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RESPONSES OF FOUR CITRUS SPECIES TO SOIL FLOODING UNDER GREENHOUSE CONDITIONS

A thesis submitted in partial satisfaction
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by

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ABSTRACT OF THE THESIS

Responses of Four Citrus Species to Soil Flooding Under Greenhouse Conditions

by

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University of California, Riverside, January 1972
Professor Walter Reuther, Chairman

Experiments were conducted in order to study the tolerance to soil flooding of four major citrus rootstocks: Carrizo citrange, Rough lemon, Sour orange and Sweet orange, under three temperature regimes. A secondary objective was to examine the root sap flow resistance as a means of evaluating root damage, in addition to direct counting of dead root tips.

Nine to ten months old seedlings growing in sterilized UC potting mixture were used in several completely randomized experiments. The experimental unit was one plant, with five replications for each treatment. Flooded and control plants were compared under three temperatures environments. Evaluation of injury was made by: 1. Counting of living and dead root tips using the TTC (2, 3, 5-triphenyl tetrazolium chloride) test, and 2. by measuring the resistance to root sap flow of decapitated seedlings with a Scholander pressure chamber, under a 10 bars pressure. Measurements were made at 7 and 14 days after flooding started.

The results obtained show that there are differences in tolerance to flooding among these rootstocks; based on percent of dead root tips the species can be ranked from resistant to susceptible as follows: Carrizo citrange, Sweet orange, Rough lemon and Sour orange.

Flooding increased resistance to root sap flow in all the species; root sap flow resistance was correlated with percent of dead root tips in some species (Rough lemon and Sour orange) but not in others. Root sap flow resistance could be a measure of injury to the water transport system of plants of Carrizo citrange and Sweet orange, where injury does not show visible symptoms. In general high temperatures increased injury by flooding in all the species tested.

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I. INTRODUCTION

Plant roots, like other living tissues, require oxygen for respiration, growth, nutrient absorption and other metabolic activities. Roots obtain oxygen from the atmosphere by diffusion through the soil or by internal aeration. When the supply of oxygen through the soil is deficient (as in flooded conditions) roots may obtain oxygen by internal aeration or may undergo anaerobic respiration.

Internal aeration during flooding apparently supplies adequate oxygen to roots of rice and a few other crops. In most other crops, however, anaerobic respiration occurs, with the consequent reduction in energy production and the production of alcohols and other metabolites which may reach toxic concentrations.

Crop plants vary in their ability to tolerate flooding. High tolerance to flooding in fruit trees is important in those areas where high water tables are common or where long rainy periods occur, as the flatwoods of Florida and the humid tropics.

Selection of citrus rootstocks tolerant to poor drainage has been mainly empirical and only in the last ten years there has been research aimed at this objective in the citrus areas of Florida. It has been very difficult to study separately the many factors affecting orchard tolerance like resistance to anaerobic conditions, rate of root regrowth, resistance to secondary fungal invasion, soil type, duration of flooding, soil temperature, etc.

The experiments reported here were conducted in an attempt to solve some simple questions. (1) Are there differences in tolerance to flooding among the following major rootstocks: 'Carrizo' citrange, 'Rough' lemon, 'Sour' orange and 'Sweet' orange, under sterilized soil conditions? (2) What is the influence of temperature? (3) Is there an influence of flooding on root permeability for water absorption? If so, is it related to root damage? It was hoped that information relating to these questions would provide a better insight into the nature of species or varietal tolerance to anaerobic conditions, and possibly provide a new approach to assaying such tolerance.

II. LITERATURE REVIEW

A. Drainage Problems in Citrus

Water management, including irrigation and drainage, has been one of the most difficult problems in producing citrus in the flatwoods of Florida (Ford, 1968). The same problems occur in the tropics on many soils where rainy periods are prolonged or high water table levels exist; indeed, wherever citrus trees are grown on imperfectly drained soils the problem is of importance (Dr. Walter Reuther, personal communication).

During and after heavy rains, surface drainage is necessary to remove excess water rapidly. The water that moves into the soil and raises the water table also has to be removed by subsoil drainage. Thus, both surface and profile drainage are necessary in most of the poorly drained soils used in citrus production (Ford, 1968; Young, 1948).

The citrus root system, like that of many other plants, is capable of rapid and deep growth in sandy soils, but will not grow into or exist long in a soil saturated with water. Thus, when the water table is within a few feet of the surface the roots are confined to a shallow zone (Ford, 1954, 1968). Limited moisture supply to the soil also determines root distribution through the profile (Aldrich, 1935).

Fluctuating water levels, as those that occur in areas with marked rainy and dry periods, have a pronounced harmful effect on the root system because they destroy feeder roots. The fluctuations of water table are dependent on rainfall, soil type, topography, type of surface and subsoil drainage and in some cases faulty irrigation practices (Ford, 1968).

Time is the critical factor in a fluctuating water table. Free water in the root zone for a few days will not do measurable damage to citrus trees, but flooding for a week will likely result in damage; on the other hand, if trees have been grown for some time over a relatively shallow water table, which during a dry period lowers more rapidly than the roots can grow, the trees may be left high and dry, and suffering severe moisture stress (Sites et al., 1964).

The damage to the roots under waterlogged conditions depends upon soil temperature and texture. Damage usually occurs faster in hot than in cool weather, and roots growing in fine textured soils are less prone to water damage than those in coarse textured soils (Stolzy et al., 1963; Young, 1948).

B. Physiological Responses of Plants Under Flooding Conditions

Many investigators have shown that the damage to roots under flooding conditions is due to lack of aeration in the root zone, inhibiting the interchange of gases (mainly oxygen and carbon dioxide) among roots, other living organisms in the soil and the aerial atmosphere.

Root respiration is the most sensitive activity of the plant influenced by soil aeration. Insufficient aeration causes reduction in activity and consequently in those root processes directly associated with respiration (Harris and Van Bavel, 1957; Letey et al., 1962; Fulton et al., 1964).

Lack of oxygen produces changes of the normal respiration pathway to anaerobic respiration or fermentation. When oxygen is present, pyruvate is oxidized through the Krebs cycle and the final product from each molecule of glucose is 6 molecules of carbon dioxide, 12 molecules of water and about 670 kg-cal of energy. This energy is used by the plant in normal metabolism and growth. When no oxygen is present, the pyruvate is not oxidized through the Krebs cycle, but is oxidized to form acetaldehyde, which is then reduced to alcohol. The final product from a molecule of glucose is 2 molecules of carbon dioxide, 2 molecules of alcohol and about 25 kg-cal of energy. This alcoholic fermentation has two disadvantages to the plant: (1) It produces only a small portion of the energy that could be produced by the normal respiration pathway. (2) It causes the formation of alcohol or other organic final products which can be toxic to the plant when present in high concentrations; in this manner it seems that the root is killing itself as long as the adverse condition of lack of oxygen remains (Woolley, 1965).

Inhibition of the Krebs cycle in roots and the accumulation of alcohols and subproducts of anaerobic respiration has been shown by several authors. However, there are differences in susceptibility to fermentation-induced damage among species (Crawford, 1967; Fulton et al., 1964; Fulton and Erickson, 1964; Grable, 1966; Hook et al., 1971; Woolley, 1965).

As early as 1938, Boynton et al. suggested that there are critical oxygen concentrations for the different stages of root development; for subsistence only, apple tree roots need a minimum of 0.1% of oxygen in the soil atmosphere, while to maintain growth of the existing root tips a concentration about 10% is necessary. Fiscus and Kramer (1970) suggested that the internal tissues of the roots are able to operate at low levels of oxygen.

The highest respiration rate occurs in the root tip (Luxmoore et al., 1970). Geisler (1965) indicated that the capacity of the roots to grow may be independent of external oxygen supply. However, Huck (1970) showed that root elongation is stopped by lack of external oxygen and the first portion of the root to die is the elongation zone. He indicated that the causes for this may be either variations in cell wall elasticity or variation in the rate of change of solute accumulation which would influence turgor pressure. Another explanation of the high degree of sensitivity of cell expansion to oxygen deficiency could be that the oxygen of the hydroxyproline extracted from cell walls is almost exclusively derived from the atmosphere. Lamport (1967), cited by Huck, proposed a role for this compound in altering the degree of cross-linkage in cell walls and hence their plasticity.

Decrease in oxygen supply to the roots causes reduction inccell division rate, in number and weight of new roots and decreased yield of foliage and fruit (Boynton and Compton, 1943; Fulton and Erickson, 1964; Varade et al., 1970; Williamson, 1968).

Since the shoot of the plant is affected relatively rapidly when plants are flooded, it seems clear that the dying root system is in some way involved in the injury to shoots. Toxic products coming from the roots and/or from microorganisms and soil and auxin accumulation could be directly responsible for some of the symptoms observed in shoots. In addition, accumulation of auxins in the root-shoot union zone is responsible for production of adventitious roots (Kramer, 1951; Phillips, 1964a, 1964b). Reduction of shoot growth is perhaps due to a reduction of gibberellic acid levels (Reid and Crozier, 1971).

Metabolic inhibitors (like ammonia compounds) reduce water uptake by roots (Stuart and Haddock, 1968). Although Larkum (1969) found that anoxia had very little effect on the water absorption of barley plants, many authors have found that deficient aeration causes a decrease of water uptake by the plant roots (Hagan, 1950; Kramer, 1951, 1969; Russell, 1959; Slatyer, 1960; Willey, 1970). In an attempt to explain this effect, Kramer and Jackson (1954) suggested that the following phenomena may be involved: (1) Deficient aeration produces an immediate, but temporary decrease in cell permeability, possibly by increased viscosity of the protoplasm. (2) Continued deficient aeration results in injury and finally death of at least some cells, resulting in an increase in permeability. (3) Plugging of the xylem vessels by decomposition products begins to occur, decreasing permeability, but decay finally results in an increase in permeability or finally in complete destruction of the root system.

Parallel with the changes in root permeability to water absorption, has been found that plants under waterlogged conditions suffer a small increase in rate of transpiration, followed by a pronounced decline (Clement, 1964; Kramer, 1969; Parker, 1950).

Lack of aeration causes unbalance of nutrient uptake by plant roots. Since this process is oxygen dependent (active), it is apparent why restricted aeration is usually followed by the appearance of nutrient deficiency and/or toxicity symptoms in plants. Many authors have shown that soil oxygen supply to the roots influences the concentration of most of the macro and micronutrients in roots and foliage (Labanauskas et al., 1965, 1966; Wallihan, 1961).

In a soil with oxygen deficient conditions, soluble iron, nitrites, iron sulfide and manganous oxides may accumulate to toxic concentrations (Buckman and Brady, 1969; Ford, 1968; Russell, 1959; Wallihan et al., 1961). Hydrogen sulfide at very low concentrations (0.2 ppm) is associated with injury to citrus roots; it is formed by bacteria (Genus *Desulfovibrio* and *Chlostridium*) in an environment without oxygen, using plant roots as substrate (Ford 1965, 1968; Ford and Calvert, 1966).

Root injury by poor soil drainage combined with attack by root-rotting fungi results in severe root decay problem. Resistant species may have the ability to minimize or isolate the infection, after good aeration is established again, by formation of wound periderm or other physiological characteristics (Stolzy et al., 1965a, 1965b, 1967; Van Gundy and Kirkpatrick, 1964).

Low oxygen conditions are more detrimental to plant growth and damage to roots is more severe as the temperature increases (Bolton and

Erickson, 1970; Kramer and Jackson, 1954; Letey et al., 1962; Letey and Stolzy, 1964; Russell, 1961; Sites et al., 1964; Valoras et al., 1964; Varade, Stolzy and Letey, 1970; Williamson and Splinter, 1969.

C. Species Resistance to Flooding Conditions

Plants in natural waterlogged conditions get adapted by development of a superficial root system or specialized roots which are functional in submerged conditions in absence of external oxygen. These adventitious roots are thicker than normal ones because of the presence of large intercellular spaces (aerenchyma), through which these roots derive oxygen from shoots. Old roots may also produce larger intercellular spaces. (Van't Woudt and Hagan, 1957; Yu et al., 1969; Varade et al., 1970).

It seems that herbaceous plants are better able to form adventitious roots than are woody plants (Heinicke, 1932; Kramer, 1951). Rice is the only major crop that is able to grow and produce while its roots are submerged (Valoras and Letey, 1966; Van't Woudt and Hagan, 1957). However, some plants that usually do not live in a wet root environment present resistance to injury by flooding, as is the case of short leaf pine, lobolly pine and pond pine (Hunt, 1951).

Evans and Ebert (1960), using radioactive oxygen in Vicia faba plants, found that oxygen moved from the cotyledons down into the main roots. Armstrong (cited by Hook et al., 1971) obtained similar results from several bog plants and from two woody genera.

Using the Jensen (1969) technique to measure root porosity, Luxmoore et al., (1971) obtained the following values of root porosity for several citrus species: 'Rough' lemon 7.2%, 'Troyer' citrange 9.8% and 'Sweet' orange 10.9%. Unfortunately the authors did not measure root porosity of 'Sour' orange which is one of the rootstocks considered resistant to poor soil aeration. The values obtained for these rootstocks are comparable to values obtained for wheat (Dr. L. H. Stolzy, personal communication).

Hook et al (1971) indicated that the assumption that tolerance of some plants to flooding is due to diffusion of oxygen from the atmosphere to the roots via the stem, seems to be inadequate because oxygen is transported through stems to the roots of onion, pea, lettuce, beet root, leek, buckwheat, turnip, barley, ryegrass, carrot, cabbage and corn, yet none of these species are tolerant to flooding. They concluded that transport of oxygen to root may be a necessity but apparently does not adequately provide for flooding tolerance in plants.

Another explanation for differences in tolerance to flooding is that roots of some species are tolerant of anaerobic respiration (Kramer, 1969; Hook, et al., 1971). Accelerated or sustained rates of anaerobic respiration have been found in rhyzomes of Nuphar advenum, and the roots of rice and several other species under low oxygen concentration or in its complete absence (Hook, Brown and Kormanik, 1971). They also indicated that in addition to increased root porosity and presence of anaerobic respiration, flooding results in the accumulation of CO_2 and other gases as well as carbonates and bicarbonates of iron, manganese and aluminum in the soil solution. Species differences in tolerance to these factors could be associated with their flooding tolerance. They found that Swamp tupelo (*Nyssa sylvatica* var. *biflora*) seedlings develop new roots that are able to tolerate anaerobic respiration, oxidize their rhyzosphere and tolerate high CO_2 concentrations. The combination of these adaptations of the new roots appears to be sufficient to account for flooding tolerance in Swamp tupelo. The absence of anyone of these adaptations would appear to reduce the flooding tolerance of a specie.

In summary, although there are many references dealing with the subject there is lack of knowledge concerning the basis or mechanism of tolerance of species to flooding conditions. There are contradictions and inconsistencies reported in the literature pertaining to plant responses to waterlogging. The problem is of importance since it involves many interrelated factors acting on the plant (such as anaerobic respiration, plant, soil and micro-organisms toxic products, pathogen invasion, etc.), and plant behavior is not consistently correlated with soil aeration. Working on the problem from all angles, including field and greenhouse experiments, water culture and biochemical investigations should give a better understanding of the phenomena associated with plants tolerance to poor drainage.

III. MATERIALS AND METHODS

A. Materials and experimental conditions

Citrus seedlings 9 to 10 months old of the following species were used: 'Rough' lemon (Citrus lemon) var. Florida, 'Sour' orange (Citrus aurantifolia) var. Rubidoux, 'Sweet' orange (Citrus sinensis) var. Koethen and Carrizo Citrange (Poncirus trifoliata x C. sinensis hybrid).

Seedlings were grown in 10 ounce (approx. 350 ml) foam cups containing sterilized UC potting mixture as modified by Nauer et al. (1968). Plants were watered normally and once a week a dilute fertilizer solution was added to the irrigation water. The potting mixture had a pH of about 5.5 and it varied with flooding between 5.5 and 6.5.

For the flooding treatment plants were placed in plastic food containers of 12 ounces (approx. 450 ml), giving a water level of about 2.5 cm above the soil level of the plant in the cup. The containers were covered with black plastic to avoid entrance of light and proliferation of algae in the water. The distilled water used for initial flooding and daily replenishment was previously treated for 12-15 hours by bubbling with nitrogen gas, to minimize the amount of oxygen present in the water.

Flooded and control plants were compared in several completely randomized experiments. Experiments were carried out in two controlled greenhouses with the following programmed temperature regimes: COLD with daily mean of 20-22 C and night mean of 10-13 C; HOT with daily mean of 30-34 C and night mean of 20-24 C. A third environment AMBIENT, was a lathouse with temperatures prevailing in Riverside during the month of September 1971, when the maximum daily was 37 C and the minimum 19 C. The experimental unit was one plant, with five replications of each treatment. Measurements were made at two dates (7 and 14 days) after flooding started.

B. Methods of evaluating injury

Degree of injury to the plants was evaluated by: (1) Measurements of root permeability or resistance to root sap flow and (2), amount of dead root tips.

Since several authors have indicated that poor aeration causes changes in root permeability to water absorption, an attempt to evaluate those changes was made by using a Scholander (1965) pressure chamber, as used by Kaufmann (1967). Plants were decapitated and placed in the pressure chamber with the cut stump exposed and the soil completely saturated with water. A pressure of 10 bars was applied, and after the pressure forced water to the surface of the stem, the rate of sap flow was measured by using a potometer (1 mm diameter) attached to the cut stem. The time required for 31.4 microliters of exudate to accumulate was recorded. The time per unit of volume provides an estimate of the resistance to root sap flow of the plant. This is related directly to the resistance of the root system to sap flow which is in turn dependent on permeability and area of absorbing surface, on resistance of the cellular and xylem transport systems, and possibly other factors.

The pressure used was selected after a comparison of resistance to sap flow at 4 different pressures (5, 10, 15 and 18 bars) in three experiments, each under different temperature conditions (COLD, HOT and AMBIENT). In all the experiments the higher the pressure, the higher was the sap flow rate, but the greatest difference between controls and flooded plants in most of the species was found at 10 bars (Table 1).

(2) The amount of dead root tips was determined as follows: The root systems of 3 of the 5 plants previously used in the pressure chamber test were washed and a random sample of 70 to 100 root tips (2 to 5 mm long) was obtained. Samples were placed in Petri dishes containing a solution 0.5% of 2,3,5-triphenyl tetrazolium chloride (TTC or Formazan), for a period of 12 to 16 hours. Root tips which acquired a red color were considered as living. The percentage of living root tips was calculated.

Analysis of variance was conducted for all the experiments and the separation of the effects of temperature and flooding was made by Multiple Range Test for each set of data.

Table 1. Resistance to root sap flow of plants flooded for 7 days as a percentage of controls (100%), under four pressures. (').

Species	Pressure (bars)			
	5	10	15	18
Carrizo Citrange	150.7	190.4	186.0	215.4*
Rough Lemon	103.4	144.1*	103.0	125.7
Sour Orange	267.0	314.2*	245.8	185.1
Sweet Orange	162.2	180.5*	131.9	149.1

('): Data average of three experiments.

* : Greatest difference between control and flooded plants.

IV. RESULTS

A. Evaluation of percent of living root tips

The data in Table 2 summarize the influence of aeration, temperature and species on the percent of living root tips. Since triphenyl tetrazolium chloride is a compound which detects tissue with high metabolic activity (Roistacher et al., 1957), the controls did not always show one hundred percent of living root tips because either some roots were not actively growing or were in fact dead. This contributed to variability and reduced precision in comparing treatment effects.

The effect of flooding in killing roots was well defined, in general, when plants were sampled after 14 days, but not when sampled after 7 days. In general the controls had greater percentage of living root tips than flooded plants. However, flooded plants of Carrizo Citrange did not show significant differences when compared with the controls at either of the two sampling periods or under any of the three temperature regimes. Similarly 'Sweet' orange showed significant difference only in one experiment under hot temperature when sampled at 14 days. Those two rootstocks seem to be relatively resistant to killing of root tips by the anaerobic conditions imposed.

On the other hand, 'Rough' lemon showed a significant influence of flooding at 14 days in both hot and ambient experiment and in one of

Table 2. The influence of flooding on the percentage of living root tips (TTC test) with four citrus species in relation to three temperature regimes and two dates of sampling (*).

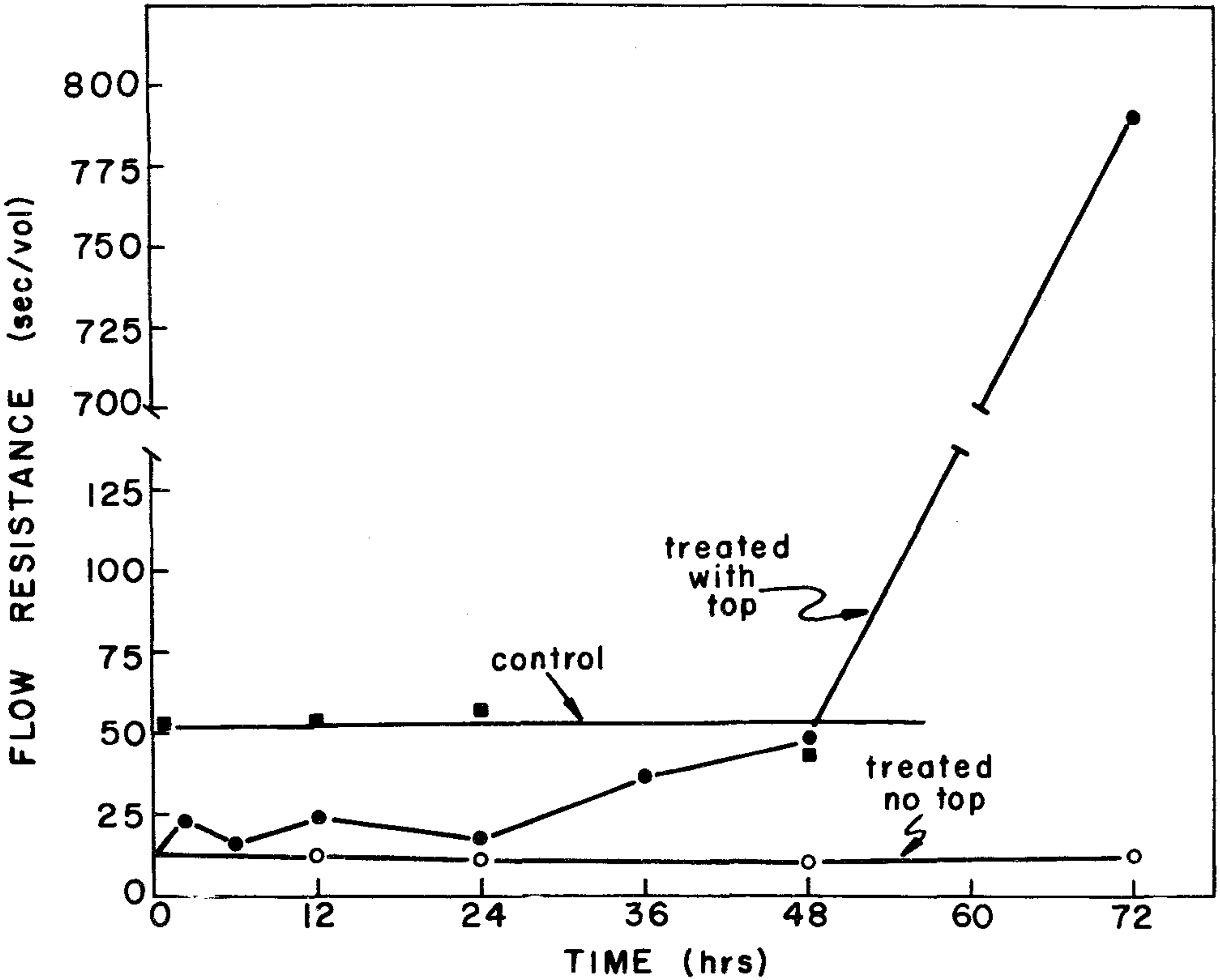
Species	Days	C O L D				H O T				AMBIENT	
		Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1	
		F†	C	F	C	F	C	F	C	F	C
Carrizo	7	85.8	98.8	99.3	100.0	99.1	99.1	97.0	96.7	92.1	79.4
Citrange	14	98.5	99.7	81.8	98.4	98.3	100.0	90.5	96.6	97.2	96.5
Rough	7	71.2	87.0	94.6	99.5	59.3**	98.2	83.2	97.7	81.7	89.9
Lemon	14	69.2**	99.3	93.6	99.2	16.0**	99.2	53.6**	99.2	12.2**	99.0
Sour	7	70.3	88.0	86.8	98.1	75.6	91.5	93.9	97.6	79.5	89.2
Orange	14	54.5**	98.9	89.8	99.2	44.0**	99.6	73.5**	100.0	17.5**	99.5
Sweet	7	91.4	76.5	97.2	100.0	96.8	96.2	99.2	98.7	64.3	82.7
Orange	14	94.6	99.5	95.7	100.0	83.1	97.8	79.0**	100.0	87.9	97.6

*: Each figure is the mean of three plants sampled.
†: Flooding treatment, C: Control.
**: Difference between F and C significant at 1%.

the cold experiments. In addition, the first hot experiment showed a significant difference after 7 days. This may indicate that this specie is even more susceptible than 'Sour' orange to flooding treatment when high temperatures prevail. After 14 days of flooding in the hot environment several 'Rough' lemon plants presented wilting symptoms and three plants were completely wilted. Similar symptoms were observed in 'Sour' orange after 18 to 22 days of flooding. Both species presented significant differences in one of the experiments in the cold environment, suggesting that their tolerance to flooding is increased at low temperatures, but not enough to approach that of Carrizo and 'Sweet' orange. Again, more replications probably would clarify this apparent interaction of species tolerance to flooding and temperature.

B. Evaluation of sap flow resistance measurements

In order to explore the relation of root sap flow measurements to root damage, a simple experiment was performed. 'Rough' lemon seedlings were subjected to irrigation with hot water (65 C) for 15 minutes; this treatment killed the entire root system of such plants, as indicated by the TTC test. A group of hot water treated and control plants were left intact while another was decapitated. Root sap flow measurements were performed in both groups of plants at different times during a period of 72 hours. Figure 1 shows the results obtained. The control plants showed uniform resistance to root sap flow during the experimental period. All plants treated with hot water presented low resistance to sap flow in the first readings as compared with the controls.



FLOW RESISTANCE (sec/vol)
800
775
750
725
700
125
100
75
50
25
0
0
12
24
36
48
60
72
TIME (hrs)
control
treated with top
treated no top

Plants that remained intact for various intervals after the hot water treatment started showing a steady increase in resistance after 24 hours, which became similar to that of the controls at about 48 hours; at 72 hours the resistance was very high in comparison with the controls. The plants decapitated immediately after hot water treatment did not show an increase in resistance; it was maintained at about the same level during the period studied.

These results show that when plants are intact killing of the root system results in an increase in resistance to root sap flow after a lag period of about 72 hours. Plants without tops may eventually show similar increase in resistance to sap flow, but after a longer period of time. The influence of the aerial part of the plant in accelerating the rise in resistance to sap flow in plants with severely injured root system is clear, but the mode of action is not. These preliminary data suggested, then, that root sap flow resistance might be a useful index of root damage.

The data on resistance to root sap flow obtained in relation to flooding, temperature and species are summarized in Figures 2, 3, 4, 5, and 6. In general, regardless of species or temperature regimes, flooded plants showed higher resistances to root sap flow than controls. In the cold temperature experiments no species showed a significant difference between control and flooded plants after 7 days, except Carrizo citrange which did show a significant difference in one of the experiments (Fig. 2). After 14 days, the flooded plants of Carrizo had significantly higher resistances than the corresponding controls in both experiments (Fig. 3). At the same date 'Sour' and 'Sweet' oranges showed significant

Figure 2. Comparison of root sap flow resistance between control and flooded plants of four citrus species, under cold temperature after 7 days of flooding in two different experiments. (N.S.: no significant difference; *: no significant difference; *: significant difference at 5% level; **: significant at 1% level. Y axis in logarithmic scale.)

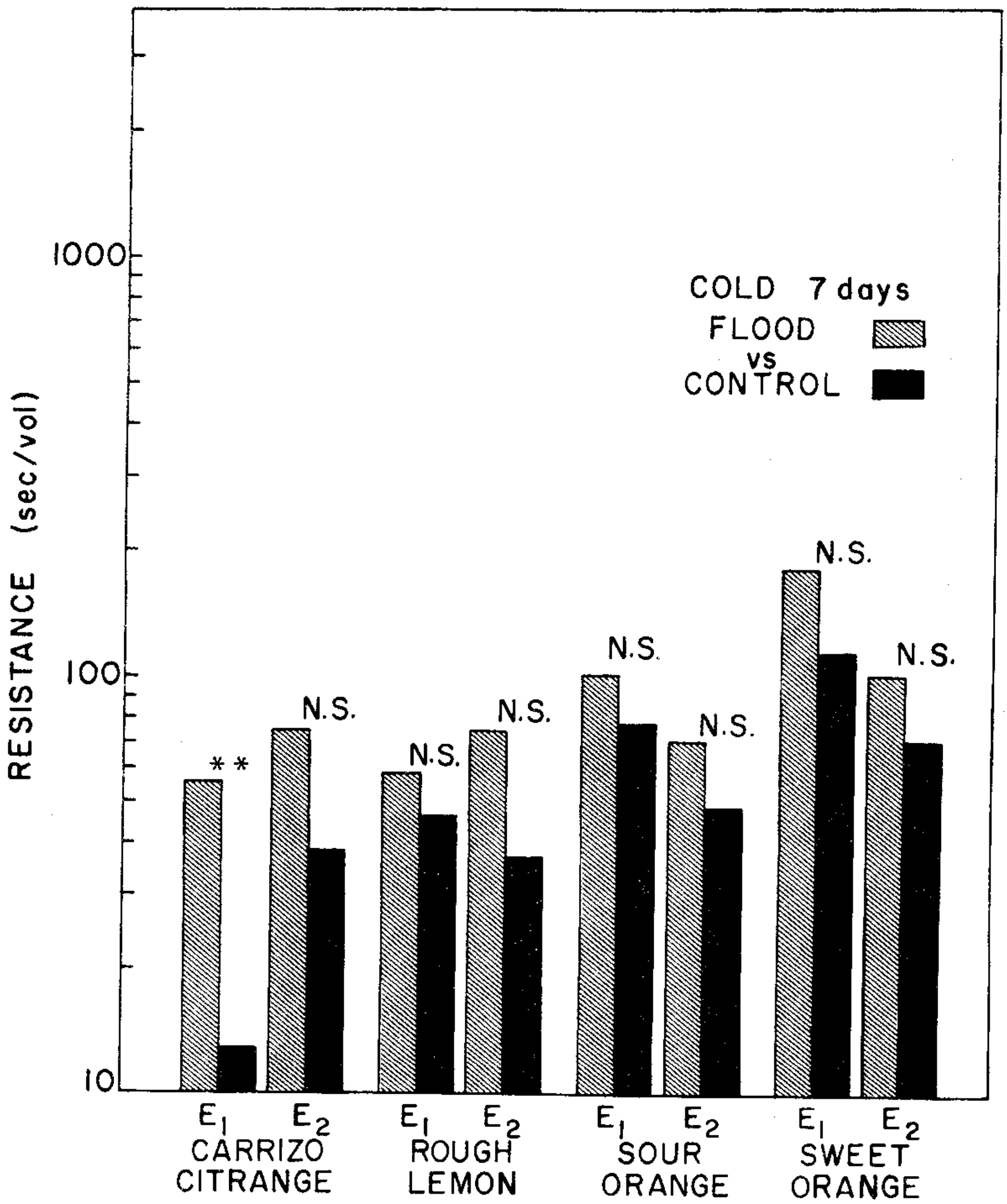
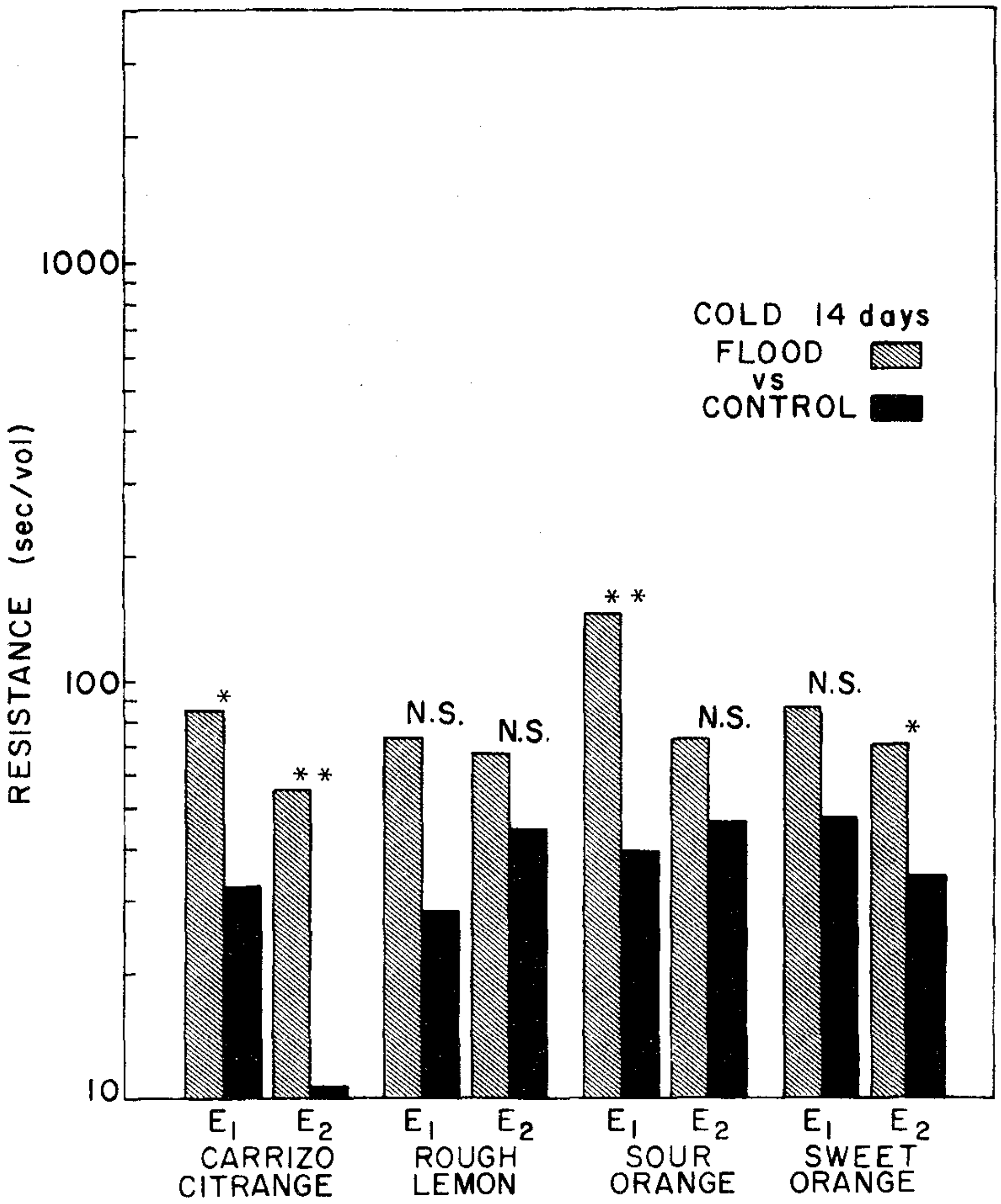

RESISTANCE (sec/vol)
1000
100
10
COLD 7 days
FLOOD
vs
CONTROL
**
N.S.
E1 E2
CARRIZO CITRANGE
E1 E2
ROUGH LEMON
E1 E2
SOUR ORANGE
E1 E2
SWEET ORANGE

Figure 3. Comparison of root sap flow resistance between control and flooded plants of four citrus species, under cold temperature after 14 days of flooding in two different experiments. (N.S.: no significant difference; *: significant difference at 5% level; **: significant at 1% level. Y axis in logarithmic scale.)



RESISTANCE (sec/vol)
1000
100
10
COLD 14 days
FLOOD
vs
CONTROL
*
* *
N.S.
N.S.
* *
N.S.
N.S.
*
E1 E2
CARRIZO CITRANGE
E1 E2
ROUGH LEMON
E1 E2
SOUR ORANGE
E1 E2
SWEET ORANGE

difference in one of the two experiments. 'Rough' lemon did not show great differences in any of the experiments in both dates.

The species tested presented a different pattern of behavior when similar experiments were carried out in the hot temperature greenhouse. After 7 days flooded plants of Carrizo citrange and 'Sour' orange had significantly higher resistances only in one of the two experiments (Fig. 4). Flooding had no detectable effect on resistance in 'Rough' lemon and 'Sweet' orange. When measurements were taken after 14 days, flooded plants of 'Rough' lemon and 'Sour' orange had very high sap flow resistance in comparison with the controls (Fig. 5). Carrizo citrange and 'Sweet' orange only showed significant differences in one of the experiments. Again it seems that more replications would be required to evaluate these differences between control and treated plants with more confidence.

The data of the experiment under ambient conditions show a high increase in resistance for flooded plants of 'Sour' orange at both 7 and 14 days (Fig. 6). Carrizo citrange and 'Rough' lemon presented significant effect of flooding.

It is apparent that there is a high variability in resistance among the control plants for each species (possibly with the exception of 'Sweet' orange), throughout the experiments under all the conditions.

Figure 4. Comparison of root sap flow resistance between control and flooded plants of four citrus species, under hot temperature after 7 days of flooding in two different experiments. (N.S.: no significant difference; *: significant difference at 5% level; **: significant at 1% level; Y axis in logarithmic scale.)

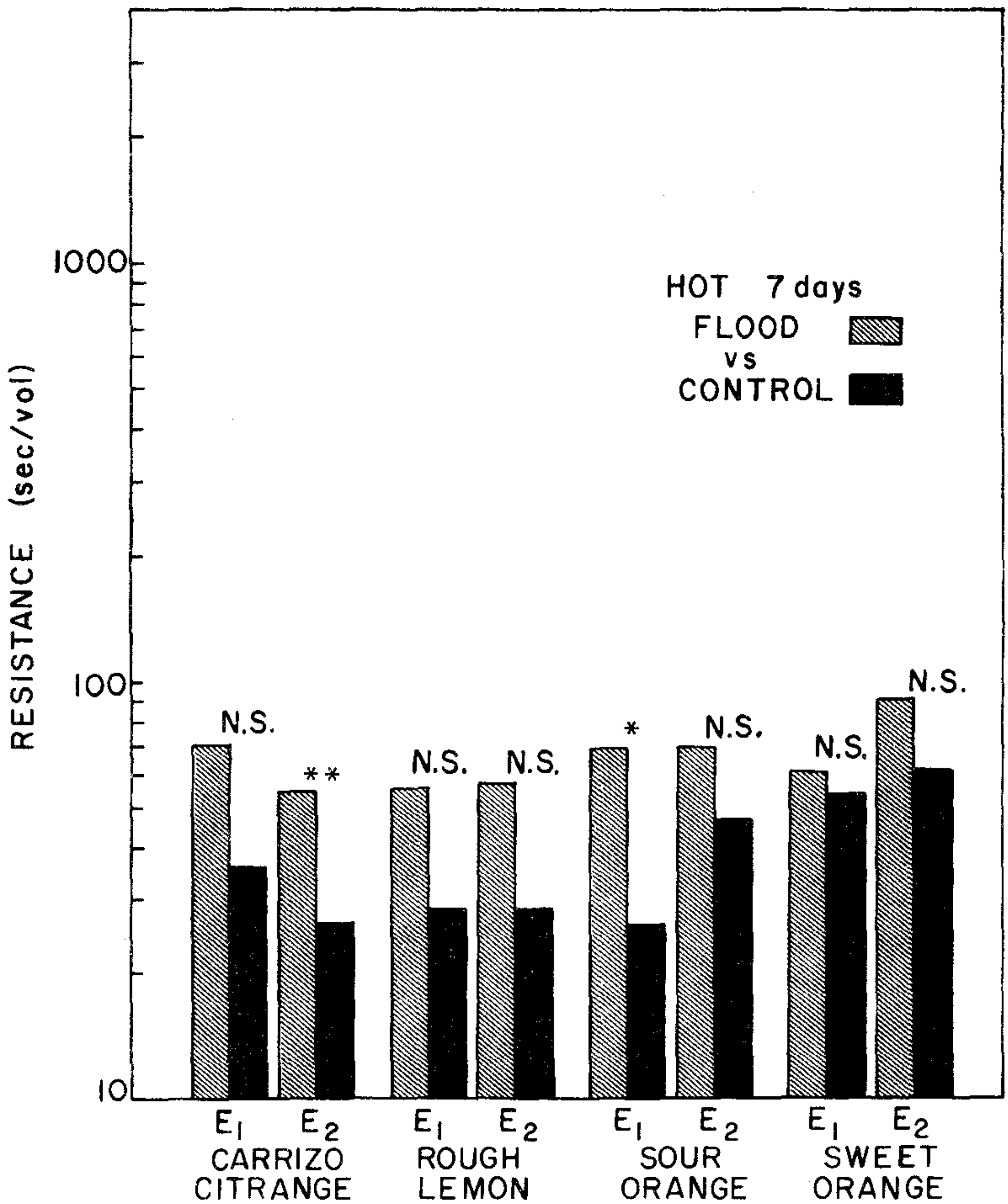
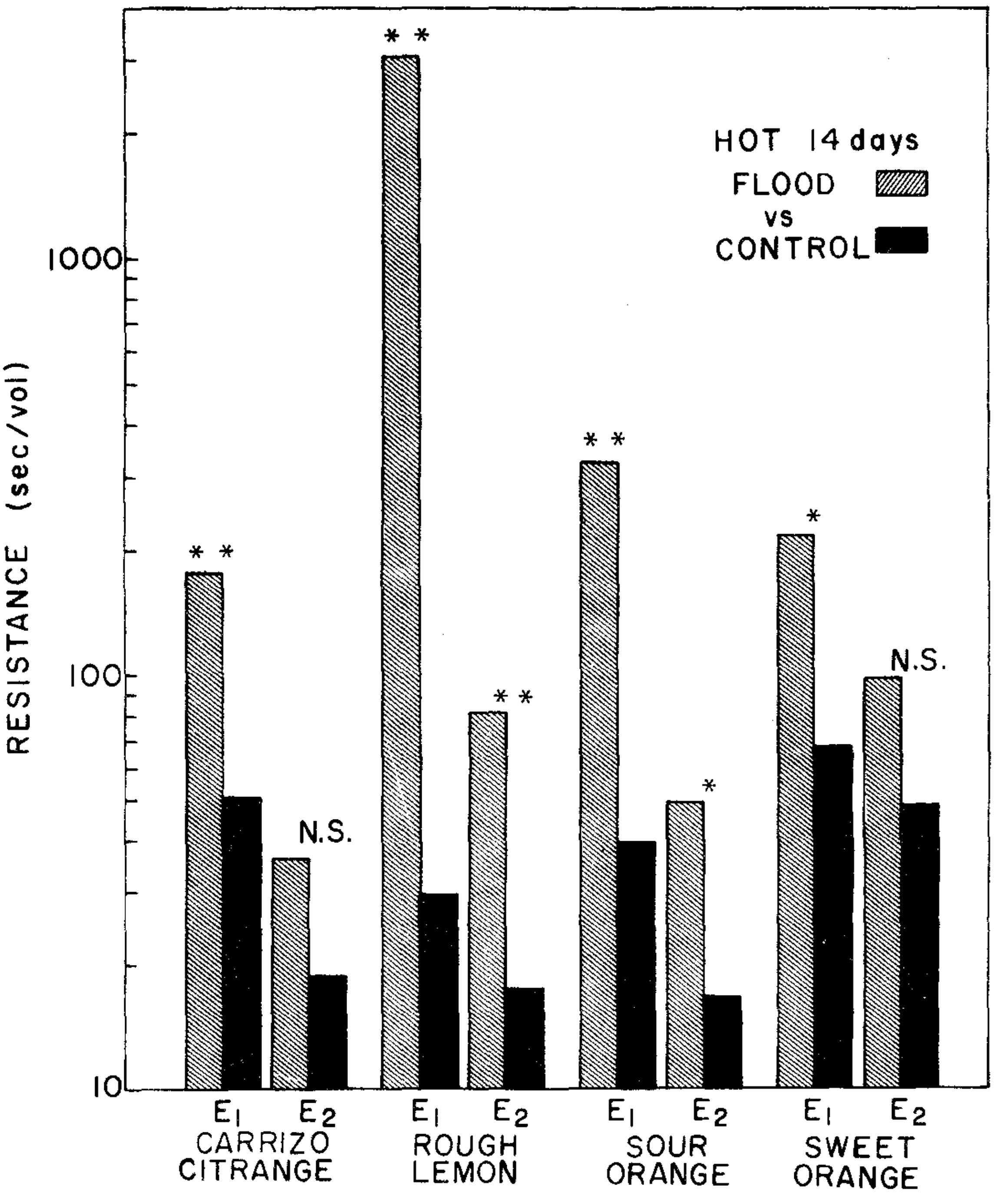
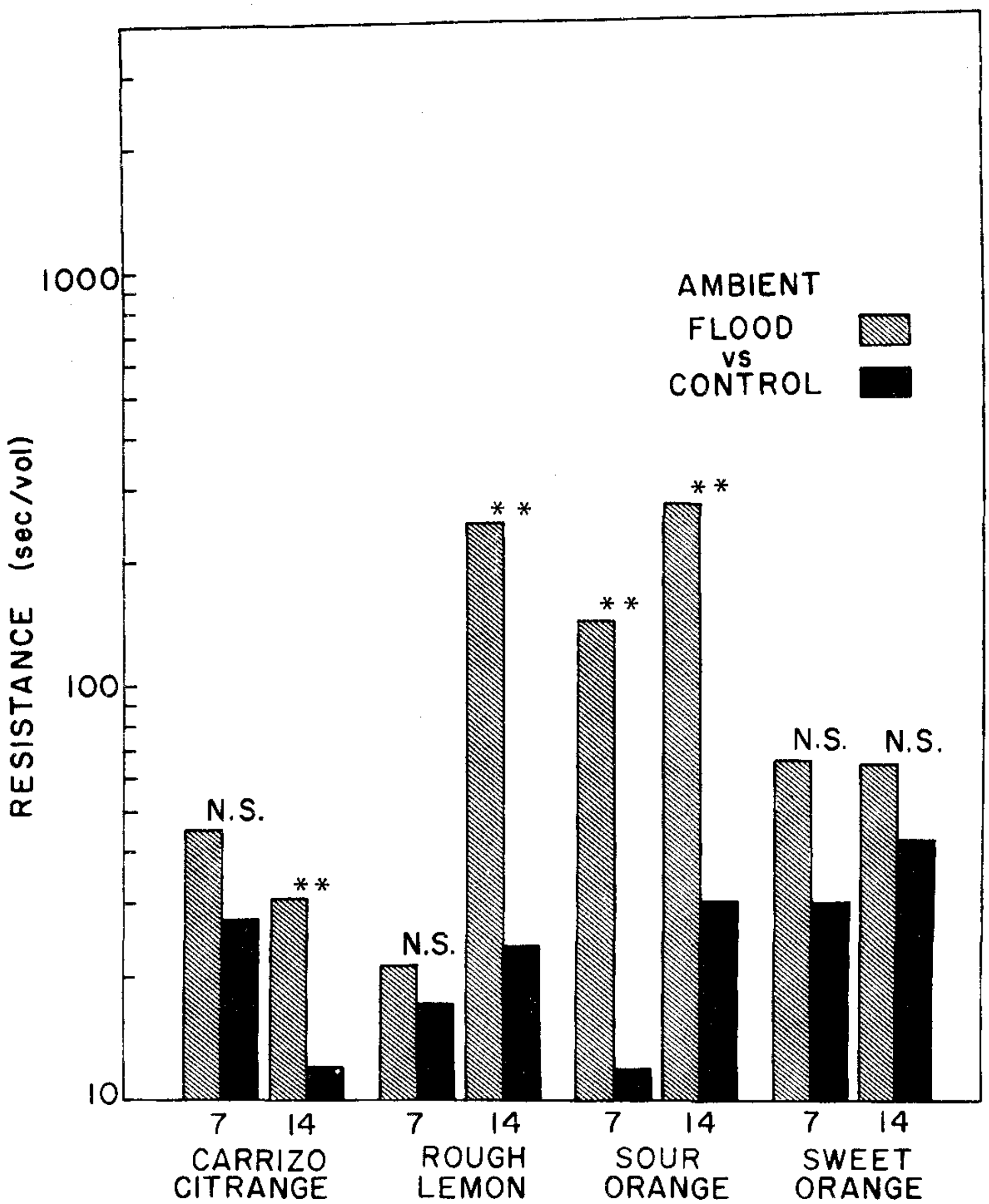

RESISTANCE (sec/vol)
1000
100
10
HOT 7 days
FLOOD
vs
CONTROL
N.S.
**
N.S.
N.S.
*
N.S.
N.S.
N.S.
E_1
E_2
CARRIZO CITRANGE
E_1
E_2
ROUGH LEMON
E_1
E_2
SOUR ORANGE
E_1
E_2
SWEET ORANGE

Figure 5. Comparison of root sap flow resistance between control and flooded plants of four citrus species, under hot temperature after 14 days of flooding in two different experiments. (N.S.: no significant difference; *: significant difference at 5% level; **: significant at 1% level. Y axis in logarithmic scale.)



RESISTANCE (sec/vol)
1000
100
10
HOT 14 days
FLOOD
vs
CONTROL
* *
* *
N.S.
* *
* *
*
*
N.S.
E1 E2
CARRIZO CITRANGE
E1 E2
ROUGH LEMON
E1 E2
SOUR ORANGE
E1 E2
SWEET ORANGE

Figure 6. Comparison of root sap flow resistance between control and flooded plants of four citrus species, under ambient conditions after 7 and 14 days of flooding (N.S.: no significant difference; *: significant difference at 5% level; **: significant at 1% level. Y axis in logarithmic scale.)



RESISTANCE (sec/vol)
1000
100
10
AMBIENT
FLOOD
vs
CONTROL
N.S.
* *
N.S.
* *
* *
* *
N.S.
N.S.
7
14
7
14
7
14
7
14
CARRIZO
CITRANGE
ROUGH
LEMON
SOUR
ORANGE
SWEET
ORANGE

V. DISCUSSION

The results of the experiment in which plants were treated with hot water showed that following treatment plants had low resistance to root sap flow (high permeability) in comparison with controls, but later high resistance was attained in a relatively short period of time when the plants were left intact until measured. When those high resistances occurred, the plants were completely wilted, suggesting a drastic decrease in water absorption and/or transport.

This behavior with time of these plants could be explained by the hypothesis proposed by Kramer (1954) suggesting that when root cells die an increase in permeability results, followed by plugging of the xylem vessels by decomposition products with the consequent decrease in permeability or increase in resistance to root sap flow under pressure. Another possible explanation of the initial effect could be that the disruption of cell membranes cause by high temperatures induced leakage of salts and soluble protoplasmic components into the xylem vessels, increasing the osmotic pressure in the xylem vessels and consequently causing an increase in permeability or water absorption. In fact, root pressure exudation was observed in some plants when decapitated immediately after the hot water treatment. When the top of the plant remained intact, with leaves normally transpiring, the tension of the water column in the xylem vessels increased as the water demand in the leaves continued. Liquid flow in the transpiration stream could result in accumulation of damage protoplasmic material of the xylem parenchyma cells in the xylem vessels causing a situation similar to the so called tyloses. Further anatomical studies in such root systems would reveal what really happens.

It seems clear that the measurement of resistance to root sap flow could be useful to evaluate root injury, even when the damage is caused by means other than hot water treatment such as injury due to anaerobic conditions or pathogens. When root injury caused by flooding on 'Rough' lemon and 'Sour' orange plants is evaluated by both percentage of living root tips and root sap flow resistance, there is close agreement between those two measurements. In both species flooded plants were badly affected by the anaerobic conditions when a hot environment prevailed and it was more apparent in the data taken at 14 days; the amount of living root tips was decreased strongly and the resistance to root sap flow was increased greatly. On the other hand the data for Carrizo citrange did not show similar concordance. No significant root damage was indicated by the TTC test under any of the three temperatures, but resistance to root sap flow was significantly higher in flooded plants in cold, hot and ambient conditions. There was no relationship between the root tip damage and sap flow resistance data in this specie. 'Sweet' orange showed slightly more concordance between these sets of data, but still the agreement is unclear.

These results suggest that root sap flow resistance and root tip damage are reasonably well correlated in some species such as 'Rough' lemon and 'Sour' orange. In the case of Carrizo and 'Sweet' orange it is possible that the root sap flow measurement is indicative of some kind of damage, other than just death of root tips, that is effectively reducing the water absorption and/or transport capacity of the root. If this is so, the root sap flow resistance could be a more sensitive measure of damage than the incidence of dead root tips in some species. However, it may be that when high sap flow resistance is associated with low percentage of living root tips (as in 'Rough' lemon and 'Sour' orange) the damage which occurred in the roots is more permanent and harmful than when high sap flow resistance occurs without being associated with dead root tips (as in Carrizo and 'Sweet' oranges).

The variability in response to flooding observed in the species between similar experiments under one temperature, may be due to the relatively low number of replications used in each treatment. In addition, the experimental system did not provide for the maintenance of a constant level of water above the soil line in the flooded plants, due to the water lost by transpiration; this fluctuation of water level could account for diffusion of some oxygen to the roots. Another observation is that plants used in the second experiment under both cold and hot environment were 15 days older than the plants used in the first experiment; this difference in age could account for part of the variability obtained. Dr. Harry Ford (personal communication) indicated that the older the plant the more tolerant it is to flooding.

Another experimental error that could induce variability of the results was the fact that none of the plants had a prior period of adaptation to the cold or hot temperatures under which the experiments were carried out. This situation may account for some variability of both control and flooded plants.

Under the conditions of the experiments, the species showed distinct capability to adapt to waterlogging. Carrizo citrange was the first in showing that ability. Although the shoot stopped growing, soon new adventitious roots started growing in the root-shoot union zone. Slight wilting was observed during the first 3-4 weeks and some plants resumed shoot growth after 4 weeks. 'Sweet' orange stopped shoot growth in the first week and no wilting symptoms appeared before the third week; however, very few plants showed adventitious root production at 14 days. Possibly the ability of this cultivar to tolerate prolonged flooded conditions depends on the ability to stop growth and maintain a low metabolic activity. None of the plants of 'Rough' lemon and 'Sour' orange showed adventitious roots in the first 4 weeks of flooding. Wilting symptoms were apparent in the first 2 weeks and some 'Rough' lemon plants were dead after 14 days. Only after 4-6 weeks were some adventitious roots observed in plants of these two species.

In ranking the species tested for tolerance to flooding there is fair agreement when they are ranked either with respect to the amount of dead root tips or with respect to sap flow resistance (Table 3). When comparing the ranking based in the present experiments with the ranking of the same species based on experiments and observations under field conditions (Ford, 1964; Ford, personal communication; Gardner, 1961) it is found that Carrizo citrange is one of the most

Table 3. Ranking of 4 citrus species in descending order of tolerance to flooding.

	PARAMETER		
Specie	Dead root tips	Sap flow resistance	Florida field experience
Carrizo Citrange	1	2	1
Sweet orange	2	1	3-4
Rough lemon	3	3-4	1
Sour orange	4	3-4	1-2

tolerant to anaerobic conditions when used as a rootstock in the orchard soil. However, 'Rough' lemon, which was one of the most susceptible in the experiments reported here, is ranked by Florida experimenters as one of the most tolerant rootstocks under field conditions. In a similar way, 'Sour' orange appears to have relatively good tolerance to flooding under field conditions while here it was the most susceptible. Finally, 'Sweet' orange was, together with Carrizo, very resistant in our experiments, but is the most susceptible under field conditions.

Several factors may account for the differences in ranking of the species between these experiments and Florida experience. The first and maybe the most important is the fact that the experiments reported here were carried out using plants that were grown in a sterilized potting mixture. It cannot be said that the soil was completely sterile at the time when the plants were flooded, but the soil mixture still was almost free of pathogens and bacteria which are commonly present in the orchard soil. Consequently the plants were subjected only to the effects of anaerobic conditions without great risk of secondary pathogen invasion or damage from toxic products of anaerobic soil bacteria. It is likely then that the main factor causing injury to the roots was the anaerobic condition with the consequent induction within the roots of anaerobic respiration and its byproducts. Resistance to the flooding conditions imposed possibly is related to the ability of the roots to undergo anaerobic respiration without irreversible injury to the root tips due to toxic byproducts and perhaps to the ability of the plants to transport oxygen through the intercellular spaces of stem and root. Probably the ability to produce adventitious roots near the better aerated free water surface in a short period of time is an additional factor accounting for tolerance of the seedlings to flooding in the greenhouse. Another very important factor possibly accounting for the apparent difference in tolerance found in Florida with orchard trees in that the seedling stage may respond differently than a mature tree, especially a budded "composite" orchard tree.

Differences in tolerance to secondary pathogen invasion may be another factor influencing species tolerance to flooding under field conditions. Thus, while 'Rough' lemon and 'Sour' orange are very susceptible to anaerobic respiration injury, they may be more resistant to secondary pathogen invasion or their roots are able to form wound periderm and regenerate new roots more rapidly, as indicated by Van Gundy and Kirkpatrick (1964), and so may tolerate better the flooding conditions in the field than 'Sweet' orange.

Another factor influencing tolerance of species to flooding in natural conditions is their relative susceptibility to chemical products naturally occurring in the flooded soil and to chemicals produced by anaerobic bacteria.

It would be desirable to conduct more refined experiments, including better control of anaerobic conditions, than those described here in order to get a clearer picture of how these rootstocks behave under completely sterile conditions. A chemical analysis of the root sap collected when measuring sap flow resistance with the Scholander pressure chamber could reveal more about the metabolic processes occurring in the root system of flooded plants. Fulton et al. (1964) reported sap analysis of flooded tomato plants. The use of older plants and the design of experiments in which the relative tolerance to individual factors influencing the root system (like soil type, duration of flooding, pathogens invasion, etc.) could be isolated and evaluated also would help to provide a clearer insight into this complex problem.

VI. CONCLUSIONS

From the experiments reported several conclusions may be drawn:

1. There are differences in tolerance to flooding under the conditions used among the rootstocks tested. With respect to root tip death, the rootstocks may be ranked in descending order of tolerance as follows: Carrizo citrange, 'Sweet' orange, 'Rough' lemon and 'Sour' orange.
2. Root injury caused by flooding is accompanied by an increase in resistance to root sap flow. There is correlation between sap flow resistance and dead root tips in 'Rough' lemon and 'Sour' orange. Although there is no correlation between these measurements in Carrizo citrange and 'Sweet' orange, sap flow resistance nevertheless may be a measure of injury to the water absorption and transport capacity of the roots of these two species.
3. High temperatures increased root injury by flooding in all the species tested when evaluated by the TTC test.
4. There seems to be a pronounced effect of high temperatures in increasing the root sap flow resistance of flooded plants.

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