

DISEASE NOTE

FIRST RECORD OF THE *APPLE STEM PITTING VIRUS* (ASPV) IN QUINCE IN GREECE**M.M. Mathioudakis¹, V.I. Maliogka¹, C.I. Dovas²,
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In autumn 2004, quince trees showing symptoms similar to those of Quince Fruit Deformation Disease (QFDD) (Nemeth, 1986) were observed in a quince orchard in northwestern Greece (Kozani area). Leaf samples and twig phloem from 18 symptomatic trees were tested for the presence of *Apple stem pitting virus* (ASPV), a member of the *Foveavirus* genus. Specific detection of ASPV was performed by reverse transcription, using an oligo d(T) 18-mer, followed by PCR using a pair of degenerate primers (forward "SPcp1": 5'-AGY-GAGCCAGTSATHHTCTCA-3' and reverse "SPcp2": 5'-AGTTTGCAGCATGAGGTTCCA-3') designed from conserved ASPV coat protein genomic regions. A 789-bp specific product was obtained in assays of all the symptomatic quince trees tested. Direct sequencing of one PCR product confirmed the specific detection of ASPV by revealing 85% amino acid sequence identity with the corresponding region of a pear isolate of ASPV (EMBL, AF491929). The obtained sequence was deposited in EMBL (AM167517). Further examination of samples from 30 asymptomatic apple and 39 asymptomatic pear trees collected from 6 fields in Macedonia region revealed a high incidence of ASPV (96% and 43%, respectively) which for apple, is much higher than that reported previously (36%) as determined by graft inoculation onto indicator plants (Syrghanidis, 1988). This is the first report of ASPV in quince in Greece.

Nemeth M., 1986. Quince deformation. In: Virus, mycoplasma and rickettsia diseases of fruit trees, pp. 253-255. Academiai Kiado, Budapest, Hungary.

Syrghanidis G.D., 1988. Problems of virus diseases of delicious fruit trees in Greece. *Acta Horticulturae* **235**: 21-25.

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DISEASE NOTE

FIRST REPORT OF *PUCCINIA MALVACEARUM* ON *ALTHEA ROSEA* IN TURKEY**H. Kavak***Department of Plant Protection, Faculty of Agriculture, Harran University, Sanliurfa 63040, Turkey*

Althea rosea is widely grown in Turkey as an ornamental in private and public gardens. During surveys carried out in early summer 2005, a rust disease of *A. rosea* was observed in the Sanliurfa province of the country (south-eastern Anatolia) where this plant is common. Disease severity was highest in June before blooming. The lower surface of the leaves of many plants was covered with dark-coloured telia, 1-3 mm in diameter, whereas tissues of the upper leaf blade above the sori were necrotic. Uredinia were not detected, notwithstanding accurate microscopical observations.

Whereas immature teliospores were single-celled, mature teliospores had two cells separated by a clear-cut septum, were asymmetrical, and brown or dark brown in colour. Upper teliospore cells were 19-28 µm long, generally obpyriform, and showed a thickened cell wall at the apex. Lower cells were 22-33 µm long, generally obovate, and ended with a hyaline pedicel 5-12 × 17-22 µm in size. Total length of teliospores ranged between 37 and 61 µm. Based on the above morphometric characters, the agent associated with the rust disease of *A. rosea* was identified as *Puccinia malvacearum* Mont. (Berks, 2001). This appears to be the first record of this fungus in cultivated hollyhock in Turkey.

Berks V.C., 2001. *Puccinia malvacearum* Mont. [<http://www.bioimages.org.uk>].

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**CUCURBITA MOSCHATA, NEW
PHYTOPLASMA HOST
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In certain Brazilian areas, pumpkin (*Cucurbita moschata*) is grown next to chayote (*Sechium edule*). In pumpkin crops at Mendanha (State of Rio de Janeiro) plants with chlorosis of the shoots and leaves, reduced leaf size, and fruit malformation were observed and assayed for the presence of phytoplasmas, using nested-PCR as previously described (Montano *et al.*, 2000). Six diseased plants contained phytoplasmal 16S rDNA, which was amplified by PCR primed by P1/P7 and re-amplified by nested-PCR primed by F2n/R2. This putative phytoplasma was identified by RFLP analysis of F2n/R2 16S rDNA amplicons and classified according to Lee *et al.* (1998). Phytoplasmas from all symptomatic plants could not be distinguished from one another based on *AluI*, *DdeI*, *EcoRI*, *HaeIII*, *HbaI*, *HpaI*, *HpaII*, *MseI*, *RsaI*, *Sau3AI*, and *TbaI* RFLP patterns of 16S rDNA. Furthermore, the collective RFLP patterns were indistinguishable from those reported previously for the chayote witches' broom phytoplasma (ChWBIII) (Montano *et al.*, 2000). Therefore, the pumpkin phytoplasma was assigned to group 16SrIII, subgroup J, and the disease named "pumpkin yellows". To our knowledge, this is the first report of phytoplasma infections of *C. moschata* in Brazil.

Lee I.-M., Gundersen-Rindal D.E., Davis R.E., Bartoszyk I.M., 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology* **48**: 1153-1169.

Montano H.G., Davis R.E., Dally E.L., Pimentel J.P., Brioso P.S.T., 2000. Identification and phylogenetic analysis of a new phytoplasma from diseased chayote in Brazil. *Plant Disease* **84**: 429-436.

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DISEASE NOTE

**FIRST REPORT OF GRAPEVINE
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AND HERZEGOVINA**D. Delić¹, M. Martini², P. Ermacora², L. Carraro²
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During autumn 2004 and summer 2005, vineyards located in the major grapevine growing areas (Mostar, Trebinje and Banjaluka) of Bosnia and Herzegovina (BiH) were surveyed for the presence of grapevine yellows. Symptoms of yellowing or reddening of leaf veins and blades and lack of lignification of the canes were observed in vineyards located in southern BiH (Mostar and Trebinje), while in the northwest (Banjaluka) typical symptoms were not encountered. Samples for laboratory tests were collected from 13 symptomatic and 20 symptomless grapevines from 12 different vineyards. Some *Daucus carota* plants showing symptoms of phytoplasma infections were collected at random during the surveys. After nucleic acid extraction, nested PCR was carried out using phytoplasma universal primers P1/P7 followed by R16F2n/R2. The R16F2n/R2 amplicons, when digested with *MseI*, showed the restriction profile typical of the 16SrXII group (Stolbur), subgroup A. The results were confirmed with a second nested PCR using 16SrXII phytoplasma group specific primer pair fStol/rStol (Maixner *et al.*, 1995). These tests showed the presence of the Stolbur phytoplasma in 10 of 13 symptomatic grapevines and in symptomatic carrot plants. This is the first report of Bois noir (Stolbur) of grapevine in BiH. The presence of this disease in the main grapevine growing areas of the country could affect the grapevine industry and the choice of grapevine cultivars. Further work will be carried out to identify natural vectors of Stolbur phytoplasma and monitor vineyards for potential outbreaks of Flavescence dorée.

Maixner M., Ahrens U., Seemüller E., 1995. Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. *European Journal of Plant Pathology* **101**: 241-250.

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DISEASE NOTE

**FIRST REPORT OF AMERICAN PLUM
LINE PATTERN VIRUS IN LEBANON**

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During a survey of the sanitary status of cherry trees, in spring 2005, leaf symptoms that ranged from mild to bright line patterns were found in several trees. The orchard was located in central Bekaa and the cherry trees were the native cv Feraouni. Fifty-four samples were collected from either symptomatic (26) or symptomless (28) trees. Collected leaf samples were tested by DAS-ELISA for the presence of *American plum line pattern virus* (APLPV) (Agdia, USA), using the manufacturer's protocol. APLPV was identified serologically in 26 samples (48% of the total). The association between virus detection and the presence of symptoms was strong: 22 out of 26 (85%) of symptomatic trees were found to be infected by APLPV. However, additional tests of some cherry trees for *Prune dwarf virus* (PDV) have indicated that this virus is also present. This finding may explain the presence of mild pattern symptoms in trees that tested negative for APLPV. RT-PCR tests (Sanchez-Navarro *et al.*, 2005) of symptomatic trees have confirmed the presence of the virus in cherry. This is the first report of APLPV in Lebanon, and this finding represents a serious threat to the cherry fruit tree industry. APLPV appears to be established in the Mediterranean region as it has also been recorded from Albania, Italy, Tunisia (Myrta *et al.*, 2002) and Palestine (Alayasa *et al.*, 2002).

Alayasa N., Al Rwahnih M., Myrta A., Herranz M.C., Minafra A., Boscia D., Pallás V., 2002. Identification and characterization of an American plum line pattern virus (APLPV) isolate from Palestine. *Journal of Plant Pathology* **85**: 3-7.

Myrta A., Abbadi H., Herranz M.C., Al Rwahnih M., Di Terlizzi B., Minafra A., Pallás V., 2002. First report of *American plum line pattern virus* (APLPV) in Albania, Italy and Tunisia. *Journal of Plant Pathology* **84**: 188 (abstract).

Sánchez-Navarro J.A., Aparicio F., Herranz M.C., Minafra A., Myrta A., Pallás V., 2005. Simultaneous detection and identification of eight stone fruit trees viruses by one-step RT-PCR. *European Journal of Plant Pathology* **111**: 77-84.

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**FIRST RECORD OF POWDERY
SCAB CAUSED BY SPONGOSPORA
SUBTERRANEA SUBSP. SUBTERRANEA
ON POTATO IN MALTA**

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Potato (*Solanum tuberosum* L.) is a major crop in Malta. *Spongospora subterranea* (Wallr.) Lagerh. f.sp. *subterranea* Tomlinson (Sss), agent of powdery scab, affects the quality of the crop and can transmit *Potato mop-top virus*. In April 2005, powdery scab was observed at harvest on tubers of potato cv Derby in a field at Qrendi. The symptoms ranged from mild (typical) to extremely severe, mimicking wart disease. Histological observations disclosed the presence of Sss cystosori and excluded that of the wart organism. The identification of the fungus was confirmed by the one-step assay based on lateral-flow immunochromatography, using monoclonal antibodies specific for *S. subterranea* (AgriStrip kit, BIOREBA AG, Reinach, Switzerland). Pathogenicity was tested on tomato seedlings (Merz, 1989). To this aim, tomato F1 Tornádo (SEMO, Smiržice, Czech Republic) plants were grown from seed on river sand watered with nutrient solution. Three weeks after sowing, the roots were trimmed to 40 mm, and the plants were transferred to nutrient solution. After one week, the roots of eight seedlings were exposed for three days to a water suspension of cystosori ($3 \times 10^3 \text{ ml}^{-1}$) scraped from tuber scab lesions and maintained for three days at 15°C in the dark. Eight control seedlings were treated in the same way with sterile nutrient solution. The plants were transferred to glass tubes containing nutrient solution kept at 15±2°C and 15h photoperiod (daylight plus fluorescent white-light) for seven days and their roots were then stained and observed under the microscope. Zoosporangia of *S. subterranea* were detected in root hairs and epidermal cells of all inoculated seedlings, whereas the roots of the controls were zoosporangia-free. This is the first record of Sss from the Maltese Archipelago. Temperatures cooler than normal that occurred in autumn and winter 2004-2005 may have favoured the powdery scab outbreak.

Merz U., 1989. Infectivity, inoculum density and germination of *Spongospora subterranea* resting spores: a solution-culture test system. *Bulletin OEPP/EPPO Bulletin* **19**: 585-592.

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DISEASE NOTE

FIRST RECORD OF *COLLETOTRICHUM ACUTATUM* ON STRAWBERRY IN MALTA

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Strawberry (*Fragaria* × *ananassa* Duch.), an expanding crop in Malta, is susceptible to black spot (anthracnose) caused by *Colletotrichum acutatum* J.H. Simmonds, which has become one of the major fungal diseases of this species worldwide (Freeman *et al.*, 2001). In December 2005 a severe black spot attack was observed on strawberry 'fruits' (receptacles) grown under plastic tunnels at St Paul's Bay, Malta. *C. acutatum* was constantly isolated from fruit lesions. A conidial suspension (3.5×10^5 conidia ml⁻¹) was prepared by pouring sterile water on a 6-day-old fungal colony grown on PDA, and gently rubbing the colony surface with a glass rod. Ripening strawberry fruits of cv Ventana were washed under running water, treated for 2 min in a water solution of sodium hypochlorite (2% active Cl), then rinsed in sterile distilled water. Six fruits were inoculated by depositing on their surface two separate drops (0.05 ml) of the conidial suspension and six fruits (controls) were treated in the same way with sterile water previously poured on a sterile PDA plate. The fruits were kept under high moisture conditions, covered with plastic bags in an air-conditioned room (20±2°C). Typical anthracnose symptoms appeared on the inoculated receptacles within 7-10 days. *C. acutatum* was re-isolated from all the inoculated fruits. No symptoms developed on the controls and attempts to isolate the pathogen failed. This is the first record of *C. acutatum* on strawberry in Malta. The epidemiology of *C. acutatum* on strawberry and other plant species, on which it could show either pathogenic or non-pathogenic lifestyles (Peres *et al.*, 2005), should be investigated in the Maltese environment.

Freeman S., Horowitz S., Sharon A., 2001. Pathogenic and nonpathogenic lifestyles in *Colletotrichum acutatum* from strawberry and other plants. *Phytopathology* **91**: 986-992.

Peres N.A., Timmer L.W., Adaskaveg J.E., Correll J.C., 2005. Lifestyles of *Colletotrichum acutatum*. *Plant Disease* **89**: 784-796.

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DISEASE NOTE

FIRST RECORD OF BACTERIAL SHEATH ROT OF WHEAT IN CHINA

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Wheat plants (cv Yangmai 158) with symptoms of sheath rot were observed in April 2003 in Xiaoshan, Zhejiang Province, China. Oblong to irregular blackish brown lesions, 6-15 cm in length and bordered by a 10-15 mm purple-black water-soaked area occurred on the leaf sheath and most obviously on flag leaf sheath. Severe infection resulted in poor spike emergence and sterility. Six bacterial isolates from infected wheat samples showed characteristics similar to those of the standard reference strains (LMG 2192, 5097) of *P. fuscovaginae* in phenotypic, pathogenicity and Biolog (V4.1) tests and gas chromatographic analysis of fatty acid methyl esters (FAMES). The isolates were all rod-shaped, Gram-negative with 1-4 polar flagella and aerobic, and produced a green-fluorescent diffusible pigment on King's Medium B. Colonies on nutrient agar were white to cream-white and translucent. Thirty-two phenotypic characteristics of the isolates showed that they were similar to those of the reference strains. The six isolates were identified as *P. fuscovaginae* with a similarity of 0.718-0.893 in the Biolog system and a FAMES similarity of 0.661-0.812. A pathogenicity test on wheat plants at the five-leaf stage (cv Yangmai 158) confirmed that these isolates produced symptoms similar to those shown by rice plants. The bacterium was re-isolated from symptomatic wheat tissues. *P. fuscovaginae* has been reported as the cause of sheath brown rot of rice three years ago in China (Xie, 2003). The characteristics of the strains pathogenic to wheat were similar to those isolated from rice, but they were different from non-pathogenic fluorescent pseudomonads of wheat and rice. To our knowledge this is the first report of sheath rot on wheat, caused by *P. fuscovaginae*, in China. The disease was previously described from Brazil (Malavolta *et al.*, 1988). This study was supported by the National Science Foundation of China (30370591).

Xie G.L., 2003. First report of sheath brown rot of rice in China and characterization of the causal organism by phenotypic tests and Biolog. *International Rice Research Notes* **28**: 5052.

Malavolta Jr. V.A., Rodrigues Neto J., Robbs C.F., Barros B.C., Cardelli M.A., 1988. Ocorrência de *Pseudomonas fuscovaginae* em trigo (*Triticum aestivum* L.) no Brasil. *Summa Phytopathologica* **14**: 219-224.

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DISEASE NOTE

FIRST RECORD OF *COLLETOTRICHUM*
sp. IN ADLAY IN CHINA

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Adlay (*Coix lacryma-jobi* var. *ma-yuen* Stapf) is grown extensively in South and East Asia, including China, where a severe disease was observed in 2005 in the Zhejiang province. Leaves and stems showed small water-soaked brownish spots which rapidly turned into longitudinal elliptic, or spindle-shaped lesions 6-8 × 2-4 cm in size, with a brown outer edge and a gray sunken central area. Coalescence of large lesions gave rise to extensive rotting and necrosis that, when stems were encircled, caused them to break, thus inducing significant yield losses. Acervuli with brown setae and falcate single-celled spores, typical of some *Colletotrichum* species (Sutton, 1992) formed on the lesions at a late stage. A fungus isolated in PDA cultures from symptomatic tissues had mycelium growing tightly close to the surface of the plate, and produced brown pigments that turned the medium brown. Fungal colonies produced acervuli containing single-celled spores like those observed in naturally infected samples. Suspensions of spores collected from cultures, were used at a concentration of 10⁶ ml⁻¹ to spray-inoculate adlay seedlings that were kept for 24 h under a polyethylene sheet cover, and grown at a temperature of 22-26°C in a greenhouse. Symptoms similar to those showed by diseased field plants developed in the inoculated seedlings, from which a fungus with the same characteristics of the isolate obtained from the original field samples was recovered. The fulfillment of the Koch's postulates allows the conclusion that the disease observed in field-grown adlay plants is caused by a *Colletotrichum* species whose identification is underway. To the best of our knowledge, this is the first report of a *Colletotrichum*-induced anthracnose in adlay in China.

Sutton B.C., 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. In: Bailey J.A., Jeger M.J. (eds.). *Colletotrichum: Biology, Pathology and Control*, pp. 1-27. CABI, Wallingford, UK.

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DISEASE NOTE

FIRST REPORT OF THE RUST
MELAMPSORA EUPHORBIAE ON
EUPHORBIA HETEROPHYLLA IN OMANM.L. Deadman¹, A.M. Al Sa'di¹, Y.M. Al Maqbali¹,
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Euphorbia heterophylla L. is a serious weed in many parts of the world and considerable efforts are made to limit its invasiveness and impact on crop productivity, including evaluation of fungi as biocontrol agents (Nechet *et al.*, 2004). In Oman, *E. heterophylla* is an introduced weed of irrigated fields. In April 2005, leaf samples of *E. heterophylla*, showing rust symptoms were collected from Mudhaibi, 100 km south of Muscat. Orange pustules covering both sides of the leaves contained urediniospores typical of *Melampsora euphorbiae* (C. Schub.) Castagne. Urediniospores were variable, mostly subglobose and echinulate, and measured 16-20 × 14-16 µm. They were intermixed with hyaline, capitate paraphyses, similar to recent reports for the pathogen on related hosts (Kavak, 2004). Identity was confirmed by nuclear ribosomal large subunit DNA analysis (voucher sequence deposited in GenBank, accession No. DQ351722, voucher specimen deposited in the U.S. National Fungus Collections, BPI 871135). *M. euphorbiae* has been reported from the Middle East (Kavak, 2004), but not from Oman. The pathogen has been suggested as a biocontrol agent against weeds including *E. esula* L. and *E. cyparissias* L. (Bruckart and Dowler, 1986). Current research is examining the ability of *M. euphorbiae* to limit growth and reproduction of *E. heterophylla* under local conditions.

Nechet K. de L., Barreto R.M., Mizubuti E.S.G., 2004. *Sphaeceloma poinsettiae* as a potential biological control agent for wild poinsettia (*Euphorbia heterophylla*). *Biological Control* 30: 556-565.

Kavak H., 2004. *Melampsora euphorbiae*, a new rust disease found on *Euphorbia rigida* in Turkey. *Plant Pathology* 53: 810.

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