

DISEASE NOTE

FIRST REPORT OF *CANDIDATUS*
PHYTOPLASMA MALI IN TURKEYG. Sertkaya¹, M. Martini² and R. Osler²¹ *Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, 31034 Antakya, Turkey*² *Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy*

In Turkey, 17 randomly selected apple orchards were surveyed from August to September 2004 in Adana (6 orchards) and Mersin (3 orchards) provinces of eastern Mediterranean region and Nigde (8 orchards) province of central Anatolia for the presence of apple proliferation disease. Since symptoms resembling those induced by this disease were observed, shoot and leaf samples from 10 different cultivars were collected from 28 symptomatic and 3 symptomless trees for laboratory testing. Total genomic DNA extracted from leaf mid-veins as described by Malisano *et al.* (1996) was used for nested-PCR with universal primers P1/P7 in direct PCR, followed by primer pair R16F2n/R16R2. RFLP analysis with *Tru1I* gave the same pattern as AP15 and PD1 reference phytoplasma strains. Nested-PCR with 16SrX group-specific primer pair f01/r01 (Lorenz *et al.*, 1995) confirmed the 16Sr phytoplasma group affiliation. RFLP patterns with *BsaAI* and *SspI* of f01/r01 PCR products were the same as that of AP15 reference strain, providing evidence that *Candidatus Phytoplasma mali* of subgroup 16SrX-A (Lee *et al.*, 1998) occurred in infected Golden Delicious trees from Adana (1/3 positive sample) and Nigde (2/2) and in cvs Scarlet Spur (2/4) and Jonathan (1/3) from Adana. Thus, 6 of 28 apples showing typical proliferation symptoms such as witches'-broom and chlorotic leaves with enlarged stipules tested positive for *C. Phytoplasma mali*. Further surveys will investigate the presence of the same pathogen in other important apple-growing regions of Turkey and of its psyllid vectors. To our knowledge this is the first report of *C. Phytoplasma mali* in Turkey.

Lee I-M., Gundersen D.E., Davis R.E., Bartoszyk M., 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology* **48**: 1153-1169.

Lorenz K.H., Schneider B., Ahrens U., Seemüller E., 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and non ribosomal DNA. *Phytopathology* **85**: 771-776.

Malisano G., Firrao G., Locci R., 1996. 16S rDNA-derived oligonucleotide probes for the differential diagnosis of plum leptonecrosis and apple proliferation phytoplasmas. *Bulletin OEPP/EPPO Bullettin* **26**: 421-428.

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DISEASE NOTE

DETECTION OF A BEGOMOVIRUS
ASSOCIATED WITH LEAF CURL DISEASE
OF ORNAMENTAL CROTON (*CODIEUM*
VARIEGATUM) IN SOUTHERN INDIAY.S. Mahesh¹, K.S. Shankarappa¹, K.T. Rangaswamy¹,
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A leaf curl disease was observed on ornamental croton plants (*Codiaeum variegatum*, family *Euphorbiaceae*) in 15 home, office and botanical gardens in and around Bengaluru, South India. Diseased plants showed thickening of leaf veins, severe leaf curling, decrease in leaf size and stunting. The causal pathogen was transmitted to healthy croton plants by grafting with infected scions, and through whiteflies (*Bemisia tabaci*) given acquisition access and inoculation access periods of 24 h and 48 h, respectively. Association of a begomovirus with the disease was confirmed by amplification of viral DNA fragments of ca. 520 bp and 575 bp of the coat protein (CP) gene in begomoviral DNA A, with the aid of degenerate primers (Deng *et al.*, 1994), from total DNA extracts of infected but not of healthy croton plants. An example of a 575 bp fragment, representing the core region of the CP gene (Brown *et al.*, 2001) was cloned and sequenced. The resulting nucleotide sequence was most similar (94-96% identity) to the equivalent sequences of four begomoviruses from Asia [*Ageratum yellow vein virus* (AYVV, Pakistan), *Crotalaria juncea leaf curl virus* (CJLCV, Lucknow), *Tobacco curly shoot virus* (TbCSV, Yunnan 1) and *Tomato leaf curl New Delhi virus* (ToLCNDV)]. The extent of the relationship between the croton virus isolates from Bengaluru and that detected in *C. variegatum* in northern India (Raj *et al.*, 1996) is not yet clear.

Brown J.K., Idris A.M., Jerez I.T., Banks G.K., Wyatt S.D., 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. *Archives of Virology* **146**: 1581-1598.

Deng D., McGrath P.F., Robinson D.J., Harrison B.D., 1994. Detection and differentiation of whitefly transmitted geminiviruses in plants and vector insects by the polymerase reaction with degenerate primers. *Annals of Applied Biology* **125**: 327-336.

Raj S.K., Srivastava K.M., Singh B. E, 1996. Evidence from nucleic acid hybridization tests for geminivirus infection of ornamental crotons in India. *European Journal of Plant Pathology* **102**: 201-203.

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DISEASE NOTE

**COMPLETE GENOME SEQUENCE
OF AN INDIAN ISOLATE
OF SUGARCANE YELLOW LEAF VIRUS**

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Yellow leaf syndrome (YLS) has recently been recognized as new disease of sugarcane causing severe decreases in the sugar content of the sugarcane. Symptoms of the disease are yellowing leaves with a bright yellow midrib, often when the rest of the lamina is still green. In India, YLS has been linked to infection by *Sugarcane yellow leaf virus* (ScYLV; genus *Polevirus*, family *Luteoviridae*) on the basis of particle morphology and serological relationships (Rao *et al.*, 2000). In this study, we determined the complete nucleotide sequence of an Indian isolate of ScYLV by using reverse transcriptase-polymerase chain reaction (RT-PCR). Virions were purified from the leaves of sugarcane (cv. CoLk 8102) showing typical midrib yellowing using the enzyme-digestion method and RNA was extracted from them by phenol extraction, each as described by Moonan *et al.* (2000). For sequencing, the RNA was polyadenylated by using yeast polyA polymerase and oligo (dt)₃₀ primer was used in RT-PCR procedures as described by Moonan *et al.* (2000). The 5899 nucleotide sequence of ScYLV-India (AY 236971) was found to be identical to that of isolate CP65-357 from Australia (AJ491288) and 98% homologous to that of the Texas isolate (AF369928). The sequence differences resulted in 5 changes in amino acid sequence. Unrooted phylogenetic tree analysis revealed that ScYLV-India was most closely related to and in the same cluster as isolates CP92-1654, Florid 1999, LHo83-153, SP71-6163B, Q136 Argentina and CP65-357.

Rao G.P., Gaur R.K., Singh Maneesha, Srivastava A.K., Virk A.S., Singh N., Patil A.S., Viswanathan R., Jain R.K. 2000. Occurrence of Sugarcane yellow leaf virus in India. *Sugar Technology* 2:37-38.

Moonan F., Molina J., Mirkov I.E. 2000. Sugarcane yellow leaf virus: An emerging virus that has evolved by recombination between luteoviral and poleroviral ancestors. *Virology*, 269: 156-171.

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DISEASE NOTE

**FIRST REPORT OF *FUSICOCCUM
AESCULI* CAUSING DIEBACK ON SWEET
CHESTNUT (*CASTANEA SATIVA* Mill.)
IN THE BLACK SEA COAST OF TURKEY**

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During a survey for chestnut blight along the Black Sea coast of Turkey conducted in September 2006, cankers without fructifications were observed on sweet chestnut (*Castanea sativa* Mill.) trees in six provinces. The cankers differed from those of chestnut blight, which usually have reddish-yellow conidiomata of the causal agent, *Cryphonectria parasitica*. To determine the cause of the cankers, isolations were made from small pieces of inner-bark tissues on potato dextrose agar (PDA). Dark brown to almost black colonies were obtained that produced hyaline, thin-walled, aseptate, fusiform, straight and irregularly guttulate conidia with an obtuse apex and a distinctly truncate base, measuring 17.5-25×5-7.5 µm. The fungus was identified as *Fusicoccum aesculi* Dda apud Strum based on the description by Sutton (1980). Pathogenicity tests on four chestnut stems of 2-year-old saplings grown in the greenhouse were done by removing a bark disc 5 mm in diameter with a cork borer and placing mycelial plugs with conidia taken from a 10-day-old culture on PDA. Inocula were protected with Parafilm to prevent dehydration. Two months after inoculation, bark necrosis averaging 1.0×2.6 cm around the disc area was observed on chestnut shoots. The fungus was reisolated from the necrotic areas, while the control shoots inoculated with PDA plugs, remained healthy. This pathogen was isolated most frequently from Zonguldak, Ordu and Rize provinces. This is the first report of *F. aesculi* [teleomorph *Botryosphaeria dothidea* (Moug:Fr.) Ces. & De Not.)] causing shoot blight on sweet chestnut along the Black Sea coast of Turkey.

Sutton M., 1980. The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, Kew, Surrey, UK.

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DISEASE NOTE

A NEW CHINESE ISOLATE
OF WATERMELON MOSAIC VIRUSY.F. Wu, T. Wei, H.N. Yue, W. Hou, J.X. Zhang,
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In May 2005 and 2006, diseased watermelon plants (cv. Giromontiina) showing mosaic symptoms and collapse of the leaves were observed in northern China. The yield losses were 30% to 50% in most fields, and occasionally the disease resulted in a complete loss of the crop. Based on the symptoms, infection with *Watermelon mosaic virus* (WMV) was suspected. A week after inoculating healthy plants with extracts from diseased plants, mosaic symptoms were observed in the top leaves and, after a further two days, the diseased plants appeared crinkled and malformed. Young leaves were collected from 80 randomly selected hosts from 10 regions in Shaanxi province and tested for the presence of WMV by using RT-PCR of total RNA extracts with primers specific for the WMV coat protein gene (Desbiez and Lecoq, 2004). In all tests, this yielded the expected 843 bp DNA amplicon. Sequences of amplicons obtained for one isolate from each region were all the same (GenBankAccession No. DQ399708), which suggests that the disease in Shaanxi province had spread from a single source. Comparison tests showed that the Shaanxi isolates were 93.0% to 95.0% identical in coat protein gene sequence to isolates from other countries (WMV-France, AY437609; WMV-USA, D13913; WMV-Japan, AB001994; WMV-Pakistan, AB127934; WMV-Australia, D00535; WMV-Israel, AF322376; WMV-Tonga, L22907; WMV-Spain AJ579524). The Shaanxi isolate coat protein gene differed from that of WMV Heilongjiang (AY464948) in 10 nucleotide positions and thus represents a distinct Chinese isolate.

Desbiez C, Lecoq H. 2004. The nucleotide sequence of Watermelon mosaic virus (WMV, *Potyvirus*) reveals inter specific recombination between two related potyviruses in the 5' part of the genome *Archives of Virology* **149**:1619-1632.

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DISEASE NOTE

FIRST REPORT OF SIMULTANEOUS
PRESENCE OF TOMATO YELLOW LEAF
CURL SARDINIA VIRUS AND TOMATO
YELLOW LEAF CURL ISRAEL VIRUS
INFECTING CROPS AND WEEDS
IN TUNISIAF. Pellegrin¹, M. Mnari-Hattab², A. Tahiri³,
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Tomato yellow leaf curl virus disease (TYLC) was first reported in Tunisia more than 20 years ago. The presence of *Tomato yellow leaf curl Sardinia virus* (TYLCSV) was detected in Tunisian crops (Fekih-Hassen *et al.*, 2003) and, more recently, *Tomato yellow leaf curl Israel virus* (TYLC-Is) was reported in tomato, pepper and bean (Gharsallah-Chouchane *et al.*, 2006). Although both viruses were present in Tunisia, their simultaneous co-existence in the same host plant was not described. A large sampling survey was done and greenhouse tomato crops with severe symptoms of TYLC were selected. Leaves were collected from tomato as well as from nearby weeds and other crops. DNA was extracted and purified by using a FastDNA Spin Kit (Q-Biogen, MP Biomedicals, Quebec, Canada). DNA was amplified by using Multiplex PCR (Qiagen, Courtaboeuf, France) with primers TY209-forward (5'-CTYGCAATWAAATATTTGCAGCTA-3'), TY575-reverse (5'-CAACACCRGTATGCTTSACG-3'), TY613-forward (5'-GAATTACTCACAGAGTSGGTAAGA-3') and TY1363-reverse (5'-GAACCACGACATCATTTCCA-3'). Amplicons derived from TYLCSV DNA were 366 bp and those derived from TYLCV-Is DNA were 750 bp. Of the 41 samples collected, 15 contained TYCV-Is or TYLCSV and 8 contained both TYLCV-Is and TYLCSV (3 tomato, 2 *Malva parviflora*, 1 *Conyza bonariensis*, 1 *Cucurbita pepo*, 1 *Vicia faba*). When assaying samples from plants thought to be infected by both viruses, no amplicons were obtained in tests made using only primers TY209-forward and TY1363-reverse, which suggests that in this region of the genomes no recombination had occurred.

Fekih-Hassen I, Gorsane F, Djilani F, Fakhafakh H, Makha M, Maxwell DP, Marrakchi M, 2003. Detection of Tomato yellow leaf curl Sardinia virus in Tunisia. *Bulletin OEPP/EPPO Bulletin* **33**: 347-350.

Gharsallah-Chouchane S, Gorsane F, Nahkla MK, Maxwell DP, Marrakchi M, Fakhafakh H, 2006. First report of Tomato yellow leaf curl virus-Israel species infecting tomato, pepper and bean in Tunisia. *Journal of Phytopathology* **155**: 236-240.

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DISEASE NOTE

A NEW CHINESE ISOLATE OF APPLE STEM GROOVING VIRUS FROM APPLES

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China is one of the largest apple producers in the world and Shaanxi province in northwestern China produced 27% of the Chinese crop in 2005. Low yields and decline of the trees are widespread problems in this area. In a survey from May to July 2006 in Yangling, Shaanxi province, we found that many apple trees of cultivar Red Fuji had long grooves on the woody stem and obvious symptoms of decline suggesting infection with *Apple stem grooving virus* (ASGV). Bark samples were collected from six symptomless and 30 symptomatic apple plants in 10 different regions in Yangling and tested for the presence of ASGV by reverse transcription RT-PCR assay of total RNA extracts (Vera *et al.*, 1998). Primers specific for the ASGV coat protein gene were designed based on comparisons among sequences available in Genbank (accession Nos. D14995, AF465354, AY886760, AF438409, AB004083, AF522459). Primer sequences were 5'-ATG AGT TTG GAA GAC GTG C-3' (ASGV-cp1) and 5'-CTA ACC CTC CAG TTC CAG A-3' (ASGV-cp2). The tests yielded a 714 bp fragment only with samples from symptomatic apple plants. Sequences of amplicons obtained for one isolate from each region were all the same (GenBank accession No. EU236258) and were identical at 657, 656 and 653 nucleotide positions, respectively, to the sequences of isolates from Brazil (AF438409), Japan (AB004063) and a Chinese pear (AY886760). To our knowledge, this is the first report characterising a Chinese isolate of ASGV from apples.

Vera L., Marinho A., Kummert J., Rufflard G., Colinet D., Lepoivre P., 1998. Detection of Apple stem grooving virus in dormant apple trees with crude extracts as templates for one-step RT-PCR. *Plant Disease* **82**: 785-790.

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DISEASE NOTE

FIRST REPORT OF *TOBACCO MOSAIC VIRUS* ON SUGAR BEET IN CHINA

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Sugar beet is a known natural host of *Tobacco mosaic virus* (TMV) (Krstic *et al.*, 1997). In 2006, samples were collected from symptomless sugar beets in a field in the Inner Mongolia province of China. Viral RNA was extracted from virus partially purified from beet roots and tested by RT-PCR using a specific primer set [TMV-480F(+): 5'-CAT GAA GGC CAG AAA GAC AG-3' and TMV-1300R(-): 5'-GCC TGG TAT GTT CGA ATG TG-3']. The primer was designed to amplify a 821 bp fragment from the TMV RdRp gene (GenBank accession No. AJ011933). Eight of the eighteen sugar beet samples tested gave the amplicon of the expected size. An isolate recovered from *Chenopodium quinoa* leaves inoculated with the partially purified TMV preparation was positively recognized by immunosorbent electron microscopy and western blotting using a TMV-specific antiserum. In addition, sequence analysis of the cloned PCR amplicon showed that the virus isolated from Chinese sugar beets had an average identity of 92.3% (range 63.0-99.7%) at the nucleotide level and 95% (range 61.6-99.2%) at the amino acid level with other available TMV sequences. In particular, our TMV isolate had the highest nucleotide identity (99.7%) and amino acid identity (99.2%) with another Chinese TMV isolate (B935A) from *Vicia faba* (Zhou *et al.*, 2000). In the course of the survey it was ascertained that TMV consistently coexisted in the same field and often in the same plant with *Beet necrotic yellow vein virus* (BNYVV) and *Beet soil-borne virus* (BSBV). To our knowledge, this is the first report of natural occurrence of TMV in sugar beet in China.

Krstic B., Stojanovic G., Vico I., Barac M., Malinaric B., 1997. Comparative investigations of some Tobacco mosaic virus isolates from different host plants. *Acta Horticulturae* **462**: 483-490.

Zhou X.P., Xue C.Y., Chen Q., Qi Y. J., Li D.B., 2000. Complete nucleotide sequence and genome organization of tobacco mosaic virus isolated from *Vicia faba*. *Science in China (Series C)* **43**: 200-208.

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DISEASE NOTE

FIRST REPORT OF *LILY MOTTLE VIRUS* INFECTING *NARCISSUS PSEUDONARCISSUS* IN CHINA

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Lily mottle virus (LMoV) (family *Potyviridae*) has been reported in lily crops in the USA, Asia, Europe, Australia (Asjes, 1972) and Taiwan as well as Fujian and Zhejiang provinces of China. Plants are frequently stunted and flowers may be malformed with color break, brown rings and necrotic spots on the bulb scales (Lawson *et al.*, 1996). In August 2007, total RNA was extracted from bulb tissue of symptomatic plants of two varieties of *Narcissus pseudonarcissus* and tested for LMoV by using a reverse transcription-polymerase chain reaction (RT-PCR) with primers L1 (5'-TGGGCACCTTGTGAATTACA-3') and L2 (5'-ACACGGAGAGGCATACAGCA-3'). Primer sequences were based on published sequences of LMoV coat protein gene (GenBank accession Nos. AJ748256; AJ748257). The predicted 553 bp amplicon was obtained from a sample of cv. Pink-charm that had brown spots on the bulbs and grew poorly in tissue culture. The nucleotide sequence of the amplicon (GenBank accession No. EU167936) showed more than 98% identity with sequences of other isolates of LMoV (AJ748256, AJ564636, AB053256, AF531458, AJ564637, AJ748257) and encoded an amino acid sequence (ABW16938) identical to that in the database for LMoV (NP-945145). This is the first report of infection of *Narcissus pseudonarcissus* by LMoV.

Asjes C.J., de Vos N.P., Slogteren D.H.M., 1972. Brown ring formation and streak mottle, two distinct symptoms in lilies associated with complex infection of lily symptomless virus and tulip breaking virus. *Netherlands Journal of Plant Pathology* **79**: 23-35.

Lawson R.H., Hsu H.T., 1996. Lily diseases and their control. *Acta Horticulturae* **414**: 175-185.

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DISEASE NOTE

NEW HOSTS FOR *SCLEROTINIA* STEM ROT OF CANOLA

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Sclerotinia sclerotiorum is an important plant pathogen that causes stem rot of different plants (Anonymous, 2005). During spring 2006 and 2007, in an epidemiological study of sclerotinia stem rot of canola (*Brassica napus*) in the Golestan province (northern Iran), symptoms of leaf, pod and stem rotting were observed on the following weeds growing in canola fields: sweet sagewort (*Artemisia annua*), wild oat (*Avena sterilis* ssp. *ludoviciana*), shepherd's purse (*Capsella bursa-pastoris*), peacock poppy (*Papaver pavoninum*), annual bluegrass (*Poa annua*), clustered dock (*Rumex conglomeratus*), blessed milkthistle (*Silybium marianum*), charlock mustard (*Sinapis arvensis*), stinging nettle (*Urtica dioica*) and volunteer wheat (*Triticum aestivum*). From all symptomatic plants a fungus identified as *S. sclerotiorum* was consistently isolated on potato dextrose agar (PDA). Pathogenicity of representative isolates was assessed by placing 8-mm disks taken from the margins of an actively growing colony on the stems and leaves of potted host plants which were then placed in a greenhouse at 25°C and more than 90% relative humidity for 3-7 days (Hollowell *et al.*, 2003). Symptoms like those observed in the field developed on all inoculated plants and the fungus was reisolated from rotten tissues. According to literature records (Anonymous, 2007), all ten species are new hosts of *S. sclerotiorum* for Iran and six of them (*A. annua*, *A. sterilis ludoviciana*, *P. pavoninum*, *P. annua*, *R. conglomeratus* and *U. dioica*) are new in the world.

Anonymous, 2005. *Sclerotinia sclerotiorum*. CAB International Crop Protection Compendium. Kew, Surrey, UK.

Anonymous, 2007. *Sclerotinia sclerotiorum*. Fungus- host distribution. USDA, USA. Online: <http://nt.ars-grin.gov/fungal-databases/fungushost/fungushost.cfm>.

Hollowell J.E., Shew B.B., Cubeta M.A. and Wilcut J.W., 2003. Weed species as hosts of *Sclerotinia minor* in peanut fields. *Plant Disease* **87**: 197-199.

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DISEASE NOTE

OCCURRENCE OF CHARCOAL ROT
ON KIWI FRUIT IN IRAN

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In July 2006 wilted kiwi fruit trees (*Actinidia chinensis* Planchon) showed severe root rotting and dry rotting of the trunks that expanded up to 30 cm above the crown in the Kordkuy area of the Golestan province (northern Iran). Numerous microsclerotia were present on rotted roots and, sometimes, the surface of rotted stems was covered with small pycnidia. Microsclerotia were black, irregular and very small (74.02×75 µm), whereas pycnidia were larger (231.6×299.2 µm), black and semi-globous. Pycnidiospores were extruded from the pycnidial pore as orange cirrhi. Conidia were hyaline, single-celled, elliptical to oval in shape and measured 5.5×13.8 µm. Culturing microsclerotia and pycnidia on potato dextrose agar (PDA) yielded colonies of a fungus identified as *Macrophomina phaseolina* (Tassi) Goid. based on morphological characteristics (Singleton *et al.*, 1993). Fungal colonies were nearly colorless at first, turning to light white during growth. Abundant microsclerotia were produced within 2-3 days of culture, which changed the colony color to black. Hyphae were mostly branched at right angle and had obvious constrictions. The toothpick method (Singleton *et al.*, 1993) was used for pathogenicity tests, following which symptoms developed which consisted of chlorosis, necrosis and shedding of the leaves, along with rotting of the stem at the inoculation points. An extensive field survey showed that most of the recently established orchards in the Kordkuy area were infected, the same as another stand in the Noshahr area of Mazandaran province. This is the first report of *M. phaseolina*-induced charcoal rot disease of kiwi fruit in Iran.

Singleton L.L., Mihail J.D., Rush C.M., 1993. Methods for Research on Soilborne Phytopathogenic Fungi. APS Press, St. Paul, MN, USA.

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DISEASE NOTE

FIRST REPORT OF STEM NECROSIS
OF OKRA BY *CORYNESPORA CASSIICOLA*
IN BANGLADESH

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In spring 2003-2005, plants with stem necrosis were observed in okra [*Abelmoschus esculentus* (L.) Moench.] fields at Savar (Dhaka, Bangladesh). Average temperature in the surveyed region ranged between 25-28°C and the average yearly rainfall was 2430-3980 mm. Symptoms on the stem first appeared as very minute, dark, water-soaked, circular spots that later coalesced forming a large necrotic sub-circular to irregular area with dark grey to black coloured lesion. A fungus isolated from the transitional zone between healthy and diseased tissue on potato dextrose agar (PDA), was identified as *Corynespora cassiicola* (Berk. et M.A. Curtis) C.T. Wei on the basis of microscopic characters and cultural behaviour (Ellis, 1971; Subramanian, 1971; Kwon *et al.*, 2003). This fungus is a well known seed-borne pathogen of okra plants. Stems of okra seedlings, grown under greenhouse condition, were surface sterilized with 0.5% NaOCl then washed with sterilized distilled water. Pathogenicity tests were performed by pricking three successive internodes, up to a depth of 0.5 mm, of each of 25 test plants, on which a mycelium plug (2.0 mm in diameter) cut from the edge of a 7-day-old monosporic fungal cultures was placed. In control plants a sterile PDA plug was placed on each wound. Symptoms similar to those shown by naturally infected plants developed on the wounded stems 6 days after inoculation. The fungus was successfully reisolated from inoculated plants. This is the first report of *C. cassiicola* causing stem necrosis on okra in Bangladesh.

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DISEASE NOTE

FIRST REPORT OF *RAMULARIA COLLO-CYGNI* IN SLOVAKIA

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In 1987, the fungus *Ophiocladium hordei* Cav. was identified in Austria as the causal agent of a new barley disease (Huss *et al.*, 1987). Sutton and Waller (1988) isolated the same fungus from triticale (*Triticum x Secale*) and other graminaceous plants, revised its taxonomic position, and renamed it *Ramularia collo-cygni*. More recently this fungus has been identified in a number of countries where is gaining importance (Frei *et al.*, 2007). During summer 2006 and 2007 ringspots of unknown nature were observed on the leaves of spring barley growing in central Slovakia (Viglas-Pstrusa area). Twenty symptomatic leaf samples were tested by polymerase chain reaction (PCR) for the presence of *R. collo-cygni*. Total genomic DNA from naturally infected barley leaves, was extracted using Dneasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according the manufacturer's protocol. Specific detection of the pathogen was done using specific primers and the amplification conditions described by Frei *et al.* (2007). Purified fungal DNA from a culture provided by Dr. P. Frei (Agroscope, RAC Changing, Nyon, Switzerland) was used as positive control. From fourteen samples of diseased barley leaves and the control, the expected product of 348 bp was amplified by PCR, confirming the presence of *R. collo-cygni* in symptomatic plants. To our knowledge, this is the first report of this pathogen in Slovakia.

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DISEASE NOTE

FIRST REPORT IN ITALY OF A TOMATO RESISTANCE-BREAKING STRAIN OF *TOMATO SPOTTED WILT VIRUS* FROM CHRYSANTHEMUMG. Bubici¹, M.M. Finetti-Sialer², T. Mascia², M. Amenduni¹, M. Cirulli¹ and D. Gallitelli²

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In 2007, samples were received from near Bari (Apulia, Southern Italy) of greenhouse-grown chrysanthemums (*Den-dranthema grandiflora* cvs. Boris Becker, Delianne, Eleonora, Euro, Il Mondo, Paso Doble Pink, Reagan, Taormina, Zembla and Snowdon) all of which showed distinct chlorotic spots on the top leaves, followed by wilting and necrosis of the leaves and stems. In cvs Delianne and Snowdon, petioles and flowerheads were also necrotic. *Tomato spotted wilt virus* (TSWV) but not *Impatiens necrotic spot virus* was detected in all samples tested by dot blot hybridization with digoxigenin-labelled riboprobes (Finetti-Sialer and Gallitelli, 2000). No amplicons were obtained in RT-PCR tests for the presence of *Chrysanthemum stem necrosis virus* using primers corresponding to positions 69-90 and 827-849 of RNA-M (accession No. AF213675). Three isolates obtained from cv. Snowdon and the wild-type strain p105 of TSWV were mechanically inoculated onto 4 plants (at 2-3 true leaf stage) of the F₁ hybrid tomato cv. Diaz, carrying the Sw5 resistance gene to TSWV, and the susceptible cv. UC82. The three Snowdon isolates, but not strain p105, overcame the resistance and induced systemic necrosis. UC82 plants became systemically infected by all 4 isolates. The results show that the Snowdon isolates are resistance-breaking. Moreover, a 670 bp DNA copy of part of the NS_M gene of each Snowdon isolate yielded a MaeI restriction pattern like that of resistance-breaking strains of TSWV A-type (Finetti-Sialer *et al.*, 2002; Ciuffo *et al.*, 2005). This is the first report of RB strains of TSWV in chrysanthemum in Italy.

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