Effects of B, Zn, Cu, S and the Acidification of Irrigation Water on Avocado Hass

*J.M.Hermoso[1], M.D.Torres[2], and J.M.Farré[3] **
[1] Estación Exp. La Mayora. C.S.I.C. 29750 Algarrobo-Costa. Málaga. Spain.
e-mail: jmhermoso@eelm.csic.es,
[2] IFAPA de Málaga. Cortijo de la Cruz. 29140. Churriana. Málaga. Spain.
[3] Tropicales Fasip, S.L. Málaga. Spain.

Abstract

The experiment was carried out on adult trees growing on slightly calcareous shale soil. Eight treatments were compared during six years:

- B application through the irrigation water acidified or not with HNO_3 to pH 6.8.
- Soil application of Zn, with or without strong initial acidification with S, of a 60 cm diameter area.
- B applied through the irrigation water plus Zn applied on initially strongly acidified soil, as before. Foliar Cu was applied only in the first two years of the experiment.
- B applied through the irrigation water and Zn to the soil, with slight acidification with S of the 60 cm diameter application area.
- Irrigation with HNO_3 acidified water (pH =6.8).
- Untreated control.

The design was on randomized blocks with four replicates and four trees per plot.

Treatments very significantly increased leaf contents of the applied micronutrients. All treatments increased number of fruits and yields relative to control but differences were not generally statistically significant. Only B+Zn with slight soil acidification and irrigation with $HN0_3$ acidified water showed statistically significant differences.

Results support the application of B and Zn to increase leaf contents above 30 mg kg^{-1}. The slight acidification of soil or irrigation water deserves further studies. Strong initial soil acidification with S does not appear promising.

Key Words: Avocado, B, Zn, Cu, S, nitric acid, growth, yield

Resumen

Efectos de B, Zn, Cu, S y la Acidificación del Agua de Riego en el Aguacate Hass

El experimento se realizó con árboles adultos en suelo pizarroso ligeramente calizo. Se compararon ocho tratamientos durante seis años:

- Aplicación de B a través del agua de riego, acidificada o no con HNO_3 , a pH 6,8.
- Aplicación de Zn al suelo, con o sin fuerte acidificación inicial del mismo con S, sobre un área de 60 cm. de diámetro.
- B aplicado a través del agua de riego más Zn aplicado en suelo inicialmente acidificado como antes. Se añadió Cu foliar sólo en los dos primeros años del ensayo.
- B aplicado a través del agua de riego y Zn al suelo, con ligera acidificación con S del área de aplicación de 60 cm. de diámetro.
- Riego con agua acidificada con HNO_3 (pH=6,8)
- Testigo no tratado.

El diseño era en bloques al azar con cuatro repeticiones y cuatro árboles por parcela elemental.

Los tratamientos aumentaron muy significativamente los contenidos foliares de los micronutrientes aplicados. Todos los tratamientos aumentaron el número de frutos y la cosecha en relación con el testigo pero las diferencias no eran normalmente estadísticamente significativas. Sólo B+Zn con ligera acidificación del suelo y riego con sólo agua acidificada mostraron diferencias estadísticamente significativas.

Los resultados apoyan la aplicación de B y Zn para incrementar el contenido foliar por encima de 30 mg kg^{-1}. La acidificación ligera del suelo o del agua de riego merecen estudios posteriores. No parece de interés la fuerte acidificación inicial del suelo con S.

Palabras clave: Aguacate, B, Zn, Cu, S, ácido nítrico, crecimiento, cosecha

Introduction

It is very common to find B and Zn leaf levels between 20 and 40 mg kg^{-1}. Regretfully there are few experiments, with adult trees, that cover this range of leaf contents obtained through soil applications. In fact, Lahav and Whiley (2002) summarized the existing information, not showing any concluding result. In two short field experiments in Australia (Whiley, Smith, Saranah, Wolstenholme 1966) increasing leaf B from 22 – 23 miligram per kilogram (mg kg^{-1}) to 56 – 43 mg kg^{-1} increased fruit size but not yields. In South Africa (Bard and Wolstenholme 1997) differences were 10 % in yield and 4 % in mean fruit size. Liming increased absorption very significantly. Kadman and Lahav (1978), in a 6 year field experiment, compared differently fertilized trees with mean leaf contents of 34 (control) 38 and 48 mg kg^{-1}. The highest level had 15 % mean higher yields than control but differences were not statistically significant. The number of trees in the experiment was rather low.

Materials and methods

This experiment started in 2002, followed another two between 1996 and 2001. Torres, Hermoso y Farré (2003) and Hermoso, Torres y Farré (2007) show the materials and methods. The soil was slightly calcareous (Table 4). Irrigation water, with pH 8.6, had high HCO_3^- contents and low electrical conductivity. Spring grown leaf blades were analysed each autumn. Roots were analysed in April 2004 and 2005 during flowering. Roots, 4 – 7 mm diameter, were sampled in the top soil (0 – 5 cm depth) within the 60 cm diameter circle around the sprinkler. After eliminating the suberized epidermis with an stainless steal scraper, cortex and stele were analysed separately.

At the start of the experiment, in August 2002, the soils that had received in previous experiments B, Zn and Cu were analysed. Samples were taken at five depths between 0 and 50 cm, where near 100 % of the roots can be found, inside the fertilised circle. Stones (Ø >2 mm), approximately 50 % of total weight, were excluded before water extraction at ambient temperature (soil: water relation 1:2.5) (Torres et al. 2003). The top layer of leaves and decomposing leaf mulch (3 cm approximately) was excluded. The treatments applied between 2002 and 2008 are shown in Table 1. A summary of fertilizers applied in previous experiments (Torres et al. 2003) is also included.

B was applied with the irrigation water, acidified or not with $HN0_3$ to pH 6.8. Zn was applied to the soil in a 60 cm diameter circle around the microsprinkler with or without previous strong soil acidification with S. The control treatment did not receive any B, Cu or Zn. A control treatment with acidified irrigation water to pH 6.8 was also included. Foliar Cu was applied at prebloom and after fruit set in 2003 and only after fruit set in 2004 in one of the combined treatments (B + Zn + foliar Cu).

Two previous Cu soil treatments in 1996/2001 (Torres et al. 2003 and 2007) had only a minor effect in plant content (roots and leaves). Because of that they were replaced from 2002 by two combined treatments. B + Zn + S replaced Cu soil application. It only received one small S application. B + Zn + foliar Cu replaced a previous Cu soil application treatment with strong soil acidification with S.

From 2000 the alley centres were subsoiled yearly to prevent root crosses. In January 2004 plastic barriers were established to 60 cm depth in the contact areas between the 4 tree plots. The alleys were kept with mowed natural weed cover. Hedgerow pruning was done, manually, every summer. All treatments received 38.5 – 39 kg ha^{-1} $year^{-1}$ of N through the irrigation system. Treatments with acidified water at pH 6.8 received an average of 350 kg ha $year^{-1}$ of commercial HNO_3 (56 % HNO_3). The others received NH_4 $N0_3$ (34.4 % N). Mean leaf macronutrient contents, similar in all treatments, were N: 1.8 %, P: 0.15 %, K: 0,68 %, Ca: 1,27 % and Mg: 0.92 %.

Planting distances were 7 x 8 m. The design was on randomised blocks with 4 trees per plot and 4 replicates. Fruits were picked by size in two passes. Net yields and fruit number were recorded per tree. Potential yield included the fruits fallen during the picking season.

Table 1. Nutrient applications (2002 to 2008) [9]

Treatments	HNO_3 [1]	B [2]	Cu [3]		Zn [4]		S [5]	
	kg ha⁻¹year⁻¹	*kg ha⁻¹year⁻¹*	*kg ha⁻¹*	*year*	*kg ha⁻¹*	*year*	*kg ha⁻¹*	*year*
B [6a]		1,79						
B+HNO_3 [6b]	325-390	1,79						
B+Zn+S [7a]		3,58			39,28	*Nov. 2002*	178	*Nov. 2002*
B+Zn+ foliar Cu [7b]		3,58	5,10 2,55	*2003* *2004*	39,24	*Nov. 2002*		
Zn [8a]								
Zn+S [8b]								
Control								
Control+HNO_3	325-390							

(1) Comercial HNO_3 (PQS. Sevilla. 56% HNO_3). Annual applications varied according to irrigation requirements.

(2) As Solubor DF® (17,5% B) (Borax Europe Ltd.). (Mixture of H_3BO_3, $Na_2B_4O_7 \cdot 5H_2O$ and $Na_2B_{10}O_{16}^{-}$ $10H_2O$).

(3) Foliar application as copper oxychloride (50% Cu). (LUQSA).

(4) Soil applied as $ZnSO_4 \cdot 7H_2O$ (Liuzhou Wenda Metal & Chemicals Factory. 22% Zn).

(5) Soil applied commercial Cepsul®. (CEPSA. Micronized sulfur 98,5% S)

(6a)-(6b) In addition they received regular B applications from 1996, acidifying (6 b) or not (6 a) to pH 6.5 with HNO_3.

(7a)-(7b) In a previous experiment (*Torres et al. 2003*) in 1996-1998 they received 2,1 kg ha^{-1} $year^{-1}$ Cu through irrigation water as $CuSO_4$, (Industrias Químicas del Vallés, 25% Cu) acidified to pH 6,5 with commercial HNO_3 (7b) or not (7a).
In 1999-2001 they received 22, 25 kg ha^{-1} $year^{-1}$ soil applied Cu in a 60 cm diámeter around the sprinkler, acidifying (7b) or not (7a) the soil with 175 kg ha^{-1} $year^{-1}$ of S.

(8a)-(8b) They received in 1996-1998 in a previous experiment 71 kg ha^{-1} of Zn through the irrigation system acidified (8b) or not (8a) with HNO_3 to pH 6,5. They also received 78,3 kg ha^{-1} of soil applied Zn, with (8b) or without (8a), S in a 60 cm diameter around the sprinkler in 1999-2001 before the start of this experiment.

(9) Further details of previous treatments in Torres et al. (2003).

Results

Table 2. Mean leaf nutrient concentration (2003-2008) (mg kg^{-1})

Treatments	B	Cu	Zn
B	40,5	8,2	23,7
B+HNO_3	32,8	8,2	25,1
B+ Zn + S	40,8	6,9	33,9
B+ Zn + foliar Cu	36,3	15,3	32,2
Control	19,3	8,0	25,4
Control+HNO_3	18,9	8,4	26,0
Zn	17,1	7,8	43,0
Zn+S	16,0	7,5	41,0

Figure 1. Leaf Zn concentration

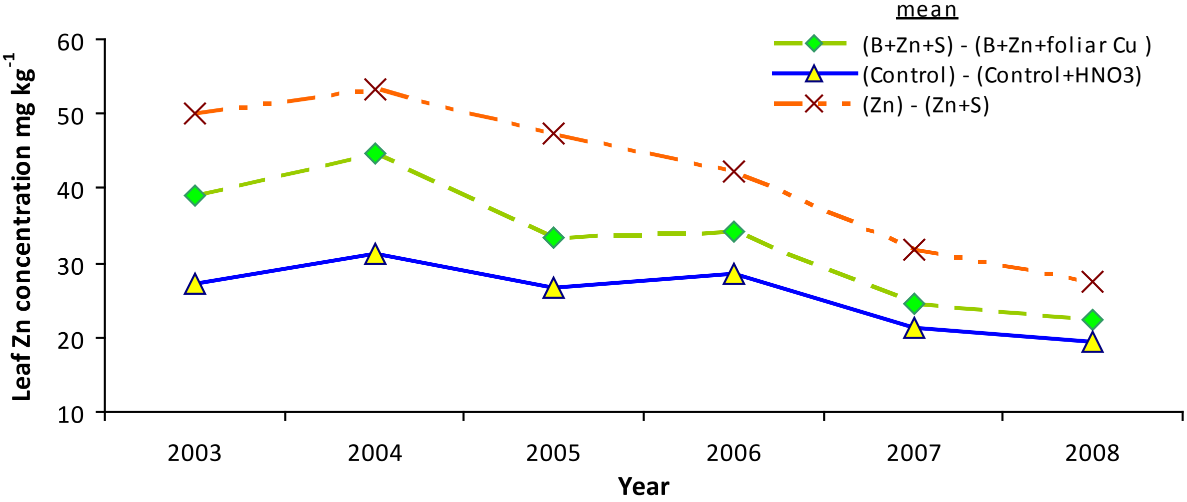
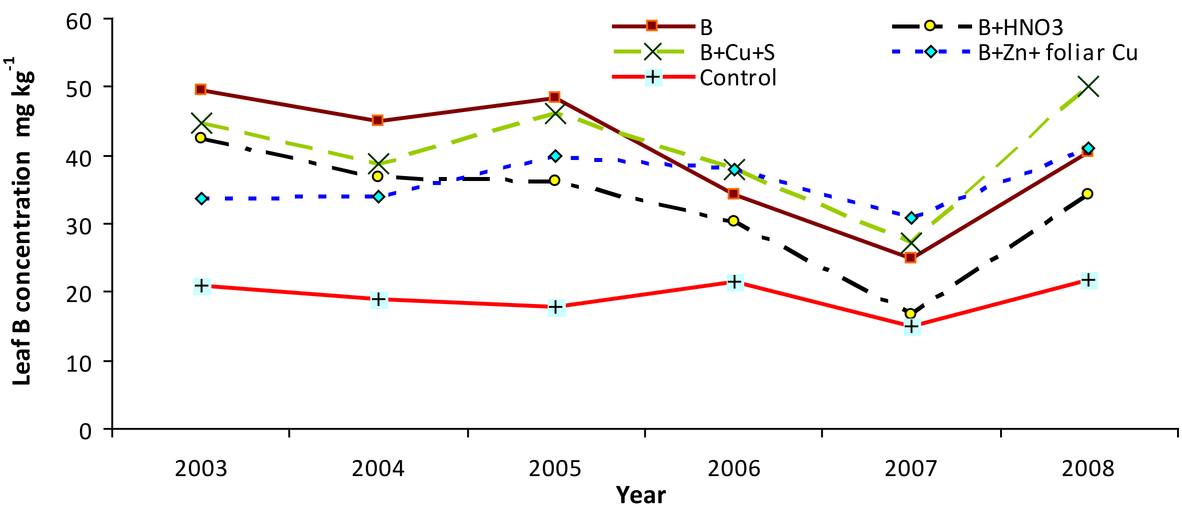


Figure 2. Leaf B concentration



Mean leaf concentrations of B, Zn and Cu can be seen in Table 2. The evolution of leaf Zn and B concentrations are shown in Figures 1 and 2. Treatments Zn and Zn + S that had received 78 kg ha^{-1} of Zn before the start of this experiment (Torres et al. 2003), showed high concentrations that persisted for 6 years. B + Zn + S and B + Zn + foliar Cu that only received a single application of 39 kg ha^{-1} at the start of this experiment had lower concentrations that persisted at a high level for only 2 years. Regarding the roots the low Zn applications increased concentration in cortex and stele (Table 3) specially in the soil strongly acidified before the experiment (B + Zn + foliar Cu). Double doses applied before this experiment, Zn and Zn + S, also increased root contents but, in this case, strong soil acidification had a smaller effect. The doses here applied (39 or 78 kg ha^{-1}) are 15.2 or 7.3 times smaller than those applied in a similar experiment in California (600 kg ha^{-1}) (Crowley, Smith, Faber and Mantley 1996). Given their 11 times larger application area (6.28 vs. 0.56 m^2 $tree^{-1}$) the application density, in grams per unit soil area, were similar in both experiments. It appears that applying reduced amounts over small areas it is possible to reduce costs and environmental impact. In both studies soils were basic and slightly calcareous. In our case, as expected, soil soluble Zn contents increased markedly after strong acidification (Table 4). This eliminated most $C0_3^{=}$ but did not increase leaf Zn contents (Table 2).

Table 3. Root nutrient concentration. Mean years 2004/2005* (mg kg^{-1})

		Treatments							
		B	B+HNO_3	B+Zn+S	B+Zn+ foliar Cu	Zn	Zn+S	Control	Control+ HNO_3
B	Cortex	23,5	24,5	30,0	31,0	15,0	15,0	14,5	14,5
	Stele	11,5	12,0	16,0	15,8	5,5	6,5	6,0	6,0
Cu	Cortex	6,0	6,0	9,5	9,3	5,0	5,6	5,6	6,0
	Stele	4,5	4,8	4,8	5,6	3,8	4,5	4,5	4,6
Zn	Cortex	28,5	30,3	35,5	50,0	56,3	48,3	31,5	30,5
	Stele	6,3	6,6	7,6	9,5	9,1	8,0	6,3	6,0

**Sampled in May-June*

Table 4. Soil analysis – August 2002

Treatment	Depth *cm*	pH	Electrical conductivity *dS m^{-1}*	Cu *mg kg^{-1}*	Zn *mg kg^{-1}*	Ca *mg kg^{-1}*	CO_3Ca *%*
B	0-5	7,41	0,37	0,17	0,09	25,50	0,22
	5-10	7,38	0,20	0,13	0,07	15,00	0,10
	10-25	7,29	0,21	0,11	0,05	19,00	0,16
	25-50	7,52	0,21	0,08	0,05	19,50	0,64
B+HNO_3	0-5	7,46	0,31	0,15	0,09	26,00	0,13
	5-10	7,40	0,22	0,12	0,07	17,50	0,09
	10-25	7,65	0,22	0,12	0,06	19,75	0,12
	25-50	7,75	0,22	0,10	0,04	21,25	0,28
B+Zn+S	0-5	7,09	0,25	3,20	0,09	16,25	0,12
	5-10	6,96	0,16	1,03	0,07	10,00	0,02
	10-25	6,93	0,16	0,68	0,07	14,25	0,00
	25-50	7,47	0,23	0,14	0,05	22,50	0,39
B+Zn+ foliar Cu	0-5	4,45	1,76	4,68	0,94	249,75	0,07
	5-10	4,16	0,36	1,34	0,25	21,25	0,07
	10-25	5,03	0,32	1,99	0,19	20,25	0,07
	25-50	4,99	0,39	1,32	0,28	36,50	0,07
Control	0-5	7,24	0,27	0,20	0,08	27,00	0,24
	5-10	7,19	0,17	0,10	0,07	15,50	0,10
	10-25	7,16	0,19	0,12	0,07	21,75	0,07
	25-50	7,46	0,21	0,08	0,04	23,50	0,22
Control+HNO_3	0-5	7,45	0,46	0,15	0,08	29,25	0,07
	5-10	7,44	0,27	0,13	0,08	19,75	0,06
	10-25	7,55	0,25	0,12	0,05	21,25	0,05
	25-50	7,65	0,27	0,10	0,04	21,75	0,30
Zn	0-5	7,13	0,37	0,20	4,09	23,50	0,23
	5-10	7,10	0,21	0,14	1,23	14,50	0,07
	10-25	7,29	0,28	0,11	0,51	22,75	0,13
	25-50	7,48	0,31	0,11	0,07	26,50	0,58
Zn+S	0-5	4,12	1,71	0,16	12,11	206,50	0,00
	5-10	4,28	0,38	0,07	6,71	25,50	0,00
	10-25	4,73	0,25	0,07	2,93	15,00	0,00
	25-50	6,13	0,52	0,06	3,20	61,50	0,30

In a previous study (Torres et al. 2003) continuous application of $ZnS0_4$ through the irrigation water, acidified or not to pH 6.5 with $HN0_3$, did not increase foliar Zn concentrations over 3 years. The increase in soil soluble Zn with the strong acidification, especially at depth, increased by about 50 times the pollution risk in underground water. Strong soil acidification should therefore not be recommended in the present experimental conditions.

Treatments B and B + $HN0_3$ received regular B applications from 1996, 6 years before the start of this experiment. On the other side B + Zn + S and B + Zn + foliar Cu only received B from 2002 but with double yearly amounts. This showed up in a progressive increase in their leaf concentrations along the experiment (Figure 2). Also in the markedly higher root concentrations in the middle of the experiment (Table 3). Decreasing pH of the irrigation water to 6.8 with $HN0_3$ (B + $HN0_3$ vs B) regularly reduced leaf levels but not root levels.

Foliar applications of Cu, 2 in 2003 and 1 in 2004 increased leaf Cu levels in autumn from 8 to 16-40 mg kg^{-1}. This big increase indicates good absorption pre and postbloom. Given the risk of toxicity no Cu treatments were applied in the last 4 years of the experiment when leaf contents stayed at 9 mg kg^{-1}, similar to controls. A small residual effect in the root stele was still apparent in 2004 – 2005 (Table 3). Soluble Cu contents in the soil in 2002, at the start of this experiment, can be seen in Table 4. They were higher in the treatments previously fertilised with Cu (B + Zn + S and B + Zn + foliar Cu) but did not affect leaf concentrations. Cu and Zn had similar behaviour in soil. Strong acidification increased soil soluble contents specially in depth.

Under treatments B + Zn + foliar Cu and Zn + S the massive application of S (525 kg ha^{-1} on 87 m^2 ha^{-1}) in the previous experiment eliminated most $C0_3^{=}$ reducing pH from 7.2 – 7.5 to 4 – 5 (Table 4). However lowering of irrigation water pH from 8.6 to 6.8 did not affect soil pH for 6 years. In November 2002, after soil analysis a single application of S (178 kg ha^{-1}) to B + Zn + S and B + Zn + foliar Cu slightly reduced soil pH (data not shown).

High S applications strongly increased electrical conductivity and soluble Ca of top soil and, to a lesser extent, the subsoil. In a parallel experiment (not shown) in calcareous soil this treatment very strongly increased soil $S0_4^{=}$.

Mean yield components in the 6 years are shown in Table 5. Data has been analysed by biennium with Statgraphics using as a base for covariance the results of the 1999/2000 biennium, before the start of the present experiment. Control trees had the lowest mean yields and fruit numbers. B + Zn + S, B + Zn + foliar Cu and T + HNO_3 had significantly larger net yields. B + Zn + S, T + HNO_3 and Zn had significantly higher fruit numbers. All treatments, including controls, had similar mean fruit weights, mean trunk areas and mean trunk area increases meaning that tree vigour was not affected.

Zn yields were larger than controls in the first biennium but similar in the other two when differences in leaf contents were smaller. B behaviour was different with yield differences increasing along the experiment (data not shown).

Table 5. Mean yearly crops 2004 – 2009

	Fruits tree^{-1} *nº*	Fruit weight *g fruit^{-1}*	Net yield *kg tree^{-1}*	potencial yield * *kg tree^{-1}*	Mean Trunk area *cm^2*	Trunk area increase *%*
Control	255 *a*	210 *ab*	49,04 *a*	49,62 *a*	1.072 *bc*	2,9 *a*
Control+ HNO_3	342 *cd*	201 *ab*	60,87 *bc*	61,32 *bc*	1.061 *abc*	3,1 *a*
B	279 *abc*	222 *ab*	55,83 *abc*	56,14 *abc*	1.070 *abc*	3,0 *a*
B+HNO_3	281 *abc*	213 *ab*	52,92 *ab*	53,59 *ab*	1.058 *abc*	3,1 *a*
Zn	324 *bcd*	193 *a*	56,73 *abc*	57,27 *abc*	1.061 *abc*	2,6 *a*
Zn+S	304 *abcd*	217 *ab*	55,27 *abc*	55,59 *abc*	1.018 *a*	2,5 *a*
B+Zn+S	368 *d*	194 *a*	64,87 *c*	65,56 *c*	1.091 *c*	2,9 *a*
B+Zn+ foliar Cu	290 *ab*	215 *b*	56,75 *b*	57,17 *b*	1.052 *b*	2,7 *a*

Different letters in the column indicate significant differences ($P \leq 0.05$)
** Including fruits fallen during the picking season*

Conclusions

It appears that, in contrast with other experiments (Coetzer, Robbertse, Barnard and Tomer 1994), none of the treatments reached toxic levels in roots or leaves. Heavy applications of Zn SO_4 to small soil areas has efficiently supplied the tree with Zn even in the presence of $C0_3^{=}$. Lowering pH with S increased by 65 % soil soluble Zn without affecting root or leaf contents. The higher soil soluble Zn, especially at depth, may increase leaching. However pollution of underground water would be reduced by the small fertilised area, only 0,86 % of the total surface area. In natural, not acidified, soil leaching would be negligible due to the low Zn contents of the subsoil. This should be therefore the recommended application technique. Similar soil behaviour had the $CuS0_4$ soil applications. But, contrary to Zn, Cu applications, with acid or basic soil pH, did not increase concentrations in the root stele or leaves. Therefore this should not be the recommended application technique. Torres et al. (2003) showed that application with irrigation water should not be recommended either. Pre or postbloom foliar applications clearly increased autumn leaf contents without, probably, a significant translocation to other tissues. In 2004, in spite of very high leaf contents, there were no differences in the root stele with controls. In addition, foliar contents from 2005 were similar to controls. B applied through the irrigation system increased root and leaf contents, increasing with the concentration applied. Reducing irrigation water pH from 8.6 to 6.8 with $HN0_3$ reduced B leaf contents may be due to a B-$N0_3^-$ antagonism (Loué 1986, p. 152). Some studies have shown a big increase in pollen tube growth with B applications (Coetzer and Robbertse 1987). But it should be considered that differences were observed between trees with 7 mg kg^{-1} (low) and 15 mg kg^{-1} (high) leaf B, well below the leaf contents in this experiment (16-22 and 33-41 mg kg^{-1} for untreated and treated trees).

Foliar Cu applications for two years, which very significantly increased leaf contents, did not affect yields. This could be because mean leaf contents in control trees were 8 mg kg^{-1}, in the sufficiency level (Lahav and Whiley 2002).

All treatments had slightly higher yields than control. This supports the results in Australia with B and Israel with Zn (see Introduction). However, only three treatments had statistically significant differences with control. B + Zn + S, with a small S application that only slightly affected soil pH, and T + HNO_3 that, applying irrigation water at pH 6.8, could slightly and temporarily affect the pH of the soil solution. B + Zn + foliar Cu, that had a very low soil pH due to previous massive high S applications, had also significantly better yields. None of the treatments increased fruit weight. It can therefore be concluded that increasing both foliar B from 19 mg kg^{-1} and foliar Zn from 25 mg kg^{-1} to the 35-41 mg kg^{-1} range may increase yield under the condition of the present experiment. This may happen even without clear deficiency symptoms in leaves or fruits.

References

Bard, Z & Wolstenholme, BN 1997, 'Soil boron application for the control of boron deficiency in Kwazulu – Natal avocado orchards'. *S. Afr.Avocado Growers' Assoc. Yearbook*, vol. 20, pp. 13-15.

Coetzer, A & Robbertse, PJ 1987, 'Pollination biology of *Persea americana* Fuerte'. *S.Afr. Avocado Growers' Assoc. Yearbook*, vol. 10, pp. 43-44.

Coetzer, LA, Robbertse, PJ, Barnard, RO & Tomer, E 1994, 'Uptake and transport of boron in avocado seedlings'. *S.Afr. Avocado Growers' Assoc. Yearbook*, vol. 17, pp. 95-98.

Crowley, DE, Smith, W, Faber, B, & Mantley, JA 1996, 'Zinc fertilisation of avocado trees'. *Hort. Science*, vol. 31, no. 2, pp. 224-229.

Hermoso, JM, Torres, MD, & Farré, JM 2007, 'Nutrición B, Zn y Cu del aguacate Hass. Influencia en cosecha'. *Actas VI Congreso Mundial del Aguacate. Viña del Mar*. http://www.avocadosource.com/wac6/es/extenso/3a-96.pdf

Kadman, A & Lahav, E 1978, 'Experiments with Zn supply to avocado trees'. *Plant Nutrition*, *Proceedings of the 8th International Colloquium on Plant Analysis and Fertilizer Problems. Auckland. N.Z.* D.S.I.R. Information Series Nº 134. Wellington Government Printer 1978, pp. 225 – 230.

Lahav, E & Whiley, AW 2002, 'Irrigation and mineral nutrition'. In: A.W. Whiley, B.Schaffer and B.M. Wolstenholme (Eds.) The avocado: Botany, production and uses. CABI Publishing. New York, pp. 259-297.

Loué, A 1986, 'Les oligo-elements en agriculture'. Agri-Natham International. Paris.

Torres, MD, Hermoso, JM, & Farré, JM 2003, 'Nutrición B, Zn y Cu del aguacate. Comparación de métodos de fertilización'. *Actas V Congreso Mundial del Aguacate. Granada.Málaga. Junta de Andalucía*, vol. 1, pp. 381-386.

Whiley, AW, Smith, TE, Jaranah, JB, & Wolstenholme, BN 1996, 'Boron nutrition of avocados'. *Talking avocados*, vol. 7, no. 2, pp.12-15.