

## **WILTING DISEASE OF YOUNG AVOCADO TREES CAUSED BY *Neonectria radiculicola* IN ISRAEL**

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Blighting and wilting of young avocado (*Persea americana* Mill.) trees accompanied by root rot were observed in the last three years in a few locations in Israel. Symptomatic trees were mostly vars. Pinkerton and Ardit, 2 – 5 years after planting in the orchard, heavily loaded with fruit. Isolations revealed a *Cylindrocarpon* sp. A survey was initiated in avocado nurseries for fungi, and *Cylindrocarpon* sp. was isolated from the roots of 10%-100% of the seedlings in all the surveyed nurseries, although the seedlings showed no wilting symptoms. Fungicides efficacy experiments in seedlings showed good control results only with Prochloraz formulations "Merag" and Sportak".

Perithecia of the fungus were observed in the lab, first on roots and later on PDA medium. Single-conidium and single-ascospore isolates were obtained for morphological identification of this *Cylindrocarpon* / *Neonectria* species. Sequences of the small subunit mitochondrial rDNA,  $\beta$ -tubulin, and ITS region revealed homologies of 99.8%, 100%, and 94% respectively with *Neonectria radiculicola* / *Cylindrocarpon destructans* complex.

*N. radiculicola* / *C. destructans* is a known agent of root rot disease in nurseries of raspberry, grapevine, ginseng and forest trees.

This is the first report of *Neonectria radiculicola* in *Persea americana*.

The questions to discuss are: is this a new soil-borne pathogen in Israel, or an indigenous one. Have changes in avocado horticulture management, (water salinity, fertigation, heavy yield on young trees etc') increased the pathogenicity of the fungus?

**Key words:** Wilting disease, *Cylindrocarpon* sp., *Neonectria radiculicola*, avocado

## **MARCHITAMIENTO DE ÁRBOLES JOVENES DE AGUACATE CAUSADO POR *Neonectria radiculicola* EN ISRAEL**

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Se observó el marchitamiento y la muerte de árboles de aguacate (*Persea americana* Mill.) jóvenes, en conjunto con la pudrición de las raíces durante los últimos tres años en distintos lugares en Israel. Los árboles con síntomas de marchitamiento fueron en su mayoría de las variedades Pinkerton y Ardit, dos a cinco años después de la plantación y que presentaban una alta carga de frutos.

El hongo aislado fue *Cylindrocarpon sp.*. Se realizó un censo de hongos en viveros de aguacate, y *Cylindrocarpon sp.* fue aislado de las raíces entre el 10%-100% de los plántones en todos los viveros, aunque los plántones no presentaran síntomas de marchitamiento.

Sólo se obtuvo buenos resultados de control en experimentos de eficacia de fungicidas en los plántones con las formulaciones de Prochloraz, del tipo "Merag" y "Sportak".

Se observaron peritecios del hongo en laboratorio, inicialmente en las raíces y posteriormente en un medio PDA. Se obtuvieron aislados monoconídicos y monoascospóricos para la identificación morfológica de las especies *Cylindrocarpon / Neonectria*. Secuencias de pequeñas sub-unidades de rDNA mitocondrial,  $\beta$ -tubulin, y la región ITS revelaron una correspondencia de 99,8%, 100%, y 94% respecto al complejo *Neonectria radicularis / Cylindrocarpon destructans*. El complejo *N. radicularis / C. destructans* es conocido por causar la pudrición de las raíces en viveros de frambuesa, vid, ginseng y árboles forestales.

Éste es el primer reporte de *Neonectria radicularis* en *Persea americana*.

Las cuestiones por debatir son:

- ¿Es éste un hongo patógeno nuevo en Israel, o es un hongo autóctono?
- Los cambios en el manejo hortícola (salinidad del agua, fertirrigación, alta producción de árboles jóvenes, etc.) ¿aumentaron la patogenicidad del hongo?

## Introduction

The fungi *Cylindrocarpon sp.* was defined in 1917. However, publications which describe its impact on agriculture started only in 1965. It includes about 30 different species with a wide and big range of hosts including field crops (peanuts, soy, potatoes and others), a wide range of fruit trees (including avocado) and ornamentals. The fungi can damage different parts of the plant. Most of the contaminations in fruit trees were found in the trunk neck and the roots. The species is close to the *Fusarium*.

During the past three years, a correlation between the fungi presence and wilting of transplanted avocado trees was found. It led to the examination of all the avocado nurseries in Israel. High degree of contamination was found in trees in the nurseries, although no visible signs of wilting or stress were evident.

## Objectives

1. Execution of a national field survey in order to quantify the problem in commercial orchards.
2. Testing different chemicals and application levels for an effective treatment against *Cylindrocarpon sp.* in avocado trees in nurseries
3. Examining the correlation between the fungi contamination and the rate of death of the seedlings in the commercial orchards.
4. Characterization of the strains isolated from the avocado wilting trees.

## Materials and methods

1. Contamination survey has been done in all the orchards that were planted during the years 2000-2006. The roots of every wilting young tree were sent to the laboratory.
2. A series of fungicide applications were carried out in 'Hass' seedlings, grafted on 'Degania 117' rootstock, four months before planting in the orchard. Pot volume was 8 liters. The growing media was mainly loam. The examined trees were naturally inoculated in the nurseries. Each tree was examined, qualitatively for the presence of the fungi. Each chemical was dissolved in 250 ml of water and was poured / applied, each pot separately, according to the concentrations described in table 1. Experimental design was randomized blocks in 4 replications. Each replication consisted of 5 seedlings. Root samples were taken 10 and/or 20 days after the chemical application and were examined in the PPIS for the presence of the fungi. In the first sampling of each treatment, one seedling in each replication was sampled and mixed samples of all replications in each treatment were examined in one bulk. In the samples no presence of the fungi was detected, roots were sampled, separately, for a second time. In this case, roots from seedlings which had not been sampled in the first one were sampled for a second round as two randomly sampled seedlings per block. Also, no presence of fungi was detected in these samples. In order to evaluate the pathogenicity of the fungi, a Koch test was carried out in 'Hass' seedlings grafted on 'Degania 117' rootstock. The seedlings were inoculated by a single spore isolated in the laboratory. The fungi developed on Agar media. A week old mycelium was cut into small pieces and each pot was inoculated with an even amount of inoculants. The pots were grown under in controlled environment and were irrigated every 2 days. Fungi presence was examined in the PPIS laboratories.
3. Growing the fungi to its reproductive stage and identification / characterization and defining by morphological means was done through conventional PCR and universal primary features for fungi.

## Results

1. The varieties that were mainly affected by the fungi were 'Ardit' and 'Pinkerton', grafted on different West Indian rootstocks, 3-4 years old trees, which bear high yields. No correlation between fungi presence and rootstock or soil type were found.
2. The efficiency of the different examined chemicals is shown in table 1 and the main results are:
  - a. The chemical **Prochloraz Zinci** ("Mirage"), 1 ml per seedling, was found effective in extermination of the fungi in seedlings planted in plastic bags.
  - b. The chemical **Prochloraz Zinci** ("Mirage"), 0.1 ml per seedling, was found efficient only in one examination and no repetition was found.

- c. The chemical **Aazoxystrobin** ("Amistar"), 0.025 ml per seedling, was found efficient only in one examination and no repetition was found.
3. The Koch test is still going on so no results are reported in this publication
4. Based on the reproductive stage, the fungi *Neonectria radicolica* was identified. This is the first finding of the fungi in avocado.

**Table 1: List of chemicals and doses applied**

| Commercial name         | Generic name              | Dosage (per seedling) | Fungi development (Y/X)**<br>Days after application |      |
|-------------------------|---------------------------|-----------------------|---|------|
|                         |                           |                       | 10  | 20   |
| "Mirage"                | Prochloraz Zinci          | 0.1 ml                |   | 0/4  |
| "Mirage"                | Prochloraz Zinci          | 0.5 ml                |   | 4/4  |
| "Mirage"                | Prochloraz Zinci          | 1.0 ml                | 0/4   | 0/20 |
| "Amistar"               | Aazoxystrobin             | 0.025 ml              |   | 0/4  |
| "Amistar"               | Aazoxystrobin             | 0.1 ml                |   | 4/4  |
| "Amistar"               | Aazoxystrobin             | 0.5 ml                |   | 4/4  |
| "Amistar"               | Aazoxystrobin             | 0.1 ml+additive       |   | 4/4  |
| "Topaz"                 | Thiophanate Methyl        | 0.175 g               |   | 4/4  |
| "Dalsan"                | Carbendazin               | 1%                    | 4/4   | 4/4  |
| "Dalsan"                | Carbendazin               | 0.5% X 2              | 4/4   | 4/4  |
| "Dalsan"                | Carbendazin               | 5%                    |   | 4/4  |
| "Dalsan"                | Carbendazin               | 10 ml                 |   | 4/4  |
| "Dalsan"                | Carbendazin               | 1%                    | 4/4   | 4/4  |
| "Dalsan"                | Carbendazin               | 0.5% X 2              | 4/4   | 4/4  |
| "Signum"                | Pyraclostrobin + Boscalid | 0.05 g                |   | 4/4  |
| "Signum"                | Pyraclostrobin + Boscalid | 1.0 g                 |   | 4/4  |
| "Signum"                | Pyraclostrobin + Boscalid | 1.0 g                 |   | 4/4  |
| Control<br>No treatment |                           |                       | 4/4   | 4/4  |

### Summary

During the past three years wilting and die back, accompanied by black and brown root rot were found in young avocado trees. The fungi that was isolated is *Neonectria radicolica*, is to be reported for the first time on avocado roots. Koch

test will verify to which extent this fungi is responsible for the wilting of the trees. Die back of young trees had occurred mainly in young trees, bearing high yields. The most effective fungicide against those fungi was found to be Prochloraz Zinci.

The above mentioned findings led to the current management which includes prevention of the fungi inoculation in the nursery and if required, treating with fungicide. Further more, young trees of high yielding varieties should not bear high yield during the first years.

The questions to discuss are: is this a new soil-borne pathogen in Israel, or an indigenous one. Have changes in avocado horticulture management, (water salinity, fertigation, heavy yield on young trees etc') increased the pathogenicity of the fungus?