



# Effectiveness of seven commercial rootstocks against verticillium wilt and their effects on growth, yield, and fruit quality of tomato



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## ABSTRACT

The susceptible to *Verticillium dahliae* Kleb. tomato (*Solanum lycopersicum* L.) cv. 'Early Pack' was evaluated in 2004 and 2005, for tolerance to verticillium wilt, after grafted on the commercial rootstocks 'He-man', 'Eldorado' (48-S-548), 'Beaufort', 'Primavera', 'Nova', 'Packmore' and 'Vigomax'. Self-grafted cv. 'Early Pack' was used as a control. The grafted plants, after artificial inoculation with a virulent isolate of *V. dahliae* during transplanting, were grown in a greenhouse and evaluated for disease tolerance, plant growth, yield, and fruit quality characteristics. In artificially inoculated plants, grafting on all rootstocks reduced disease severity significantly, in comparison to self-grafted cv. 'Early Pack', but effectiveness was repeated in both years only in 'Beaufort'. In addition, in 2004, under high disease pressure, grafting on 'Vigomax', 'Beaufort', 'Nova', and 'He-man' increased root weight, while grafting on the first three increased also above ground fresh weight of tomato. In 2005, grafting on 'Nova', 'Beaufort' and 'Vigomax' increased total marketable yield, while 'Beaufort' increased early marketable yield also. Grafting on 'Beaufort', 'Nova', and 'Vigomax' decreased total soluble solids (<sup>o</sup>Brix) and dry matter of tomato fruits, in both years, as compared to self-grafted cv. 'Early Pack', however, values recorded are acceptable for tomato. None of the rootstocks had any significant effect on flesh firmness, pericarp thickness, number of locules, equatorial diameter, and polar diameter. These results strongly support that grafting on commercial rootstocks increases tomato tolerance to verticillium wilt, plant growth and productivity, without affecting any fruit quality characteristics.

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## 1. Introduction

*Verticillium dahliae* Kleb. is the cause of verticillium wilt that is one of the most destructive diseases of the crops. The occurrence of this disease on tomato (*Solanum lycopersicum* L.) has been reported in almost all countries of the temperate and tropical regions (Sherf and MacNab, 1986). Symptoms include expanded yellow-bronze areas between the leaf veins, wilting and vascular discoloration. Plant growth and yield can be dramatically reduced (Bletsos et al., 2003; Oda, 1999; Sherf and MacNab, 1986) and concerning Greece, the disease is a serious problem in vegetable production systems, causing yield losses that reach 50% (Thanassouloupoulos and Kitsos, 1972). The fungus forms microsclerotia that persist in soil and can be viable for many years (Wilhelm, 1955), making the pathogen

extremely difficult to control.

Management of verticillium wilt relies primarily on preventive measures, since there are not any effective curative fungicide treatments especially after the establishment of the pathogen in the plant xylem system. Grafting of vegetable varieties on commercial rootstocks is amongst the most popular alternative methods that have been widely implemented during the last two decades for the control of soil-borne pathogens (Lee et al., 2010; Louws et al., 2010; Miguel, 1997). Growing grafted vegetables was first launched in Japan and Korea (Lee, 1994), where watermelons were grafted to gourd rootstocks. This technique is now applied to several vegetables including eggplant, melon, watermelon, cucumber, tomato, and pepper in many countries around the world (Bletsos and Olympios, 2008; Lee et al., 2010) and it is considered as one of the most important for the sustainable production of vegetables for both conventional and organic growers.

Conferring resistance to soil-borne diseases is the major reason for using rootstocks. However rootstocks may also promote

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earliness, increase yield of the cultivars used as scions, and, combining such properties, they are widely accepted among growers and consumers (Flores et al., 2010; Rouphael et al., 2010). Rootstocks, in most cases, do not degrade important fruit quality characteristics such as: (i) visual appearance defined by size, shape and color; (ii) texture, described by firmness and pericarp thickness; flavor compounds, mainly total soluble solids (TSS), and juice pH and titratable acidity accounting for sugar and acid content, respectively; (iii) health related compounds such as vitamins, carotenoids and minerals (Flores et al., 2010; Rouphael et al., 2010).

Rootstocks or grafted vegetables are provided to the growers by commercial enterprises. These commercial rootstocks, designed to be resistant to pathogenic fungi by their breeders, offer a practical tool widely applied in many countries, including Greece, for the control of vascular wilts. Although a number of rootstocks available in the market are widely used as a means to control verticillium wilt in tomato, comparative studies on their effectiveness against the disease and their influence on plant growth, yield and fruit quality are sparse and limited to few rootstocks each time. The aims of the present study were: (i) to evaluate and compare the effectiveness of seven commercial rootstocks against verticillium wilt in tomato and (ii) to investigate their effects on growth, yield, and fruit quality of plants grown in the presence and in the absence of *V. dahliae*.

## 2. Materials and methods

### 2.1. Plant material, fungus, and experimental set up

The experiment was conducted twice in April–July 2004 and April–August 2005, in a non-heated plastic greenhouse covering a 250 m<sup>2</sup> area, located in Thessaloniki, Greece, and particularly in the Agricultural Research Center of Macedonia and Thrace of the Hellenic Agricultural Organization-Demeter (HAO-Demeter, ex National Agricultural Research Foundation, NAGREF). The soil was sandy loam, with pH 7.25, free CaCO<sub>3</sub> 2.65%, organic matter 1.75%, electric conductivity 3.12 mmhos/cm, Olsen's phosphorus >200 ppm, and exchangeable potassium 895 ppm.

In order to acquire a high level of verticillium wilt severity, the highly susceptible cv. 'Early Pack' of a self-pollinated tomato was used in this study. This cultivar produces a 150–170 g of round red fruits. Tomato plants of cv. 'Early Pack' were grafted on the following seven commercial rootstocks, known as resistant to verticillium wilt: 'He-man' (*S. lycopersicum* x *S. habrochaites* S. Knapp & D.M.Spooner), 'Eldorado' (48-S-548) (*S. lycopersicum*), 'Beaufort' (*S. lycopersicum* x *S. habrochaites*), 'Primavera' (*S. lycopersicum*), 'Nova' (*S. lycopersicum*), 'Packmore' (*S. lycopersicum*), and 'Vigomax' (*S. lycopersicum* x *S. habrochaites*). As a control to the grafting procedure and because a vascular pathogen was to be inoculated, grafting was applied to all treatments. Plants of cv. 'Early Pack' grafted on rootstocks were compared for resistance to verticillium wilt with the self-grafted cv. 'Early Pack'. Thirty days old tomato seedlings were grafted on the above commercial rootstocks, by the cleft method (Oda, 1999), in the establishments of the company "Agrotikos Oikos Spirou AEEV" (Vateri, Thiva, Greece). Grafted seedlings were grown in 50 ml pots, in a perlite:peat moss, 10:90 (v:v) mixture, for extra 30 days, before they were inoculated with the fungus and transplanted in the greenhouse.

A virulent isolate of *V. dahliae* isolated from a diseased eggplant, re-inoculated on and re-isolated from tomato every year, was used to artificially inoculate tomato plants in the present study. For massive inoculum production, the fungus was grown in 1000 ml Erlenmeyer flasks, containing 500 ml of Czapek-Dox broth (Duchefa, Haarlem, The Netherlands), for 10 days, at 24 °C, on a rotary shaker, at 160 rpm. The contents of the flasks were then

homogenized in a Waring blender, and the inoculum was adjusted to 10<sup>6</sup> cfu ml<sup>-1</sup>, using a haemocytometer.

Plants were artificially inoculated just before transplanting, by dipping their whole root system, in inoculum prepared as described above, for 10 min. A hundred ml of inoculum at a concentration of 10<sup>5</sup> cfu ml<sup>-1</sup> was used for each plant. Half centimetre trimming of the lower root edges was applied to facilitate infection of each plant. Plants of cv. 'Early Pack' self-grafted and grafted on the above 7 rootstocks comprised 8 treatments. Three replications of nine plants were used for each treatment. The whole soil area of the greenhouse was disinfected by methyl bromide (Bromine Compounds Ltd., Israel) 4 weeks ahead of transplanting, and then divided into 3 blocks of equal sizes. Each block was subdivided in 2 sub-blocks (inoculated and non-inoculated), outlining 6 sub-blocks (3 inoculated and 3 non-inoculated) in the greenhouse. Care was taken to avoid mix of inoculated with non-inoculated plants in adjacent rows or plots due to high risk of fungal inoculum transfer. Inoculated plants were transplanted in the upper 3 sub-blocks, and for each of the 8 treatments the same number of grafted tomato plants was transplanted in the remainder 3 sub-blocks without inoculation, after trimming and dipping their roots in distilled water, for 10 min. In each sub-block, the 8 rootstock treatments were randomly distributed. Planting distances between rows were 80 cm, and 50 cm between plants on each row.

### 2.2. Recording disease severity, tomato growth, yield and fruit quality characteristics

Plants were grown in the greenhouse for about 12 weeks, from April 12 to July 8, 2004 and from April 22 to July 27, 2005 receiving all necessary cultural care for their normal growth. Irrigation and pruning were normally applied and foliar sprays against insects and foliar pathogens were conducted when necessary. Disease severity was expressed using three scales. First, a leaf symptom index (LSI) to rank external symptoms and a vascular discoloration index (VDI) to rank internal symptoms in the scions were set up as described by Bletsos et al. (2003). LSI was determined at 10-day intervals, starting 30 days after transplanting, and using the following 1 to 6 scale: 1 = apparently healthy plant; 2 = slight chlorosis of the lower leaves, erratic interveinal yellow-bronze wilted area; 3 = leaf spot necrosis, defoliation of lower leaves, yellow-bronze wilted area in the upper leaves, slight wilt; 4 = serious wilt, lower leaves dead, partial death of plants, extensive defoliation, discoloration of the petiole vascular tissue in the upper leaves; 5 = plants with a leaf tassel only on the top, clear vascular discoloration in the upper part of the stem; 6 = practically dead plant. A total of 6 LSI estimations were made, on all plants. At the end of the experimental period, all the plants were uprooted and VDI was recorded for each plant, using the following 1 to 4 scale: 1 = white root; 2 = vascular discoloration only in root system; 3 = brown discoloration of the vascular bundles up to the first stem knob; 4 = discoloration above the first knob. Finally, a 1–6 disease index (DI) was calculated for each plant, by multiplying LSI with VDI, as described by Bletsos et al. (2003). Infection of rootstocks and scions by *V. dahliae* was verified by isolations of the pathogen from roots and the main stem, on potato dextrose agar (PDA, Lab M Limited, Heywood, Lancashire, UK).

Stem length, total above-ground fresh weight of whole plant and total root weight were recorded at the end of the experimental period. Produced tomatoes were harvested at acceptable market maturation stage, once a week from June 1 to July 5, 2004 and from June 13 to July 18, 2005. In addition, fruit lacking market value were discarded and then total marketable yield weight, number of marketable fruits and fruit weight were recorded for each plant, each time. At the end of the experimental period data of marketable

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