DISEASE NOTE

FIRST REPORT OF *PEPINO MOSAIC VIRUS* IN TOMATO IN TURKEY

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Pepino mosaic virus (PepMV), genus *Potexvirus*, was first observed in pepino crops (*Solanum muricatum*) from Peru and described by Jones *et al.* (1980). During spring 2008 and 2009 unusual virus-like symptoms were observed in greenhouse-grown tomato (*Lycopersicon esculentum*) plants in Dalaman (Mugla, south-western Turkey). Affected plants showed symptoms ranging from interveinal leaf chlorosis, mosaic, leaf distortion, mottling and uneven ripening of fruits. Since the involvement of PepMV in this disease was suspected, to verify the presence of the virus 74 leaf and 13 flower samples were collected from symptomatic tomato plants and tested by DAS-ELISA using a commercial antiserum to PepMV (Bioreba, Switzerland). *Potato virus X* (PVX), which is serologically related to PepMV, was also surveyed by DAS-ELISA using reagents from the same company. Infection rates of 68% and 100% were obtained for PepMV in leaf and flower samples, respectively, whereas PVX was not detected. The presence of PepMV was confirmed by mechanical inoculations to tomato, *Nicotiana rustica* and *Datura stramonium*. Leaf samples of tomato plants that were positive for PepMV in DAS-ELISA induced mosaic symptoms in indicator plants and the presence of the virus was confirmed serologically. To our knowledge, this is the first report of PepMV in Turkey.

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DISEASE NOTE

SEVERE OUTBREAKS OF TOMATO POWDERY MILDEW CAUSED BY *LEVEILLULA TAURICA* IN THE MARMARA REGION OF TURKEY

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The main production areas of tomato (*Solanum lycopersicum*) in Turkey are in the South Marmara and Aegean regions, with a production of about 45% and 20% of the total crop at the national level, respectively. Powdery mildew, caused by *Leveillula taurica* (Lév.) G. Arnaud, is a major problem in several countries. Until recently, the disease was not considered serious in the Batak plain (south Marmara region), where about 14,500 ha are planted with tomato. During 2007-2009 severe outbreaks of powdery mildew, affecting as much as 100% of the plants, occurred in this area, resulting in *ca*. 45% yield loss and lower quality of the crop. Disease was first detected in early summer and spread rapidly afterwords. Newly infected leaves exhibited fungal colonies on abaxial leaf surfaces, but frequently both surfaces of older leaves were completely covered. Severely infected leaves developed chlorotic and necrotic patches prior to dropping. *L. taurica* was identified because of the presence of endophytic mycelium, branched conidiophores and dimorphic conidia borne singly or in short chains (Boesewinkel, 1980). Conidia were pyriform or cylindrical averaging 64×20 µm and 62×18 µm, respectively. The teleomorph was not observed. To determine pathogenicity, a suspension of 5×10^4 conidia/ml from infected leaves was applied to both leaf surfaces of 6-week-old tomato plants that were were maintained in a growth chamber at 25/21°C (day/night) and 80% relative humidity. Control plants were not inoculated. After 21 days, inoculated plants developed powdery mildew symptoms on the abaxial leaf surface, including sporulation similar to that of naturally infected plants. Fungus localization on the leaves, presence of endophytic mycelium, and morphological characteristics of the imperfect stage confirmed the identity of the pathogen (Palti, 1988). This is the first report of a significant outbreak of *L. taurica*-induced powdery mildew on tomato in Turkey.

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Palti J., 1988. The *Leveillula* mildews. *Botanical Review* **54**: 423-535.

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DISEASE NOTE

FIRST REPORT OF AN OLD WORLD BEGOMOVIRUS INFECTING JUTE IN INDIA

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Jute (*Corchorus capsularis* L. and *Corchorus olitorius* L., Tiliaceae), is one of the most important bast fibre crops in India. Recently a yellow mosaic of *C. olitorius* was noticed in the fields of Baharich district of Uttar Pradesh. Field-infected symptomatic plants were used as initial source of virus inoculum for whitefly (*Bemisia tabaci*) transmission. Upon transmission, typical symptoms of the disease were produced on healthy *C. olitorius* plants grown under glasshouse conditions, suggesting the probable association of a whitefly-vectored begomovirus with the disease. Total DNA was extracted from symptomatic leaves of experimentally infected *C. olitorius* plants using a Qiagen DNeasy kit (Qiagen, USA). Begomovirus-specific universal primers PAL1v1978 and PAR1c496 (Rojas *et al*., 1993) which span from partial AC1 ORF to partial AV1 ORF including the intergenic region of DNA-A were used to amplify the corresponding genomic fragments of the putative virus. The expected 1.2 kb segment of DNA-A was obtained from all samples. Sequencing of a representative clone from each of 10 amplicons revealed that all were 1158 nt in length. One representative sequence, deposited in GenBank (accession No. EU368668) shared 88.6% and 84.8% identity with two Old World begomoviruses, *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Papaya leaf curl virus* (PaLCuV), respectively. Earlier, the association of a New World begomovirus inducing a similar disease in *C. capsularis* (Ghosh *et al*., 2006) was reported. This, however, constitutes the first report of a whitefly-vectored Old World begomovirus infecting *C. olitorius* in India.

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DISEASE NOTE

FIRST REPORT OF *FUSICLADIUM LEVIERI* ON PERSIMMON IN TURKEY

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In late summer 2009, a severe leaf spotting was observed on a local genotype (cv. Harbiye) of persimmon (D*iospyros kaki*) grown in some orchards at Kırıkhan (Hatay, Turkey). Subcircular brown spots surrounded by a yellowish halo, 1 to 4 mm in diameter, were present on the leaf surface of 10-30% of the plants. Conidiophores (15-60×2-8 µm) with conspicuous and often denticle-like conidiogenous loci, bearing branched chains of ellipsoid to ovoid or fusiform, 0-1 septate (13-30×3-6 µm) conidia, were observed under the light microscope. A conidial suspension from leaf lesions was spread onto the surface of water agar and incubated for 24 h. Single germinated conidia were transferred to potato dextrose agar and incubated for two weeks. Mycelial colonies were brown and bore conidiophores and conidia similar to those described above. Pathogenicity tests were performed by spraying three healthy young persimmon plants with a conidial suspension of a representative fungal isolate (1×10^6 conidia/ml of sterile distilled water). After 20 days, symptoms resembling those exhibited by naturally infected plants appeared on inoculated plants. Control plants sprayed with sterile distilled water did not show any symptoms. Re-isolations yielded fungal colonies identical to the original ones. Based on morphological characters, the fungus was identified as *Fusicladium levieri* (Schubert, 2001). *F. levieri* infections to D*iospyros virginiana*, *D. lotus* and *D. kaki* have been reported from USA, China, Japan, Georgia and Romania (Scholler *et al*., 2003; Schubert, 2001). However, to our knowledge, this is the first record of *D. kaki* leaf spot caused by *F. levieri* in Turkey.

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DISEASE NOTE

FIRST REPORT OF *PYTHIUM SPLENDENS* ON GINGER

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In August, 2009, a new disease was observed on ginger (*Zingiber officinale* Roscoe) at Ronhat, Chandni and Mashu areas in the low hills of Himachal Pradesh (India). Symptoms were first seen on the leaves which became pale yellow and drooped later along the pseudostem. Initially, the symptoms affected a few pseudostems, but later the whole plant was diseased and the pseudostems showed soft rot at the ground level, which affected also rhizomes and roots. A fungus isolated consistently from small pieces of diseased rhizomes on potato dextrose agar (PDA) at 25°C produced a cottony white mycelium. This fungus grew well in single spore culture on PDA (90 mm diameter in 5 days at 28±2°C) and produced abundant long tapering sporangia (12-38 µm long), which released evanescent vesicles. Spherical oospores (24.9-34.8 µm diameter) with smooth wall were found in the scales of diseased rhizomes. Based on these morphological characters, the fungus was identified as *Pythium splendens* Braun (Waterhouse, 1967). The identity of the culture was also established by PCR amplification and sequencing of the internal transcribed spacer (ITS) region (White *et al*., 1990) (GenBank accession No. GQ121298). To the best of our knowledge, this is the first record of *P. splendens* on ginger rhizomes worldwide.

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DISEASE NOTE

FIRST REPORT OF OKRA LEAF CURL DISEASE IN CHINA

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In October 2009, many okra plants (*Abelmoschus esculentus*), an important vegetable crop growing in the surroundings of Nanning (China), showed symptoms consisting of upward curling of the leaves. Leaf samples from four symptomatic plants were collected and tested by PCR for the presence of geminiviruses. Amplicons 2.7 kb in size, obtained form all samples using the universal begomovirus-specific primers BM-V and BM-C (Luan *et al*., 2006), which amplify nearly full-length DNA-A, were cloned and sequenced. The obtained sequences were almost identical to one another. The complete DNA-A sequence of an isolate (OKRA1) was then determined and found to be 2,738 nt in size (GenBank accession No. GU574208). A comparison with other begomoviruses showed that DNA-A of isolate OKRA1 shares 99.8% nucleotide sequence identity with *Cotton leaf curl Multan virus* (CLCuMV) isolate GX1 (CQ924756), reported to possess DNA β (Cai *et al*., 2010). Thus, isolate GX1 DNA β-specific primers were used to identify and PCR-amplify DNA β of isolate OKRA1. The 1,347 bp full-length sequence of isolate OKRA1 DNA β was obtained (GU574207) and shown to have 99.5% identity at the nucleotide level with DNA β of CLCuMV isolate GX1 (GQ906588). Based on symptomatology and sequence information, it is plausible to conclude that the Chinese okra leaf curl disease is associated with CLCuMV. To our knowledge, this is the first report of okra leaf curl disease in the People's Republic of China.

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DISEASE NOTE

FIRST REPORT OF BACTERIAL WILT OF COMMON BEAN CAUSED BY *CURTOBACTERIUM FLACCUMFACIENS* pv. *FLACCUMFACIENS* IN MATOGROSSO DO SUL

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Common bean (*Phaseolus vulgaris*) is an important crop in the state of Mato Grosso do Sul (mid-western Brazil), especially because it is grown in rotation before cotton (*Gossypium hirsutum*). In the 2009/2010 season, in a farm located in the municipality of Chapadão do Sul where a 360 ha surface was given over to common bean cv. Pérola, patches of plants at the reproductive stage were observed, which showed yellowing, dwarfing and withering. From the stems of these plants a small rod-shaped, Gram-positive bacterium was isolated, whose colonies were slightly convex, non-viscous and yellow. To fulfill Koch´s postulates, bean seeds were sown in the soil inside plastic bags and inoculated after 10 days as described by Theodoro and Maringoni (2006), using a 72 h old nutrient-sucrose agar culture. Sterile distilled water was used as control. Symptoms comparable to those observed in the field were observed 5 days post inoculation and severe wilt after 11 days. Control plants remained symptomless. Re-isolations from diseased plants yielded bacterial strains with the same morphological characteristics as the original ones. Several features of these isolates were analysed, i.e. reaction to Gram stain and KOH test, growth on nutrient-sucrose-agar plus 7% NaCl (Camara *et al.*, 2009), O/F test, casein and esculin hydrolysis. The MicroLog 4.2.05 System (Biolog, USA) was used for ultimate identification. Re-isolated bacteria were rod-shaped, Gram-positive, KOH test negative, hydrolized casein and esculin, grew in nutrient-sucrose-agar with 7% NaCl, showed oxidative metabolism of glucose and were identified as *Curtobacterium flaccumfaciens* by MicroLog 4.2.05 System. Bacterial wilt of bean, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, had already been reported from some Brazilian states (Theodoro and Maringoni, 2006), but this is the first record of this important disease from Mato Grosso do Sul.

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DISEASE NOTE

FIRST REPORT OF BOTRYTIS BLIGHT CAUSED BY *BOTRYTIS CINEREA* ON SWEET BASIL IN TURKEY

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In recent years, the cultivated area of sweet basil (*Ocimum basilicum* L.) has expanded markedly in Turkey, due to increasing demand of the market. In February 2008, significant blossom and leaf blight symptoms were observed on sweet basil plants in Antalya province. Initially, symptoms consisted of small, round or elliptic, brown lesions on the leaves, followed by stem infection, death of the leaves and secondary buds. In severe attacks, infection progressed along the leaf blade and petiole and expanded upward and downward towards the stem. In wet or humid conditions, diseased leaves and stems produced a grayish-brown mold. Diseased tissues taken from surface-sterilized infected leaves were plated on potato dextrose agar (PDA) and incubated for 7-10 days at 20ºC. Microscopic examination of greyish brown mycelial growth revealed that conidia were smooth, hyaline, obovoid or slightly elipsoidal and measured 9.5-15 µm (average 11.7 µm) × 5.0-9.2 µm (average 7.5 µm). Sclerotia were dark, spherical or irregular, 2-4×1-2 mm in size. The causal organism was identified as *Botrytis cinerea* Pers. based on morphological characteristics (Ellis, 1971). For pathogenicity tests PDA plugs from 10-day-old mycelial cultures were placed on wounded leaves of 8-week-old potted sweet basil seedlings. Plants were covered with polyethylene bags for 3 days at 20±2ºC and 75-80% relative humidity. Control plants were treated with sterile PDA plugs. The first foliar lesions identical to those observed on naturally infected leaves of sweet basil became visible 5 days after inoculation. No symptoms developed on control plants. This disease has been previously reported from Italy (Garibaldi *et al*., 1997), Greece (Holevas *et al*., 2000) and Hungary (Nagy, 2007). To our knowledge, however, this is the first report of the presence of *Botrytis cinerea* on sweet basil in Turkey.

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DISEASE NOTE

ROSEMARY POWDERY MILDEW CAUSED BY *GOLOVINOMYCES BIOCELLATUS* IN TURKEY

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Rosmarinus officinalis (Lamiaceae), a perennial evergreen bush native to Mediterranean, is grown for ornamental, medicinal and food purposes in parks, home and public gardens in warm and temperate areas of Turkey, including Sanliurfa (south-east Turkey) in one of whose parks, powdery mildew symptoms were observed in spring 2009. Dust-like whitish formations covered all parts of lower rosemary branches. Amphigenous mycelium was present on both surfaces of infected leaves and on stems. Branched hyphae were superficial, septate, and hyaline. Conidiophores, generally starting from a long food cell were linear or, more rarely, helicolidal, bore chains of 4-8 hyaline conidia, and measured 100-180×10-12 µm. Generally, three types of conidia were present, i.e. ovoid, doliiform and sub-cylindrical, from the apex down in the conidial chain, the ovoid type being most common. Conidia were 29-44×15-20 µm in size and had no clear fibrosin bodies. Germ tubes emerged from conidial shoulders or poles. Teleomorph structures were not detected. Based on anamorph morphology (Braun, 1987), the fungus was identified as *Golovinomyces biocellatus*. For pathogenicity tests several heavily infected rosemary branches were cut and shaken several times onto three healthy plants that were incubated in a 70-90% changeable humidity chamber for two days at 23-25ºC, then left to grow normally. Control plants were not inoculated. First powdery mildew symptoms developed 10 days after inoculation. A specimen was deposited in the Herbarium of the Plant Protection Department at the Agricultural Faculty, Harran University. *G. biocellatus* had been previously reported form different plant species, e.g. *Mentha* spp. (Liberato and Cunnington, 2007) and was first observed on potted rosemary plants in Korea (Park *et al.,* 2009). However, there is apparently no record of powdery mildew infections on natural rosemary stands. To my knowledge, this is the first report of *G. biocellatus* on naturally growing rosemary plants in Turkey.

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DISEASE NOTE

RHIZOCTONIA SOLANI AG 4 CAUSES LEAF BLIGHT ON WOODLAND SAGE (*SALVIA NEMOROSA*) IN NORTHERN ITALY

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In summer 2009, in a public park of the city of Torino (northern Italy), a leaf blight was observed on plants of *Salvia nemorosa* cv. Blau Koenigin. Symptoms consisted of dark-brown lesions at the crown level and semi-circular, water-soaked lesions on the leaves just above the ground. Blighted leaves turned brown, withered, and clung to the shoots. A fungus with the morphological characters of *Rhizoctonia solani* (Sneh *et al.*, 1991) was consistently recovered from infected leaves. Twenty-day-old mycelium grown on potato dextrose agar (PDA) at 22±1°C, was light-brown and rather compact. Isolates of *R. solani* obtained from affected plants were successfully anastomosed with tester isolate AG 4 (AG 4 RT 31). The ITS region of rDNA was amplified using primers ITS4/ITS6 and sequenced. BLASTn analysis (Altschul *et al.*, 1997) of the 679 bp amplified product showed 100% homology with the sequence of *Rhizoctonia solani* AG-4 and the nucleotide level (GenBank accession No. HM044763). Artificial infections with the pathogen were carried out on *S. nemorosa* cv. Blau Koenigin plants using an inoculum consisting of an aqueous suspension of PDA and mycelium fragments (1g/mycelium/plant) placed on the leaves. Inoculated plants were covered with plastic bags for three days and maintained in a glasshouse at 20-25°C. The first symptoms, similar to those observed in the park, developed seven days post inoculation. *R. solani* was consistently reisolated from infected leaves.

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DISEASE NOTE

FIRST REPORT OF *PHOMOPSIS AMYGDALI* CAUSING SHOOT BLIGHT OF PEACH IN CHINA

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In the summers of 2007 to 2009, cankers were observed on peach plants (*Prunus persica*) in Jiaxing city (Zhejiang province, China). Symptoms appeared in early summer as elongate, brown, sunken cankers, often with a zonate pattern, that constricted the base of the shoots, which eventually wilted and died. Gumming was commonly associated with cankers. A fungus consistently isolated from cankers on potato dextrose agar (PDA) produced pycnidia containing both α conidia, measuring 6.61×2.53 µm (average of 60 conidia), and β conidia, measuring 15.99×1.84 µm (average of 60 conidia). The morphological features of the fungus and the symptoms of the disease with which it is associated, match the description of *Phomopsis amygdali* infection to peach (Uddin *et al.*, 1997). To fulfill Koch's postulates, 40 current-year shoots of peach cv. Hujing were inoculated with one of the fungal isolates (JX-1) by placing a mycelial plug 5×5 mm in size on a 1 mm deep wound made with a scalpel, which was then wrapped with a piece of sterile moist cotton and sealed with parafilm. Control shoots were inoculated with sterile PDA plugs. Lesions 12 to 40 mm in length developed 10 days post inoculation (dpi) in all inoculated shoots, most of which wilted 30 dpi. By contrast, controls showed no signs of disease. The fungus was re-isolated from diseased shoots. To further confirm that *P. amygdali* was the causal agent of the observed disease, the internal transcribed spacer region of the fungus was amplified with ITS4 and ITS6 universal primer pair (Cooke and Duncan, 1997). The determined sequence (GU133063) showed a 100% match with that of *P. amygdali* from GenBank (AF102998) (Farr *et al.*, 1999). To the best of our knowledge, this is the first report of *Phomopsis* shoot blight on peach in China.

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DISEASE NOTE

INULA VISCOSA NEW HOST OF *CUCUMBER MOSAIC VIRUS*

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Inula viscosa (Asteraceae) is a perennial medicinal herb that grows wild in the Mediterranean area and has multiple uses in folk and traditional medicine. In spring 2007, almost 40% of *I. viscosa* plants grown in the Herb Garden "Augusto Rinaldi Ceroni" of Casola Valsenio (Emilia Romagna, Italy) showed chlorotic mottling of the leaves. Symptomatic samples were collected and analysed. No virus particles were observed with the electron microscope in leaf dips. However, *Cucumber mosaic virus* (CMV) was detected by PAS-ELISA in all symptomatic plant (more than 40) tested with a polyclonal antiserum to this virus (PVAS 30, American Type Culture Collection, USA). Virus was mechanically transmitted from *I. viscosa* to *Nicotiana tabacum* Samsun, which developed systemic mosaic symptoms. To confirm the association of CMV with diseased plants, total RNA was extracted from the same samples with RNeasy Plant Mini Kit (Qiagen, Germany) and analyzed by RT-PCR using CMV-specific primers MP+ and MP- (Lin *et al.*, 2004). The expected 842 bp fragment was amplified from samples of symptomatic tissues while no amplification products were obtained when water or healthy plants were used as template. RT-PCR products were cloned and sequenced. The sequence obtained (GenBank accession No. EU432181) had 99% identity with CMV-TN (AB176847) which induces tomato necrosis, and several other isolates of subgroup II. To our knowledge, this is the first report of CMV infecting *I. viscosa,* adding a new host to the list of more than 1,000 species infected by this virus.

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DISEASE NOTE

FIRST REPORT OF *KENAF LEAF CURL VIRUS* AND *MALVASTRUM YELLOW VEIN BAOSHAN VIRUS* ASSOCIATED WITH YELLOW VEIN DISEASE OF *SIDA ACUTA*

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Samples from two *Sida acuta* plants (Y340 and Y341) showing yellow vein symptoms were collected in Yunnan (China) in 2008. To identify possible begomoviruses, total DNA was extracted and PCR was performed with begomovirus degenerate primers PA/PB (Xie *et al.*, 2003). A 500 bp DNA fragment was obtained from both samples. Sequence analysis revealed that Y340 contained two begomoviruses (designated as Y340-I and Y340-II) and Y341 contained one begomovirus which was identical to Y340-I. Complete DNA-A sequences of Y340-I and Y341 were both 2,750 nt in size (accession No. FN806777 and FN806778), and shared the highest nucleotide sequence identity (>90.0%) with *Kenaf leaf curl virus* (KeLCuV-[IN:Kai:07], EU822321) while Y340-II DNA-A was 2,745 nt long (FN806779) and was most closely related to *Malvastrum yellow vein Baoshan virus* (MaYVBsV-[CN:Yn281:04], FN386460) (89.2% identity). Universal abutting primers beta01/beta02 were used to detect betasatellite (Zhou *et al.*, 2003) and a 1.3 kb product was obtained from both samples. Betasatellites of Y340 and Y341 were 1,342 and 1,341 nt in length (FN806780 and FN806781), respectively, sharing the highest sequence identity (83.4% and 82.4%, respectively) with Malvastrum yellow vein Yunnan betasatellite (MaYVYnB-[CN:Yn308:03], AM236778). Alphasatellite amplified from Y340 with primers UN101/102 (Bull *et al.*, 2003) was 1,370 nt (FN806782) in size and had 61.4-82.2% identity at the nucleotide level with other previously reported alphasatellites. These results show that the yellow vein disease of *S. acuta* is associated with two begomoviruses (KeLCuV and MaYVBsV) together with satellite molecules.

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DISEASE NOTE

CELERY MOSAIC IN CELERY IN POLAND

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A virus survey was conducted in celery (*Apium graveolens* L. var. *rapaceum*) in Poland in September 2008. Out of 28 cultivars tested, 16 (Albin, Brilant, Denar, Diaman, Fenix, Ilona, Luna, Makar, Mentor, Monarch, Neon, President, Printz Rex, Talar, Tango and Zagloba) did not show apparent symptoms. Deformation, chlorosis, chlorotic or necrotic spots of the leaves and stunting were observed on 11 cultivars: Dukat, Snow White, Maxim, Eden, Cisko, Helios, Zefir, Gol, Jablkowy, Edward and Verden Pascal. Symptoms were similar to those induced by *Celery mosaic virus* (CeMV) (Pemberton and Frost, 1974; Walkey and Ward, 1984), genus *Potyvirus*, family *Potyviridae*. The presence of CeMV was confirmed by DAS-ELISA of leaf extracts from individual plants using a commercial kit (Loewe Biochemica, Germany). The virus was detected in all 11 symptomatic cultivars and in the symptomless cvs Makar and Mentor with mean A_{405} values of 1.4-1.9 compared to 0.145 of healthy plants. These results were confirmed by bioassays in a greenhouse. Inoculated plants of *A. graveolens* cvs Odrzanski and Tina showed green to light green mottling and malformations, *Daucus carota* developed chlorotic spotting on the young leaves and *Chenopodium amaranticolor* produced occasional small chlorotic lesions. To the best of our knowledge, this is the first report of CeMV in celery in Poland.

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DISEASE NOTE

BLACK ROT OF *BRASSICA* spp. CAUSED BY *XANTHOMONAS CAMPESTRIS* pv. *CAMPESTRIS* IN MID-WESTERN NEPAL

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Brassica spp. are the most widespread vegetable crops in Nepal, accounting for 26% of the total area given over to vegetables. During autumn 2009, a devastating disease damaged *Brassica* crops in 12 different fields of Parsapur, Sitapur of Banke, Motipur, Belawa of Bardia and Rampur, Chandanpur of Dang districts in the Rapti zone (Nepal), resulting in 55-65% yield losses. V-shaped necrotic lesions with blackened veins were observed on the edge of cauliflower (*Brassica oleracea*) and cabbage (*Brassica oleracea* var. *capitata*) leaves, from which bacteria were isolated and purified on yeast dextrose $CaCO_3$ (YDC) medium after streaking and incubation at 28±1°C for 48 h. Twenty isolates were identified as *Xanthomonas campestris* pv. *campestris* (*Xcc*). All grew at 36°C, were aesculin positive, produced H_2S from peptone and acid from arabinose, galactose, trehalose and maltose. Isolates were further streaked on Fieldhouse-Sasser media (Fukui *et al*., 1994) to observe growth. Pathogenicity was ascertained on 1-month-old cabbage and cauliflower by spraying 10 healthy potted plants (10^8 CFU/ml) per isolate/host in a greenhouse. A known bacterial strain (CFBP 1119) and sterile distilled water were used as positive and negative controls respectively. All strains caused symptoms similar to those observed in the field within a week. Bacteria re-isolated from the necrotic lesions proved identical to the original strains. A 1339 bp region of the 16S rDNA from all strains was amplified and sequenced (GenBank accession No. GU373652, strain NEP XCC10). A BlastN search revealed that almost all strains had 99% identity with the 16S rDNA sequence of *Xcc* type strains available in databases. *Xcc* had previously been reported from the central region of Nepal (Adhikari and Basnyat, 1999; Jensen *et al*., 2010) but this is believed to be the first record in the country's mid-western region.

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Jensen B.D., Vicente J.G., Manandhar H.K., Roberts S.J., 2010. Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable *Brassica* fields in Nepal. *Plant Disease* **94**: 298-305.

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DISEASE NOTE

FIRST REPORT OF *CUCUMBER MOSAIC VIRUS* IN *CENTELLA ASIATICA* IN MADAGASCAR

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Centella asiatica (Asiatic pennywort) is an edible plant of the family Apiaceae used mainly as a medicinal herb in traditional Chinese and Ayurvedic medicine. In 1998, plants of *C. asiatica* from an industrial crop in the province of Antananarivo (Madagascar), showed mosaic, mottling and yellowish ringspot symptoms in the leaves. Inoculated plants of *Chenopodium quinoa*, *C. amaranticolor*, *Nicotiana tabacum* cvs. Xanthi and Samsun, *Cucumis sativus* and *Vigna unguiculata* reacted with symptoms typical of *Cucumber mosaic virus* (CMV). Occurrence of CMV in one sample was confirmed by the observation under the electron microscope of isometric particles *ca.* 30 nm in diameter in leaf dips, the positive reaction in DAS-ELISA with polyclonal antibodies to CMV (Devergne and Cardin, 1975) and the nonpersistent transmission to virus-free tobacco cv. Xanthi plants by *Myzus persicae*. In double-immunodiffusion analysis, the viral isolate was shown to belong to CMV subgroup II. To determine if CMV was responsible for the symptoms observed, the isolate was multiplied in Xanthi plants after isolation from local lesions on *V. unguiculata* and mechanically inoculated to 20 plants of CMV-free *C. asiatica* eight months after sowing. Two months after inoculation, all inoculated plants showed mosaic symptoms and were CMV positive in DAS-ELISA, while mock-inoculated plants were DAS-ELISA negative and showed no symptoms. CMV was previously detected by DAS-ELISA in samples of *C. asiatica* collected in 1980 in the same region (unpublished data) and, more recently, in adventive *C. asiatica* found in vanilla plantations from Leeward Islands (French Polynesia) (Richard *et al.,* 2009).

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Richard A., Farreyrol K., Rodier B., Leoce-Mouk-San K., Wong M., Pearson M., Grisoni M., 2009. Control of virus diseases in intensively cultivated vanilla plots of French Polynesia. *Crop Protection* **28**: 870-877.

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DISEASE NOTE

FIRST REPORT OF PHYTOPLASMA INFECTIONS IN SEVERAL TEMPERATE FRUIT TREES AND VEGETABLE CROPS IN AZERBAIJAN

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From 2003 to 2008, surveys were conducted to evaluate for the first time the presence of phytoplasma diseases in temperate fruit crops and vegetables of Azerbaijan. Twigs from stunted pear trees exhibiting leaf-roll and reddening, peach, apricot, cherry, cherry-plum (*Prunus myrobolan*) and common medlar (*Mespilus germanica*) trees showing yellowing and decline were collected. For solanaceous crops, yellowing and stunted peppers and eggplants as well as stunted tomatoes with purplish leaves were collected. DNAs were classically extracted by CTAB extraction method (Maixner *et al.*, 1995) and submitted to nested PCR with the universal 16S rDNA primers for phytoplasmas (Gundersen and Lee, 1996). While symptomless plants gave no amplification, positive results were obtained for 14 plants. The 16S rDNA PCR products amplified from four pear trees had sequences 100% identical to the 16S rDNA sequence of '*Candidatus* Phytoplasma pyri' (accession No. AJ542543). The 16S rDNA sequence of the phytoplasma detected in the peach tree shared 100% identity with the 16S rDNA sequence of '*Ca.* P. brasiliense' (AF147708) whereas those of a plum tree, an apricot tree and a cherry-plum tree had sequences 99.9% identical to the 16S rDNA sequence of '*Ca.* P. prunorum' (AM933142). The 16S rDNA PCR products amplified from the cherry tree sample, one pepper and one eggplant, two tomato plants and a common medlar had sequences 100% identical to the 16S rDNA sequence of the stolbur phytoplasma (EU552453). This is the first detection of the stolbur phytoplasma, '*Ca.* P. pyri', '*Ca.* P. prunorum' in Azerbaijan and the first report of '*Ca.* P. brasiliense' both in peach and outside Brazil.

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DISEASE NOTE

FIRST REPORT OF '*CANDIDATUS* PHYTOPLASMA ASTERIS' ASSOCIATED WITH YELLOWING OF *JATROPHA CURCAS* IN INDIA

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Jatropha curcas L., a multipurpose tree of family Euphorbiaceae, is an emerging biodiesel crop of tropical and subtropical regions of the world. Its wood and fruits are a source of fuel, while the viscous oil derived from seeds is used in the manufacture of candles, soaps and cosmetics. During February 2010 plants showing an unique disease were seen in a field at New Delhi (India), 99% of the plants being affected. Symptoms included yellowing of leaves and premature leaf fall, leaving the plants with bunch of fruits on bare erect stems. Genomic DNA extracted from stem tissues of five symptomatic and non-symptomatic plants each were subjected to PCR with phytoplasma 16S ribosomal DNA universal primers P1/P7 (Deng and Hiruki, 1991). The diluted PCR products (1:30) were further amplified by the nested primer pair R16F2/R2 (Gunderson and Lee, 1996) yielding ~1.25 kb amplicons only from symptomatic samples. One of the amplified products was purified using QIAquick gel extraction kit (Qiagen, USA) and sequenced. BLAST comparisons of the obtained partial 16S rDNA sequence revealed a 99% sequence identity with the phytoplasmas belonging to '*Candidatus* Phytoplasma asteris', 16rI group (Accession Nos AB558132.1, AB551736.1, GQ365729.1, GQ249410.1 and GQ240827.1). The sequence was deposited in GenBank (accession No. HM467912). This is the first record of *Jatropha curcas* as a new host of '*Ca.* Phytoplasma asteris' in India and in the world.

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DISEASE NOTE

FIRST REPORT OF *OLPIDIUM BORNOVANUS* AND *O. VIRULENTUS* IN TUNISIA

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Two surveys were conducted in 2007 and 2008 on melon and tomato crops grown in plastic houses at Monastir (northwest Tunisia) and Kebili (southeast Tunisia) to look for soil-borne fungal vectors of viral diseases. To this aim, five soil samples, representative of each region, were collected in four plastic houses from the root zone of plants showing virus-like symptoms. *Olpidium* spp. were isolated on homologous bait plants, total DNA was extracted from plant roots and tested by multiplex PCR for the simultaneous detection and identification of *Olpidium* spp. (Herrera-Vásquez *et al*., 2009). Mixed infections with *O. bornovanus* and *O. virulentus* were detected in two melon plants, and a single *O. virulentus* infection in one tomato plant. No amplicons were produced from healthy melon and tomato root extracts or water used as negative control. To confirm the identity of the *Olpidium* spp., amplified PCR products were directly sequenced. BLAST analysis of sequences of a previously characterized *O. bornovanus* isolate (Herrera-Vásquez *et al*., 2010) from Monastir (GenBank accession No. GU344684) and *O. virulentus* from Kebili (HQ008862) showed 100% nucleotide homology with reference sequences deposited in database. *O. bornovanus* has recently been reported as a root pathogen of melons (Stanghellini *et al*., 2010), and *O. bornovanus* and *O. virulentus* are economically important because they act as vectors of several destructive plant viruses (Alfaro-Fernández *et al*., 2009). To our knowledge, this is the first report of *O. bornovanus* and *O. virulentus* as potential root pathogens and vectors of plant viruses in Tunisia.

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DISEASE NOTE

FIRST REPORT OF *HOP STUNT VIROID* IN APRICOT IN TUNISIA

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A survey for the presence of viroids was carried out in the major stone fruit orchards of central and north Tunisia. A total of 214 samples were collected from peach (79), almond (31), apricot (35), plum (31) and cherry (38). All samples were tested by tissue printing hybridization according to Pallás *et al*. (2003), pressing three replicates of freshly cut ends of leaf petioles onto positively charged nylon membrane (hybond N+) and using digoxigenin labelled riboprobes to *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd). All samples were tested twice in 2008 and 2009. Results showed that 47 of 79 peach trees (59.5%) were infected with PLMVd, four (5%) with HSVd and two with both PLMVd and HSVd. Nine of 35 apricot trees (25.7%) were infected with HSVd. No viroid infection was found in almond, plum and cherry. In Tunisia, PLMVd was previously detected in peach and almond (Fekih-Hassan *et al*., 2004, 2005). HSVd in peach, almond and pear (Fekih-Hassan *et al*., 2004). To our knowledge, this is the first report on the presence of HSVd in apricot in Tunisia.

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DISEASE NOTE

FIRST REPORT OF KENAF LEAF CURL VIRUS INFECTING TOMATO IN YUNNAN, CHINA

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During a survey in November 2006, a virus isolate (YN377) was obtained from tomato (*Solanum lycopersicum*) showing severe leaf yellowing, curling, and stunting at Yuanmou (Yunnan province, China). To identify possible begomoviruses, total DNA was extracted from symptomatic tomato leaves using the Qiagen DNeasy kit (Qiagen, USA) and amplified by rolling circle amplification (RCA) using the TempliPhi Amplification kit (Amersham Bioscience, UK). The digested RCA products were inserted into the Hind III site of pGEM-3Z (Promega Biotech, USA) and the insert was sequenced. Sequence analysis confirmed that two clones of YN377 contained begomovirus DNA-A sequences. The complete DNA-A consisted of 2,751 nucleotides (accession No. HM448898) and contained the conserved nonanucleotide sequence (TAATATTAC), two open reading frames (ORFs V1 and V2) in the virion-sense and four ORFs in the complementary sense. The complete DNA-A sequence of YN377 had the highest nucleotide sequence identity (94.8%) with Kenaf leaf curl virus (KLCV), isolate Y340 (accession No. FN806777.1). When the total DNA was amplified using degenerate primers for DNA-B (PBL1v2040 and PCRc1) components of whitefly-transmitted geminiviruses (Rojas *et al*., 1993) and the universal abutting primer pair (beta01/beta02) (Briddon *et al*., 2002) to detect DNA-B or DNA β, no amplicon was obtained. Consequently, on the basis of the currently accepted begomovirus species demarcation threshold of 89% nucleotide identity, the symptomatic *S. esculentum* plants were infected by KLCV. To our knowledge, this is the first report of KLCV infecting *S. esculentum* in the People's Republic of China.

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DISEASE NOTE

FIRST REPORT OF BOLL AND LINT ROT DISEASE OF COTTON CAUSED BY *EXSEROHILUM ROSTRATUM* IN IRAN

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In October 2008, a disease causing lint and boll rot of cotton (*Gossypium hirsutum* L.) cv. Varamin was observed in Nehbandan region (south Khorasan, Iran). Symptoms included internal lint rot within multiple locules and olive to dark-brown rot of bolls associated with spiny bollworm damage. Symptomatic tissues were surface-disinfested and cultured on potato dextrose agar (PDA) at 26°C in the dark. Velvety, dark brown colonies with a black reverse developed after 5-7 days. Following transfer to tap water agar with sterile pieces of wheat straw or cotton lint embedded in the agar surface and incubation at 26°C, a gray to black mycelium with olivaceous brown, smooth, geniculate and simple conidiophores, up to 200 µm long was produced. Conidia were straight to slightly curved, ellipsoid to narrowly obclavate or rostrate, brown, with basal septum darker and thicker than intermediate septa, up to 12-distoseptates (mostly 7), 12.5-20×27.5-105 µm in size, with a distinctly protuberant hilum. Germination was bipolar, germ tubes grew semi-axially, displacing the hilum. Based on these morphological characteristics, the fungus was identified as *Exserohilum rostratum* (Drechsler) K.J. Leonard et Suggs (Leonard, 1976; Sivanesan, 1987). A culture is preserved at the Iranian Fungal Culture Collection (IRAN 1652C). Pathogenicity tests were performed three times with two isolates on each of 10 mature bolls approximately 4-4.5 and 2.5-3 cm in size. A suspension of 1×10^5 conidia/ml of sterile distilled water was injected into boll rinds, or agar plugs (5 mm in diameter) of each fungal isolate were placed into wounds. Sterile PDA plugs or distilled water served as controls. Bolls wrapped in parafilm and incubated at 25°C in the dark for one week reacted with extensive rotting in 5-6 days. The same fungus was re-isolated from the symptomatic tissues. No symptoms occurred on controls. This is the first report of a cotton disease caused by *E. rostratum* in Iran.

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DISEASE NOTE

FIRST REPORT OF RICE BLACK-STREAKED DWARF VIRUS INFECTING BARLEY IN JIANGSU, CHINA

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Rice black-streaked dwarf virus (RBSDV; genus *Fijivirus*, family *Reoviridae*) causes rice black-streaked dwarf, maize rough dwarf and wheat dark-green dwarf diseases, which cause severe economic damage to rice, maize, barley and wheat (Lee *et al.*, 2005). RBSDV is transmitted to cereal crops by the small brown planthopper *Laodelphax striatellus*. In the spring of 2010, barley (*Hordeum vulgare*) plants showing extreme dwarfing were found in Jiangsu province (China). Twelve leaf samples were collected from symptomatic plants in a barley field and tested for the presence RBSDV by enzyme-linked immunosorbent assay (ELISA) using RBSDV-specific monoclonal antibodies. The presence of RBSDV was also ascertained by RT-PCR using total RNA extracted with Trizol and RBSDV-specific primers 376f (5'-GATAGACAGGCAAATATAAGCGT-3') and 1462r (5'- GGATTACAACACACACAACGAAA -3'). RBSDV was detected by ELISA and amplicons of the expected 1200 bp in size were obtained from infected but not from healthy leaf samples. Alignment of the sequences of two barley isolates showed 98% sequence identity at the nucleotide level with RBSDV isolates from rice (accession No. AJ297430.1) (Zhang *et al.*, 2003) and maize (accession No. AF536564.2), respectively. These results indicate that the virus associated with dwarf disease of barley in Jiangsu is an isolate of RBSDV. To our knowledge, this is the first report of RBSDV infecting barley in the People's Republic of China.

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DISEASE NOTE

FIRST REPORT OF THE SPOT FORM OF NET BLOTCH OF BARLEY CAUSED BY *PYRENOPHORA TERES* f.sp. *MACULATA* IN EGYPT

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In summer 2010, ovoid necrotic spots with chlorotic halo 15×20 mm in size were observed on barley (*Hordeum vulgare* L.) in a commercial farm in Tahreer province (Bohara Governorate, Egypt). The disease affected about 40% of the plants. Conidiophores from diseased tissues were dark brown, single or in small groups and bore several hyaline to olive-brown, almost cylindrical conidia 75.5-100.0×16.6-18.8 µm in size, with three to seven pseudosepta. Cultures were gray to olive green, cottony, and did not form conidia and sexual structures on potato dextrose agar (PDA). These characteristics indicated that the pathogens belonged to the genus *Pyrenophora*. Species identity was confirmed by PCR assays with primers developed for *Pyrenophora* spp pathogenic to barley (Taylor *et al.*, 2001). For pathogenicity tests, the fungal isolate, identified as *Pyrenophora teres* f. *maculata*, was grown on two 9 cm PDA plates at 24°C in the dark. After 10 days, aerial mycelium was scraped off, blended in 100 ml of sterile distilled water, and filtered through two layers of cheesecloth. Twenty seedlings at the three-leaf stage were sprayed with the mycelial suspension and a water control until runoff. Seedlings were kept in a growth chamber at 100% relative humidity and 20°C in the dark for 24 h, then at 70% relative humidity and 24/20°C (day/night) with a 12 h photoperiod. Within 3 weeks, one to four brownish ovoid spots, typical of the spot form of the net blotch syndrome, developed on the inoculated leaves. The fungus was reisolated and identified by specific PCR and according to Kingsland (1991), thus fulfilling Koch's postulates. To my knowledge, this is the first report of the occurrence of *P. teres* f. *maculata* in Egypt.

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DISEASE NOTE

BLUE MOLD EPIDEMICS CAUSED BY *PERONOSPORA TABACINA* ON TOBACCO IN THE AEGEAN REGION OF TURKEY

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Blue mold is a devastating disease of tobacco (*Nicotiana tabacum* L.) caused by *Peronospora tabacina* Adam. In the Aegean region of Turkey approximately 70,000 ha of oriental tobacco are grown annually. During June and July 2010, there was a serious blue mold epidemic in commercial tobacco fields, that resulted in a *ca*. 90% yield loss in some areas. The affected area was difficult to determine because it included many fields of small growers, but at least 17,000 ha were involved. Disease symptoms were yellowish round spots on the upper surface of the lower leaves with corresponding purplish to grayish sporulation on the lower surface. Microscopic observations revealed conidiophores between 244 to 463 µm in length and 9.5 to 14.8 µm in diameter. Conidiophores were arborescent and dichotomously branched 6 to 8 times at acute angles. Conidia were elliptical, grayish in mass and measured 16-30×13-20 µm. No oospores were found. This morphology conforms to that of *P. tabacina* (Johnson, 1989; Trigiano *et al*., 1985). Three factors are thought to have contributed to this epidemic: (i) undetected infected plants within seedbeds, which may have been transplanted into commercial fields; (ii) infection by a fungal strain possibly resistant to routinely used fungicides; (iii) environmental conditions favourable to disease development. *P. tabacina* is an A1 quarantine pest by the IAPSC (Inter-African Phytosanitary Council) and a quarantine pest for India and China. In the EPPO region, blue mold of tobacco has the potential to cause substantial losses, except in hot (above 25°C) and dry conditions. *P. tabacina* was first recorded in Thrace (Marmara region) in 1961 (Türkmenoglu, 1963). To our knowledge, this is the first report of a blue mold epidemic on tobacco in the Aegean region of Turkey.

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DISEASE NOTE

FIRST REPORT OF NATURAL INFECTION OF *BROAD BEAN WILT VIRUS 2* ON *LACTUCA INDICA*

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During a survey of viral diseases conducted in 2008 in vegetable crops of Linan (Zhejiang province, China), broad bean (*Vicia faba* L.) plants were observed displaying mosaic and distortion of the upper leaves and wilting of the middle and bottom leaves which resembled symptoms of *Broad bean wilt virus 2* (BBWV-2) infections (Zhou *et al*., 1995). Nine symptomatic and a few apparently healthy broad bean plants and five different species of weeds with mottled leaves present within and around the broad bean field were tested by DAS-ELISA with monoclonal antibodies to BBWV-2 (Quing *et al*., 2000). All symptomatic broad bean plants reacted strongly, whereas no reaction was observed with sap from healthy plants. Among the tested weeds, only an Indian lettuce (*Lactuca indica* L.) plant with mottled leaves reacted with the same antibody. Total RNA was extracted from leaf tissues of both healthy and diseased plants with TRIzol (Invitrogen, USA) and reverse transcribed into cDNA with Oligo d(T)18 primers (TaKaRa, Dalian, China). PCR was performed with the degenerate primer pair Fab5´R1F and Fab5´R1R, designed to amplify the 5´ terminal sequences of all members of the genus *Fabavirus* (Ferrer *et al*., 2007). Fragments of about 350 bp were amplified from diseased samples, whereas no amplification was obtained from healthy plants. Two amplicons from Indian lettuce were recovered and sequenced (GenBank accession No. HQ172160 and HQ172161). Both amplicons showed the highest nucleotide sequence identity (98.5 and 97.4%, respectively) with BBWV-2 isolate B935 (AF149425). To our knowledge this is the first report of natural infection of Indian lettuce by BBWV-2. This plant (family *Compositae*), is a common tuberous-rooted perennial weed, that may serve as an important overwintering host of BBWV-2.

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DISEASE NOTE

FIRST REPORT OF FIG MOSAIC VIRUS FROM FIG TREES IN IRAN

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Fig trees (*Ficus carica*) were surveyed for the presence of Fig mosaic virus (FMV) in Iran in 2009 and 2010. A total of 40 leaf samples showing mosaic and discolouration patterns were collected in the province of Tehran (Varamin district) and Lorestan (Khorram Abad district). Crude extracts from symptomatic leaves were mechanically inoculated to herbaceous hosts, i.e. *Nicotiana tabacum*, *Phaseolus vulgaris*, *Cucumis sativus*, *Chenopodium amaranticolor* and *Petunia hybrida*, but none of them showed visible symptoms. dsRNA extraction from symptomatic fig leaves according to Valverde *et al*. (1990) yielded bands ranging from 0.6 to *ca.* 7 kbp, indicating the presence of multiple RNAs of viral origin. Total RNA extracted from both symptomatic fig leaves and inoculated indicator plants was used for random primed cDNA synthesis and RT-PCR assays with specific FMV primers, as described by Elbeaino *et al*. (2009a,b). A DNA fragment of 302 bp in size, amplified from symptomatic fig leaves, originated from the RNA-dependent RNA polymerase (RdRp) gene encoded by viral RNA-1. An amplicon of similar size was obtained from fig samples infected with a FMV isolate from Italy but there was no amplification from inoculated indicator plants. Viral amplicons were sequenced and nucleotide sequences compared with FMV sequences available in database. The sequence of the Iranian FMV isolate was 93% identical to the RdRp gene sequence reported for a FMV isolate from Turkey (accession No. FN666274). To our knowledge, this is the first report of FMV in Iran.

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DISEASE NOTE

OCCURRENCE OF *CLERODENDRUM GOLDEN MOSAIC VIRUS* IN *CLERODENDRUM BUNGEI* IN YUNNAN CHINA

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Rose glorybower (*Clerodendrum bungei*) is a small deciduous shrub with large, heart-shaped leaves, native to China and northern India. During a survey in 2009, a virus isolate (YN1095) was identified and characterized from *C. bungei* plants showing chlorosis and yellow mosaic in Jinghong municipality (Yunnan Province, China). Total DNA was extracted from symptomatic leaves and tested for the presence of begomoviral DNA-A and DNA-beta by PCR using begomovirus-specific degenerate primer pairs PA/PB (Deng *et al*., 1994) and Beta01/Beta02 (Briddon *et al*., 2002), respectively. The expected 0.5 kb DNA-A PCR product was obtained from isolate YN1095 but no DNA-beta product was amplified. Based on the 0.5 kb DNA-A sequence and *Clerodendrum golden mosaic virus* (GlGMV) DNA-B sequence (accession No. DQ641693), two primer pairs, ClGMV-AF (5'-GCACACGAAAACAGTTATGGGCCAA-3')/ClGMV-AR (5'-TCAGAGGACCTATGCGGATTGGTTGTTT-3') and ClGMV-BF (5'-TAAGCGTCCTGTATGGATAGTC-3')/ClGMV-BR (5'-AACAGATTGGACCCAGCA-3'), were designed to amplify the remaining DNA-A and the full-length DNA-B of isolate YN1095. Sequence of isolate YN1095 contained the conserved nonanucleotide sequence (TAATATTAC) in DNA-A and DNA-B (HQ317134 and HQ317135) and the expected six (AV1, AV2, AC1, AC2, AC3, and AC4) and two (VA1 and BC1) open reading frames of DNA-A and DNA-B, respectively. BLASTn analysis showed that DNA-A and DNA-B of isolate YN1095 had the highest nucleotide sequence identity (99%) with ClGMV (DQ641692 and DQ641693). Consequently, according to the currently accepted begomovirus species demarcation threshold of 89% nucleotide identity, symptomatic *C. bungei* plants were infected with ClGMV. To our knowledge, this is the first report of ClGMV infecting *C. bungei* in China.

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DISEASE NOTE

FIRST REPORT OF *TOMATO SPOTTED WILT VIRUS* IN TOMATO AND TOBACCO IN CHINA

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Tomato (*Solanum lycopersicum)* and tobacco (*Nicotiana tabacum*) are important crops in Yunnan province (south-west China). In 2008-2010, field surveys were conducted for virus diseases in Kunming (Yunnan), where the occurrence of *Impatiens necrotic spot virus* (INSV) and *Tomato zonate spot virus* (TZSV) had previously been reported (Cheng *et al*., 2010; Dong *et al*., 2008). Field-grown symptomatic tomato and tobacco plants were collected and crude leaf extracts were tested for the presence of tospoviruses using commercial DAS-ELISA detection kits (Agdia, USA). Extracts of 78 samples reacted with *Tomato spotted wilt virus* (TSWV) antibodies. Total RNA was extracted from TSWV-infected leaves (Dong *et al*., 2008) and tested with a single-step RT-PCR kit (TaKaRa, Dalian, China) using specific primers (Tosp-1T: 5'-AGAGCAATTGTGTCAATTTTATTC-3'; Tosp-2T: 5'-TCACTGTAATGTTCCATAGCAA-3') designed on the 3'-terminal sequence of TSWV S RNA. cDNA fragments of the expected size (860 bp) were amplified, cloned and sequenced. A high nucleotide sequence identity (99.8%) was obtained for TSWV isolates from tomato and tobacco indicating that both crops were infected with the same virus. The complete S RNA sequence of the TSWV tomato isolate, denoted KM-T, was determined to be 2,971 nt long (accession No. HQ402595). Analyses of S RNA sequences showed that KM-T is closely related to TSWV isolates from South Korea (AB190819), USA (AY744478) and Italy (DQ431237). The NSs and N proteins encoded by KM-T shared 97.6% and 98.5% amino acid identities with those of South Korean TSWV. To our knowledge, this is the first report of TSWV in tomato and tobacco in China.

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DISEASE NOTE

MULTIPLE VIROID INFECTIONS IN IRANIAN GRAPEVINES

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Hop stunt viroid (HSVd), *Grapevine yellow speckle viroid 1* (GYSVd-1), *Grapevine yellow speckle viroid 2* (GYSVd-2), *Australian grapevine viroid* (AGVd) and *Citrus exocortis viroid* (CEVd) are known to infect the grapevine in nature (Little and Rezaian, 2003). Except for CEVd, the other viroids have been previously detected in vines grown in southern Iran (Izaki-Aghl and Izadpanah, 2009). However, little information is available regarding their combination in mixed infections as well as their presence in other Iranian regions. During summer 2010, leaf samples were collected from 57 grapevine vines grown in north-west Iran and tested for viroid infections by RT-PCR using primers specific for each viroid species. Viroid identity was conclusively established by sequencing the amplified cDNAs. The large majority (96,2%) of the 53 positive samples had mixed infections, whereas single infections by HSVd or AGVd were detected in only two samples. HSVd, AGVd, GYSVd-1, and GYSVd-2 occurred in various combinations in 87.7%, 78.9%, 71.9% and 45.6% of the samples, respectively. HSVd was present in most double- and triple-infected vines, representing 15.8% and 38.5% of the samples respectively. Specifically, HSVd/GYSVd-1 and HSVd/AGVd were found in 7% of the samples, whereas the AGVd/GYSVd-2 combination was less frequent (1.8%). Vines contemporarily infected by HSVd, GYSVd-1 and AGVd were 29.8%, whereas the combinations HSVd/GYSVd-1/GYSVd-2 (3.5%) and HSVd/AGVd/GYSVd2 (5.3%) were less frequent. In contrast, double infections by AGVd/GYSVd-1 or GYSVd-1/GYSVd-2 and triple infections by AGVd/GYSVd-1/GYSVd.2 were not detected. Interestingly, 18 the 57 tested grapevines (31.6%) contained four different viroids species, so that *ca.* 70% of the tested grapevines were infected by three and four viroids. It was not possible to associate any of the viroid combinations with a specific symptomatology. Altogether, these data show that most grape-infecting viroids are widespread in north-west Iran. To our knowledge this is the first report of multiple infections by three and four viroids to grapevine in Iran.

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DISEASE NOTE

FIRST RECORD OF *APRICOT LATENT VIRUS* IN KOSOVO

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Apricot latent virus (ApLV), first detected in Moldova in symptomless apricot cv. Silistra introduced from Bulgaria (Nemchinov and Hadidi, 1998), was assigned as definitive species to the genus *Foveavirus* (Martelli and Jelkmann, 1998). During a small-scale survey in autumn 2009 to evaluate the sanitary status of stone fruit trees in Kosovo, dormant cuttings were randomly collected in old orchards from four localities (Ferizaj, Gjilan, Prishtina and Viti) from a total of 39 trees (28 European plum, 8 apricot and 3 peach). Buds from all samples were grafted onto healthy GF305 seedlings grown in a greenhouse at 22-24ºC. After about two months, ELISA was carried out on extracts from fully expanded leaves for the presence of *Plum pox virus* (PPV), *Apple mosaic virus* (ApMV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV) and *Apple chlorotic leaf spot virus* (ACLSV). Apricot and peach trees were also analysed moleculary for the presence of *Apricot latent virus* (ApLV), subjecting total nucleic acid extracts from leaves of both species to RT-PCR using the ApLV-specific primers HALV1: GGAATAGAGCCCCAAGAAG and CALV1: AGC AAGGTAAACGCCAAC (Nemchinov and Hadidi, 1998). ELISA results showed a high incidence of PPV (12 of 28 plums, one peach and three apricots). One plum was positive to PDV, one peach to PNRSV and one plum to ACLSV. ApMV was not detected in any of the 39 plants tested. RT-PCR showed that two peach and three apricot trees, all from the Gjilan area, were infected with ApLV. To the best of our knowledge, this represents the first record of ApLV in Kosovo.

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DISEASE NOTE

INCIDENCE OF VIRUSES AFFECTING "CUMARI" HOT PEPPER (*CAPSICUM BACCATUM var. PRAETERMISSUM*) IN CENTRAL BRAZIL

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"Cumari" [*Capsicum baccatum* L. var. *praetermissum* (Heiser et Smith) Hunziker] is a semi-domesticated hot pepper variety native to Brazil, growing in the southeastern and midwestern regions of the country. Cumari plants produce small yellow fruits and have characteristics that make them appreciated also as ornamental plants. In 2009-2010, virus-like symptoms were observed on field-grown Cumari plants in Brasilia (DF Brazil). A couple of months after transplanting, affected plants showed stunting, accompanied by yellow and green mottling, vein banding, deformation and reduced size of the leaves. Incidence of diseased plants was estimated to 80%. Leaves from symptomatic (35 samples) and symptomless (9 samples) plants were collected and tested for the presence of viruses using polyclonal antibodies to *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV), *Potato virus Y* (PVY), *Pepper yellow mosaic virus* (PepYMV), and *Pepper mild mottle virus* (PMMoV) by DAS-ELISA, and *Cucumber mosaic virus* (CMV) by dot-ELISA. Results showed that 98% (43 of 44) of the samples tested were virus-infected. PepYMV (66%; 29 samples) and TCSV (55%; 24 samples) were the prevailing viruses followed by PMMoV (34%), TSWV (23%), PVY (18%) and GRSV (7%). CMV was not detected. These data show that Cumari hot peppers are susceptible to infections by multiple viral species under field conditions. Although all the above viruses have been reported from *Capsicum* spp. (Pernezny *et al.,* 2003; Nagata *et al.*, 2002), this is the first record of TSWV, GRSV, TCSV, PVY, PepYMV and PMMoV in Cumari hot peppers in Brazil.

Pernezny K., Roberts P.D., Murphy J.F., Goldberg N.P., 2003. Compendium of Pepper Diseases. APS Press, St. Paul, MN, USA.

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