Detection of Huanglongbing (Citrus Greening) Disease by Nucleic Acid Spot Hybridization

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Z. Naturforsch. **64c**, 711–716 (2009); received February 12/April 30, 2009

Polymerase chain reaction (PCR) amplification with primers specific to the rDNA region successfully amplified the 1160-bp DNA fragment from a Huanglongbing (HLB)-infected sweet orange sample with mottling symptoms leaves, but not from healthy sweet orange plants. The PCR product of 1160-bp was used as probe labeled with biotin for detection of the HLB pathogen in the nucleic acid spot hybridization (NASH) test. It was found that the HLB pathogen could be detected up to 1:100 dilution in HLB-infected tissue. Total DNA extracted from HLB-infected tissue was diluted 2-fold as 900 ng in TE buffer and spotted on a nitrocellulose membrane. Strong signals were observed up to 225 ng of DNA dilution, whereas a moderate signal was recorded at 112 ng. No hybridization signal was observed in the healthy samples, while strong signals were observed in the positive control.

Key words: Huanglongbing, PCR Detection, Nucleic Acid Spot Hybridization, Non-Radioactive Probes