

INFLUENCE OF AUTUMN FERTILIZATION WITH NITROGEN AND PHOSPHORUS ON  
ROOT ACTIVITY AND DEVELOPMENT IN AVOCADO

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Abstract

Autumn fertilization with nitrogen (N) and phosphorus (P) for eight years increased yield by 50% in avocado (*Persea americana*). The supplementary NP-treatment was compared with normal spring and summer fertilization alone. The yield increase was accompanied by increased root mass and activity in the spring, as monitored by increased white root tip density. The roots of NP-treated trees had higher levels of calcium (Ca) and endomycorrhiza.

The concentration of P in roots was higher in NP-treated trees, while no differences were found in the concentration in leaves. The mineral composition was different on roots of different diameters. In thin roots (<1-mm-wide) of NP-treated trees, the P concentration was twice as high and there was a change in the quantitative relationship between the cations. NP treatment decreased the concentration of potassium and magnesium and increased the concentration of Ca. Also, in wider roots, there was a change in element concentration with trends similar to those in thin roots although not always as great as in thin roots. The concentration of microelements in roots was not affected by NP autumn fertilization.

Further work must be done to determine the element concentration in roots during the growth season to verify the possibility of using these data as a measure of the nutrient status of the tree.

Additional index words: yield, mycorrhiza, *Persea americana*, nutrition, root morphology

Introduction

Root morphology and root-shoot relationships are affected by mineral nutrition (Aung, 1974; Hackett, 1968). In general, nitrogen (N) causes an increase in root mass and number of root hairs, phosphorus (P) and potassium (K) cause and increase in ramification of the roots, and calcium (Ca) improves root hardiness (Vogt et al., 1991).

Sub-optimal mineral nutrition, especially P and N, often results in an increase in root/shoot ratio (Alberda, 1965; Barley, 1970; Bourn, 1967; Taylor et al., 1976). Sometimes elongation of the main root occurs at the expense of the lateral roots at sub-optimal K (Jensen, 1982) and N (Clement et al., 1979) levels.

A different kind of root morphology modification can be found when only a limited volume of roots of plants with mineral deficiencies are fertilized (Russel, 1977). In this case there is an increase in number of lateral roots, especially with N and P fertilization (Drew et al., 1973; Drew et al., 1975). With K there is also a general increase in the number of lateral roots, and not only in limited fertilized volume (Drew et al., 1975). The mineral nutrition, especially of N, and P, can also increase the length and density of the thin roots.

The form of N supplied to the root can influence the growth and viability of the root. Ammonium leads to a lower root turnover and biomass, and nitrate leads to a higher root turnover and biomass (Aber et al., 1985; Smucker, 1984).

Root mass is in part controlled by the photosynthate source and competition between sinks that determine the carbohydrate distribution. There is a slow turnover of the thick roots (Vogt et al., 1991) and a fast turnover of the thin roots (Cox et al., 1978; Reynolds, 1970; Vogt et al., 1991).

Thin roots are generally associated with mycorrhizal fungi (Vogt et al., 1981) that can influence their longevity. In forest trees the lack of mycorrhiza can shorten root longevity to a few weeks; with mycorrhiza the roots can remain active for some years (Vogt et al., 1991).

In a previous work we reported an highly significant yield increase in avocado by autumn fertilization with N and P (Reuveni et al., 1989). There was an increase in yield despite the similar concentrations of N and P as determined by standard leaf samples of NP-treated and control trees. The highest yield in NP-treated trees was accompanied by greater leaf number during the spring, and reduced leaf abscission during fruiting season (Reuveni et al., 1989). An occurrence of extremely hot winds in May 1988, which caused a large decrease in Israel avocado yield, lead to a more rapid old-leaf drop in control trees compared with NP-treated trees (Reuveni et al., 1989).

Preliminary observations on the rapidity of root renewal in the spring by NP-treated trees in the present work, lead us to hypothesize that the increased yield is a result of the nutrient treatment effect on root growth and morphology. In the present work we determined the effect of autumn fertilization with N and P on the element composition of roots and the rapidity of root renewal in the spring.

## Materials and methods

An avocado (*Persea americana* Mill.) plantation growing on sandy-loam soil, pH 7, in Kibbutz Nir-Eliahu in the Coastal Plain of Israel was selected. The trial was initiated in November 1985 when the plantation was 7-years-old. The trees were cv. 'Hass' on Nabal stock. A group of thirty trees was selected for the trial. Ten completely randomized blocks of 3 consecutive trees in a row were established. Five blocks were subjected to an NP-fertilizer treatment during November of each year. Five blocks served as non-treated controls.

*Standard horticultural treatments.* The irrigation was effected with mini-jets twice a week. During spring and summer the plantation fertilizer was distributed through the irrigation system.

A total of 25 kg N/ 1000 m<sup>2</sup> was applied each season. Potassium was applied only during 1988 and 1989 at a rate of 25 kg K/ 1000 M<sup>2</sup> per season.

*Autumn fertilization treatment* The NP-fertilizer treatments were applied through the irrigation system twice each year during two successive irrigations of 6 mm. An "8-26-0" fertilizer was prepared with ammonium nitrate and phosphoric acid and injected into the irrigation water; the mean amount applied each autumn during the eight years of experiment was 2 kg N and 6.5 kg P<sub>2</sub>O<sub>5</sub> / 1000 M<sup>2</sup> per season.

*Root and soil sample.* Soil samples were taken in March each year with a 0.5-liter- volume metal tube (8-cm-diameter X 10-cm-length). The samples came from the irrigated region under the tree canopy at a distance of 40 to 50 cm from a mini-jet. Leaf debris was cleared away prior to taking the core which contained soil and roots. Six to 8 cores were sampled from the central tree of each replicate. The soil was separated from the roots by sieving through a 2 mm screen taking care to prevent loss of small roots. The screened soil was taken for chemical analysis. Roots were used for determination of root number, mass and mineral composition.

*Determination of root dry weight and mineral composition.* The total number of white-colored root tips visible during the spring flush served as the measure of root activity. Because so many roots tips were counted at the first counting in May 1991, we decided to determine root activity earlier (March) in the following years. After counting the root tips, all the roots were classified and separated according to their diameter and color: white roots (a) 0 to 0.9 mm, (b) 1 to 1.9 mm, (c) 2 to 3 mm, and dark roots.

After washing the roots with distilled water they were prepared for mineral determination. Roots were placed in sulfuric acid 10% (v/v) for a few seconds, rinsed with distilled water and dried at 60 °C for a few days (until there was no change in sample weight). The light colored roots were ground in a Wiley mill and mineral composition was determined in the Plant Nutrition Laboratory of the Faculty of Agriculture, Rehovot.

*Association of thin roots with mycorrhizal fungus.* Root samples for evaluating the percent of root colonized by mycorrhizal fungi were collected on 30 July 1992, in a similar way to that of the spring root sampling. A composite sample from the several soil cores around each central-tree was screened, washed under running water, and the remaining light-colored roots were randomly separated into 10 g aliquots.

The preparation of the roots for mycorrhizal determination was done as described by Haas et al. (1986). The roots were cleared in 2% KOH at 95 °C rinsed in 1% HCl and stained with Trypan-Blue.

The percent of roots colonized with endomycorrhizal fungus was estimated by the visual evaluation method as described by Giovannetti et al. (1980).

*Leaf analysis.* In October 1993, 15 mature leaves were sampled from the central tree of each block and nutrient analysis was performed in the Plant Nutrition Laboratory of the Faculty of Agriculture, Rehovot.

*Yield.* Avocado fruits from the three trees of each block were collected separately and weighed during each year of the trial.

*Statistical analysis.* Analysis of variance was performed separately on the data from each sampling. Means separation was by F-test or Duncan's Multiple Range Test, as appropriate.

## Results

*Soil and leaf analysis.* In the spring of 1992, seven years after the start of autumn NP-treatments, there was no significant fertilizer effect on the N or K concentrations or electrical conductivity in the soil. There was 3.0 mg NI kg soil, 4.3-4.6 mg K/ kg and conductivity was 0.35-0.36 dS/ in. The available P under NP-treated trees was 27 mg/ kg and that under control trees was 13 mg/ kg.

Concentrations of N, P, K and Ca were similar in leaves of NP-treated and control trees sampled in October 1993, the last year of the trial. On a dry weight basis the mean of NP-treated and control roots was 1.82 and 1.88 % N, 0.196 and 0.188 % P, 1.01 and 1.06 % K, and 2.10 and 2.17 % Ca, respectively.

*Mineral composition of the roots.* The mineral composition of the thin (up to 3-mm- wide), light-colored roots sampled in March 1992 and 1993 was similar in both years and only the 1993 results are presented (table 1). The concentration of some elements differed in roots of different width. The N, Mg, Zn, Mn and Cu concentrations were higher in the thinnest roots (<1-mm width) and decreased with the increase in root width. The autumn NP-fertilization greatly increased the concentration of P and Ca in the roots ( $P < 0.01$ ), especially those up to 2-mm diameter. The autumn NP-fertilization caused a decrease in K concentration of the thinnest roots (<1-mm width) and of the widest roots (2 to 3-mm width). There were no significant differences in the concentration of the other elements measured.

*Root mass and activity.* The number of root tips in the 4-liter soil collected served as a measure of root activity in spring. The autumn NP-fertilization stimulated earlier and increased root activity. In the mid-March and early May there were two to five times more root tips in NP-treated trees than in control trees (table 2).

The dry weight of roots in the sample was considered a measure of the total tree root mass. The autumn NP-fertilization resulted in a doubling or tripling in the small root mass (table 3). The weight of larger, dark roots was increased by 50% by the fertilization treatment (table 3).

*Yield* The average yield of the 8-harvests from 1986 to 1993 was significantly higher in NP-treated trees. There were 29.5 kg fruit/ tree/ year in control trees and 44.2 kg in treated plants.

*Mycorrhizal symbiosis.* Autumn NP-fertilization caused an increase in the association of thin roots with mycorrhizal fungi . The mycorrhizal fungus colonization was doubled by the NP-treatment (table 4).

## Discussion

Supplementary fertilization with N and P for an eight-year-period from 1986-1993 caused a 50% increase in mean yield of avocado. This increase was recorded despite the lack of evidence

of N or P deficiency in leaves, based on the levels generally accepted in Israel (2% N and 0.12% P, dry weight basis).

The K concentration was higher in leaves of control trees during the first years of the trial (1986 to 1989) (Reuveni et al., 1989). This difference in K concentration was annulled by fertilizing both treatments with K in 1988 and 1989.

The increased yield following autumn NP-fertilization challenges the validity of the standard leaf analysis recommendations for sufficiency of these two elements. In a previous report (Reuveni et al., 1989) we suggested the need for selecting a different plant tissue for nutrient status analysis of avocado. This is one consideration that lead us to examine the mineral composition of roots and to determine the kinetics of the changes in mineral composition of leaves and flowers during spring (Yoel Bar and Leo Winer, paper in preparation).

In the present work we found differences in nutrient concentration of roots depending upon the root width. The most important differences in the nutrient concentration in roots following autumn NP-fertilization were found in the thin roots (<1-mm-width). The P concentration in roots was increased and K concentration decreased by autumn NP fertilization.

More work must be done to determine the time-course of changes in element concentrations in roots during the season, before these data can be employed as additional parameters for determining the mineral nutrition status of avocado.

One of the important findings of the present work is the effect of autumn NP- fertilization on the greater intensity of root activity in the spring and, after eight years of the trial, a very significant increase in total root mass. The higher total root mass may be the result of the prolonged root longevity. Many factors are known to influence root development and longevity. Brown et al. (1984) thought that soil parameters are paramount to genetic parameters in determining the rate of root turnover. This would lead to more intensive root development in the parts of the soil mass with less water or nutrient deficiency and to greater nutrient uptake by the roots (Eissenstat et al., 1988; St. John et al., 1983). This, in contrast with more rapid aging and root death in the adequately watered and fertilized parts of the soil.

The autumn NP-fertilization increased the symbiosis of thin roots and mycorrhizal fungi. The mycorrhizal symbiosis is known to positively affect the longevity of roots (Vogt et al., 1991), and cause increased concentrations of Ca, an element known to strengthen roots (Vogt et al., 1991). An important effect of mycorrhiza is to improve the transfer of available-P to roots and this was evident in our results. High levels of P in roots can inhibit mycorrhizal development but not at the levels found in the treated-tree roots.

We postulate that the increased root growth and avocado yield was largely due to the greater absorption of P in treated trees and that the mycorrhizal fungi contributed significantly to this absorption.

## References

- Aber, J.D., Melillo, J.M., Nadelhoffer, K.L., McClaugherty, C.A., and Pastor, J., 1985. Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: a comparison of two methods. *Oecologia* 66:317-321.
- Alberda, Th., 1965. Influence of temperature, light intensity, nitrate concentration on dry matter production and chemical composition of *Lolium perenne* L., *Neth. J. Agric. Sci.* 13:335-360.
- Aung, H.L., 1974. Root-shoot relationships. In: *The Plant Root and its Environment*. E.W. Carson, Ed., University Press of Virginia, Charlottesville, pp. 29-61.

- Barley, K.P., 1970. The configuration of the root system in relation to nutrient uptake. *Adv. Agronomy* 22:159-201.
- Bouma, D., 1967. Nutrient uptake and distribution in subterranean clover during recovery from nutritional stress. 1. Experiments with P. *Aust. J. Biol. Sci.* 20:601-612.
- Brown, D.A. and Scott, H.D., 1984. Dependence of crop growth and yield on root development and activity. Ln: *Roots, Nutrient and Water Influx, and Plant Growth*, Publication No. 49 \* American Society of Agronomy, Madison, Wis., pp. 101 - 136.
- Cox, T.L., Harris, W.P., Ausmus, B.S. and Edwards, H.T., 1978. The role of roots in biogeochemical cycles in an eastern deciduous forest. *Pedologia* 18:264-271.
- Clement, C.R., Jones, L.H.P. and Hopper, M.J., 1979. Uptake of nitrogen from flowing nutrient solution: Effect of terminated and intermittent nitrate supplies. In: *Nitrogen Assimilation of Plants*. (E.J. Hewitt, C.V. Cuttings, Eds.). Academic Press, London. pp. 123-133.
- Drew, M.C., and Saker, L.R., 1975. Nutrient supply and the growth of the seminal root system in barley. 11. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. *J. Exp. Bot.* 26:79-90.
- Drew, M.C., Saker, L.R. and Ashley, T.W., 1973. Nutrient supply and the growth of the seminal root system in barley. I. The effect of nitrate concentration on growth axes and laterals. *J. Exp. Bot.* 24:1189-1202.
- Eissenstat, D.M., and Caldwell, M.M., 1988. Seasonal timing of root growth in favorable microsites. *Ecology* 69:870-873.
- Giovannetti, M. and Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* 84:489-500.
- Haas, J.H., Bar-Tal, A., Bar-Yosef, B., and Krikun, J., 1986. Nutrient availability effects on vesicular arbuscular mycorrhizal bell pepper (*Capsicum annuum* L.) seedlings and transplants. *Ann. App. Biol.* 108:171-179.
- Hackett, C., 1968. A study of the root system of barley. 1. Effects of nutrition on two varieties. *New Phytol.* 67:287-299.
- Jensen, P., 1982. Effects of interrupted K supply on growth and uptake of K, Ca, Mg, and Na in spring wheat. *Physiol. Plant.* 56:259-265.
- Orlov, A.Y., 1957. Observations on absorbing roots of spruce (*Picea excelsa* Link) in natural conditions. *Bot. Zh. SSSR* 42:1172-1181.
- Reuveni, B., Winer, L., and Shnior, I., 1989. Autumn fertilization for yield increase in avocado. *Alon Hanotea* 3:191-198 (in Hebrew).
- Reynolds, E.R.C., 1970. Root distribution and the cause of its spatial variability in *Pseudotsuga tasifiblia* (Poir.) Brit. *Plant Soil* 32:501-517.
- Reynolds, E.R.C., 1975. Tree rootlets and their distribution. In: *The Development and Function of Roots* (J.G. Torrey, D.T. Clarkson, Eds.). Academic Press, London, pp. 163-177.
- Russel, R.S., 1977. *Plant Root Systems*. McGraw-Hill, New York. pp. 53-61, 82-89. Smucker, A.J.M., 1984. Carbon utilization and losses by plant root systems. In: *Roots, Nutrient and Water Influx, and Plant Growth*, Special Publication No. 49. American Society of Agronomy, Madison, Wis., pp. 27-46.
- St. John, T.V., Coleman, D.C., and Reid, C.P.P., 1983. Growth and spatial distribution of nutrient-absorbing organs: selective exploitation of soil heterogeneity. *Plant Soil* 71:487-493.
- Taylor, B.K., and Goubran, F.H., 1976. Effects of phosphate and pH stress on the growth and function of apple roots. *Plant Soil* 44:49-162.

- Vogt, K.A., Bloomfield, J., 1991. The root turnover and senescence. *In: Plant Roots, the Hidden Half.* (Y. Waisel, A. Eshel, U. Kafkafi, Eds.). Marcel Dekker, New York. pp. 287-306.
- Vogt, K.A., Edmonds, R. L., and Grier, C.C., 1981. Seasonal changes in biomass and vertical distribution of mycorrhizal and fibrous-textured conifer fine roots in 23 and 180-year-old *subalpine Abies amabilis* stand. *Can. J. For. Res.* 11:223-229.

Table 1: Elemental composition (dry weight basis) of three root-width categories of avocado. Sampling was on 17 March 1993 following eight years with or without supplemental fertilization treatments with N and P each autumn. Different letters beside the means in a row indicate significant statistical differences ( $P < 0.05$ ).

Element	<u>Thinner than 1 mm</u>		<u>1 - 1.9 mm</u>		<u>2 - 3 mm</u>	
	Control	NP-fert.	Control	NP-fert.	Control	NP-fert.
N (%)	1.9 a	1.9 a	1.5 b	1.6 b	1.5 b	1.2 b
P (%)	0.21 b	0.42 a	0.16 b	0.33 a	0.17 b	0.24 a
K (%)	2.05 b	1.50 a	1.87 b	1.82 b	2.31 b	1.69 a
Ca (%)	0.50 b	0.70 a	0.40 b	0.63 a	0.41 b	0.52 a
Mg (%)	0.53 a	0.46 a	0.34 b	0.35 b	0.28 c	0.25 c
B (ppm)	22.1	21.8	32.5	27.7	26.9	24.8
Fe (ppm)	612 a	663 a	446 b	380 b	525 a	378 b
Zn (ppm)	93 a	114 a	61 b	88 a	46 b	62 b
Mn (ppm)	55 a	64 a	35 b	42 b	33 b	30 b
Cu (ppm)	55 a	60 a	33 b	36 b	18 c	31 b

Table 2: Number of root tips in a 4-liter sample of soil in which avocado were growing. The supplementary N and P fertilization was applied each autumn beginning in 1986. Different letters beside the means in a row indicate significant statistical differences ( $P < 0.01$ ).

<u>Date of sampling</u>	<u>Control</u>	<u>NP-fertilization</u>
3 May 1991	52 a	200 b
16 Mar 1992	22 a	108 b
17 Mar 1993	36.2 a	79.2 b

Table 3: Root dry weight (g) in size- and color-categories of roots from avocado trees treated with autumn supplementary fertilization with N and P initiated in 1986. The roots were contained in a 4 liter sample of soil. Different letters beside the means in a row indicate significant statistical differences between them ( $P < 0.05$ ).

<u>Root category</u>	<u>Date of sampling</u>	<u>Control</u>	<u>NP-fertilized</u>
White, 0-0.9 mm	16 Mar 1992	0.72 a	1.66 b
	17 Mar 1993	0.93 a	1.87 b
White, 1-1.9 mm	16 Mar 1992	0.42 a	1.38 b
	17 Mar 1993	0.71 a	1.18 b
White, 2-3 mm	16 Mar 1992	0.30 a	1.68 b
	17 Mar 1993	0.68 a	1.57 b
Total white, 0-3 mm	16 Mar 1992	1.44 a	4.72 b
	17 Mar 1993	2.43 a	4.61 b
Total dark	16 Mar 1993	8.91 a	12.91 b
	17 Mar 1993	9.67 a	16.60 b

Table 4: Mycorrhizal fungus colonization of thin roots of avocado. The roots were sampled on 30 July 1992. Different letters beside the means indicate a significant statistical difference ( $P < 0.05$ ).

<u>Treatment</u>	<u>Root length colonized (%)</u>
Control	23.6 a
NP-treatment	44.0 b