Comparative Anatomy of Citrus and Cold-Hardiness[1]

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INTRODUCTION

Cold-tolerance is one of the important properties sought in the selection of citrus hybrids and introductions. It may be a deciding factor when comparing otherwise similar varieties. Testing citrus for cold-hardiness has been done by exposing the plants to cold, either naturally or through the use of freezing tests. The plants may or may not have been preconditioned before the test, although they usually are, in an attempt to duplicate the cold days of autumn before winter freezing.

The recognized cold-tolerance of the better known varieties has been the result of numerous and repeated observations throughout the years. The condition of the plants before a freezing test may definitely determine the results obtained. It has been found that the behavior of a certain variety may be quite misleading from a given test. Thus, plants out of the greenhouse or the field, grown under high temperatures, may be more cold-tolerant than cold-preconditioned plants (*6,28*).[3]

Anything which curtails growth to the point of inducing shoot, root, and cambial dormancy, tends to produce cold-tolerance. Such treatment should not have caused a great increase in respiration or in the breakdown of elaborated foods in the tissues of the plant, but should have permitted their accumulation and the development of the physiological conditions associated with cold-hardening. The difficulty in artificially obtaining such a state before conducting a freezing test is probably one of the causes of contradictory results with the same variety.

The detection of physical traits which do not depend upon a given physiological balance, and which may be consistently associated with cold-tolerance, would simplify the separation of hardy and tender varieties.

REVIEW OF LITERATURE

The correlation of cold-hardiness with physical traits has not been very successful. According to Levitt (*14*), the reported correlations of various traits with cold-hardiness often include a number of exceptions, or have been found by others not to be so correlated. The similarity of the effects

[1] Taken from the author's Dissertation "Comparative Anatomy of Citrus in Relation to Cold Hardiness," University of Florida, Gainesville, Fla., April 1965.

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[3] Italic numbers in parentheses refer to Literature Cited, pp. 334–6.

of freezing, drought, and heat on cell dehydration has been discussed by Scarth (*22*) and Levitt (*13,14*).

Those physical factors involved in the retardation of water loss have been considered as favorable or conducive, directly or indirectly, to increased tolerance to freezing. Levitt has, in fact, combined in the term "*dehydration resistance*" the tolerance to cold, drought, and heat (*13*).

Small cell-size has been found by Scarth (*23*), Shields (*24*), Siminovitch and Briggs (*26*), Levitt (*13,14*), and Parker (*18*) to be the only constant and reliable anatomical feature associated with cold-hardiness. The ability of small cells to resist the injurious effects of freezing is due largely to their great surface-to-volume ratio. The large surface is favorable for rapid exosmosis and imbibition during freezing and thawing (*2,15*). This reduces the dehydration and rehydration stresses involved. Small cells possess small or no vacuoles (*15*), a factor which considerably reduces the amount of water exchanged during freezing and thawing. According to Parker (*18*), the reduced water movement of small cells maintains cellular organization close to a normal level during low temperatures.

Small cell-size may be a result of environmental conditions (*4*). Moisture stress, nutrient deficiencies, abundant illumination, or cold during development have been found to be factors inducing the formation of smaller cells. The effect of the stresses mentioned is responsible, within certain limits, for the development of the xeromorphic type of leaf. Such leaves have a thick cuticle, a well-developed palisade, increased overall leaf thickness, increased stomatal density, and a well-developed vascular system (*3,4,33*). These attributes may be associated with a rapid metabolic rate, facilitating the accumulation of photosynthates, increase in osmotic concentration, and protection against desiccation (*13,14*).

A well-developed palisade has been recognized by Turrell (*30*) as a feature which implies a large internal-to-external surface ratio, a characteristic of xeromorphs. Plants of this type have been found to have a lower water content than mesomorphs, and a larger percentage of dry matter (*24*). Smith and Reuter (*29*) have found a larger percentage of dry matter in leaves of 'Valencia'[4] orange in winter than in summer. Curtis and Clark

[4] Single quotes are used for varietal names in this paper following the International Code of Nomenclature for Cultivated Plants-1961, *Regnum Vegetabile* vol. 22, International Commission for the Nomenclature of Cultivated Plants of the International Union of Biological Sciences; Utrecht, Netherlands. Article 17 of said code reads in part: "A cultivar (variety) name, when immediately following or preceding a botanical or common name, must be distinguished clearly from the latter, either by placing the abbreviation cv. before the cultivar (variety) name, or, for example, by enclosing within single quotation marks. * * * Note: Double quotation marks must not be used to distinguish cultivar (variety) names."

(*2*) have recognized that a lower water content in the tissues is conducive to resistance to ice formation.

Citrus leaves have been found to possess two or three layers of palisade, depending upon the variety (*7,32*). Halma found that palisade-layer number is not influenced by the stock upon which the scion is grafted (*7*). This proportion of the leaf corresponding to palisade tissue in citrus foliage was found to range from 21 to 30 percent, thus leaving a large proportion of the leaf mesophyll to the spongy parenchyma. Such a distribution of foliar mesophyll would appear characteristic of mesomorphic leaves, but Turrell (*30*) classified citrus leaves as xeromorphic.

Whether the amount of palisade in citrus is a favorable factor in the development of cold-hardiness is open to argument. Palisade thickness in citrus was found to increase with age of the leaf, from a 2-month-old fully expanded leaf to a 12-month-old mature leaf. This amounted to 5½ percent in 'Eureka' lemon (*7*).

Hirano (*9*) observed a certain relation between stomatal density in the citrus leaf and the climatic distribution of the varieties. He concluded that densities of 500 stomates per square millimeter or less were associated with cooler climates and hardier varieties. There are some noticeable exceptions to this conclusion. The hardy calamondin (*32*) with 665 stomates, and the tender 'Tahiti' and 'Bearss' limes with 372 and 326 stomates per square millimeter, respectively, may be mentioned as such. Hirano (*9*) conceded nevertheless, that stomatal density may be more a result of moisture than of temperature. Thus, in hot, arid regions, stomatal densities have been found higher for the same variety than in cooler and moister areas (*31*). Similar observations have been made by Miller (*17*), Shields (*24*), and Maximov (*16*) for other plants.

A thick cuticle has been mentioned by Vasil'yev (*31*) as a feature of plants exposed to constant low temperatures. Maximov (*16*), Shields (*25*), and Philpott (*19*) have associated a thick cuticle with xeromorphic leaves. Harvey (*8*) found that a waxy covering provided protection against ice-nucleation during freezing.

A small surface-to-volume ratio has been observed by Shields (*25*) as a characteristic of leaf xeromorphy. This implies a smaller, thicker leaf, which is associated with a well-developed palisade and a relatively compact spongy parenchyma. Leaves with such characteristics usually have a more dense network of veins. This adaptation to drought implies an easier water transport from cell to cell, and to the intercellular spaces. The large internal surface and the numerous points of contact between the cells of this type of leaf accounts for the ease of water movement (*24*).

MATERIALS AND METHODS

With the object of establishing possible relations between anatomical traits and cold tolerance, four varieties of citrus representing a wide variation in recognized cold-hardiness were selected. The plants used were grown from seed, and all variants appearing during the initial 5-month period of growth were eliminated. Criteria for elimination were departures from typical foliage characteristics and unusually slow or rapid development.

The varieties selected were, in descending order of observed cold-tolerance: 'Changsha' mandarin (*Citrus reticulata*), 'Taiwanica' sour orange (*C. taiwanica*), 'Parson Brown' sweet orange (*C. sinensis*), and 'Mexican' ('West Indian') lime (*C. aurantifolia*). This rating is based on observations reported by various investigators (*1,5,10,11,32,34*). 'Taiwanica' sour orange has been observed by Krezdorn (*12*) to be hardier under certain conditions than 'Changsha' mandarin.

The seedlings were grown in 10-cm. plastic pots in a greenhouse bench. After the plants were 5 months old, a series of them was placed in a controlled-climate chamber for preconditioning treatment. This was done to investigate whether there occurred any anatomical or growth changes as a result of the treatment, and to determine the degree of cold-hardiness developed in each variety. The preconditioning cycle included a 12-hour photoperiod at 26°C. and a 12-hour nyctoperiod at 2°C. The night low was gradually reached after 10 progressively cooler nights, starting at 14°C. the first night. The total preconditioning treatment lasted 5 weeks, although freezing tests were made at the end of 2, 4, and 5 weeks.

The growth of the plants was recorded at 5, 5½, and 6 months of age. Total height, foliation, and branching were observed. Leaf areas were determined at the time of leaf sampling for microscopic examination. Specific gravity and dry matter were determined from the eleventh emerging leaf of each seedling. Microscopic examinations were made from the tenth emerging leaf of each plant.

Leaves were picked at 8:00 a.m. and placed immediately in a fold of moist filter paper in a plastic bag. They were stored in a refrigerator for the very short period between collecting and processing. Each leaf was surface-cleaned of possible deposits of dust and foreign matter with distilled water and a No. 6 camel's-hair brush, dried with bibulous paper, and weighed. The volume of the leaf was then determined by immersion and measurement of the displaced liquid. After rinsing in distilled water, the leaves were placed in individual paper bags and dried in a forced draft oven at 70°C. After storage in a desiccator for 48 hours they were weighed again. From the data obtained, specific gravity and dry matter were calculated.

Vascularization was studied from whole cleared leaves after removal of the pigments by lactic acid (*27*). Microscopic venation patterns were examined from small disks of similarly cleared tissue cut from the midportion of the blade, approximately equidistant from the margin and the midrib. Disks from the tenth emerging leaf of each seedling were killed and fixed for the preparation of stained mounts. Anatomical study was carried on from transections and paradermal sections of leaves and from transverse, radial, and tangential sections of stems. Fixing fluids for leaves were formalin-aceto-alcohol and chromic-aceto-formalin, and for stems formalin-aceto-alcohol only. Staining was routinely done with safranin-fast green (*21*).

Features which were amenable to measurement were selected so that the anatomical characteristics of the varieties under study could be compared. These included densities of stomates, crystal idioblasts, oil gands, and cells of epidermis, upper palisade, and lower palisade, as well as vein-termination numbers, areolar numbers, and interveinal intervals. Lamina widths, central-vein widths, leaf thicknesses, palisade-tissue thicknesses, and spongy mesophyll thicknesses were also measured, as were several other minor characteristics which may have a bearing upon cold-tolerance.

RESULTS AND DISCUSSION

The freezing tests conducted after 2, 4, and 5 weeks of preconditioning revealed results which did not conform in all respects to the accepted cold-tolerance rating of the varieties under study. After 2 weeks all plants survived a freezing temperature of −4°C. for 1 hour. After 4 weeks the freezing temperature used was −7°C. for 3 hours. An additional test was also made at 5 weeks. The results of the second and third tests appear as test I and test II in table 1. Control plants in test I had been kept in a controlled-climate chamber similar in illumination to the preconditioning chamber, but under a cycle including favorable temperatures for growth. Control plants for test II were brought directly from the greenhouse.

The preconditioning sequence, the growth conditions, and the freezing tests as carried out in this investigation apparently induced a marked degree of cold tolerance in 'Mexican' lime, but did not do so in 'Parson Brown' sweet orange. The overall score for hardiness obtained by the four varieties placed them in the following order: 'Changsha' mandarin first, followed by 'Mexican' lime and 'Taiwanica' sour orange with quite similar scores, and 'Parson Brown' sweet orange last. The natural hardiness of plants direct from the greenhouse placed these varieties in descending order as follows: 'Taiwanica', 'Changsha', 'Mexican', and 'Parson Brown'.

Plants from the controlled-climate chamber, kept at favorable growth temperatures, showed cold-tolerance in the order: 'Changsha', 'Mexican', 'Taiwanica', and 'Parson Brown'. Finally, the combined numerical score obtained from nonhardened plants in the two tests, was in the following

TABLE 1.—*Cold-tolerance rating of 6-month-old seedlings of 4 varieties of citrus subjected to −7°C. for 3 hours*

Variety and condition	Test[1]	Survival	Score	Recovery	Score	Total rating
		Percent		*Percent*		
'Mexican'						
Preconditioned	I	100	10	85	34	
	II	60	6	35	14	
Control	I	80	8	45	18	
	II	30	3	12.5	5	
			27		71	2
'Taiwanica'						
Preconditioned	I	60	6	42.5	17	
	II	40	4	32.5	13	
Control	I	70	7	42.5	17	
	II	60	6	57.5	23	
			23		70	3
'Parson Brown'						
Preconditioned	I	90	9	45	18	
	II	50	5	27.5	11	
Control	I	50	5	15	7	
	II	10	1	2.5	1	
			20		37	4
'Changsha'						
Preconditioned	I	100	10	75	30	
	II	60	6	52.5	21	
Control	I	100	10	77.5	31	
	II	40	4	32.5	13	
			30		95	1

[1] Tests I and II after 4 and 5 weeks of preconditioning, respectively.

Survival rating schedule:

A—Living .... 1 point

B—Dead .... 0 point

Percent Survival = A × 1 × 100/number of plants

Recovery rating schedule:

A—No wood or foliage damage .... 4 points

B—Partial or complete defoliation or less than 1 inch of wood killed .... 3 points

C—One-third to three-fourths wood killed .... 3 points

D—Killed to ground level, but sprouting .... 1 point

E—Dead .... 0 point

$$\text{Percentage Recovery} = \frac{25\,((A \times 4) + (B \times 3) + (C \times 2) + (D \times 1))}{\text{Number of plants}}$$

Total rating = points Recovery + points Survival order: 'Changsha' mandarin first, followed rather closely by 'Taiwanica' sour orange, 'Mexican' lime third, and 'Parson Brown' sweet orange last.

The low degree of cold-tolerance shown by 'Parson Brown' sweet orange and the relatively high score of the normally tender 'Mexican' lime seem to indicate that different varieties require a different set of growth conditions before the onset of freezing temperatures, which is not identical for all varieties. When these conditions are not met, cold-hardening does not take place.

In table 2 are summarized the growth in height, and the leaf expansion in seedlings of the four varieties when kept under favorable growing conditions

TABLE 2.—*Development of control and preconditioned seedlings of 4 citrus varieties between 5 and 6 months of age*

Variety and condition	Height			Expanded leaves		
	5 mo.	6 mo.	Change	5 mo.	6 mo.	Change
	Cm.	*Cm.*	*Cm.*	*Number*	*Number*	*Number*
'Mexican'						
Control	37.2	39.6	2.4	29.0	30.9	1.9
Preconditioned		39.1	1.9		30.7	1.7
'Changsha'						
Control	18.4	22.8	4.4	17.5	21.7	4.2
Preconditioned		19.2	.8		20.2	2.7
'Parson Brown'						
Control	33.2	37.1	3.9	18.4	22.6	4.2
Preconditioned		35.5	2.3		21.3	2.9
'Taiwanica'						
Control	31.6	36.7	5.1	20.3	24.6	4.3
Preconditioned		33.7	2.1		22.3	2.0

and under preconditioning temperatures. The greatest stem elongation was made by 'Taiwanica' and 'Changsha' under control conditions, and the most marked depression was also observed in these two varieties under preconditioning temperatures (fig. 1). This seems to indicate that these varieties initiate dormancy soon after the temperature begins to fall. 'Parson Brown' and 'Mexican' were depressed less in their growth by low night temperatures.

Thus, under cold nights 'Changsha' made only 18 percent as much growth as the control plants, 'Taiwanica' 41 percent, 'Parson Brown' 58 percent, and 'Mexican' lime 78 percent as much, respectively. A direct relationship appears to exist between the depression of growth under cold nights and the cold-tolerance of the variety.

Oil glands are found frequently in leaves and stems of citrus. They are generally close to the epidermis and may be observed as translucent spots. Total oil-gland density in seedling leaves was found to bear no apparent relationship to cold-hardiness. The proportion of oil glands between the upper and the lower epidermises was variable, according to the variety. Thus, 'Mexican', the tenderest variety, had 3 times as many oil glands in the ventral epidermis as in the dorsal, 'Parson Brown', and 'Taiwanica' had twice as many, while 'Changsha', the hardiest variety, had an almost equal number in both faces of the lamina (fig. 2). It is not known what effect oil-gland distribution may have upon cold-hardiness, but the fact remains

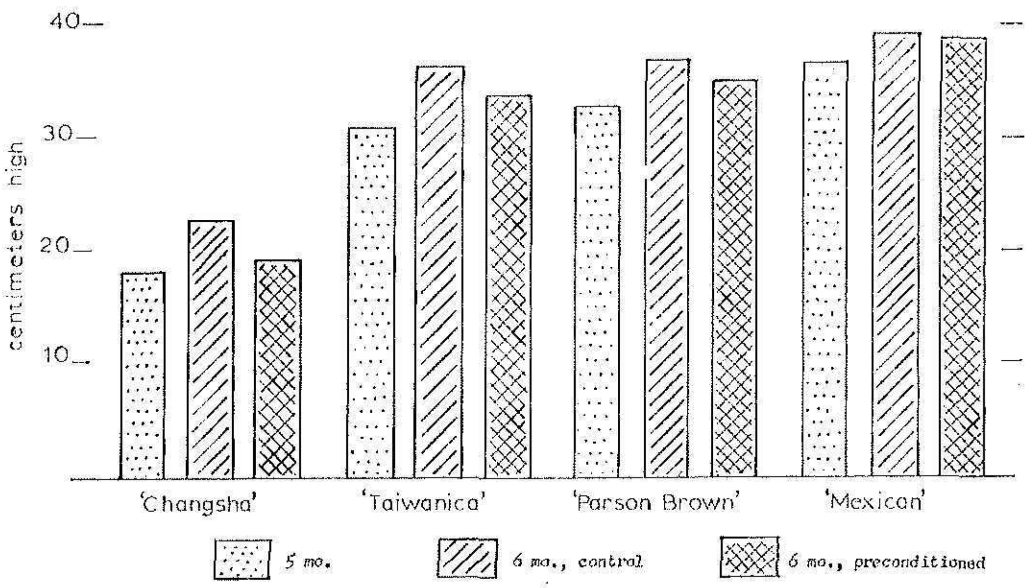

FIG. 1.—Effect of preconditioning on stem elongation in seedlings of 4 citrus varieties.

that the more evenly distributed the oil-glands, the more cold-tolerant the variety appears to be. The total number of oil-glands per square millimeter in these varieties were: 'Mexican' 4.46, 'Changsha' 2.74, 'Parson Brown' 1.91, and 'Taiwanica' 1.37.

Stomatal counts made on control plants showed a slight increase between 5 and 6 months in 'Changsha', 'Parson Brown', and 'Mexican'. This was not observed in 'Taiwanica'. Preconditioned plants had stomatal counts similar to the means obtained from the control plants. This supports the observations made by Reed and Hirano (*20*) to the effect that stomates are formed early in the development of the leaf. The relatively low stomatal counts obtained were attributed to the lowered illumination under which the seedlings were grown. No correlation seemed to exist between stomatal counts and cold-hardiness, as the hardiest and the tenderest varieties had similar counts (fig. 3). The average number of stomates per square millimeter in these varieties were: 'Mexican'-424, 'Changsha'-412, 'Parson Brown'-398, and 'Taiwanica'-362.

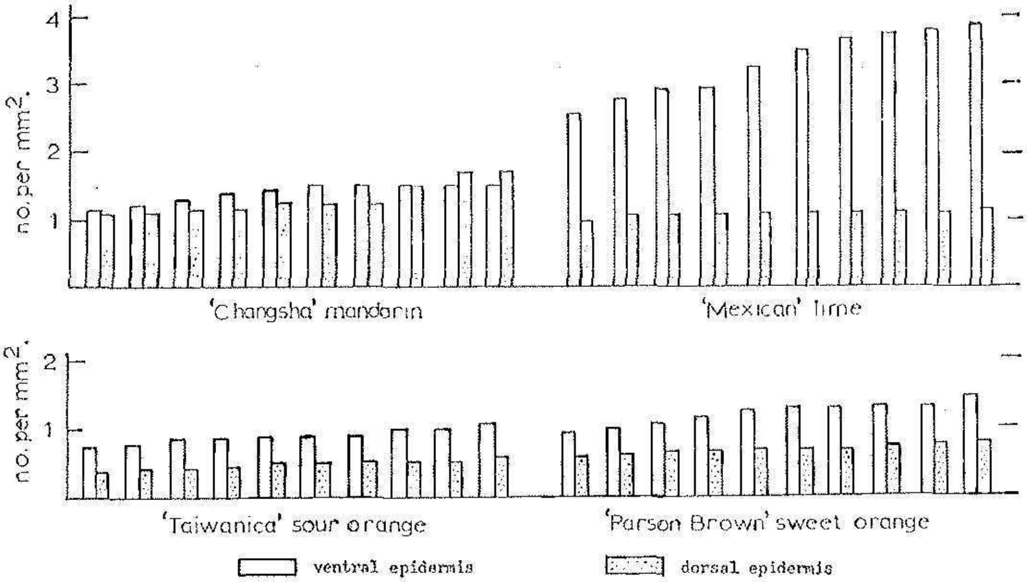

FIG. 2.—Oil-gland densities in leaves from 10 different seedlings of each of 4 citrus varieties.

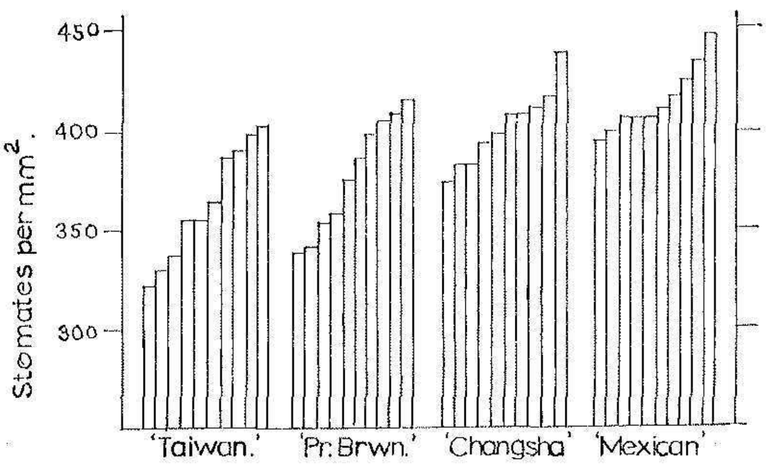

FIG. 3.—Stomatal densities in dorsal epidermis of leaves from 10 different seedlings of each of 4 citrus varieties.

Crystal idioblasts occurred in parenchymatous tissues of all aerial parts of the plant. These idioblasts are very conspicuous because of their size and the thick cellulose wall which stains deeply. The tenderest variety, 'Mexican', had 947 idioblasts per square millimeter, twice as many as the hardiest variety, 'Changsha,' which had 455. The other two varieties, 'Taiwanica'

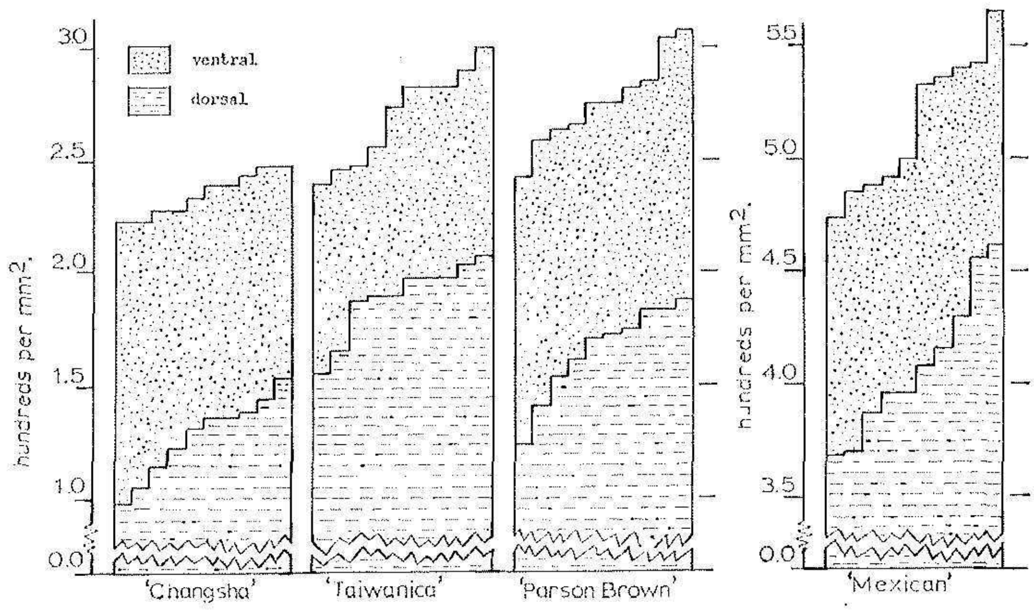

FIG. 4.—Crystal-idioblast densities in ventral and dorsal epidermises of leaves from 10 different seedlings of each of 4 citrus varieties.

TABLE 3.—*Mean cell densities in tissues of the 10th emerging leaf of 5- and 6-month-old seedlings of 4 citrus varieties*

Tissue and age (*months*)	Number of cells per sq. mm. for—			
	'Changsha'	'Taiwanica'	'Parson Brown'	'Mexican'
Ventral epidermis				
5	3,940	3,447	3,143	2,917
6	3,907	3,244	3,333	2,860
Average	3,923	3,345	3,238	2,888
Upper palisade				
5	16,238	16,190	15,524	11,666
6	17,107	16,845	14,583	12,162
Average	16,672	16,517	15,053	11,914
Lower palisade				
5	10,375	6,428	6,619	7,520
6	9,630	6,527	7,083	7,420
Average	10,002	6,447	6,851	7,470
Total average	30,597	26,339	25,142	22,272

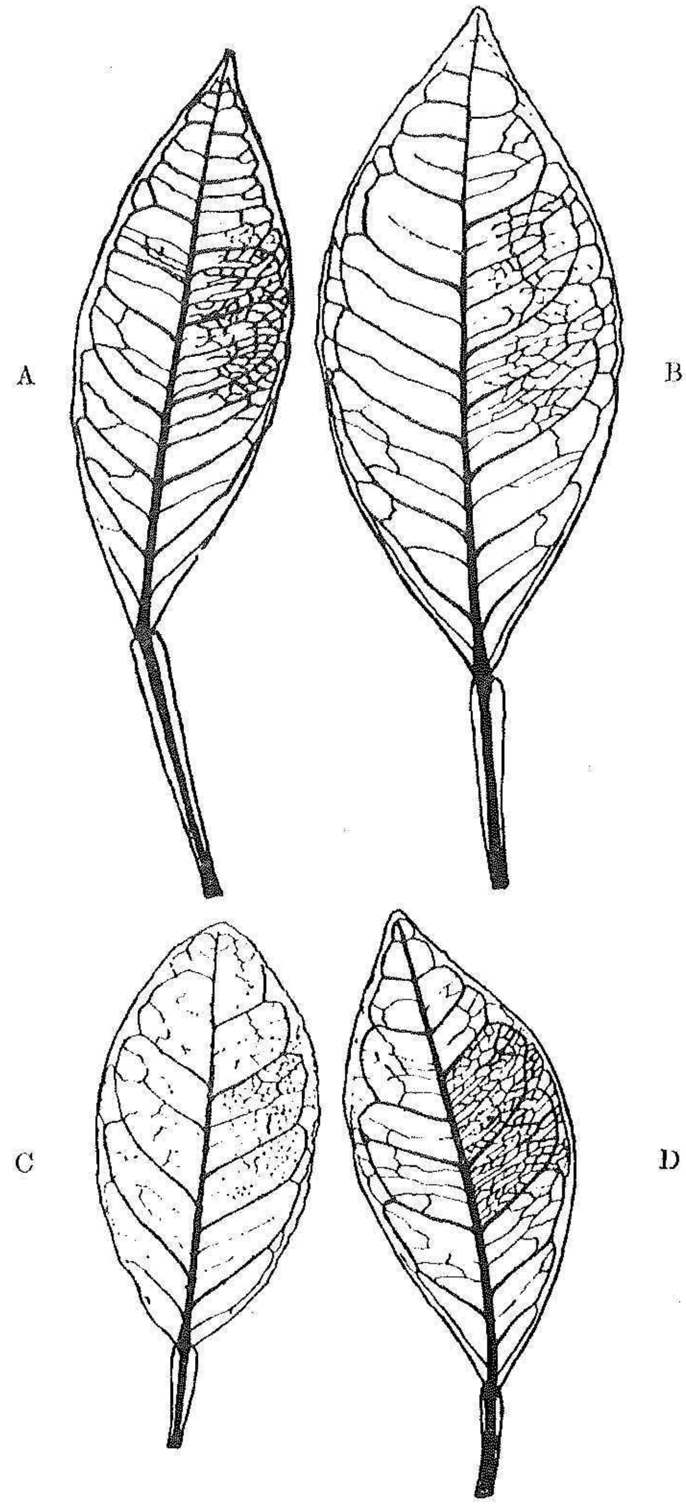

FIG. 5.—Main venation in seedlings of 4 citrus varieties: A, 'Taiwanica' sour orange (*C. taiwanica*); B, 'Parson Brown' sweet orange (*C. sinensis*); C, 'Mexican' lime (*C. aurantifolia*); D, 'Changsha' mandarin (*C. reticulata*).

and 'Parson Brown', had 524 and 476, respectively (fig. 4). Crystal-idioblast densities showed an apparent inverse correlation with cold-tolerance. Preconditioning by cold seemed slightly to reduce idioblast formation, as suggested by the consistently lower means of cold-treated plants as compared with the controls.

Three tissues of the leaf having a relatively orderly organization were used for cell-density determinations. These were the ventral epidermis, the upper

TABLE 4.—*Ratios of central-vein width to lamina width in the leaves of seedlings of 4 citrus varieties*

Variety and age (months)	Vein width	Lamina width	Ratio
	μ	*Mm.*	
'Changsha'			
5½	404.2	19.65	46.13
6	404.65	20.2	49.92
Average			48.02
'Taiwanica'			
5½	415.6	23.9	57.45
6	415.19	25.15	60.57
Average			59.01
Parson Brown'			
5½	375.9	34.4	91.4
6	435.75	34.65	79.92
Average			85.46
'Mexican'			
5½	296.0	28.65	90.5
6	297.82	26.6	89.31
Average			89.9

palisade and the lower palisade. The values observed are included in table 3. The total cell-density of the three tissues mentioned, as well as that of the upper epidermis or the upper palisade taken alone, appear to bear a direct relationship with cold-tolerance.

At 6 months of age leaf-thickness averages were: 'Parson Brown'-241 μ, 'Changsha'-226, 'Mexican'-211, and 'Taiwanica'-209, while palisade thickness averages were: 'Mexican'-69, 'Taiwanica'-65, 'Parson Brown'-53, and 'Changsha'-62 μ. The proportion of the leaf occupied by the palisade as seen in transection was: 'Mexican'-32 percent, 'Taiwanica'-31 percent,

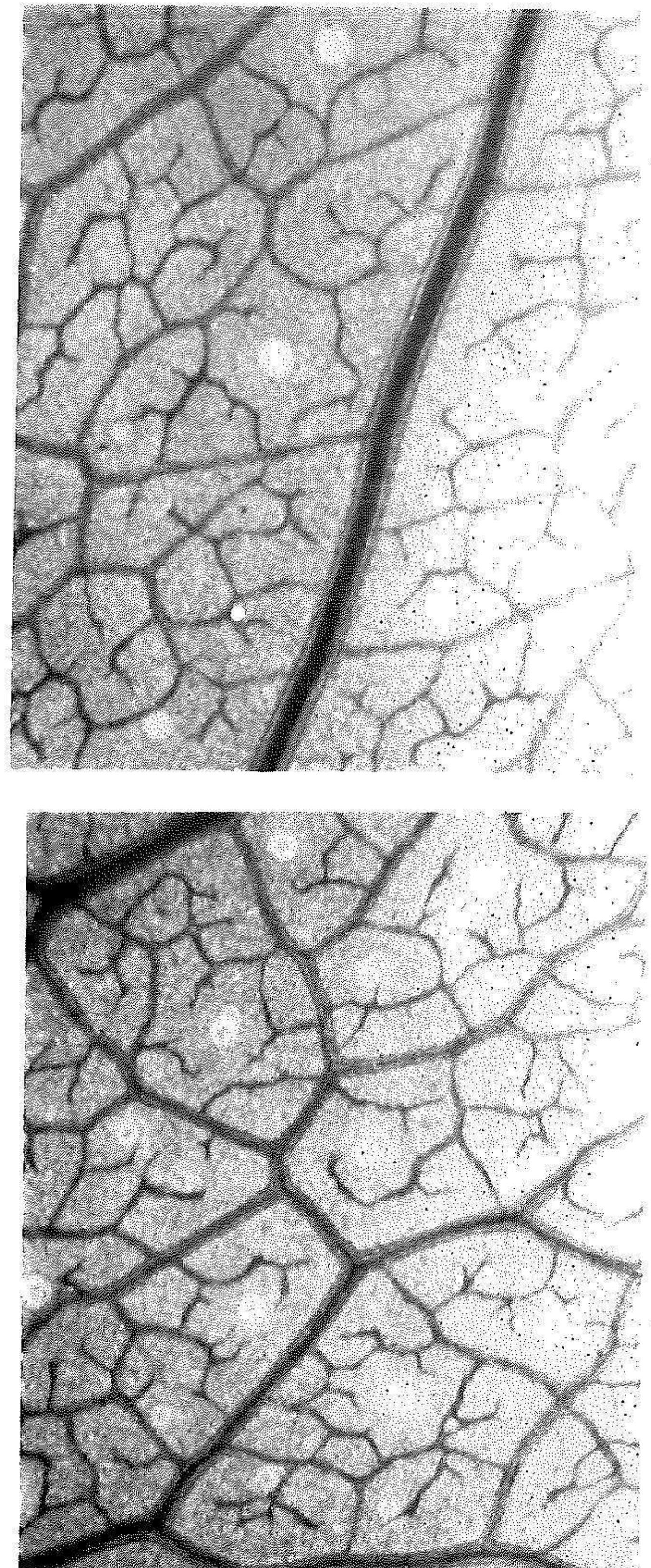

FIG. 6.—Microscopic venation patterns of seedlings of four different citrus varieties. (All 26×).

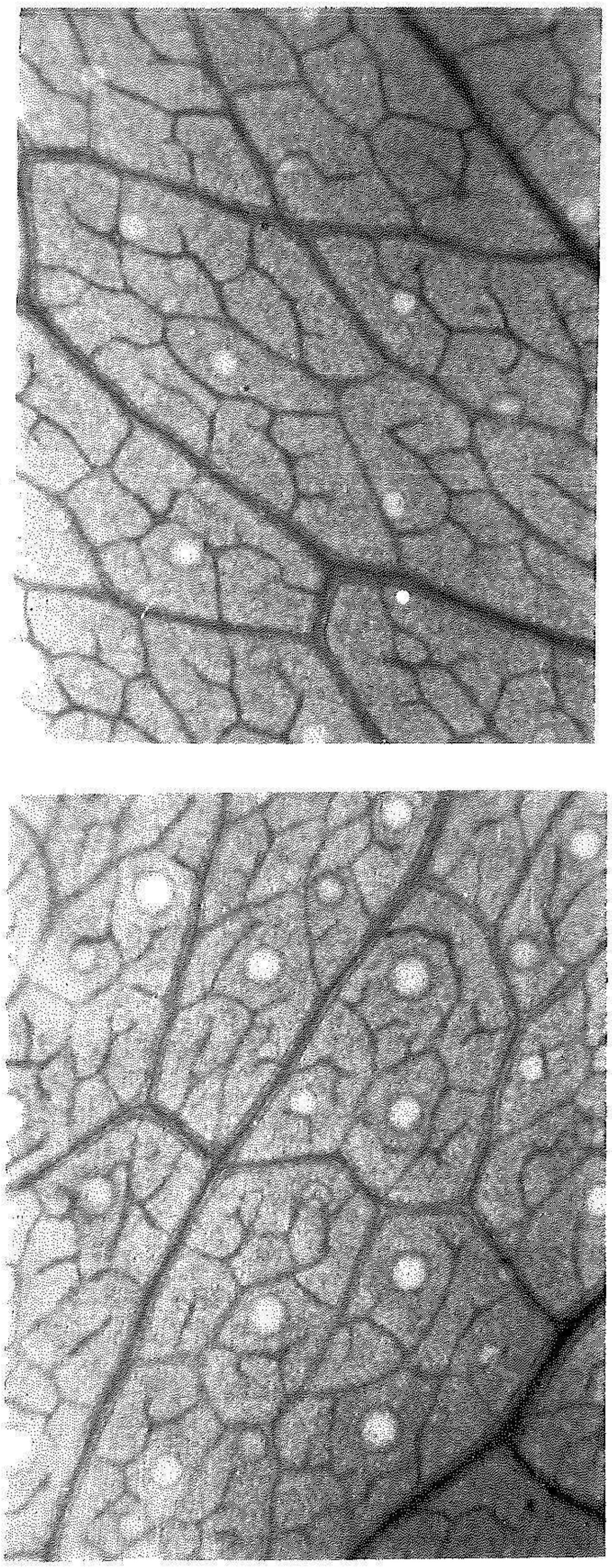

Fig. 6—*Continued*

'Changsha'-17 percent, and 'Parson Brown'-26 percent. From the above figures it appears that leaf- or palisade-tissue thicknesses are not related to cold-hardiness of the varieties concerned.

Vascularization characteristics as to vein distribution, interveinal spacing, vein terminations per square millimeter, and areolar frequencies, did not have a consistent relationship to cold tolerance (figs. 5 and 6). The width of the central vein, nevertheless, was found to be proportionately thicker in the hardier varieties. Comparing the vein width to the width of the lamina, it was found that this ratio was narrower in the hardier varie-

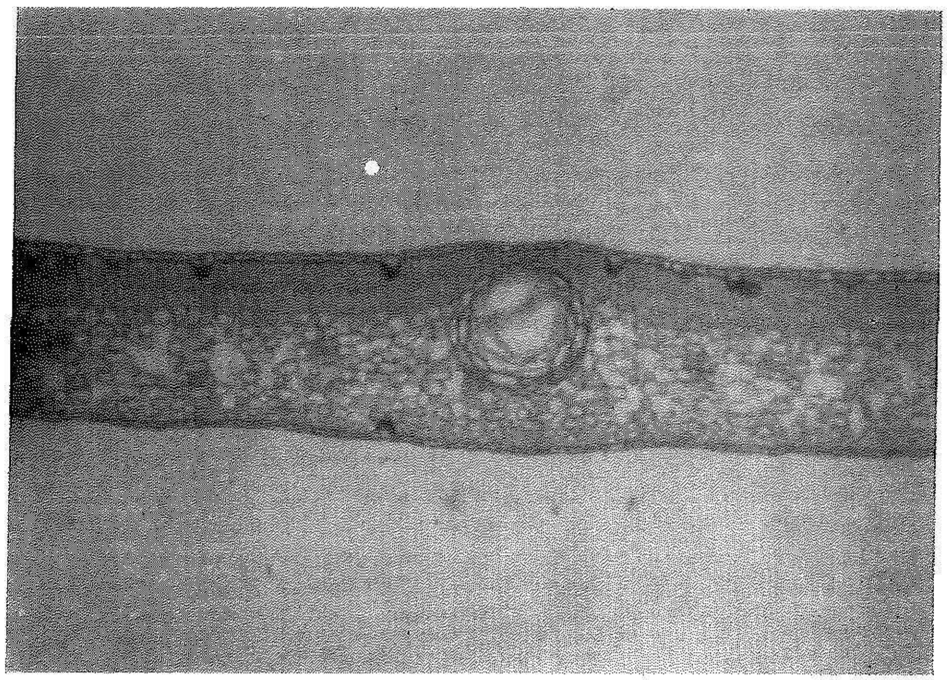

FIG. 7.—Leaf transection of 'Changsha' mandarin: Large oil gland, small-celled palisade, and compact spongy mesophyll; crystal idioblasts relatively near to oil-glands. (75×)

ties. Table 4 shows the values observed and the calculated ratios. As will be seen, the narrowest ratio is that in 'Changsha' mandarin, the hardiest variety, and the widest ratio in the tenderest variety, 'Mexican' lime. The other two varieties also have ratios proportional to their accepted hardiness when compared with 'Changsha' and 'Mexican'.

Both the specific gravity and the dry matter of all the varieties increased under favorable growing conditions from 5 to 6 months of age. Plants under cold nights surpassed the controls of comparable age in specific gravity and in dry matter. The highest increase in specific gravity after preconditioning was shown by 'Changsha' mandarin and 'Mexican' lime. These were also the two varieties with the highest cold-tolerance score in the freezing tests.

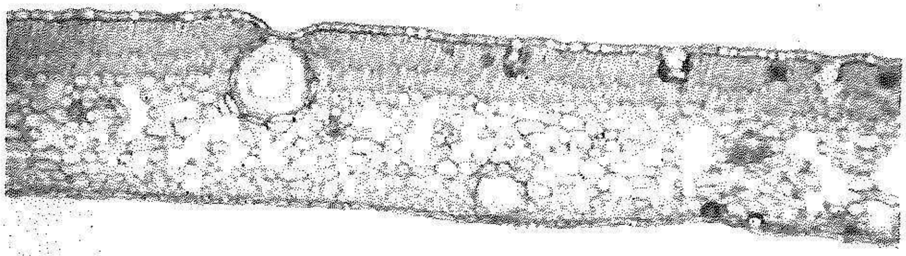

FIG. 8.—Leaf transection of 'Parson Brown' sweet orange: Medium-sized oil glands and loose spongy mesophyll; crystal idioblasts away from oil-glands. (75×)

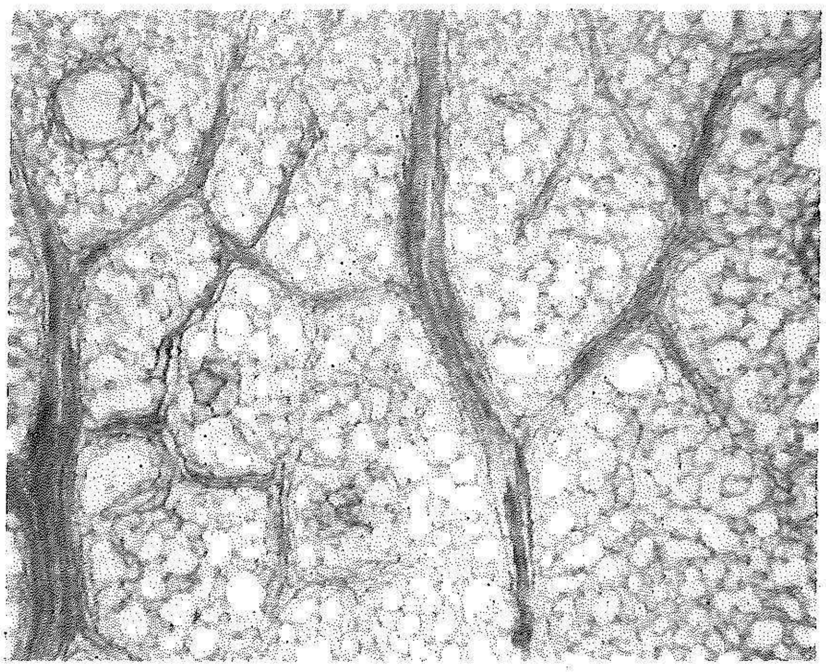

FIG. 9.—Paradermal section of leaf of 'Changsha' mandarin: Midleaf section showing relatively compact spongy mesophyll. (75×)

Preconditioning induced a higher accumulation of dry matter in three of the four varieties in the following order: 'Changsha', 'Taiwanica', and 'Mexican'. 'Parson Brown' plants had a lower dry-matter content at 6 than at 5 months. Dry-matter accumulation followed the rating obtained by these varieties in respect to survival and recovery when frozen without preconditioning, as can be seen in table 1.

In figures 7, 8, 9, and 10 the more compact nature of the leaf tissues of 'Changsha' mandarin as compared with those of 'Parson Brown' sweet orange and the 'Mexican' lime can be appreciated.

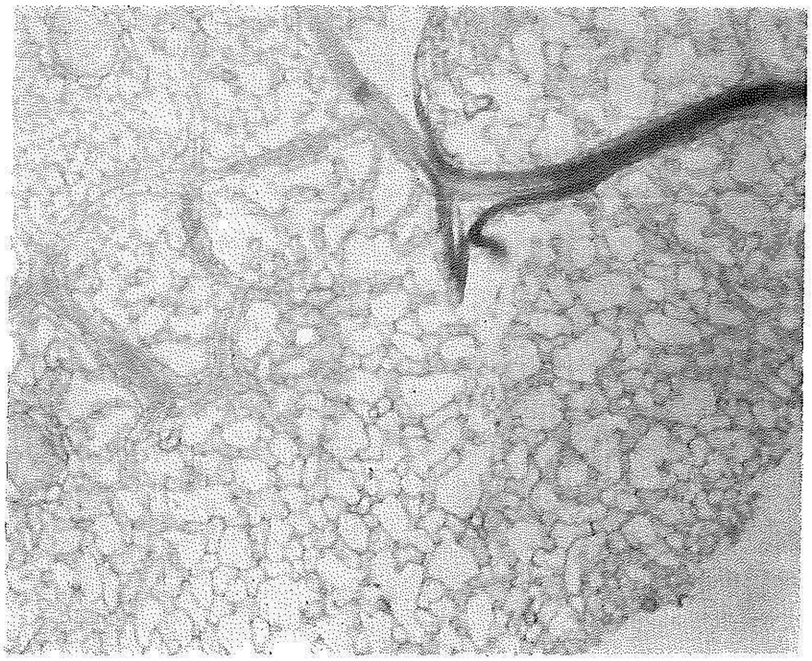

FIG. 10.—Paradermal section of leaf of 'Mexican' lime: Midleaf section showing open type of spongy mesophyll. (75×)

SUMMARY AND CONCLUSIONS

Seedlings of four varieties of citrus were used, representing four species with a range of cold-tolerance from high to very low. They were examined anatomically and the findings related to the accepted cold-tolerance of the varieties and with their performance in freezing tests.

The varieties selected were, in descending order of cold-tolerance: 'Changsha' mandarin (*Citrus reticulata*); 'Taiwanica' sour orange (*C. taiwanica*); 'Parson Brown' sweet orange (*C. sinensis*); and 'Mexican' ('West Indian') lime (*C. aurantifolia*).

It was concluded from the results of these tests that a given preconditioning treatment may induce in the plants subjected to it an ability to endure cold, which may not be consistent with their accepted tolerance to cold.

A definite inverse relationship between growth-rate and cold-tolerance was found in the plants under investigation. This growth-rate was more depressed in the hardier varieties when they had been subjected to a preconditioning treatment.

There appeared to be no relationship between total oil-gland content and cold-hardiness, but it was found that, if ventral and dorsal numbers were nearly equal, the plants were more resistant to cold.

Stomatal densities, under the conditions of this investigation, did not show any relation to the accepted tolerance to cold of these varieties, or to their tolerance in the freezing tests.

Calcium oxalate idioblasts appeared to occur in the leaves of Citrus in quantities inversely proportional to accepted tolerance to cold.

There was apparently no correlation between the thickness of palisade as percentage of total thickness and cold-tolerance in the varieties studied.

The number of cells per square millimeter in the upper palisade parenchyma of the leaf, as well as the combined total cell content of the palisade tissues and of the ventral epidermis, appeared to bear a direct relationship to the accepted cold-tolerance when comparing the four varieties studied.

Among the varieties in this investigation, there were no appreciable differences in the pattern of the major veins of the leaf. It was found, nevertheless, that within a variety there was a relatively constant ratio of the width of the central vein to the width of the lamina. The numerical value *of this ratio was found to be inversely proportional to the cold-tolerance of* the variety.

There appeared to be no relation in the varieties studied between cold hardiness and areolar frequencies, intervascular intervals, or vein-termination numbers.

Preconditioning by cold brought about a greater increase in specific gravity and dry matter in the hardier varieties, between the ages of 5 and 6 months, as compared with the controls.

Early branching and early formation of lenticles and cork in the 'Mexican' lime and the 'Parson Brown' sweet orange, as compared with 'Taiwanica' sour orange and 'Changsha' mandarin, seemed to be inversely correlated with cold-resistance.

RESUMEN Y CONCLUSIONES

Se estudiaron plantitas de cuatro variedades del género *Citrus*, las cuales representaban cuatro especies, cuya tolerancia al frío variaba de muy alta a muy baja. Se hizo un examen de las plantitas y se asociaron los resultados obtenidos a la reconocida tolerancia al frío de las variedades y a su comportamiento en las pruebas de congelación.

De los resultados de estas pruebas, se concluyó que al aplicárseles un tratamiento preacondicionador a las plantitas puede inducirse en éstas una capacidad de resistencia al frío que no coincida con su reconocida tolerancia.

En las plantas bajo estudio se encontró una relación inversa definitiva entre su ritmo de crecimiento y su tolerancia al frío. El ritmo de crecimiento era menos pronunciado en las variedades más resistentes cuando se sometieron a un tratamiento preacondicionador.

Aparentemente no existe relación alguna entre el contenido total de glándulas oleaginosas en las hojas y la resistencia al frío, pero se encontró que cuando el número de estas glándulas era casi igual en ambos lados de la hoja-el ventral y el dorsal las plantas eran más resistentes al frío.

Tampoco se encontró, bajo las condiciones de este estudio, ninguna relación entre la densidad de estomas y la tolerancia al frío reconocida de estas variedades ni con su tolerancia en las pruebas de congelación.

Los idioblastos de oxalato de calcio en las hojas del género Citrus, parecían ocurrir en cantidades inversamente proporcionales a su reconocida tolerancia al frío.

Aparentemente no existía correlación alguna entre el espesor de los tejidos en forma de palisada y la tolerancia al frío en las variedades estudiadas

El número de células por milímetro cuadrado en el parenquima de palisada superior, tanto como el contenido total combinado de las células de palisada y de la epidermis ventral de la hoja, pareció tener una relación directa con la reconocida tolerancia al frío al compararse las cuatro variedades bajo estudio.

Entre las variedades bajo estudio no se observaron diferencias apreciables en el diseño de las venas principales de la hoja. No obstante, se encontró que dentro de una misma variedad existía una proporción constante entre la anchura de la vena central y la anchura de la lámina. Se encontró que el valor numérico de esta proporción es inversamente proporcional a la tolerancia al frío de la variedad.

No pareció existir relación alguna en las variedades estudiadas entre su resistencia al frío y las frecuencias areolares, intervalos intervasculares, o el número de terminaciones de venas.

El preacondicionamiento por enfriamiento provocó un aumento mayor en la gravedad específica y la materia seca en las variedades más resistentes, entre las edades de 5 a 6 meses, al compararlas con las plantas testigos.

La formación temprana de ramas, lentículos y corcho en la variedad 'Mexican' de limón y en la china dulce 'Parson Brown', pareció estar inversamente correlacionada con la resistencia al frío, al compararse con la variedad 'Taiwanica' de naranja agria y la mandarina 'Changsha'.

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