

Phymatotrichopsis root rot and its biological control in the pecan tree in Mexico

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Abstract

In Mexico, phymatotrichopsis root rot is regarded as the most important disease in the pecan tree. Its causative agent is the fungus *Phymatotrichopsis omnivora* (Duggar) Hennebert, and the percentage of diseased trees ranges between 3 to more than 25% in a plantation. Symptoms vary from mild damages to the death of the infected tree. The roots invaded by the fungus die with the consequent reduction of the foliage. Chemical control is one of the most used methods to control this pathogenic fungus, but it is expensive and not very efficient, so it is necessary to try other methods such as antagonistic fungi, resistant varieties, plant extracts, among others that have been shown to have some effectiveness, and to design an integrated management program for the pest. For all the reasons stated, the objective of this review was to address the most current aspects of the disease and its biocontrol in Mexico.

Keywords: antagonistic; chemical control; fungus; root disease

Introduction

The importance of the pecan nut in Mexico is well documented, being Chihuahua the first producing state with 68,500 t, followed by Coahuila (16,800 t) and Sonora (15,000 t) (Comenuz, 2021).

In nut production, diseases are a constraining factor; phymatotrichopsis root rot or Texas root rot, caused by the fungus *Phymatotrichopsis omnivora* (Duggar) Hennebert, is a severe disease that attacks pecan tree [*Carya illinoensis* (Wangenh.) K. Koch] roots, which when infected and destroyed by the fungus, lead the foliage to dry up and die. The fungus can attack seedlings less than one-year-old up to trees older than 45, being more frequent the death of trees less than seven years old (Hu, 2020).

The opportunity to develop tools for the biological control of phymatotrichopsis root rot is high, since there are several soil microorganisms that are strongly antagonistic to *P. omnivora*, such as the fungus

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Trichoderma (Samaniego-Gaxiola *et al.*, 2019). According to a recent report, the use of chemical pesticides diminished in 2% per year, and biopesticides application increased to a 15% because of restriction laws (Damalas and Koutroubas, 2018). Agronomic practices based on respect for the environment, which at the same time guarantee the sustainability of farms and income for producers, are the future of agriculture (Fenibo *et al.*, 2021).

The present review was done by searching in Google Scholar, Research Gate, PubMed/MEDLINE, Web of Science and Scopus databases, being the aim to give information about the phymatotrichopsis root rot diseases and its control, focusing on disease control with antagonistic fungi. It is important to note that there is not much recent literature on this disease affecting pecan tree.

Pecan tree [*Carya illinoensis* (Wangenh.) K. Koch]

The pecan tree, commonly called pecan, is a deciduous fruit tree of the Juglandaceae family, genus *Carya* that includes nearly 20 species (Chávez *et al.*, 2009) that reaches a height of 75-100 feet tall with a large, typically wide spreading crown (canopy) and a longevity of more than 100 years (Madero, 2000). The reproductive age of these trees begins from the fifth or sixth year. This species is native to Mexico and the United States of America (Orona *et al.*, 2007), being Mexico the second largest producer of pecan nuts and the first exporter of this product in the world (Orona-Castillo *et al.*, 2019).

In Mexico, the approximate total area of the pecan tree in irrigation conditions was 144,649.50 ha in 2021. The states with the highest pecan nut production at the national level are Chihuahua, Coahuila and Sonora with a total of 92.36%, particularly the states of Chihuahua and Coahuila together contribute 82% of the national nut production (SIAP, 2022).

Pecan is one of the most important horticultural nut crops in the world and the nut has an extraordinary calorific value (~680 calories/100 g kernel). Pecan is better to walnut in quality (8-10% proteins, flavour, 65-70% fats, high in potassium, phosphorous, and vitamins A, C, E and B complex) and succeeds best in the areas which are lower and hotter for nut cultivation (Sparks, 2000; Singh *et al.*, 2009).

In addition to the nutritional characteristics that the nut has, the shells of the pecan also have important properties that are useful for industry by-products (Dunford *et al.*, 2022). Several studies reported the existence of health-beneficial phytochemicals compounds in pecan shells (do Prado *et al.*, 2009; Sevimli-Gur *et al.*, 2021). Greater levels of tannins and phenols were found in the shells compare to the edible part of pecans (do Prado *et al.*, 2009), those compounds have medicinal and nutritional significance because of their powerful antioxidant properties. Huge amounts of shells produced during the edible process represent about 40 to 50% of the whole nut weight, for this reason pecan nut shelling industry by-products have commercial impact and possible for valorisation (Sevimli-Gur *et al.*, 2021; do Prado *et al.*, 2009).

The antioxidant activity of phenolic compounds is associated with preventing the incidence of chronic-degenerative diseases such as arteriosclerosis, Alzheimer's, Parkinson's, obesity, diabetes, cardiovascular problems, cholesterol and some types of cancer (Flores-Córdova *et al.*, 2017). In addition, it protects and strengthens the human body, delaying aging by preventing cell oxidation by neutralizing free radicals (Flores-Córdova *et al.*, 2021).

Phymatotrichopsis root rot generalities

The disease has been reported in Mexico since 1922, being widely detected in the North and northwest states such as Chihuahua, Sinaloa, Tamaulipas, Sonora, Nuevo León, Durango, Baja California and Baja California Sur (Reyes, 2015).

Phymatotrichopsis root rot is a disease that destroys the roots of pecan trees and other agricultural crops, the causal agent is the fungus *P. omnivora* (Samaniego-Gaxiola *et al.*, 2001). The infection is influenced by

several physiological and environmental factors, including the maturity of the host, quantity and location of the primary inoculum, soil temperatures and soil texture (Rush *et al.*, 1984).

One of the main characteristics of phymatotrichopsis root rot is that the leaves turn brown and wither without previous symptoms (Figure 1), permanent wilting occurs in a couple of days, then the tree dries up, and the leaves remain firmly attached to the plant (Figure 2). At the time of incipient wilting, the roots are already completely invaded by the fungus (Hu, 2020). The cortical tissue of the roots is destroyed, so they will rapidly flake off and they are covered by the mycelium of the fungus. Almond and pecan trees often exhibit a brown colour before wilting and one or several branches of the tree may die even if few roots have been invaded (Goldberg, 2005).



Figure 1. Wilting (bronzing) and leaf necrosis of pecan trees affected by phymatotrichopsis root rot
Source: Authors



Figure 2. Pecan tree that suffered sudden death from phymatotrichopsis root rot, where the brown foliage remains attached for the first few weeks
Source: Authors

Root colonization can occur 14 d before symptoms can be observed (Rush *et al.*, 1984). Alternatively, it has been observed that in pecan trees when the plantation density is high, there is more mortality of the trees, however, according to Samaniego-Gaxiola *et al.* (2014) the presence or development of the disease is closely associated with the susceptibility of the plant to the pathogen.

In aboveground tissues, root rot produces vascular discoloration of the stem and sudden wilting of the leaves, without abscission and eventual death of the plant. Characteristic branching mycelial cords woven by anastomoses around the central hyphae (creamy-yellowish) of the pathogen are typically visible on the root surface, which help diagnosis. Different long tapered hyphae arise from this structure (opposite and at right angles) forming a cruciform figure, an irrefutable taxonomic characteristic. Old trees show decreased growth and vigor (Reyes, 2015).

Generally, plants that acquire diseases caused by pathogens like *Fusarium*, *Rhizoctonia*, *Phymatotrichopsis* and others increase their severity with the time. In the specific case of pecan trees affected by *P. omnivora* is possible that some affected trees show no change in symptoms during four years (Samaniego-Gaxiola *et al.*, 2002).

In annual or perennial crops, phymatotrichopsis root rot through the years, expands radially and the area devastated by the disease is called blotch. When the distance of the plants between rows does not favor contagion between plants, the incidence of the disease will be in function of the number of points of infection, multiplied by the average number of diseased plants in each point (Jeger *et al.*, 1987).

Taxonomy, ecology, and biology of *P. omnivora*

The major group of plant fungal pathogens are necrotrophic fungi causing substantial crop losses worldwide (Shao *et al.*, 2021). *Phymatotrichopsis omnivora* is a soil-borne necrotrophic fungal pathogen that can infects over 2,000 dicotyledonous plants (Kankanala *et al.*, 2020). The definition of necrotrophic pathogens is that they kill host plant cells and use nutrients to support their own live. Necrotrophic fungi also may induce apoptosis in host cells rather than breaking plant cell walls, or induce their own apoptosis (Shi *et al.*, 2020).

The Index taxonomical identification of the fungus is given in the NCBI database by Schoch *et al.*, (2020) as following:

Current scientific name: *Phymatotrichopsis omnivora* (Duggar) Hennebert, 1973

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Pezizomycotina

Class: Pezizomycetes

Order: Pezizales

Family: Rhizinaceae

Genus: *Phymatotrichopsis*

P. omnivora is a fungus with no known sexual phase, it was named *Phymatotrichum omnivorum* (Shear) because of the conidial characteristics, which are produced in spore mats that grow near the diseased plant (Duggar, 1916). It was found within the class Hyphomycetes, order Hyphales (Moniliales), and was associated within the Phylum Basidiomycota. Hennebert (1973) renamed it *Phymatotrichopsis omnivora* (Duggar) Hennebert, due to the affinity that he observed that this fungus had with Botrytis, within the division Ascomycota. The life cycle of the fungus is represented in Figure 3.

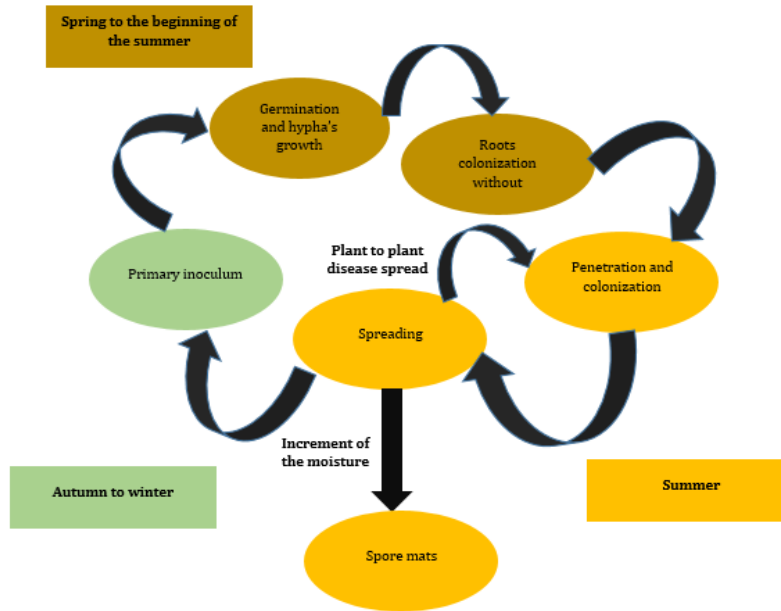


Figure 3. The life cycle of *P. omnivora*
Source: Authors

Kankanala *et al.* (2020) founded that *P. omnivora* infects the susceptible plant as a traditional necrotroph. However, it infects the partially resistant plant as a hemi biotroph triggering salicylic acid-mediated defence pathways in the plant. Further, the infection strategy in partially resistant plants is determined by the host responses during early infection stages.

Agrios (1997) explained that this fungus survives and reproduces by sclerotia or hyphae and by this one the fungus moves through the soil or over the root tissue. It spreads slowly from plant to plant when a fungal thread from an infected root grows through the soil and reaches a healthy root. However, the sclerotia are resistance structures through which the fungus can survive during the winter without a host or when the conditions of the environment are not favourable. Also, these structures can survive for at least five years in the soil (Riggs, 2008) up to 20 years if there are no limiting factors (Samaniego-Gaxiola, 2008).

Reyes (2015) mentioned that for the infection the mycelium, by contacting the roots, invades and penetrates the tissues (Figure 4).



Figure 4. Root of pecan tree infected with *P. omnivora*
Source: Authors

This fungus produces a mass of conidia (white crusty formations) on the soil surface near the host when hot, humid weather prevails. These conidia have been considered sterile, but it is suspected that they may serve to establish new sources of infection. The way that this pathogen penetrates the roots is not yet clear, but the fungus colonizes the interior of this organ, obstructing the vascular system and blocking the movement of water (Riggs, 2008).

Control

The sclerotia of *P. omnivora*, as already mentioned, are capable of living in the soil in adverse periods for many years, making this pathogen control very difficult (Rush *et al.*, 1984).

The main control methods are based on integrated crop management, which includes crop rotation, reductive soil disinfestation (Momma *et al.*, 2006), elimination of diseased plants, the addition of fertilizers that, in addition to reducing the soil pH, when decomposed, favour the proliferation of populations of microorganisms that can present antagonism to the fungus (Agrios, 1997), chemical control (Martínez-Escudero *et al.*, 2016) and biological control (Guigón-López *et al.*, 2015).

Reductive soil disinfestation

This is a method where a carbon (C) source such as molasses, wheat straw or carbohydrate-rich compounds are added to the soil, then the soil is saturated with water and covered with a plastic (Momma *et al.*, 2006).

According to Samaniego-Gaxiola *et al.* (2019) sclerotia of *P. omnivora* died in supernatants at pH ~4 that came from the soil with 2.0 and 4.0 mg g⁻¹ of added glucose. The pH and oxidation-reduction potential reached in soils with glucose and molasses are characteristic of reductive soil disinfestation. Another way of reductive soil disinfestation is to use various substrates as a source of C for the control of a single pathogen (Serrano-Pérez *et al.*, 2017).

An additional experiment demonstrated that after the reductive soil disinfestation was possible to reduce the populations of fungi in soil cultivated with watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) (Liu *et al.*, 2018).

Chemical control

For many years, synthetic fungicides have been used as the major control against fungal plant pathogens. Nevertheless, recently, while the chemical method is still predominant over other controls, the application of synthetic fungicides has been gradually diminishing because of the global preoccupation on risks due to residues in soil, water, air and foods (Palmieri *et al.*, 2022).

Less success with the application of several chemicals have been achieved; nevertheless, Rush and Lyda (1984) worked with methyl bromide and they found that it was effective but huge amounts of this chemical must be added deeply into the soil, making it costly and consequently not commercially practical.

Even though there is no effective fungicide to control phymatotrichopsis root rot in pecan trees, the chemical flutriafol has been used with effectiveness in controlling *P. omnivora* in cotton (Isakeit *et al.*, 2012). Rhyme is the common name of flutriafol in Arizona and it is used for the control of powdery mildew and scab (foliar fungal diseases) on pecan, but not for the control of phymatotrichopsis root rot (Hu, 2018).

Systemic fungicides, like benzimidazoles and sterol biosynthesis inhibitors, have been used with good results to reduce the incidence of *P. omnivora* (Whitson and Hine, 1986). Because most systemic fungicides are relatively expensive, do not translocate well, and exhibit poor soil penetration and persistence, the efficacy of these products must be prudently evaluated (Uppalapati *et al.*, 2010).

Biological control

For plant diseases, biological control is frequently defined as direct or indirect inhibition of the pathogen responsible for the disease or a disease, by another organism or group of them (Cook, 1985). A wider meaning also includes specific metabolites, isolated from interactions or plant extracts (attractant activities or antibiotic) that can be beneficial for controlling diseases (Roberts and Taylor, 2016). Plant pathologists have inclined to use the term to denote control methods including soil pH alterations, crop rotation, use of organic amendments, etc (Baker, 1991).

The influence of oilseed meals [0%, 1%, and 5% (w/w)] from both brassicaceous plants including mustard (*Brassica juncea* L.) and camelina (*Camelina sativa* L. Crantz) as well as non-brassicaceous plants including jatropha (*Jatropha curcas* L.), flax (*Linum usitatissimum* L.), and Chinese tallow (*Triadica sebifera* L.) was evaluated on *P. omnivora* sclerotial germination and hyphal growth in Branyon clay soil and as results all tested brassicaceous and jatropha seed meals at 5% and 1% application rates respectively were capable to inhibit *P. omnivora* sclerotial germination and hyphal growth, with mustard seed meal being the best treatment (Hu *et al.*, 2011).

Valero-Galván *et al.* (2005) used essential oils produced by plants such as oregano (*Origanum vulgare* L.) in different concentrations (50, 100, 150, 200, 250, 500 ppm) to control *P. omnivora* and they found that when the essential oil was used in concentrations of 150, 200, 250, 500 ppm, the fungus was completely inhibited.

According to Rush and Lyda (1982) another form to control *P. omnivora* was by the application of anhydrous ammonia (NH₃) that was toxic to the mycelium and sclerotia of the fungus at 28 µg mL⁻¹ and this was proportional to the period of exposure, giving 3, 23, 34, and 59% of kill of sclerotia after 1, 12, 24, and 48 h respectively. Exposure of sclerotia to NH₃ concentrations of 42, 56, or 84 µg mL⁻¹ for 12 h resulted in 100% control of the pathogen *in vitro* but for the *in-situ* control higher concentrations of NH₃ were necessary so 138 and 276 µg g⁻¹ resulted in 35 and 79% control, respectively.

Soil microorganisms play an important role in pathogen control because plants through the roots produce exudates that provide a perfect environment for the growth of microorganisms with which the roots establish associations, either for the stimulation of plant growth, exchange of nutrients, or protection against pathogens, and some others (Whipps, 2001; Sarabia *et al.*, 2010).

There are several biocontrol products listed in the Environmental Protection Agency (EPA), and one of the most used microorganisms in the registered products correspond to fungi belonging to the genus *Trichoderma* spp., particularly *T. harzianum*, *T. lignorum* and *T. viride* (Vinchira-Villarraga and Moreno-Sarmiento, 2019). The application of these antagonistic microorganisms is because the mechanisms of action that they use to control pathogens such as mycoparasitism, competition, production of volatile compounds and antibiosis (NawRocka *et al.*, 2017; Awad *et al.*, 2018).

Trichoderma spp. have the capacity to produce hydrolytic enzymes capable of invading the sclerotia of *P. omnivora*, preventing them from germinating (Samaniego-Gaxiola, 2008; Guigón-López *et al.*, 2014; Guigón-López *et al.*, 2015).

López *et al.* (2015) tested three native isolates of *T. asperellum* as antagonists of *P. omnivora*, finding an antagonistic capacity of between 61.4% and 90.1%, with a daily mycelial rate of 2.23 mm. Also, Guigón-López *et al.* (2010) analysed the effect of six *Trichoderma* spp. native strains and they found that *T. asperellum* TC74, T341 and T359 inhibited the *P. omnivora* growth from 28% to 37%.

Commercial plant protection products have been developed that contain special strains of live *Trichoderma* species, primarily *T. harzianum* and have been formulated so that farmers can easily apply them in the field. It is a safe and effective biocontrol agent that can control a variety of fungal (and bacterial) diseases, including wilt, stem rot, and downy mildew (Singh *et al.*, 2010).

Molecular research of the topic

Despite the extensive investigation achieved on *P. omnivora* over the past 100 years, several areas of the physiology and molecular biology of this pathogen and its relationship with host plants are still poor understood (Uppalapati *et al.*, 2010).

Marek *et al.* (2009) reported the molecular systematics of *P. omnivora* using the ribosomal DNA and RNA polymerase II subunit 2 loci and they confirmed that this fungus belongs to the phylum Ascomycota not Basidiomycota. Also, through this research authors found that the acknowledgement exclusively to Duggar (Hennebert, 1973) was an error because this is in conflict with the 'one fungus one name' definition, and left the taxon without a type specimen. Their proposal was that the attribution should be to Shear (1907), with his type specimen as holotype. The Panel notes that this proposal has not been taken into account by indexfungorum.org and mycobank.org, which still use *P. omnivora* (Duggar) Hennebert.

Macmil (2009) constructed a cDNA library containing expressed genes from the three different structures that characterized distinct morphological stages in the life cycle of *P. omnivora* (mycelia, conidial spore and sclerotia) and on exposure to three different nutrient conditions (media deprived of either carbon or nitrogen; *Medicago truncatula* Gaertn and *Medicago sativa* L. root exudates and non-host root exudates of *Sorghum bicolor* Moench. Unique expressed sequence tags (ESTs) were compared with homologs sequences in GenBank, the Consortium for the functional Genomics of Microbial Eukaryotes (COGEME), Eukaryotic Orthologous Genes (KOG) and Kyoto Encyclopedia of Genes and Genome (KEGG) databases using the Blast alignment program and characterised into groups based on biological function assignment. Results from the genomic sequence showed that the ~74 Mbp assembly was around twice the dimension expected by electrophoretic gel karyotyping from *P. omnivora* protoplasts supporting the theory that this fungus is an obligate heterokaryon with numerous heterokaryotic nuclei. The comparison with several databases allowed the author to found that *P. omnivora* encode greater numbers of heavy metal and calcium transporting P-type ATPases and ABC-type transporters than *Magnaporthe grisea* (T.T. Hebert) M.E. Barr and *Neurospora crassa* Shear&Dodge, 1843, which allow the pathogen to survive in calcareous heavy metal containing soils and on exposure to plant toxins and fungicides.

Microarrays research was developed by Uppalapati *et al.* (2009) to analyse the host defence signals under *P. omnivora* infection and ethylene was induced by ROS (Reactive Oxygen Species) together with the fungus. In this study when roots of *M. truncatula* were infected with the pathogen, chitinases Class I and Class IV, genes involved in ROS generation and phytohormone such as ethylene and jasmonic acid were identified as upregulated genes.

An expression study was done by Guigón-López *et al.* (2015) between *T. asperellum* strains TC74 (great antagonistic capacity) and Th1 (little antagonistic capacity) with *P. omnivora* and as results both strains were capable to express their β -1,3-exoglucanase and chitinases activities when grown on the different carbon sources (*P. omnivora* powdered sclerotium (MM-PO) and in *Agaricus bisporus* (J.E.Lange) Imbach, 1946 plus fiber (MM-AF). Mycoparasitism-related genes were primary expressed when both *T. asperellum* strains were tested with *P. omnivora*.

No molecular study was found in the reviewed literature that refers to the pathogen in the pecan tree but the articles read offer a better understanding about this important fungus and help with the improvement of the management strategies and resistant germplasm to control the disease.

Conclusions

Phymatotrichopsis root rot is the fungal disease that causes the greatest damage to pecan trees in Mexico, with losses that in some cases exceed a third of the plantation. Until now, the most used control methods are reductive soil disinfestation and chemical control; however, the efficiency of these techniques is not high, they require some procedures for their use, they cause contamination in the soil and water, and they are very expensive. In the past two decades, biological control methods have begun to be used in the fight against this disease; the use of essential oils from plants and microorganisms, mainly *Trichoderma* sp., exhibits promising results in inhibiting the development of the fungus, and additional advantages such as its harmlessness to the environment and its low cost, which makes it a viable alternative to conventional chemical methods. Molecular biology has shown incipient results on the pathogen, plant defence mechanisms and host-pathogen interaction, which will undoubtedly support the implementation of these technologies in the future.

Authors' Contributions

SPA and MDG Conceptualization; SPA, EFHA and ES writing original draft; SPA, EFHA and EGR writing, review and editing.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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