H.Sh. Susuri⁴
and A. Myrta⁵**

¹ *Kosova Academy of Sciences and Arts, Emin Duraku 1,
Prishtina, Kosova*

² *Faculty of Agriculture, University of Prishtina, Kosova*

³ *Dipartimento di Protezione delle Piante e Microbiologia
Applicata, Università degli Studi and Istituto
di Virologia Vegetale, Sezione di Bari,
Via Amendola 165/A, 70126 Bari, Italy*

⁴ *Ministry of Agriculture, Forestry and Rural Development,
Mother Teresa stree 35, Prishtina, Kosova*

⁵ *Istituto Agronomico Mediterraneo Via Ceglie 9, 70010
Valenzano, Bari, Italy*

A survey was carried out in 2005 to assess the presence of *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd) and evaluate their incidence in peach and apricot trees in Kosova. Leaves were collected in November in orchards from 11 localities, i.e. Bërnjak, Prizren, Prishtinë, Vlashnjë, Suharekë, Ferizaj, Vushtrri, Zhur, Korishë, Mushtisht and Komoran, from a total of 176 trees (116 peaches and 60 apricots). All samples were tested by tissue-printing hybridization (Pallás *et al.*, 2003). A fresh cut end of the leaf petiole was pressed onto Hybond N⁺ nylon membranes and hybridized at 55°C with the SP6 and T7 RNA polymerase-generated full-length cRNA digoxigenin-labelled probes (Shamloul *et al.*, 1995; Astruc *et al.*, 1996). PLMVd was detected in 20 (17.2%) of the peach samples tested. PLMVd infections occurred in Korishë, Prizren and Vlashnjë. HSVd was detected only in one apricot in Korishë and one peach in Zhur, representing 1,1% of the total samples tested. To the best of our knowledge, this is the first record of PLMVd and HSVd in Kosova.

Astruc N., Marcos J.F., Macquaire G., Candresse T., Pallás V., 1996. Studies on the diagnosis of hop stunt viroid in fruit trees: identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. *European Journal of Plant Pathology* 102: 837-846.

Pallás V., Torres H., Myrta A., Gomez G., 2003. Validation of the 'tissue-printing' technique for detecting stone fruit viroids. *Options Méditerranéennes, Série B* 45: 135-137.

Shamloul A.M., Minafra A., Hadidi A., Waterworth H.E., Giunchedi L., Allam E.K., 1995. Peach latent mosaic viroid: nucleotide sequence of an Italian isolate, sensitive detection using RT-PCR and geographic distribution. *Acta Horticulturae* 386: 522-530.

Corresponding author: A. Myrta
Fax: +39.080.4606302
E-mail: myrta@iamb.it

Received March 15, 2006
Accepted June 20, 2006

DISEASE NOTE

STEM AND CROWN ROT OF *ASTER ERICOIDES* VAR. *ERICOIDES* CAUSED BY *SCLEROTINIA SCLEROTIUM*

S.M. Wolcan^{1,2}, M.C. Rollán¹, L. Ronco¹ and G. Lori^{1,2}

¹ CIDEFI, Facultad de Ciencias Agrarias y Forestales, UNLP, 60 y 119, (1900) La Plata, Buenos Aires, Argentina

² Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina

In Argentina, *Aster ericoides* var. *ericoides* L. (heath aster), a cut flower crop, is grown in the outskirts of La Plata and Buenos Aires. Death of heath aster plants during flowering was observed since 1997 in commercial greenhouses. Affected plants exhibited stems bleaching and wilting of the leaves, followed by total necrosis. Under humid conditions, cottony white micelium developed on stem surfaces, soon followed by the appearance of black, round or oblong sclerotia, also in the pith cavity. Isolations were made on potato dextrose agar from plants tissues and sclerotia. Typical colonies of *Sclerotinia sclerotiorum* were isolated from plants and sclerotia, sometimes together *Fusarium oxysporum*, *F. solani* or *Rhizoctonia* sp. Because any of these fungi could cause basal rot, pathogenicity tests were made with two isolates of each of them. Ten 2-month-old potted plants of cvs Suncity (violet flowers), Suncarlo (white), and Sun-top (pink) were inoculated by adding rice kernels colonized with each fungal isolate in the soil in contact with the roots and stems. Plants were grown in a greenhouse at 25-32°C except for those inoculated with *S. sclerotiorum*, which were kept in a growth chamber at 18-20°C. Only *S. sclerotiorum* was pathogenic after 8-10 days. The other tested fungi did not cause symptoms after two months. *Sclerotinia* rot has been recorded from *Aster* sp. in the USA (Bolland and Hall, 1994) and *A. pilosus* in Japan (Takeuchi and Horie, 1999). To the best of our knowledge, this seems to be the first record of *S. sclerotiorum* on *A. ericoides* var. *ericoides*.

Boland G.J., Hall R., 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **16**: 93-108.

Takeuchi J., Horie H., 1999. First occurrence of *Sclerotinia* rot in *Aster* and strawflower in Japan. *Annual Report of the Kanto Tosan Plant Protection Society* **46**: 57-59.

Corresponding author: S.M. Wolcan
Fax: +54.221.4252346
E-mail: swolcan@speedy.com.ar

Received March 30, 2006
Accepted June 20, 2006

DISEASE NOTE

OCCURRENCE OF *XANTHOMONAS ARBORICOLA* pv. *CORYLINA* IN HAZELNUT ORCHARDS IN SARDINIA AND SICILY

G. Cirvilleri¹, M. Fiori², A. Bonaccorsi¹, G. Scuderi¹, S. Virdis² and M. Scortichini³

¹ Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Via S. Sofia 100, Catania, Italy

² Dipartimento di Protezione delle Piante, Università degli Studi di Sassari, Via E. De Nicola, Sassari, Italy

³ C.R.A. - Istituto Sperimentale per la Frutticoltura, Via di Fioranello 52, 00040 Ciampino aeroporto, Roma, Italy

In the course of field surveys carried out in hazelnut (*Corylus avellana*) orchards of Sardinia [Barbagia di Belvì (Nuoro)] and Sicily [Nebrodi (Messina) and Etna (Catania)] symptoms resembling those incited by *Xanthomonas arboricola* pv. *corylina* Vauterin *et al.* were observed. Brownish, elliptical, water-soaked necrotic spots were present on fruit husks and, sometimes, twigs showed partial die-back. Tissues from lesion margins were ground in a mortar containing physiological sterile saline. Aliquots (0.1 ml) of serial ten-fold dilutions of the homogenate were plated on Yeast extract-dextrose-calcium carbonate agar and incubated at 25-27°C for three days. The resulting circular, mucoid, yellowish colonies were subjected to biochemical and pathogenicity tests, as well as repetitive-sequence PCR and fluorescent AFLP analysis in comparison with the reference strain of *X.a.* pv. *corylina* NCPPB 2896 (National Collection of Plant Pathogenic Bacteria, York, UK). All isolates from Sardinia and Sicily were starch and esculin-positive, had an oxidative glucose metabolism, and grew at 35°C. Genetic fingerprinting showed a strong similarity of the isolates with NCPPB 2896. Pathogenicity tests, made according to Scortichini *et al.* (2002), showed that all tested isolates induced wilting of inoculated hazelnut twigs. Re-isolations yielded the same colony type as in the primary isolations. We conclude that the agent of the disease observed in hazelnut orchards in Sardinia and Sicily is *X. a.* pv. *corylina*. This is the first report of this pathogen in both islands.

Scortichini M., Rossi M.P., Marchesi U., 2002. Genetic, phenotypic and pathogenic diversity of *Xanthomonas arboricola* pv. *corylina* strains, question the representative nature of the type strains. *Plant Pathology* **51**: 374-381.

Corresponding author: G. Cirvilleri
Fax: +39.095.7147264
E-mail: gcirvil@unict.it

Received April 11, 2006
Accepted April 19, 2006

DISEASE NOTE

OUTBREAKS OF *PEPPER MILD MOTTLE VIRUS* IN GREENHOUSES IN SANLIURFA, TURKEY

M.E. Güldür¹ and B.K. Çağlar²

¹ Department of Plant Protection, Harran University, Sanliurfa, Turkey

² Department of Plant Protection, Çukurova University, Adana, Turkey

The tobamovirus *Pepper mild mottle virus* (PMMoV) was first identified in Turkey in 1994 in fields of commercial pepper (*Capsicum annuum*) (Guldur, *et al.*, 1994; Palloix *et al.*, 1994). There was no evidence of its presence in the southeastern Anatolian province of the country until, in February 2006, very severe symptoms were observed in greenhouse-grown peppers of cv Charlee in the vicinity of Sanliurfa. Plants were stunted, had mottled, puckered, and malformed leaves and bore fruits that were small, deformed and marked by off-colored sunken areas. Disease incidence ranged from 60 to 95% and resulted in a 75 to 95% yield loss. Fruit and leaf tissues from several plants were tested by ELISA using commercial kits for PMMoV, *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) (Loewe Phytodiagnostica Biochemica, Sauerlach, Germany), and *Tobacco etch virus* (TEV) (Agdia Incorporated Elkhart, IN, USA). Of 42 symptomatic samples from five greenhouses, all gave a positive result for PMMoV but no reaction for the other viruses tested. Seed samples from twelve pepper plants (25 seeds from each infected plant) also gave consistently positive ELISA result for PMMoV. Seeds from symptomatic ELISA-positive plants were individually planted in pots containing an autoclaved commercial soil-less mix: horticultural sand (1:1) in a greenhouse at 25°C. Of 120 seedlings tested for PMMoV 34 were positive, confirming transmission through seed. Mechanical inoculation of several healthy seedlings of cv Charlee peppers with sap extracted from symptomatic leaves and fruit resulted in 100% infections as determined by symptom observation and ELISA tests. This record, the first of PMMoV in southeastern Anatolia, appears to be particularly threatening because of the environment (greenhouses) in which the outbreak developed and the high rates of seed transmission of PMMoV.

Güldür M.E., Özaslan M., Baloğlu S., Yılmaz M.A., 1994. Pepper mild mottle virus in pepper in Türkiye. *Proceedings of the 9th Congress of the Mediterranean Phytopathological Union, Kusadası 1994*, 465-467.

Palloix A., Abak K., Daubeze A.M., Güldür M.E., Gebre K.G., 1994. Survey of pepper diseases affecting the main production regions of Turkey with special interest in viruses and potyvirus pathotypes. *Capsicum and Eggplant Newsletter* 13: 78-81.

Corresponding author: M.E. Güldür

Fax: +90.414.2474480

E-mail: mguldur@harran.edu.tr

Accepted May 24, 2006

Received June 13, 2006

DISEASE NOTE

FIRST REPORT OF BACTERIAL BLIGHT OF HAZELNUT CAUSED BY *XANTHOMONAS ARBORICOLA* pv. *CORYLINA* IN IRAN

M.N. Kazempour, B. Ali and S.A. Elahinia

Department of Plant Pathology, Faculty of Agricultural Sciences, Guilan University, Rasht, Iran

A severe disease of hazelnut (*Corylus avellana*) was observed in 2004 in orchards of the Guilan province (Rahim-Abad of Roudsar and Amlash) of Iran. Water-soaked spots were present on the leaves, that later withered and desiccated, and on the shoots, which soon expanded into dark brown to black strip-like lesions (Scortichini *et al.*, 2002). Extracts (100 µl) from symptomatic tissues crushed in peptone water were plated on NA and YDC media containing cycloheximide (50 µg.MI⁻¹). Bacterial colonies that developed within 48 to 72 h were yellow on YDC medium or mucoid and convex on NAS medium. All of 32 selected isolates were rod-shaped, obligate aerobic, gram negative, oxidase, indole, and pectinase negative, H₂S, catalase, and levan positive. In addition, they were ice-nucleation negative, caused hypersensitive reaction on tobacco and pepper leaves, and hydrolysed starch, gelatin, and aesculin. All isolates used arabinose, glucose, trehalose, cellobiose, glycerol, galactose, and sucrose as carbon sources but none of them utilized rhamnose, lactose, raffinose, mannitol, and sorbitol. Pathogenicity tests were done by inoculating leaves, fruits and 1-year-old twigs of healthy hazelnut plants with suspensions of 10⁸ CFU/ml of each isolate (Gardan and Devaux, 1987). Inoculated plants and the controls inoculated with sterile water were maintained in a greenhouse at 25 to 28°C. Within 14 to 21 days, necrotic spots developed on the leaves and fruits of inoculated plants but not in the controls. The pathogen was reisolated from lesions of inoculated plants. Based on the results of the above mentioned tests the bacterium recovered from diseased hazelnuts was identified as *Xanthomonas arboricola* pv. *corylina*. This is the first report of bacterial blight of hazelnut in Iran.

Gardan L., Devaux M., 1987. *Xanthomonas campestris* pv. *corylina* on hazelnut. *Bulletin OEPP/EPPO Bulletin* 17: 241-250.

Scortichini M., Rossi M.P., Marchesi U., 2002. Genetic, phenotypic and pathogenic diversity of *Xanthomonas arboricola* pv. *corylina* strains question the representative nature of the type strain. *Plant Pathology* 51: 374-378.

Corresponding author: M.N. Kazempour

Fax: +98.131.6690281

E-mail: nikkazem@yahoo.fr

Received May 29, 2006

Accepted June 12, 2006

DISEASE NOTE

FIRST REPORT OF MALVASTRUM LEAF
CURL VIRUS INFECTING PAPAYA

C.Z. Wu and X.P. Zhou

*Institute of Biotechnology, Zhejiang University,
Hangzhou 310029, People's Republic of China*

Virus isolates G100 and G136 were obtained from papaya plants showing leaf curl symptoms in Nanning, Guangxi Province, China, in October 2004 and in January 2005, respectively. PCR of total DNA extracts from plants infected by these isolates using the degenerate primer pair PA and PB, which are designed to amplify part of the intergenic region and the AV2 gene of DNA-A of begomoviruses (Zhou *et al.*, 2003), yielded a 500 bp DNA fragment. The fragments were cloned in pGEM-T and sequenced. Alignment of the 500 bp sequences showed that DNA fragments of G100 and G136 (AM260699 and AM260700) have 90% nucleotide sequence identity, suggesting that the sources were infected by the same virus. The full-length sequence of DNA-A of G100 was obtained by PCR, using primers G100/F (5'-CTGATGTTCTCGCGGATGTGAAG-3') and G100/R (5'-CAAGATTGGACAACAGCGCGTG-3'). The complete DNA-A sequence of G100 comprised 2742 nucleotides (AM260699). A comparison with DNA-A of other begomoviruses showed that G100 is closely related with Malvastrum leaf curl virus (MLCV, AJ971263) with a 98% nucleotide sequence identity. These sequence results confirm that the papaya samples were infected by MLCV, a virus first identified in *Malvastrum coromandelianum* in Heinan Province (Huang and Zhou, 2006). To the best of our knowledge this is the first report of MLCV infecting papaya.

Huang J.F., Zhou X.P., 2006. Molecular characterization of two distinct begomoviruses from *Ageratum conyzoides* and *Malvastrum coromandelianum* in China. *Journal of Phytopathology* **154**: 2006.

Zhou X.P., Xie Y., Zhang Z.K., 2003. Malvastrum yellow vein virus, a new *Begomovirus* species associated with satellite DNA molecule. *Chinese Science Bulletin* **48**: 2205-2209.

Corresponding author: X.P. Zhou
Fax: +86.571.86971498
E-mail: zzhou@zju.edu.cn

Received June 26, 2006
Accepted July 11, 2006

DISEASE NOTE

INTERNAL BROWN ROT OF ONION
CAUSED BY AN OPPORTUNISTIC
BACTERIAL PATHOGEN
(*PSEUDOMONAS AERUGINOSA*)
IN CHINA

X.J. Hao and G.L. Xie

*State Key Laboratory of Rice Biology, Institute
of Biotechnology, Zhejiang University, Hangzhou 310029,
People's Republic of China*

Onion (*Allium cepa*) bulbs of cv Fujia with internal brown rot were observed in June 2004 in Hangzhou (Zhejiang Province, China). Rotting was initially confined to one to two of the inner fleshy scales, which turned brown but remained firm, while the adjacent scales were not visibly affected. Eventually, tissues collapsed and the exuded juices infected adjacent bulbs, resulting in substantial economic losses. Five bacterial isolates from infected onions showed characteristics similar to those of the standard reference strains of *Pseudomonas aeruginosa* (LMG 1242^T from Belgium; IR07358, originally from the Philippines) in phenotypic tests (including results of the Biolog Identification System, version 4.1), pathogenicity tests, and gas chromatographic analysis of fatty acid methyl esters (FAMES) using the Microbial identification System (MIDI Company, USA) with aerobic bacterial library (TABA50). Isolates were aerobic, rod-shaped, gram-negative, had 1-4 polar flagella, and produced a green-fluorescent diffusible pigment on King's Medium B. Colonies on nutrient agar were pale dirty-white, slightly raised with smooth margins, then translucent and flat with irregular margins within 3 days. No hypersensitive reaction was observed on tobacco. All isolates were identified as *Pseudomonas aeruginosa* with Biolog similarity of 0.726-0.972 and FAMES similarity of 0.702-0.764. Inoculation of intact onion bulbs of cv Fujia reproduced the symptoms observed in natural infections. The bacterium was re-isolated from symptomatic bulbs. Lesions and rotting were only induced when high inoculum concentrations (10^9 cfu/ml) were used, tissues were wounded and kept under high moisture conditions. *P. aeruginosa* has been first reported from Australia as the cause of internal brown rot of onion (Cother *et al.* 1976). To our knowledge this is the first report of internal brown rot on onion caused by *P. aeruginosa* in China.

This study was supported by the National Science Foundation of China (30370951).

Cother E.J., Barbyshire B., Brewer J., 1976. *Pseudomonas aeruginosa*: cause of internal brown rot of onion. *Phytopathology* **66**: 828-834.

Corresponding author: G.L. Xie
Fax: +86.86.049815
E-mail: glxie@zju.edu.cn

Received July 3, 2006
Accepted July 20, 2006