DISEASE NOTE

FIRST REPORT OF *TOMATO SPOTTED WILT VIRUS* IN GLOBE ARTICHOKE IN GREECE

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In February 2005 and November 2007, globe artichoke (*Cynara scolymus* L.) plants showing virus-like symptoms were observed in Argolida (southern Greece, Peloponnese) and Komotini (northern Greece, Thrace), close to lettuce and tobacco fields both infected with *Tomato spotted wilt virus* (TSWV). The incidence of diseased plants ranged from 3% to 7% in Argolida and Komotini, respectively. Leaf samples from symptomatic plants were analyzed in DAS-ELISA with polyclonal antisera to TSWV, *Cucumber mosaic virus* (CMV) and *Turnip mosaic virus* (TuMV). Plants exhibiting chlorotic and/or necrotic rings, line patterns of the leaves and mild to severe stunting reacted only with the antiserum to TSWV. Out of 40 samples tested, TSWV was detected in 25 and 30 samples from Argolida and Komotini, respectively. In RT-PCR, amplicons of the expected size (*ca*. 270 bp) were obtained from all samples that tested positive for TSWV in ELISA with primers: upstream 5'-GTC GAA ATG GTC GGC A-3' and downstream 5'-AAT TGC CTT GCA ACC AAT TC-3' (Weekes *et al.*, 1996). Sequenced PCR products (AM940436) showed nucleotide sequence identity ranging from 94% to 96% with other TSWV isolates (AY070218 and AB198742, respectively). Adult thrips collected from infected plants in Argolida were identified as *Frankliniella occidentalis,* but only *Thrips tabaci* adults were found on symptomatic plants in Komotini. A number of samples exhibiting vein clearing, leaf deformation and crinkling did not react serologically to TSWV, CMV or TuMV. TSWV is a known pathogen of globe artichoke (Gallitelli *et al.*, 2004). Although it is found in several crops in Greece (Chatzivassiliou *et al.*, 2000), to our knowledge, this is the first natural infection of globe artichoke by TSWV in Greece.

Chatzivassiliou E.K., Weekes R., Morris J., Wood K.R., Barker I., Katis N.I., 2000. *Tomato spotted wilt tospovirus* (TSWV) in Greece: its incidence following the expansion of *Frankliniella occidentalis*, and characterization of isolates collected from various hosts. *Annals of Applied Biology* **137**: 127-134.

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DISEASE NOTE

FIRST REPORT OF ROOT ROT CAUSED BY *ROSELLINIA NECATRIX* TO ALMOND NURSERY TREES AND FIG ORCHARD TREES IN GREECE

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Rosellinia spp. have been recorded all over the world as root rot pathogens of many plants, mainly trees. *Rosellinia necatrix* Prill. (anamorph: *Dematophora necatrix* Hartig) is among the best known species. This fungus is a pathogen that causes root rot of many orchard trees, such as almond, peach, plum, apple, pear, olive, cherry and avocado (Sousa *et al.,* 1995). In spring and summer 2008, almond nursery trees in Thessaly (central Greece) and fig orchard trees (*Ficus carica*) in the Greek island of Evoia were found to be affected by a soil-borne pathogen that formed a white cottony mycelium and mycelial strands at the crown of the plant, or on the main roots, and induced leaf yellowing. Almond nursery trees died in 5-6 days. Fig trees were killed in a single season or in a couple of years. Diseased almond trees occurred in patches in the nursery plots due to the pathogen spread to neighbouring plants. The identification of the casual agent of the disease was based on microscopic observation of the vegetative mycelial structures, isolated from affected tissues and grown in artificial culture onto potato dextrose agar plates. Mycelia obtained from different sources consistently showed the pear-shaped hyphae and synnemata typical of *R. necatrix* (Sivanesan and Holliday, 1972). To the best of our knowledge, this is the first record in Greece of *R. necatrix* attacks to almond trees in the nursery and to fig trees in commercial orchards.

Sivanesan A., Holliday P., 1972. *Rosellinia necatrix*. *C.M.I. Descriptions of Pathogenic Fungi and Bacteria.* No. 352.

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DISEASE NOTE

FIRST REPORT OF PHOMA STEM CANKER (BLACKLEG) OF OILSEED RAPE CAUSED BY THE SPECIES COMPLEX *LEPTOSPHAERIA MACULANS* AND *L. BIGLOBOSA* IN GREECE

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Phoma stem canker (blackleg) is an internationally important disease of oilseed rape (*Brassica napus* L.) to which it causes serious crop losses. Symptoms develop on true leaves and, at various levels, on the stems. Epidemics are initiated in autumn by air-borne ascospores released from infected stubbles of previous crops (Lo-Pelzer *et al.,* 2009). In 2008, phoma stem canker was observed in experimental *B. napus* plots at the Technological Education Institute (TEI) of Larissa (central Greece). Early symptoms of the disease appeared as small grey lesions on the leaf surface, which were studded with black specks, i.e. the fruiting bodies (pycnidia) of the pathogen. At later stages, necrotic symptoms developed in the form of cankers at the crown of the plant and lesions on the upper stem. Small circular pycnidia were observed on the surface of infected stems. According to our observations, based primarily on the morphology of fungal colonies and spores, the blackleg disease observed at Larissa is caused by the species complex *Leptosphaeria maculans*/*Leptosphaeria biglobosa*, as reported in the literature (Fitt *et al*., 2006). *L. maculans*, the prevailing species, is associated with the production of crown cankers, whereas *L. biglobosa* is associated with upper stem lesions. As reported by Fitt *et al*. (2006), fungal colonies grown on potato dextrose agar plates were characterised by a brown mycelium that produced no pigment (*L. maculans*) or a dark-brown pigmented mycelium (*L. biglobosa*). This seems to be the first record of blackleg of oilseed rape in Greece.

Lo-Pelzer E., Aubertot J.N., David O., Jeuffroy M.H., Bousset L., 2009. Relationship between the severity of phoma stem canker (*Leptosphaeria maculans*/*L biglobosa* species complex) and subsequent primary inoculum production on oilseed rape stubble. *Plant Pathology* **58**: 61-70.

Fitt B.D.L., Brun H., Barbetti M.J., Rimmer S.R., 2006. Worldwide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology* **114**: 13-15.

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DISEASE NOTE

GENOTYPIC CLASSIFICATION OF ATYPICAL SYMPTOM-INDUCING *XANTHOMONAS AXONOPODIS* pv. *CITRI* STRAINS IN TAIWAN

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Citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*) is one of the most important diseases in many citrus-producing countries. This disease is characterized by the formation of erumpent, callus-like lesions with a water-soaked margin. Several distinct phenotypes of *Xac* have been reported internationally. Three novel atypical symptom-inducing strains, designated *Xac*-A^f, -A^r and -A^p were reported recently from Taiwan (Lin *et al*., 2005, 2008). In this study, DNA fingerprintings generated by REP-PCR and amplified fragment length polymorphism (AFLP) (Ah-You *et al*., 2007) were optimized to compare 36 strains, including the atypical symptom-inducing strains *Xac*-A^f, -A^p and -A^r from Taiwan, and the additional reference strains *Xac*-A, -A*, -A^w, *X. axonopodis* pv. *aurantifolii* and *X. axonopodis* pv. *citrumelo*. Cluster analysis by combining the band patterns of ERIC- and REP-PCR clearly grouped the atypical symptom-inducing strains in types *Xac*- A^f, -A^r and -A^p into the same cluster with typical symptom-inducing strains in type *Xac*-A. These three types of atypical symptom-inducing *Xac* strains could be excluded from strains of *Xac*-A* and -A^w in this combined analysis. Based on AFLP analysis, all type *Xac*- A^f, -A^r and -A^p strains were also grouped into the same cluster with strains in type *Xac*-A. Strains of *Xac*-A* and -A^w were closely related to *Xac*-A strains in our results. No Taiwan isolate was related to *X. axonopodis* pv. *aurantifolii* or *X. axonopodis* pv. *citrumelo*. The results further confirm the atypical symptom-inducing *Xac* strains in Taiwan belong to the A type of *Xac*.

Ah-You N., Gagnevin L., Chiroleu F., Jouen E., Neto J.R., Pruvost O., 2007. Pathological variations within *Xanthomonas campestris* pv. *mangiferaeindicae* support its separation into three distinct pathovars that can be distinguished by amplified fragment length polymorphism. *Phytopathology* **97**: 1568-1577.

Lin H.C., Chang H., Tzeng K.C., 2008. Characterization of novel strains of citrus canker bacteria from citrus in Taiwan. *Journal of Taiwan Agricultural Research* **57**: 265-278.

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DISEASE NOTE

FIRST REPORT OF THE TRANSMISSION OF CITRUS YELLOW VEIN CLEARING BY *APHIS CRACCIVORA* KOCH

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Citrus yellow vein clearing (CYVC) disease was first recorded from lemon (*Citrus lemon* (L.) Burm. f.) in Pakistan (Bové, 1989), then observed again in the same country on 11 lemon varieties (Catara *et al*., 1993). More recently, the disease was found in the Abhar and Punjab regions of India and a virus was mechanically transmitted from infected lemons to seedlings of *Phaseolus vulgaris* cv. Saxa, which were invaded systemically (Alshami *et al*., 2003). In 2007, CYVC was observed in the Çukurova region of Turkey on lemon and sour orange (*C. aurantium* L.) which showed characteristic foliar vein clearing, yellow flecks and crinkling (Önelge *et al*., 2007). The rapid natural spread of CYVC in lemon trees suggested transmission of the causal agent by a vector. To verify this hypothesis, adults of *Aphis craccivora* Koch maintained on infected lemon and bean seedlings were fasted 24 h, transferred in groups of 10 to healthy *P. vulgaris* cv. Dermason plants, and allowed to feed for a 24 h period. Plants were maintained in a greenhouse for two months after exposure to aphids. Systemic symptoms, consisting of severe mosaic, blotching and necrosis, developed on aphid-inoculated bean plans. *A. craccivora* transmitted CYVC from infected lemon to 62 of 90 bean plants (69%) and, at a lower rate (52%), from infected to healthy bean plants (26 of 50). These observations were taken as evidence that there is a biological relationship between CYVC and *A. craccivora* and, to the best of our knowledge, represent the first report of CYVC transmission by an aphid.

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Catara A., Azzaro A., Davino M., Polizzi G., 1993. Yellow vein clearing of lemon in Pakistan. *Proceedings of the 12th Conference of IOCV, New Delhi 1993*: 364-367.

Önelge N., Bozan O., Gök M., Satar S., 2007. Yellow vein clearing of lemons in Turkey. *Proceedings of the 17th Conference of IOCV, Adana 2007*: 176.

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DISEASE NOTE

FIRST REPORT OF *GARLIC COMMON LATENT VIRUS* IN GARLIC IN TURKEY

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Garlic (*Allium sativum*), one of the most important vegetables used in Turkish cuisine, hosts several viruses of the genera *Potyvirus*, *Carlavirus* and *Allexivirus* (Van Dijk, 1994). In Turkey, garlic is grown in different geographic areas with nearly 20% of the production coming from the Kastamonu (Tasköprü) region, followed by the Kahramanmaras (Narlı) and Gaziantep (Araban) areas. During previous surveys, typical virus symptoms were observed in commercial garlic fields, in which the presence of *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) was recorded (Fidan and Baloglu, 2009). In 2007-2008, during further surveys, plants showing stunting, deformity and mild to severe mosaic symptoms were collected in garlic fields of the Kastamonu (Tasköprü), Kahramanmaras and Gaziantep regions. DAS-ELISA with specific antibodies (Agdia, USA) showed these plants to be infected by OYDV, LYSV and *Garlic common latent virus* (GCLV). The occurrence of GCLV was confirmed by RT-PCR using total RNA extracted from leaf tissues (100 mg) with the RNeasy Plant Mini kit (Qiagen, USA). The expected 481 bp fragment was amplified using primers specific to the GCLV coat protein gene (GCLV-F GCACCAGTGGTTTGGAATGA and GCLV-R AGCACTCCTAGAACAAC CATTA). The amplicon was obtained from the 25 samples that were ELISA-positive for GCLV. The sequenced amplicon of one of the Turkish GCLV isolates had 97% identity at the nucleotide level with the coat protein gene of a GCLV isolate from Japan (accession No. AB004805.1). To the best of our knowledge, this is the first report of GCLV from garlic in Turkey.

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DISEASE NOTE

FIRST REPORT OF *PESTALOTIOPSIS* sp. ON *PROTEA CYNAROIDES* IN ITALY

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Protea sp. (Proteaceae), originating from the southern hemisphere, is becoming an economically important ornamental crop in Europe. During summer 2007, plants of *Protea cynaroides* cv. Miniking showing stem die-back and leaf desiccation were observed in a nursery at Pescia (Tuscany, Italy). Acervuli were present on stem lesions. A fungus isolated from these lesions on potato carrot agar + streptomycin sulphate (0.05 g l^{-1}) and bacitracin (0.1 g l^{-1}) had a white mycelium that differentiated acervuli containing black, slimy spore masses. This fungus was morphologically identified as *Pestalotiopsis* sp. Conidia were five-celled with the three middle cells brown and darker than those at both ends, and measured 19.97 (22.40 ± 1.82 SD) 25.85 µm × 5.87 (7.12 ± 0.54 SD) 9.40 µm. Apical conidial cells were hyaline with two or three, rarely four, simple widely divergent setulae, 14.10 (35.10 ± 6.83 SD) 49.35 µm long. Basal cells were also hyaline with a single pedicel 2.35 (5.56 ± 1.7 SD) 9.40 µm long. Koch's postulates were fulfilled by inoculating mycelial plugs form a 7-day-old single spore culture into incisions made on disinfested stems of healthy *P. cynaroides* plants. Wounds inoculated with plugs of sterile PDA served as control. The field syndrome, i.e. die-back of the stem and leaf desiccation, was reproduced on inoculated plants and the same fungus was re-isolated from symptomatic but not from control stems, which remained symptomless. *Pestalotiopsis* sp. has been isolated from different *Proteaceae*, including *Protea* sp. (Swart *et al.*, 1999; Crous *et al.*, 2000) but, to our knowledge, this is the first report of *Pestalotiopsis* sp. on *P. cynaroides* in Italy.

Crous P.W., Summerell B.A., Taylor J.E., Bullock S., 2000. Fungi occurring on *Proteaceae* in Australia: selected foliicolous species. *Australasian Plant Pathology* **29**: 267-278.

Swart L., Crous P.W., Petrini O., Taylor J.E., 2000. Fungal endophytes of *Proteaceae*, with particular emphasis on *Botryosphaeria proteae*. *Mycoscience* **41**: 123-127.

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DISEASE NOTE

FIRST REPORT OF *PUCCINIA IRIDIS* ON *IRIS CROATICA* IN CROATIA

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During summers of 2007 and 2008, in the Nature park Zumberak-Samoborsko gorje and the Botanical Garden of the University of Zagreb leaf spots with evident rust pustules were observed on the leaves of Croatian iris (*Iris croatica* Horvat et Horvat), an endemic and protected plant species. Uredinia were amphigenous, brown-orange, while telia were black and hypophyllous. Based on the morphological characteristics of urediniospores and teliospores, as seen under the light microscope, the fungus was identified as *Puccinia iridis* (DC) Wallr. (Gäumann, 1959). Urediniospores were yellow-brown, echinulate, sphaerical to ovoid 28-35×20-25 µm in size. Teliospores were bicellulate, golden-brown, clavate, cylindric, 35-50×14-20 µm in size, with pedicels 20-30 µm long. *P. iridis* is heteroecious with aecial stage developing on species of the genera *Urtica* and *Valeriana,* whilst uredinia and telia appear mostly on taxa of the genus *Iris,* but also on some species of the family Iridaceae (e.g. *Dietes, Crocus, Belamcanda*). To date, *P. iridis*, a new record from Croatia, has been reported from 69 *Iris* taxa (Farr and Rossman, 2009), but not from *Iris croatica* which, therefore, is also a new host for this rust. Plant material with uredinia and telia of *P. iridis* is stored, as a herbarium specimen, in the Department of Plant Pathology at the Faculty of Agriculture of Zagreb.

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DISEASE NOTE

SURVIVAL OF *CANDIDATUS* LIBERIBACTER ASIATICUS, CAUSAL AGENT OF HUANGLONGBING DISEASE, IN REMNANTS OF 'VOLKAMER' LEMON ROOTS IN VIETNAM

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Citrus production is considerably affected by Huanglongbing (HLB) disease, caused by a sieve tube-restricted α-proteobacterium (Bové, 2006). In the Mekong delta, the Asian HLB pathogen *Ca.* Liberibacter asiaticus is disseminated by the citrus psyllid *Diaphorina citri* (Kuwayana) and/or the distribution of infected propagating material (seedlings and budsticks). In this area, *Citrus limonia* 'Volkameriana' (volkamer lemon) is frequently used as a rootstock. In May 2001, a naturally infected mandarin grove at Lai Vung (Don Thap province, Vietnam), was treated following the guidelines of the Southern Fruit Research Institute (Long Dinh, Tien Giang province, Vietnam), i.e. the trees were cut, the volkamer rootstocks were dug and burned and an insecticide was applied. Volkamer lemon roots regenerate quickly, hence new buds were differentiated in the remaining roots, from which new shoots soon sprouted. We have analysed the presence of the HLB pathogen in these shoots. Six shoots (3-5 cm long) were collected from different roots in June and six in November 2001. DNA was extracted according to Rogers and Bendich (1988) and PCR amplification was performed using primers OI2 and 23S1 (Jagoueix *et al*., 1997) to amplify an 800 bp band of the 16S/23S intergenic region of *Ca*. Liberibacter asiaticus. The result of the analysis was positive in all cases. This is a first report about the survival of *Ca*. Liberibacter in root remnants and its transmission to new shoots. We propose that the control of HLB should involve the careful destruction of both canopy and roots of infected citrus plants.

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DISEASE NOTE

FIRST REPORT OF *HYDRANGEA RINGSPOT VIRUS* IN HYDRANGEA IN BRAZIL

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Hydrangea is a very popular ornamental plant in Brazil. Hydrangea plants showing chlorotic and necrotic rings on the leaves were observed in Aruja (São Paulo). Electron microcopy observations of leaf dips disclosed the presence of filamentous virus-like particles 490 nm in length, suggesting the presence of a potexvirus. For identifying the virus species associated with infected plants, total RNA was extracted (Bertheau *et al*., 1998) and used as template for one step RT-PCR with AMV reverse transcriptase. PCR primers Hydsens (5' GGAGACAATCAAGGCTAGGC 3') and Hydant (5' TGGGATTGGTCGAAGGCGG 3') were designed in the course of this work on the RNA-dependent RNA polymerase gene sequence of *Hydrangea ringspot virus* (HdRSV), a potexvirus found in different parts of the world (ICTVdB). The expected 550 bp fragment was successfully amplified and sequenced. A 96% identity at the nucleotide level was obtained with a comparable HdRSV sequence available in database (accession No. AY707100), thus providing unambigous identification of the virus. To the best of our knowledge, this is the first report of HdRSV from Brazil.

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ICTVdB. The Universal Virus Database, version 4. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/

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DISEASE NOTE

CLERODENDRUM YELLOW MOSAIC CHINA VIRUS IS A DISTINCT BIPARTITE BEGOMOVIRUS ISOLATED FROM *CLERODENDRUM CYRTOPHYLLUM* IN CHINA

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Samples of *Clerodendrum cyrtophyllum* Turcz. leaves showing yellow mosaic were collected in the Fujian province of China. Total DNA was extracted from symptomatic leaves to amplify begomoviral DNA-A and DNA-B components according to Haible *et al.* (2006). An isolate denoted Fz7 was selected for further sequence analysis. The complete DNA-A (GenBank accession No. FJ011668) and DNA-B (GenBank accession No. FJ011669) sequences of Fz7 comprised 2776 and 2739 nucleotides, respectively. Genome organization was typical of begomoviral species with a bipartite genome. DNA-A of Fz7 was most closely related to that of an isolate of *Clerodendrum golden mosaic viru*s (ClGMV 8VN:Son:05, GenBank accession No. DQ641692) with 78.9% nucleotide sequence identity. Phylogenetic analyses showed that the DNA-A sequence of Fz7 clustered together with that of ClGMV (VN:Son:05). According to the demarcation criteria for identifying begomovirus species (Fauquet *et al.*, 2008), Fz7 appears to be a distinct bipartite begomovirus for which the name *Clerodendrum yellow mosaic China virus* (ClYMCNV) is proposed. This appears to be the first report of a bipartite begomovirus infecting *Clerodendrum cyrtophyllum* in a geographical area like China, where a large prevalence has been found up to now of monopartite begomovirus species.

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DISEASE NOTE

FIRST REPORT OF *HOP STUNT VIROID* FROM ALMOND TREE IN CHINA

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A survey for the presence of *Hop stunt viroid* (HSVd) was carried out in early spring 2008 to assess its presence and incidence in almond trees in China. The orchards investigated were located in the Shanxi and Shandong provinces where several trees with yellow leaves were found. Fifty-eight leaves were collected from both symptomatic and symptomless trees. Total RNA was extracted (Li, 1995) and tested for the presence HSVd by Northern blot using digoxygenin-labeled riboprobes. HSVd was detected in 17 out of 58 samples (29.3%), but there was no correlation between the presence of the viroid and leaf yellowing. These results suggest that the infection of HSVd in almond trees is latent. A 297 bp DNA fragment was amplified from positive samples by RT-PCR using previously designed primers (Yang *et al*., 2007). Two different sequence variants were identified and deposited in GenBank (Accession No. EU937524 and EU937525). Sequence analysis of the amplified products revealed 98.66–99.33% nucleotide sequence identity with previously identified HSVd isolates in Spanish almonds (AJ011813 and AJ011814). This is the first report of HSVd in almond trees in an Asian country. The present results call for the establishment of guidelines to prevent or minimize the transmission of HSVd from almond trees to other susceptible crops.

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DISEASE NOTE

STEMPHYLIUM LEAF BLIGHT OF BROAD BEAN IN IRAN

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During spring 2009, broad bean (*Vicia faba*) plants in several commercial fields in the Gorgan area (Iran) showed symptoms of blight, consisting of irregularly shaped dark spots that started mostly from the leaf margins, then coalesced covering large areas of the blade. Fungi isolated from diseased tissue were identified as *Stemphylium botryosum* Wallroth and *S. vesicarium* (Wallroth) Simmons based on different morphological and biometrical traits of conidia. *S. botryosum* conidia were subspherical, oblong or broadly ovoid, 23.8-35.4×19.2-25.0 µm in size, usually constricted at the median transverse septum, brown to dark brown, with walls minutely warted or echinulate. *S. vesicarium* conidia were also oblong or broadly oval, 23.5-42.3×11.1-16.5 µm in size, constricted at one or more (commonly three) of the major transverse septa, light brown, with external walls conspicuously and densely verrucose. Species were easily separated based on the length/width ratio of conidia (average 1.4 and 2.4 for *S. botryosum* and *S. vesicarium*, respectively) which represents a trait of high diagnostic value (Simmons, 1969). For pathogenicity tests, 8 mm disks taken from the margins of actively growing colonies were deposited on the leaves of potted host plants placed in a greenhouse at 25°C and more than 90% relative humidity for 3-7 days. Symptoms were similar to those observed in commercial fields. The fungi were reisolated from lesions of inoculated plants, but not from tissues of any of the control plants. Broad bean infections by *S. botryosum* and *S. solani* have been recorded from Saudi Arabia (Abdel-Hafez, 1984) and Mauritius (Orieux and Felix, 1968), respectively. This is the first report in the world of *S. vesicarium* on broad bean and of *S. botryosum* in Iran.

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DISEASE NOTE

A NEW OUTBREAK OF *RALSTONIA SOLANACEARUM* ON TOMATO IN SARDINIA

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A new outbreak of bacterial wilt by *Ralstonia solanacearum* was detected in 2009 in a tomato crop cv. Cuore di bue grafted on Beaufor, grown in a greenhouse in Sardinia. Surveys were conducted in four greenhouses. In the first (As-1) the disease had been already detected in 2007 (Loreti *et al.*, 2008); in the others (As-2, As-3 and As-4) plants with bacterial wilt symptoms were observed as in the first greenhouse. The percentage of affected plants ranged from 10% to 50%. Samples from plants, soil and water were collected from As-1 (eighteen) and from As-2 (twenty-two) whereas, only plant samples were taken from As-3 (six) and AS-4 (eight). All 54 samples were used for isolation on SMSA and TZCA semi-selective media. Typical bacterial colonies were recovered only from plant and soil samples collected from As-2. The isolates elicited a hypersensitive reaction in tobacco leaves, induced wilting of tomato plantlets, did not produce fluorescent pigment on KB and levan on NSA, gave a positive reaction in immunofluorescence tests using a polyclonal antiserum (Loewe, Germany), and yielded the expected band of 288 bp in PCR assays (Seal *et al.*, 1993). From inoculated plants showing typical symptoms, the same colony type as in the primary isolates was recovered. Phylotype characterization (Fegan and Prior, 2005) showed that all isolates belonged to phylotype II. *R. solanacearum* had been eradicated from greenhouse As-1 in 2007, by uprooting and burning tomato plants, methyl bromide treatments and decontamination of stored water with a commercial sodium chloride solution. The pathogen was not recovered from any of the samples collected from As-3 and As-4, but a new outbreak was detected in the neighbouring As-2 greenhouse. This finding is alarming, because the causes underlying the introduction and dissemination of *R. solanacearum* in Sardinia are still unknown.

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DISEASE NOTE

FIRST REPORT OF *BOTRYOSPHAERIA DOTHIDEA* CAUSING CANKER AND BRANCH DIEBACK ON *QUERCUS SUBER* IN ITALY

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In winter 2007 canker and branch dieback on cork oak trees were observed in a declining forest in south-central Sardinia (Italy). Fungal isolates obtained from symptomatic twigs and branches were identified as *Fusicoccum aesculi* Corda [teleomorph: *Botryosphaeria dothidea* (Moug.: Fr.) Ces. et De Not.] on the basis of morphological characters (Phillips *et al*., 2005). On oatmeal agar (OA) at 25°C, fungal isolates developed white colonies with dense aerial mycelium turning dark grey after 4 to 6 days, and formed pycnidia after two weeks. The hyaline, fusiform and aseptate conidia measured 21.4-27.7×3.8-6.3 µm, with a length/width ratio of 4.9 (n = 50). Identity was confirmed by analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2) of rDNA. BLAST searches in GenBank showed 99-100% identity with reference sequences of *B. dothidea*. The representative sequence of the *B. dothidea* strain S4, obtained in this study was deposited in GenBank (accession No. GQ281660). Pathogenicity of the strain S4 was tested by stem inoculation on seven 2-year-old cork oak seedlings maintained in a greenhouse at 25°C. Stem cankers developed on infected seedlings within 1 month from inoculation. The pathogen was reisolated from all inoculated but not from control seedlings, thus fulfilling Koch's postulates. These results show the active role played by *B. dothidea* in the aetiology of oak decline, as previously reported in Spain (Sanchez *et al*., 2003) and in Italy (Turco *et al*., 2006). Whereas several other species of *Botryosphaeria* species were shown to cause damaging diseases to cork oak in Sardinia, to our knowledge this is the first report of *B. dothidea* causing canker disease to cork oak in Italy.

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DISEASE NOTE

FIRST REPORT OF CROWN GALL OF *PRUNUS* spp. CAUSED BY *AGROBACTERIUM TUMEFACIENS* BIOVAR 1 IN NEPAL

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During a spring 2008 survey in different fruit orchards of Kirtipur (Kathmandu, central Nepal), a high incidence (nearly 70%) of crown-gall like symptoms was observed at the root level of 8-year-old plums of cv. Maithili grafted on *Prunus cerasifera* and *P. salicina*. Non-fluorescent, gram-negative bacteria were isolated onto YMA+C medium (Moore *et al*., 2001), five strains of which were characterised as aerobic, non-sporing, non-pigmented, rod-shaped and oxidase positive. Pathogenicity was tested on 3-week-old tomato plants of cv. San Marzano (three plants per strain) by needle inoculation of a bacterial suspension (10^8 CFU/ml). A reference *Agrobacterium tumefaciens* strain (CG 634) was used as positive control. All plants were maintained in a greenhouse. Three of the five isolates and the reference strain induced galls within 3 weeks from inoculation. No symptoms were observed on water-inoculated negative controls. Bacteria re-isolated from symptomatic plants were identical to the original strains. To identify the biovar (Moore *et al*., 2001), the isolates were subjected to oxidase, growth on 2% NaCl, 3-ketoglycoside and reaction on litmus milk. The positive reactions obtained indicated their belonging to *A. tumefaciens* biovar 1. The sequence of the 16S rDNA region (Weisburg *et al*, 1991) of one of these isolates (GenBank accession No. FJ666055) had 98.48% (1429/1451 bp aligned) to 100% (1400/1400 bp aligned) identity with comparable sequences from about 60 *A. tumefaciens* strains available in databases. This disease is of regulatory importance, since Nepal shares boundaries with China and India, from which crown gall has been reported. No reports came, instead, from neighbouring countries Bhutan and Bangladesh. Infected Nepalese orchards were established with imported propagating material, which is the likely cause of the introduction of the pathogen. This the first report of crown gall disease on plum trees in Nepal.

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DISEASE NOTE

TRANSMISSION OF *CANDIDATUS* PHYTOPLASMA AURANTIFOLIA TO MEXICAN LIME BY THE LEAFHOPPER *HISHIMONUS PHYCITIS* IN IRAN

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Witches' broom disease of lime (WBDL) caused by *Candidatus* Phytoplasma aurantifolia is a serious disease of *Citrus aurantium* in Oman and southern Iran. WBDL phytoplasma was experimentally transmitted to bakraee (*Citrus reticulate* hybrid) seedlings by feral *Hishimonus phycitis* (Salehi *et al*., 2007) but not to Mexican lime with earlier trials (Salehi *et al.*, 2002). The failure of these experiments and the very low rate of natural infection of young lime trees in the field, prompted a repetition of the trial. To this aim, five healthy 15-20- year-old lime trees, previously checked by PCR, were selected in a WBDL free-area of Bandar Abbas (Hormozgan province). During March and April batches of 1000 *H. phycitis*, collected from WBDL-affected limes in Rudan (Hormozgan province) were transferred to four trees covered by a two-layered insect-proof net for a 8 weeks inoculation access period. Moreover, almost 500 *H. phycitis* (some examined by PCR) collected from non-infected trees in disease-free areas were similarly released on a healthy Mexican lime as negative control. The leafhoppers were then killed by an insecticide treatment (metasystox) and the lime trees inspected for WBDL symptom appearance. After about 8 months, typical WBDL symptoms were observed on 3 of the 4 lime trees exposed to leafhoppers collected from infected trees, but not on the negative control. Infection was confirmed by direct PCR using R16F2n/R16R2 primer pair. Amplified PCR products (1250 bp) from experimentally infected lime trees, *H. phycitis* captured in the field on symptomatic trees, and a naturally infected lime tree, were separately digested with endonucleases *Alu*I, *Hha*I, *Rsa*I and *Taq*I, obtaining identical patterns, comparable to those of *Ca.* Phytoplasma aurantifolia (Lee *et al.*, 1998). This is the first report of experimental transmission of *Ca*. Phytoplasma aurantifolia by *H. phycitis* to Mexican lime.

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DISEASE NOTE

PHOMOPSIS THEAE ON *CAMELLIA SINENSIS* IN TURKEY

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Blight symptoms starting from the leaf edges were first observed on tea [*Camellia sinensis* (L.) O. Kuntze] plants in Central, Iyidere and Çayeli districts of Rize Province, (Turkey). Diseased areas were yellow-cream in colour and were dotted with pycnidia. Isolations in culture from diseased tissues yielded fungal colonies with a whitish mycelium that produced pycnidia in Petri plates. Yellowish spore droplets oozed from the pycnidia in old cultures. Both alpha and beta conidia were present, the former being more common. Alpha conidia were hyaline, non-septate, fusiform to ellipsoidal, 2-3 guttulate and measured (1.75) 2.15 (2.5)×(6.25) 7.27 (8.75) µm. Beta conidia were hyaline, elongate, filiform, curved and measured (0.5) 0.75 (1.25)×(18) 22.4 (29.5) µm. Based on morphological characters, the presumed causal agent of the disease was identified as *Phomopsis theae* Petch. (Punithalingam and Gibson, 1972). Pathogenicity tests were done by placing agar pieces 5 mm in diameter from 10-day-old cultures, on wounded leaves of 2-year-old tea plants cv. Fener-3 at 18/23ºC (night/day) in a controlled growth room. In the controls, only sterile agar plugs were used. Inoculated and control plants were covered with plastic bags for 5 days to ensure high humidity, then left uncovered. Disease symptoms began to appear ten days after inoculation. The fungus was consistently reisolated from diseased tissues. No disease developed on control plants. *P. theae* has been recorded from tea in Ethiopia, Kenya, Malawi, Tanzania, Uganda, Zimbabwe, India, Malaysia, Nepal, Sri Lanka, Thailand and Papua New Guinea (Punithalingam and Gibson,1972; Anonymous, 1993) but, apparently, not from Turkey, this being the first report.

This study was supported by The Scientific and Technical Research Council of Turkey (Project No: 107 O 661).

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DISEASE NOTE

SHOOT AND UMBEL BLIGHT OF CARAWAY CAUSED BY *PHOMOPSIS DIACHENII* IN HUNGARY

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Caraway (*Carum carvi* L.) is an important medicinal and aromatic plant in Hungary cultivated on *ca.* 650 ha. During field surveys conducted between 1999 and 2007 significant shoot and umbel blight symptoms were observed in plant stands located near Budapest and at Herencsény. Disease frequency and incidence reached 94% and 61%, respectively, in a two-year-old plant stand at Herencsény. Reddish-brown discolouration and occasional wilting was observed on umbels and shoots. Fruits often failed to set. Stromatic pycnidia, 100-275 µm in diameter, were found on necrotic umbel stalks, stems and fruits belonging to a fungus was identified as *Phomopsis diachenii* Sacc. Pycnidia produced two types of hyaline conidia (± and ≤). The ± conidia were one-celled, fusoid and measured 10.8×2.7 µm (7.5-15.0×2.1-3.3 µm), ≤ conidia were one-celled, falcate or sigmoid, 11.7-25.1 µm in length. On plants, β conidia were rarely produced. The fungus was isolated on malt extract agar from diseased stems and umbels. Pathogenicity was evaluated by placing 5 mm agar discs colonized by the fungal mycelium on stems and umbels of healthy plants, which were then placed in moist chambers at 16-28°C with a 12 h photoperiod. Half of the plant parts were wounded with a needle prior to inoculation. After 12-13 days elongated brownish, slightly sunken necroses developed around the agar discs. Symptoms developed only on wounded stems and umbels. To our knowledge this is the first report of Phomopsis blight on caraway in Hungary. In Europe the disease has previously been observed in Germany (Gabler and Ehrig, 2000), Czech Republic (Ondrej, 1997) and Bulgaria (Rodeva and Gabler, 2004).

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DISEASE NOTE

FIRST REPORT OF *HEDERA CANARIENSIS* WILT CAUSED BY *FUSARIUM SOLANI* IN INDIA

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Hedera (*Hedera canariensis*) is an important ornamental plant that has become popular as an indoor and outdoor potted plant in the Kashmir valley of the Jammu and Kashmir State (India) over the last few years. In September 2007 and 2008, wilting of Hedera plants was observed in some commercial nurseries and private houses in Srinagar (Kashmir). The disease manifested itself as yellowing of the lower leaves, which gradually spread upwards and ultimately the whole plant showed irreversible wilting symptoms leading to death. Isolations from the roots and crown of diseased plants yielded fungal colonies with thick white mycelia on potato dextrose agar medium. The colonies produced macro- and micro-conidia within 3-4 days at 25±1°C. The size of macro-conidia was in the range of 28.0-38.5×3.5-5.25 µm (mean 32.48×4.48 µm) and the micro-conidia were in the range of 10.5-17.5×3.5-5.25 µm (mean 14.98×4.66 µm). The pathogen culture was identified on the basis of colony and spore morphology as *Fusarium solani* (Synder and Toussoun, 1965; Matuo and Synder, 1973; Grewal *et al.*, 1974) and the identification was confirmed by the Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi. The culture was deposited at ITCC, IARI, New Delhi under accession number ITCC-6334. For pathogenicity tests, sterilized soil was inoculated with 7-day-old pathogen cultures (bulked up in sand-corn meal medium) in 10 pots, followed by planting of healthy Hedera plants in each pot. Five pots with uninoculated plants served as controls. All pots were placed in a moist chamber at 25±2°C. Every inoculated plants showed symptoms within 10-15 days and, on re-isolation, yielded the original fungus. The control plants remained healthy. This is the first report of wilt of *H. canariensis* caused by *F. solani* in India.

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DISEASE NOTE

FIRST REPORT OF *PAPAYA LEAF CURL VIRUS* NATURALLY INFECTING TOBACCO IN INDIA

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Papaya leaf curl disease, first reported from India in 1939 (Thomas and Krishnaswamy, 1939), is caused by the begomovirus *Papaya leaf curl virus* (PaLCV) (Saxena *et al.*, 1998). Since 2001, a leaf curl disease of tobacco, accounting for a yearly yield loss of 50%, was consistently observed in the tobacco-growing areas of Bihar (eastern India). Infected plants showed typical curling of the leaves, vein thickening and enations. The whitefly *Bemisia tabaci* was present in diseased fields. To identify the causal agent of the disease, likely a virus, total DNA was extracted from infected leaves and used to amplify the coat protein gene (CP) using the begomovirus-specific primer pair P1 (5' GGGATTTGATTTCAGTAATAAGG 3') and P2 (5' GAGCATGTTGTATATGTAGA CCA 3') designed by us. The resulting PCR amplicon (850 bp), cotaining 771 bp of the CP sequence, was subjected to Southern hybridization with DNA probes prepared from the CP gene of *Cotton leaf curl Multan virus* (CLCuMV), another begomovirus (accession No. DQ191160), giving a strong signal. To investigate the relationship of the unknown begomovirus under study with other begomoviruses, the PCR amplicon was cloned and sequenced (accession No. GQ139516). BlastN results showed 85% and 91% sequence identity at the nucleotide level with two different isolates of PaLCV from India (accession No. DQ376039 and DQ376036). Interestingly, the same CP fragment showed a higher similarity (97% nucleotide sequence identity) with a PaLCV isolate from Pakistan (accession No. AJ436992). Further experiments are in progress to identify additional viral DNA components and to develop infectious clones. To the best of our knowledge, this is the first record of natural PaLCV infections to tobacco.

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DISEASE NOTE

FIRST REPORT OF *CUCUMBER MOSAIC VIRUS* IN *AMARYLLIS BELLADONNA* IN INDIA

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The majority of *Amaryllis belladonna* plants growing in the Botanical garden at NBRI, Lucknow, India, and suburbs exhibit foliar symptoms characterized by severe chlorotic stripes, mosaic and yellow mottling, stunting of the spikes, colour breaking of the flowers and reduction of their size. The occurrence of *Cucumber mosaic virus* (CMV) was suspected in symptomatic plants. For virus characterization, total RNA was recovered from leaves of infected plants as described by Asif *et al*. (2000) and used in RT-PCR assay with primer pairs CMV/CPF and CMV/CPR (Gutiérrez-Villegas *et al.*, 2004). Amplicons of 656 bp obtained from infected plants were cloned and sequenced (GenBank accession No. EF187825). No amplification was obtained from healthy plants. BlastN analyses showed 96% nucleotide sequence similarity with CMV strains infecting Indian long pepper (GenBank accession No. AY690621) and betel vine (GenBank accession No. AY690620) in South India. This is the first definite report of CMV infecting *A. belladonna* in India.

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DISEASE NOTE

FIRST RECORD OF FIG LATENT VIRUS 1 IN SPAIN

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Leaf tissues from a mosaic-affected rooted cutting from a fig tree of Spanish origin were examined by electron microscopy (thin sections, leaf dips) and by RT-PCR. The cutting was collected years ago from a diseased tree of undetermined cultivar in the vicinity of Murcia (Spain). Thin-sectioned mesophyll cells contained: (i) clusters of the so-called "double-membrane bodies", which are consistently associated with fig mosaic and were recently identified as particles of the putative disease agent (Fig mosaic virus, FMV), a virus with a negative-sense RNA genome, proposed as a possible member of the newly etablished genus *Emaravirus* (Elbeaino *et al*., 2009); (ii) filamentous virus-like particles, scattered in the ground cytoplasm or arranged in discrete bundles. The same particles were abundantly present in leaf dips and were clearly decorated by an antiserum to Fig latent virus 1 (FLV-1), a recently described putative member of the genus *Trichovirus* (Gattoni *et al*., 2009). No virus particles were apparently present in phloem tissues. RT-PCR assays from leaf extracts using FLV-1-specific primers (CPtr1 CCATCTTCACCACACAAATGTC; CPtr2 CAATCTTCTTGGCCTC CATAAG) designed on the viral coat protein gene, yielded the expected amplicon of 380 bp. Whereas the presence FMV particles has previously been reported from Spanish figs affected by mosaic (Martelli *et al*., 1993), to the best of our knowledge this represents the first record of FLV-1.

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DISEASE NOTE

FIRST REPORT OF *BEET SOIL-BORNE VIRUS* IN RHIZOMANIA-INFESTED SOILS OF SUGAR BEET FIELDS IN LEBANON

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During spring 2007, 15 sugar beet (*Beta vulgaris* subsp. *vulgaris* var. *altissima*) fields with a history of rhizomania disease (Choueiri *et al.*, 2001), were surveyed in the Bekaa Valley (Lebanon) to look for soil-borne fungal vectors of viral diseases (Mouhanna *et al.*, 2008). Five soil samples were collected from each field, regardless of the crop planted at the time of collection. Each soil sample was distributed among three 12×12 cm pots, then placed in a greenhouse at 22-25°C, RH between 80-85%, and 16 h photoperiod. Sugar beet cv. Hilma seeds were sown in each pot. After eight weeks, leaves were harvested and the roots were left in the soil until they dried. After additional three weeks, sugar beet seeds were re-sown in the same pots. Eight weeks later the plants were collected, their roots were washed thoroughly and ELISA-tested using polyclonal antisera (DSMZ Plant Virus Collection, Germany) to *Beet soil-borne virus* (BSBV) and *Beet necrotic yellow vein* (BNYVV). BSBV was detected in the roots of plants grown in 9 soil samples primarily from the central and west Bekaa. Roots extracts in 0.1 M phosphate buffer, pH 7 and 2.5% nicotine were mechanically inoculated to *Chenopodium quinoa*. Necrotic ringspots developed on the leaves and BSBV presence was confirmed by ELISA. BNYVV was identified in the roots of plants grown in 32 soil samples. In the BSBV-infected soil samples, BNYVV was detected in seven samples whereas two samples were infected with BSBV alone. BNYVV has previously been reported from Lebanon (Choueiri *et al.*, 2001). To our knowledge, this is the first report of BSBV occurring in Rhizomania-infested soils of sugar beet fields in Lebanon.

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DISEASE NOTE

TRACHEOMYCOSIS CAUSED BY *VERTICILLIUM TRICORPUS* ON *ACANTHUS MOLLIS* subsp. *MOLLIS* IN ITALY

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Bear's breech (*Acanthus mollis* subsp. *mollis,* family Acanthaceae) is a wild perennial plant grown as ornamental in private and public gardens. In the last three years, plants from gardens of the Bari and Foggia provinces (southern Italy) showed a disease condition characterized by light stunting, yellow leaves, brown streaking of the vascular tissues of stem, crown and roots, and wilting. A fungus with orange-yellow prostrate hyphae was consistently isolated from discoloured xylem tissues which, on malt agar (MA) produced abundant hyaline verticillated and branched conidiophores, with 3-4 phialides arising from each node. Ellipsoid to irregularly sub-cylindrical, hyaline conidia 4-11×1.2-2.7 µm in size, were produced singly at the apex of the phialides. Resting mycelium consisted of dark brown to blackish, swollen and torulose cells. Chlamydospores 8-11 µm in diameter were differentiated, as well as irregularly shaped microsclerotia 58×82 µm in diameter. According to these morphological features, this fungus was identified as *Verticillium tricorpus* (Isaac, 1953; Gould *et al*., 2003). Molecular analyses encompassing amplification and sequencing of the ITS1-5.8S-ITS2 region of ribosomal DNA of our fungal isolates (accession No. GQ258661, GQ258662) showed 99 to 100% similarity with *V. tricorpus* sequences from the database. Healthy 70-day-old bear's breech plants, potted in a steam disinfested soil mix in a greenhouse at 23-26°C were used for pathogenicity tests. Ten plants were inoculated by dipping roots into a conidial suspension (1.5×10^6 cfu ml^{-1}) of each of two isolates of *V. tricorpus.* Ten uninoculated plants served as control. First symptoms of wilting were observed 90 days after inoculation. Both isolates caused vascular discoloration, stunting, wilting and plant death. The pathogen was consistently re-isolated from infected plants, fulfilling Koch's postulates. Non-inoculated plants remained healthy. To our knowledge, this is the first report in Italy of a wilt condition of *A. mollis* subsp. *mollis* caused by *V. tricorpus.*

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DISEASE NOTE

FIRST REPORT OF WHEAT POWDERY MILDEW AND ITS SEVERITY IN THE GRAND-DUCHY OF LUXEMBOURG OVER THE 2003-2009 PERIOD

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In the framework of a study aiming at the monitoring and prediction of cryptogamic diseases of winter wheat, *Blumeria graminis* (DC.) E.O. Speer (= *Erysiphe graminis* f. sp. *tritici* E. Marchal), the powdery mildew agent, was identified for the first time in the Grand-Duchy of Luxembourg (GDL) in 2003, based on morphological characters. The disease was monitored weekly in wheat crops (starting from Zadoks' growth stage 30, pseudostem erection) in 2003-2009. Disease severity (leaf area affected) was recorded in four plots in four sites, representative of the different GDL agroclimatological zones, located in two regions: (i) Gutland (southern and central GDL) comprising the sites of Everlange (cv. Achat in 2003 and 2009), Burmerange (cvs Dekan in 2003 and Cubus in 2009) and Christnach (cvs Flair in 2003 and Boomer in 2009) and (ii) Oesling (northern GDL), comprising the Reuler site cropped with cvs Bussard in 2003 and Schamane in 2009. Cultivar susceptibility to powdery mildew according to the German scale is: Achat=5, Boomer=4, Bussard=4, Cubus=2, Dekan=1, Flair=4; Schamane=4; with most resistant=1 and most susceptible=9 (Anonymous, 2008). A significant difference in severity was observed between sites ($P<0.001$), cultivars ($P<0.001$) and years ($P<0.001$). The 2003 and 2009 cropping seasons showed the highest disease severity in Oesling (15% and 40%, respectively), whereas in Gutland the disease was negligible (less than 1% severity in both years). The disease appeared much earlier in Oesling (GS 30, pseudostem erection) than in Gutland (GS 39, flag leaf ligule visible). Two major climatic factors favoured the 2003 and 2009 disease outbreaks: a daily mean temperature comprised between 15°C and 22°C and relative humidity of at least 80% during May-June. Since these parameters complemented by rainfall data proved sufficient for modeling *Septoria tritici* leaf blotch on winter wheat (El Jarroudi *et al*., 2009), predicting wheat powdery mildew outbreaks may appear feasible on the same ground.

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DISEASE NOTE

FIRST REPORT OF NEEDLE BLIGHT CAUSED BY *PESTALOTIOPSIS GUEPINII* ON *PINUS WALLICHIANA* IN INDIA

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During a 2008-2009 survey for mycorrhizal diversity in conifer-dominated forests of western Himalaya (Jammu and Kashmir, India) small reddish-brown spots were observed on needles of blue pine (*Pinus wallchiania*). These gradually enlarged and coalesced, so that the entire needle became brown to reddish-brown usually starting from the tip. Premature defoliation could follow. Small black acervuli were present on symptomatic needles which, following eruption, released septate conidia measuring 20-26.2×6.1-7.9 (mean 21.1×6.2) µm. The conidia bore apical and basal appendages, were fusiform, smooth and exhibited concolourous median cells. The fungus was cultured on PDA at 25±1°C and, after 10-12 days incubation, produced a creamish-white, dense, mycelium with abundant distinct black acervuli. The mycelium was immersed, branched, septate and hyaline to pale-brown. Acervuli from culture released conidia morphologically similar to those observed on the host. Apical appendages of conidia were 2 to 5 (mostly 3), unbranched, apically obtuse and measured 16-33 (mean 20.2) µm in length. Basal appendages were 4 to 12 (mean. 6.4) µm long. Koch's postulates were fulfilled by inoculating needle on 1- to 2-year-old seedlings of *P. wallchiania.* Reddish brown spots developed on the needles two weeks after inoculation from which the pathogen was successfully reisolated. Based on symptomalogy and morphological characters, the fungus was identified as *Pestalotiopsis guepinii* (Desm.) Stey (Mordue, 1971), confirmed by Dr. P. N. Choudhary (National Centre for Fungal Taxonomy, New Delhi). Since there is no record of this fungus in the fungal flora of India (Bilgrami *et al.* 1991; Jamaluddin *et al.* 2004; Sarboy *et al.* 1996) this appears to be its first report of in the country on *Pinus wallichiana.*

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DISEASE NOTE

FIRST REPORT OF *BEAN YELLOW MOSAIC VIRUS* IN GLADIOLUS IN INDIA

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The majority of gladiolus plants growing in the botanical garden at National Botanical Research Institute of Lucknow (India) and adjoining area, shows symptoms of mosaic, colour breaking, stunting of the spikes, and reduction in flower size. Since the occurrence of *Bean yellow mosaic virus* (BYMV) in symptomatic plants was suspected, total RNA was isolated from the leaves of infected plants (Singh *et al*., 2002) and used in RT-PCR assays. A primer set (forward primer BYU-5'-TCTGACCAAGAA CAACTCAATGCAGG-3' and reverse primer BYD-5'CTAAATACGAACAC CAAGCATGGTGT-3') designed in the BYMV coat protein region was employed. Amplicons of the expected size (820 bp) were obtained from infected plants but not from symptomless, presumably healthy control plants. Viral amplicons were cloned and sequenced (accession No. EU868830). BlastN analyses showed 97% nucleotide sequence homology with BYMV strains infecting gladiolus from Japan (accession No. AB029439). This appears to be the first definite report of BYMV infecting gladiolus in India.

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DISEASE NOTE

FIRST REPORT OF *MURRAYA PANICULATA* AS HOST OF *COLLETOTRICHUM ACUTATUM*

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Postbloom fruit drop (*Colletotrichum acutatum*) is one of the most important citrus diseases in São Paulo, Brazil. The pathogen causes blossom blight and fruit drop, reducing yield. *Murraya paniculata* is a herbaceous perennial from the family Rutaceae. commonly used for fencing citrus groves. The objective of this work was to verify if *M. paniculata* could be a host for *C. acutatum.* Five monosporic isolates from diseased flowers of cv. Valencia sweet orange (*Citrus sinensis*) were identified by PCR using universal primer ITS4 (White *et al*., 1990) coupled with primer CaInt2 specific for *C. acutatum* (Sreenivasaprasad *et al*., 1996). Region ITS1-5.8S-ITS2 was amplified from all monosporic isolates using PCR with primers ITS1 and ITS4 (Talhinhas *et al*., 2002). BLAST analysis showed a 98-100% identity in the nucleotide level with known sequences of *C. acutatum*. A sequence from one monosporic isolate was submitted to GenBank (accession No. GQ994099). Five 2-year-old potted plants of *M. paniculata* and cv. Valencia, bearing 50 flowers each, were inoculated in a greenhouse, spraying blossoms with a conidial suspension of *C. acutatum* (10^5 conidia/ml). Blossoms were kept in a moist chamber for 48 h, at temperatures of 25-32 ºC. Typical lesions showing acervuli of the fungus developed on the petals of inoculated plants within 4 days on both hosts. No symptoms were observed in control blossoms sprayed with distilled water. The fungus reisolated from *M. paniculata* blossoms was identified as *C. acutatum.* This is the first report of blossom blight caused by *C. acutatum* to *M. paniculata.* This plant could be a source of inoculum for the pathogen in citrus orchards, but additional epidemiological studies are necessary to determine its importance.

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DISEASE NOTE

MULTIPLE VIRUSES IN LANZHOU LILY (*LILIUM DAVIDII* var. *UNICOLOR*) IN THE PEOPLE'S REPUBLIC OF CHINA

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Mixed infections by *Lily symptomless virus* (LSV), *Cucumber mosaic virus* (CMV), and *Lily mottle virus* (LMoV) were detected in Lanzhou lily (*Lilium davidii* var. *unicolor*) in the Gansu province of western China. The presence of the three viruses in infected plants was suspected following electron microscope observation of virus particles, and confirmed by DAS-ELISA and RT-PCR. Of 94 samples tested, 84 (89%) were infected with LSV and 90 (96%) with CMV, as shown by DAS-ELISA. All LSV-infected plants were co-infected with CMV, even though this latter virus was also found in single infections. The occurrence of LSV and CMV was confirmed by RT-PCR using primers designed in the coat protein sequence of the two viruses, which amplified products of 408 bp and 483 bp, respectively (Niimi *et al.*, 2003). LMoV was detected in a subset of plants co-infected with LSV and CMV (3 of 5) by RT-PCR with previously designed specific primers (Niimi *et al.*, 2003), which amplified a 570 bp coat protein gene fragment. To the best of our knowledge, this is the first report of multiple virus infection in Lanzhou lily.

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DISEASE NOTE

RHODOCOCCUS FASCIANS: CAUSAL AGENT OF FALSE BROOMRAPE OF TOBACCO IN GUATEMALA

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White succulent outgrowths of tobacco cv KY-17 roots observed at Zacapa (Guatemala) and diagnosed as "False broomrape", appeared first as tumour-like protuberances, that developed into leafy shoots when emerging from soil. Whereas tumour extracts observed in the electron microscope did not contain virus particles, several bacteria were isolated on Yeast-Dextrose-Agar (YDA). One, denoted C-66, caused a hypersensitive response in infiltrated tobacco leaves. Bacterial colonies were small, orange, round and smooth after 3-day incubation at 27°C. C-66 was Gram positive and rod-shaped, nitrate reduction, catalase and urease positive, oxidase and casein hydrolisis negative, non motile and strictly aerobic, as described for the genus *Corynebacterium* (Goodfellow, 1986). Testing by API Coryne system (BioMérieux, Italy) confirmed that C-66 belongs in the *Corynebacterium* genus. DNA from C-66 and the *Rhodococus fascians* strain CECT-3001 was tested by PCR (Ruimy *et al*., 1995), obtaining the expected 320 bp 16S ribosomal fragment. A subsequent analysis disclosed that C-66 rDNA sequence had a 99% nucleotide identity with *R. erythropolis* (accession No. EU070938) or *R. globerulus* (accession No. FM208198). For pathogenicity tests, six healthy tobacco plants were watered with an extract of 100 g tumor tissues in 1 liter of water and six plants with a bacterial suspension (10^8 CFU ml^{-1}) from a of C-66 48 h culture on YDA. Outgrowths like those of naturally infected plants developed on the roots of inoculated but not of control plants, and the bacterium isolated from them was identified by PCR as *R. fascians*. Positive results were obtained when PCR (Stange *et al*., 1996) was used to determine the presence in C-66 of the fas-1 gene of the linear plasmid pFi, which is present only in virulent strains of *R. fascians*. To our knowledge, this is the first report of *R. fascians* in Guatemala and its first identification as the causal agent of False broomrape.

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