SOME RESPONSES OF VALENCIA ORANGE SEEDLINGS TO VARYING CONCENTRATIONS OF CHLORIDE AND HYDROGEN IONS [1]

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MUCH OF the citrus produced in the western and southwestern states is grown under conditions involving some degree of salinity of the soil solution. It has seemed desirable to extend the investigations noted below to include the anatomical and physiological responses of citrus seedlings to controlled concentrations of chloride and hydrogen ions. Since the root is in most intimate contact with the soil solution, emphasis has been placed on the structural and functional behavior of that organ in relation to the conditions imposed upon the experimental plants.

The nutritional requirements of citrus seedlings have been investigated with respect to nitrogen and the absorption of other essential ions (Breazeale, 1919; Chapman and Liebig, 1937, 1940); and the response of seedlings grown in water cultures to various environmental factors-temperature, aeration, and hydrogen ion concentration-has been studied (Girton, 1927). The absorption of chloride by rough lemon and ***Poncirus trifoliata*** seedlings in relation to the intake of nitrate, calcium, and sodium ions has been investigated by Haas and Reed (1926), and the same authors have reported on the effects of sodium, potassium and calcium on young orange trees (1923). The anatomy of citrus roots has been examined in relation to osmotic pressure and periodicity of growth (Cossmann, 1939), and the developmental anatomy of the seedling and roots of the Valencia orange has been described by Hayward and Long (in press).

METHODS AND EXPERIMENTAL DESIGN.-The seedlings were grown in water culture to permit periodic observation of the developing roots and to determine their condition with respect to lateral root formation, production of root hairs and other growth responses. ***Since it*** has been noted (Hoagland and Arnon, 1938) that aeration of the culture solution may have a marked effect on the development of the plant, half of the cultures under each treatment were aerated by a low pressure continuous flow system while the other half were maintained without aeration.

Selected Valencia orange seeds were germinated in ***Sphagnum*** moss in September, 1940, and in November 288 of the most uniform were divided into thirty-six groups of eight each. Each lot was assigned by chance to one of the designated cultures which were randomized on greenhouse benches.

Three culture solutions were used: (1) the base nutrient solution, Hoagland's number two (1938),[2] (2) the base nutrient solution plus 50 m.e. chloride per liter, (3) the base nutrient solution plus 100 m.e.

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2 This solution contains the following salts:

Salts	m.e./l.
$NH_4H_2PO_4$	3
KNO_3	6
$Ca(NO_3)_2$	8
$MgSO_4$	4

TABLE 1. *Number of leaves per plant and percentage of chlorosis and tip burn.*

Series	Salt treatments	No. of plants	Leaves per plant	Chlorosis,[a] per cent	Tip burn,[b] per cent
A (pH 5.5-6.0, unaerated)	1-B. N.	25	8	0	1
	2-50 m.e. Cl-/1	23[c]	7	1	7
	3-100 m.e. Cl-/l	18	*6.3*	*23*	16
AO (pH 5.5-6.0, aerated)	I-B. N.	24	*7.4*	*0*	0
	2-50 m.e. Cl-/l.	22	*6.6*	10	7
	3-100 m.e. Cl-/l	11	*5.0*	*58*	38
B (pH 7.5-8.0, unaerated)	1-B. N.	24	*4.8*	*3*	0
	2-50 m.e. Cl-/l	21	*5.3*	*26*	22
	3-100 m.e. Cl-/l.	10	*4.0*	*62*	50
BO (pH 7.5-8.0, aerated)	I-B. N.	24	*6.8*	*12*	1
	2-50 m.e. Cl-/l.	22	*5.1*	*40*	22
	3-100 m.e. Cl-/l	11	*3.5*	*73*	71

[a] Based on average severity of symptoms for three replications.
[b] Percentage of leaves showing symptoms.
[c] See mortality figures, table 4.

chloride per liter. The chloride for solutions 2 and 3 was supplied as NaCl 50 per cent, $MgCl_2$ 25 per cent and $CaCl_2$ 25 per cent. Micro-elements were added in the following concentrations: boron, 0.5 ppm. ; manganese, 0.5 ppm. ; zinc 0.05 ppm. ; copper 0.02 ppm.; molybdenum, 0.01 ppm. Five ppm. of iron was added initially as citrate, and further additions were made as needed,

Two levels of hydrogen ion concentration were maintained by daily adjustment of the pH values with HNO_3 or KOH: Series A-pH value within range 5.5 to 6.0; Series B-pH value within range 7.5 to 8.0. Half of the cultures in each series (designated with an 0) were aerated with a carbon pipe aerator operated under a continuous pressure of approximately 8 pounds. The other half (without the 0 designation) were unaerated. Each treatment was replicated three times.

All the seedlings were started in the base nutrient solution, and the pH value was adjusted within the

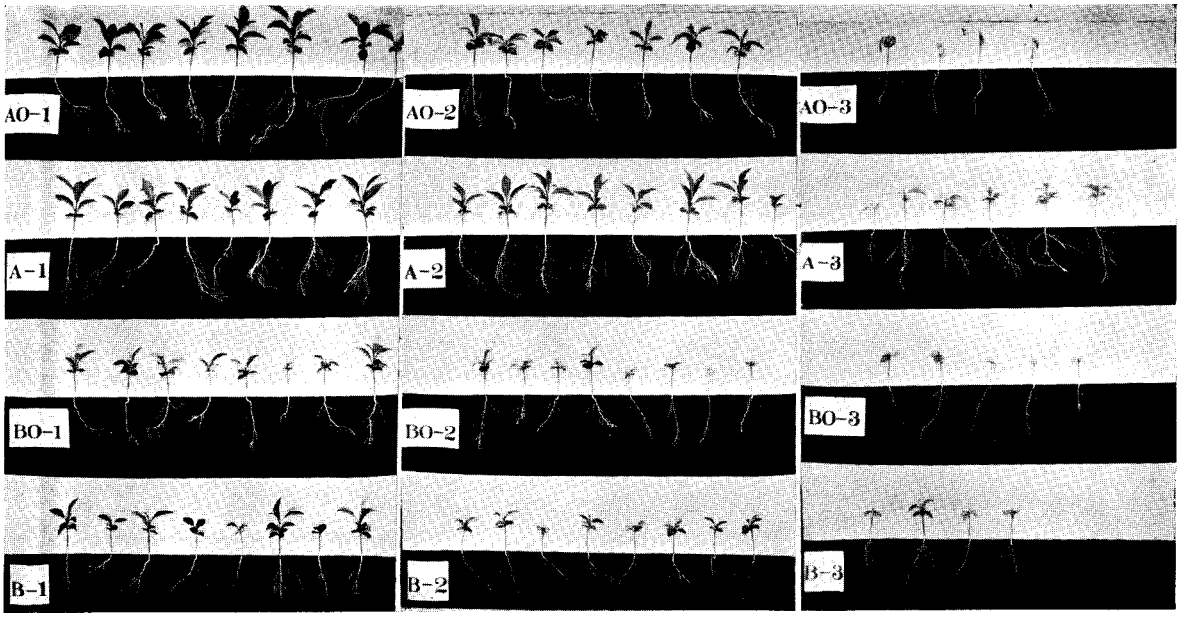
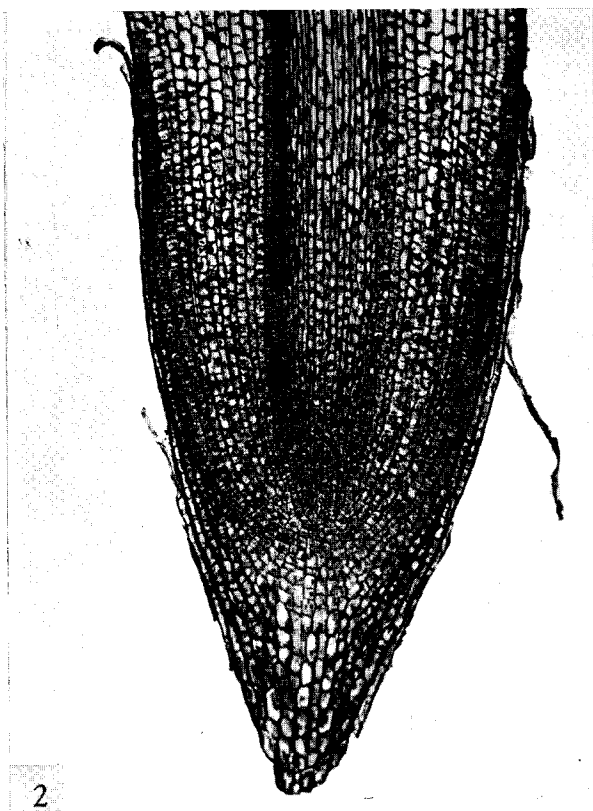


Fig. 1. Valencia orange seedlings at the conclusion of the experimental period showing relative development under the various treatments. Each panel represents the seedlings in one replication. The A and AO series were maintained at pH 5.5-6.0, the B and BO series at pH 7.5-8.0. **The cultures designated with an 0 were aerated. No. 1 cultures contained the base nutrient solution, No. 2 the base nutrient solution with 50 m.e. chloride per liter added, No. 3 with 100 m.e. chloride per liter added. The smaller number of plants in the No. 3 cultures represents the approximate mortality incurred under that treatment.**



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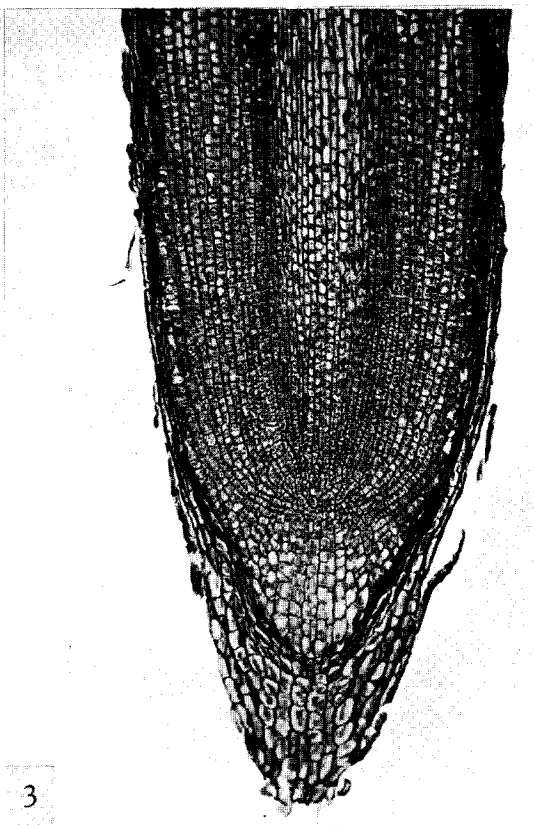

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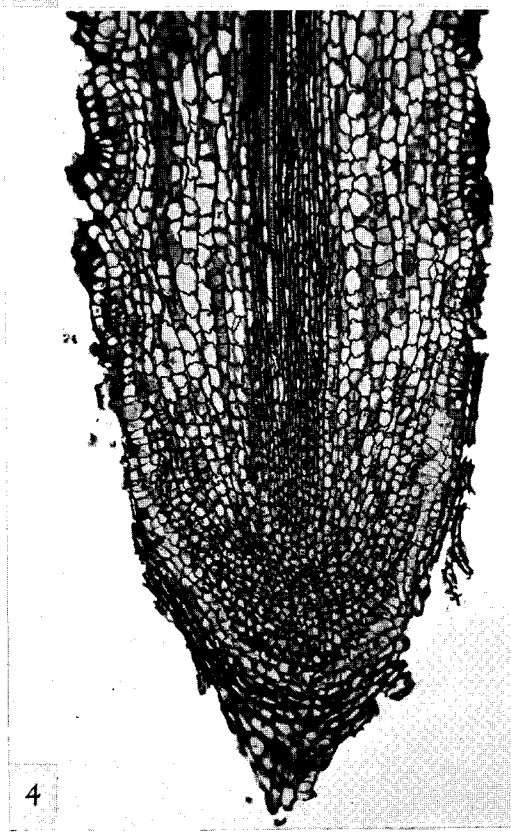

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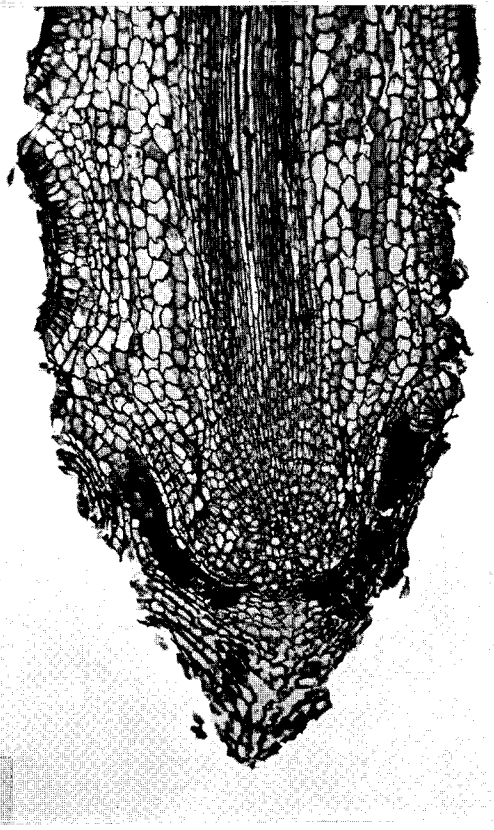

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TABLE 9. Harvest data. Summary.

Series	Salt treatments	No. of plants	Total length cm.	Average per plant: Fresh wt. mg.	Dry wt. mg.	Fresh wt. roots mg.	Dry wt. roots mg.	No. lateral roots	No. white root tips
A (pH 5.5-6.0, unaerated)	1-B. N.	25	22.7	1,750	360	520	90	20.8	8.8
	2-50 m.e. Cl-/l.	23	22.4	1,580	290	420	70	17.5	6.4
	3-100 m.e. Cl-/l.	18	19.9	1,130	180	320	50	15.8	9.6
AO (pH 5.5-6.0, aerated)	1-B. N.	24	22.9	1,730	340	550	100	23.0	10.5
	2-50 m.e. Cl-/l.	22	20.3	1,310	230	370	60	17.5	5.5
	3-100 m.e. Cl-/l.	11	18.0	750	130	260	40	13.4	2.2
B (pH 7.5-8.0, unaerated)	1-B. N.	24	18.0	940	170	360	60	11.2	6.7
	2-50 m.e. Cl-/l.	21	17.6	840	130	300	40	14.9	.6
	3-100 m.e. Cl-/l.	10	17.3	640	100	240	30	14.4	.3
BO (pH 7.5-8.0, aerated)	1-B. N.	24	20.6	1,220	230	440	80	20.9	2.0
	2-50 m.e. Cl-/l.	22	18.8	910	140	310	60	16.7	.5
	3-100 m.e. Cl-/l.	11	16.8	500	100	210	30	12.9	.1

specified range of the series. In treatments 2 and 3, the chloride salts were added by increments of 25 milliequivalents per liter at two-day intervals until full concentration was reached, and complete changes of all solutions were made as required. The plants were grown for about two months, the final harvest being January 28, 1941.

GROSS MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES.--Differences in leaf color were detectable between the A and B Series within a week after the solutions were up to full concentration; and, at the end of two weeks, the leaves of plants grown at the lower pH value were definitely darker green. Chlorosis was more pronounced at the high chloride levels in all series, but was intensified by high pH values (table 1). Under equivalent salt treatments, the plants in aerated cultures ivere somewhat more chlorotic.

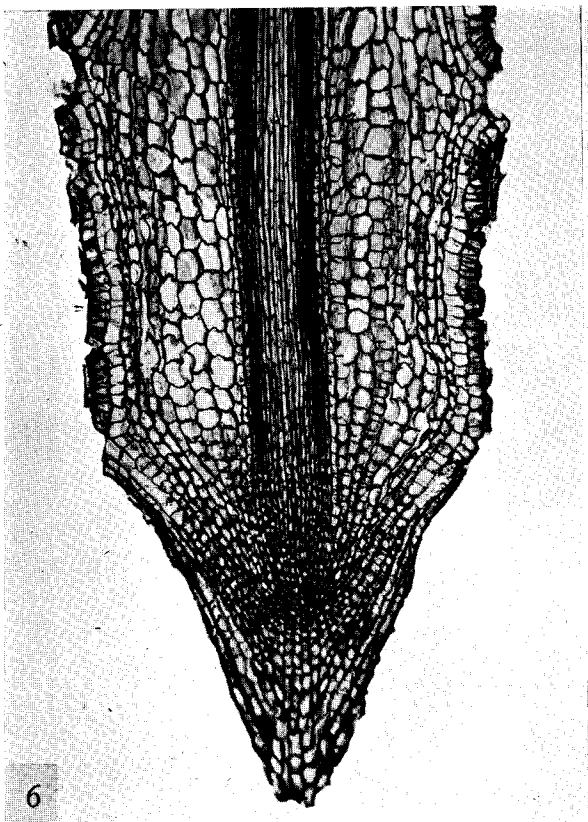
Chloride injury was indicated by tip burn of the leaves. This symptom was noted first in the high chloride cultures two weeks after the solutions were brought to full concentration. The degree of burning was conditioned by the hydrogen ion concentration of the solution and by aeration at the highest chloride level. Burning was most severe in the aerated cultures of the 100 m.e. chloride solution maintained at pH **7.5-8.0** and least, in base nutrient solutions regardless of aeration (table 1). The tip burn was progressive and tended to involve more and more of the blade until the entire leaf became brown and desiccated. This was most pronounced in the high salt-high pH cultures.

Growth was inhibited at the high chloride levels, and this was intensified by low hydrogen ion concentration. There were fewer, smaller leaves and fewer, shorter, lateral roots on plants grown under these conditions (fig. 1). A highly significant reduction in the total length of plants, total fresh and dry weights, number of lateral roots and white root tips occurred under the high chloride treatments. The effect of the higher pH value was also highly significant; in fact, the response to this factor was greater than to increased chloride treatment. On the basis of all treatments, no significance could be determined for aeration, although, at the lower pH value, aeration did influence the dry weight of seedlings significantly. The failure to get significant differences between aerated and non-aerated cultures might have been due to the presence of enough oxygen in the non-aerated solutions to meet the requirements of the slowly growing roots. The harvest data are shown in table 2.

ROOT DEVELOPMENT.--The response of a plant to varying conditions of the soil or nutrient solution is intimately associated with the anatomy and physiology of its roots. Numerous investigators have observed that the extent of the absorbing zone of a root will vary with changes in external conditions, such as soil moisture, temperature, and aeration. Scott (1928) refers to the effect of temperature on the water roots of *Salix,* and Plaut (1910, 1919) reports the closure of the absorbing zone during conditions unsuitable for growth in forest trees. The development of a protective suberized layer, the metacutis, which closes the absorbing zone has been described for a number of plants by Kroemer (1903-4), Plaut (1919), and Muller (1906). Cossmann (1939) has pointed out that suberization in the root of citrus belongs to a simple type where a metacutis to two to three rows of cells is formed in the root cap adjacent to the growing point.

The roots of citrus grow slowly and may exhibit alternating phases of activity and dormancy (Reed and MacDougal, 1937). During the active period,

Fig. 2-5.-Fig. 2. Median longitudinal section of an active root tip showing meristematic region and root cap.-Fig. 3. **The same, showing the initiation of the metacutis, indicated by the zone of deeply stained cells extending through the root cap.-Fig. 4 and 5. Later stages in growth inhibition showing roots in the dormant state with heavily cutinized epidermis and metacutis and reduced root cap.**



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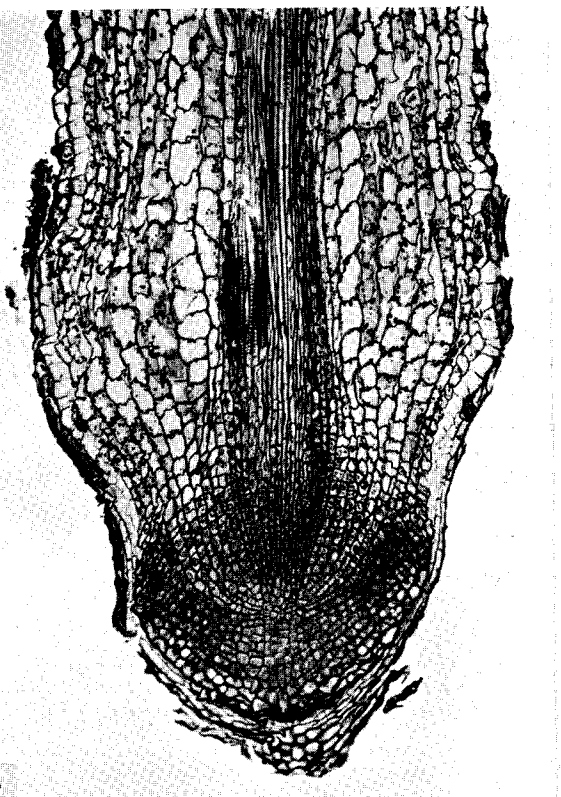

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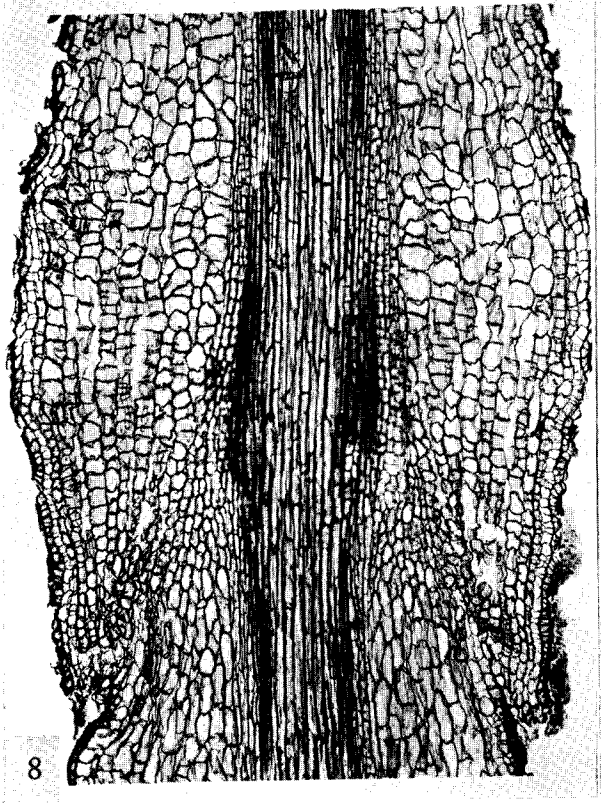

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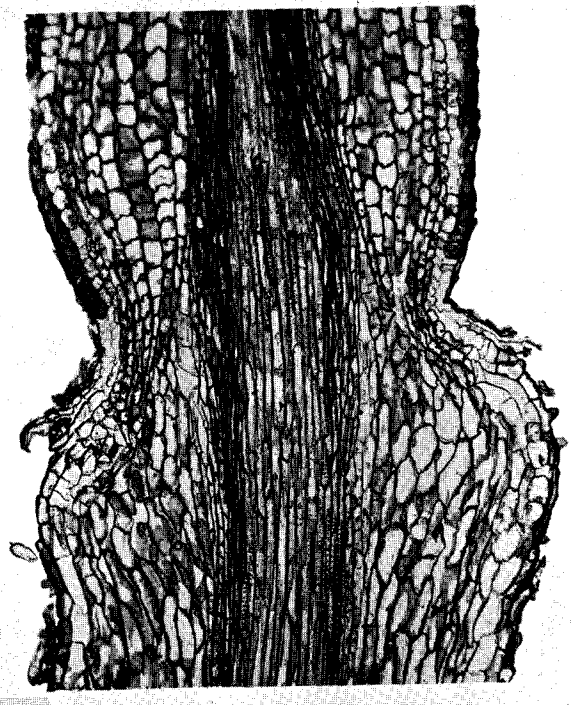

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the cells of the terminal meristem divide, adding new cells to the root cap distally and to the epidermis, cortex, and stele proximally (Hayward and Long, in press). The root cap is acute to sub-acute, and the outermost cells have thick heavily suberized or lignified walls. As the root elongates the peripheral cells of the root cap are lost, and the underlying ones form the suberized protective layer (fig. 2).

Under the influence of environmental factors which induce dormancy, the cells of the epidermis adjacent to the root cap become filled with deeply staining substances, and a similar condition develops in the cells of the hypodermis. There is a progressive extension of this reaction that involves one or two layers of root cap cells in a zone lying two or three cell layers outside the meristematic region (fig. 3). The cells of the deeply-stained layers become thick-walled, and there is a deposition of suberin on their inner faces. As a result, the cells of the distal portion of the root cap outside the suberized layer die (fig. 4). Coincident with these changes in the cells of the protective tissues, the activity of the meristematic cells diminishes. Continued differentiation results in mature stelar elements adjacent to the much reduced meristematic region (fig. 5).

The root tips in the resting stage are brown with flattened root caps in contrast to the actively growing root tips that are white with conical caps (fig. 2, 5). Senescent root tips resemble those of resting roots and may become gelatinized as necrosis progresses.

The resumption of active growth by a dormant root is initiated in the meristematic cells of the growing point and the cells of the pericycle adjacent to it (fig. 7). The combined activity of these cells produces a broadened growing point which forms new root cap cells inside the metacutis. As growth proceeds, the old root cap and metacutis are ruptured and displaced laterally, forming a shoulder of dead and broken cells which marks the point of renewed activity (fig. 8). There is no break in the continuity of the stelar elements, and the new ones are differentiated against the older ones, but at the point of resumption of activity the newly formed epidermis can be distinguished from the older epidermal cells by the degree of suberization and the presence of root hairs (fig. 9). The activation of the pericyclic cells forces the endodermal laver toward the periphery of the root, and a separating zone is formed between the cortical parenchyma of the old and new growth. Cossmann (1939) describes a somewhat similar development as the formation of "a direct link of suberized cells ... formed between endodermis and exodermis."

ANATOMICAL RESPONSES TO TREATMENTS.--Although brown roots, indicative of the dormant phase that commonly occurs in the ontogeny of the citrus seedling, may resume active growth and form new white tips under normal circumstances, they will not do so if sufficiently high salt concentration and high pH values are maintained. In such cases, the inhibiting effects of high concentrations of chloride salts and low hydrogen ion concentration were reflected in an increased number of brown roots and a reduction in white root tips (table 2). In some instances, no white root tips were found on seedlings grown at the highest chloride level. In the most severe treatments, the brown inhibited condition was frequently followed by a breakdown of the meristematic tissue of the root and death of the seedling.

The histological picture accompanying progressive inhibition involves : (1) the reduction of meristematic activity in both the growing point and the pericyclic zone, (2) the continued maturation of the stelar tissues until they abut the meristematic zone, and (3) a marked increase in the deposition of suberin on the walls of the epidermal, hypodermal, and root cap cells.

The inhibition of meristematic activity results in the formation of fewer cells by the histogens and a slower growth rate in the cells produced. In the periblem, the potential cortical cells may not enlarge to the size they attain in the base nutrient solution, and in consequence the root may be smaller in diameter than those of the control plants. The stelar tissues continue to differentiate, and mature xylem and phloem elements ultimately abut the reduced meristematic zone thus eliminating the regions of elongation and differentiation that usually occur between the meristem and the mature primary tissues of the stele. This means that the processes of cell differentiation and maturation have proceeded at a relatively more rapid rate than that of cell division.

Another effect of high chloride and low hydrogen ion concentration is the reduction of pericyclic activity in the formation of lateral roots. Except in the B series (unaerated, high pH value), there was a highly significant reduction in the number of lateral roots formed at the high chloride levels (fig. 1) (table 2).

Suberization of the walls of epidermal and hypodermal cells which normally accompanies dormancy was accentuated under high chloride conditions, and this was more pronounced in cultures where high chloride concentrations and high pH values were combined. The retardation in meristematic activity and consequent reduction in the production of new root cap cells, plus the development of a heavily suberized metacutis, results in a blunt root cap with few peripheral cells. One or two layers of cells may remain between the metacutis and the dermatogen-calyptrogen layer (fig. 5).

THEDEVELOPMENT OF ROOT HAIRS.-Theeffect of hydrogen ion concentration, temperature and aera-

Fig. 6-Y.-Fig. 6. Longitudinal section of semi-dormant root showing reduced growth activity.-Fig. 7. Root tip showing resumption of activity of the meristematic and pericyclic cells.-Fig. 8. Longitudinal section of portion of root showing collar formation at point where growth activity was resumed.-Fig. 9. Longitudinal section of root at point of growth resumption showing the blocking off of the older cortical cells from the new. The old epidermis is heavily cutinized and root hair primordia have been formed by the younger epidermal cells.

TABLE 3. *Frequency* of *root hairs.*

Series	Salt treatments	None	Few	Many
A (pH 5.5-6.0, unaerated)	I-B. N.	17"	50	33
	2-50 m.e. Cl-/l.	0	58	42
	3-100 m.e. Cl-/l.	31	50	19
AO (pH 5.5-6.0, aerated)	1-B. N.	6	31	63
	2-50 m.e. Cl-/l.	40	20	40
	3-100 m.e. Cl-/l	92	8	0
B (pH 7.5-8.0, unaerated)	1-B. N.	81	19	0
	2-50 m.e. Cl-/l.	89	11	0
	3-100 me. Cl-/l.	77	23	0
BO (pH 7.5-8.0, aerated)	1-B. N.	63	37	0
	2-50 me. Cl-/l.	100	0	0
	3-100 m.e. Cl-/l.	100	0	0

a Percentage of total number of root tips examined.

tion on root hair formation has been noted by Girton (1927). Under the conditions of his experiments, he found the most favorable conditions at pH 5.0 and 33°C. and demonstrated that continuous aeration greatly enhanced production. Cossmann (1939) observed that the length and development of root hairs was "directly affected by soil moisture conditions."

With respect to hydrogen ion concentration, our results confirm those of Girton. Seedlings growing in solutions maintained at the lower pH values (5.5-6.0) produced more root hairs than those growing in solutions having equivalent amounts of chloride salts but with pH values ranging from 7.5 to 8.0. Although low hydrogen ion concentration was the most important factor in inhibiting root hair formation, high concentration of the chloride ion was also a vital one. With one exception (the 100 m.e. chloride culture in the B series), there was a significant reduction in the number of root hairs at the high chIoride levels. No root hairs occurred on plants in the aerated cultures at the high pH level in the 50 and 100 m.e. chloride solutions. In the base nutrient cultures, more root hairs were produced by the aerated than the unaerated plants as noted by Girton, but at both levels of hydrogen ion concentration, the high chloride seedlings in the aerated cultures produced fewer root hairs than those in the unaerated solutions (table 3).

The treatment also influenced the length of root hairs. In general, the root hairs formed by this variety of orange are tubular, although some papillate, spatulate, and irregular forms do occur. In no instance were long root hairs developed, and under the most favorable conditions they seldom exceeded 100 μ in length. High hydrogen ion concentration favored development of the longest root hairs, but they were observed only in the control cultures of the A and AO series. The depressant effect of chloride was indicated by the shorter root hairs found in the 50 m.e. chloride cultures in both the A and AO series, and at the 100 m.e. level of the A cultures. At the high pH values, the development of root hairs was limited to the formation of primordia confirming Girton's results with respect to the effect of low hydrogen ion concentration.

The persistence of root hairs of the Valencia orange has been noted in a previous report (Hayward and Long, in press). The root hairs may become suberized and lignified, and in such cases persist for some time, although it seems unlikely that they remain functional. We have noted cases where

TABLE 4. *Mortality data.*

Series	Culture and treatment	Mortality no. of plants	Per cent of culture	Ave. no. days full treatment to death
A (pH 5.5-6.0, unaerated)	I--B.N.	0	0	0
	2-50 m.e. Cl-/l.	1	4	14
	3-l-00 m.e. Cl-/l.	6	25	36
AO (pH 5.5-6.0, aerated)	I--B. N.	0	0	0
	2-50 m.e. Cl-/l.	0	0	0
	3-l-00 m.e. Cl-/l	13	54	47
B (pH 7.5-8.0, unaerated)	1--B.N.	0	0	0
	2-50 m.e. Cl-/l.	3	12.5	42
	3-I-00 m.e. Cl-/l.	13	54	44
BO (pH 7.5-8.0, aerated)	1--B.N.	0	0	0
	2-50 m.e. Cl-/l.	2	8.5	49
	3-l-00 m.e. Cl-/l	13	54	48

root hairs have remained intact until the underlying hypodermal layer has divided periclinally to initiate a phellogen. The development of such hairs was more common with low hydrogen ion concentration than with high as might be expected on the basis of the relative degree of suberization of epidermal and hypodermal cells occurring under such conditions.

MORTALITY.--During the course of the experiment, 51 plants, or 17.7 per cent of the total population, died. Except for three seedlings in the A series that died at the end of two weeks while apparently in good condition, death was preceded by a slow progressive chlorosis and burning of the leaves accompanied by a gradual browning of the lateral roots. The color of the root tips changed from a glistening white to dull brown, and frequently this was accompanied by a gelatinization of the tissues of the root tip so that they appeared semi-translucent.

The major portion of the fatalities occurred between 35 and 50 days after the seedlings had received full treatment. No deaths occurred in the base nutrient cultures in either series and few at the 50 m.e. chloride level. At the 100 m.e. level, over 50 per cent of the population died in each of the AO, B, and BO treatments. The effect of high chloride was intensified by low hydrogen ion concentration. With high hydrogen ion concentration, the mortality rate at the high chloride level was significantly lower in the unaerated cultures (table 4).

SUMMARY

Valencia orange seedlings were grown in water cultures under three salt treatments: (1) a four-salt base nutrient solution containing no chloride except for a small amount occurring in the tap water (0.35 to 0.55 m.e./l.), (2) the nutrient solution plus 50 m.e. chloride per liter, and (3) the nutrient solution plus 100 m.e. chloride per liter. Chloride was supplied as NaCl 50 per cent, $MgCl_2$ 25 per cent, and $CaCl_2$ 25 per cent. Solutions were adjusted to pH 5.5-6.0 in one series and to pH 7.5-8.0 in the other. Half of the cultures of each series were aerated.

Citrus roots may exhibit alternating phases of activity and dormancy in their usual growth cycle. When in the dormant condition, meristematic activity is diminished and the walls of the cells of the epidermis and root cap adjacent to the meristem become suberized, forming a metacutis.

The anatomical response of the root to high chloride concentrations and high pH values resembles that of dormancy, but may be accentuated. In extreme cases, the root may remain in the dormant condition until death ensues.

Few root hairs developed on roots grown under conditions of low hydrogen ion concentration, and these were usually short or primordial in character.

The frequency and length of root hairs was reduced by high concentrations of chloride salts.

Chlorosis and tip burn of leaves may be induced by high chloride or low hydrogen ion concentration; the most severe symptoms occurred when the two treatments were combined.

Growth was inhibited under high chloride treatment and high pH values, fewer leaves and lateral roots being produced. The plants were shorter and lighter under such conditions.

Mortality was highest at the 100 m.e. chloride level in all series; few plants died under the 50 m.e. chloride treatment and none in the control cultures.

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