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Physiological influence of N in preventing of alternate-bearing of pistachio (*Pistacia vera* cv. Kalleh-ghuchi)

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Abstract

The physiological influence of N in preventing of alternate-bearing of pistachio (*Pistacia vera* cv. Kalleh-ghuchi) was studied in the Ghazel Ozen (south-west of Zanjan), Iran, during four years (2003-2006). Nitrogen as urea ($CO(NH_2)_2$, 46% N) at 100, 150 and 200 g actual N per tree were soil surface applied for both fruiting and non-fruiting of the 25 year-old trees. Floral bud abscission was influenced by the level of N application. It increased, through both in the low N and high N application, as compared to medium (150 g actual N $tree^{-1}$) levels. There was a strong relationship between leaf protein and N application, similarly as leaf protein increased, floral bud abscission decreased. Fresh weight of fruit per tree, as annual yield, was significantly ($P = 0.05$) influenced by growth season, regardless of N application. “Off” year was the main factor that decreased nut yield (up to 30%) in control, but fruit quality (nut size, blanked %, protein and fat %) was less affected. The rate of fruit growth (dry weight) varied during fruit growth season for individual treatments. The rate of growth was associated with floral bud abscission, however, as the N application increased, percent of bud abscission decreased. Minerals and protein concentrations in leaves from fruiting branches were in most cases lower than that of non-fruiting ones. The floral bud abscission per cluster was very low during nut fill in “off” year trees compared with “on” year trees, there was a relationship between bud drops and consumption of N (protein concentration of kernel). This indicates that reproductive growth (nut growth) demand more N, especially in the “on” year.

Key words: Alternate-bearing, mineral concentration, nut quality, pistachio fruit characters.

Introduction

The yield and fruit characteristics of pistachio (*Pistacia vera* cv. Kalleh-ghuchi) orchards in the Ghazel Ozen (south-west of Zanjan), Iran, are not consistence in every growth season. The highest yields per tree were recorded in some years, while the lowest yields were obtained in some growth seasons with favorable conditions and longer growing period. Many reports indicate fluctuation of pistachio yields [1-4]. These fluctuations were partly due to inherited alternate-bearing [5, 6]. Pistachio produces a large amount of floral buds every year, but in the “on” year, during active embryo growth, most of them abscise [1, 7-9]. The competition for nutrients between the ovary and the floral buds is responsible for the abscission of them [3, 10, 11], especially during seed development [7, 12]. As the number of nuts per inflorescence increases, the nutrient competition increases [8, 13]. The effect of crop load reduction treatments, performed at different dates, clearly demonstrated that floral bud retention is positively correlated with amount of reproductive organs removed, and with the earliness of the removal [8]. Furthermore, Crane and Al-Shalan reported a rapid decline in concentration of nutrition in pistachio branches during flowering, shoot growth and nut development and lower contents of nutrient in fruiting pistachio trees compared with non-bearing trees [14]. Polyamines could have an important physiological function in the development of flower bud abscission [15].

In alternate-bearing trees, the recovery of sufficient nutrient reserve status for the next year is prevented by the utilization of available source for fruit production. Accumulation of minerals in the kernel during the nut fill period was also influenced by the presence of fruits (yield). In “on” year trees this situation is critical, while, in “off” year trees, which had neither fruits nor such an intensive vegetative growth, did not have depletion in nutrient reserve. On the other hand, other experiments have indicated that unbalanced nutrition was not the primary cause of bud abscission, and attention should be given to the involvement of plant growth regulators [6, 7, 10, 16, 17].

Fertilizer management in pistachio orchards have largely ignored the possibility that mineral demands and tree capacity for nutrients uptake from the soil vary with crop load over the alternate-bearing cycle [18, 19]. Nitrogen plays an important role in plant growth, and usually is needed in greater amounts than other minerals. Consequently, much attention has been paid to the physiological function of nitrogen in pistachio. On the other hand, nitrogen excess does not show characteristic symptoms in the tree, but it is accumulated in fruits [11, 18, 19]. Nitrogen (as a substance) is involving in growth regulator biosynthesis, which may be related to floral bud abscission [6, 7, 10, 15, 16].

Although nitrogen metabolism (polyamines) could be a possible messenger of bud abscission, but not the cause [20], the magnitude and patterns of tree nitrogen utilization in “off” and “on” years are not similar [21, 22]. Lack of nitrogen (N) reserves has also been proposed to be limiting after large crops, thus reducing return bloom. It can be assumed that nitrogen status and plant growth are concurrent processes, with increased reproductive growth. This hypothesis, however, has not been adequately tested. An understanding of the effects of alternate-bearing on nitrogen utilization and more reproductive growth is a prerequisite to developing best management practices for pistachio fertilization.

Materials and Methods

This research was conducted in a pistachio orchard in the Ghazel Ozen (south-west of Zanjan), Iran. A randomized complete block design was used to investigate the influence of N on alternate-bearing of pistachios (*Pistacia vera* cv. Kalleh-ghuchi) during four growth seasons (2003-2006). The specific climatic characteristics of the orchard site, where the study was carried out is as follows: the latitude north is 33°; the height above the sea level (altitude) is 200-350 m; the average temperature of the zone is 16.2°C; the average maximum temperature is 33.4°C (July); the average minimum temperature is 2.5°C (January); the average annual rainfall is 360 mm and the annual total chilling hours (≤7.2°C) ≈ 450). The cumulative effective temperatures (17–28°C) were satisfied for different stage of fruit growth and development. The sufficient daily mean temperature and total temperature (degree-day) were satisfied during nut maturation. The physical and chemical characteristics of the soil were clay 18%, silt 20% and sand 62%, limestone <4.5%, pH = 7.9, electric conductivity (EC_e) 5.5 dS m^{-1}, and organic matter less than 1%.

Treatments received N as urea ($CO(NH_2)_2$, 46% N) at 0 (as control), 100, 150 and 200 g actual N $tree^{-1}$. All fertilizers were surface applied at two times, split soil application at bud break, and 30-days after fruit set. Besides, all trees were fertigated with other essential minerals based on the leaf mineral nutrient analyses. Management factors such as irrigation, pruning, and weed control were done the same as local standards. Trees were trained with an open-center system and distance of trees was 4 m x 6 m.

Ten uniform trees were selected in each fruiting state “on” and “off” years. Five uniform shoots were selected from different sides of the tree in each fruiting state. Eight clusters were collected from each shoot. Bud abscission (calculated as a percentage of flower buds abscised from the shoot, out of the initial ones) was recorded in “off” years for control, and “on” years for all treatments every 15 days during five months of fruit growth and development. On each date of observation, buds were touched lightly with a fingertip to distinguish those that had formed an abscission layer but remained lodged between the leaf petiole and the shoot according to Wolpert and Ferguson [8]. Simultaneously, the rate of fruit growth (calculated as increasing dry weight per g 100 nuts) was recorded every 15 days during five months of nut growth. The fruit was considered mature when several nuts in the cluster were light color and the hull was easily separated from shell. Characteristics of yield per tree, kernel percentage (by weighing the amount of kernel present in 100 g of whole fruit), splitting percentage and the weight of 1000 nuts, and some other characteristics related to kernel quality (weight, size, protein and fat percentage) were determined.

Samples of 10 leaflets from the mid-section of current year shoots and 400 mature fruits were collected. Fruits and leaflets were washed with mild detergent, rinsed with distilled water (the pericarp of fruits and kernels were removed) and dried in a forced air drying oven at 70°C to constant weight. They were ground to pass a 40 mesh screen. One g of dried ground leaf sample was dry ashed at 550°C for 5 h. The ash was then dissolved in 5 ml of 20% HCl. These samples were analyzed for P, K, Mg Ca, Fe, Zn and Cu by atomic absorption spectrophotometer [23]. For determination of protein content in kernel and leaf, nitrogen was determined in 0.5 g dried plant tissues using the micro-Kjeldahl technique as described by Tandon [23]. The protein was calculated by using the factor of N×6.25. Samples of dried kernel, used for crude fat determination, were ground to a fine powder and the crude fat extracted with ether in a Soxhlet-type extractor. The percentage of ether-extractable fat was determined according to Horwitz [24]. Data were evaluated by analysis of variance with SPSS. When the F-test was significant, means were separated by Duncan’s Multiple Range Test ($P \leq 0.05$).

Results

The alternate-bearing of pistachio (*Pistacia vera* cv. Kalleh-ghuchi) trees was significant influenced by N utilization. Fresh weight of fruit per tree, as annual yield, in “off” year was significantly (P = 0.05) decreased up to 0.28% (in control). However, application of N (100, 150 and 200 g N $tree^{-1}$) did not prevent from alternate-bearing, the yield declining was significantly (P = 0.05) less (23, 18 and 13%) in N used treatments, respectively (Fig. 1). However, the pattern of fruit growth (as dry weight g 100 nut^{-1}) for all treatments was double sigmoid, the lowest rate of fruit growth and the highest rate were occurred in control (0 g N $tree^{-1}$), and 200 g N $tree^{-1}$, respectively (Fig. 2). Protein concentration in leaves in “on” year trees was lower than that in “off” years trees (non-fruiting ones) (Table 1). Leaf protein concentration declined during the period that coincides with that of kernel development in “on” year trees.

It was revealed that floral bud abscission during the growth and development of the fruit (kernel) on “off” year was significantly less than “on” year. As Fig. 3 shows “off” year trees made very low percentage of bud abscission during the development of the kernel on the nearby (on next primary branch) fruiting branches. On the other hand, the pattern of bud abscission rate was similar to the rate of fruit growth (dry weight). Interestingly, the highest rate of bud abscission was coincided with the highest rate of kernel development (Figs. 2 and 3).

Mineral contents in fruit and leaf of cropping “on” trees were different from non-cropping “off” trees (Table 2). The concentrations of minerals (P, K, Ca, Mg, Fe, Zn and Cu) in leaves in “on” year trees are less than in leaves in “off” year trees (Table 2). It can be said that tree nutrient contents and, presumably, the size of nutrient storage pools in dormant trees varied between “on” and “off” years. Mineral contents of pistachio kernels changed year by year. Concentrations of some minerals (P, K, Mg, Ca, Fe, Zn, and Cu) of kernel were higher in the “on” years (Table 3). As

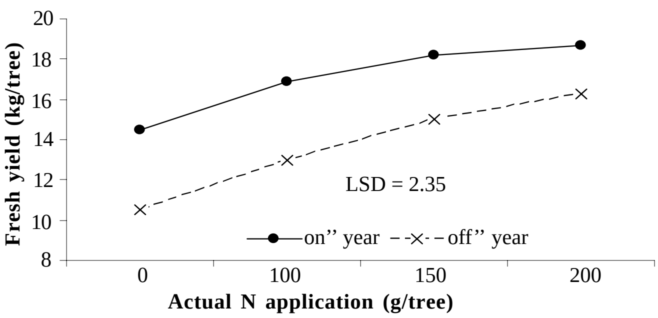

Figure 1. Changes of yield (fresh fruit kg/tree) of pistachio (*P. vera* cv. Kalleh-ghuchi) grown in Ghazel-Ozen, Zanjan, Iran. The mean of each 2 years “on” and 2 years “off”, and LSD was calculated at P = 0.05.

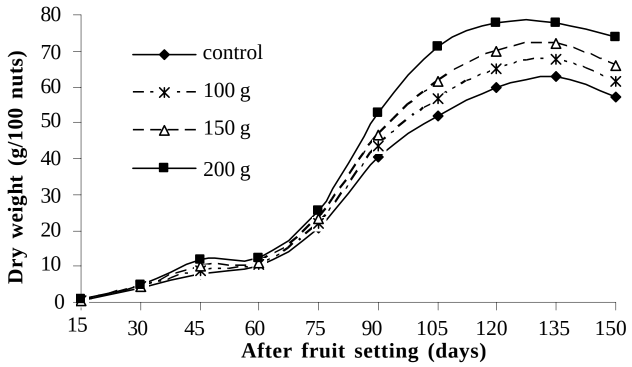

Figure 2. The rate of fruit growth (as dry weight g 100 nut^{-1}) of pistachio (*P. vera* cv. Kalleh-ghuchi) were fertilized at different levels (0, 100, 150 and 200 g actual N per tree). The mean of each 2 years "on" and 2 years "off", and LSD was calculated at P = 0.05.

Table 1. Changes of the leaf protein concentration (%) during the development of the kernel of pistachio grown in Ghazel-Ozen, Zanjan.*

Treatments	N-applied (g tree^{-1})	Leaf protein (%)
"on" year	control	6.80
	100	7.05
	150	7.25
	200	7.80
"off" year	control	7.15
	100	7.85
	150	8.25
	200	9.50
LSD		0.84

*the mean of each 2 years "on" and 2 years "off". **Mean separation in each column by LSD at 5% level.

N (0, 100, 150 and 200 g N tree^{-1}) application increased, protein (in leaf and fruit) and fat (in fruit) increased (Tables 1and 4). The greater N accumulation in "off" year trees is depleted in support of the large fruit demand for N during "on" years. In contrast to N, there was less some minerals (P, Ca and Zn) accumulation in leaves during "on" years than during "off" years (Table 2). Protein and crude fat also differed over time (Table 4). The composition of pistachio kernels were within the following ranges, fat 49.8 - 54.8%; protein 18.2 - 22.3%; nut weight 570 – 740 g/1000 nuts; blank nut 27–15% (Table 4).

Discussion

The phenomenon of pistachio alternate-bearing cv. Kalleh-ghochi is due to mainly the flower bud abscission during the summer (of the previous "on" year) during the period of fruit growth and kernel development (Figs 2 and 3). It can be said that the bud abscission is due to removal minerals and proteins from leaves to fruits during the period that coincides with that of kernel development, which occurred from mid-July till late September in fruiting branches [15]. On the other hand, bud abscission in the pistachio has been attributed to the genetic characteristics of the varieties, rather than decline of leaf nitrogen metabolism (leaf polyamines and proteins) [5, 6]. Assimilate status of nitrogen, conditioned by the leaf to bud ratio and by the time of floral differentiation, had crucial influence on bud abscission [15]. Nitrogen metabolism (proteins) can also influence on the "quality" of flowers produced and their ability to set and retain fruits [25].

Nitrogen metabolism (polyamines) has been shown to stimulate growth of several higher plant species, suggesting that the endogenous concentrations of amines can be growth limiting. Nitrogen application has been found to play a significant role in bud development and floral initiation [26-28]. Leaf nitrogen content plays an important role in the recycling of C and N during bud induction, and the activities of several enzymes regulating nucleic acid synthesis are affected by amines [25, 29]. Polyamines have been reported to have an important function in reproductive organ development [30], and it could be suited to serve either as nitrogenous sources or as signal molecules regulating the fruitlet abscission processes in grapevine [31]. Therefore, N application could play a crucial role in preventing the flower bud abscission of pistachio. It can be inferred that nitrogen metabolism (polyamines) may be acted as anti-senescence agents and counteract the activity of abscisic acid (ABA) and ethylene[27, 32]. The reduction of polyamines in leaves led to senescence with subsequent enhancement of the biosynthesis of either ABA and/or ethylene and bud abscission. Barnett and Mielke suggested that nutrition may be involved in regulation of pistachio alternate-bearing [33]. The involvement of N has been supported by several studies[34-36]. Scientists suggested that a critical N shortage develops in the fall during years with large crops, ''on-years,'' that contributes to alternate-bearing.

Furthermore, some reports indicated that leaf protein content decreased below a specific level in "on" year, it could trigger the abscission mechanism and could trigger senescence, thus presenting a hormone-like action [15, 32]. If initial large pools of polyamines can affect similar to those of nitrogen fertilizers applications on various plant [27], decreasing of N concentration below a crucial value (during a specific bud developmental stage) induces such alterations, with an immediate effect on bud abscission (Fig. 3).

Since bud growth ceases early and any outgrowth does not occur in the current year in "off", it is possible that reduced leaves protein contents can be associated with reduction in biosynthesis rate and by conjugation with nitrogen compounds, such as hydroxy cinnamic acids and amines [27, 37]. N could be limiting during on-years because developing fruit may deplete leaf polyamines that simultaneously accumulates in shuck and kernel tissue during fruit ripening[38]. Nitrogen is obviously essential to maintain growth and orchard productivity; so adequate N may ensure subsequent flowering and reduced alternate bearing. Nitrogen deficiency is often identified in pistachio trees with leaf N level below 0.8%, however, leaf N status varies greatly with crop load, growth season, other chemical fertilization and nutrient interactions [11, 19, 21, 39]. Nitrogen consumption is more than N uptake during the "on" year, indicating that some of the N demand for optimum growth is supplied by redistribution from storage. Therefore, more (150 to 200 g actual N tree^{-1}) must be used during the "on" year to ensure cropping in the following year, however, the pronounced effect of alternate-bearing on tree N demand and capacity for uptake has important implications for fertilizer program.

Table 2. Changes of leaf mineral concentrations of pistachio (*P. vera* cv. Kalleh-ghuchi) grown in Ghazel-Ozen, Zanjan.*

Treatments	N-applied (g tree^{-1})	P (%)	K (%)	Mg (%)	Ca (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)
"on" year	control	0.136	1.74	0.64	4.47	110.5	9.49	8.9
"	100	0.138	1.28	0.83	3.55	89.75	8.95	4.7
"	150	0.153	1.72	1.09	4.03	128.5	8.32	9.9
"	200	0.146	1.51	0.90	3.39	89.16	10.62	4.2
"off" year	control	0.181	2.69	1.72	5.62	118.75	10.92	5.63
"	100	0.171	2.2	1.25	3.95	133.64	9.28	7.19
"	150	0.161	1.93	1.18	4.88	89.17	7.56	4.23
"	200	0.163	2.06	1.09	3.15	101.93	9.61	4.22
LSD		0.036	0.35	0.52	0.54	40.56	2.4	1.8

*the mean of each 2 years "on" and 2 years "off". **Mean separation in each column by LSD at 5% level.

Table 3. Changes in macro nutrient (g/100 g) and micro nutrient (mg kg^{-1}) of kernel pistachio (*P. vera* cv. Kalleh-ghuchi) grown in Ghazel-Ozen, Zanjan.*

Treatments		P (%)	K (%)	Mg (%)	Ca (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)
"on" year	control	0.22	0.95	0.25	0.67	7.29	10.79	3.43
"	100	0.38	1.05	0.73	1.31	17.07	22.91	7.98
"	150	0.40	1.45	0.35	0.75	7.98	11.36	4.83
"	200	0.51	1.65	0.91	1.81	18.11	23.11	8.18
"off" year	control	0.21	0.62	0.39	0.41	8.97	14.85	3.95
"	100	0.19	0.76	0.38	0.52	9.85	12.43	5.12
"	150	0.22	0.66	0.34	0.39	9.54	15.49	4.34
"	200	0.17	0.71	0.32	0.59	9.74	12.19	4.73
LSD		0.16	0.55	0.48	0.45	7.6	11.2	3.7

*the mean of each 2 years "on" and 2 years "off". **Mean separation in each column by LSD at 5% level.

Table 4. Changes in kernel quality (protein%, fat %), yield (nut weight g/1000 nut, nut size mm) and blank nut% of pistachio (*P. vera* cv. Kalleh-ghuchi) grown in Ghazel-Ozen, Zanjan.*

Treatments		Protein (%)	Fat (%)	Nut weight (g/1000 nuts)	Nut size (mm)	Blank nut (%)
"on" year	control	18.4	51.2	570	10.2	17
"	100	18.7	52.5	620	11.5	20
"	150	19.5	53.7	660	12.3	18
"	200	22.3	56.2	740	13.3	15
"off" year	control	18.2	49.8	610	11.2	27
"	100	19.7	50.1	670	12.4	29
"	150	17.2	53.7	690	12.8	21
"	200	19.4	54.8	720	13.1	28
LSD		1.7	3.5	59	1.5	2.4

*the mean of each 2 years "on" and 2 years "off". **Mean separation in each column by LSD at 5% level.

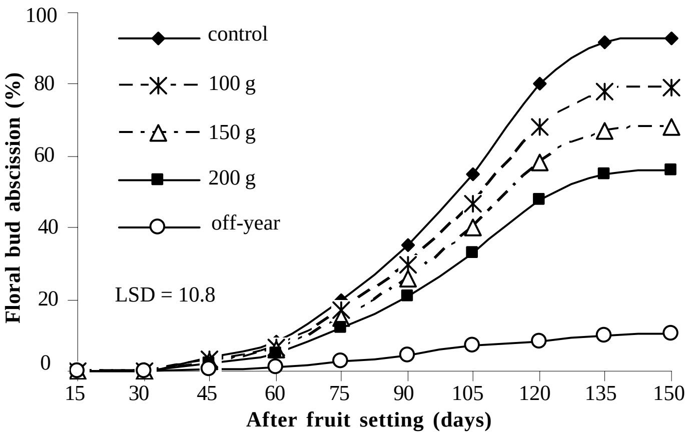

Figure 3. The comparison of course of floral bud abscission on fruiting branches of pistachio 'Kalleh-ghuchi' trees treated (0, 100, 150, and 200 g actual N per tree), and non-fruiting branch in "off" year (open circle symbols). The mean of each 2 years "on" and 2 years "off", and LSD was calculated at P = 0.05.

Conclusions

Leaf protein concentration depends upon crop load in the current and previous years. Nuts were the major sink for nutrients (protein). Very early competition effects between growing nut (kernel) and floral buds were not remarkable, but this competition was important during nut fill period. The nutritional status of the trees affects upon the alter-bearing phenomenon. Nutrition application has an important effect on nutrient contents of leaves and kernels of pistachio. When the nitrogen compounds (protein) content decreases below a specific level, it could trigger the abscission mechanism and could trigger senescence, thus presenting a hormone-like action.

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