Diagnosing *Cannabis sativa* crop diseases in their early and late stages through systematic examination and holistic approaches

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ABSTRACT

Diagnosing plant diseases requires a complete examination of whole plants and looking into the network formed by various pathogens, pests, and the plant system to determine the cause. Oftentimes, a diagnosis is made solely from a small pieces of tissue sample or a single test targeting a specific speculative pathogen, and such a diagnostic approach may not find the real cause during the early stages of the disease nor obtain a complete set of pathogens/pests involved throughout the disease development. By using systematic examination and inclusive diagnosis, an indoor cannabis crop with mild foliar symptoms was found to be in the early stage of crown rot caused by *Fusarium oxysporum*, evidenced by the necrosis of internal crown tissue and 100% recovery of *F. oxysporum*. Similarly, an outdoor hemp crop with mild leaf chlorosis was found to have been infected with *Plectosphaerella cucumerina*, a pathogen causing collar rot. The diagnostic outcomes from these two cases were far from the growers' hypothesis of the cause being hop latent viroid infection or a certain nutrient deficiency. In another case, a hemp crop prior to harvest was found to be infected by seven *Fusarium*, six *Pythium*, one *Rhizoctonia*, and one phytoplasma species, among which *F. equiseti* and *P. ultimum* were most dominant at the time of isolation. Fifty percent of sampled plants had dual-organism infection, 16.7% had tri-organism infection, 16.7% had quad-organism infection, and only 25% had single-organism infection. The holistic approach used in this case revealed the full spectrum of organisms involved in the disease complex leading to crop failure.

CONCEPT

The term 'holistic' is often used in medicine but rarely in plant diagnostics. It defines an approach that treats a thing as a whole, instead of considering it as a sum of different unrelated parts. In plant diagnostics, the holistic approach has three layers of meaning. First, a plant is a system, and all parts of the system should be viewed as a whole. Diagnosis should be based on an entire plant instead of on a single piece of plant material. Secondly, the approach of 'all things considered' should be practiced when finding pests, pathogens, or factors that contribute to an observed plant problem. This approach considers the diagnosis process to be an investigation instead of a specific test for a single pathogen. Thirdly, all factors found in a disease are related and each of them may have a unique role in disease initiation and progression. The relationships among various pathogens, pests, and the plant system form a unique network that may define the type, complexity, or severity of a crop disease (Fig. 1). A holistic approach investigates this network and often finds broader or more in-depth causes and such findings help growers to develop more effective pest management (Wang, 2021).
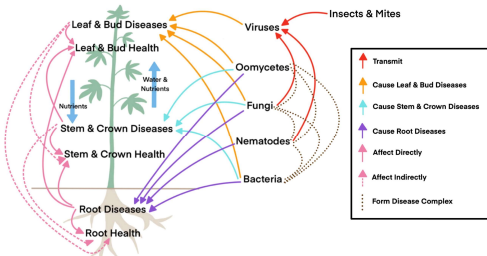
Fig. 1. Overall plant health impacted by pathogen types, organ diseases, pests, and their interactions. (Wang, 2021. "*Diagnosing Hemp and Cannabis Crop Diseases*" CABI Publishing. DOI: 10.1079/9781789246070.0000)

APPROACHES

Systematic Examination: Affected plants are systemically examined both visually and under a microscope from the top to bottom, including the root system and internal tissue of all organs to reveal pathological discoloration, necrosis, decay, pathogens, or pests and their leftovers.
Problem Classification: Based on the examination, plant symptoms are diagnostically classified, following the problem to cause chart (Fig. 2).
**Laboratory Analysis:** Based on problem classification, pertinent tissue samples are collected from specific portions of the plants and analyzed separately to confirm the presence of each hypothesized pathogen or pest. All encountered pathogens or pests were further identified through their biological characteristics and/or their DNA/RNA sequences.

Fig. 2. Problem-cause chart of plant diagnostics (Wang, 2021. "*Diagnosing Hemp and Cannabis Crop Diseases*" CABI Publishing. DOI: 10.1079/9781789246070.0000)

CASE #1

Early Stage of Internal Crown Rot Caused by *Fusarium oxysporum*
Symptom described by the grower: "I am seeing leaf tip curl as well as leaf cupping. Also, I am seeing nodes that are growing horizontal from the stem. Leaves also have taken on a "Leathery" or "Crinkled" look to them." (**Fig. 3A**)
Diagnosis: *F. oxysporum* (**Fig. 3F**) infected cuttings *via* wound (**3B**) and progressed to internal tissue causing lesions and decay inside the base of young cannabis plants (**3C**). Root and stem were healthy (**3D, 3E**). Plants were tested negative for *hop latent viroid*.
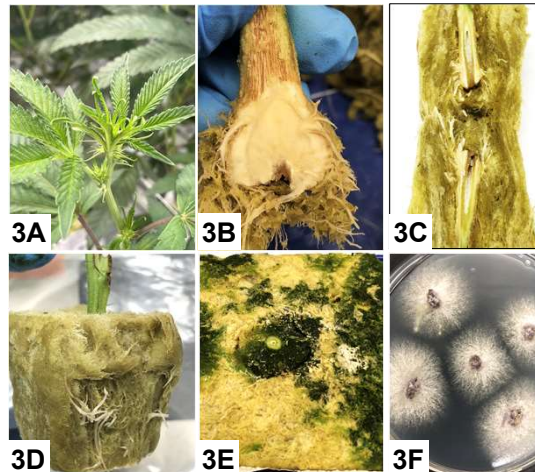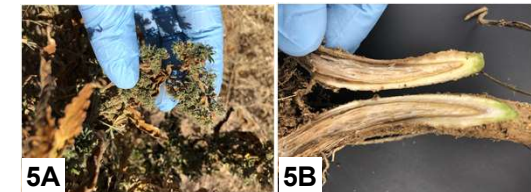
CASE #2

Hidden Collar Rot Caused by *Plectosphaerella cucumerina*
Symptoms concerned by the grower: Narrowed and yellowing new leaves and slightly browning along older leaf edges (**Fig. 4A**).
Diagnosis: Internal collar tissue was infected by *P. cucumerina* causing soft rot (**Fig. 4C**). Massive spores (**4D**) were produced inside rotted tissue shown in **4C**. But roots and external crown tissue appeared normal (**4B**).

CASE #3

Hemp Crop Failure due to the Infections by a List of Pathogens
Problem: Entire pivot of hemp crop failed with up to 70% plant mortality. Most plants had stem and root rot (**Fig. 5B**), and some had witches' broom (**Fig. 5A**).
Diagnosis: Fifteen species of *Fusarium*, *Pythium*, *Rhizoctonia,* and *Phytoplasma* were detected from 24 sampled plants (Tables below).

Organism	Plants infected
Fusarium equiseti	58.3%
Pythium ultimum	45.8%
Fusarium oxysporum	29.2%
Rhizoctonia solani	12.5%
'*Candidatus* Phytoplasma trifolii'	12.5%
Pythium conidiophorum	12.5%
Fusarium solani	12.5%
Pythium salpingophorum	8.3%
Fusarium tricinctum	8.3%
Fusarium incarnatum	4.2%
Fusarium redolens	4.2%
Pythium perplexum	4.2%
Pythium arrhenomanes	4.2%
Fusarium proliferatum	4.2%
Pythium heterothallicum	4.2%

Infection by organisms	Plants infected
Single-organism infection	25.0%
Dual-organism infection	50.0%
Tri-organism infection	16.7%
Quad-organism infection	16.7%
Single *Fusarium* species infection	20.8%
Dual *Fusarium* species infection	4.2%
Single *Pythium* species infection	4.2%
Dual *Pythium* species infection	4.2%
Dual-infection by *Fusarium* and *Pythium*	54.2%
Tri-infection b *Fusarium*, *Pythium* and *Rhizoctonia*	12.5%

LITERATURE CITED

1. Wang, S. 2021. "*Diagnosing Hemp and Cannabis Crop Diseases*" CABI Publishing. August 2021 | ePUB 9781789246094 | ePDF 9781789246087 | September 2021 | Hardback | 304 Pages | 9781789246070.