Avocado root rots in Andalucia: A review

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ABSTRACT

This work summarises the studies conducted in Andalucia (southern Spain) on avocado root rots. During the initial period of this investigation, samples were taken from symptomatic trees in orchards located in this area. The fungi isolations and pathogenicity tests were carried out and Phytophthora cinnamomi *and* Rosellinia necatrix *were identified initially as casual agents of these symptoms. Currently, the percentage incidence of the diseases of the total orchards visited, is 35% for* P. cinnamomi *and 39% for* R. necatrix. *These diseases are considered as the most important in avocado crops in southern Spain.*

Studies of virulence, as well as the morphological and cultural characteristics of each pathogen and its control by physical (solarization) and chemical methods, have been undertaken. The effectiveness of the solarization on root rot control has been demonstrated. Additionally a relative control in experiments under greenhouse and fields conditions with different chemicals has been obtained for R. necatrix.

Currently an evaluation programme of avocado material from different origins to select rootstocks tolerant to the cited pathogens is being developed. An investigation on biological control of these diseases incorporating antagonistic fungi and bacteria to both pathogens has been initiated.

Key words: *biological control, chemical control,* Persea americana*, physical control,* Phytophthora cinnamomi, Rosellinia necatrix.

INTRODUCTION

Avocado is a relatively recent crop in Spain; the orchards are located in a specific area of Andalucia, the coastal region of Malaga and Granada provinces, and in the Canary Islands, which have a subtropical climate. In Andalucia the earliest avocado orchards were established during the 1970's and 1980's and now they have reached stable acreage. In general the rootstocks are plants from Topa Topa seeds and the main cultivar used is Hass (75%).

In the eighties, symptomatic trees were detected in these orchards. Wilting, general decline, leaf drop, chlorosis and small fruit size were observed. To solve this problem, samples from symptomatic trees were analysed and the results indicated that the avocado root rot caused by *Phytophthora cinnamomi* Rands and the white root rot caused by *Rosellinia necatrix* Prill. (asexual stage *Dematophora necatrix* Hart.) were the most important diseases in this crop. We also noticed that these diseases were widely dispersed in this area (López Herrera, 1989).

Due to the importance of the problem a fundamental investigation was initiated and the following goals were set:

i) to establish the distribution and incidence of each one of the diseases in commercial orchards in Southern Spain;

ii) to study different aspects related to the virulence and morphological and cultural characteristics of the causal agents: *P. cinnamomi* and *R. necatrix*; and

iii) to control these diseases using physical (soil solarization), and chemical (systemic fungicides) methods.

MATERIALS AND METHODS

Distribution and incidence of avocado root rots

During the initial period of this investigation, orchards with trees in decline and dead trees were sampled. Root samples from these trees were taken and afterwards analysed in the laboratory to isolate and identify the fungi. Later on, we kept on prospecting new orchards in this area in a routine way in order to establish a more accurate distribution of each one of the pathogens. As a whole, between 1986 and 2003, a total of 1397 samples from avocado roots of 481 different orchards with symptomatic trees were analysed.

Virulence and cultural and morphological characteristics of *Phytophthora cinnamomi*

Initially we carried out a virulence study with artificial inoculations using 11 isolates of *P. cinnamomi* coming from this area. We then completed a morphological description of the mycelium of 25 isolates using two different culture media: corn meal agar (CMA) and eight vegetables juice agar (V8A). For each isolate, morphological descriptions of sporangia and sexual stage structures: antheridia, oogonia and oospores, and mating type definition (A^1 o A^2), were carried out.

Virulence and cultural and morphological characteristics of *Rosellinia necatrix*

As for *P. cinnamomi* we conducted a virulence study using eight isolates from this area. Cultural characteristics under different pH and temperatures and their effect on fungal development were defined. Also, two different cultured media – potato dextrose agar (PDA) and malt agar 1% (MA) – were used to describe the morphology of the fungus.

Morphological descriptions of sexual structures of *R. necatrix* formed on a dead avocado tree located in a commercial orchard were completed. In this study measurements of stroma, ascocarps, asci and ascospores were registered. In an attempt to reproduce the typical infection symptoms caused by the asexual stage and to recover the characteristic mycelium of this stage, ascospores coming from these naturally formed ascocarps, were germinated, monoascosporic isolates were obtained and then inoculated onto avocado plants.

Finally the existence of a somatic incompatibility system in this species was studied, which would allow, firstly, to differentiate individuals and, secondly, to determine the degree of genetic variation existing in the *R. necatrix* avocado pathogenic population. In order to achieve this, intraspecific crosses between fungus massal isolates (63 isolates coming from 56 trees located in 23 different orchards) and between monoascosporic isolates (75 isolates coming from 10 different ascocarps) were made.

Physical control

The effect of soil solarization on avocado root rot fungus was studied. Soil treatments were carried out in established commercial avocado orchards severely affected by *P. cinnamomi* or *R. necatrix*. In the summer the soil was covered with 75 µm transparent polyethylene for an eight-week period. Also, soil treatments were carried out to determine the effect of temperature reached by the solarization to negate the viability of the pathogen inocula.

Chemical control

For a three-year period, field trials were carried out to study chemical control of *P. cinnamomi* in a naturally infected commercial avocado orchard with Hass/TopaTopa 10-year-old trees. Four fungicide applications were made during July and November in 1992 and 1993. These fungicides were: phosphorous acid (20% phosphonate solution adjusted to pH 5.8) and fosetyl-Ca (EF 2008-B) trunk injected; phosphorous acid and fosetyl-Al (80WP) in foliar applications and Metalaxyl added to the soil.

Preliminary studies of chemical control of *R. necatrix* were carried out *in vitro* and *in vivo* using different fungicides: Benomyl, Carbendazim, Fluazinam and Thiophanate Methyl.

RESULTS

Distribution and incidence of avocado root rots

The surveys conducted confirmed the preliminary results regarding the wide distribution of *P. cinnamomi* and *R. necatrix* in avocado orchards of Malaga and Granada and they allowed to update a distribution map for these pathogens in the sampling area (Pérez Jiménez, 1997). This information shows that the incidence of *P. cinnamomi* in symptomatic orchards is around 35% and for *R. necatrix* is near 39%, while the incidence of symptomatic trees in orchards infested with both pathogens is increasing by 7% (Pérez Jiménez, 2003).

Virulence and cultural and morphological characteristics of *Phytophthora cinnamomi*

Virulence experiments showed significant differences in virulence of pathogenic *P. cinnamomi* population in orchards of Andalucia (Pérez Jiménez, 1997).

The mycelial diameters of 25 isolates, growing on CMA and AV8, were very similar (6.7 µm) and typical collaroid hyphae and spherical hyphal swellings were present in both media. Chlamydospores were only formed on AV8. In most of the isolates, abundant ovoid or ellipsoid sporangia with zoopores were formed; they were papillate or semipapillate with internal proliferation. The antheridia were anphigynous and unicelular, the oogonium spheric and the oospores plerotic, with a mean size of 35.8 µm (34.0-36.5). The fusion organs were only formed in paired cultures, and all *P. cinnamomi* isolates studied, were A^2 mating type (López Herrera and Pérez Jiménez, 1995).

Virulence and cultural and morphological characteristics of *Rosellinia necatrix*

Inoculation experiments differentiated isolates with high, medium and low virulence. All isolates had an optimum growth temperature of 25ºC; they did not grow at 35ºC, and at 5ºC the growth was at a minimum. Growth was optimal at pH = 9 and at a minimum at pH = 4. On PDA the mycelial growth was radial, very dense, with a white colour and a with very high growth rate. On MA the mycelia were scarce, less dense than in PDA, irregular, and had a slower growth rate than in PDA (Pérez Jiménez, 1997).

Morphological descriptions of sexual structures of *R. necatrix* formed in nature (ascocarps, asci and ascospores) were compared with those referred to in the literature on different hosts and from different geographic locations. Furthermore, we confirmed the existence of the sexual cycle of this fungus in natural conditions and we also confirmed the relationship between the perfect and imperfect stages of the fungus (Pérez Jiménez *et al*., 2003). Finally, the existence of a somatic incompatibility system in this species was confirmed. Two types of mycelial interactions were differentiated after pairing massal isolates of *R. necatrix*:

a) intermingling mycelia, whereby the two colonies merged into each other and the colonie's surface had an uniform aspect which was interpreted as a somatic compatibility reaction; and

b) a mycelial interaction whereby a barrage zone was formed at sites of contact between colonies, which was considered a somatic incompatibility reaction (Pérez Jiménez *et al*., 2002).

Physical control

In our experiments, soil temperatures in solarized avocado orchards reached a maximum of between 35°C and 42ºC, depending on the soil depth and year. At unshaded locations of solarized plots, maximum mean hourly soil temperatures were between 4°C and 8ºC higher than in the controls, and at shaded locations between 2°C and 4°C.

Viability of *P. cinnamomi* evaluated, on root and soil samples and on artifical inoculum buried at different depths, was severely reduced after 6-8 weeks of solarization at unshaded and at shaded locations (Fig. 1). The pathogen remained undetected on treated trees until 14 months after solarization. Treatment did not affect tree growth and yield was higher than in controls (López Herrera *et al*., 1997).

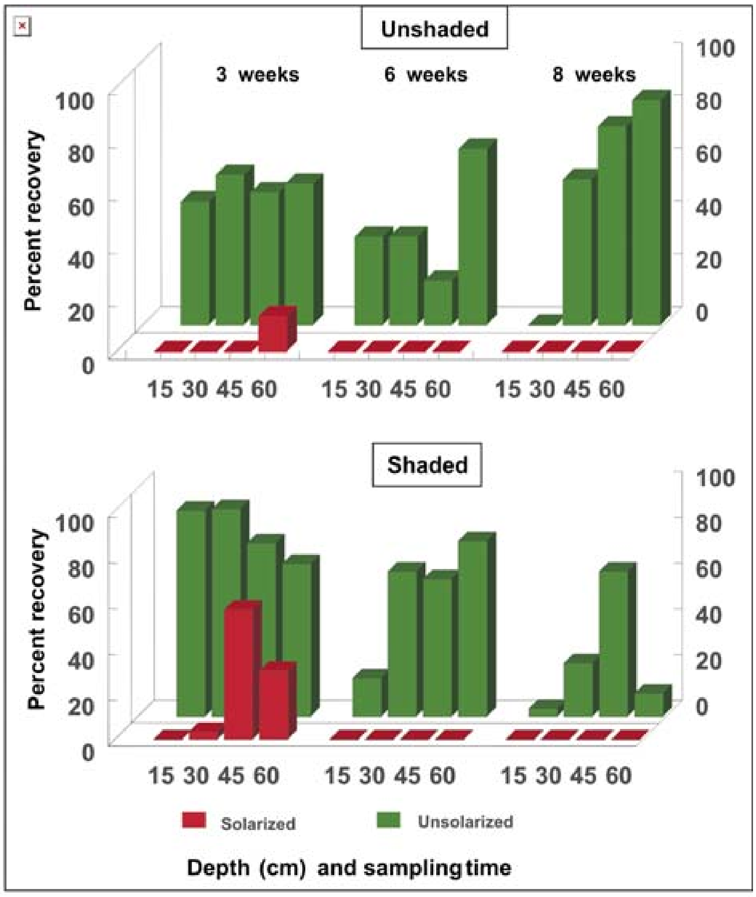

Figure 1. Effect of soil solarization on the viability of *Phytophthora cinnamomi* at shaded and unshaded locations and different soil depths.

Viability of *R. necatrix* evaluated on infected root samples buried at unshaded locations at 15-30 cm was inactive after 3-5 weeks of treatment, and the ones buried at 45-60 cm were inactive after 4-8 weeks (Fig. 2). *R. necatrix* did not colonize the solarized soil after 9 months after solarization (López Herrera *et al.*, 1998).

Results of soil solarization experiments, to establish the relationship between soil temperature and viability loss of *P. cinnamomi* and *R. necatrix*, suggested that the loss of inoculum viability in naturally infected roots could be related to accumulated temperature-time (degree-hours) and to a minimum threshold temperature of 30ºC being adequate for regression fitting. It seems that the accumulation of degree-hours is crucial in the eradication of *P. cinnamomi* and *R. necatrix* inocula in solarization treatments (López Herrera *et al.*, 1997; López Herrera *et al.*, 1999).

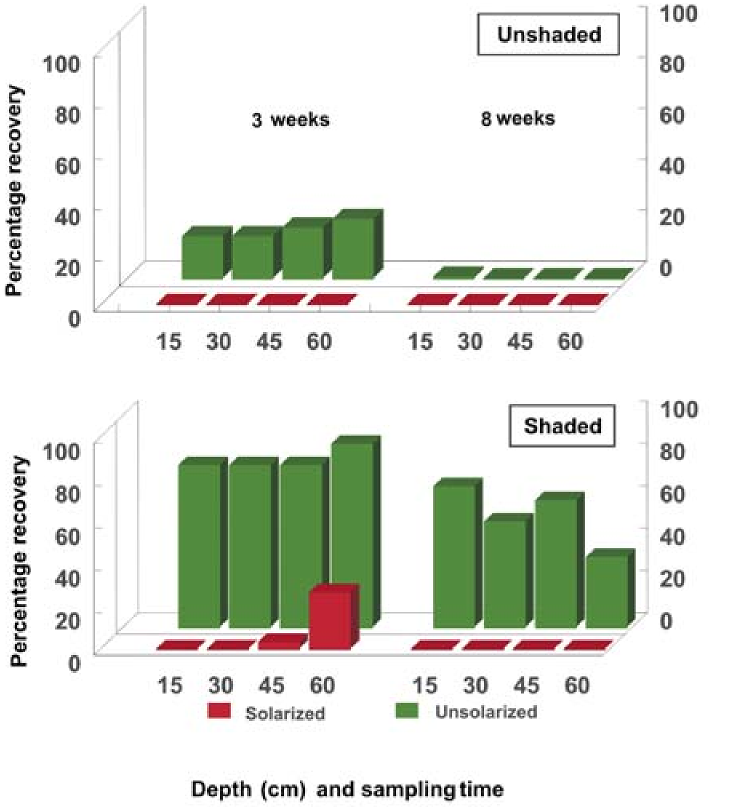

Figure 2. Percentage recovery of *Rosellinia necatrix* after 3 and 8 weeks of solarization in unshaded and shaded locations at different soil depths.

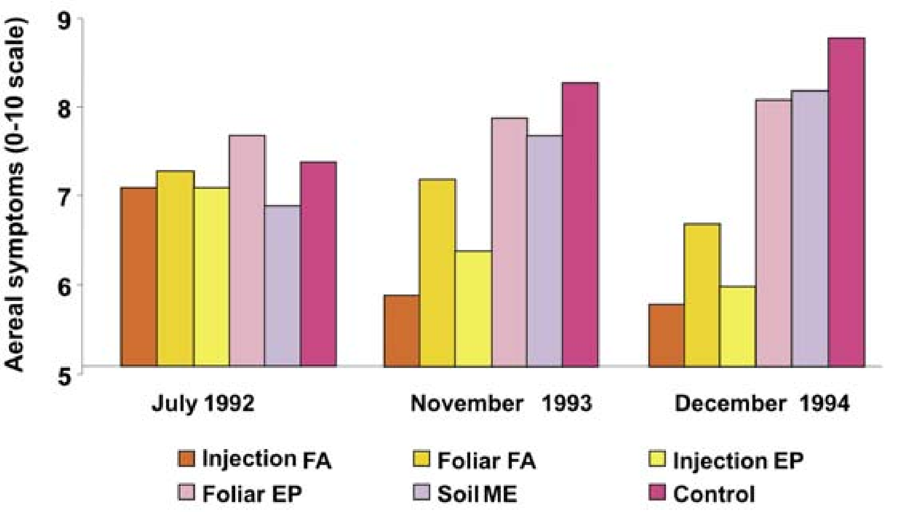

Figure 3. Effect of different treatments on avocado aerial symptoms based on a scale 0-10 (FA: Phosforous acid, EP: ethil phosphite-Al, ME: metalaxyl).

Chemical control

Decreasing order in effectiveness of fungicide treatment to control *P. cinnamomi* avocado root rot under field conditions was: injected phosphorous acid, injected fosetyl-Ca, foliar phosphorous acid, foliar fosetyl- Al and metalaxyl (Fig. 3) (López Herrera *et al.*, 1995).

Preliminary results in chemical control of *R. necatrix* showed that benzimidazoles could affect the development of the disease caused by this fungus (López Herrera *et al.*, 2003).

CONCLUSIONS

Nowadays the most important avocado diseases in Andalucia are root rots caused by *P. cinnamomi* and *R. necatrix*. These pathogens have been studied in relation to their virulence and cultural and morphological characteristics in order to characterize their populations in our area. Different studies have been conducted to achieve control using solarization and chemical control, and right now our investigations are mainly focused on the evaluation of avocado germplams to detect tolerance to *R. necatrix*, including in this project *P. cinnamomi* tolerant rootstocks (Pérez Jiménez *et al.*, 2003), and in the biocontrol of these diseases using antagonistic bacteria and fungi (Gónzalez Sanchez, *et al.*, 2003; Pliego *et al.*, 2003; Ruano Rosa *et al.*, 2003).

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