

LIME-INDUCED CHLOROSIS IN AVOCADO AND A POSSIBLE METHOD OF CONTROL

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SUMMARY

Edranol, a Guatemalan variety, in glasshouse tests with seedlings in soil limed with calcium carbonate was found to be very susceptible to a leaf chlorosis. Since very young leaves of chlorotic plants were greened with iron sprays and since the iron content of leaves, stems, and roots of chlorotic plants was considerably reduced, the chlorosis was believed to be the result of iron deficiency. Manganese in the plants was greatly reduced by liming, but iron was reduced only on those plants that became chlorotic. Of the test plants in limed soil, 78 per cent of the Edranol seedlings became chlorotic within ten months while 30 per cent of Topa Topa seedlings, a Mexican variety, became chlorotic. There was very little difference in the inorganic composition between green Edranol and Topa Topa and between chlorotic Edranol and chlorotic Topa Topa. Chlorotic plants appeared to be unable to use nitrogen fertilizer without leaf burning. Susceptibility to lime-induced chlorosis is very possibly inherited and open pollinated seedlings very likely contribute to the variability within a variety. The study demonstrated that in experimental work with seedling avocados a very large number of replications are necessary. Control of the chlorosis has been achieved by application to the soil of ethylene-diamine-tetracetic acid (EDTA) containing iron, both in the glasshouse and in the field. There are difficulties in keeping this iron complex in solution at pH values above 7.5. These studies are of insufficient duration to make sound recommendations for its practical use.

INTRODUCTION

Halma and White (3) in avocado rootstock trials in Santa Barbara and Orange counties observed that scions on Guatemalan rootstocks often became chlorotic, while those on Mexican and West Indian rootstocks were much less susceptible. Since they did not study the nature or cause of the chlorosis and since an observation was reported from San Diego County concerning- a possible relationship of soil lime to root rot, a project was started for the purpose of studying the effects of lime in soil on avocados. This report covers the first of these studies .

MATERIALS AND METHODS

Mexican and Guatemalan seedlings were grown from November 1, 1951, until August 13-15, 1952, in a control soil and in six levels of artificially limed soil. The concentrations of lime were 0, $\frac{1}{4}$; 1, 2, 5, 10, and 15 per cent of U.S.P calcium carbonate as per cent of weight of oven-dry soil. The sandy loam soil was virgin and from a hilltop near U.C.L.A., and before any treatments it had a pH of 6.3 and an exchangeable calcium content of about 10. It was sieved through a $\frac{1}{2}$ -inch screen and placed in gallon glazed pots without bottom drainage. The calcium carbonate was weighed and mixed separately for each pot.

Edranol and Topa Topa varieties were chosen to represent the Guatemalan and Mexican races, respectively, because seed of them were available. One hundred seeds of each were germinated and 72 of each were planted, one per pot, directly into the treated soils after germination. Nine replications were used for each variety and treatment except that there were two sets of control plants for each variety. An effort was made to place the same number of large and small seedlings into each treatment. The plants were grown on benches in a well-lighted glasshouse and irrigated with distilled water as needed. Since there was no bottom drainage extreme care was taken to avoid waterlogging and excess drying. No difficulties were experienced with water and it is believed that all the results were independent of water complications. Ammonium nitrate was the only fertilizer used and was uniformly applied to all plants when the need was indicated by some. Applications of $\frac{1}{2}$ gram of nitrogen after five months and again after eight months were made.

Only five plants of each treatment were harvested for chemical analysis. The remainder were kept for additional studies. Height (from 1.5 inches above point of cotyledon attachment) and diameter (at the same point) measurements of all plants were made at that time. Fresh weight measurements were made of the harvested plants and they were divided into leaves, stem:-, and roots. All these measurements were analyzed statistically. All fractions were washed thoroughly with tap water, 0.3N H_2SO_4 to remove contaminating iron (5), and distilled water, dried at 70°C and ground in a Wiley mill to pass a 60-mesh screen. The methods used in inorganic analyses have been reported (10), except that both 80 per cent alcohol soluble and insoluble nitrogen determinations were made of leaf material.

Small and large chlorotic avocado trees in Orange County were used for control studies. The calcium carbonate content of the soil on which these trees were growing varied from one to 2.5 per cent and the pH from 7.1-7.6.

RESULTS AND DISCUSSION

Within five months after planting of the seedlings in the limed pots, those in the 10 and 15 per cent treatments were noticeably shorter and lighter green in color than the control plants. Between then and the harvest date

Table 1
Diameter, height, and fresh weight measurements of avocado seedlings
grown ten months in the glasshouse with different levels of
calcium carbonate in the soil
Coefficient

Treatment CaCO ₃	Diameter	Height	of variation		Relative height	Fresh Weight ^b	
			of height ^a	height		whole plant	stems
%	mm.	cm.	%	%	gm.	gm.	
TOPA TOPA							
Mean of all	12.5	112	30.0	72	207	90	
0	15.9	145	8.7	100	311	144	
¼	12.4	103	47.4	71	188	84	
1	12.1	117	30.1	81	181	87	
2	11.6	102	30.7	70	225	93	
5	11.9	108	31.3	75	194	78	
10	12.7	118	25.7	82	233	102	
15	10.8	90	36.1	62	115	45	
F. value ^c	4.8**	5.8**			2.7*	3.1*	
L.S.D. .01	3.2	30			NS	NS	
L.S.D. .05	2.5	23			119	56	
EDRANOL							
Mean of all	12.2	93	41.4	62	160	74	
0	16.9	150	30.0	100	346	188	
¼	14.3	112	32.3	75	211	102	
1	11.1	83	48.4	56	256	82	
2	12.1	87	39.0	58	111	50	
5	9.8	71	48.2	47	56	24	
10	10.2	70	48.3	47	83	48	
15	10.7	76	53.8	51	58	24	
F. value ^c	10.5**	3.7**			5.3**	5.4**	
L.S.D. .01	3.3	31			209	113	
L.S.D. .05	3.5	23			155	84	

^a Coefficient of variation is standard deviation expressed as per cent.

^b Weights were taken on only five of the nine seedlings in each treatment.

^c F. values marked * are significant at the .05 level and those marked ** are significant at the .01 level. L.S.D. means least significant difference.

progressively more plants and at lower lime levels became chlorotic and those at the high lime levels became more severely chlorotic. At the time of harvest marked differences in tolerances to lime were apparent between and within the two varieties (figure 1). An observational estimation resulted in 42 of 54 (78 per cent) Edranol and 16 of 54 (30 per cent) Topa Topa being classed as chlorotic. This corresponds closely with estimates made by Halma and White (3) in their rootstock trials. Of course lime is not uniformly distributed in field soils and for this reason such estimates have limited meaning. Since Edranol, a Guatemalan, was more susceptible to the chlorosis than was Topa Topa and since iron spray in the very new leaves resulted in greening, it is quite certain that the chlorosis observed by Halma and White was lime-induced chlorosis. There are similarly tolerant and susceptible grape rootstocks (11) and pear (1) and

citrus rootstocks (2) to lime-induced chlorosis.

The lack of uniform responses to lime by avocados indicates rather large genetic variation of these particular seedlings within each of the two varieties. This is apparent both in figure 1 and in the values for coefficient of variation of height given in table 1. The coefficient of variation was lowest for the control seedlings and in general much higher for limed soils. Edranol was, of course, more variable than was Topa Topa because more of them were chlorotic.



Fig. 1. (Top.) The Topa Topa seedlings grown on artificially limed soil in pots in the glasshouse; from left to right the lime content of the soil was 0, $\frac{1}{4}$, 1, 2, 5, 10, and 15 per cent. (Bottom.) The Edranol seedlings placed in the same order. Only one set of the control seedlings is shown.

Height, diameter and fresh weight measurements are also in table 1 with their statistical evaluation. The effect of lime was very apparent and significant for the $\frac{1}{4}$ per cent lime treatment for both varieties, indicating that some avocados are extremely sensitive to lime. Chlorotic avocados on Guatemalan rootstocks have been observed in the field on soils equally low in lime. The pH of the potted soil at $\frac{1}{4}$ per cent lime was about 7.0 when the seedlings were planted and had dropped slightly by the end of the experiment. The data in table 1 indicate quite clearly that the Edranol seedlings were in general more severely injured at lower lime levels than were Topa

Topa seedlings. More Edranol seedlings grown in 5 and 10 per cent lime were severely injured than Topa Topa in the same lime concentrations.



Fig. 2. Chlorotic *Edranol* seedlings with burned leaves following application of ammonium nitrate to the soil.

The content of leaf nitrogen is in table 2. Nitrogen composition will be discussed first because of special and differential effects of nitrogen when it was added as a fertilizer. When nitrogen was added to the soil the green plants responded with new growth and improved color. About one week after the addition of the second nitrogen application there was a severe leaf burn similar to excess ammonium nitrogen damage (figure 2) on most of the chlorotic plants. None of the green plants were similarly affected. It is quite possible that chlorotic plants are unable to use nitrogen properly and this was partially confirmed by the finding of large amounts of water-soluble nitrogen in chlorotic leaves. This phenomenon has been observed by other workers (4, 9, 11). Leaves of small green plants contained less total nitrogen than small chlorotic plants. Since the stems and roots have not yet been analyzed for nitrogen, it is not yet known whether the effect is the result of different distribution among plant parts or of greater absorption of either nitrate or ammonium nitrogen by chlorotic plants. There were large increases of total nitrogen as well as soluble nitrogen by chlorotic leaves. Some but not all of this is the result of the small size of chlorotic plants. With very few exceptions there were no real differences between the composition of *Edranol* and *Topa Topa* and there were no special differences for nitrogen. The manganese and iron contents of the plants were of special interest. Liming always resulted in reduced manganese content of leaves, stems, and roots, reductions being greater for the chlorotic plants than for green ones. Liming did not result in reduced iron content of plants that remained green but tended to result in reduced iron on those that became chlorotic. This was especially noticeable when all elements in the leaves were calculated as the ratio to nitrogen to eliminate some of the complications resulting from plant stunting (table 2). Since greening was

obtained for iron sprays and not for manganese sprays, the deficiency is undoubtedly one of iron. Stems of the low-lime Topa Topa seedlings contained more manganese than those of Edranol, and roots of chlorotic Edranol contained less iron than roots of chlorotic Topa Topa. Both these factors could be important. There was no evidence of iron accumulation in roots and stems of chlorotic plants, and if this is true the chlorosis is caused by lime resulting in insufficient iron absorption. Only the fleshy portions of the roots, however, were analyzed because of the difficulty in removing soil particles from the fine roots, and there is a possibility that iron could have accumulated there in the chlorotic plants.

The calcium content was increased by liming (table 3) and was greater for chlorotic plants than for green ones. Relative to nitrogen (table 2) the increase was less pronounced. The Ca-N ratios are smaller for chlorotic than for green plants and could be the result of real as well as relative nitrogen increases in chlorotic leaves. Calcium is usually decreased (7, 9) but not always (9) in chlorotic plants. Decreased calcium is usually associated with increased potassium in chlorotic plants (7, 9). In the present study potassium as well as calcium in all limed plants increased, but chlorotic plants increased more than did non-chlorotic plants so that the K-Ca ratio for leaves was higher for the chlorotic plants than for green ones. This is typical of chlorotic plants and is believed to be a result rather than a cause of chlorosis. The U.S.P. calcium carbonate used to lime the soil in this study was possibly more soluble than that naturally found in soil, and this could partly account for the tendency for calcium in plants to increase when the soil was limed.

In general Topa Topa seedlings contained less phosphorus than did Edranol seedlings. It is not known whether this had any bearing on susceptibility to lime-induced chlorosis. There was very little difference in magnesium between varieties or for the lime treatments.

Table 2

Nitrogen content and ratio of other nutrient elements to nitrogen for some of the leaves of avocado seedlings described in figure 1 and table 1 but arranged into fewer groupings for simplicity

Treatments as relative amounts of CaCO ₃ and plant condition	No. analyzed	Nitrogen		Total %	Ratio of percentages						
		Alcohol soluble %	Alcohol insoluble %		Fe/N	Mn/N	Ca/N	K/N	P/N	Mg/N	
					Topa Topa						
Control	5	.11	1.40	1.51	.0069	.0285	1.05	0.64	.11	.27	
Low-green	4	.13	1.55	1.68	.0066	.0135	1.33	0.54	.07	.24	
Low-chlorotic	4	.52	2.14	2.66	.0035	.0047	1.01	0.52	.01	.15	
High-green	3	.12	1.44	1.56	.0063	.0053	1.45	0.77	.07	.31	
High-chlorotic	4	.63	2.21	2.84	.0018	.0020	1.32	0.62	.04	.19	
					Edranol						
Control	5	.10	1.33	1.43	.0073	.0394	1.09	0.63	.06	.28	
Low-green	6	.13	1.55	1.68	.0063	.0148	1.41	0.65	.07	.28	
Low-chlorotic	7	.55	1.82	2.37	.0028	.0024	0.98	0.61	.03	.17	
High-green	1	.08	1.27	1.35	.0086	.0059	1.94	0.73	.06	.33	
High-chlorotic	6	.48	1.67	2.15	.0033	.0017	1.42	1.14	.05	.29	

Table 3
Inorganic nutrient content of leaves, stems, and roots of avocado seedlings grouped as in table 2^a

Treatments as relative amounts of CaCO ₃ and plant condition	Fe		Mn		Ca		K		P		Mg		Ca/K ^b	
	T.	E.	T.	E.	T.	E.	T.	E.	T.	E.	T.	E.	T.	E.
	ppm		ppm		%		%		%		%		Ratio	
	105	104	430	563	1.59	1.56	0.97	0.90	.167	.081	.41	.40	0.61	0.58
Control	111	106	227	249	2.24	2.18	0.91	1.09	.118	.119	.41	.47	0.41	0.50
Low-green	92	67	125	58	2.67	2.32	1.37	1.44	.103	.060	.39	.40	0.51	0.62
Low-chlorotic	98	116	83	80	2.27	2.61	1.18	0.98	.109	.076	.48	.44	0.52	0.38
High-green	52	71	57	36	3.74	3.05	1.77	2.45	.118	.100	.55	.61	0.47	0.80
High-chlorotic	Leaves													
	ppm		ppm		%		%		%		%		Ratio	
	51	58	122	189	0.68	0.88	0.63	0.68	.073	.063	.10	.18	0.93	0.77
Control	47	60	145	41	0.98	0.90	0.75	0.74	.137	.079	.16	.19	0.77	0.82
Low-green	50	63	106	11	1.86	1.32	0.80	0.75	.044	.030	.20	.19	0.43	0.57
Low-chlorotic	51	84	9	6	1.11	1.58	0.87	0.97	.087	.052	.17	.26	0.78	0.61
High-green ^c	54		6	5	1.45	1.58	0.91	0.97	.041	.062	.21	.26	0.63	0.61
High-chlorotic	Stems													
	ppm		ppm		%		%		%		%		Ratio	
	375	378	60	100	0.20	0.25	0.47	0.47	.091	.056	.08	.09	2.35	1.88
Control	237	328	12	5	0.36	0.39	0.42	0.48	.116	.071	.09	.11	1.17	1.23
Low-green	208	161	10	5	0.79	0.55	0.48	0.54	.048	.037	.12	.13	0.62	0.98
Low-chlorotic	205		6	6	0.37	0.36	0.44	0.65	.088	.062	.09	.11	1.19	1.81
High-green	230	127	4	4	0.56	0.36	0.55	0.65	.044	.062	.11	.11	0.98	1.81
High-chlorotic	Roots													

^a T. is Topa Topa; E. is Edranol.

^b The Ca/K is the ratio of the percentages.

^c Leaf analysis only was made of high-lime green Edranol because there was only one plant in that category and it was kept growing for further study.



Fig. 3. *A chlorotic Edranol seedling ten days after soil treatment with ammonium nitrate, the same seedling 30 days later and on day of soil application of 250 mgm. of iron in the EDTA complex, and the same seedling 74 days later, reading from left to right.*

When it was learned that the chlorosis was a lime-induced iron disorder, attempts were made to control the deficiency by the use of ethylene-diamine tetracetic acid (EDTA) as a chelating agent for iron to keep applications of it soluble in the soil. This chemical had been successfully used by Stuart and Leonard (8) to control iron chlorosis of citrus on acid soil after Jacobson (6) had shown that EDTA would keep iron in solution in nutrient cultures.

Excellent recovery (figure 3) of chlorotic avocados on artificially limed soil was obtained in the glasshouse by the application of 250 mgm. of iron with EDTA, which was prepared according to the method of Jacobson (6). Trials were made in the field on trees in three different groves with the dry commercial sodium salt of EDTA, containing 13 per cent iron. Thirty-gram applications to small trees resulted in excellent recovery and in much new growth. One pound of the salt washed into the soil under a fairly large eight-foot tree resulted in greening in about three weeks' time. These studies are not of sufficient duration to know how much of the salt should be applied or how long the effect will last. The pH of the soil in one grove was 7.6 and was less in the others. In laboratory tests it has been demonstrated that EDTA begins to lose its effectiveness in keeping iron in solution somewhere between pH 7.5 and 8 and becomes progressively ineffective at higher pH values. It is possible that other chelating agents will be more effective than EDTA. Soil applications of EDTA do have some promise for keeping micronutrients available in the soil. Leaf sprays of iron containing EDTA on chlorotic avocados caused greening in spots but were no better than other iron sprays.

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