White Root Rot

Rosellinia necatrix

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1. Abstract

Rosellinia necatrix Prill. (anamorph *Dematophora necatrix* R. Hartig), is a soil-borne ascomycete that causes white (or dematophora) root rot in a large number of economic fruit tree species, including pear trees (*Pyrus* spp.), apple trees (*Malus* spp.) and grapevines (*Vitis vinifera*). Over 170 plants in 63 genera can be affected by this fungus, some being economically relevant like coffee (*Coffea* spp.), olive (*Olea europaea* L.), avocado (*P. americana* Mill.), stone fruit (*Prunus* spp.) and citrus (*Citrus* spp.). In the European Union, *R. necatrix* is listed as regulated non-quarantine pests (RNQPs) and has been reported all over the world, with substantial losses in yield and quality of host plants. The pathogenic cycle is summarized in the production of three types of spores: chlamydospores and conidiospores, which are developed during the asexual life cycle, and ascospores, which are the sexual reproductive structures. White root rot has a limited spread capability, being restricted to the soil and infected material. The fungus is capable of living in the ground in a dormancy stage and disease emerges only when environmental conditions are favourable. The detection of the disease is the most important step to control the spread. Thus, early diagnosis and identification of symptoms is key. Some of the macroscopic symptoms caused by R. necatrix that allow its detection in the field include: abnormal colours and shapes of the leaves, hairy root and plant weakening. In recent years, large efforts have been made to facilitate the detection of *R. necatrix* because of the difficulties of in-field diagnosis. The use of molecular techniques, such has Scorpion-PCR, is the current gold standard to detect and identify *R. necatrix* in the lab because these methods are rapid and sensitive. Furthermore, fungicides are normally used to control this fungus infection, for example, thiophanate-methyl that is used by the Japanese pear growers. In the same way, the use of fluazinam was show to be more efficient than other fungicides. Besides, some antagonist species of bacteria and fungi are recognized as a biological control, this microorganism can compete for niche and nutrients present in the soil. To conclude, *R. necatrix* is a pathogen that need to be control because it has high ranges of host plant targets, and most of the targets are economic fruit tree species, use field and laboratory detection should improve the control and could optimize the prevention methods.

2. Resumo

Rosellinia necatrix Prill. (anamorfo *Dematophora necatrix* R. Hartig) é um fungo ascomicete causador da podridão radicular em várias espécies, incluindo pereira (*Pyrus* spp.), macieira (*Malus* spp.), videira (*Vitis vinifera*), cafeeiro (*Coffea* spp.), oliveira (*Olea europaea* L.), abacateiro (*P. americana* Mill.), *Prunus* spp. e citrinos (*Citrus* spp.). Mais de 170 espécies distribuidas em 63 géneros, são afectadas por este fungo. O género *Rosellinia* pertence à família Xylariaceae e inclui outros agentes patogénicos importantes, como as espécies *Rosellinia arcuata*, *Rosellinia desmazieresii* e *Rosellinia pepo.* A podridão radicular está presente em vários países por todo o mundo, em regioẽs com clima temperado, subtropical e tropical, com grande impacto no rendimento e qualidade da planta afectada. Na década de 90, estima-se que 42% dos pomares de macieiras na zona de Alcobaça (Portugal) estavam infectados com *R. necatrix,* sendo que 14% das árvores apresentavam sintomas severos. Na União Europeia, a *R. necatrix* é considerada uma praga regulamentada não sujeita a quarentena (RNQPs).

Este agente patogénico infecta tanto a superfície das raízes como os tecidos por baixo da casca das árvores, e a sua capacidade de dispersão está restrita ao solo e material infectado. *R. necatrix* pode sobreviver nas raízes mortas e na matéria orgânica presente nos solos dos pomares em estado dormente e emergir quando as condições ambientais são favoráveis. Este fungo apresenta reprodução sexuada e assexuada no seu siclo de vida, produzindo três tipos de esporos: clamidosporos e conidiospore durante a fase assexual, e ascosporos na fase sexual. Os clamidosporos são raramente encontrados na natureza. *R. necatrix* dispersa-se no solo através do crescimento dos micélios ou pelo contacto directo entre as raízes de plantas infectadas e saudáveis. Quando em contacto com raízes saudáveis, a rede de micélios prolifera, a infecção primária ocorre e as hifas invadem o xilema primário e secundário. A solarização e a temperatura são essenciais para o desenvolvimento do fungo, sendo a temperatura óptima de crescimento do micélio 22.5-25.5°C. A luz tem um efeito inibitório no desenvolvimento do fungo. A detecção da doença é essencial para o controlo. Os sintomas incluem a coloração e formato anormal das folhas, desfoliação, redução do tamanho dos frutos e a presença de micélios brancos nas raízes. Para a detecção e identificação do fungo em laboratório são usadas técnicas moleculares como o Scorpion-PCR. Para o controlo da doença, são usados fungicidas como o fluazinam. Em Portugal, nenhum fungicida está aprovado pela DGAV, especificamente contra o fungo *R. necatrix.* Além disso, algumas espécies de bactérias e fungos antagonistas têm sido avaliados como agentes de controlo biológico, como por exemplo *Trichoderma harzianum, Agrobacterium* e *Pseudomonas*. Estes microorganismos competem por nutrientes presentes no solo. Os métodos culturais também são essenciais no combate à doença, como por exemplo o uso de substratos arejados e secos tratados com vapor ou fungicidas, as sementes devem ser tratadas com água quente, os materiais de uso agricola devem ser desinfectados. Para além disso, é recomendado fazer uma inspecção periódica das raízes de forma a detectar precocemente o fungo.

Em suma, *R. necatrix* é um agente patogénico com elevada importância uma vez que afecta várias espécies, sendo que grande parte têm alto valor comercial, como é o caso de árvores de fruto. Desta forma, os meios de detecção no campo e no laboratório devem ser melhorados, assim como os meios de prevensão da doença.

3. Introduction

Rosellinia necatrix Prill. (anamorph *Dematophora necatrix* R. Hartig), is a soil-borne ascomycete that causes white root rot, also known as dematophora root rot disease, in a large number of plant species, including economically important fruit trees such as pears, apples, olive, orange, grape, coffee and avocado. This pathogen as been reported all over the world, in temperate, subtropical and tropical regions. White root rot is specially important in the Mediterranean region due to the co-occurrence of favourable environmental conditions for fungal development and susceptible hosts (Pliego et al., 2012). This disease leads to substantial losses of yield and quality for host plants with economic importance (Sawant et al., 2021). In 1963, estimated losses caused by the fungus for apple production in the state of Himachal Pradesh, India, were at least $272,000 (US), corresponding to $1.6 million dollars in the present time (Agarwala and Sharma, 1966). In Japan, the losses reach $4 million (US) per year for both *Vitis vinifera* and Japanese pear (*Pyrus pyrifolia*) (Ten Hoopen and Krauss, 2006). In the early 90s in Portugal, it was estimated that in the Alcobaça region, 42% of the orchards were infected and 14% of the apple trees presented advanced symptoms

(Teixeira de Sousa et al., 1995). *R. necatrix* is considered a regulated non-quarantine pests (RNQPs) (Commission Implementing Regulation (EU) 2019/2072, Annex IV).

R. necatrix infects both the surfaces of roots and the tissue under the bark. White root rot disease leads to multiple degrees of canopy decline, followed by leaf drop, wilting, and possibly the death of infected plants. *R. necatrix* can survive on dead roots and other plant debris on the ground, although fresh debris rich in cellulose is required for its continued survival (Sawant et al., 2021). *R. necatrix* has both sexual and asexual reproduction phases in its biological cycle, producing three types of spores: chlamydospores and conidiospores, developed during the asexual phase, and ascospores, produced in the sexual phase (Pliego et al., 2012). This pathogen spreads in the soil via mycelial growth, which proliferates and aggregates on the roots of host plants, and thought direct root-to-root contact between infected and healthy plants (Pliego et al., 2009).

White root rot disease can be detected in the field through the aerial symptoms: top leaves show bronzing, and eventually defoliation, reduction in fruit size and drying of the tree; and through root symptoms: presence of a white cottony mycelium and mycelial strands that surrounds the roots (Ten Hoopen and Krauss, 2006). Detection in the lab can be achieved through molecular assays. Disease control strategies usually consist of an integrated approach.

4. The pest and its biology

4.1. Taxonomy

Name: *Rosellinia necatrix* Prill. (teleomorph)

Synonyms: *Dematophora necatrix* R. Hartig (anamorph)

Taxonomic tree:
Domain: Eukaryota
Kingdom: Fungi
Phylum: Ascomycota
Subphylum: Pezizomycotina
Class: Sordariomycetes
Subclass: Sordariomycetidae
Order: Xylariales
Family: Xylariaceae
Genus: *Rosellinia*
Species: *Rosellinia necatrix*

Common names: white root rot of trees (english), *Dematophora* root rot, podridão radicular (portuguese), llaga blanca (spanish), pourridié blanc (french)

EPPO code: ROSLNE (Rosellinia necatrix)

The genus *Rosellinia* belongs to the family Xylariaceae, which includes several species including some economically important root rot pathogens, such as *Rosellinia arcuata*, *Rosellinia desmazieresii* and *Rosellinia pepo* (Pérez-Jiménez, 2006).

On culture media, young mycelium of *R. necatrix* is initially white and cottony and over time it becomes brown–black (Pérez-Jiménez, 2006). *R. necatrix* grows fast in the culture medium when incubated in the dark at 20-24°C (Pérez-Jiménez, 2006). An important morphological characteristic of the fungal hyphae is the presence of pear-shaped or pyriform swellings can be found above the hyphal septum, with a diameter of up to 13 µm (Pérez-Jiménez, 2006; Pliego et al., 2012). Sclerotia are black, hard and spherical nodules, with several millimetres in diameter, and it develops mainly on invaded roots. Black sclerotia crusts may also develop on roots (Pérez-Jiménez, 2006; Pliego et al., 2012) . On potato dextrose agar (PDA) medium in the light, *R. necatrix* forms microsclerotia, which are irregular, rough bodies composed of a compact mass of melanized, interwoven hyphae with no differentiated cells (Pérez-Jiménez, 2006; Pliego et al., 2012). Chlamydospores are rarely found under natural conditions, and they are almost spherical with 15 µm in diameter (Pérez-Jiménez, 2006; Pliego et al., 2012). Synnemata, also named coremia (0.5–1.5 mm in length), can be formed from both sclerotia or mycelial masses. Conidia are 3–5 µm in length and 2.5–3 µm in width and they are very difficult to germinate *in vitro*. Ascospores are monostichous, situated inside acylindrical, long-stalked ascus. They are ellipsoidal and cymbiform, with 36–46mm in length and 5.5–6.3mm in width (Pérez-Jiménez, 2006; Pliego et al., 2012).

R. necatrix grows poorly on synthetic media but the growth is vigorous on natural plant extract media containing peptone. Moreover, vitamins biotin, thiamine and inositol, asparagine, glucose and some mineral salts are essential for fungal growth (Pérez-Jiménez, 2006). Fungal development is encouraged when vegetable debris is present in the soil, since organic matter decomposition slowly activates the saprotrophic and parasitic activity (Pérez-Jiménez, 2006). Nevertheless, green amendments, such as soybean, bean or pea, restrict fungal development (Pérez-Jiménez, 2006).

One draft genome is available at NCBI database (GCA_001445595.3).

4.2. EU and PT regulatory status

In the European Union (EU), *R. necatrix* is listed as regulated non-quarantine pests (RNQPs) (Commission Implementing Regulation (EU) 2019/2072, Annex IV). A RNQP is defined as ‘a non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party’.

4.3. Disease distribution

R. necatrix has a wide range of host species, being widely distributed across five continents in temperate, subtropical and tropical regions (Figure 1). In Europe, *R. necatrix* is present in Austria, Bulgaria, Cyprus, former Federal Republic of Yugoslavia, France (main land and Corsica), Germany, Greece, Hungary, Italy, Portugal, Romania, Russia, Spain (main land and Canary Islands), Switzerland, Ukraine, United Kingdom (CABI, 2021). In Portugal, *R. necatrix* is widely distributed, with high impact in the Oeste region (Teixeira de Sousa et al., 1995). In early 90s, it was estimated that 42% of apple orchards in Alcobaça region were infected with *Rosellinia* (Teixeira de Sousa et al., 1995).

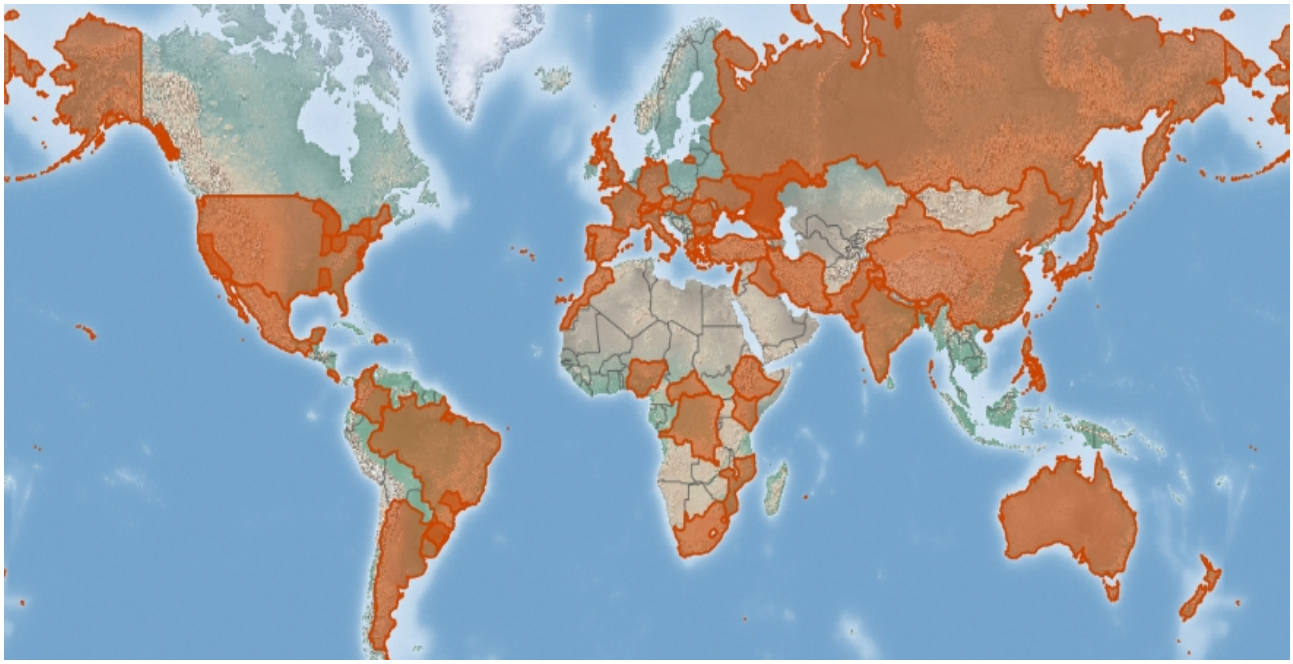

Figure 1: *Rosellinia necatrix* distribution (CABI, 2021).

4.4. Disease cycle

Rosellinia necatrix produces three types of spores: chlamydospores and conidiospores, which are developed during the asexual life cycle, and ascospores, which are the sexual reproductive structures (Pliego et al., 2012). Chlamydospores are rarely found under natural conditions (Pérez-Jiménez et al., 2003). Conidia are formed at the end of synnemata of conidiogenous cells, that are produced from either sclerotia or brown mycelial masses. Ascospores, which are easily found in found in infected tissues, are developed inside the perithecium. When mature, they are expelled into a mucilaginous mass from the pore of the papilla located at the top of the perithecium (Pliego et al., 2012) . With ageing, ascospores contract and acquire a brown–black colour and a dry aspect as a result of hyphal and cell melanization. The asci are projected towards the interior of the perithecium (Pérez-Jiménez et al., 2003). Perithecia takes a long time to form under natural conditions. Diseased roots develop a synnemata, which produces conidia as spermatia. Based on microscopy analysis on infected avocado roots, it was observed that *R. necatrix* is spread throughout the soil via mycelial growth or via direct root-to-root contact between host and healthy plants (Pliego et al., 2009). When in contact with healthy roots, the mycelia network proliferates, covering the root surface either as a diffuse mycelium or in the form of hyphal strands. Then, mycelial aggregates are formed in areas covered by a diffuse mycelium, penetrating the roots through natural openings (lenticels), wounds or directly by forming a penetration sclerotium. Primary infection of the roots occurs and hyphae invade and penetrate into the primary and secondary xylem (Pliego et al., 2009). *R. necatrix* can survive on the residues of susceptible plants for many years and can also survive as saprophytes on dead roots and other plant debris in the soil, although fresh vegetable debris rich in cellulose is required for its continued survival (Sawant et al., 2021). Nevertheless, green amendments, such as soybean, bean or pea, restrict fungal development (Pérez-Jiménez, 2006).

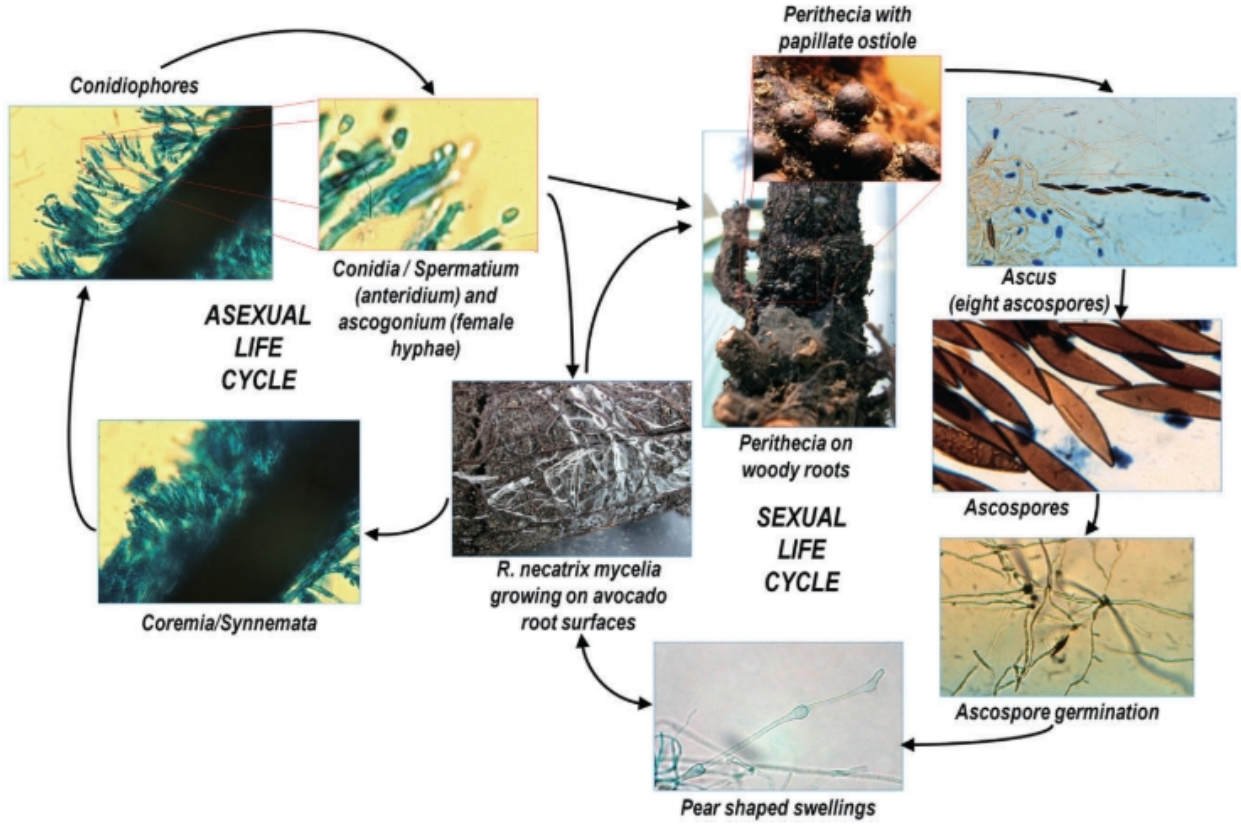

Figure 2: *Rosellinia necatrix* life cycle (Pliego et al., 2012).

5. Host Plant Target population

5.1. Host range and main hosts

The dematophora root rot disease has a wide range of hosts particularly affecting fruit tree crops, such as apple trees (*Malus* spp.), pear trees (*Pyrus* spp.) and grapevines (*Vitis vinifera*) (Pliego et al., 2012). Over 170 plants in 63 genera can be affected by this fungus, some being economically relevant like coffee (*Coffea* spp.), olive (*Olea europaea* L.), avocado (*P. americana* Mill.), stone fruit (*Prunus* spp.) and citrus (*Citrus* spp.) (Arakawa et al., 2002).

Host list: *Abies alba, Acacia, Acer, Acer caudatifolium, Actinidia chinensis, Aesculus, Annona, Ardisia elliptica, Aronia melanocarpa, Asparagus officinalis, Aucuba japonica, Begonia, Berberis, Beta vulgaris var. saccharifera, Boehmeria nivea, Bombax ceiba, Brassica oleracea, Camellia, Camellia sinensis, Carya, Castanea sativa, Ceanothus megacarpus, Cedrus atlantica, Citrus, Citrus aurantium, Coffea, Corylus avellana, Cotoneaster, Crocosmia x crocosmiiflora, Cryptomeria japonica, Cyclamen, Cydonia oblonga, Cynara cardunculus var. scolymus, Cyperus esculentus, Daucus carota, Dianthus, Diospyros, Ehretia microphylla, Eriobotrya japonica, Eucalyptus, Fagus, Feijoa sellowiana, Ficus, Ficus carica, Fragaria, Fragaria vesca, Geranium, Gladiolus hybrids, Helianthus annuus, Humulus lupulus, Hyacinthus, Ilex aquifolium, Iris, Ixia, Jasminum, Juglans, Juglans regia, Larix decidua, Laurus nobilis, Lavandula, Ligustrum vulgare, Macadamia integrifolia, Malus, Malus domestica, Malus sylvestris, Mangifera indica, Manihot esculenta,*

Medicago sativa, Mimosa, Morus, Narcissus, Narcissus tazetta, Olea, Olea europaea, Olea europaea subsp. Europaea, Paeonia, Passiflora edulis, Pelargonium, Persea americana, Phaseolus, Picea abies, Pinus, Piper nigrum, Pistacia Pistacia vera, Platanus, Poaceae, Populus, Populus nigra var. italica, Protea, Prunus, Prunus armeniaca, Prunus avium, Prunus cerasifera, Prunus cerasus, Prunus domestica, Prunus dulcis, Prunus persica, Prunus salicina, Punica granatum, Pyracantha, Pyrus, Pyrus communis, Pyrus pyrifolia, Rhododendron, Rhus verniciflua, Ribes, Rosa, Rosa damascena, Rubus, Rubus idaeus, Rumex, Salix, Serissa foetida, Solanum tuberosum, Sorbus aucuparia, Spiraea thunbergii, Theobroma cacao, Tulipa, Ulmus, Viburnum, Vicia, Viola, Vitis, Vitis vinifera, Zantedeschia, Zea mays, Ziziphus jujuba [1].

In Portugal, white root rot disease was mostly identified in apple (*Malus* spp.), sweet cherry (*Prunus cerasus* L.) and pear (*Pyrus* spp.) orchards (Teixeira de Sousa et al., 1995).

There is limited knowledge of tolerant rootstocks species, some apple trees such as *M. toringoides* and *M. floribunda* are considered to be tolerant (Teixeira de Sousa, 1985), sour orange in Corsica were found to be more resistent to poncirus and troyer citrange (Laville and Vogel, 1984).

5.2. Environmental suitability

Solarization and temperature are crucial environmental factors for the development of *Rosellinia necatrix* in plant host. *In vitro* studies have shown that the optimal growth temperature is 22–24°C, not growing below 4°C or above 32°C (Anselmi and Giorcelli, 1990a; Mantell and Wheeler, 1973). *R. necatrix* grows well i*n vitro* at pH 5–8, and can even develop at pH 4 or 9 (Anselmi and Giorcelli, 1990a; Ruano-Rosa, 2006), while growth in soil occurs between a pH of 6-8 (Gupta and Gupta, 1992). Soil moisture is a key condition influencing the growth of the fungus, *R. necatrix* optimal development is at field capacity and is reduced at soil wilting point (Anselmi and Giorcelli, 1990a).

An anaerobic environment is adverse for the occurrence of the disease, growth of *R. necatrix* mycelium is reduced when the O2 content of the air is less than 10% (Araki, 1967) and solarization has a strong inhibitory effect on its growth (Anselmi and Giorcelli, 1990a; Makambila, 1976). High organic matter levels were also found to be needed for mycelial growth (Agarwala and Sharma, 1976).

5.3. Spread capacity

White root rot has a limited spread capability, being restricted to the soil and infected material. The fungus is capable of living in the ground in a dormancy stage and disease emerges only when environmental conditions are favourable (López-Herrera, 2000). In some cases, the disease arises after the establishment of new crops in soils in which vulnerable crops have been cultivated previously and where infected debris remained in the ground. This was observed in Mediterranean areas in fields where susceptible crops like olive and grapevine were followed by new plantings of avocados (López-Herrera and Zea-Bonilla, 2007).

1 https://www.plantwise.org/knowledgebank/datasheet/47860#ImpactSection

Long distance transmission of *R. necatrix* mainly occurs with infected propagating materials, this soils can be spread by cultural practices or through rivers and water irrigation (Anselmi and Giorcelli, 1990b). Loose soils are particularly beneficial for fungal spreading.

The pathogen progresses through soil cavities as a diffuse mycelium or as hyphal strands and after contacting with the root of a new plant the hyphae attaches, it to its surface (Mantell and Wheeler, 1973).

5.4. Risk factor identification

The detection of risk factors is necessary to identify and estimate the probability of the infection by a pathogen in a delimited area. As a result, some abiotic factors are used to evaluate the risk. For example, for *R. necatrix* the texture and structure of the soil is an important factor because the fungus spreads easily on loose soil characterized by high sand content. In clayey soil, the spread of the mycelium is affected and there is a reduction in the growth rate of the mycelium. In addition, the water content of the soil and the moisture content >45% allows the fungus to grow in the best conditions. If the infection occurred, the pathogen develops more rapidly in trees under moisture stress, and without irrigation and symptoms in affected trees become more serious after dry periods. Additionally, the fungus grows better at pH 5-8 and temperature has a strong effect on the development of the mycelium, with the range of optimal growth being 22.5-25.5°C and light has a strong inhibitory effect on the mycelium of the pathogen. Furthermore, the quantity of inoculum is important for determining the speed of colonization and the intensity of attack (Anselmi and Giorcelli, 1990a). A yet unexplored risk factor is the soil microbiome. Whereas there is some evidence for the role of specific microbes in protection against *Rosellinea necatrix* (particularly in avocados), the community still requires a better understanding of the multitrophic interactions established among bacterial, plant rhizosphere and the mycelia of soil-borne pathogens (Pliego et al., 2019).

5.4.1. Predictive models

Predictive disease models have been used to simulate real pathogen plant host dynamics in accord to environmental conditions. Meteorological data like temperature, humidity, precipitation and even biotic conditions like soil microbiome can be included as important factors for prediction of an increased risk of infection or of disease outbreak in plant crop (Rossi et al., 2010).

Risk models are currently being used for different diseases, like downy mildew in Italy (Caffi et al., 200AD) and to make sure that preventive measures like the application of fungicide can be taken in an optimal timing.

To the best of our knowledge no predictive models for white root rot have been published to this date.

6. Detection and identification

6.1. Detection and identification in the field

The detection of the disease is the most important step to control the spread. For this reason, it is necessary to perform the diagnosis in presence of symptoms. *R. necatrix* is know to cause white root rot and some of the macroscopic symptoms caused by *R. necatrix* allows its detection in the field. For example, the disease shows aerial and root symptoms that are accompanied by a progressive weakening of the plant (Pliego et al., 2012). The aerial symptoms can be identified by the colour of the leaves, the top leaves show bronzing and eventually the symptoms can be the defoliation, reduction in fruit size and drying of the tree. Besides that, on the roots the main characteristic is the presence of a white cottony mycelium and mycelial strands that surrounds the roots. Furthermore, the fungus develops typical mycelium fans, those fans invade the whole root and causes general rooting. When the diseases progress, the mycelium turns grey and eventually black (Ten Hoopen and Krauss, 2006).

The symptoms can be observed in all the above-ground parts of the plant and the most common are (CABI, 2021):

- Leaves: abnormal colours and shapes and presence of wilting;
- Roots: hairy root, soft rot of cortex and rot of wood;
- Stems: dieback, discolouration of bark, gummosis or resinosis;
- Vegetative organs: dry/soft rot;
- Whole plant: dieback, weakening and unusual odour.

One of the most traditionally method used to detect *R. necatrix* in the soil is baiting. The procedure is performed by the insertion of twigs into the soil at a depth of 15 cm for 2 months, then check the presence of the pathogenic fungus white cottony mycelium (Tanaka, 1965). This technique has been optimized (Eguchi et al., 2008), in these methods the insertion of twigs is near trunk bases at soil depths of 6-20 cm. The baiting methods can be use to detect the pathogen in trees at early stages of infection, also, is recommended to detect the recurrence of the fungus after fungicide treatment (Pliego et al., 2012).

6.2. Detection and identification in the lab

In recent years, large efforts have been made to facilitate the detection of *R. necatrix* because of the difficulties on diagnosing it in field. The detection and identification in the lab allows for early detection and treatment of the pathogen. Moreover, some methods were developed to differentiate healthy plants from those with a latent infection but with no visible symptoms (Pliego et al., 2012). The lack of selective media and the presence of saprophytic microorganisms make difficult to use microbiological techniques to isolate the pathogen from infected roots. Despite this, the use of molecular techniques is the gold standard to detect and identify *R. necatrix* in the lab because these methods are rapid and sensitive. For example, with real-time Scorpion-polymerase chain reaction (PCR), which is based on the sensitivity of conventional PCR with a specific fluorescent signal.

Scorpion-PCR provides real time analysis of the reaction, allows the quantification of specific DNA target and is more sensitive and reliable that traditional methods. A nested Scorpion-PCR is performed with conventional primers (R3-R8) and a Scorpion probe (R15 Scorpion R18) to detect *R. necatrix* in infected soils, this procedure takes six hours to generate results (Schena and Ippolito, 2003). The Scorpion-PCR primers are design from the internal transcribed spacer (ITS) regions ITS1 and ITS2 from *R. necatrix* (Ruano-Rosa et al., 2018; Schena et al., 2004). The table below shows the primers that are used for the identification of *R. necatrix* and to determinate genetic diversity.

Table 1. Primers utilized for the identification of *Rosellinia necatrix* and the generation of Polymorphisms (Armengol et al., 2010; Ikeda et al., 2005; Kanda et al., 2003; Ruano-Rosa et al., 2018; Schena et al., 2002; Schena and Ippolito, 2003).

Primer	Sequence	Used for	Reference
R2F	CAAAACCCATGTGAACATACCA	^{I}PCR	Schena *et al*. (2002)
R3F	CGAAGTGCCCTACCCTGTTA	^{I}PCR	Schena *et al*. (2002)
R8R	CCGAGGTCAACCTTTGGTATAG	^{I}PCR	Schena *et al*. (2002)
R15F	CCATAGGCGAGATGAGAAATC	$^{I\text{-Scorpion}}$PCR	Schena and Ippolito (2003)
S18R	CAGCCCCTCGAAGTCAGT	$^{I\text{-Scorpion}}$PCR	Schena and Ippolito (2003)
ISSR1	5′HBH$(AG)_7$A	^{P}ISSR-PCR	Armengol *et al*. (2010)
ISSR2	5′DBDA$(CA)_7$	^{P}ISSR-PCR	Armengol *et al*. (2010)
ISSR4	5′YHY$(GT)_7$G	^{P}ISSR-PCR	Armengol *et al*. (2010)
ISSR5	5′BDB$(ACA)_5$	^{P}ISSR-PCR	Armengol *et al*. (2010)
CA8CT	GCGCGCGCGCGCGCCT	^{P}ISSR-PCR	Armengol *et al*. (2010)
CA8G	CACACACACACACACAG	^{P}ISSR-PCR	Ikeda *et al*. (2005)
GTG	GTGGTGGTGGTGGTG	^{P}ISSR-PCR	Ikeda *et al*. (2005)
AS4	TGTGGGCGCTCGACAC	^{P}UP-PCR	Ikeda *et al*. (2005)
OPC10^{P}	5′-TGTCTGGGTG-3′	^{P}RAPD	López *et al*. (2008)
OPC13	AAGCCTCGTC	^{P}RAPD	López *et al*. (2008)
OPF3	CCTGATCACC	^{P}RAPD	López *et al*. (2008)
OPF12	ACGGTACCAG	^{P}RAPD	López *et al*. (2008)
BaLccF	GGNCANTTYTGGTAYCAYWSNCA	^{P}RFLP	Kanda *et al*. (2003)
BalccR	TGNCCRTGNARRTGRAANGGRTG	^{P}RFLP	Kanda *et al*. (2003)

I, used for identification; ISSR, inter-simple sequence repeat; P, used to generate polymorphisms; PCR, polymerase chain reaction; RAPD, random amplification of polymorphic DNA; RFLP, restriction fragment length polymorphism; UP, universally primed.

7. Economic and social impact

Rosellinia necatrix can cause extensive wilting and tree death across an extensive host range. The severity of the disease makes it one of the most harmful fungal pathogens infecting the economically important fruit tree species, such as pears, apples, olive, orange, grape, coffee and avocado (López et al., 2008; Pasini et al., 2016). The disease can be present in nurseries and

orchards resulting in substantial loss of yield and quality for host plants with economic importance growers (Sawant et al., 2021).

Quantitative data on losses caused by *R. necatrix* are lacking. However, according to Agarwala and Sharma (1966) estimated losses caused by the fungus pathogen in apple in the state of Himachal Pradesh, India, was at least $272,000 (US) in 1963 ($1.6 million dollars present time). On the other hand, in Colombian *Coffea arabica*, the losses reported were $469 (US) ha^{-1} yr^{-1} (Bautista and Magdiel, 2000). In Japan, the losses reach $4 million (US) yr^{-1} for *Vitis vinifera* and similar losses for Japanese pear (*Pyrus pyrifolia*) (Ten Hoopen and Krauss, 2006). Pears, corresponding to the genus *Pyrus*, are a host plant of the pathogen with significant economic losses caused by the fungus. Pear production worldwide is second to that of apples, and the world production for 20/21 will reach over 1.0 million tons to 22.2 million tons. However, the production levels and number of pear orchards are progressively decreasing, a major reason is the prevalence of the disease that causes tree mortality (Sawant et al., 2021).

In Portugal, according to APAS (*Associação dos Produtores Agrícolas da Sobrena*), the average disease incidence is 5%, with annual losses of 5 775 000€ to the producers and 16 500 tonnes in production. Moreover, disease progression is higher in irrigated orchards (APAS, personal communication, November 2021).

Table 2: Economical impact of *Rosellinia necatrix* in Portugal.

	APAS (2021) [2]
Average incidence	5%
Annual economical losses for the producers (€)	5775000
Annual economical losses for the value chain (€)	22275000
Annual production losses (tonnes)	16500
Annual cost of control measures (pruning, phytochemicals, etc.; €)	192500

8. Available control/prevention methods

Rosellinia necatrix can survive in the soil in a dormancy state in infected debris for several years. The disease can develop following the establishment of new crops in orchards in which susceptible crops have been cultivated previously and where infected debris has remained in the soil. Therefore, preplanting treatments must have a long-term effect (Pliego et al., 2012).

8.1. Chemical

Fungicides are normally used to control *R. necatrix* infections. Diseased roots are exposed by digging the soil and drenching with fungicides. Most of the studies were performed in avocado and very few were found in pear. In Japan, glasshouse grapevine and Japanese pear growers have successfully controlled *R. necatrix* using thiophanate-methyl, but this compound has been later

2 Calculated by João Azevedo (from Associação dos Produtores Agrícolas da Sobrena) based on the assumptions:
- Total orchard area in Portugal – 11 000 ha;
- Average productivity – 30 ton/ha
- Average value paid to producer – 0,35€/kg
- Average value paid by retailers – 0,85€/kg
- Retail selling margin – 100%
- Average value for the remaining value chain – 1,35€/kg

replaced with phenylpiridinamine fluazinam (Kanadani et al., 1998; Nitta et al., 1998). Phenylpiridinamine fluazinam remains stable in the soil for much longer than thiophanate-methyl (Ten Hoopen and Krauss, 2006). Soil fumigant dazomet has been used to eradicate the *Rosellinia* inoculum in soil before crops are planted (Nitta et al., 2002).

Benomyl, carbendazim, fluazinam and thiophanate methyl were evaluated against *R. necatrix*, and fluazinam was shown to be more efficient than the others fungicides (López-Herrera and Zea-Bonilla, 2007). Integrated control with low concentrations of fluazinam and *Trichoderma* spp. has been shown as a good strategy to effective control of avocado white root rot and avoid the development of resistance to the fungicide (Ruano-Rosa et al., 2018).

In Portugal, no chemical treatment has been specifically approved by DGAV against *R. necatrix* infection.

8.2. Biological

Multiple biological control strategies have been investigated to control the dissemination of *R. necatrix* and antagonists species of bacteria and fungi are recognized. Trichoderma harzianum is well known for its mycoparasitic capabilities and proven to protect avocado plants (Ruano-Rosa et al., 2010) when applied, however its effectiveness against the disease is dependent on soil type (Sztejnberg et al., 1987). A similar effect was observed in apple orchards infected by *R. necatrix* (Freeman et al., 1986), it was concluded that the control mechanism could either be nutrient competence or mycoparasitic behaviour of the fungus.

Likewise bacterial species of *Agrobacterium* and *Pseudomonas* isolated from soil or roots of peach and apple, have been show to have positive effects on disease mitigation (Yasuda and Katoh, 1989). The novel use of mycoviruses (RnMYRV-3/W370) as virological control and double-stranded RNA (dsRNA) present in some fungal strains have been identified to reduce the disease virulence (Kanematsu et al., 2004; Matsumoto et al., 2002).

Although the application of biological control agents (BCAs) has yielded variable rates of success, possibly from varying biotic and abiotic conditions, it is thought that their application is better suited for controlled glasshouse conditions than in the open field (Paulitz and Bélanger, 2001).

In Portugal, no BCA has been approved by DGAV against *R. necatrix* infection.

8.3. Cultural

To control and prevent the growing and spread of *R. necatrix* it is necessary to obtain healthy planting material from nurseries. For example, use of aerated and dry substrates treated with steam or fungicides. In addition, plant seeds should be treated with hot water. Certainly, the use of sterile tools, soil and organic manure is obligatory into the nurseries. In addition, is recommended to test plant roots periodically to check the presence of the pathogen (Pérez-Jiménez, 2006). In general terms, the following cultural practices are necessary:

- **Before planting**: removal of plants, stumps and roots from all sites where the fungus was present. After that, expose them to light and air. Perform a treatment of the soil by stream sterilization or with chemicals (Pérez-Jiménez, 2006).

- **After planting:** avoid water stress, soil drench or drought. Check the roots and other symptoms, if the infection occurs is necessary to remove immediately all diseased plants and to isolate diseased areas. In addition, prevent contact between infected and healthy roots. Evidently, it is recommended that in infested areas, when it is practicable, resistant crops should be grown and susceptible crops avoided (Pérez-Jiménez, 2006).

8.4. Breeding

No study has been found regarding breeding programs in pear. Nevertheless, preliminary studies have shown resistance/tolerance to *R. necatrix* in some wild apple species and avocado rootstocks (Johnson, 2000; Zumaquero et al., 2019).

8.5. AgroEcological

Little is known about the effect of agroecological practices on the control of *R. necatrix*, but a recent study (Bonilla et al., 2015) suggested that organic soil amendments (e.g. almond shell and yard waste) could induce protection against this pathogen.

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