

# First report of cucumber mosaic virus in Edgeworthia chrysantha in France and Italy

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#### **DISEASE NOTE**

## THE REMOTE CITROID FRUIT TREE GLYCOSMIS PENTAPHYLLA IS A HOST OF CITRUS LEPROSIS VIRUS C AND EXHIBITS NOVEL LEPROSIS SYMPTOMS

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Citrus leprosis virus C (CiLV-C), the causal agent of one of the main virus disease of citrus in Brazil, is considered to have a narrow host range. However, recent studies have shown that some weeds, hedgerow and windbreak plants can host CiLV-C, thus may play a role in the epidemiology of the disease (Bastianel et al., 2006). We now report that CiLV-C can infect Glycosmis pentaphylla Retz. DC., a rutaceous shrub native to tropical Asia. G. pentaphylla is grown for its edible ripe fruits in gardens that can be near citrus groves. G. pentaphylla leaves were infested with presumably viruliferous Brevipalpus phoenicis mites, the vector of CiLV-C, which had been reared on leprosisinfected plants for 72 h. Twenty-four days after infestation, localized symptoms appeared as conspicuous dark spots, with a darker center and irregular borders surrounded by a small chlorotic halo. These spots differed significantly from the well-defined chlorotic to necrotic lesions often seen in leprotic Citrus spp. tissues. The symptoms remained unchanged for 110 days. CiLV-C was detected in symptomatic leaves by RT-PCR using specific primers that amplify a region of its putative movement protein gene (Locali et al., 2003). Amplified products had the expected 344 bp size. Thirty-three amplicons were cloned and sequenced (GenBank accession No. EU257507). Their deduced amino acid sequence was 93% identical to that of a Brazilian isolate of CiLV-C (YP\_654542.1), confirming the identification of the virus transmitted. This is the first report of CiLV-C infecting a remote citroid fruit tree, and G. pentaphylla is the only so far known CiLV-C host exhibiting such particular symptoms.

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#### DISEASE NOTE

## FIRST REPORT OF *PHOMOPSIS ACTINIDIAE* CAUSING CANKERS ON SHOOTS OF KIWIFRUIT IN GREECE

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In June and through summer 2007, wilted and blighted shoots with distinct dark cankers were observed on kiwifruit plants of cvs Hayward and Tsechelidis in the province of Imathia (Greece). Isolations made on acidified potato dextrose agar yielded a fungus which was identified as Phomopsis actinidiae (Henn.) Died. on the basis of morphological and sporulation characters. Circular chalk white-colored aerial mycelial mats developed in culture after incubation at 25°C for 7 days. After additional 3-4 weeks, black, spherical or bluntly conical pycnidia 230-500 um in size bearing a- and b-conidia were formed all over the mycelial mats. The conidia, extruded as yellowish or milky conidial masses, were hyaline, unicellular, fusiform, filiform to hamate. Koch's postulates were fulfilled by inoculating with agar plugs from fungal colonies, 20 segments (6 cm in length and 1.5-2 cm in diameter) of 1-yearold woody shoots of kiwifruit cv. Hayward as described by Jeffers et al. (1981). Ten segments, used as controls, were wounded and inoculated with an agar disk without fungal mycelium. Shoot segments inoculated with the fungus developed cankers similar to those observed in the field, from which the same pathogen as that used for inoculations was recovered. Controls remained symptomless. P. actinidiae has been reported as the causal agent of leaf blight and fruit rot of kiwifruit in other countries (Jeong et al., 2008) but, to our knowledge, this is the first report of its occurrence in Greece, on kiwifruit showing cankers on the shoots.

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#### **DISEASE NOTE**

## FIRST REPORT OF *PHOMOPSIS DIOSPYRI* CAUSING SHOOT BLIGHTS ON PERSIMMON TREES IN GREECE

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In July and through summer 2006, persimmon trees (Diospyros lotus) showing wilted and blighted shoots were observed in commercial orchards of the Imathia prefecture (northern Greece). Close examination of the cortical tissues of affected shoots revealed the presence of darkcoloured cankers. Isolations from the lower margins of these cankers made by plating tissue fragments ca. 3 mm in size on acidified potato dextrose agar, yielded a fungus which, based on morphological and sporulation characteristics, was identified as Phomopsis diospyri (Sacc.) Trav. & Spessa. Fungal colonies in culture had a white-coloured mycelium on whose surface dark-pigmented, flask-shaped pycnidia developed, oozing one-celled, ovoid to fusoid curved conidia as milky or yellowish mucilaginous drops. Koch's postulates were fulfilled by inoculating with agar plugs from fungal colonies 20 segments (6 cm in length and 1.5-2 cm in diameter) of 1-year-old woody persimmon shoots as described by Jeffers et al. (1981). Ten segments, used as controls, were wounded and inoculated with an agar disk without fungal mycelium. Shoot segments inoculated with the fungus developed cankers similar to those observed in the field, from which the same pathogen as that used for inoculations was recovered. Controls remained symptomless. P. diospyri has been reported as the causal agent of shoot cankers and fruit rots of persimmon in other countries (Horst, 2008). However, to our knowledge, this is the first report from Greece documenting attacks of P. diospyri to persimmon.

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#### **DISEASE NOTE**

## FIRST REPORT OF A 16SrV-B GROUP PHYTOPLASMA ASSOCIATED WITH A LEAFROLL-TYPE DISEASE OF APRICOTS IN NORTHERN CHINA

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In July 2007, a phytoplasma-like disease was observed on apricot plants (Prunus armeniaca L.) grown in the Chinese province of Shaanxi. Affected trees were stunted and showed yellow, small and upward curled leaves. Incidence of this disease was less than 15%. From samples collected from 14 symptomatic and six symptomless plants in six different orchards, total DNA was extracted from *ca*. 0.5 g of leaf midrib or stem phloem tissue using a modified cetyltrimethyl-ammonium bromide (CTAB) method (Qi et al., 2004). DNA extracts were analyzed by nested PCR, using the 16S rRNA gene universal primer pairs R16mF2/R16mR1 followed by R16F2n/R16R2 (Gundersen et al., 1996), which amplified a 1.4 kb and a 1.2 kb product, respectively, from symptomatic plants only. RFLP analysis of the 1.2 kb products with AluI, MseI, HhaI, HpaI, RsaI, BfaI, HinfI and TaqI endonuclease (Lee et al., 1998), showed that all symptomatic plants were infected by a phytoplasma belonging to the subgroup B of the elm yellows group (16SrV-B) (Candidatus Phytoplasma ziziphi). The nucleotide sequence of cloned 16SrDNA (Gen-Bank Accession No. FJ572660) showed the highest identity (99%) with comparable sequences of subgroup 16SrV-B, thereby confirming the results of RFLP analysis. Phytoplasmas of 16SrV-B subclade have previously been detected in cherry in China and in peach in India (Lee et al., 2004), and in Poland in apricot imported from China (Cieslinska et al., 2004). However, to our knowledge, this is the first molecular evidence of the presence of a phytoplasma of the 16SrV-B subgroup associated with a leafroll-type disease of apricot in northern China.

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#### **DISEASE NOTE**

## POWDERY MILDEW OF ORIGANUM VULGARE CAUSED BY GOLOVINOMYCES BIOCELLATUS

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Oregano (Origanum vulgare L.), an aromatic perennial herb native to Mediterranean, is widely grown throughout the world. In 2003, plants cultivated in La Plata (Buenos Aries province) showed the upper leaf surface and part of the stems covered by a whitish fungal mycelium, under which light to dark brown necrotic tissues were observed. Affected plants underwent defoliation. The fungus had an amphigenous mycelium bearing indistinct appressoria. Conidiophores tapering towards the base, frequently twisted, sometimes branched, 44-78 (average 57.7) x10-15 (average 11) µm long, followed by 1 to 3 or 4 shorter cells. Foot-cells normally straight. Chains of up to six immature conidia showing sinuate edge line. Fibrosin bodies absent. Mature conidia doliiform, sometime subcylindric, 24-36 (average 31) x13-16 (average 14) µm in size. One or two germ tubes on the shoulder of the conidia, usually with indistinct appressorium. Chasmothecia absent. Based on the above, the pathogen was identified as Golovinomyces biocellatus (Ehrenb.) V.P. Gelyuta. To test its pathogenicity, leaves from a diseased oregano plant were gently pressed on those of ten healthy plants of O. vulgare and ten of Origanum x hybrid. Inoculated and control plants were covered with plastic bags and maintained in different greenhouse compartments for 48 h at 22-25°C. Symptoms appeared on inoculated O. vulgare plants after one week, but not on controls and Origanum x hybrid plants, suggesting a possible pathogenic specialization of G. biocellatus on these hosts. Powdery mildew of oregano was reported from Europe (Braun, 1995; Amano, 1986) and China (Amano, 1986) as caused by Erysiphe galeopsidis and, recently, in Switzeland as incited by Neoerysiphe galeopsidis and G. biocellatus (Bolay, 2005). This is the first report of G. biocellatus on oregano in Argentina.

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#### **DISEASE NOTE**

### FIRST REPORT OF *APPLE STEM GROOVING VIRUS* FROM POME AND STONE FRUITS IN INDIA

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Apple stem grooving virus (ASGV), the type member of the genus *Capillovirus*, induces severe pitting and grooving of the xylem, brown line and graft union abnormalities, reduced vigour of the canopy and an overall decline in susceptible Malus species (Yoshikawa, 2000). It also infects apricot, cherry, and pear. Infection rate in apple can be as high as 80-100% and yield losses my reach 40%. During a survey in the temperate fruit-growing Indian districts of Himachal Pradesh (Shimla, Solan and Palampur) and Jammu & Kashmir (Srinagar), preliminary evidence for the presence of ASGV was obtained by DAS-ELISA, using a commercial kit (Adgen Phytodiagnostics, UK). To confirm the record, total RNA was extracted using RNeasy Plant Mini kit (Qiagen, Germany) from one of the positive apple samples (cv. Starkrimson) which had reacted strongly in ELISA. PCR primer pair ASGV6396 (reverse), 5'-CTG CAA GAC CGC GAC CAA GTT T-3' and ASGV5641 (forward), 5'-ATG AGT TTG GAA GAC GTG CTT C-3', yielding an amplicon of 755 base pairs (bp) was used to amplify the complete ASGV coat protein (CP) gene, as described by Nickel et al. (2001). ASGV was detected in eleven different cultivars of apple, nectarine, plum, cherry, quince and apricots by DAS-ELISA and dot blot hybridization using the cloned CP of the present isolate as a probe. Multiple alignment of CP sequences of the Indian ASGV isolate showed 100% identity at the amino acid level with a Brazilian isolate from apple (accession No. AF438409) whereas identities of 95-98% and 95-100% were found with a citrus strain (Citrus tatter leaf virus) and other isolates, respectively. To our knowledge, this is the first report of ASGV infection to pome and stone fruit trees in India.

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#### **DISEASE NOTE**

## FIRST REPORT OF CUCUMBER MOSAIC VIRUS IN EDGEWORTHIA CHRYSANTHA IN FRANCE AND ITALY

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In 2004 and 2008, symptoms of mosaic and oak-leaf patterns were observed on the leaves of Edgeworthia chrysantha Lindl. (Giant leaf paper plant), family Thymelaeaceae, in the botanical gardens of Villa Taranto (Verbania, northern Italy) and Lyon (France). Symptoms on Nicotiana tabacum cvs. Xanthi and Samsun, Chenopodium quinoa, C. amaranticolor, Vigna unguiculata cv. Black and Cucumis sativus cv. Poinsett inoculated mechanically with extracts of infected E. chrysantha plants were as those typically induced by Cucumber mosaic virus (CMV). Sap extracts from these plants contained isometric particles 30 nm in diameter and reacted positively with polyclonal antibodies specific to CMV in DAS-ELISA (Devergne and Cardin, 1975). To fulfil Koch's postulates, one Italian Edgeworthia isolate was propagated in Xanthi tobacco plants after isolation from local lesions of Vigna unguiculata and used to inoculate a set of two three-year-old healthy E. chrysantha plants either manually or with aphids (Myzus persicae Sulz.) that were allowed to acquire the virus from infected tobaccoes. Controls consisted of mock-inoculated healthy Edgeworthia plants. Ten months after inoculation, mosaic and yellow spots developed on the young leaves of the four inoculated plants, from which a positive response for CMV was obtained in DAS-ELISA. Controls remained symptomless and DAS-ELISA was negative for CMV. To the best of our knowledge, this is the first report of CMV in *E. chrysantha*.

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#### DISEASE NOTE

## FIRST REPORT THE CHERRY STRAIN OF CHERRY LEAFROLL VIRUS ON WALNUT AND PECAN TREES IN SYRIA

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During a field survey carried out in Syria in spring and autumn 2008, dieback of some shoots and branches and chlorotic to pale vellow discolorations were observed on the leaves of local walnut (Juglans regia L.) trees. In Hama governorate, some of trees grafted on Juglans nigra rootstocks showed a black line at the graft union. No obvious symptoms were seen on pecan [Carya illinoensis (Wangenh) K. Koch] trees except for leaf shedding. A total of 339 samples of leaves, shoots and flower clusters from five local walnut cultivars, Balahseen, Kalesh, Bukaei, Baladi and Kastal Gandar (297 samples), four pecan cultivars, Riverside, GraTex, GraZona and Choctaw (24 samples) and from J. nigra rootstocks (18 samples), were collected during May-October 2008 from seven Syrian governorates: Damascus-countryside, Al-Qunaitara, Homs, Hama, Idleb, Aleppo and Lattakia. All samples were tested for the presence of Cherry leaf roll virus by DAS-ELISA (Clark and Adams, 1977) using a commercial kit (Bioreba, Switzerland). CLRV was detected in 22 walnut samples (7.4% infection) of cvs Balahseen and Baladi, and in four samples (16.7% infection) of the four pecan cultivars. CLRV infections were found mainly in the north western and coastal regions of the country (Idleb and Lattakia, with 13 and 3 infected samples, respectively), and in the central region (Hama and Homs, with 6 and 4 infected samples, respectively). No infected trees were found in Damascus-countryside and Al-Qunaitara in the southern region, and Aleppo in the northern region. This is the first record of CLRV from walnut and pecan trees in Syria. The presence of his virus, however, had been previously reported on Syrian olive trees (Alabdullah et al., 2005).

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#### **DISEASE NOTE**

## FIRST REPORT OF BLACK ROT OF APPLE CAUSED BY *DIPLODIA SERIATA* IN INDIA

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Apple is a predominant fruit crop in Jammu and Kashmir state (India), grown over an area of 107,925 ha, with an annual production of 1.09 million metric tonnes (Anonymous, 2005). In July-August 2006 and 2007, rotting fruits were observed in various orchards of the Kashmir valley. Incipient symptoms were small brown spots on the fruit surface, which enlarged forming reddish-brown concentric rings with scattered pycnidia. The fruits finally turned black. Isolations on oat meal agar consistently yielded cottony grayish-white colonies that produced black pycnidia within 10-12 days of incubation at 24±1°C. Conidiogenous cells were hyaline, smooth and thin-walled. Conidia were cylindric to ellipsoid, rounded at both ends, some truncate at the base, initially hyaline but dark-brown when mature, smooth walled, finely ornamented on the inner surface, and measured 20.9-28.5 × 10.64-15.20 µm (average  $22.2 \times 12.8 \,\mu$ m). A few conidia developed unusual median septa (not true septa) at a later stage. Pathogenicity was proved by inserting 3 mm mycelial disc inside 5 mm long and 2-3 mm deep cuts on each of ten surfacesterilized fruits of cv. Golden delicious. Five non-inoculated fruits served as control. Fruits were incubated at 24±1°C. Symptoms developed within 3-4 days and re-isolations yielded the original fungus. Control fruits remained healthy. Based on morphological characters (Phillips, 2006) the fungus was identified as Diplodia seriata De Not (culture deposited at the National Center of Fungal Taxonomy, New Delhi, vide accession no. 2835.09). D. seriata has been reported as the cause of black rot of apple in Holland, Belgium and northern Germany but, to our knowledge, this is the first record of it from India.

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## FIRST REPORT OF FUNGI ASSOCIATED WITH WOOD DISCOLOURATION OF GRAPEVINE ROOTSTOCKS FROM LEBANESE NURSERIES

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Self-rooted and grafted grapevine rootstocks are supposed to be the main sources of inoculum for several fungal pathogens in new vineyards. Phaeomoniella chlamydospora (W. Gams, P. Crous, M.J. Wingf. & L. Mugnai) P. Crous & W. Gams and Phaeoacremonium spp. are frequently recovered from young vines and are supposed to be the main causal agents of young vine decline (Mugnai et al., 1999). Cylindrocarpon spp. and Botryosphaeria spp., the causal agents of black-foot and black dead arm diseases, respectively, have also been associated with young vine decline (Phillips, 2002; Petit and Gubler, 2005). In January 2008, at uprooting time, a total of 115 samples of one-year-old grafted and self-rooted rootstocks were randomly collected in six nurseries in the Bekaa valley (Lebanon). Each vine was transversely cut in three points (1 cm above the crown, in the middle, and 1 cm below the apex or the graft union), and observed for the presence of wood alterations. The severity of wood discoloration was assessed using an empirical scale with six classes, and the McKinney's Index (IMK) was then calculated. Discoloured wood fragments were placed on malt extract agar additioned with 0.5 g l<sup>-1</sup> streptomycin sulphate for mycological analysis. Wood alterations were more severe at the crown level (IMK mean value: 38.8%) and at the apex (IMK: 33.1%), whereas IMK was 18.0% in the middle of the rootstocks. Cylindrocarpon spp. proved to be the most common fungi associated with wood discoloration (11% of samples), while P. chlamydospora, Botryosphaeria spp. and Phaeoacremonium spp. were associated with discoloured tissues in 4, 3.5 and 1% of the samples, respectively. To our knowledge, this is the first report of fungi responsible for wood discolouration of self-rooted and grafted grapevine rootstocks in Lebanese grapevine nurseries.

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#### DISEASE NOTE

## OCCURRENCE OF *PSEUDOMONAS VIRIDIFLAVA* ON TOMATO IN BRAZIL

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During March to May 2008, a severe disease characterized by leaf necrosis was observed in Brazil on tomato cvs Saladinha, Debora Max and Carmen, grown in Mucugê (State of Bahia) and Apiaí and Botucatu (State of São Paulo). Fluorescent, opaque and yellowish colonies of a rod-shaped Gram-negative bacterium were obtained on King' B medium following isolations from diseased tissue. Three bacterial strains were analysed with the MicroLog 4.2.05 System (Biolog, Hayward, USA) and identified as Pseudomonas viridiflava with a similarity index ranging from 66 to 83%. Biochemical and physiological characterization showed that all strains were aerobic, catalase positive, oxidase, levan, arginine dihydrolase, and nitrate reduction negative. They also showed pectinolitic activity, induced hypersensitive reactions in tobacco leaves, and produced acid from mannitol and sorbitol but not from sucrose. These results are in line with those reported for tomato strains of P. viridiflava (Goumas et al., 1999). For pathogenicity tests bacterial suspensions (107 CFU ml-1) were sprayed on the leaves of tomato plants of cv. Santa Clara in which they induced necrotic spots within five days from inoculation. This was taken as evidence that the bacterial isolate used for inoculation is pathogenic to tomato. In Brazil, P. viridiflava has previously been reported a the agent of diseases of several cultivated plants (Malavolta Júnior et al., 2008) but not of tomato. Thus, to the best of our knowledge, this is the first report of P. viridiflava infections to tomato in Brazil.

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#### DISEASE NOTE

## FIRST REPORT OF *TRICHODERMA PLEUROTUM* ON OYSTER MUSHROOM CROPS IN SPAIN

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In Spain, oyster mushroom (*Pleurotus ostreatus*) is the second commercially most important edible mushroom. In March 2008, a severe outbreak of compost green mould was observed in oyster mushroom farms in southeast Spain (Albacete province), which resulted in substantial crop losses. The fungus was isolated and its morphological and cultural characteristics were consistent with those of Trichoderma pleurotum (Komon-Zelazowska et al., 2007). A Trichoderma isolate (P4) from P. ostreatus substrate was sequenced after PCR amplification. Sequence analysis of ITS1 and ITS2 was performed with the aid of the program TrichOKey 2 available online (www.ISTH.info) and identified P4 as Trichoderma pleurotum (accession No. FJ418567). When compared with DNA sequences available in databases, the P4 sequence showed 99% similarity with a strain of T. pleurotum (EU280069.1). In the first of two tests, the pathogenicity of the fungus was tested by spraying several pots containing five-day-old P. ostreatus substrate (150 g) with a 5 ml suspension of 10<sup>6</sup> conidia/ml sterile water. Sterile water was sprayed on controls. Pots were sealed with parafilm and placed in a growth chamber at 25°C. Eighteen days later, the pathogen was successfully recovered from 50% of the inoculated pots. Controls did not show symptoms. In the second test, blocks containing ten-day-old P. ostreatus substrate (3 kg) were sprayed with 10 ml of the same conidial suspension as above. Sterile water was sprayed on controls. The blocks were covered with a polyethylene film and placed in a mushroom growing room at spawn-run temperature. Two weeks later, T. pleurotum was re-isolated from 50% of the inoculated blocks. Controls remained symptomless. This is the first report of T. pleurotum causing green mould disease in oyster mushroom farms in Spain.

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#### **DISEASE NOTE**

## FIRST REPORT OF WHITE MOLD (Sclerotinia minor) OF SOYBEAN IN IRAN

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In summer 2008, some wilted soybean plants, scattered throughout the field, were observed in the Kordkuy area of the Golestan province (northern Iran). Watersoaked brownish lesions were initially present on the stems, extending upward and downward. Then, lesions appeared on other organs and turned withish, so that large bleached areas developed on stems, shoots and pods. Cottony and white mycelial growth was observed on all diseased plant organs. Black, round to irregularly shaped sclerotia of various size (525-575 x 1425-2050 µm) formed on the stems, in the stem pith (becoming visible only when the stem is opened) and, abundantly, on pods and seeds. Infected seeds were flattened and shriveled, and had a cottony appearance. The fungus grew on potato dextrose agar (PDA) from plated sclerotia, and was identified as Sclerotinia minor Jagger, based on symptoms and morphological characteristics (Singleton et al., 1993). Fungal colonies on PDA were nearly colorless at first, then turned white and acquired a cottony appearance. Small- to average-sized black sclerotia were produced within a week from culturing. Pathogenicity of the fungus was assessed by placing mycelial disks or sclerotia on the stems of healthy soybean seedlings and observing symptom development on inoculated plants. White mold (or Sclerotinia stem rot), a major disease of soybean in some areas of the world mainly caused by S. sclerotiorum (Sinclair and Backman, 1993), to the best of my knowledge, has not been reported from Iran. S. minor has a wide host range (Melzer et al., 1997). Soybean is the fourth host of this fungus in Iran, after eggplant, sunflower, and tomato (Ershad, 1995).

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## A NEW CHINESE ISOLATE OF *PRUNUS NECROTIC RINGSPOT VIRUS* FROM SWEET CHERRY

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The cherry industry is greatly expanding in China, but low yields and a degenerative condition of the crop are widespread problems caused primarily by Prunus necrotic ringspot virus (PNRSV) (Zhou et al., 1996). Surveys carried out in May and June 2008 in cherry orchards of Shaanxi province, disclosed the presence in most plantations of symptoms, such as necrotic rings and tatter leaf, typically induced by PNRSV infections. The presence of this virus was ascertained by ELISA, also in symptomless trees, using a commercial kit (Agdia, USA). Total RNAs, extracted from cortical scrapings of diseased cherry trees, served as template for RT-PCR assays, using the virus-specific primer pairs PNRSV-CP1:5'-ATGGTTTGCCGAATTTG-CAAT-3', PNRSV and CP2:5'- CTAGATCTCAAGCAG GTCCTCAT-3) designed on a highly conserved stretch of the coat protein sequence. The expected 681 bp product was amplified, cloned in pMD18-T vector (TaKaRa, Dalian, China) and transformed into competent cells of E.coli strain JM109. Some PCR-positive clones were selected and sequenced. This sequence, which comprised the complete CP gene of the PNRSV ShaaXi isolate was deposited in GenBank under the accession No EU869295. A BLAST analysis showed that our sequence had 98.2%, 97.7% and 93.4% identity at the amino acid level, respectively, with comparable sequences of PNRSV isolates from different hosts from Poland (accession No. DQ983498), India (AM493717), and Yunnan (China) (AY684271). Since the Yunnan virus isolate came for rose, ours is the first report of a complete CP sequence of PNRSV from cherry trees in China.

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