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Disease Notes

**First Report of Aster Yellows Phytoplasma in Alfalfa**

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Samples of alfalfa (*Medicago sativa* L.) leaves and stems showing symptoms of inter-veinal chlorosis and purpling, commonly associated with insect feeding, were collected from 8 sites in central and southern Wisconsin in autumn of 1998. Samples were frozen within 24 h of collection. Approximately 0.3 g of plant tissue from each sample was used for total DNA extraction according to the protocol of Zhang et al. (4), with minor modifications in grinding procedures and reagent volumes to optimize results. Nested polymerase chain reaction (PCR) was carried out by amplification of 16S rDNA with the universal primer pairs R16mF2/R16mR1 followed by R16F2n/R16R2 as described by Gunder-sen and Lee (1). Undiluted total sample DNA was used for the first amplification; PCR products were diluted (1:30) in sterile water prior to final amplification. Alfalfa DNA and sterile water were used as negative controls; DNA from phytoplasma causing X-disease in peach (CX) served as a positive control. Fragments of 16S rDNA from putative phytoplasmas amplified by PCR with the primer pair R16F2n/R16R2 were characterized by restriction endonuclease digestion (3). The resulting restriction fragment length polymorphism (RFLP) patterns were compared with patterns for known phytoplasmas described by Lee et al. (3). Products of nested PCR were also purified and sequenced with primers R16F2n/R16R2 and an automated DNA sequencer (ABI 377XL; C. Nicolet, Biotechnology Center, University of Wisconsin-Madison). Of 51 samples of alfalfa assessed, one sample from Evansville, WI, yielded a nested PCR product of the appropriate size (1.2 kb), indicating the presence of phytoplasma. Digestion of this product with various restriction enzymes produced RFLP patterns that were identical to those for phytoplasmas in the aster yellows phytoplasma subgroup 16SrI-A (3). Alignment of the DNA sequence of the nested PCR product from the positive sample with sequences found in the GenBank sequence data base (National Center for Biotechnology Information, Bethesda, MD) with the BLAST sequence similarity function confirmed this result. Although other phytoplasma strains (particularly those causing witches'-broom) have been reported to infect alfalfa (2), this is the first report of the presence of the aster yellows phytoplasma in the alfalfa crop. Vectors involved in transmission and the potential agronomic impacts of aster yellows phytoplasma in alfalfa are topics of current investigation.

*References*: (1) D. E. Gundersen and I.-M. Lee. Phytopathol. Mediterr. 35:144, 1996. (2) A.-H. Khadhair et al. Microbiol. Res. 152:269, 1997. (3) I.-M. Lee et al. Int. J. Syst. Bacteriol. 48:1153, 1998. (4) Y.-P. Zhang et al. J. Virol. Methods 71:45, 1998.