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SELECTION PROGRAM FOR AVOCADO ROOTSTOCKS TOLERANT TO WHITE ROT CAUSED BY *Rosellinia necatrix* IN SOUTHERN SPAIN (1995-2007)

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This paper shows the current results of the selection program for avocado rootstocks tolerant to white rot caused by *Rosellinia necatrix*, one of the most important diseases affecting this crop in Southern Spain. Tests for material selection have been carried out through artificial inoculations of the pathogen on seedlings from first-inoculation seeds as well as on preselected plants, multiplied *in vitro* or through conventional semi-woody cutting in second inoculation.
During this four-year period (2003-2006), 2,612 seedlings from seeds of local trees acclimatized to the zone have been subject to first inoculation. Pre-selected seedlings together with selections from previous years are being propagated to undergo a second inoculation, after their vegetative multiplication. Surviving selections are being placed on a plot artificially infested with *R. necatrix* for evaluation prior to a final one in commercial orchards infested by the pathogen. At the same time, propagation of trees selected from infested areas (escape trees) is being carried out to evaluate its performance following artificial inoculations with the pathogen.

Keywords: *Persea* americana, germplasm, artificial inoculation, avocado rootstocks, susceptibility.

PROGRAMA DE SELECCIÓN DE PORTAINJERTOS DE AGUACATE TOLERANTES A LA PODREDUMBRE BLANCA CAUSADA POR *Rosellinia necatrix* EN EL SUR DE ESPAÑA (1995-2007)

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En este trabajo se presentan los resultados actuales del programa de selección de portainjertos de aguacate tolerantes a la podredumbre blanca causada por *Rosellinia necatrix*, una de las enfermedades más importantes que afectan a este cultivo en el sur de España.

Se han continuado realizando los tests de selección de material, mediante inoculaciones artificiales del patógeno sobre plantas procedentes de semillas en primera inoculación y sobre plantas preseleccionadas, multiplicadas *in vitro* o mediante estaquillado semileñoso convencional, en segunda inoculación. Durante este cuatrienio (2003-2006) se han sometido a primera inoculación 2.612 plantas procedentes de semillas de árboles locales aclimatados a la zona. Los clones supervivientes (preselecciones) han pasado, junto con las preselecciones de años anteriores, al programa de propagación para, una vez multiplicados vegetativamente, ser sometidos a la segunda inoculación con *R. necatrix*. El material superviviente (selecciones), está siendo plantado en una parcela infestada artificialmente con *R. necatrix* para su evaluación previa a la definitiva en plantaciones comerciales infestadas por el patógeno. Paralelamente, se ha continuado con la propagación de los árboles seleccionados en campo como "posibles escape", y con la evaluación de su tolerancia a *R. necatrix* mediante inoculaciones artificiales con el patógeno.

Palabras clave: *Persea* americana, germoplasma, inoculación artificial, patrones de aguacate, susceptibilidad.

1. Introduction

White root rot caused by *Rosellinia necatrix* Prill. is a very destructive disease in several crops (Khan, 1959; Sztejnberg y Madar, 1980). In Spain, it was detected for the first time in 1987 (López-Herrera, 1989) and currently, the incidence of *R. necatrix* in orchards with trees showing disease symptoms is approximately 40%. Control of the disease is based upon the use of adequate cultural practices such as reduction of irrigation frequency, isolation drenches, weed elimination and removal of soil at tree bases. Soil solarization, although it shows several drawbacks in practical aplications, is very effective to control the disease since this fungus is very sensitive to high temperatures (López-Herrera *et al.,* 1998; 1999a). For chemical control, several fungicides have been tested with fluazinam showing to be the most effective as a contact fungicide as well as in terms of persistence in the soil (López-Herrera and Zea- Bonilla, 2007). For biological control, several T*richoderma* spp. have been isolated for its effectiveness under *in vitro* (Ruano *et al*., 2003) and *in vivo* conditions (López-Herrera *et a*l., 1999b). *Pseudomonas* spp. have also been used as promising biocontrol agents (Pliego *et al.,* 2007). However, control of this disease needs to be carried out following integration of the different approaches; along this line, it is of great interest to have rootstocks tolerant to this fungus to be used for replacement of diseased trees as well as for new plantings. In this investigation, the advances for the 2003-2006 period on material selection showing tolerance to this disease are presented; these results are based on previous investigations carried out by our group (López-Herrera *et al*.,1999b; Pérez-Jiménez *et al*., 2003).

2. Material and Methods

Evaluation of tolerance to white root rot in seedlings

Using artificial inoculations, the susceptibility to *Rosellinia necatrix* of avocado material from different sources has been tested.

At "La Mayora" Experimental Station C.S.I.C. (Algarrobo-Malaga, Spain), there exists a plot of avocado rootstocks derived from seeds of diverse origin, used in the first avocado plantings carried out at Southern Spain. These trees were used as seed source for the present investigation.

Seeds were obtained from trees not previously tested as well as from 3 trees (La Piscina, C.A. Bueno and Consula 9) whose progenies had previously shown promising results in terms of survival rates after the first inoculation with *R. necatrix.* Prior to sowing, carried out in the winter, seeds were surface disinfested through washing with water at 50^{o}C for 30 min; after that, they were sown in 5 l pots with a sustrate mixture containing sand:peat moss:soil (1:2:1), previously sterilised with water vapor.

Six months after germination, the seedlings were inoculated with a highly virulent isolate of *R. necatrix* (López-Herrera *et al.,* 1999b). The inoculum of *R. necatrix,* sterile wheat inoculated with the fungus (3,75 g of colonized wheat /l sustrate), was directly incorporated into the pot at different depths. These inoculations were carried out each spring under screenhouse conditions, trying that the different factors affecting disease development (inoculum, plant material and environmental conditions) were similar in all experiments.

After determination of the fungus viability in the roots of surviving plants, they were transplanted to plastic bags of bigger size. The preselections were grown for another six months before going into the propagation phase.

Multiplication of pre-selected seedlings

To confirm the observed tolerance in the seedlings following the first inoculation with *R. necatrix*, they were multiplied following two different techniques: through micropropagation (Barceló-Muñoz *et al*., 1990) or by conventional semi-hardwood cuttings (Hartmann *et al*., 1997), e.g., twelve cm long cuttings, with 3-4 leaves, were rooted under a plastic tunnel, in a greenhouse under controlled temperature, following immersion of the cuttings for 10 s in a indole-3-butyric acid solution (5000 rpm).

The vegetative progenies were left to grow in a screenhouse, until they reached 125 cm, and they were ready to undergo the second inoculation.

Selection of escape trees

A total of seventeen trees without symptoms had been selected as escape trees, in diversed spots of several commercial avocado plantings in Southern Spain. Nine, out of the seventeen, have been micropropagated (Barceló-Muñoz and Pliego-Alfaro, 1997). Their vegetative progenies, are artificially inoculated, after reaching 125 cm, as previously described for the progenies of preselected seedlings.

Evaluation in pots of the degree of tolerance of the vegetative progenies from preselected seedlings or from escape trees

After reaching the size previously indicated, e.g., 125 cm, the vegetative progenies from preselected seedlings or escape trees, were grouped in the so called **Inoculation Groups** (Table 1), and they were evaluated following the same methodology indicated for the first inoculation. In this case, plants were maintained in a greenhouse with controlled temperature. Following appearance of the first symptoms, evaluation of aerial damage was carried out at weekly intervals. After 6 months, selection of surviving plants (advanced selections), was carried out, after checking the presence of inoculum in the soil and the mycellium in the roots, and after isolating the fungus from roots with symptoms.

Table 1. Second inoculation on surviving plants from first inoculation.

Year	Inoculation Groups	Total inoculated plants
2003	G-IV	54
2003	G-V	11
2003	G-VI	19
2004	G-VII	18
2004	G-VIII	59
2004	G-IX	106
2004	G-X	77
2005	G -XI	30
2005	G-XII	22
2005	G-XIII	53

Evaluation of the degree of tolerance to R. necatrix of advanced selections planted in an artificially infested plot

The vegetative progenie of advanced selections is being evaluated in the following phase of the selection programme, in simulated field conditions. To achieve this goal, back in 2001, at the IFAPA, Centro de Churriana, (Malaga, Spain), a plot was prepared using a 14 x 12 x 1.5 m artificial hole, and filling it up with slated soil from the Velez-Malaga area where most avocado plantings are

located. The soil was adequately prepared and fertilized and it was also solarized using a plastic film over.

To artificially inoculate this plot, back in 2001, 92 one year old avocado seedlings cv. Topa-Topa, a cultivar very susceptible to *R. necatrix*, were planted and they were allowed to grow for three years. Once plants had a well developed root system they were artificially inoculated with two highly virulent isolates (CH 53 and CH 320) of *R. necatrix*. The inoculation was carried out after incorporating to the sustrate 18,75 g of colonized wheat seeds/plant, from a 1:1 mixture of the two fungal isolates. After the death of the plants, they were cut at the trunk base and the root systems were kept buried, keeping an adequate soil moisture, in order to increase the level of *R. necatrix* inoculation in the infected roots.
The advanced selections, derived from seeds of diverse origins or from escape trees, are being planted in holes of the plot located between plants previously inoculated, to evaluate its performance under these conditions. The infected root systems of not surviving plants will be kept incorporated to the soil to increase the inoculum level and surviving plants will be considered tolerant to the fungus

3. Results and Discussion

Within the four years period 2003-2006 seeds from different tress have been collected: 2002: 20-XIX-La Consula 5; 18-XIX-La Consula 7; 33-XVIII, 27-XVIII-La Piscina; 23-XVIII-Alhaurin and 21-XVIII-La Consula 12.
2003: XVIII-17, XVIII-25, XVIII-28, XVIII-33, XIX-17, XIX-18, XIX-20, XIX-21, XIX-24, XIX-25, XIX-26, XIX-29, XIX-30, XX-17 y XX-40.
2004: XIX-17 Consula 9 y XVIII 28 A. Bueno.
2005: XVIII-27 La Piscina, XIX-17 Consula 9 and XVIII-28 A. Bueno.

Preselections tolerant to *R. necatrix* are shown in table 2.

Table 2. Seedlings inoculated within the period 2003-2006 and resulting preselections showing tolerance to *R. necatrix*.

Year	Inoculated plants	Preselections
2003	318	0
2004	604	4
2005	949	32
2006	741	4
TOTAL	2612	40

In the first inoculations carried out in this four years period, we keep detecting tolerance in plants obtained from 3 mother trees (La Piscina, C.A. Bueno and Consula 9) as observed in previous years.

Following the second inoculation, also within this four years period, three new selections have been obtained: BG-108, BG-89 and BG-7. Moreover, one of the escape trees, survived the artificial inoculation.

The advanced selections as well as the progeny from escape trees surviving the artificial inoculations were planted in the artificial plot (Table 3).
Clones planted in the infested plot are shown in table 2. Currently, the survival of these plants is under evaluation.

Table 3. Selections planted in artificially infested plot.

Clones	Nº of inoculated plants
BG 41	4
BG 42	6
BG 83	6
BG 181	6

In apple, Lee *et al.* (2000) are carrying out similar investigations to select rootstocks tolerant to *R. necatrix* (Lee *et al.,* 2000); these authors find, in general, high susceptibility to the fungus in the seedlings derived from most clones studied in the collection; however, as we have observed, they detect clones whose progenies show consistent tolerance. In the avocado, the survival rate detected in the progenies of several trees located at "La Mayora" Experimental Station, seems to indicate that this material could be used as initial source to obtain rootstocks tolerant to *R. necatrix*; these clones will be crossed, in a directed manner, in the near future with the hope of increasing tolerance to the fungus on the resulting seedlings.

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