Disease Note

Diseases Caused by Bacteria and Phytoplasmas

First Report of '*Candidatus* Phytoplasma trifolii' and *Spiroplasma citri* in *Cannabis sativa* in Washington State, U.S.A.

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In 2022 and 2024, field surveys were conducted in the Columbia Basin of Washington State, U.S.A., for pathogens transmitted by the beet leafhopper, *Circulifer tenellus* (recently reclassified as *Neoaliturus tenellus*), including beet curly top virus (BCTV), '*Candidatus* Phytoplasma trifolii' (CPt), and *Spiroplasma citri*. For these pathogens, an association of symptoms with a causal agent is often complicated by coinfections and the inability to culture CPt (Hu 2021; Rivedal et al. 2022, 2024; Schoener and Wang 2023). In 2022, 35 hemp (*Cannabis sativa*) plants were observed at the Washington State University (WSU) Research Station in Othello, WA, with typical symptoms of BCTV (a finding published by Jarugula et al. 2023), and we further tested these samples for *C. tenellus*-associated bacteria. In 2024, 23 and 15 hemp plants were observed in Prosser, WA, from the WSU Irrigated Agriculture Research Center and WSU Roza Farm, respectively, with symptoms of CPt infection, including stem fasciation and purpling. Leaf tissue from each plant was ground in liquid nitrogen, DNA was extracted using the DNeasy Plant Mini Kit (Qiagen), and a multiplex real-time PCR protocol was used to detect the *C. tenellus*-associated pathogens (Swisher Grimm et al. 2023). CPt was identified in 8.6 and 65.8% of plants in 2022 and 2024, respectively, while *S. citri* was not detected in 2022 but was found in 15.8% of plants in 2024. Coinfections were common. Nested PCR (primers P1/P7 and R16F2n/R16R2, Crosslin et al. 2006) and conventional PCR (primers Trif-SecY-163F, 5′-AGCAGCTAAAAAAGTTAGAAAAA ACCTC-3′/Trif-SecY-1040R, 5′-AAATCTAGCGAAAATGATTTTTTG TTTTCA-3′) targeted the CPt 16S rRNA and *secY* genes, respectively. *S. citri* infection was confirmed by PCR targeting the s*piralin* gene (primers SpiralinF/R, Yokomi et al. 2008). All targets were amplified using PrimeSTAR HS DNA Polymerase (Takara Bio). Thermal cycle conditions for CPt 16S rRNA and *S. citri spiralin* genes consisted of 30 or 40 cycles, respectively, at 98°C for 10 s, 55°C for 15 s, and 72°C for 10 s, followed by a final elongation at 72°C for 5 min. Conditions for CPt *secY* consisted of 35 cycles at 98°C for 15 s, 55°C for 30 s, and 72°C for 10 s, followed by a final elongation at 72°C for 5 min. Amplicons of size 1,250 bp, 877 bp, and 675 bp for the 16S rRNA, *secY*, and *spiralin* genes, respectively, were visualized on an agarose gel stained with GelRed (Sigma-Aldrich) or ethidium bromide. For CPt, sequencing of 16S rRNA from seven infected samples (2 from 2022, 5 from 2024) revealed identical sequences (GenBank accession PV983691) with 100% identity to CPt in U.S. hemp (OQ597521), and sequencing of *secY* in one sample from 2022 and one from 2024 revealed identical sequences (PX725980), with 100% identity to CPt in U.S. periwinkle (GU004317). For *S. citri*, two sequenced samples had identical s*piralin* sequences (PV955037), matching 100 and 99.85% with *S. citri* found in Oregon cabbage (PV099668) and hemp (OQ969984), respectively. This is the first confirmed report of CPt and *S. citri* in *C. sativa* in Washington State. These findings highlight the need to evaluate effects of mollicutes on hemp and underscore the need to develop integrated pest management strategies to reduce vector transmission. In addition, these findings suggest that hemp could serve as a reservoir of the *C. tenellus*-transmitted pathogens, leading to higher pathogen prevalence across the region and negatively impacting economically important vegetable and seed crops grown in Washington State that are susceptible to these pathogens.

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