Effect of Grafting on Growth, Yield, and Verticillium Wilt of Eggplant

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Abstract. Eggplant (*Solanum melongena* L.) seedlings ('Tsakoniki') were grafted by hand on the *Verticillium dahliae* Kleb. resistant wild species *Solanum torvum* Sw. (GST) and *Solanum sisymbriifolium* Lam. (GSS). Grafted and nongrafted eggplants were transplanted to a fumigated soil with methyl bromide and to infested soil with microsclerotia of *V. dahliae*. Grafted plants were more vigorous, as measured by plant height, main stem diameter, and root system weight, than the nongrafted 'Tsakoniki'. This resulted in an increased early production (GST, 45.5%; GSS, 18.4%) and late production (GST, 69.3%; GSS, 59.2%) as compared to the noninfected controls. The mean yield reduction (over years) in early production caused by the disease, as compared to the controls grown in fumigated soil, was 29.4%, 36.6%, and 77.9% for eggplant grafted on *S. torvum*, *S. sisymbriifolium*, and nongrafted plants, respectively. This yield reduction in total production was 6.9%, 20.5%, and 56.8%, respectively. The disease incidence in ungrafted plants was 96% and 100% during early and late harvest periods. In contrast, the disease incidence in grafted plants was significantly lower, averaging 28.1% (GST) and 52.6% (GSS) in early production, and 37.6% and 79.3%, respectively, in late production. *Solanum torvum* was found more resistant than *S. sisymbriifolium*, because grafted infected plants developed mild symptoms, as indicated by significantly lower leaf symptom index (average value 1.2 and 2.22) and disease index (average value 1.55 and 3.38), respectively. In conclusion, grafting of eggplant on either wild species had positive effects on growth, production, and verticillium wilt control.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is one of the most destructive diseases of eggplant, causing an estimated yield reduction of up to 50% (Bletsos et al., 1997a). Symptoms of this disease are yellow-bronze wilted areas, mainly between the leaf veins, and vascular discoloration. Plant growth (O'Brien, 1983) and fruit yield and quality are reduced (Bletsos et al., 1999). Continuous cropping of eggplant is commonplace in greenhouses. More than 68% of the failure in Japanese vegetable production under continuous cropping has been ascribed to diseases caused by soilborne pathogens, primarily verticillium wilt (Takahashi, 1984, referred by Oda et al., 1997). In greenhouse cultivation, verticillium wilt is controlled by soil fumigation with methyl bromide. Methyl bromide is considered a dangerous air pollutant

because of its effect on the ozone layer. It is both toxic to humans and animals during its application and dissipation periods (Ristaino and Tomas, 1997) and has been detected in groundwater (Wheeler and Kwar, 1997). For these reasons, 168 countries, including Greece (1997), have agreed to gradually reduce methyl bromide production and to phase out its use in agriculture by the year 2005.

Grafting plants on resistant rootstock could be an alternative control of soilborne diseases. Grafting watermelon on gourd rootstock has been used for protection from *Fusarium* wilt since the 1900s. Grafting has been expanded to other vegetables, including melon, cucumber, tomato, and eggplant (Lee, 1994; Oda, 1995). Thus, Ferrari (1998) grafted melon to control *Fusarium oxysporum* f. sp. *melonis* and Klose et al. (1980) grafted cucumber to control *F. oxysporum* f. sp. *cucumerinum*. Grafting susceptible tomato cultivars on resistant F₁ tomato hybrid rootstock type KVFN was an economic method of controlling verticillium and fusarium wilt, corky root (*Pyrenochaeta lycopersici*), and root-knot nematodes (*Meloidogyne* sp.) (Ginaux et al., 1979).

All commercial eggplant cultivars are susceptible to verticillium wilt. Furthermore, production and breeding for resistance has

been difficult (Kalloo, 1993; Nicklow, 1983). Grafting eggplant on resistant tomato rootstocks was found an effective control method for verticillium wilt (Ginaux and Douple, 1985; Lockwood et al., 1970). The wild species *Solanum torvum* Sw. and *Solanum sisymbriifolium* Lam. are resistant to verticillium wilt (Alconero et al., 1988; Sakata et al., 1989). Susceptible eggplants were grafted on rootstock of scarlet eggplant (*Solanum integrifolium* Poir.) early in the 1950s with satisfactory results (Yamakawa, 1983, referred by Oda, 1995). Since then, the cultivation of grafted fruit-bearing vegetable plants has increased greatly. Grafting of eggplant on *S. torvum*, which grows in tropical and subtropical areas and has a vigorous root system and vegetative growth (Tachibana, 1994), is extensively used in the vegetable industry in Japan (Sakata et al., 1989), accounting for 50% of the total acreage (Oda, 1995). There are no reports on the effectiveness of grafting eggplant on *S. sisymbriifolium*.

Disadvantages of grafting are the required time, space, materials, and high labor costs. Sometimes the incompatibility between rootstocks and scions also reduces fruit quality (Oda, 1995). In Greece, grafting of vegetables is practiced in the south, where early watermelon, melon, and eggplant are grown under low plastic tunnels. About 5% to 10% of the tunnels are planted with grafted eggplant. Grafting is considered an important technique for the sustainable production of fruit-bearing vegetables.

The present work was undertaken to investigate the agronomic behavior of the most important Greek eggplant cultivar, 'Tsakoniki', when grafted on rootstocks of *S. torvum* and *S. sisymbriifolium*.

Materials and Methods

The experiments were carried out during 1998 and 1999 at the Agricultural Research Centre of Macedonia and Thrace, Greece. The soil was a sandy loam and had a pH of 7.25, free CaCO₃ = 2.65%, organic matter 1.75%, electric conductivity 3.12 mmhos/cm, Olsen's phosphorus >200 ppm, and exchangeable potassium 895 ppm.

The Greek eggplant 'Tsakoniki' was planted in soil artificially infested with *V. dahliae*. Isolates of *V. dahliae* from tomato, potato, and eggplant were grown on potato dextrose agar and were used in a mixture (1:1:1) throughout. Inoculum was prepared by growing each isolate for 8 d at 20 ± 2 °C in plastic petri dishes, 5.5 cm in diameter. A quantity of ≈5 mL of sterile distilled water was added per dish and the colonies were scraped with a sterilized needle. The contents of each dish were filtered through cheesecloth; the filtrates were combined; and the inoculum suspension consisted of microconidia adjusted to 10⁶ spores/mL. Five milliliters of the above inoculum suspension was used to inoculate a 1-L glass jar containing 200 g growth medium made of 1 cornmeal : 1 perlite : 4 water (by weight). The jars were kept at 20 ± 2 °C and the fungus grew during the first 7 d at daylight; then they were covered with black plastic during the following 23 d in order to

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promote microsclerotia production (Bletsos et al., 1997b).

In the first year (1998) the soil was fumigated with methyl bromide 98%–chloropicrin 2% (by weight) (Bromine Compounds Ltd., Israel) at the rate of 68 g·m⁻². A total of 200 g of inoculum containing 15 × 10⁴ microsclerotia/g (Bardas, unpublished data) was used to infest each hill in one field. The other noninfested field, where the controls were planted, was fumigated in both years. Eggplants, 30 d old, were grafted (on the first week of April each year) onto rootstock from plants of the wild species *S. torvum* (50 d old) and *S. sisymbriifolium* (30 d old), applying the cleft grafting method (Oda, 1995). Thirty days later the grafted and nongrafted seedlings were transplanted. The experiment was a two-factor factorial trial with soil treatments (soil fumigated, and soil fumigated + infested with *V. dahliae*) and rootstock treatments (grafting on *S. torvum*, grafting on *S. sisymbriifolium*, and nongrafting). Analysis of variance was performed with MSTAT-C program. The treatments were arranged in a randomized complete-block design with three replications for each treatment (infested and noninfested soil). There were 10 or seven plants per replication in 1998 and 1999, respectively. The distance between rows was 100 cm, and among hills within the row was 50 cm. The percentage of infection was converted by square root transformation $\sqrt{x} + 0.5$ prior to statistical analysis.

A leaf symptom index (LSI), ranging from 1 to 6 (1 = apparently healthy plants; 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 = severely diseased plants) was used to estimate disease severity and a vascular discoloration index (VDI), ranging from 1 to 4 (1 = tap-root apparently healthy and white in color; 2 = vascular discoloration only in root system; 3 = brown discoloration of the vascular bundles up to the first stem knot; 4 = brown discoloration in internodes up to the top of the plant) was used to estimate the severity of vascular bundle discoloration. Finally, the disease index (DI) was calculated as the product of the two above indices (LSI × VDI) and it ranged from 1 to 24. According to their product value, plants were divided into six DI groups: 1) plants with a product value of 1; 2) plants with a value of 2; 3) plants with values of 3 to 5; 4) plants with values of 6 to 9; 5) plants with values of 10 to 15; and 6) plants with values of 16 to 24 (Bletsos et al., 1999; see Table 1 for more details). The LSI and VDI were determined for each plant and the DI (Table 1) was calculated for the final production period.

The total and marketable fruit yield was measured for two time intervals: early production period (3–31 July 1998 and 12–29 July 1999) and final harvest (3 July–22 Sept. 1998 and 12 July–9 Sept. 1999). Number and weight of total and marketable fruit weight were determined. Plant height (cm) from the cotyledons to the apex, main stem diameter (cm) at the height of the cotyledons, total biomass (g), aboveground biomass (g), and root biomass (g) were measured. The pH was measured in 5 mL of fruit juice using a digital pH meter (model 196, WTW, Weilheim i. OB, Germany), and the total soluble solids (°Brix) were measured in a few drops of the juice using the digital refractometer ATAGO PR-1 (ATAGO Co., Ltd., Tokyo, Japan). Plant infection by *V. dahliae* was verified by isolations from roots and the main stem on potato dextrose agar.

Results

Success in grafting. The percentage of successfully grafted eggplants on *S. torvum* was 84.4% and 80.8% in 1998 and 1999, re-

spectively, while on *S. sisymbriifolium* it was 77.2% and 74.8%.

Performance of grafted vs. nongrafted plants in fumigated soil; early and late harvest periods. In fumigated soil, all the parameters of vigor, yield, and fruit quality (except mean fruit weight and plant height) during early production in 1998 had significantly higher values for grafted eggplant (Table 2). In contrast, in 1999 the grafted plants on fumigated soil produced lower early yields. In the late production period in fumigated soil, significant differences were observed in root weight, number of marketable fruit, and marketable yield in 1998, while in 1999 only in stem diameter for both grafted treatments compared to nongrafted plants (Table 3). The grafted plants on *S. torvum* compared to nongrafted plants, grown in fumigated soil with methyl bromide (Table 3), were significantly taller in 1999, root weight was higher, and main stem diameter was larger in both years, while the grafted plants on *S. sisymbriifolium* had significantly higher root weight and main stem diameter in 1999 (Table 3). In fumigated soil, the advantages of the grafted plants were clear in 1998 and variable in 1999.

Performance of grafted vs. nongrafted plants in nonfumigated, inoculated soil; early and late harvest periods. In the infested soil, all the parameters in 1998 had higher values for plants grafted on *S. torvum* than nongrafted plants (Table 2). In 1999 during the early production period on infested soil, significant differences were observed in number of marketable fruit and plant height. In the late production period, however, in the presence of *Verticillium* the differences were significant for all the measured characteristics in both years (except number of marketable fruits and marketable yield in 1999). Grafting on both rootstocks provided better performance than nongrafted plants grown on infested soil. Averaging over both years, early marketable yield of GST, GSS, and nongrafted plants grown in infested soil was reduced by 29.4%, 36.7%, and 77.9% compared to plants grown in fumigated soil. Total marketable yield was reduced by 6.9%, 20.5%, and 56.8%, respectively. However, none of the qualitative fruit traits (pH and °Brix) was affected. Their values ranged from 5.70 to 5.96 and from 4.55 to 5.27, respectively. Grafting on GST had higher values for many parameters studied in both

Table 1. Mode of calculation of verticillium wilt index.

LSI ^a	VDI ^b	LSI × VDI	DI ^c
1	1	1	1
2	2	2	2
3	3	3, 4, 5	3
4	4	6, 8, 9	4
5		10, 12, 15	5
6		16, 18, 20, 24	6

^aLSI = Leaf symptom index.

^bVDI = Vascular discoloration index.

^cDI = Disease index.

The numbers 7, 11, 13, 14, 17, 19, 21, 22, and 23 can not exist as products of columns 1 and 2.

Table 2. Effect of grafting eggplant on verticillium wilt resistant rootstocks (*S. torvum* and *S. sisymbriifolium*) compared with nongrafted eggplant, on scion plant height and several fruit and yield parameters, during the early production period of 1998 and 1999, when grown on both soil fumigated with methyl bromide and soil infested with *Verticillium*.

Grafting on/Year	Characteristics													
	Total early yield (g)		Marketable yield (g)		No. of fruit		No. of marketable fruit		Mean wt of marketable fruit		Mean fruit wt (g)		Plant ht (cm)	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
<i>Fumigated soil</i>														
<i>S. torvum</i>	2867 a	993 bc	2294 a	894 b	20.3 a	6.0 bc	14.0 a	4.5 b	159.7 a	182.3 a	137.7 ab	226.5 a	124 a	116 ab
<i>S. sisymbriifolium</i>	2217 b	1240 b	1516 b	1080 b	18.3 a	9.4 a	10.3 b	7.5 a	142.7 a	162.1 ab	119.7 ab	158.7 b	103 ab	124 a
Not grafted	914 cd	1599 a	722 c	1469 a	6.7 b	10.5 a	4.3 cd	9.3 a	136.0 b	139.8 bc	118.3 ab	130.6 bc	92 b	115 b
<i>Infested soil</i>														
<i>S. torvum</i>	2491 ab	452 d	1848 ab	404 c	18.0 a	2.8 d	11.3 ab	2.0 c	160.0 a	110.7 cd	140.7 a	106.1 bc	115 b	99 c
<i>S. sisymbriifolium</i>	1285 c	879 c	863 c	782 b	10.0 b	6.3 b	6.3 b	5.3 c	125.0 ab	121.4 cd	108.0 b	130.0 bc	86 bc	99 c
Not grafted	534 d	413 d	175 d	310 c	6.3 b	3.9 cd	3.9 cd	1.3 d	83.3 b	96.6 d	76.5 c	90.0 c	64 d	67 d

Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

infested and noninfested soil. Total and marketable yield in 1998 decreased significantly on nongrafted as compared to GST when the plants were grown in fumigated or infested soil. Furthermore, root biomass and stem diameter decreased significantly in the control compared to GST. A significant decrease was also observed during the late production period for all parameters studied (except number of marketable fruit and marketable yield in 1999) on the plants grown in infested soil (Table 3). Finally, nongrafted and noninfected plants had marketable yield and plant height significantly higher than nongrafted and infected ones in the early production period, while all values were significantly higher (except number of marketable fruit in both years, marketable yield in 1998, and weight of marketable fruit in 1999) in the late production period (Table 3).

Differences in performance between *S. torvum* and *S. sisymbriifolium*. In fumigated soil, during the early production period, the total early yield, marketable yield, and number of marketable fruit in 1998 had significantly higher values for plants grafted on *S. torvum* than on *S. sisymbriifolium*. In 1999, however, significantly higher values for GST were observed for mean fruit weight and for GSS for number of fruit and number of marketable fruit (Table 2). In the late production period, only stem diameter in 1998 and biomass and root biomass in 1999 were significantly higher for plants grafted on *S. torvum* when compared to GSS. In infested soil and during the early pro-

duction period, GST gave significantly higher total early yield, marketable yield, number of fruit, and mean fruit weight in 1998 when compared to GSS, while in 1999 the results were reversed for total early yield, marketable yield, and number of fruit. In the late production period, plants grafted on *S. torvum* produced significantly higher values for biomass and aerial biomass in 1998 and root biomass and height in 1999 when compared to GSS. Root biomass in 1998, however, was significantly higher for plants grafted on *S. sisymbriifolium*. Over the 2 years in early production, plants grafted on *S. torvum* outyielded the ones grafted on *S. sisymbriifolium* by 22.8% and 37.0% in fumigated and infested soil, respectively, and in late production by 6.3% and 24.5%, respectively.

Isolations from all plants showed positive results only on the susceptible eggplant scion, whereas the resistant *Solanum* rootstocks apparently remained free of infection, as evidenced by lack of vascular discoloration and the failure to isolate the pathogen from rootstock vascular tissue. The pathogen occurred at epidemic levels in nongrafted (control) plants, with essentially 96% and 100% disease incidence in the early and late production periods, respectively. In addition, the average value of LSI during early production and DI during late production were 3.78 and 5.23, respectively. Grafted plants that were infected by the pathogen generally developed mild symptoms, as indicated by a very low LSI (over years 1.2

and 2.22) and DI (over years 1.55 and 3.38) for GST and GSS, respectively.

The resistance of the grafted plants was indicated by the increased percentage of the healthier plants observed in the grafted group as compared to the nongrafted ones (Table 4). The percentage of the severely diseased plants for GST, GSS, and nongrafted were 0.0%, 22.9%, and 79.0%, respectively. Healthy plants were observed among nongrafted plants only in the early production period.

Discussion

The high percentage of successful grafting observed for both *Solanum* species with no change in fruit quality indicated that both rootstocks are suitable for eggplant grafting. The yield advantage of the grafted plants was obvious when grown on infested soil (Tables 2 and 3). Yet, the advantage of the grafted plants was not justified in 1999 when the plants were grown in pathogen-free soil. The taller plants and the larger main stem diameter of the grafted plants were attributed to the vigorous root system (Table 3) of the rootstock. Similarly, the increased yield of grafted plants is believed to be due to enhanced water and mineral uptake (Lee, 1994). According to Young (1989), the rootstock's vigorous root system is often capable of absorbing water and nutrients more efficiently than scion roots. Thus, the significantly higher marketable yield of the plants grafted on *S. torvum* and *S. sisymbriifolium*

Table 3. Effect of grafting eggplant on verticillium wilt resistant rootstocks (*S. torvum* and *S. sisymbriifolium*) compared with nongrafted eggplant on plant parameters and fruit yield quality, during the final harvest period in 1998 and 1999, when grown on both soil fumigated with methyl bomide and soil infested with *Verticillium*.

Grafting on/Year	Characteristics															
	Marketable yield (g)		No. of marketable fruit		Wt of marketable fruit (g)		Biomass (g)		Aerial biomass (g)		Root biomass (g)		Stem diam (cm)		Ht (cm)	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
<i>Fumigated soil</i>																
<i>S. torvum</i>	4560 a	1912 a	29.3 a	12.6 ab	158 a	161 a	2090 ab	2514 a	1923 ab	2225 a	167 ab	289 a	3.16 a	2.96 a	154 a	165 a
<i>S. sisymbriifolium</i>	3979 a	2108 a	26.3 a	15.0 a	157 a	157 a	1995 ab	1936 b	1798 b	1719 ab	198 a	171 cd	2.36 c	2.80 a	137 ab	156 ab
Not grafted	1601 b	2222 a	9.7 b	13.0 ab	153 a	143 ab	1960 b	1693 b	1838 ab	1543 b	122 c	150 d	2.60 bc	2.26 b	145 ab	136 bc
<i>Infested soil</i>																
<i>S. torvum</i>	4719 a	1310 ab	30.0 a	7.6 b	159 a	152 a	2327 a	2109 ab	2144 a	1938 ab	134 bc	262 ab	3.06 a	2.60 ab	129 b	156 ab
<i>S. sisymbriifolium</i>	3549 a	1291 ab	23.0 a	8.6 ab	149 a	150 a	1192 c	2357 a	1508 c	2095 ab	183 a	171 cd	2.80 ab	2.30 b	137 ab	133 c
Not grafted	882 b	768 b	6.7 b	6.3 b	96 b	119 b	692 d	789 c	613 d	719 c	79 d	70 e	1.47 d	1.76 c	86 c	98 d

Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 4. Distribution of the eggplants in each grade of leaf symptom index and disease index during a) the early production period and b) at the final production period during the 2 years (1998 and 1999), when grafted on *S. torvum* and *S. sisymbriifolium* or not grafted.

Grafting on /Year	Leaf symptom index in the early production period													
	1	2	3	4	5	6	$\bar{X}$	1	2	3	4	5	6	$\bar{X}$
	Percentage of plants in each grade of leaf symptom index													
	1998							1999						
	86.7 a	10.0 a	3.3 a	0.0 a	0.0 a	0.0 a	1.27 c	57.1 a	28.6 a	0.0 b	14.3 a	0.0 a	0.0 b	1.13 c
<i>S. sisymbriifolium</i>	56.7 a	10.0 a	6.7 a	21.4 a	3.3 a	0.0 a	2.47 b	38.1 a	28.6 a	20.8 a	14.4 b	0.0 a	0.0 b	1.97 b
Not grafted	3.3 b	13.3 a	30.0 a	50.0 a	3.4 a	0.0 a	3.93 a	4.8 b	14.3 a	28.6 a	38.0 a	0.0 a	14.3 a	3.63 a
Grafting on /Year	Disease index in the late production period													
	1	2	3	4	5	6	$\bar{X}$	1	2	3	4	5	6	$\bar{X}$
	Percentage of plants in each grade of disease index													
	1998							1999						
	86.7 a	0.0 a	0.0 a	13.3 a	0.0 b	0.0 b	1.40 c	57.1 a	19.1 a	19.1 a	4.7 b	0.0 b	0.0 b	1.70 c
<i>S. sisymbriifolium</i>	36.7 a	3.3 a	23.3 a	10.0 a	13.3 a	13.4 b	3.00 b	4.8 b	0.0 b	33.3 a	42.8 a	14.3 ab	4.8 b	3.77 b
Not grafted	0.0 b	0.0 a	3.3 a	10.0 a	26.7 a	60.0 a	5.43 a	0.0 b	0.0 b	4.8 a	23.8 a	33.3 a	38.1 a	5.03 a

Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

and grown in fumigated soil as compared to the nongrafted ones observed in 1998 in the present study, could be attributed to their higher root biomass (Table 3). In addition, the reduction of marketable yield (early and total) from plants grafted on *S. torvum* was 29.3% and 8.7%, while the ones grafted on *S. sisymbriifolium* exhibited a yield reduction of 52.8% and 42.3%. This supports the idea that *S. torvum* is markedly resistant, while *S. sisymbriifolium* is less resistant to verticillium wilt (Bletsos et al., 1998a). However, germination was delayed (≈ 20 d) when eggplants were grafted on *S. torvum*, and the early growth before grafting was low due to the very small seeds. Tachibana (1994) reported that the plants grafted on *S. torvum* sometimes suffer from Mg deficiency mainly due to the low ability of the stock roots to absorb Mg ions.

The fact that the grafted plants produced better results than the nongrafted ones when grown on infested soils indicates the potential economic value of growing grafted plants for the growers. Grafting is extremely laborious and time consuming, and growers would like to reduce the labor input required. Grafting robots have been developed for grafting eggplant, tomato, and pepper (Kurata, 1994; Oda, 1995). Robot grafting is ≈ 10 times faster than conventional hand grafting. Eggplant grafted by robot produced a similar fruit yield to that of plants grafted by conventional methods (Oda et al., 1997). Since grafting gives increased disease tolerance and vigor to crops, it should be useful for the low-input sustainable horticulture of the future (Table 4). In conclusion, grafting of eggplant on either of the two wild species had a positive effect on the growth, production, and observed disease incidence.

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