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The effect of nitrogen and phosphorus fertilization on yield, some fruit characteristics, hormone concentrations, and alternate bearing in pistachio

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Abstract: This research was carried out in order to determine the effects of nitrogen and phosphorus fertilization on yield, some fruit characteristics, hormone concentrations, and alternate bearing in pistachio (*Pistacia vera* L.) cv. 'Kırmızı' grafted on *P. vera* over 3 consecutive years. Nitrogen was applied at the rate of 0 (control), 800, 1000, and 1200 g tree^{-1}, and phosphorus was applied at the rate of 0 (control), 200, 400, and 600 g tree^{-1} into the soil. As a result, although yield mostly decreased and nut weight increased, especially in the 2nd year ('off' year), considerable interactions among fertilizer rates were determined for only nut weight, auxin, gibberellic acid, and zeatin concentrations ($P \leq 0.05$). For all parameters investigated in this study, a stable and regular effect of fertilizers applied into the soil was not observed, probably because of this species' characteristics. Therefore, further research, including longer treatment periods, is needed to determine the effects of fertilizers.

Key words: Alternate bearing, fertilization, GA_3, IAA, nut weight, *Pistacia vera* L.

Antepfıstığında azotlu ve fosforlu gübrelemenin verim, bazı meyve özellikleri, hormon konsantrasyonları ve periyodiste üzerine etkisi

Özet: Bu çalışma, *P. vera* üzerine aşılı 'Kırmızı' Antepfıstığı (*Pistacia vera* L.) çeşidinde azotlu ve fosforlu gübrelemenin verim, bazı meyve özellikleri, hormon konsantrasyonları, periyodisite üzerine etkilerini belirlemek amacıyla 3 yıl boyunca yürütülmüştür. Azot 0 (Kontrol), 800, 1000 ve 1200 g ağaç$^{-1}$ oranlarında ve fosfor ise 0 (Kontrol), 200, 400 ve 600 g ağaç$^{-1}$ oranlarında toprağa uygulanmıştır. Çalışma sonunda, özellikle periyodisite yılı olan 2. yılda, verim önemli ölçüde düşmüş, meyve ağırlığı artış göstermiş olmasına rağmen, gübre oranları arasındaki interaksiyonlar, sadece meyve ağırlığı, oksin, gibberellin ve zeatin konsantrasyonları için önemli bulunmuştur ($P \leq 0.05$). Muhtemelen bu türün bitkisel özellikleri nedeniyle topraktan uygulanan gübrelerin çalışmada incelenen bütün parametreler üzerine sabit ve düzenli bir etkisi, gözlenmemiştir. Bu nedenle Antepfıstığında gübreler ve gübre oranlarının etkilerini belirlemek amacıyla daha uzun süreli uygulama periyotları içeren çalışmalara gereksinim duyulmaktadır.

Anahtar sözcükler: Periyodisite, gübreleme, GA_3, IAA, meyve ağırlığı, *Pistacia vera* L.

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Introduction

Pistachio nut (*Pistacia vera* L.) is a widely growing species especially in southeast Anatolia in Turkey, the third highest pistachio producing country (14.2%) after Iran and USA in the world (FAO 2007). In Turkey, there is low and irregular yield problem due to pistachio being an alternate bearing tree as well as insufficient cultural management in terms of fertilization and irrigation. It has been reported that irrigation, fertilization, and pruning have been very important cultural practices in obtaining high and quality yield. However, studies carried out regarding the effect of these practices on alternate bearing in pistachio are limited.

In fruit production, alternate bearing, the occurrence of a high yield 'on' year followed by low yield 'off' year (Monselise and Goldschmidt 1982), is undesirable because it affects management factors such as cash flow, labor needs, and use of farm machinery. In pistachio, while fruit are borne on 1 year old fruiting wood, buds are borne on current year shoots. If a new shoot is subtended by 1 year old wood with fruit, then the flower buds on the current year shoot abscise (Crane and Iwakiri 1985). As such, flower bud abscission on these shoots is the cause of alternate bearing. The physiological mechanisms of this phenomenon are unknown (Nzima et al. 1997). In some studies it has been suggested that alternate bearing has been affected by not only genetic factors but also some other factors such as cultural management, the carbohydrate and nitrogen balance of the tree (Monselise and Goldschmidt 1982; Crane and Iwakiri 1985), and amounts and rates of plant growth regulators, which have a direct effect on plant physiology (Monselise and Goldschmidt 1982; Pontikis 1990). Crane and Nelson (1971) reported that alternate bearing in pistachio was directly related to the amount of assimilates synthesized by a plant during the preceeding summer period. Recently, Baninasab and Rahemi (2006) indicated that soluble sugars in the bud, leaf, and root of a pistachio tree could be related to the alternate bearing, and there is a high and significant negative correlation between bud, leaf and root sucrose and fruit glucose concentrations, and flower bud abscission. Contrary to this, alternate bearing increases nitrogen, phosphorus, and potassium as well as starch storage pools in pistachio trees (Rosecrance et al. 1998). Crane et al. (1982), investigating the effects of different ethephon doses on flower bud abscission, did not find a significant effect of ethephon.

There are 5 accepted groups of natural hormones including 3 auxins, several cytokinins, many gibberellins, abscisic acid, and ethylene. The functioning of a plant depends on specific levels of natural hormones. Generally, auxins, gibberellins, and cytokinins are called stimulators; they regulate a number of physiological activities. Abscisic acid and ethylene are known as growth retardants; they restrict or completely impede some physiological activities but hasten ripening (Westwood 1993; Davies 2004). Occurrence, increase, decrease, or interaction of these growth regulators in fruit trees change based on months. Moreover, they have been synthesized at different times. It has been reported that the amount and rates of plant growth regulators in a plant affected the occurrence and severity of alternate bearing (Yılmaz 1990). There has been limited research about the effect of cultural management, such as irrigation, pruning, and fertilization, on the growth regulator levels in the plant.

In some studies about the fertilization of pistachios, only yield and some fruit criteria were taken into consideration. For example, potassium fertilization increased soil and leaf potassium content, nut yield, and quality of mature pistachio trees in California (Zheng et al. 2001). Kuru (1993) found that soils in Gaziantep, Turkey, used for pistachio growing had poorer levels of phosphorus than nitrogen and potassium. Additionally, they mentioned that calcium, magnesium, and copper content in leaves were satisfactory and an application of 60 kg P ha^{-1} increased the yield at least 3 fold. Similarly, Ashworth et al. (1985) mentioned that insufficient levels of potassium and phosphorus caused poor growth and low yield. Weinbaum et al. (1995) reported that nitrogen fertilization increased yield in pistachio in the 'on' year, but decreased it in the 'off' year, and caused late leaf abscission.

In this research, the aim was to determine the effect of nitrogen and phosphorus fertilization on some generative characteristics such as alternate bearing and yield and some fruit characteristics as well as on some hormone concentrations in mature pistachio trees.

Materials and methods

Research area and plant material

This research was conducted in a commercial pistachio orchard located in the Fırat valley in Nizip, Gaziantep, where pistachio was the species produced in the largest quantities in 1999-2002. Based on the average values from 1990-2002, annual precipitation in this district was 48.38 mm and precipitation values higher than 60 mm were observed from November to April. The average relative humidity and annual average temperature were reported as 55.42% and 16.44 °C, respectively (T.C. Çevre ve Orman Bakanlığı, Devlet Meteoroloji İşleri Genel Müdürlüğü, 2009). Pistachios were grown by using traditional methods common in this region. 'Kırmızı' pistachio cultivar trees, 35 years old and grafted on *Pistacia vera* L., were used as plant material.

Soil characteristics of the pistachio orchard based on texture characteristics, pH and $CaCO_3$ content, plant available phosphorus and total nitrogen are presented in Table 1.

Fertilizer applications

Nitrogen application was split, half in March and the other half in April, using ammonium sulfate at the rate of 0 (control, N_0), 800 (N_1), 1000 (N_2), and 1200 (N_3) g tree^{-1}, while phosphorus from TSP was applied as a basal dressing at the rate of 0 (control, P_0), 200 (P_1), 400 (P_2), and 600 (P_3) g tree^{-1}, in February, at a soil depth of 25-30 cm. Each treatment was replicated 3 times.

During the experimental period (3 years), irrigation was implemented as flooding, begun in the second half of July and continued until harvest time at intervals of 20 days. All trees included in this research were pruned based on the traditional pruning technique common in this region.

Harvest

Pistachios were harvested during the commercial harvest period in Gaziantep when the fruit reached a physiological maturity stage signalled by a reddish hull. All pistachio nuts were removed from the trees by hand so as to determine the gross yield of each tree. Nuts were harvested by shaking trees and collecting by hand.

Yield and nut characteristics

Yield per tree for each treatment was determined by weighing the red, fresh, and unshelled nuts. In these fruits, the splitting rate was determined with the naked eye by counting and rating split nuts to unsplit ones. Nut weight was measured by weighing 100 in-shell nuts. The last 2 parameters were observed in 100 nuts with 3 replications.

Flower bud abscission

At the beginning of the vegetation period, trees were marked, 12 shoots in each direction, totally 48 shoots. This parameter was determined by counting flower buds on these shoots at the beginning of June and also in November.

Analyses of natural growth regulators

In May, June, and September, for the first ('on' year) and second year ('off' year), leaf samples were taken from the tree from each treatment, frozen in liquid nitrogen and kept at –80 °C until extraction as described by Ergun (1997). Briefly, 1 g of homogenized leaf tissue was kept in 20 mL of extraction solvent including methanol, chloroform, 2 N ammonium hydroxide, and BHT (12:5:3, V:V:V,

Table 1. Soil characteristics of experimental orchard.

Depth (cm)	Sand (%)	Clay (%)	Silt (%)	pH (1:2.5 water)	Total salt (%)	$CaCO_3$ (%)	Organic matter (%)	Plant available P (kg ha^{-1})	Extractable K (kg ha^{-1})	Bulk density (g cm^{-3})
0–30	14.15	12.10	74.21	7.60	0.050	20.8	1.50	20	1155	1.2
30–60	14.10	13.50	72.35	7.55	0.055	22.5	0.95	9.0	959	1.2
60–90	14.20	15.24	69.70	7.25	0.090	25.7				1.4
90–120	14.50	15.50	70.03	7.40	0.075	27.8				1.4

0.001% BHT) within an amber colored bottle at –18 °C for 3 weeks. Each week, the solvent was filtered and collected in another bottle (combined extract), and 40 mL of solvent was added to the samples. Both of them were kept at –18 °C. At the end of 3 weeks, 25 mL of DDW was added to the combined extract in a separating funnel and the aqueous methanol on the top was put into a round-bottom flask. All organic solvents were evaporated under vacuum at 45 °C. The pH value of the aqueous extract was equal to 2.5 for the extraction of indole-3-acetic acid (IAA), gibberellic acid (GA_3), and abscisic acid (ABA). Growth regulators in this extract were transferred into ethyl acetate by washing with 15 mL of ethyl acetate 3 times. All ethyl acetate solvents were collected in a round-bottom flask. For zeatin extraction, after the pH value was put at 7.0, the same procedure was repeated. At the end 90 mL of ethyl acetate was evaporated under vacuum and the residue was solved in 1 mL of methanol and kept at –18 °C for thin layer chromatography. In this stage, all regulators were separated onto thin layers and were collected separately in 1 mL of absolute methanol. All of the extraction was done under dark conditions. A 20 µL sample was analyzed using high pressure liquid chromatography (HPLC) (Bio Rad Model 2800) equipped with C_{18} column (Phenomenex Luna, 5 m) and UV detector (Bio Rad UV-1806) following straining the samples through 0.45 mm pore size filters (Millipore, SLGV013NL). All reagents used in this procedure were HPLC-grade obtained from detector wavelength settings. Solvents and their flow rates are presented in Table 2. Obtained peaks were defined according to retention times using external standards and quantified by the ratings of external standard peak areas. External standards such as IAA (I-2886), GA_3, (G-7645), zeatin (Z-0164) and ABA (A-1049) were obtained from Aldrich Co Ltd.

Statistical analysis

This research was planned in a randomized block design. While some parameters such as yield, nut characteristics and flower bud abscission were determined for 3 years, hormone analyses were done for the 1st ('on') and the 2nd ('off') years. Multifactorial variance analysis (ANOVA) was performed on the data by Minitab Software (MINITAB Inc.) and data in each year were separately evaluated. Nitrogen and phosphorus rates were taken into consideration as variables for all parameters, apart from hormones. Besides these variables, sampling months were evaluated for hormone concentrations. Means were compared by Duncan's multiple range test ($P \leq 0.05$) using the MSTATC program. Arcsin transformations were used for all percentage data (Winer et al. 1991).

Results

Yield

For all 3 years, there was no significant effect of fertilizer applications on yield ($P \geq 0.05$) (Table 3). Almost all trees had lower yield values in the 2nd year than in the 1st and the 3rd due to their alternate bearing characteristic. Generally, the highest yield was obtained with the N_0P_3 treatment for all 3 years, 12.75 kg, 8.14 kg, and 16.40 kg, respectively. The lowest yield was observed in the controls in the 1st (2.10 kg) and 2nd (1.50 kg) year; however, it was also observed in the N_1P_1 treatment in the 3rd(1.99 kg)

Nut weight

The highest nut weight was obtained in the 2nd year ('off' year) and was lower in the 1st and 3rd years (heavy crop years or 'on' years) (Table 3). Measures for nut weight were, on the whole, parallel to fruit size. The nitrogen × phosphorus interactions were

Table 2. Solvents and conditions applied during HPLC analysis.

Hormones	Mobile phase	Wavelength (nm)	Flow rate (mL min^{-1})
IAA	CH_3CN:DDW (v/v, 1:1), %0.5 CH_3COOH	280	1.5
ABA	CH_3CN:DDW (v/v, 1:1), %0.5 CH_3COOH	265	1.5
GA_3	CH_3CN:DDW (v/v, 3:2)	208	3
Zeatin	0.02N CH_3COOH:MeOH (v/v, 60:40), pH= 4.18	254	2

Table 3. The effects of nitrogen and phosphorus applications on yield and nut size of pistachio nut trees.

Treatments		Yield (Fresh, red, unshelled kg tree^{-1})			Nut size (g 100 nut^{-1})		
Nitrogen	Phosphorus	1st year ('on')*	2nd year ('off')	3rd year ('on')	1st year ('on')	2nd year ('off')	3rd year ('on')
N_0	P_0	2.10***	1.50	3.91	82.93 A,ab**	90.43 A,a	79.77 A,a
	P_1	9.18	4.93	11.89	81.27 A,ab	88.60 A,a	80.50 A,a
	P_2	9.77	6.35	11.03	67.40 C,c	73.50 B,b	66.60 B,c
	P_3	12.75	8.14	16.40	74.47 B,b	84.50 A,a	77.33 A,a
N_1	P_0	6.28	4.42	7.58	79.07 BC,b	82.87 A,ab	77.90 B,a
	P_1	3.33	2.73	1.99	84.40 AB,a	87.67 A,a	83.57 AB,a
	P_2	7.37	5.27	8.75	86.47 A,a	88.93 A,a	88.57 A,a
	P_3	11.63	7.98	15.23	76.70 C,b	80.80 A,a	80.07 AB,a
N_2	P_0	3.60	2.77	5.40	87.57 A,a	77.43 A,b	79.50 A,a
	P_1	6.22	4.85	5.75	85.37 A,a	81.83 A,a	79.07 A,a
	P_2	9.85	7.47	12.60	77.83 B,b	81.57 A,ab	79.13 A,b
	P_3	5.58	4.02	6.08	75.73 B,b	80.53 A,a	76.70 A,a
N_3	P_0	10.13	6.78	13.92	84.53 A,ab	92.13 A,a	86.47 A,a
	P_1	7.58	5.83	12.15	75.80 B,b	78.67 B,a	74.07 B,a
	P_2	4.00	3.57	6.63	84.60 A,a	90.23 A,a	79.07 AB,ab
	P_3	11.45	7.32	15.68	82.63 A,a	89.63 A,a	80.60 AB,a

* 'on'; heavy cropping year, 'off'; subsequent light cropping year.

** Capital letters in each column show phosphorus doses in comparison to each nitrogen dose, lower case letters in each column show nitrogen doses in comparison to each phosphorus dose at $P \leq 0.05$ error level according to Duncan's multiple range test.

*** Non-significant at $P \leq 0.05$ error level.

significant ($P \leq 0.05$). For all nitrogen rates, increases in phosphorus rates did not greatly affect nut weight for all years with the exception of N_2P_2 and N_2P_3 treatments in the 1st year. In the control trees, those free from phosphorus fertilizers (P_0), nitrogen rates with the exception of N_1 for the 1st year and N_2 for the 2nd year resulted in significantly higher nut weight. In the 3rd year, the effect of nitrogen rates was not significant ($P \geq 0.05$). Similarly, for trees treated with P_1 and P_3 rates, the effect of nitrogen fertilizers was not significant ($P \geq 0.05$) for the 2nd and 3rd years. In the P_1 treatments, the higher nut weights were recorded in N_0 (81.27 g 100 nut^{-1}), N_1 (84.40 g 100 nut^{-1}), N_2 (85.37 g 100 nut^{-1}) and at dose of P_3 in N_3 (82.63 g 100 nut^{-1}) for the 1st year. For P_2 treatments, the lowest nut weight values were observed in control trees (N_0) in all years. Although the fertilizer combinations caused higher and lower nut weight changes depending on year, the lowest nut weights were determined in N_0P_2 treatments for all 3 years. However, the highest values were recorded in N_2P_0 for the 1st, N_3P_0 for the 2nd, and N_1P_2 treatments for the 3rd year.

Splitting rate

The effect of nitrogen and phosphorus treatments on the split nut rate was not significant for any of the years ($P \geq 0.05$) (Table 4). The highest rates determined in the same nitrogen-phosphorus combination (N_3P_2) were 53.33% in the 1st year, 61.00% in the 2nd year, and 49.67% in the 3rd year. The lowest rates were recorded for N_2P_2 treatment as 22.67% in the 1st, as 23.67% in the 2nd year, and for N_0P_3 treatment as 24.00% in the 3rd year. Generally, N_0 applications caused the lowest splitting rate.

Table 4. The effects of different nitrogen and phosphorus applications on splitting rates and flower bud abscission.

Treatments		Splitting rate (%)			Flower bud abscission (%)		
Nitrogen	Phosphorus	1st year ('on')*	2nd year ('off')	3rd year ('on')	1st year ('on')	2nd year ('off')	3rd year ('on')
N_0	P_0	28.00***	30.67	27.33	10.17	12.50	14.49
	P_1	29.33	31.67	28.00	0.00	43.40	24.60
	P_2	34.67	38.33	33.00	20.97	47.20	16.50
	P_3	25.67	26.00	24.00	26.20	12.50	20.11
	Average				14.33 B**	28.90	18.93
N_1	P_0	44.00	49.67	41.33	24.83	17.77	14.88
	P_1	36.00	39.33	34.00	36.33	44.70	24.51
	P_2	25.67	27.33	24.67	18.75	47.30	23.33
	P_3	31.00	34.00	29.67	39.67	56.30	34.52
	Average				29.90 A	41.52	24.31
N_2	P_0	45.70	54.30	42.70	29.83	41.70	25.06
	P_1	32.67	36.33	31.33	31.10	32.80	25.08
	P_2	22.67	23.67	25.67	25.80	35.20	23.52
	P_3	36.67	40.33	34.67	27.10	45.60	29.77
	Average				28.45 A	38.82	25.86
N_3	P_0	36.67	40.67	34.67	53.50	33.94	29.71
	P_1	36.67	40.67	34.67	27.80	21.30	22.72
	P_2	53.33	61.00	49.67	33.33	40.90	9.13
	P_3	36.70	40.70	36.33	24.80	55.60	24.02
	Average				34.86 A	37.95	26.40

* 'on'; heavy cropping year, 'off'; subsequent light cropping year.
** Capital letters show nitrogen doses in comparison at $P \leq 0.05$ error level according to Duncan's multiple range test.
*** Non-significant at $P \leq 0.05$ error level.

Flower bud abscission

The N_1P_3 application caused higher flower bud abscission for all 3 years (Table 4). Significant differences were found only for nitrogen doses and only for the 1st year ($P \leq 0.05$). In addition, statistically lowest flower bud abscission rates were measured in controls (N_0). However, the effect of the phosphorus treatment was not significant for this year. When flower bud abscission rates were compared with yield values, it was observed that flower bud abscission rates occurred at lower levels in the 1st year and the 3rd year, being 'on' years.

Hormone concentrations

For the first 2 years of this study, significant interactions among nitrogen doses × phosphorus doses × sampling months were considerable for IAA, GA_3 and the zeatin concentration in the pistachio nut leaf ($P \leq 0.05$) (Tables 5–7). The highest and the lowest IAA, GA_3, and zeatin values determined in different phosphorus doses for the same month and the same nitrogen rate showed a coincidental distribution. A similar situation was determined for different nitrogen rates for the same month and the same phosphorus dose. Additionally, changes in the IAA and GA_3 content of leaves based on the month for the same nitrogen and phosphorus dose were not regular. In both years, treatments causing the highest and the lowest IAA, GA_3, and zeatin levels changed depending on the sampling months. Furthermore, similarities among combinations having the highest and the lowest growth regulator levels were not clear.

Table 5. The effects of different nitrogen and phosphorus applications on IAA content (μg g^{-1} fresh weight) of pistachio nut leaf.

Treatments		1st year ('on')*			2nd year ('off')		
Nitrogen	Phosphorus	May	July	September	May	July	September
N_0	P_0	248.85 A,a,*a***	79.08 B,b,*b*	200.31 B,a,*ab*	122.48 A,ab,*a*	137.04 B,b,*a*	78.08 A,b,*a*
	P_1	164.04 A,a,*b*	50.90 B,a,*b*	379.99 A,a,*a*	89.77 A,a,*a*	121.77 B,b,*a*	157.16 A,a,*a*
	P_2	209.05 A,a,*ab*	310.92 A,a,*a*	154.41 B,ab,*b*	123.76 A,b,*b*	428.38 A,a,*a*	28.24 A,b,*b*
	P_3	118.97 A,a,*a*	38.26 B,a,*a*	79.33 B,b,*a*	117.31 A,a,*a*	333.37 AB,ab,*a*	166.97 A,a,*a*
N_1	P_0	229.81 A,a,*a*	182.00 A,b,*a*	278.32 A,a,*a*	185.34 A,ab,*a*	377.68 A,a,*a*	327.54 A,a,*a*
	P_1	244.48 A,a,*a*	164.96 A,a,*a*	218.55 A,b,*a*	319.79 A,a,*a*	438.57 A,a,*a*	50.16 B,a,*b*
	P_2	67.11 B,a,*b*	168.79 A,ab,*ab*	256.97 A,a,*a*	300.14 A,ab,*a*	342.00 A,a,*a*	276.48 AB,a,*a*
	P_3	127.53 AB,a,*b*	164.25 A,a,*b*	300.29 A,a,*a*	196.42 A,a,*a*	361.55 A,ab,*a*	196.74 AB,a,*a*
N_2	P_0	59.29 A,b,*a*	67.91 A,b,*a*	205.22 A,a,*a*	311.44 A,a,*a*	372.71 A,a,*a*	185.75 A,ab,*a*
	P_1	110.74 A,a,*a*	164.84 A,a,*a*	169.84 A,b,*a*	299.81 A,a,*a*	315.88 A,ab,*a*	209.96 A,a,*a*
	P_2	114.19 A,a,*a*	217.19 A,ab,*a*	211.13 A,a,*a*	293.09 A,ab,*a*	319.03 A,a,*a*	222.79 A,ab,*a*
	P_3	112.08 A,a,*a*	121.54 A,a,*a*	101.58 A,ab,*a*	206.38 A,a,*b*	482.39 A,a,*a*	155.66 A,a,*b*
N_3	P_0	108.55 A,ab,*b*	334.58 A,a,*a*	132.21 AB,a,*b*	61.23 B,b,*a*	239.08 AB,ab,*a*	80.13 A,b,*a*
	P_1	125.23 A,a,*a*	153.56 B,a,*a*	222.16 A,b,*a*	131.85 B,a,*b*	439.56 A,a,*a*	44.36 A,a,*b*
	P_2	101.42 A,a,*a*	95.15 B,b,*a*	41.55 B,b,*a*	459.94 A,a,*a*	222.55 AB,a,*b*	77.65 A,ab,*b*
	P_3	92.49 A,a,*a*	187.51 B,a,*a*	193.38 AB,ab,*a*	203.22 B,a,*a*	187.92 B,b,*a*	44.86 A,a,*a*

* 'on'; heavy cropping year, 'off'; subsequent light cropping year.

** Capital letters in each column show phosphorus doses in comparison to each nitrogen dose, lower case letters in each column show nitrogen doses in comparison to each phosphorus dose, italic letters in each column represent sampling months in comparison to each nitrogen and phosphorus dose at $P \leq 0.05$ error level according to Duncan's multiple range test.

For ABA content, there were significant interactions between nitrogen doses × sampling months ($P \leq 0.05$) (Table 8). The ABA content decreased with increasing nitrogen doses in May and July in the 1st year, apart from IAA, GA_3, and zeatin. For the samples in September, the opposite was observed. In the 2nd year, similar changes were observed for ABA levels, but the N_2 treatment for May and July, and the N_3 treatment for September caused an increase in ABA levels (Table 8).

Discussion

In this study, there was no significant effect of fertilization treatments on the yield. The lower yield values in the 2nd year may be a result of alternate bearing in pistachio nut trees, because the 1st and the 3rd years of this study were 'on' years (Table 3). Additionally, the highest yield caused by the N_0P_3 treatment for all years could be an indicator of a stimulatory effect of a phosphorus treatment on the yield. However, a non-significant effect of fertilization has been mentioned in some other studies. Aydeniz et al. (1982) reported that it was not possible to follow of the effect of fertilization on yield and some nut characteristics in short periods such as 1 or 2 years in pistachios because of the deep root characteristic of mature trees. Similarly, some researchers mentioned that the effect of a foliar iron chelate application could be observed in the same year, but it would take a long time for soil applications in pistachios being 30 years old (Hokmabadi et al. 2007).

Weinbaum et al. (1995) determined that nitrogen fertilization increased the yield of pistachio in 'on' years. In this study, our findings were not similar.

Table 6. The effects of different nitrogen and phosphorus applications on GA_3 content (µg g^{-1} fresh weight) of pistachio nut leaf.

Treatments		1st year ('on')*			2nd year ('off')		
Nitrogen	Phosphorus	May	July	September	May	July	September
	P_0	462.92 A,b,*b***	297.49 A,a,*c*	815.56 A,a,*a*	345.68 A,b,*b*	301.24 A,a,*b*	1289.91 B,a,*a*
N_0	P_1	615.93 A,b,*a*	295.25 A,ab,*b*	379.97 B,a,*b*	410.10 A,b,*b*	298.13 A,a,*b*	2404.72 A,a,*a*
	P_2	725.88 A,a,*a*	341.21 A,ab,*b*	278.01 B,a,*b*	357.92 A,b,*ab*	318.06 A,a,*b*	681.32 C,a,*a*
	P_3	684.83 A,a,*a*	310.88 A,a,*b*	291.88 B,a,*b*	379.96 A,c,*b*	331.86 A,a,*b*	755.39 C,a,*a*
	P_0	657.59 AB,a,*a*	196.05 A,a,*b*	326.44 A,b,*b*	365.06 A,b,*a*	290.61 A,a,*a*	184.96 A,b,*a*
N_1	P_1	814.64 A,a,*a*	257.25 A,ab,*b*	384.68 A,a,*b*	450.09 A,b,*a*	311.68 A,a,*a*	434.61 A,b,*a*
	P_2	643.23 B,a,*a*	194.41 A,b,*b*	351.69 A,a,*b*	425.30 A,b,*a*	278.08 A,a,*a*	498.49 A,a,*a*
	P_3	517.60 B,b,*a*	236.71 A,a,*b*	282.13 A,a,*b*	430.32 A,c,*a*	296.60 A,a,*a*	388.94 A,b,*a*
	P_0	410.93 A,b,*a*	230.19 B,a,*b*	276.56 A,b,*ab*	483.83 A,b,*a*	276.97 A,a,*a*	331.63 A,b,*a*
N_2	P_1	506.18 A,b,*a*	412.44 A,a,*ab*	283.29 A,a,*b*	460.03 A,b,*a*	438.59 A,a,*a*	298.71 A,b,*a*
	P_2	571.33 A,a,*a*	456.40 A,a,*ab*	341.56 A,a,*b*	665.78 A,b,*a*	220.10 A,a,*b*	102.42 A,b,*b*
	P_3	487.79 A,b,*a*	239.13 B,a,*b*	356.19 A,a,*ab*	973.47 A,b,*a*	251.56 A,a,*b*	252.79 A,b,*b*
	P_0	344.89 A,b,*a*	236.95 A,a,*a*	343.09 A,b,*a*	2364.22 A,a,*a*	370.91 A,a,*b*	400.97 A,b,*b*
N_3	P_1	511.42 A,b,*a*	198.56 A,b,*b*	295.65 A,a,*b*	2492.37 A,a,*a*	234.65 A,a,*b*	242.81 A,b,*b*
	P_2	383.04 A,b,*a*	210.53 A,b,*b*	320.91 A,a,*ab*	1057.51 C,a,*a*	226.78 A,a,*b*	418.33 A,ab,*b*
	P_3	365.56 A,b,*ab*	236.89 A,a,*b*	407.56 A,a,*a*	1556.53 B,a,*a*	515.19 A,a,*b*	480.07 A,ab,*b*

* 'on'; heavy cropping year, 'off'; subsequent light cropping year.

** Capital letters in each column show phosphorus doses in comparison to each nitrogen dose, lower case letters in each column show nitrogen doses in comparison to each phosphorus dose, italic letters in each column represent sampling months in comparison to each nitrogen and phosphorus dose at $P \leq 0.05$ error level according to Duncan's multiple range test.

Nut weight was largely parallel to fruit size. The highest nut weight was observed in the 2nd year ('off' year) (Table 3) and this result is parallel with Westwood (1993), who reported that, generally, in most fruit trees, fruit size tends to decrease in heavy crop years and sometimes fruit thinning is required not only to decrease crop load but also to increase fruit size. In this study, fertilizer combinations causing higher and lower nut weight changed depending on the year. The highest values were recorded in N_2P_0 for the 1st, N_3P_0 for the 2nd, and N_1P_2 for the 3rd year. Our results regarding this parameter are not parallel with the findings of other researchers, who have reported that nitrogen increased nut weight in pistachio (Kanber et al. 1993). This may show that fertilization does not affect nut size alone, and that some other factors such as nut load, irrigation, and pruning mostly affect nut size in pistachio (Crane 1978; Karaca 1990; Kuru 1993). The effect of nitrogen and phosphorus treatments on the split nut rate was not significant for any of the years ($P \geq 0.05$) (Table 4). Generally, the N_0 treatment caused the lowest rates. This is in contrast with the result of Pontikis (1977), who reported that a higher nitrogen dose caused an increase in shoot growth but a decrease in nut growth and delay in splitting in pistachio. The main reason for this difference may be cultivar behavior. Crane and Takeda (1979) and Crane et al. (1982) reported that the splitting of pistachio nuts is a genetic property and there have been some other factors, such as rootstock, cultivar, nutrition level, alternate bearing, ecology, cultural management and also pollen source, that affect the splitting rate. Zheng et al. (2001) determined that the application of K obviously improved nut

Table 7. The effects of different nitrogen and phosphorus applications on zeatin content (mg g^{-1} fresh weight) of pistachio nut leaf.

Treatments		1st year ('on')*			2nd year ('off')		
Nitrogen	Phosphorus	May	July	September	May	July	September
	P_0	332.92 B,a,*a***	15.02 A,a,*c*	21.40 B,c,*b*	34.46 A,a,*b*	39.30 A,b,*a*	3.69 A,a,*c*
N_0	P_1	385.89 A,a,*a*	0.00 B,d,*c*	15.32 D,b,*b*	24.06 B,a,*b*	27.81 B,b,*a*	2.86 AB,b,*c*
	P_2	199.93 D,c,*a*	15.92 A,a,*b*	17.55 C,c,*b*	34.31 A,a,*a*	22.26 C,b,*b*	1.63 BC,ab,*c*
	P_3	205.25 C,c,*a*	0.00 B,c,*c*	31.49 A,c,*b*	33.19 A,a,*a*	16.77 D,b,*b*	0.99 C,a,*c*
	P_0	128.23 D,c,*a*	4.53 A,b,*c*	24.85 C,b,*b*	31.63 A,b,*a*	10.84 A,c,*b*	0.77 A,b,*c*
N_1	P_1	164.57 C,c,*a*	2.58 A,c,*c*	10.66 D,c,*b*	19.66 C,b,*a*	11.32 A,c,*b*	1.56 A,c,*c*
	P_2	235.34 B,a,*a*	3.68 A,c,*c*	32.57 B,b,*b*	22.79 B,b,*a*	11.00 A,c,*b*	1.38 A,ab,*c*
	P_3	1360.94 A,a,*a*	3.78 A,b,*c*	99.02 A,a,*b*	18.12 D,c,*a*	12.04 A,c,*b*	0.88 A,a,*c*
	P_0	170.28 C,b,*a*	15.07 A,a,*c*	75.37 A,a,*b*	9.80 D,d,*a*	10.53 A,c,*a*	3.11 B,a,*b*
N_2	P_1	351.74 A,b,*a*	10.87 B,a,*c*	69.97 B,a,*b*	11.38 C,c,*a*	9.84 A,d,*b*	8.32 A,a,*c*
	P_2	212.56 B,b,*a*	7.37 C,b,*c*	55.93 C,a,*b*	17.83 B,c,*a*	7.87 B,d,*b*	0.53 C,b,*c*
	P_3	138.01 D,d,*a*	3.75 D,b,*c*	44.63 D,b,*b*	33.12 A,a,*a*	9.69 A,d,*b*	0.97 C,a,*c*
	P_0	73.02 B,d,*a*	4.85 C,b,*c*	12.86 A,b,*b*	19.88 B,c,*b*	74.72 C,a,*a*	1.53 AB,b,*c*
N_3	P_1	70.82 C,d,*a*	5.47 C,b,*c*	14.04 A,b,*b*	11.22 D,c,*b*	70.16 D,a,*a*	0.56 B,c,*c*
	P_2	74.05 B,d,*a*	7.73 B,b,*b*	9.43 B,d,*b*	17.28 C,c,*b*	144.37 A,a,*a*	2.17 A,a,*c*
	P_3	299.93 A,b,*a*	10.09 A,a,*b*	3.82 C,d,*c*	23.86 A,b,*b*	106.76 B,a,*a*	1.16 AB,a,*c*

* 'on'; heavy cropping year, 'off'; subsequent light cropping year.

** Capital letters in each column show phosphorus doses in comparison to each nitrogen dose, lower case letters in each column show nitrogen doses in comparison to each phosphorus dose, italic letters in each column represent sampling months in comparison to each nitrogen and phosphorus dose at $P \leq 0.05$ error level according to Duncan's multiple range test.

quality in pistachio with an increased percentage of split nuts and 100-nut weight and a reduced percentage of blank and stained nuts.

In this study, apart from phosphorus treatments, nitrogen rates significantly affected flower bud abscission, but only in the 1st year ($P \leq 0.05$). The 2nd year of this research was an 'off' year; the abscission in buds being responsible for the yield of this year was more severe. It has been mentioned that flower bud abscission has been a basic reason for the alternate bearing of pistachio (Nzima et al. 1997; Stevenson et al. 2000). In some studies, insufficient irrigation and cultural management and also insufficient carbohydrate accumulation in plant tissues have been reported as the reasons for alternate bearing in pistachio (Kanber et al. 1993; Nzima et al. 1997). Because the plant cannot offer the nutrient support for the flower buds, due to a deficiency in its carbohydrate accumulation, flower bud abscission occurs in the next year following the 'on' year. As a result, it has not been possible to obtain a sufficient yield for the next year and, therefore, pistachio nut trees have shown this alternate bearing characteristic. In pistachio, as in other fruit crops, the principal member of carbohydrate accumulation is the leaf. Vemmos (1994) tested for differences in leaf photosynthesis, stomatal conductance, chlorophyll concentration, and specific leaf weight associated with alternate bearing, but no clear differences were found.

In the current study, significant interactions were determined for the IAA, GA_3, and zeatin content of the pistachio nut leaf ($P \leq 0.05$) (Tables 5–7). However, none of the treatments caused a regular increase or decrease in internal growth regulators.

Table 8. The effects of different nitrogen and phosphorus applications on ABA content (mg g^{-1} fresh weight) of pistachio nut leaf.

Treatments		1st year ('on')*			2nd year ('off')		
Nitrogen	Phosphorus	May	July	September	May	July	September
	P_0	121.68	55.04	27.71	6.50	126.09	6.56
N_0	P_1	60.46	48.31	15.76	0.86	30.09	3.59
	P_2	45.94	62.17	8.76	0.60	19.25	2.20
	P_3	148.15	50.70	5.17	1.98	45.90	1.49
Average		94,06 A,a**	54,06 A,b	14,35 A,c	2,48 A,b	55,33 A,a	3,46 A, b
	P_0	42.59	37.67	4.17	0.88	9.55	4.81
N_1	P_1	7.66	10.75	41.18	10.58	10.55	1.43
	P_2	8.36	20.68	66.41	9.47	6.69	3.83
	P_3	8.15	19.07	40.82	10.80	6.76	2.24
Average		16,69 B,a	22,04 B,a	38,14 A,a	7,93 A,a	8,39 C,a	3,08 A,a
	P_0	5.91	11.36	21.68	4.60	8.69	1.43
N_2	P_1	78.14	25.17	11.29	21.41	48.23	1.41
	P_2	15.40	11.85	20.36	6.96	4.95	1.93
	P_3	15.79	13.55	16.35	4.49	3.58	1.28
Average		28,81 B,a	15,49 B,a	17,42 A,a	9,37 A,a	16,36 BC,a	1,51 A,a
	P_0	9.64	16.37	16.95	3.19	5.88	3.81
N_3	P_1	12.81	25.02	14.09	3.69	55.66	9.86
	P_2	3.72	11.53	14.05	2.68	9.36	2.67
	P_3	32.93	11.39	26.47	2.55	63.21	1.27
Average		14,78 B,a	16,08 B,a	17,89 A,a	3,03 A,b	33,53 AB,a	4,40 A,b

* 'on'; heavy cropping year, 'off'; subsequent light cropping year.

** Capital letters in each column show phosphorus doses in comparison to each nitrogen dose, lower case letters in each column show nitrogen doses in comparison to each phosphorus dose, italic letters in each column represent sampling months in comparison to each nitrogen and phosphorus dose at $P \leq 0.05$ error level according to Duncan's multiple range test.

Furthermore, changes in the IAA and GA_3 content of the leaf, depending on the month, at the same nitrogen and phosphorus dose were not regular. Salisbury and Ross (1991) and Westwood (1993) reported that IAA levels being positively related to meristematic activities were higher at the beginning of the vegetation period. Our data do not support these studies because we observed, in some combinations, significantly higher IAA levels in the May samples. Similar changes were also determined for the ABA levels (Table 8). Wolpert and Ferguson (1990) proposed that hormone concentrations were related to the bud abscission process. In this study, discriminative differences were not observed in hormone concentrations based on 'on' and 'off' years. Similarly, many physiological studies seeking to understand the bud abscission process in pistachio have investigated hormones causing bud abscission such as auxin (Crane and Nelson 1972), gibberellins (Lin et al. 1984), ABA (Takeda and Crane 1980) but found no definitive results.

In conclusion, there was no significant effect of fertilizers on yield, split nut ratio, or flower bud abscission. Since pistachio is a very long-life species and has a very deep root system, future research over longer periods about the physiology of the bud abscission, hormonal regulations, and optimum fertilizer rates is needed to find a clearer physiological mechanism.

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