The effects of nitrification inhibition and phosphonate on 'Hass' avocado yield and *Botryosphaeria* susceptibility

L. Winer

Extension Service, Ministry of Agriculture, Israel.

Abstract

Phosphonate is globally used in avocado production for the control of root rot caused by *Phytophthora cinnamomi*. The phosphonate mechanism of action is to increase the plant's resistance to different groups of fungi. Recently it was reported to be effective in avocado against *Botryosphaeria* as well. The present research was performed in Israel on a 'Hass' avocado orchard planted in clay soil of high pH, that displayed no symptoms of *Botryosphaeria*. Use of a nitrification inhibitor, phosphonate or a combined treatment with the two components were compared with a control treatment. All four treatments were administered together with fertilizers through the irrigation system at constant concentrations during the entire irrigation season. The fertilizer components were uniform in all four treatments, using ammonium as the main source of nitrogen. Inhibition of the nitrification process increased the soil concentrations of nitrogen approximately 3-fold, mostly by increasing the ammonium component. The water soluble phosphorous in soil solution was increased by 3.7- to 5.5-folds in treatments that included K-phosphonate. The availability of potassium as represented by its concentration in the saturated paste of the soil increased from 14.0 to 22.6 mg kg^{-1}. Phosphonate or nitrification inhibitor caused an increase in the mean yield of three consecutive seasons from 11.3 to 14.9 and 14.7 t ha^{-1}, respectively. Combined treatment of the nitrification inhibitor with phosphonate when the trees were fertilized with ammonium resulted in a significant mean yield increase to 16.4 t ha^{-1} (about 45% above the control). Trees started developing branch dieback symptoms related to *Botryosphaeria* only during the third season of the trial. The combined treatment of phosphonate and nitrification inhibitor dramatically decreased the susceptibility of the trees to those symptoms. The administration of phosphonate alone was only slightly effective.

Keywords: ammonium-N, nitrate-N, *Persea americana*

INTRODUCTION

The nitrification process is inherently a biological process in which ammonium (NH_4^+) becomes nitrate (NO_3^-) in oxidation processes involving different groups of bacteria. Nitrite (NO_2^-) is also formed in the intermediate phase of the process. The uptake of ammonium by the roots is followed by a secretion of H^+ ions acidifying the rhizosphere.

Nitrogen fertilization significantly impacts the nitrous oxide atmospheric emission rate formed during the process of the chemical transformation of ammonium to nitrate in the nitrification process. Under high soil pH and relatively high temperatures (Singer, 2007), the application of ammonia or ammonium-based fertilizers can result in high rates of nitrification and therefore losses of N in the form of N_2O. Nitrification is an inherent biological process in which ammonium (NH_4^+) is converted into nitrate (NO_3^-) in oxidation processes involving bacteria from different groups. In the first phase of the process, nitrifying bacteria use ammonium as an electron donor and oxygen as an electron receptor, with the oxidation product being nitrite. Another type of nitrification bacteria is involved in the second stage of the process, which involves the oxidation of nitrite, which serves as an electron donor and oxygen as an electron receptor, with the end result of the process being the formation of nitrate. Atmospheric nitrogen losses affect and impair the efficiency of the fertilizer and constitute a pollutant of the atmosphere through ozone oxidation (Crutzen, 1976). Most of the nitrogen found in the rhizosphere among actively growing roots, when soil temperature is relatively high, is nitrate. Excess nitrate, when fertilization exceeds crop usage, can seep to the groundwater becoming a pollutant factor.

The mode of action of K-phosphanate involves the decrease of plant sensitivity to pathogenic fungus (Perez-Jimenez, 2008). Steinberg found effectiveness against *Botryosphaeria* symptoms when spraying K-phosphanate on the canopy, but very limited or no effect when administrating it through the roots with the irrigation (Steinberg et al., 2016). The cankers caused by *Botryosphaeria* exudes a white powder and the inner wood is brown and the most evident symptom of *Botryosphaeria* infection is the dieback of tree branches. The low or not effectiveness of K-phosphanate with root administration may be due to the basic soils of Israel which inactivate the phosphonate.

The present research hypothesizes that the inhibition of the nitrification process will create a more acidic environment in the rhizosphere due to the process of ammonium uptake and secretion of hydrogen ions by the roots. The acidic environment should improve the availability and absorption of microelements by the tree. Acidification of the rhizosphere will also promote phosphonate availability improving the resistance of the tree to *Botryosphaeria* fungus. We suppose those factors will improve yield.

MATERIALS AND METHODS

The present research was performed in an avocado (*Persea americana* 'Hass') commercial orchard at Kibbutz Nachshonim located near the coast of the Mediterranean Sea, planted during the spring of 2014. The clay soil contains a very low percentage of active calcium carbonate. The 'Hass' trees are grafted on West-Indian Degania 117 rootstocks. The amount of irrigation water applied during one season was about 8,600 m^3 ha^{-1} with an average of 500 mm of rain per winter season. The trial was performed during the 2018, 2019 and 2020 seasons.

The experimental plot was fertilized proportionally to the irrigation water with a rate of 0.5 L of liquid fertilizer m^{-3} of water irrigation. The liquid fertilizer used in the research plot was composed of ammonium sulfate, ammonium nitrate, phosphoric acid and potassium chloride with a proportion of 5-1-5 (NPK). This composition of fertilizer gave a concentration of 30 ppm nitrogen with ammonium as the main component (70%), 6 ppm phosphorous oxide and 30 ppm potassium oxide in the irrigation water. An amount of 258 kg of nitrogen, 51.6 kg of phosphorous oxide and 258 kg of potassium oxide was applied annually ha^{-1}.

Four treatments were tested:

a) Control. The fertilizer used contained ammonium sulfate, ammonium nitrate, phosphoric acid and potassium chloride in a proportion of 5-1-5 of the elements (N-P-K) fertigated with the irrigation – Basic;
b) Basic fertigation combined with K-phosphonate at a rate between 11.7 and 35.1 g m^{-3} of irrigation water;
c) Basic fertigation combined with nitrification inhibitor at a rate of 3 g m^{-3} of irrigation water;
d) Basic fertigation combined with K-phosphonate and nitrification inhibitor.

The commercial product 'KeeP' from Soiltech company, Colombo, Sri Lanka was used as the source for K-phosphonate. The product contains 780 g L^{-1} K-phosphonate (equivalent to 500 g L^{-1} phosphonic acid). 'KeeP' was administered in treatments b and d) by adding it at a proportion of 3% to the liquid fertilizer during 2018 and 2020. During 2019 'KeeP' was added at a proportion of 6% to the liquid fertilizer. The commercial product 'Green' from 'ICL-fertilizers company of Haifa, Israel' was used as the source for the nitrification inhibitor. 'Green' contains dicyandiamide (DCD), a known and effective inhibitor of the nitrification process (Lan et al. 2013). 'Green' was administered in the treatments c and d by dissolving DCD in the liquid fertilizer solution at a rate of 0.6%. All the treatments were administered at constant concentrations proportionally to the irrigation water. The experimental design was random with 4 replications of 0.1 ha per replicate.

Leaf element analysis

Adult leaves from the spring vegetative flush were collected during October of each season for element analysis of the concentrations of nitrogen, phosphorous, potassium, calcium, magnesium, zinc, iron, manganese and boron in leaves by dry weight.

Soil analysis

Soil in two different depths (0-20 and 20-40 cm) were sampled in the end of July 2019 to determine the concentration of nitrogen, phosphorous and potassium elements. Soil was dried during 48 h at 70°C. The dried soil was then grinded before element extractions. Nitrate was extracted from a mixture of soil and water (1:5). Ammonium was extracted from a mixture of soil and 1 N KCl solution (1:5). The concentration of both nitrate and ammonium was calculated based on soil dry weight. K was extracted from the saturated paste of the soil or extracted using $CaCl_2$. The soluble portion of P and also the Olsen phosphate were determined.

Yield

The yield of each replicate was harvested separately before the performance of research treatments and in each of the three following seasons during the research.

Botryosphaeria infection

A sample of 20 trees per replicate was used for the evaluation of branch dieback related to *botryosphaeria* infection. The trees were distributed in two categories: a) Mild infected: the trees included in this category had very mild *Botryosphaeria* symptoms with maximum one branch suffering of dieback; b) Acute infected: the trees including in this category had more than two branches suffering of dieback.

RESULTS AND DISCUSSION

Leaf analysis

1. Macroelements.

'KeeP', 'Green' and the combined treatment of those two products increased significantly the nitrogen concentration in the leaves. The combined treatment of 'KeeP' + 'Green' caused the sharpest effect increasing the concentration of nitrogen from 1.67 to 1.91% of leaf dry weight. 'Green' and 'KeeP' individually, caused an increase of nitrogen concentration to only 1.74 and 1.80%, respectively (data not shown). On the other hand 'KeeP', 'Green' and the combined treatment of those products did not change significantly the concentration of P, K and Mg in the leaves that ranged from 0.13 to 0.14%, 0.66 to 0.71% and 0.62 to 0.67%, respectively (data not shown).

2. Microelements.

A uniform concentration of Fe of about 65 ppm was recorded in the leaves of all treatments. The Zn concentration in all treatments ranged from 18.8 to 20.4 ppm without statistical differences between the treatments. The concentrations of Mn in the leaves of 'KeeP', 'Green' and the combined treatments increased sharply from 109 to 178 ppm, 139 and 171 ppm, respectively (data not shown).

Soil analysis

The nitrification inhibition by 'Green' caused a sharp increase in ammonium concentration of more than 3-fold in the soil solution at 0-20 cm depth with an increase from 17.07 to 72.18 and 54.38 mg kg^{-1} soil in the treatments 'Green' and the combined treatment of 'Green' with 'KeeP', respectively (Figure 1). The concentration of nitrate found in the soil solution ranged from 8.82 to 14.75 mg kg^{-1} soil in the shallow soil layer (0-20 cm) and 4.65 to 8.30 mg kg^{-1} in the deeper soil layer without any statistically significant differences between treatments at each of the different layers (Figure 1).

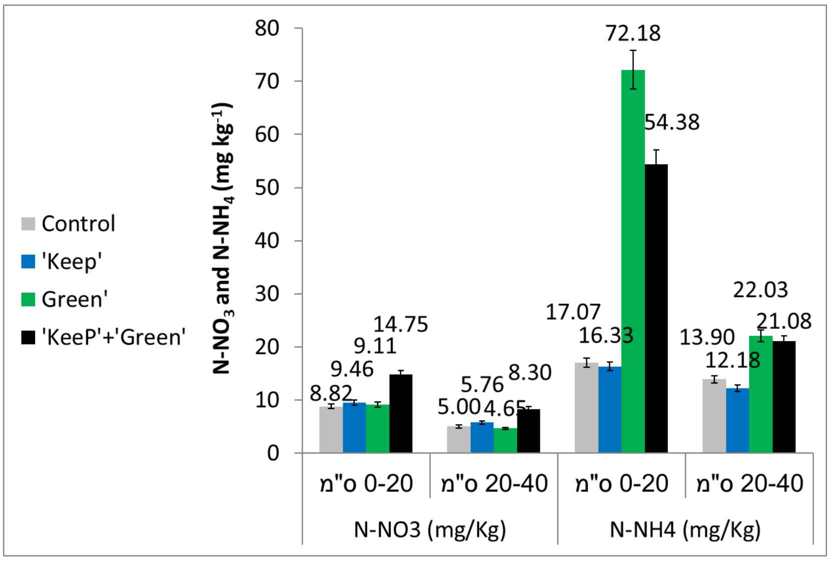

Figure 1. Ammonium and nitrate concentrations in soil sampled on July 30, 2019 at Kibbutz Nachshonim affected by 'KeeP', 'Green' and the combined treatment of 'KeeP' + 'Green'.

'KeeP' increased significantly the concentration of potassium in the soil solution in the shallow (23.3 mg kg^{-1}) and also in the deeper (16.4 mg kg^{-1}) soil layers in relation to the concentration of potassium in the treatment without additions, where we recorded 14.0 and 9.6 mg kg^{-1} in the shallow and deeper soil layers, respectively (Figure 2). A similar increase in the potassium soil solution concentration was recorded also in the combined treatment of 'KeeP' and 'Green' (Figure 2). The increase in available potassium in the soil in the 'KeeP' treatment and in the combined treatment of 'KeeP' and 'Green' was found also in the potassium concentration extracted using calcium chloride (Figure 3). Since 'KeeP' only without 'Green' increased the concentration of the available potassium in the soil, we can conclude that this increase is due, at least the major part of it, from the potassium component of 'KeeP'.

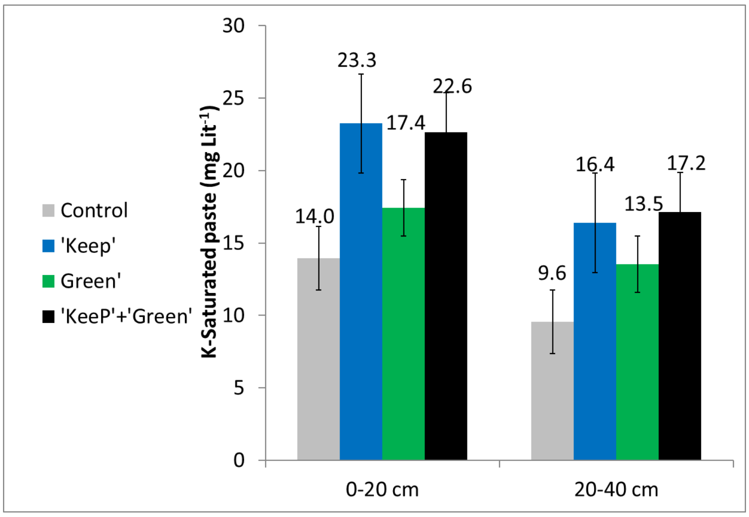

Figure 2. Potassium concentration in the saturated paste of the soil sampled on July 30, 2019 at Kibbutz Nachshonim affected by 'KeeP', 'Green' and the combined treatment of 'KeeP' + 'Green'.

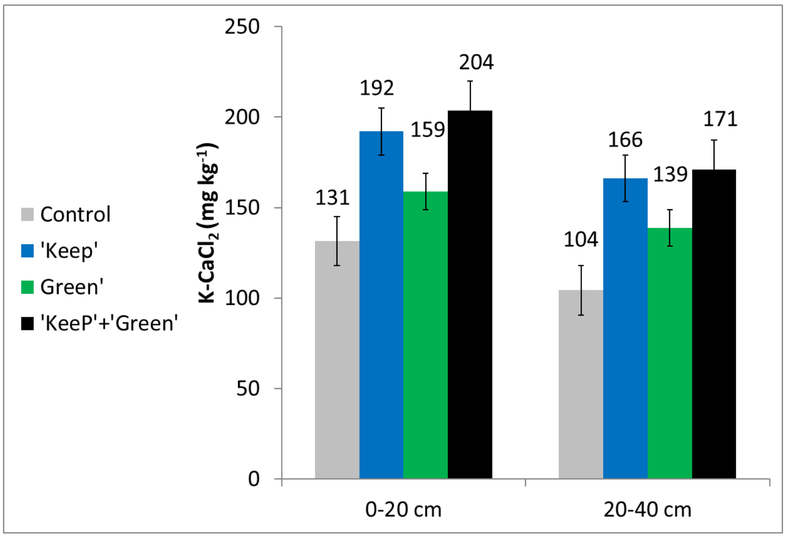

Figure 3. The concentration of potassium in extractions of calcium chloride in soil sampled on July 30, 2019 in Kibbutz Nachshonim affected by 'KeeP', 'Green' and the combined treatment of 'KeeP' + 'Green'.

'KeeP' increased significantly the concentration of dissolved phosphorous in the soil solution from 0.54 to 2.02 mg L^{-1} (by about 4-folds). Also, in the combined treatment of 'KeeP' and 'Green' a significant increase in dissolved phosphorous was found (Figure 4). In contrast, the 'Green' treatment did not change the phosphorous concentration in the soil solution (Figure 4). Therefore, we can conclude that 'KeeP' is the responsible element for the increase in the dissolved portion of phosphorous in the soil solution. The concentrations of dissolved phosphorous in the different treatments corresponded to the phosphorous tested using the Olsen method for basic soils (Figure 5) expressing the potential portion of phosphorous in the soil that can be exchanged to the soil solution.

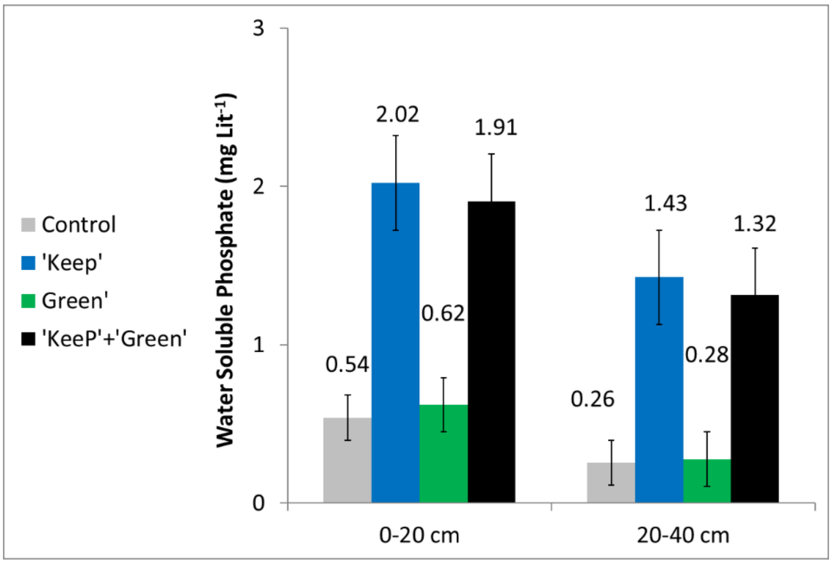

Figure 4. The portion of water-soluble phosphate (mg kg^{-1}) in soil sampled on July 30, 2019 at Kibbutz Nachshonim affected by 'KeeP', 'Green' and the combined treatment of 'KeeP' + 'Green'.

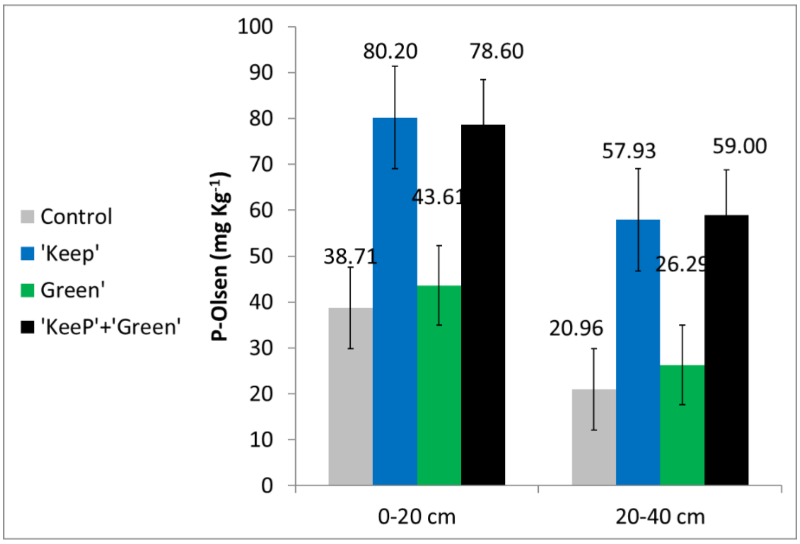

Figure 5. The Olsen Phosphate concentration in soil sampled on July 30, 2019 at Kibbutz Nachshonim affected by 'KeeP', 'Green' and the combined treatment of 'KeeP' + 'Green'.

Yield

Three years after planting, before the start of the present research, the yield in the research trial plot was about 10 t ha^{-1} (season of 2017) with similar average yields for the different replicates of the area.

After one season of running the trial, 'KeeP' and 'Green' increased significantly the total yield from 13,710 to 16,200 and 15,820 kg ha^{-1}, respectively (Figure 6). The combined treatment of 'KeeP' + 'Green' increased the total yield to 18,860 kg ha^{-1} (Figure 6), i.e., an addition of about 5,000 kg ha^{-1} and this in the fourth year after planting.

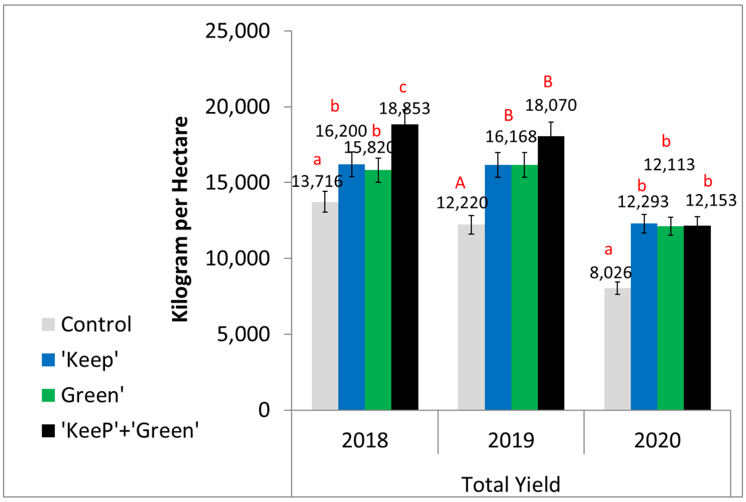

Figure 6. Total yield by 'KeeP', 'Green' and the combined treatment of 'KeeP' + 'Green' during three consecutive seasons.

The yield after the second season of the treatments, reinforced the results obtained during the first season. The highest total yield was achieved in the combined treatment with 'KeeP' + 'Green' with an increase from 12,220 to 18,070 kg ha^{-1} (Figure 6), i.e., 6,000 kg ha^{-1} more than the control. Therefore, the combined treatment increased the total yield consistently and significantly in two consecutive seasons. The treatments with 'KeeP' or

'Green' separately also increased yield significantly in both consecutive seasons but at a lower extent compared with the combined treatment (Figure 6).

After the third season the combined treatment of 'KeeP' + 'Green' caused a significant increase in the total yield from 8,026 to 12,153 kg ha^{-1} (Figure 6), i.e., 4,127 kg ha^{-1} more than the control. The treatments with 'KeeP' or 'Green' separately also increased yield significantly in relation to the control but not significantly different from the combined treatment (Figure 6).

'KeeP' or 'Green' caused an increase in the mean yield of three consecutive seasons from 11.3 to 14.9 and 14.7 t ha^{-1}, respectively (increment of about 30% above the control). Combined treatment of the nitrification inhibitor with phosphonate when the trees were fertilized with ammonium resulted in a significant mean yield increase to 16.4 t ha^{-1} (about 45% above the control).

***Botryosphaeria* infection**

Botryosphaeria branch dieback symptoms became evident only during 2020 season with 41.4% of the trees showing acute infection (Figure 7). 'KeeP' caused a decrease on *Botryosphaeria* expression lowering from 41.4 to 33.3% of the trees with acute infection (Figure 7). The combined treatment of 'KeeP' + 'Green' was effective with a dramatic decrease in the percentage of trees with acute infection from 41.4 to 6.1% (Figure 7).

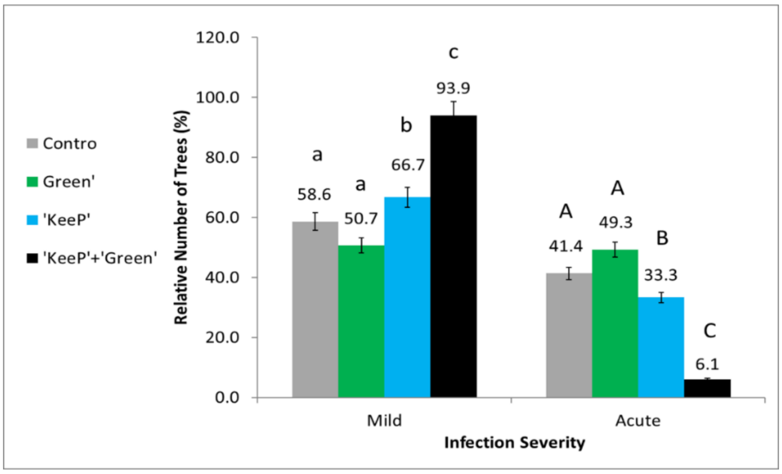

Figure 7. Relative number of trees showing mild or acute branch dieback related to *Botryosphaeria* infection during 2020.

CONCLUSIONS

A high crop level was recorded in the experimental section in 2017 before starting the trial and also during the three consecutive seasons of the research (the fourth, fifth and sixth years after planting). Proportional application of 'Green' or 'KeeP' increased significantly the total yield during three consecutive seasons by about 30%. The combined treatment with 'KeeP' and 'Green' increase the yield more significantly than every treatment alone (by 45%).

Part of the positive effects on the yield may be explained by the sharp increase in nitrogen in the soil caused by 'Green', by means of the accumulation of ammonium in the shallow soil layer. The rise in availability of potassium and phosphate by treatment with 'KeeP' may also explain part of the positive effects in yield. The evident effect of the combined effect of 'KeeP' + 'Green' on decreasing the sensitivity of the trees to the dieback related to *Botryospheria* symptoms may be also a factor determining improved yield.

In summary, application of 'KeeP' and 'Green' improves the fertilizer efficiency, decreasing sensitivity to *Botryosphaeria* symptoms causing an increase in crop yield.

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Literature cited

Crutzen, P.J. (1976). Upper limits on atmospheric ozone reductions following increased application of fixed nitrogen to the soil. Geophys. Res. Lett. *3* (*3*), 169–172 https://doi.org/10.1029/GL003i003p00169.

Lan, T., Han, Y., Roelcke, M., Nieder, R., and Cai, Z. (2013). Effect of nitrification inhibitor dicyandiamide (DCD) on gross transformation rates and mitigating N_2O emission in paddy soils. Soil Biol. Biochem. *67*, 174–182 https://doi.org/10.1016/j.soilbio.2013.08.021.

Perez-Jimenez, R.M. (2008). Significant avocado diseases caused by fungi and oomycetes. Eur. J. Plant Sci. Biotechnol. *2*, 1–24 http://www.globalsciencebooks.info/Online/GSBOnline/images/0806/EJPSB_2(1)/EJPSB_2(1)1-24o.pdf.

Singer, A. (2007). The Soils of Israel (Berlin, Heidelberg, New York: Springer), p.8–9 https://doi.org/10.1007/978-3-540-71734-8.

Steinberg, D., Freeman, S., Shulchani, R., Burstein, M., Simenski, E., Golani, M., and Maoz, I. (2016), Confrontation with *Botriosphaeria* in Avocado – Chapter 1. Research report publication (Israel: Department of Plant Pathology and Weed research, ARO the Volcani Center) (in Hebrew).