



Effect of Interstocks In Photosynthesis and Growth Rates for 'Navelina' Orange And 'Kütdiken' Lemon

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Abstract

We studied the effects of interstocks in dwarfing properties and photosynthesis ratio during the growth period. Combinations were set up by grafting 'Navelina' orange (N) and 'Kütdiken' lemon (K) cultivars grafted on sour orange; and, Flying Dragon trifoliolate (FD), Citrumelo 1452 (C), Star Ruby grapefruit (SR), Rubidoux trifoliolate (R) were used as interstock. Experimental plants were grown in controlled greenhouses. Rootstock, interstock and scion stem diameters and sprout growth were measured following grafting on a monthly bases. Aboveground parts of plants, leaf and root dry weight, number of leaves, leaf area, photosynthesis rate and transpiration ratio were determined on plants at four periods (July 06, October 06, January 07 and April 07). The greatest dwarfness effect determined at final measurement of various grafting combinations was at FD interstock for 'Naveline' orange and at R interstock for 'Kütdiken' lemon. The highest dry weight of aboveground parts of plants for both cultivars was determined at C interstock combinations and control, whereas lowest values were determined at SR interstock combinations. The photosynthesis rate was highest in summer period. The photosynthesis rate was highest at 'Navelina' cultivar having dwarfing effect at N/FD/SO and N/SR/SO combinations while the highest photosynthesis rate for 'Kütdiken' was achieved at K/C/SO combinations, which has no dwarfing effect.

Introduction

Turkey has a great potential for citrus production when its ecological and other characteristics were considered. Turkey's total citrus production is 3,556,407 tons out of which 1,662,000 tons is orange, 889,253 tons is mandarin, 759,711 tons is lemon, 243,267 tons is grapefruit and 2,136 tons others (FAO, 2012).

In Turkish citrus production, the common rootstock used for citrus production in the country is sour orange (95%) followed by trifoliolate orange, Carrizo and Troyer citranges. The use of dwarf rootstocks is preferred in today's fruit growing since its use enables more yields with better quality from a unit area. The management of orchards established with dwarf rootstocks is easier thus dwarf rootstocks should be compatible with cultivars for rootstock studies. However, besides several advantages for using dwarf rootstocks, there is no commercially available dwarf rootstock (Lliso *et al.*, 2004). Some rootstocks with dwarfness potential can be used as interstock for certain plants (Simon, 1987). Ashkenazi *et al.* (1992) reported that such dwarfness can be advantageous for harvesting,

decreasing shading problems, enhancing early fruit development which all in turn result high income.

It is crucial for sustainable citrus production to develop and introduce rootstocks having dwarf properties and resistant to ecological conditions along with pest and diseases. However, the adaptation of rootstocks with current and new citrus cultivars with their relation between rootstock-scion have not been studied extensively. The objective of this study was to determine the effect of Sour orange rootstock, Star Ruby, Citrumelo 1452, Flying Dragon and Rubidoux trifoliolate interstocks on the dwarfness of widely produced 'Navelina' orange and 'Kütdiken' lemon cultivars along with seasonal photosynthesis rate.

Materials and Methods

Plant materials: The study is carried out in a research greenhouse at Çukurova University located in Eastern Mediterranean Region of Turkey. While Sour orange (SO) is used as rootstock, 'Flying Dragon' trifoliolate (FD), 'Citrumelo 1452' (C), 'Star Ruby' grapefruit (SR), 'Rubidoux' trifoliolate (R) were used as interstock for 'Navelina' orange (N) and 'Kütdiken' lemon (K) cultivars.

Rootstock, interstock and cultivar combinations are given in Table 1. Saplings are planted to plastic bags filled with 2:1 volcanic tuff and peat mixture. The interstocks were grafted on sour orange seedlings in March of 2004. 'Navelina' and 'Kütdiken' cultivars were grafted to interstocks and sour orange rootstock as control in March of 2005. Irrigation, fertilization, pest and disease management of all experimental plants were practiced in a similar way. Seasonal measurements were realised at four periods in July 2006 for summer, in October 2006 for autumn, in January 2007 for winter and in April 2007 for spring with 5 plants for each period.

Diameter and shoot measurements:

Rootstock, lower part of interstock, upper part of interstock and scion stem diameters were measured following sprouting of buds on monthly bases from June 2005 to March 2007 with a total of 22 surveys. The measurements were done at 5 cm below and upper part of the grafting point by digital caliper. Considering age differences among plants may affect shoot length, at the time of interstock grafting, N and K saplings grafted to SO rootstocks' shoot length after crowning in final stage were measured. These data are used for determination of dwarfness effect of interstocks compared to control.

Measurement of above-ground parts of plants: The dry weight (g), root dry weight (g), leaf dry weight (g), number of leaves, leaf area (cm²) above-ground parts of five plants were measured for each period. Plant tissues were dried at 68 °C for 48 h for dry weight measurement.

Measurements of leaf photosynthesis rate (leaf CO₂ exchange ratio) and transpiration ratio: CO₂ exchange rate (CER) and transpiration rate (Ts) were measured with a portable photosynthesis analyzer (Model LCA-4, ADC Bioscientific Ltd, Hoddesdon, UK) from fully developed four youngest leaves for each replicates of combinations. Measurements were done from 09.30 to 14.00 hours at clear weather days. Photosynthesis active radiation (PAR) values obtained during periodical measurements undertaken by LCA-4 photosynthesis equipment are provided in Table 2.

Statistical Analyses: Data were subjected to analysis of variance by SAS statistical program and means were compared by Duncan's test at P< 0.05 significance level.

Table 1. Rootstock / interstock / cultivar combinations

Cultivar	Interstock	Rootstock	Abbreviations
Navelina	----	Sour orange	N/SO (control)
Navelina	Flying Dragon Trifoliolate	Sour orange	N/FD/SO
Navelina	Rubidoux Trifoliolate	Sour orange	N/R/SO
Navelina	Citrumelo 1452	Sour orange	N/C/SO
Navelina	Star Ruby Grapefruit	Sour orange	N/SR/SO
Kütdiken	----	Sour orange	K/SO (control)
Kütdiken	Flying Dragon Trifoliolate	Sour orange	K/FD/SO
Kütdiken	Rubidoux Trifoliolate	Sour orange	K/R/SO
Kütdiken	Citrumelo 1452	Sour orange	K/C/SO
Kütdiken	Star Ruby Grapefruit	Sour orange	K/SR/SO

Table 2. PAR values of photosynthesis measurement periods

Measurement intervals	PAR (μmol m ⁻² s ⁻¹)
4 th Period (July)	1797
5 th Period (October)	1330
6 th Period (January)	993
7 th Period (April)	1631

Results and Discussion

In 'Navelina', effect of interstocks on sour orange stem diameter growth found to be statistically significant. While N/SR/SO combination resulted in largest stem diameter value with 9.45 mm, the N/SO combination yielded smallest stem diameter with 6.32 mm. As for stem diameter values at the lower part of interstock, the combinations set up by trifoliolate hybrids of R, FD and CD statistically appeared in same group and manifested highest values 8.94, 8.84, 8.62 mm. The lowest stem diameter was 7.79 mm, and observed at SR combinations. The stem diameter of the upper part of interstock is largest at N/FD/SO combination (8.76 mm) while N/SR/SO combination revealed lowest with 7.97 mm. Cultivar shoot diameter was greatest at N/C/SO (5.69 mm) combination and was minimum at N/FD/SO combination (3.65 mm) (Table 3).

Differences among rootstock stem diameter averages were statistically significant at 'Kütdiken'. The maximum value was obtained at K/SR/SO combination with 9.32 mm, 7.34 mm which was lowest was determined at K/SO combination. The stem diameter of the lower part of interstock was highest at K/FD/SO (9.32 mm) and K/R/SO (9.06 mm) combinations, however, K/C/SO (8.51 mm)

and K/SR/SO (8.28 mm) combinations yielded lowest averages. The stem diameter of the upper part of interstock was largest at K/FD/SO combination with 9.31 mm, and the lowest diameter was measured at K/C/SO with 8.15 mm which was followed by K/SR/SO with 8.37 mm. Shoot diameters of the cultivar at K/SR/SO and K/C/SO combinations were high with 6.55 mm and 6.44 mm, but at K/R/SO combination the value of 5.42 mm was the lowest for all combinations (Table 4).

For both cultivar shoot lengths of plants after crowning were measured at the latest sampling period (April 2007) and dwarfing effect of interstocks compared to control were given in Table 5. The highest dwarfing effect for Navelina orange was 41.13% at FD interstock combination, and lowest was at C combination with 3.63%. The interstock R used for Kütdiken lemon cultivar has the highest dwarfing effect by 34.71%, the lowest effect was determined at C interstock with 20.29%.

Table 3. Stem growth of Navelina combinations

Combination	Rootstock diameter (mm)	The stem diameter of lower part of interstock (mm)	The stem diameter of upper part of interstock (mm)	Stem diameter of the scion (mm)
N/SO	6.32 d	-----	-----	4.43 c
N/FD/SO	8.31 c	8.84 a	8.76 a	3.65 d
N/R/SO	8.84 b	8.94 a	8.54 ab	4.23 c
N/C/SO	8.67 b	8.62 a	8.30 b	5.69 a
N/SR/SO	9.45 a	7.79 b	7.97 c	5.06 b
Significance	*(2)	*	*	*

(1): The differences among averages are provided by different letters.

(2): * : p<0.05

Table 4. Stem growth of Kütdiken combinations

Combination	Rootstock diameter (mm)	The stem diameter of lower part of interstock (mm)	The stem diameter of upper part of interstock (mm)	Stem diameter of the scion (mm)
K/SO	7.34 d	-----	-----	5.76 bc
K/FD/SO	8.50 bc	9.32 a	9.31 a	5.85 b
K/R/SO	8.62 b	9.06 a	8.81 b	5.42 c
K/C/SO	8.29 c	8.51 b	8.15 c	6.44 a
K/SR/SO	9.32 a	8.28 b	8.37 c	6.55 a
Significance	*(2)	*	*	*

(1): The differences among averages are provided by different letters.

(2): * : p<0.05

Table 5. The dwarfing effect of interstocks to cultivars (%)

Interstock	Cultivar	
	Navelina orange (%)	Kütdiken lemon (%)
Flying Dragon	41.13	22.50
Citrumelo 1452	3.63	20.29
Rubidoux	33.00	34.71
Star Ruby	38.42	26.47

The plants dry weight aboveground parts of 'Navelina' and 'Kütdiken' combinations found to be statistically significant for four measurement periods. The heaviest above ground dry weight average for 'Navelina' orange for all periods was determined at N/C/SO (68.07%) and N/SO (64.89%) combinations respectively, while the lowest aboveground dry weight was at N/SR/SO combination with (55.89%). Heavy aboveground weights for 'Kütdiken' cultivar were at K/C/SO

(74.45%) and K/SO (70.76%) combinations, and K/SR/SO combination yielded lowest with (65.22%) (Fig. 1).

For dry root weight, while N/SR/SO combination has the highest dry root weight (% 44.11), N/SO and N/C/SO combinations have the lowest weights with 35.12% and 31.94%, respectively for 'Navelina'. 'Kütdiken's highest dry root weights were determined at K/SR/SO (34.78%) and K/R/SO (33.55%), whereas K/C/SO

(25.55%) and K/SO (29.25%) combinations have yielded lowest values (Fig. 2).

The heaviest dry leaf weight of Navelina cultivar was observed at N/SO (18.78 g) and lightest was at N/SR/SO (7.47 g) combination. For 'Kütdiken', K/C/T revealed highest value with 23.44 g and K/R/SO gave lowest with 6.36 g (Fig. 3).

The average number of leaves for the plant for all periods was highest at N/SO combination (95.85) and was lowest at N/SR/SO (32.80) for 'Navelina'. The similar results were obtained when measurement periods were analyzed separately. The number of leaves for the plant at 'Kütdiken' was high at K/SO (90.05) and K/FD/SO (91.65) respectively and lowest was determined at K/R/T (77.75) combination (Fig. 4).

As for leaf area, while largest was at N/SO (1874.70 cm²), N/SR/SO has the smallest area (752.60 cm²) for 'Navelina', and for 'Kütdiken', the highest value with 3531.80 cm² was determined at K/SO, while K/R/SO combination yielded 2079.10 cm² which was the smallest area (Fig. 5).

Although the summer period photosynthesis speed, July, has the fastest of all combinations, statistically significant differences were determined among combinations. The fastest value for Navelina was determined at N/SR/SO with 3.83 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and lowest was at N/R/SO with 2.13 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For Kütdiken, the fastest rate was obtained from K/C/SO (1.82 $\mu\text{mol m}^{-2} \text{s}^{-1}$) while slowest from K/FD/SO (1.11 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for all periods (Fig. 6).

Transpiration rates of 'Navelina' were found to be highest at N/FD/SO (1.60 $\text{mmol m}^{-2} \text{s}^{-1}$) and N/SR/SO (1.32 $\text{mmol m}^{-2} \text{s}^{-1}$) and were lowest at N/SO, the control, with 0.92 $\text{mmol m}^{-2} \text{s}^{-1}$ and N/C/SO with 0.83 $\text{mmol m}^{-2} \text{s}^{-1}$. For all periods average, the highest rate of transpiration was 0.98 $\text{mmol m}^{-2} \text{s}^{-1}$ at K/R/SO and lowest with 0.65 at K/R/SO and K/SR/SO combinations (Fig. 7).

The high competition in citrus market necessitates establishment of dense orchards for increasing production from a unit area. Although dwarfing rootstocks like Flying Dragon and Rubidoux trifoliolate are present in Turkey, it is not possible to use them as rootstock in alkali soils of Mediterranean where meets 90% of the country's citrus production. The use of these rootstocks for dwarfing, which are also resistant to cold weather, increase yield and quality, provide earliness and decrease juvenile period, can only be possible by using them as interstock in high pH soils of Mediterranean and Aegean regions. However, growth, dwarfing and compability of rootstock – interstock – cultivar combinations are not well described yet. The growth potential and photosynthetic activity of cultivars grafted to various interstocks based on seasonality are determined in the study.

N/SO and K/SO were smaller than their combinations with interstock in respect of the monthly rootstock stem diameter growth. It is most probably due to transportation of carbohydrates and plant nutrients without any trouble and having one grafting point. The larger rootstock stem diameters for other treatments with two grafting points may be attributed to particularly interstocks limiting effect, and double grafting points reducing effect on vascular tissues phloem which cannot transport sufficient carbohydrate flux. Moreover N/FD/SO, N/R/SO, K/FD/SO and K/R/SO combinations with FD and R interstocks of 'Navelina' sweet and 'Kütdiken' exhibited the smallest shoot diameter growth compared to other interstocks. Similar results were determined at Aoshima, Outsu no 4, Sweet spring and Kiyomi grafted to Flying Dragon yielded lower stem and rootstock circumference and canopy compared to trifoliolate (Takishita *et al.*, 2000).

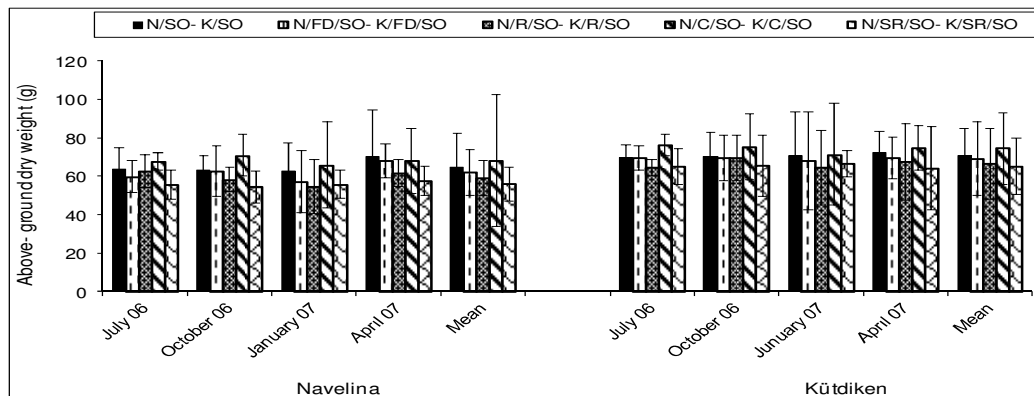


Fig. 1. Above-ground biomass dry weight of combinations according to periods $P \leq 0.05$ (Duncan).

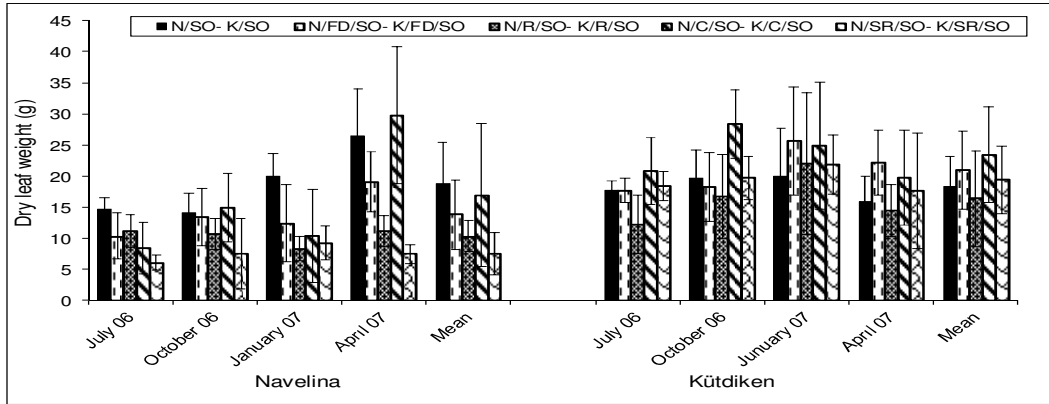


Fig. 2. Leaf dry weight of combinations according to periods $P \leq 0.05$ (Duncan).

The combinations of ‘Navelina’ and ‘Kütdiken’ with R and FD interstocks had highest values for the diameters of the lower and upper part of interstock. Kaplankiran (1984) reported that trifoliolate exhibit small growth on itself but the growth was quite high when used as rootstock for sour orange and volkameriana. He concluded that the growth was not as fast as rootstock when it was used as scion for sour orange and volkameriana.

Highest dwarfness for ‘Navelina’ in terms of shoot lengths at last sampling period following crowning of plants was obtained at FD. However, combination set up with R interstock has high dwarfing effect at ‘Kütdiken’. Bitter *et al.* (1977) concluded that in general average tree size was reduced by 50%; in some instances 75% and in one or two cases by only 25% by using of citrus relatives either as rootstock or as interstock for dwarfing. Ashkenazi *et al.*, (1992) measured 30 to 70% canopy volume decrease at ‘Michal’ and ‘Nova’ mandarins grafted to Flying Dragon. The finding of these two studies were similar to those

of our findings for ‘Navelina’ grafted to Flying Dragon interstock with 41.13% decrease in young tree size when compared to control. Our results are also supported by Ferguson and Chaparro (2005). Researchers also reviewed other studies and informed that in an experiment undertaken at 11 years old trees in Italy in 1989, where mandarin, orange and grapefruit were grafted to sour orange with interstock of Flying Dragon exhibited 1/3 dwarfing and higher production than controls. K/SO and N/SO combinations which were used as control produced more leaves and larger area than combinations with interstock. Also, the number of leaves of ‘Kütdiken’ was higher than ‘Navelina’ combinations which may be attributed to early sprouting of lemon buds at cooler temperatures. In our study, the higher aboveground parts of plants dry weight, leaf number and photosynthesis capacity and lower root weight in K/C/SO, K/SO, N/SO applications could indicate that most of the carbohydrate produced by plant was used for vegetative growth.

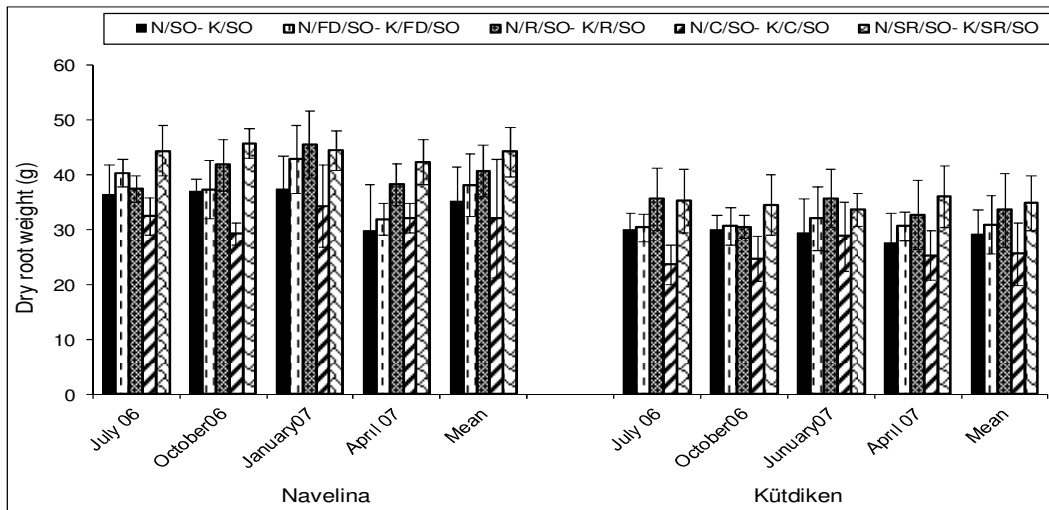


Fig. 3. Root dry weight of combinations according to periods $P \leq 0.05$ (Duncan).

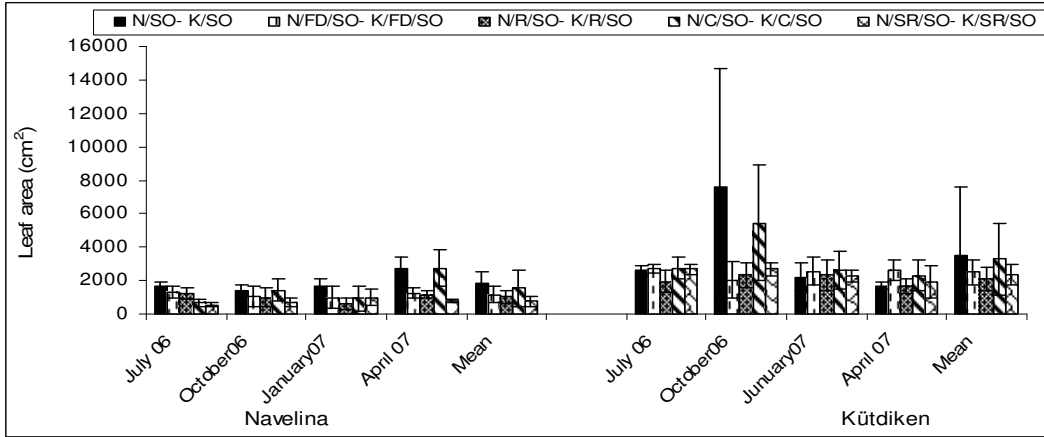


Fig. 4. Number of leaves of combinations according to periods $P \leq 0.05$ (Duncan).

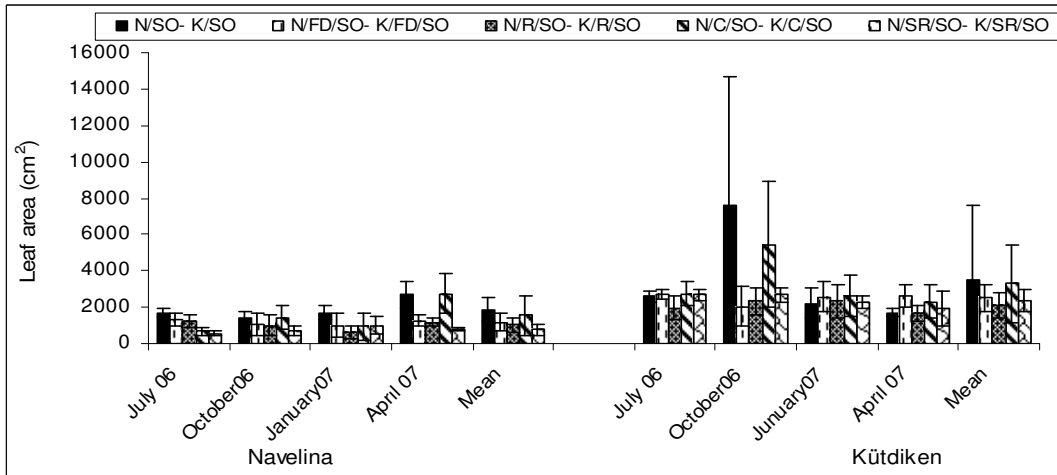


Fig. 5. Area of leaves of combinations according to periods $P \leq 0.05$ (Duncan).

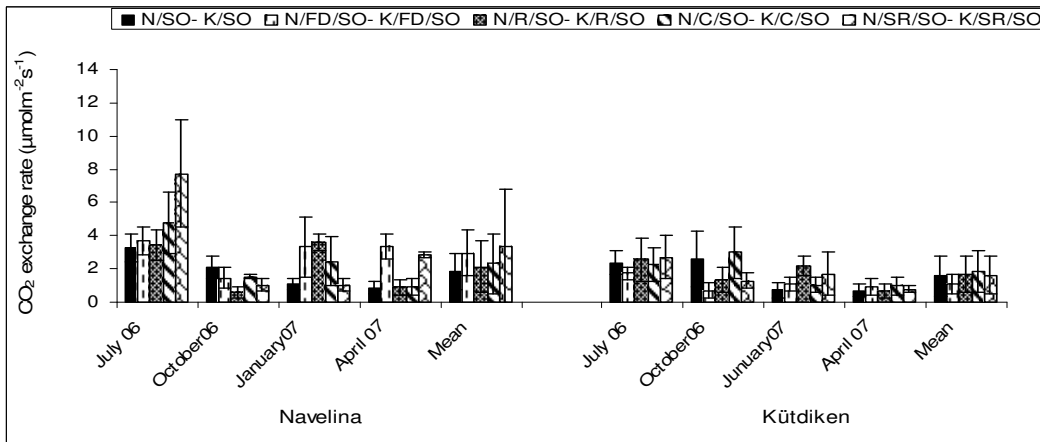


Fig. 6. The photosynthesis rate of combinations according to periods $P \leq 0.05$ (Duncan).

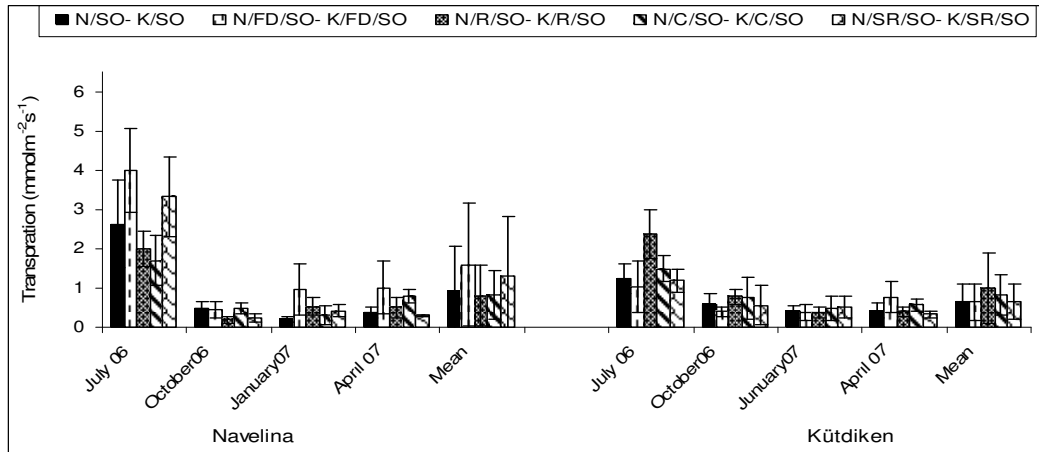


Fig. 7. Transpiration rate of combinations according to periods $P \leq 0.05$ (Duncan).

The fastest photosynthesis at all plants for all measurement periods was determined at 4th period which correspond summer period. Riberio *et al.* (2009), determined higher photosynthesis rate in summer than winter for 'Valencia' orange grafted to 1-year-old Cleopatra mandarin in subtropic regions. The authors suggested that reduction in photosynthesis rate in winter was due to cold temperatures, and decrease in photosynthesis in winter afternoons caused by lessening of stoma conductivity and renewing of RuBP (ribulose -1,5- biphosphate). Guang- Hui *et al.* (2006) defined that low temperature in winter limited photosynthetic carbohydrate metabolism and photochemical reactions caused low photosynthesis. On the other hand, Tsokankunku *et al.* (2008) determined increasing transpiration rate and photosynthesis speed at leaves having 25-30°C during summer of 2007 for 5-year-old 'Navel' and 'Bahianinha' oranges. Combinations of 'Navelina' revealed higher photosynthesis speed than 'Kütdiken' combinations probably due to thicker leaf structure of 'Navelina' than 'Kütdiken'. Close relationship between leaf anatomy and photosynthesis were determined by several studies. Germana and Sardo (1996) marked photosynthesis capability differences among species. The net photosynthesis ratio of *C. natsudaidai* (Hay) and *C. grandis* (L) Osbeck were found significantly higher than *C. unshiu* (Marc), *C. junos* (Sieb.ex.Tan) ve *C. reticulata* (Blanco) (Morinaga & Ikeda 1990). The researchers attributed to several factors such as chlorophyll content, stoma density, enzyme activity and leaf weight by the authors. Germana *et al.* (2002) determined differences in photosynthesis of kamkat and grapefruits and showed that larger and greener grapefruit leaves have higher CO₂ exchange ratio due to their effect on PAR and Vapour Pressure Deficit (VDP). The net CO₂

exchange ratio was found higher than kamkat at grapefruits.

When the interstocks effect on photosynthesis was evaluated, N/SR/T and N/FD/T with dwarfing effect found to have high influence for 'Navelina' combinations which is in accordance with Walt & Davie's (1995) findings. The authors determined that during fruit growth period, the photosynthesis activity of 'Marsh Seedless' grapefruits grafted to Flying Dragon, which causes dwarfness was higher than grafted to rough lemon. However, some studies suggested that photosynthesis rate is not affected from dwarfing mechanism. Lliso *et al.* (2004) measured leaf photosynthesis activity on 'Navelina' cultivars grafted to #23 (Troyer citrange × Kleopatra mandarin), # 24 (Troyer citrange × Kleopatra mandarin) and F&A 418 (Troyer citrange × local mandarin) rootstocks. They found same photosynthesis activity was for all treatments of Navelina cultivar even F&A 418 and #23 provided dwarfing. Takishita *et al.* (2000) reported lower photosynthesis at Otsu no 4 variety grafted to Flying Dragon rootstock than the ones grafted to trifoliate during two years. On the other hands, no difference was observed at between 'Aoshima' and 'Sweet spring' grafted to 'Flying Dragon' and Trifoliate. In our study, while photosynthesis rate for K/C/SO combination was the highest, it was lowest for K/FD/SO combination when 'Kütdiken' photosynthesis rate was investigated. In general, transpiration rate determined highest at 'Navelina' combinations than 'Kütdiken' combinations.

Conclusions

The photosynthesis speed was highest at summer period for all combinations in seasons. 'Navelina' combinations have faster photosynthesis than 'Kütdiken' cultivar

combinations. The interstocks effect on photosynthesis for 'Navelina' orange was high at N/FD/SO and N/SR/SO which have also dwarfing effect. However, highest values for photosynthesis of 'Kütdiken' was achieved at K/C/SO, without dwarfing effect, and lowest was measured at K/FD/SO combinations. The FD used as an interstock has a positive dwarfness effect on 'Navelina' but has no dwarfness effect on 'Kütdiken'. On the other hand, the shortest shoot length obtained with 'Rubidou' trifoliolate rootstock treatments for both cultivars particularly its reducing effect for 'Kütdiken' aboveground biomass, number and area of leaves suggested a potential use for dwarfing. Although having high photosynthesis speed of combinations particularly for 'Navelina' where 'Star Ruby' interstock was used, this interstock is not suggested for 'Navelina' due to observation of undeveloped plants at field.

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