Disease Note

FIRST REPORT OF ELSINOE LEAF AND FRUIT SPOT AND *ELSINOE PYRI* ON APPLE IN DENMARK

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An apple disease, known as "Topaz spot" in northern Europe (Trapman and Jansonius, 2008) has since year 2000 become widespread in Danish organic apple orchards (*Malus domestica*). Characteristic symptoms are small spots (black on fruits, brown on leaves) having a silvery-grey centre. The associated pathogen has not previously been identified, but symptoms are identical to those described for elsinoe leaf and fruit spot (ELFS) caused by the ascomycete, *Elsinoe pyri* (Scheper *et al*., 2013). In 2012, DNA from fruit skin of apples was purified from two cultivars, Pigeon fra Juellinge and Rifbjerg Skarlagen Pearmain growing near Copenhagen that showed severe symptoms of Topaz spot. Fungal DNA was analyzed by pyrosequencing using PCR primers targeting the *ITS2* region of ascomycetes (Louarn *et al*., 2012). Several fungal species were identified, but only a single DNA sequence of 182 bp was found to show consistent and specific accumulation in all symptomatic skin samples (n=6). A BLAST search revealed 100% identity only to sequences of *E. pyri* (isolates from New Zealand, GenBank accession Nos. KC626006, KC626007). Two independent fungal isolates with morphology identical to *E. pyri* (Scheper *et al*., 2013) were recovered by inoculating Topaz-spot infected fruit skin onto potato dextrose agar. Sequencing of the *ITS1-5.8S-ITS2* region revealed two identical 628 bp sequences (GenBank KC928079, KC928080) with 99% sequence identity to the previously published sequences of *E. pyri*. Our findings suggest that Topaz spot is identical to ELFS and show that the pathogen, *E. pyri*, is hereby reported in Denmark and in Scandinavia for the first time.

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Disease Note

FIRST REPORT OF A '*CANDIDATUS* PHYTOPLASMA ZIZIPHI'- RELATED STRAIN ASSOCIATED WITH PEACH DECLINE DISEASE IN INDIA

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In mid 1980s, peach (*Prunus persica*) trees growing in Himachal Pradesh (India) showed phytoplasma-like symptoms consisting of yellowing, marginal rolling and scorching of the leaves that conferred a burnt-like appearance to the trees. Fruits shrivelled and dropped prematurely and affected plants declined and died. Although the association of a phytoplasma with this disease had already been established (Thakur *et al*., 1998), the species of the putative agent had remained unidentified. Total DNA isolated from nine diseased peach trees was subjected to nested PCR employing universal primers P1/P7 followed by P1/Tint primers, and yielded fragments of 1.6 kb. PCR products from five diseased trees were digested with *Alu*I, *Bfa*I, *Dra*I and *Eco*RI. The RFLP profile for each enzyme was identical, indicating that all trees contained a similar phytoplasma. PCR amplicons from three trees were cloned and sequenced. 16S rDNA sequences from all clones were identical and the consensus sequence, archived in GenBank under accession No. JQ695914 was shown by BLAST analysis to be nearly identical (99%) to that of '*Candidatus* Phytoplasma ziziphi' (16SrV-B). A dendrogram drawn using 16S rDNA sequences was in line with BLAST comparisons and confirmed that the peach decline phytoplasma is related to members of the 16SrV-B subgroup. A virtual RFLP pattern employing 14 restriction enzymes also showed its relatedness to 16SrV-B subgroup members (Wei *et al*., 2007). On the basis of sequence identity, phylogenetic analysis and virtual RFLP of 16S rDNA, the phytoplasma associated with peach decline disease in Himachal Pradesh can be identified as a strain of '*Ca.* P. ziziphi'. This is the first definitive identification of a 16SrV-B subgroup phytoplasma associated with peach decline disease in India.

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