Disease Notes

First Report of *Pantoea* sp. Induced Soft Rot Disease of *Pleurotus eryngii* in Korea. M. K. Kim, J. S. Ryu, and Y. H. Lee, Division of Plant Environmental Research, Gyeongsangnam-do Agricultural Research and Extension Service, Jinju 660-360, Korea; and H. D. Yun, Research Institute of Life Science, Gyeongsang National University, Jin Ju 660-701, Korea. Plant Dis. 91:109, 2007; published on-line as DOI: 10.1094/PD-91-0109A. Accepted for publication 2 October 2006.

The king oyster mushroom, *Pleurotus eryngii*, has become a popular crop because of its unique flavor and texture and is cultivated in many areas in Korea. In 2003, symptoms of water-soaked lesions and soft rot in the stipes and pileus of cultivated *P. eryngii* was observed in Jinju, Korea. Diseased tissue was plated on nutrient media. Dominate colonies were yellow, convex, circular with smooth margins, and had a shiny texture. Computer analysis of the data gathered, using the API kit (50CHE, bioMérieux, Marcy-l'Etoile, France), showed that the strain belongs to the *Enterobacteriaceae*. Although the API system did not give an exact identification, the metabolic profile of the bacterial strain closely resembled the database profile of *Pantoea* sp. (positive for acid production from the fermentation of D-fructose, D-galactose, D-glucose, D-trehalose, and D-ribose and negative for oxidase, urease, pectate, and thiosulfate). The 16S rDNA sequence of the bacterium was determined (GenBank Accession No. AY530796). When compared with those in GenBank, the bacterium was determined to belong to the *Enterobacteriaceae* family of the *Gammaproteobacteria*, and the highest degree of sequence similarity was found to be with *Pantoea ananatis* strain BD 588 (97.4%) and *Pantoea ananatis* strain Pna 97-1 (97.3%). In the phylogenetic tree, the bacterium clearly related to the *Pantoea* lineage, as evidenced by the high bootstrap value. A BLAST search with 16S rDNA sequence of the bacterium supported the API results that the isolate belongs to a species of *Pantoea*. Pathogenicity tests of this new *Pantoea* isolate were carried out with bacterial suspensions (approximately 1×10^6 CFU/ml) that were grown for 24 h in Luria-Bertani broth cultures. These were used to inoculate directly on the mycelia of *P. eryngii* that had been cultivated for 35 days in a plastic bottle. The water and broth were also inoculated to another set of bottles as a control experiment. Inoculated bottles were incubated in a cultivation room at 16 to 17°C with relative humidity between 80 and 95%. Early symptoms of the disease included a dark brown water drop that developed on hypha and primordium of the mushrooms after 5 to 7 days. After 13 days, water-soaked lesions developed on the stipes and pileus, and the normal growth of the mushrooms was inhibited. An offensive odor then developed along with a severe soft rot that was similar to the disease symptoms observed under natural conditions. Mushrooms in control bottles did not develop symptoms. Koch's postulates were fulfilled by isolating bacteria from typical lesions from inoculated mushrooms that were identical to the inoculated strain in colony morphology and biochemical characteristics. *Pantoea ananatis* was first reported as a pathogen of pineapple fruit causing brown rot (3). Several bacterial diseases, such as brown blotch on cultivated mushrooms by *Pseudomonas tolaasii* (2) and bacterial soft rot on winter mushroom by *Erwinia carotovora* subsp. *Carotovora*, causing severe damage to mushrooms are known (1). However, no *Pantoea* sp. induced disease of edible mushroom has been previously reported. To our knowledge, this is the first report of soft rot disease on *P. eryngii* caused by *Pantoea* sp.

References: (1) H. Okamoto et al. Ann. Phytopathol. Soc. Jpn. 65:460. 1999. (2) S. G. Paine. Ann. Appl. Biol. 5:206. 1919. (3) F. B. Serrano. Philipp. J. Sci. 36:271, 1928.

***Phytophthora infestans* Mating Types on Tomato (*Solanum lycopersicum*) in Southern Spain.** J. M. Segura, M. de Cara, M. Santos, and J. Tello, Universidad de Almeria, Departamento. Producción Vegetal, 04120 Almeria, Spain. Plant Dis. 91:109, 2007; published on-line as DOI: 10.1094/PD-91-0109B. Accepted for publication 6 October 2006.

During 2004, an unusual spread of *Phytophthora infestans* on tomato plants in greenhouses located in Almería and Granada provinces, southern Spain, was observed. Infected plants had water-soaked, brown spots on leaves and stems and necrotic areas with white mold on the surface of fruits. Three isolates were obtained by plating diseased tissue on V8 juice agar medium and maintained on rye agar at 18°C. These isolates were analyzed for the mating type. Crosses were carried out using V8 juice agar and rye agar. The two parental isolates US1 (A1) and US8 (A2) were both provided by W. E. Fry, Cornell University, Ithaca, NY. Two of the Spanish isolates were homothallic and the other isolate belonged to the uncommon mating type A1A2. To confirm the occurrence of the two mating types, 43 single-sporangium progeny were produced and analyzed from the A1A2 mating type. Thirty eight isolates were A1, two were A2, one was A1A2 mating type, and two were sterile. Assessment of five single-sporangium progeny from the homothallic type resulted in two A1, two homothallic, and one sterile isolate. A1A2 isolates produced oospores when crossed with either A1 or A2, but not when self-crossed. Previously, the A1A2 mating type has been found in Israel in the field and was obtained from oospores produced on tomato seeds (2,3). Since 2003, mating types of *P. infestans* isolates recovered from potato (60) and tomato (8) in southern Spain have been characterized. Seventy-five percent of the isolates recovered from potato were A1 and 25% were A2 mating types. Isolates recovered from tomato were 50% A1 and 50% A2 (1). To our knowledge, this is the first report of the occurrence of the A1A2 mating type and homothallic *P. infestans* isolates on tomato in Spain.

References: (1) E. Andujar et al. Congr. Sociedad Española de Fitopatol. 12:244, 2004. (2) E. Rubin and Y. Cohen. Phytoparasitica 32:237, 2004. (3) E. Rubin and Y. Cohen. Plant Dis. 90:741, 2006.

First Report of Southern Blight Caused by *Sclerotium rolfsii* on Lily in China. X. M. Yang, J. H. Wang, S. P. Qu, and L. H. Wang, Supervision and Testing Center for Flower of Agriculture Ministry, Yunnan Academy of Agriculture Science, Kunming 650205, China. Plant Dis. 91:109, 2007; published on-line as DOI: 10.1094/PD-91-0109C. Accepted for publication 3 October 2006.

Lily (*Lilium* spp.) is an economically important cut flower cultivated in China. The soilborne fungus, *Sclerotium rolfsii*, is a major pathogen on many plants. During July 2005, severe basal stem rot and bulb rot symptoms were observed on an oriental lily cultivar (Sorbonne) in some commercial fields in northern Kunming (China). Disease incidence ranged from 20 to 30% across fields. Leaves of infected plants were chlorotic initially. As the disease progressed, stems and bulbs rotted and plants wilted. In the presence of abundant moisture, a white mycelium occurred on infected tissues. White or light-to-dark brown sclerotia (1 to 3 mm in diameter) developed from mycelium. Fungal isolates from infected bulbs grown on potato dextrose agar (PDA) produced white mycelia and 1- to 2-mm diameter dark brown sclerotia. Sclerotia were nearly round with smooth surfaces and distributed over the entire colony. Isolates were identified as *S. rolfsii* on the basis of mycelial characteristics and color, size, and distribution of sclerotia. Pathogenicity was tested in the greenhouse on oriental lily cv. Sorbonne grown in pots (1 plant per pot, five replicates). Inoculum that consisted of 1 g per pot of wheat kernels infested with mycelium and sclerotia was placed at the base of each inoculated plant. Five noninoculated plants served as controls. The inoculation trial was repeated once. After inoculation, all plants were covered with a polyethylene bag for 72 h and kept at temperatures ranging between 25 and 27°C. Inoculated plants developed symptoms of leaf yellowing within 12 days, soon followed by the appearance of white mycelium and sclerotia, and then eventually wilted. Control plants remained symptomless. *S. rolfsii* was reisolated from inoculated plants. To our knowledge, this is the first report of southern blight caused by *S. rolfsii* on lily in China. Infection of lily bulbs by *S. rolfsii* may cause losses in production fields in China, and the presence of infected bulbs may also interfere with bulb shipment.

(*Disease Notes* continued on next page)

Detection of *Poinsettia mosaic virus* by RT-PCR in *Euphorbia* spp. in New Zealand. B. S. M. Lebas, F. M. Ochoa-Corona, D. R. Elliott, J. Z. Tang, and B. J. R. Alexander, Investigation and Diagnostic Centre, Biosecurity New Zealand, Ministry of Agriculture and Forestry, P.O. Box 2095, Auckland 1140, New Zealand. Plant Dis. 91:110, 2007; published on-line as DOI: 10.1094/PD-91-0110A. Accepted for publication 12 October 2006.

Euphorbia pulcherrima (poinsettias) are commonly infected with *Poinsettia mosaic virus* (PnMV), which resembles the *Tymovirus* genus in its morphology and viral properties (2) but is closer to the *Marafivirus* genus at the sequence level (1). Symptoms induced by PnMV range from leaf mottling and bract distortion to symptomless (2). The presence of PnMV in plants imported into New Zealand had never been proven. Leaves of 10 *E. pulcherrima* samples and six samples from other *Euphorbia* spp. (*E. atropurpurea*, *E. lambii*, *E. leuconeura*, *E. mellifera*, *E. milii*, and *E. piscatorial*) were collected in the Auckland area, North Island in 2002. Isometric particles of 26 to 30 nm in diameter were observed with electron microscopy in 3 of 10 *E. pulcherrima* samples. These three samples produced systemic chlorosis and crinkling symptoms on mechanically inoculated *Nicotiana benthamiana*, which tested PnMV positive by double-antibody sandwich (DAS)-ELISA (Agdia, Elkart, IN). No particles or symptoms on *N. benthamiana* were observed with the other *Euphorbia* spp., which were also PnMV-negative by DAS-ELISA. A reverse transcription-polymerase chain reaction (RT-PCR) was developed to further characterize PnMV. Specific primers were designed from the PnMV complete genome sequence (Genbank Accession No. AJ271595) using the Primer3 web-based software (4). Primer PnMV-F1 (5′-CCTGTATTGTCTCTTGCCGTCC-3′) and primer PnMV-R1 (5′-AGAGGAAAGGAAAAGGTGGAGG-3′) amplified a 764-bp product from nt 5291 of the 5′-end RNA polymerase gene to nt 6082 of the 3′-untranslated region (UTR). Total RNA was extracted from leaf samples using the Qiagen Plant RNeasy Kit (Qiagen Inc., Chastworth, CA). RT was carried out by using PnMV-R1 primer and MMLV reverse transcriptase (Promega, Madison, WI). The PCR was performed in a 20-µl volume reaction containing 2 µl cDNA, 1× *Taq* reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM PnMV-F1 primer, and 1 U of *Taq* polymerase (Promega) with a denaturation step (94°C for 5 min), 30 amplification cycles (94°C for 30 s; 55°C for 30 s; 72°C for 1 min), and a final elongation (72°C for 5 min). The sequence of the RT-PCR product (Genbank Accession No. DQ462438) had 98.7% amino acid identity to PnMV. PCR products were obtained from two of three PnMV ELISA-positive *E. pulcherrima* and three of three PnMV ELISA-positive symptomatic *N. benthamiana*. The failure to amplify the fragment from all ELISA-positive PnMV is likely because of the presence of inhibitors and latex in *E. pulcherrima* (3) that make the RNA extraction difficult. Thus, while RT-PCR may be useful for further characterizing PnMV isolate sequences, ELISA may be more reliable for virus detection. In conclusion, to our knowledge, this is the first report of PnMV in *E. pulcherrima* but not in other *Euphorbia* spp. in New Zealand. *E. pulcherrima* plants have been imported into New Zealand for nearly 40 years, and the virus is probably widespread throughout the country via retail nursery trading.

References: (1) B. G. Bradel et al. Virology 271:289, 2000. (2) R. W. Fulton and J. L. Fulton. Phytopathology 70:321, 1980. (3) D.-E. Lesemann et al. Phytopathol. Z. 107:250, 1983. (4) S. Rozen and S. Skaletsky. Page 365 in: Bioinformatics Methods and Protocols: Methods in Molecular Biology. S. Krawetz and S. Misener, eds. Humana Press, Totowa, NJ, 2000.

First Report of Tomato (*Lycopersicon esculentum*) Pith Necrosis Caused by *Pseudomonas fluorescens* and *P. corrugata* in the Kingdom of Saudi Arabia. Y. Molan and Y. Ibrahim, Plant Protection Department, Faculty Of Food and Agriculture Sciences, King Saud University, Kingdom Of Saudi Arabia. Box 2460, Riyadh 11451. Plant Dis. 91:110, 2007; published on-line as DOI: 10.1094/PD-91-0110B. Accepted for publication 3 October 2006.

From 2002 to 2004, tomato (*Lycopersicon esculentum*) plants with external stem lesions, adventitious roots, and necrotic pith that was hollowed or chambered were received by the Clinical Lab of the Plant Protection Department from eight greenhouses in the Riyadh, Abha, and El-Kharj regions of Saudi Arabia. Bacteria were isolated on nutrient agar or King's medium B (KMB) from the stems of tomato plants cv. Red Gold, the cultivar most commonly grown in greenhouses. Gram-negative, rod-shaped bacteria were consistently isolated from stems with symptoms of pith necrosis. They were identified as *Pseudomonas fluorescens* (biotype I) and *P. corrugata* on the basis of morphological, physiological, and biochemical tests (2). Isolates of *P. fluorescens* isolated from Abha and El-Kharj were fluorescent on KMB, aerobic, and positive for oxidase, arginine dihydrolase, and gelatin liquefaction. Furthermore, all isolates produced levan-type colonies on sucrose nutrient agar and utilized glucose, 2-ketogluconate, sucrose, and sorbitol. They were negative for tobacco hypersensitivity and nitrate reduction. The strains of *P. corrugata* isolated from Riyadh were nonfluorescent, aerobic, and positive for oxidase, nitrate reductase, arginine dihydrolase, and utilization of malonate, alanine, trehalose, arginine, mannitol, and m-inositol. They were negative for levan, pectinase, tobacco hypersensitivity, and utilization of cellobiose and sorbitol. The identity of bacterial species was confirmed by Biolog analysis (carbon source utilization at 37°C), with a similarity index of 0.75 for *P. corrugata* and 0.71 for *P. fluorescens*. Four-week-old tomato plants (cv. Red Gold) were inoculated by injecting 50 µl of a bacterial suspension into the axils of the first true leaves. The bacterial suspension was prepared from 24-h-old cultures with sterile distilled water. Sterile distilled water was used as the negative control. After inoculation, plants were covered with polyethylene bags for 24 h to maintain high humidity at 25°C (1). Necrotic lesions surrounding injection points were observed 14 days after inoculation. At 4 weeks after inoculation, all inoculated plants showed symptoms of necrotic pith similar to those observed on the samples received. Control plants injected with water remained healthy throughout the experiments. Isolates of *P. fluorescens* (biotype I) and *P. corrugata* were reisolated from inoculated plants and were identical to the original strains on the basis of Biolog analysis. To our knowledge, this is the first report of tomato pith necrosis in Saudi Arabia.

References: (1) G. Demir. J. Turk. Phytopathol. 19:63, 1990. (2) R. A. Lelliott and D. E. Stead. Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications. Oxford, UK, 1987.

First Report of Fire Blight on *Pyrus elaeagrifolia* and *Amelanchier* sp. in Bulgaria. S. G. Bobev, Agricultural University, 4000 Plovdiv, Bulgaria; and J. Van Vaerenbergh and M. Maes, Institute for Agricultural and Fisheries Research, Plant-Crop Protection, 9820 Merelbeke, Belgium. Plant Dis. 91:110, 2007; published on-line as DOI: 10.1094/PD-91-0110C. Accepted for publication 3 October 2006.

In 2005, a fire blight epidemic occurred for the second time within the last 3 years, and severe damages were observed on pome fruits trees in many regions of Bulgaria. For the first time, we found fire blight on *Pyrus elaeagrifolia* and *Amelanchier* sp. grown in a park area (Plovdiv Region), providing evidence of continuing spread of the pathogen in Bulgaria. The symptoms on *P. elaeagrifolia* were necrotic, immature fruitlets and progressive necrosis toward the adjacent branches, thus forming cankers and leading to death of the plant above the canker. Many *Amelanchier* sp. shrubs had severely blighted flowers, fruitlets, shoots, and branches and dried, amber ooze droplets on the shoots. All the isolations made from blighted hosts' shoots and cankers on King's medium B (2 to 3 days, 26 to 27°C) yielded whitish, glistening, round bacterial colonies. Infiltration of the suspensions of randomized isolates from both hosts into tobacco leaves resulted in a typical hypersensitive reaction. Subsequently, some strains showed a typical ooze production on immature pear fruits (cv. Conference) and were also successfully reisolated from artificially inoculated quince shoots (1.2×10^9 CFU, cv. Portugalska, three replicates), where typical fire blight symptoms were observed, thereby fulfilling Koch's postulates. No symptoms or bacteria were found within any of the shoots from the same plant species injected with sterile water. The identity of the isolates was confirmed as *Erwinia amylovora* by an antibody-based slide agglutination test (Neogen_Express; Neogen Europe, Ltd., UK) and PCR test with primers derived from the *ams* region (1). On the basis of the symptoms, cultural characteristics, and positive results in pathogenicity, serological, and PCR tests, the isolates were considered to be *E. amylovora*. To our knowledge, this is the first report of fire blight on *P. elaeagrifolia* and *Amelanchier* sp. in Bulgaria.

Reference: (1) S. Bereswill et al. Appl. Environ. Microbiol. 61:2636, 1995.

First Report of a "*Candidatus* Phytoplasma australiense"-Related Strain in Lucerne (*Medicago sativa*) in Australia. M. A. Getachew, Pest Biology and Management Group, Faculty of Rural Management, University of Sydney, Orange. Leeds Parade 883 Orange NSW 2800, Australia; A. Mitchell, Orange Agricultural Institute, NSW Department of Primary Industries, Forest Road, Orange NSW 2800, Australia; G. M. Gurr, Pest Biology and Management Group, School of Rural Management, Charles Sturt University, Orange. Leeds Parade 883 Orange NSW 2800, Australia; M. J. Fletcher, Orange Agricultural Institute, NSW Department of Primary Industries, Forest Road, Orange NSW 2800, Australia; L. J. Pilkington, Gosford Horticultural Institute, NSW Department of Primary Industries, Research Road, Gosford NSW 2250, Australia; and A. Nikandrow, Orange Agricultural Institute, NSW Department of Primary Industries, Forest Road, Orange NSW 2800, Australia. Plant Dis. 91:111, 2007; published on-line as DOI: 10.1094/PD-91-0111A. Accepted for publication 4 October 2006.

Australian lucerne yellows (ALuY), a phytoplasma-associated disease, is a major problem in Australia that causes the pasture seed industry millions of dollars in losses annually (3). Samples were collected from lucerne (*Medicago sativa* L.) plants exhibiting symptoms indicative of ALuY (4) in a seed lucerne paddock (cv CW 5558) at Griffith, southwestern New South Wales (NSW), Australia, in November 2005 and again in January 2006. Samples were kept at 4°C and processed within 36 h of collection. Total DNA was extracted from approximately 0.3 g of leaf midribs and petioles of each plant sample and used as template in a nested PCR assay with phytoplasma universal primer pairs P1/P7 and fU5/m23sr. PCR products resulting from the first amplification were diluted (1:30) with sterile distilled water (SDW) before reamplification with fU5/m23sr. DNA of Australian tomato big bud (TBB) phytoplasma and SDW were used as positive and negative assay controls, respectively. Ten of fifteen plant samples collected in November tested positive for phytoplasma DNA. Restriction digestion profiles of nested PCR amplicons with *Hpa*II endonuclease were the same for all symptomatic plants but differed from the control. Phytoplasma identity was determined by sequencing two nested PCR products that yielded identical sequences. One was deposited in the GenBank database (Accession No. DQ786394). BLAST analysis of the latter sequence revealed a >99.6% similarity with "*Candidatus* Phytoplasma australiense" (L76865) and related strains papaya dieback (Y10095), phormium yellow leaf (U43570), strawberry green petal (AJ243044), and strawberry lethal yellows (AJ243045). Direct PCR with primers FP 5′-GCATGTCGCGGTGAATAC-3′ and RY 5′-TGAGCTATAGGCCCTTAATC-3′ designed to specifically amplify DNA of "*Ca.* P. australiense" detected the phytoplasma in 8 of 40 samples collected in January. Whether this phytoplasma is the etiological agent solely responsible for ALuY is currently under investigation. "*Ca.* P. asteris" and stolbur group (16SrXII) phytoplasmas have been reported in lucerne in the United States (2) and Italy (1), respectively. Within the stolbur group 16SrXII, "*Ca.* P. australiense" and stolbur phytoplasma are regarded as separate species and both are distinct from "*Ca.* P. asteris", a group 16SrI strain. To our knowledge, this is the first report of a "*Ca.* P. australiense" related strain in lucerne.

References: (1) C. Marzachi et al. J. Plant Pathol. 82:201, 2000. (2) R. D. Peters et al. Plant Dis. 83:488, 1999. (3) L. J. Pilkington et al. Australas. Plant Pathol. 28:253, 1999. (4) L. J. Pilkington et al. First report of a phytoplasma associated with 'Australian lucerne yellows' disease. New Disease Report. Online publication at http://www.bspp.org.uk/ndr/jan2002/2001-46.asp.

First Report of the Occurrence of *Wheat dwarf virus* in Wheat in China. J. Xie, X. Wang, Y. Liu, Y. Peng, and G. Zhou, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2, West Yuan Ming Yuan Road, Beijing 100094, P.R. China. Plant Dis. 91:111, 2007; published on-line as DOI: 10.1094/PD-91-0111B. Accepted for publication 11 October 2006.

In May of 2004 and 2005, several diseased wheat (*Triticum aestivum* L.) plants showing extreme dwarfing, various types of yellowing, and reduced or no heading were found in the breeding fields of the Institute of Crop Science, Shanxi Academy of Agricultural Sciences, Taiyuan, Shanxi Province, China. On the basis of these symptoms, infection with *Wheat dwarf virus* (WDV) was suspected. Total DNA was extracted from diseased plants with the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). Primers were designed based on the WDV-Enkoping1 genome sequence (NC_003326) (1), including: 245f, 5′-CGACTACGCCTGGCGAACATTTG-3′ (residues 245–267); 806r, 5′-TCTGGCATTGCCTGTTTCGG-3′ (complementary to residues 787–806); 1381f, 5′-CAGTGACATCTTCGCCGGAG-3′ (residues 1381–1400); and, 1886r, 5′-ACTCCGTAAGCCTCGAATCC-3′ (complementary to residues 1867–1886). With primer pairs 245f/806r, 1381f/1886r, 245f/1886r, and 1381f/806r, PCR products of 560, 506, 1642, and 2,275 bp were expected, respectively. After amplification, fragments of the expected sizes were seen on 1% (w/v) agarose gels. The fragments were purified by using a DNA gel extraction kit (TaKaRa, Dalian, China) and cloned into the pGEM-T vector (Promega, Madison, WI). The plasmids were transformed into *E. coli* strain DH5α, and plasmid DNA was isolated from overnight cultures by alkaline lysis. Insert sequences were determined using the dideoxynucleotide chain termination method with an automated sequencer (ABI BigDye 3.1, Applied Biosystems, Foster City, CA). At least three independently isolated clones were analyzed for each PCR product. The compiled 2,750 nt sequence (GenBank Accession No. DQ868525) was 98.1, 98.5, 97.8, and 97.9% identical to WDV-Enkoping1 (NC_003326), WDV-SE (X02869), WDV-B (AM040732), and WDV-F (AM040733), respectively. Therefore, the virus isolate (WDV-TY) was identified as WDV (genus *Mastrevirus*, family *Geminividae*). Wheat samples collected from different provinces from 2004–2006 were also infected with WDV as indicated by PCR using the same primer pairs. For Shijiazhuang (Hebei Province), Yangling (Shanaxi Province), Kunming (Yunnan Province), Yuncheng (Shanxi Province), Tianshui (Gansu Province), Gangu (Gansu Province), and Zhenzhou (Henan Province), 13 of 14, 6 of 6, 5 of 5, 4 of 4, 2 of 3, 1 of 2, and 1 of 1 samples were positive, respectively, indicating a broad distribution of WDV in China. To our knowledge, this is the first report of WDV in wheat in China.

Reference: (1) A. Kvarnheden et al. Arch Virol. 147:205, 2002.

First Report of Soybean Rust Caused by *Phakopsora pachyrhizi* on *Phaseolus* spp. in Argentina. A. J. Ivancovich and G. Botta, INTA, Pergamino, Argentina; M. Rivadaneira, INTA Yuto, Argentina; E. Saieg, Facultad Agronomia, Santiago del Estero, Argentina; L. Erazzú, INTA, Famaillá, Argentina; and E. Guillin, INTA, Castelar, Argentina. Plant Dis. 91:111, 2007; published on-line as DOI: 10.1094/PD-91-0111C. Accepted for publication 10 October 2006.

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd., has been reported in Argentina on soybean (*Glycine max*) and kudzu (*Pueraria lobata* and *Pueraria javanica*) since the 2002 growing season (1–4). On 29 May 2006, plants of *Phaseolus* spp. were found to have tan ASR-like rust lesions on leaves at eight different field plots located in the northwestern province of Salta, Argentina. Growth stages of infected bean plants within plots were between pod setting and physiological maturity. Diagnosis of ASR on bean leaves was performed with a stereoscopic microscope to view rust pustules, and suspected uredinia of *P. pachyrhizi* were observed, furthermore, typical ASR urediniospores also were also observed at ×400. ELISA and PCR methods gave positive results for ASR. Rust spores from these plants were used to inoculate soybean plants at the V3 growth stage with rust spores from field bean plants produced under greenhouse conditions. Typical ASR tan pustules developed within 21 days of inoculation. Bean rust caused by *Uromyces phaseoli* also was seen in some of the bean plots but was easily differentiated from ASR because the uredinia were much darker and affected the upper leaves, while the ASR uredinia were lighter and spread from the lower leaves to the upper leaves. This finding is of significance in Argentina because bean is an important crop grown in the northwestern region of the country and is planted approximately 2 months after soybean planting. Given this planting time difference, bean may provide an overwintering host for the survival of ASR spores, thereby providing a green bridge for infection of soybean plants during the following growing season.

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(*Disease Notes* continued on next page)

First Report of *Fusarium verticillioides* on Kenaf in South Africa. W. J. Swart and G. Tarekegn, Center for Plant Health Management, Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Plant Dis. 91:112, 2007; published on-line as DOI: 10.1094/PD-91-0112A. Accepted for publication 4 October 2006.

Kenaf (Malvaceae; *Hibiscus cannabinus* L.) is being commercially cultivated in Winterton, South Africa for its high-quality cellulose fibers with approximately 2,000 ha currently under cultivation. In 2004, 25% of 1-month-old kenaf plants grown from seed were observed in the field with severe wilting followed by lodging and mortality within 1 week. Isolations from diseased stem and root tissue on malt extract agar (MEA) consistently yielded *Fusarium verticillioides* (Sacc.) Nirenberg (2). Pathogenicity tests were conducted by inoculating kenaf seedlings with inoculum prepared from barley grains that had been colonized by the pathogen in vitro for 2 weeks prior to being finely ground in a laboratory mill. Fifty seeds from each of eight kenaf cultivars were incubated at 25°C on sterile filter paper to ensure germination and the absence of pathogens. Germinated seeds were sown in pots (400 cm^3) containing steam sterilized loam soil (200 g) by placing 20 germinated seeds from each cultivar, with four replicates (5 seeds per pot), on the soil in each pot and covering them with 100 g of the same soil. Inoculum powder was sprinkled on the surface of the soil in each pot and covered by 100 g of soil. Pots were maintained in a glasshouse at an ambient temperature of 25°C. Sterile ground barley seeds served as the control treatment. Pots were watered daily with 20 ml of water and observed periodically for seedling emergence. The percentage of diseased seedlings was recorded after 3 weeks and the experiment was repeated. Wilting had occurred in 85% of seedlings when they were approximately 4 cm high and all diseased seedlings had died within 1 week thereafter. Subsequent examination revealed dark brown lesions girdling the stem and decayed roots in all instances. No symptoms developed on control plants. From means of combined data, the greatest seedling mortality was observed for cv. Gregg (65%) and the least for cv. Cuba108 (5%). Mean mortalities for the remaining six cultivars ranged from 30 to 55%. The pathogen was reisolated on MEA from all diseased seedlings. To our knowledge, this is the first report of *F. verticillioides* occurring on kenaf in South Africa. The only other report of *Fusarium* sp. causing serious damping-off of kenaf is from Iran (1). The potential impact of the pathogen on kenaf production in South Africa must be considered in the implementation of disease control measures.

References: (1) J. M. Dempsey. Kenaf. In: Fiber Crops. The University Press of Florida, Gainesville, 1975. (2) J. F. Leslie and B. A. Summerell. The Fusarium Laboratory Manual. Blackwell Publishing, Ames, IA, 2006.

***Tobacco ringspot virus* Found in the Cardboard Cycad (*Zamia furfuracea*) in Florida.** C. A. Baker, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL 32614; and S. Adkins, USDA-ARS-USHRL, Fort Pierce, FL 34945. Plant Dis. 91:112, 2007; published on-line as DOI: 10.1094/PD-91-0112B. Accepted for publication 14 October 2006.

Zamia furfuracea (Zamiaceae) is native of coastal Mexico. It is a popular houseplant and easy to grow outdoors in warm climates. In November 2005, a plant of *Z. furfuracea*, originally from Texas, was received at the Division of Plant Industry in Gainesville, FL. The plant had numerous chlorotic spots on the leaves that eventually became necrotic. Leaves were ground in phosphate buffer (pH 7.2) with Carborundum and used to inoculate a host range that included *Chenopodium amaranticolor*, *C. quinoa*, *Gomphrena globosa*, *Datura stramonium*, and *Nicotiana benthamiana*. Systemic symptoms were seen in *C. quinoa* (necrotic lesions), *G. globosa* (stunting), *D. stramonium* (chlorotic ringspots), and *N. benthamiana* (wavy line patterns) 1 to 2 weeks after inoculation. *C. amaranticolor* showed only small necrotic local lesions. In further host range studies, systemic infections of *Beta vulgaris, D. metaloides*, *Lactuca sativa, N. clevelandii, Pisum sativus*, *Petunia hybrida*, *Zinnia elegans* (symptomless), and *Cucumis sativa* were observed. However, no infection of *Zea mays*, *Verbena hybrida*, *Glycine max*, *Phaseolus vulgaris*, *Catharanthus roseus*, *Arachis hypogaea*, *Trifolium* spp., *Vigna unguiculata*, *Vicia faba* or *Digitalis* spp. was detected. Inclusions observed in leaf strips of *N. benthamiana* and *D. stramonium* indicated a possible infection of this plant with a nepovirus (1). A 337-bp fragment was amplified from total RNA isolated from an inoculated *D. stramonium* using reverse transcription-PCR with nepovirus group primers provided by Agdia Inc. (Elkhart, IN). Sequence analysis indicated that the nucleotide (nt) and deduced amino acid (aa) sequences of the fragment were 89 to 91% and 91 to 95% identical, respectively, to sequences of the RNA-dependent RNA polymerase gene for *Tobacco ringspot virus* (TRSV) contained in GenBank (Accession Nos. U50869 and AJ698718). This region was only 50% (nt) and 38% (aa) identical to *Cycas necrotic stunt virus* (GenBank Accession No. NC_003791), a nepovirus previously reported to infect cycads (2). The original plant, symptomatic inoculated hosts, and the symptomless zinnia tested positive by double-antibody sandwich-ELISA using commercially available antiserum for TRSV (Agdia, Inc.), further confirming the presence of TRSV. Although the virus infecting *Z. furfuracea* has a more restricted host range than that reported for TRSV, the serology and gene sequence indicates that this virus is a unique isolate of TRSV.

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First Report of Bacterial Blight of Rutabaga (*Brassica napus* var. *napobrassica*) Caused by *Pseudomonas syringae* pv. *alisalensis* in California. S. T. Koike and K. Kammeijer, University of California Cooperative Extension, Salinas 93901; C. T. Bull, USDA, Agricultural Research Station, Salinas, CA 93905; and Doug O'Brien, Doug O'Brien Agricultural Consulting, Santa Cruz, CA 95062. Plant Dis. 91:112, 2007; published on-line as DOI: 10.1094/PD-91-0112C. Accepted for publication 12 October 2006.

In 2005, commercial, organically grown rutabaga (*Brassica napus* var. *napobrassica*) in San Benito County, CA showed symptoms of a previously undescribed disease on approximately 30% of the plants. Initial symptoms consisted of small (1 to 2 mm in diameter), angular, water-soaked flecks that often were surrounded by chlorotic haloes. These flecks enlarged and coalesced into large, irregularly shaped, gray brown lesions that could be as long as 10 mm. Lesions were visible from both adaxial and abaxial leaf surfaces and generally retained the chlorotic borders. A blue-green fluorescing pseudomonad was consistently isolated from lesions on King's medium B. Eight isolates were characterized and were levan positive, oxidase negative, and arginine dihydrolase negative. Isolates did not rot potato slices but induced a hypersensitive reaction in tobacco (*Nicotiana tabacum* cv. Samsun). These data indicated that the bacteria belonged to Lelliot's LOPAT group 1 (2). This was confirmed with data from fatty acid methyl ester analysis (MIS-TSBA version 4.10; MIDI, Inc., Newark, DE) that showed that the isolates were highly similar (similarity = 0.922 or greater) to *Pseudomonas syringae*. Amplification of repetitive bacterial sequences (rep-PCR) using the BOXA1R primer and the polymerase chain reaction resulted in identical banding patterns for the rutabaga isolates and the *P. syringae* pv. *alisalensis* pathotype strain. Pathogenicity was demonstrated by growing inocula of six isolates in nutrient broth shake cultures for 48 h (24°C), adjusting the bacterial suspension to 10^6 CFU/ml, and misting the resulting suspensions onto rutabaga (cv. American Purple Top). Plants were enclosed in plastic bags for 24 h and then incubated in a greenhouse (24 to 26°C). Control plants were misted with sterile water and treated the same way. After 5 to 7 days, foliar symptoms similar to symptoms seen in the field developed on all inoculated plants, and reisolated bacteria were characterized and found to be *P. syringae* pv. *alisalensis*. Control plants remained symptomless. The results of two sets of pathogenicity tests were the same. To our knowledge, this is the first report of commercially grown rutabaga as a host of *P. syringae* pv. *alisalensis* and the first report of a *B. napus* host of this pathogen. This bacterial pathogen has previously been reported on commercial plantings of arugula (*Eruca sativa*), broccoli (*Brassica oleracea* var. *botrytis*), and broccoli raab (*Brassica rapa* var. *rapa*) in California and under experimental conditions it causes disease on additional hosts, including members of the Poaceae (1).

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First Report of Leaf Rust by *Puccinia triticina* on Wheat in Oman. M. L. Deadman, A. Al Sa'di, and Y. Al Maqbali, Department of Crop Sciences, Sultan Qaboos University, Box 34, Al Khod 123, Oman; and M. C. Aime, USDA-ARS, SBML, 10300 Baltimore Ave, Beltsville, MD 20705. Plant Dis. 91:113, 2007; published on-line as DOI: 10.1094/PD-91-0113A. Accepted for publication 4 October 2006.

Wheat (*Triticum aestivum* L.), cultivated for forage and grain production, is an important crop in the Sultanate of Oman. In April 2005, leaf samples of an unknown local variety showing rust symptoms were collected from Rustaq, 100 km southwest of Muscat. Circular-to-oval, red-brown pustules, typical of uredinia, occurred mostly on the upper surface of leaves on plants nearing maturity. Telia with teliospores were observed on leaf sheaths. The disease was widespread in many fields and was likely to be limiting the yield. Urediniospores typical of *Puccinia triticina* Erikss. (=*P. recondita* Rob. ex Desm. f. sp. *tritici*) were roughly subglobose, measuring 18 to 28 × 20 to 25 µm, echinulate, with 3 to 8 scattered germ pores; teliospores were 2-celled, 34 to 50 × 15 to 17 µm, apex is chestnut brown, lower cell is light yellow, no germ pores (1,2). Pathogen identity was confirmed by nuclear ribosomal large subunit and internal transcribed spacer region-2 DNA analysis (voucher sequence deposited in GenBank, Accession No. DQ664194, voucher specimens deposited in the U.S. National Fungus Collections, BPI 872158 and 872159). Wheat is grown during the winter months in Oman and harvested in May. Although the disease was observed again in 2006, pathogen survival mechanisms are not presently clear, and current research is attempting to confirm its presence on alternate hosts, including grass weeds, and determine the distribution of the pathogen on local wheat land races and imported varieties. To our knowledge, this is the first documented report of *P. triticina* on wheat in Oman.

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First Report of *Iris yellow spot virus* in Commercial Leek (*Allium porrum*) in the United States. H. F. Schwartz and K. Otto, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins 80523-1177; and H. R. Pappu, Department of Plant Pathology, Washington State University, Pullman 99164-6430. Plant Dis. 91:113, 2007; published on-line as DOI: 10.1094/PD-91-0113B. Accepted for publication 12 October 2006.

Iris yellow spot virus (IYSV; family *Bunyaviridae*, genus *Tospovirus*) has a wide host range, with onion (*Allium cepa* L.) being one of the most economically important hosts. IYSV has been widely reported from this species throughout most onion-production regions of the United States and many areas of the world in recent years. A relative of onion, leek (*Allium porrum* L.), has been reported to be a host of IYSV in countries such as the Netherlands, Reunion Island, and Australia (1,4). A related tospovirus, *Tomato spotted wilt virus* (TSWV), was recently reported causing necrotic lesions and extended bleaching of leaf tips of leek in Georgia (2). In September of 2006, disease symptoms suspected to be caused by IYSV were observed on central and outer leaves of plants in a 2.6-ha section of commercial leeks being grown from seed (cvs. Tadorna and King Richard). The leek plants were adjacent to a 3.1-ha section of seeded onion (cv. Exacta) that had been harvested 2 weeks earlier. Twenty-five to thirty percent of unharvested onion plants next to the leek section also exhibited IYSV-type disease symptoms generally on the central leaves. Both *Allium* spp. were seeded 5 months earlier and grown under certified organic, pivot-irrigated conditions in Larimer County in northern Colorado. Disease symptoms on leek and onion leaves appeared as dry, white-to-straw-colored, spindle- or diamond-shaped lesions that ranged in size from 5 to 10 × 25 to 50 mm or larger depending on lesion age. Lesion centers, especially on leek, often had green centers with concentric rings of alternating green and straw-colored tissue. Green tissue near necrotic lesions of a single symptomatic leaf from 10 plants each of leek and onion was sampled and analyzed using a double-antibody sandwich (DAS)-ELISA (Agdia, Inc., Elkhart, IN). Five of ten leek and nine of ten onion samples were positive for IYSV. Using reverse transcription (RT)-PCR and primers specific to the small RNA of IYSV (5′-TAA AAC AAA CAT TCA AAC AA-3′ and 5′-CTC TTA AAC ACA TTT AAC AAG CAC-3′), the complete nucleocapsid (N) gene was amplified from symptomatic leek plants and then sequenced (3). Comparisons with IYSV N gene sequences available in the GenBank confirmed the identity of the virus as IYSV. Leek samples were negative for TSWV when tested by RT-PCR with TSWV-specific primers. In addition, three specimens of the presumed thrips vector recovered from five IYSV-infected leek plants were identified as *Thrips tabaci* (L. A. Mahaffey and W. S. Cranshaw, *personal communication*). Earlier in the season, *T. tabaci* was observed in the nearby planting of onion that also exhibited IYSV in September. To our knowledge, this is the first report of natural infection of commercial leek with IYSV in the United States. The incidence of plants (25 to 30%) with foliar lesions on multiple leaves and stunting of 5% of infected plants in both leek cultivars suggests that IYSV could seriously reduce leek stem development and marketability.

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