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Kern Pistachio Notes

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PHYTOPLASMAS

Phytoplasma are specialized bacteria that are obligate parasites of plant phloem tissue and transmitting insects (vectors). They were discovered by scientists in 1967 and were named mycoplasma-like organisms or MLOs. They cannot be cultured in vitro (i.e. outside of the plant in petri dishes, for example) in cell-free media. They are characterized by their lack of a cell wall, a pleomorphic (i.e changing) or filamentous shape, normally very small size with a diameter less than 1 micrometer, and their very small genomes. Although a phytoplasma has been reported in pistachio in Iran, to my knowledge, no plant infection or disease of pistachio by phytoplasma have been scientifically reported or published in the U.S.A. as of this writing. Nevertheless, an alert, informed and vigilant industry is a prepared industry, and should be knowledgeable of all potential problems that can impact economic returns.

SYMPTOMS

Phytoplasmas have been identified as pathogens of some agriculturally-important plants. While many of the diseases they cause are in the tropics, potatoes, apples and pears suffer significant losses in the temperate zones from phytoplasmas. Phytoplasmas induce symptoms that suggest interference with plant growth and development. Typical symptoms include: witches' broom (clustering of branches); usually shortening of internode length; phyllody (change of flower parts to leaf-like tissue); virescence (green coloration of non-green flower parts); bolting (growth of elongated flower stalks); flower sterility; reddening of leaves and stems; generalized yellowing, decline and stunting of plants; phloem necrosis; formation of bunchy fibrous secondary roots; and death in some instances.

TRANSMISSION

To spread naturally, phytoplasmas, generally, require a vector to be transmitted from plant to plant, and this normally takes the form of sap-sucking (i.e. phloem sucking) insects in which the phytoplasma may also be able to survive and replicate. Usually phytoplasmas are spread by a specific species of insect in the families Cicadellidea (leafhoppers), Fulgoridea (planthoppers) and Psyllidae (jumping plant lice). These insects feed on the phloem tissues of infected plants, picking up the phytoplasmas and transmitting them to the next plant they feed on. The concentration of the infectious agent (called ‘titer’) may not be enough in the crop plant to infect the vector sufficiently to cause crop- plant to crop-plant infection. However, sometimes the cycle of infection includes neighboring weed species that are host to the vector and, in which, titer can reach concentrations high enough to infect the insect vector, even if titer in the crop plan of concern never reaches these levels. Thus feeding on the weed species may be necessary first, for the insect vector to spread the disease to additional crop plants.

Many phytoplasmas are graft transmissible, either through human interventions or, more rarely, through natural root grafting. Phytoplasmas are not easily transmitted through pruning. Tissue culture, possibly, could provide a mechanism of transfer for some phytoplasmas and can be used as a tool for removing them from plant tissue.

IDENTIFICATION OF INFECTION OF AN UNKNOWN PHYTOPLASMA

In talking with experts, such as Dr. Georgios Vidalakis (University of California Plant Pathologist), who directs the California Citrus Clonal Protection Program, great care must be taken to be able to positively conclude that a plant specimen is infected by a phytoplasma of unknown origin. Accurate determination that the plant problem under investigation is caused by the phytoplasma requires patience. The process of identifying an unknown phytoplasma usually begins with PCR techniques. General primers exist for identifying a 'possible' infection by a phytoplasma; however, interpreting the results from their use can be tricky. Since many plant cellular organelles may have descended from free-living bacteria and since phytoplasmas are bacteria, these primers may falsely identify normally occurring cell mitochondrial DNA as being that of a phytoplasma. Specifically, for example, a phytoplasma will have sequence homology in the 16S-spacer regions to chloroplasts and plastids increasing the risk of false positives. Ribosomal DNA and the DNA of naturally-occurring plant endophytic bacteria can also cause false positives. Unfortunately, some primers can induce dimers or unspecific bands that are amplified in the PCR process complicating analysis. To further complicate the identification procedure, some phytoplasma-infected tissue is able to produce inhibitors, such as polysaccharides, that interfere with the ability of the PCR process to amplify the existing phytoplasma DNA, and increase the chance of a false negative.

Thus, the meticulous plant pathologist will use a range of PCR primers and more sensitive gels to increasingly rule-out false positives and negatives and eventually sequence the genome of the phytoplasma if present. Sequencing an unknown pathogen as a phytoplasma is necessary for ensuring that the cause of the disease is a phytoplasma. If a phytoplasma can be sequenced from an unhealthy plant and not healthy plants, the pathologist is probably on the right track. Additionally, light or electron microscopy should be used to show the presence of the phytoplasma in infected phloem cells and not those in normal phloem tissue. Eventually, transmission studies using grafting and, possibly, insect vectors are conducted to prove and further understand the disease.

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