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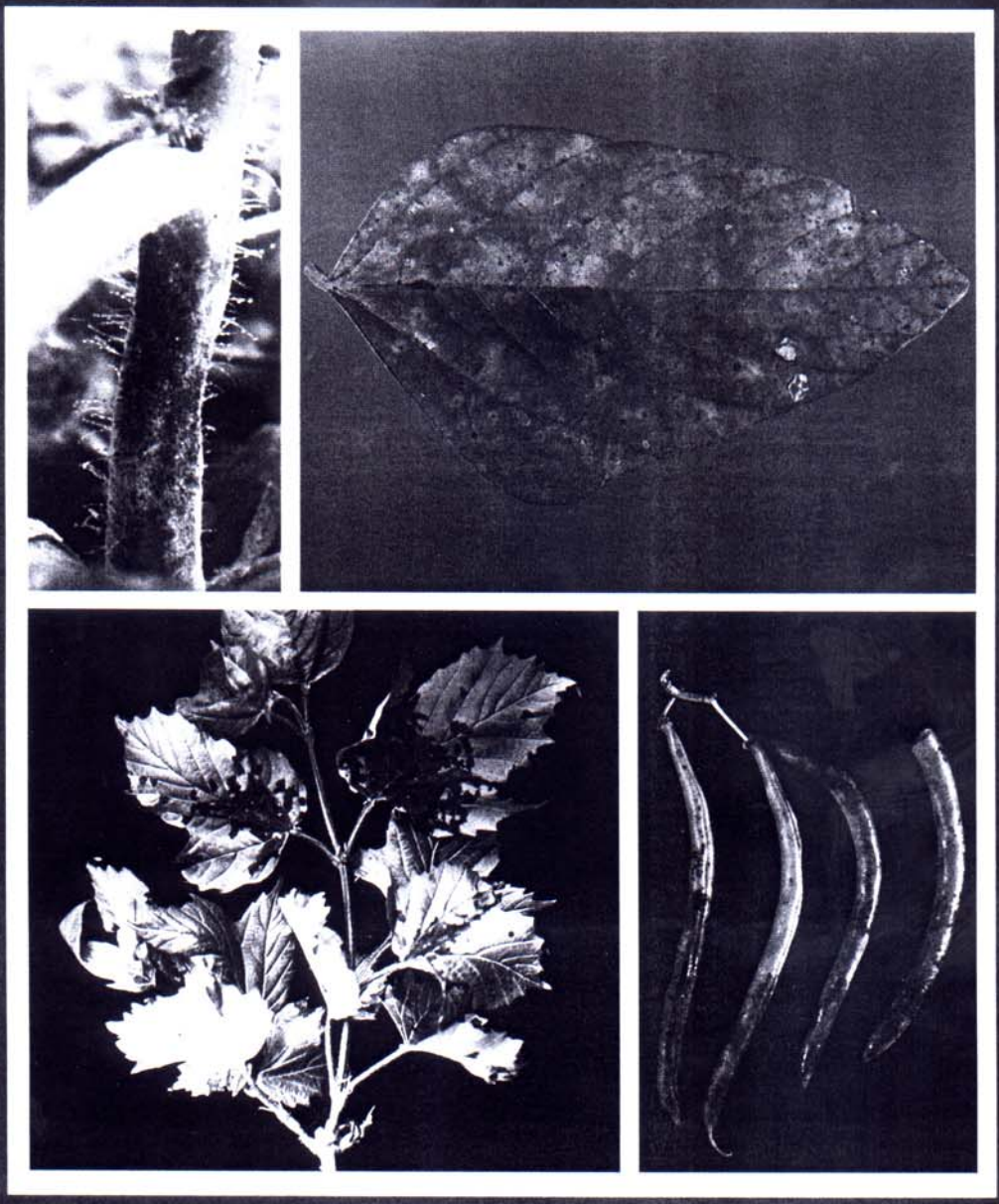

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DISEASE NOTES

Disease Notes (*continued*)

First Report of Soybean Rust Caused by *Phakopsora pachyrhizi* in the Continental United States. R. W. Schneider, C. A. Hollier, and H. K. Whitam, Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, Baton Rouge 70803, M. E. Palm and J. M. McKemy, USDA/APHIS/PPQ/NIS, Beltsville, MD 20705, J. R. Hernández, USDA/ARS, Systematic Botany and Mycology Laboratory, Beltsville, MD 20705; and L. Levy and R. DeVries-Paterson, USDA/PPQ/CPHST/NPGBL, Beltsville, MD 20705. Plant Dis. 89:774, 2005; published on-line as DOI: 10.1094/PD-89-0774A. Accepted for publication 1 April 2005.

Asian soybean rust, caused by *Phakopsora pachyrhizi* Sydow, has been known to occur in the eastern hemisphere for nearly a century. More recently, it was reported from Hawaii in 1994, eastern and southern Africa from 1996–1998, Nigeria in 2001, and Brazil and Paraguay in 2002. Aerobiological models suggested that urediniospores of the pathogen would be disseminated on wind currents to the continental United States in association with tropical storms if the disease became established north of the equator during hurricane season (U.S. Soybean Rust Detection and Aerobiological Modeling online publication at www.aphis.usda.gov/ppq/ep/soybean_rust/). Since soybean rust was observed at approximately 5°N latitude in South America before several hurricanes impacted the continental United States in September 2004, it seems likely that the introduction was associated with at least one of these tropical storms, especially hurricane Ivan. Symptoms of the disease were first observed on soybean (*Glycine max* (L.) Merr.) in the continental United States on November 6, 2004 in a field near Baton Rouge, LA. Typical pustules and urediniospores on infected leaves were readily apparent when viewed with a dissecting microscope. Urediniospores were obovoid to broadly ellipsoidal, hyaline to pale yellowish brown with a minutely echinulate thin wall, and measured 18 to 37 × 15 to 24 µm. Paraphyses were cylindric to clavate and slightly thickened at the apex, colorless to pale yellowish brown, and 25-50 × 6-14 µm in size. This morphology is typical of *Phakopsora pachyrhizi* and *P. meibomiae*, a less aggressive, western hemisphere species (2). DNA was extracted from leaves containing sori using the Qiagen DNeasy Plant Mini kit. *P. pachyrhizi* was detected using a real-time polymerase chain reaction (PCR) protocol (1) that differentiates between *P. pachyrhizi* and *P. meibomiae* performed in a Cepheid thermocycler with appropriate positive and negative controls. The PCR master mix was modified to include OmniMix beads (Cepheid). The field diagnosis of *P. pachyrhizi* was confirmed officially by the USDA/APHIS on November 10, 2004, and this was followed on November 11, 2004 by a wide-ranging survey of soybean and kudzu (*Pueraria* sp.) in soybean production areas in southern and central Louisiana. Collections from this survey also were assayed as described above, and six soybean specimens from five sites were confirmed positive. The disease was not found on kudzu samples. To our knowledge, this is the first report of *P. pachyrhizi* in the continental United States. Voucher specimens have been placed in the USDA National Fungus Collection.

References: (1) R. D. Frederick et al. Phytopathology 92:217, 2002. (2) Y. Ono et al. Mycol. Res. 96:825, 1992.

Detection of Soybean Rust caused by *Phakopsora pachyrhizi* in Northwestern Argentina. L. D. Ploper, V. González, M. R. Gálvez, N. V. de Ramallo, M. A. Zamorano, G. García, and A. P. Castagnaro, Estación Experimental Agroindustrial "Obispo Colombres", C.C. 9, (4101) Las Talitas, Tucumán, Argentina. Plant Dis. 89:774, 2005; published on-line as DOI: 10.1094/PD-89-0774B. Accepted for publication 1 April 2005.

Asian soybean rust, caused by *Phakopsora pachyrhizi*, is regarded as one of the most destructive diseases of soybean (*Glycine max* (L.) Merr.). In Argentina, it was first detected in the province of Misiones in the northeast near Paraguay and Brazil during the 2001–02 growing season (2). The following season, it also was found in the neighboring province of Corrientes. However, it did not reach major soybean production areas in northern Argentina until the end of the 2003–04 season. During April 2004, as soybean crops were nearing maturity, the disease was found throughout the region of northwestern Argentina, which includes the provinces of Tucumán, Salta, Jujuy, Catamarca, and Santiago del Estero, where approximately 6% of the soybean crop of Argentina is produced. During February and March, the area had a severe drought and above average temperatures, but in April, rainfall was abundant, particularly during the first half of the month. Soybean rust was first observed on 16 April in several locations of the departments (counties) of Moreno and Jiménez in the province of Santiago del Estero, and the following week in the departments of Alberdi, Burruyacú, Cruz Alta, Famaillá, La Cocha, and Leales in the province of Tucumán, in the department of Santa Rosa in the province of Catamarca, and in the departments of Anta, Metán, Rosario de la Frontera, and San Martín in the province of Salta. In those fields where the disease was detected, nearly all plants showed symptoms. Affected crops were mostly in growth stages R7 to R8, except for a few fields that had been planted late and were in a late R5 stage. Yield losses as much as 28% and premature defoliation occurred in these fields only. Disease severity, measured as percentage of affected leaf area, ranged from 45 to 50% in untreated fields and 0.9 to 39% in fungicide-treated fields. Leaf lesions were reddish brown, irregularly shaped, and were more abundant on the abaxial surface. Under the dissecting microscope, uredinia were observed as erumpent pustules with a conspicuous central pore. Masses of urediniospores were expelled through the pore and covered the pustules. Urediniospores were hyaline to pale yellow-brown, sub globose to ovoid, with finely echinulate, hyaline walls, and an average size of 27.8 × 18.5 µm. Because there are two morphologically similar species of *Phakopsora* that infect soybean, *P. pachyrhizi* (the Asian species) and *P. meibomiae* (the New World species), a molecular differentiation was carried out using the polymerase chain reaction (PCR) assay described by Frederick et al. (1). DNA extracted from 37 samples from different locations was amplified with specific primers for both species of *Phakopsora* and specific primers for *P. pachyrhizi* and for *P. meibomiae*. Twenty-eight samples amplified with the two species primers and with the *P. pachyrhizi* primer. None of the samples amplified with the *P. meibomiae* primer. Specimens have been deposited at Instituto Miguel Lillo, Tucumán, Argentina. These results confirmed the presence of *P. pachyrhizi* in the provinces of Catamarca, Tucumán, Salta, and Santiago del Estero, Argentina.

References: (1) R. D. Frederick et al. Phytopathology 92:217, 2002. (2) R. L. Rossi. Plant Dis. 87:102, 2003.

First Report of Pear Decline Phytoplasmas on Pear in Serbia. B. Duduk, M. Ivanović, and A. Obradović, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade-Zemun, Serbia; and S. Paltrinieri and A. Bertaccini, DiSTA, Patologia Vegetale, Alma Mater Studiorum, University of Bologna, via F. Re 8, 40126 Bologna, Italy. Plant Dis. 89:774, 2005; published on-line as DOI: 10.1094/PD-89-0774C. Accepted for publication 11 April 2005.

During August of 2004, pear (*Pyrus communis* L.) plants with typical symptoms of pear decline (PD) were observed in orchards in central Serbia. The affected plants showed premature reddening and upward rolling of leaves that often showed down-turned petioles. In some cases, premature defoliation was observed. Although a similar decline of pear was observed earlier, until now, the causal agent had not been identified. DNA was extracted with a chloroform/phenol procedure from fresh leaf midribs and branch phloem scrapes of four symptomatic and one asymptomatic pear plants separately. A nested polymerase chain reaction assay (PCR) was used for phytoplasma detection (first PCR round with P1/P7 (4) phytoplasma universal primer pair, followed by nested PCR with group 16SrX specific primers f01/r01) (3). With these primers, the expected products from phloem scrapes and midrib extracts of symptomatic plant samples were obtained. Restriction fragment length polymorphism (RFLP) analyses of the f01/r01 amplicon, with *Rsa*I and *Ssp*I restriction enzymes, discriminating among 16SrX subgroup phytoplasmas, showed profiles corresponding to those of the apple proliferation phytoplasma group, 16SrX-C subgroup, "*Candidatus* Phytoplasma pyri" (2). A 1,155-bp sequence of 16S rDNA gene for one of the PA2f/r (1) amplicons obtained in nested PCR on P1/P7 products from one of the leaf midrib samples was deposited in GenBank (Accession No. AY949984); both strands of the fragment were sequenced with the Big Dye Terminator reaction kit (Applied Biosystems, Foster City, CA). The sequences were analyzed with the Chromas 1.55 DNA sequencing software (Technelysium, Queensland, Australia) and aligned with BLAST software (http://www.ncbi.nlm.nih.gov). The blast search showed 100% homology of this sequence with that of PD strain Y16392, confirming the identity with PD of the phytoplasma detected. To our knowledge, this is the first report of pear decline phytoplasmas in Serbia.

References: (1) M. Heinrich et al. Plant Mol. Biol. Rep. 19:169, 2001. (2) IRPCM Phytoplasma/Spiroplasma Working Team–Phytoplasma Taxonomy Group. Int. J. Syst. Evol. Microbiol. 54:1243, 2004. (3) K.-H. Lorenz et al. Phytopathology 85:771, 1995. (4) Schneider et al. Pages 369-380 in: Molecular and Diagnostic Procedures in Mycoplasmology. Vol I. S. Razin and J. G. Tully, eds. The American Phytopathological Society, 1995.