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RESUME OF INVESTIGATIONS CONCERNING THE OXYGEN REQUIREMENTS OF AVOCADO SEEDLINGS INCLUDING A STUDY OF INTERRELATIONS TO NITRITE AND PHYTOPHTHORA CINNAMOMI

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The sensitivity of avocado trees to over-moist soil conditions is well known. The reason for this is not that the water in itself is detrimental, for avocados can be grown and maintained in a healthy condition in water cultures; rather the difficulty is that proper soil aeration is hindered when the soil is waterlogged or over-moist, and not only are plant roots deprived of needed oxygen but carbon dioxide, resulting from the respiration of plant roots and microorganisms, accumulates and a whole chain of other chemical and biological changes occurs. All of these, singly or combined, may influence and alter the normal course of root growth, absorption, and metabolism.

Because of the extreme complexity of the soil, and the difficulty of studying one factor at a time in this medium, many workers in an attempt to understand better the role of a given factor have chosen to study the behavior of plants in water or sand cultures where one or more factors can be varied at will and their effect on the plant noted.

As part of a general investigation of the cause or causes of avocado root rot, and the inference that, because of the plant's sensitivity to over-moist soil conditions, its oxygen requirement must be different than that of other trees such as citrus, the Division of Soils and Plant Nutrition, of the University of California Citrus Experiment Station, was asked to investigate the oxygen requirement of the avocado. Because of the intimate relation of oxygen supply to nitrite formation in soils, and the possibility also that avocado roots might be more or less sensitive to invasion by the fungus *Phytophthora cinnamomi* Rands, depending upon the oxygen level in the nutrient medium, these aspects were also studied.[2]

[1] *All of this work has been published in scientific journals and for a full account the reader is referred to the following papers: "Effect of oxygen supply in nutrient solution on avocado and citrus seedlings" by Denzel S. Curtis, Soil Sci. 67:253-260; "Nitrite injury on avocado and citrus seedlings in nutrient solution" by Denzel S. Curtis, Soil Sci. (In Press): and "Effect of oxygen supply on Phytophthora root rot of avocado in nutrient solution" by Denzel S. Curtis and George A. Zentrnyer, Amer. Jour. Bot. 36:471-474.*

[2] *For purposes of convenience, the three aspects of this work are presented separately. In this section the oxygen investigations only are discussed.*

EXPERIMENTAL PROCEDURE

Young avocado and citrus seedlings (the latter for comparison) were grown under controlled conditions in water cultures that were aerated to provide the roots with different amounts of dissolved oxygen. When a stream of air is bubbled through a nutrient solution about 8 parts per million of oxygen go into solution, and this concentration can be essentially maintained so long as air continues to be passed into the solution. By adding nitrogen gas to this stream of air the oxygen percentage in the gas mixture is reduced and the level of maintained oxygen in the nutrient solution correspondingly reduced; by adding more oxygen gas, the oxygen supply in the nutrient solution is increased. Through an application of this principle, water culture experiments were carried out as shown in (Fig. 1) in which oxygen levels varying from .05 to 32 p.p.m. O_2 were maintained.

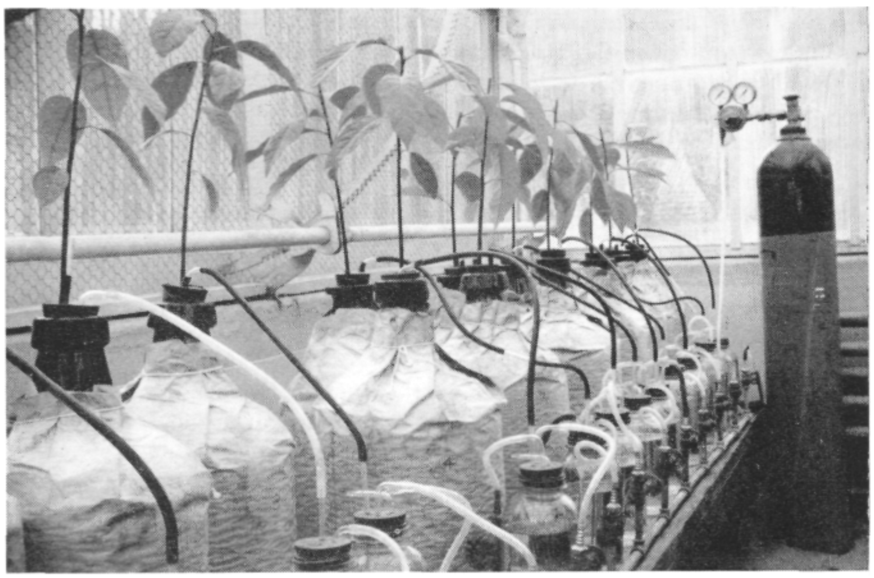

Fig. 1. *Aeration Experiment for Oxygen Studies. Avocado seedlings (Dickinson variety) growing in nutrient solution in 20-liter bottles (left). Aeration tubes leading into these bottles are connected to the 2-quart mixing bottles (right). The compressed gases which make up the aeration mixture are carried in 2 separate pipelines and are released into the mixing bottles through brass needle valves. A cylinder of compresssed nitrogen gas (extreme right) with pressure reducing valve attached is connected with one of the gas lines below; the other line carries compressed air.*

RESULTS

Wilting.—Low oxygen levels (0.7 p.p.m. or less) caused avocado seedlings to show symptoms of distress as early as 2 hours after treatment. At this time the immature leaves began to wilt, but the mature leaves kept their normal appearance. Within the next few hours, however, most of the wilted leaves had regained their normal turgor and it was not until several days later that they showed further signs of distress. After a period of 6 to 10 days the leaves began to wilt again but this time wilting persisted and became more and more severe until both mature and immature leaves drooped to a vertical position and the seedlings finally withered and died. The citrus seedlings, on the other hand, did not wilt at any time even at the lowest oxygen level.

Root Injury at Low Oxygen Level.—Roots of both avocado and citrus seedlings were injured in cultures having an oxygen concentration of 0.7 p.p.m or less. Injury began at the root tips and progressed up along the plump, white feeder roots, becoming more extensive as the time of exposure to low oxygen level was continued, and progressing more rapidly at the lowest oxygen level.

Injured avocado root tips (Fig. 2) became constricted or shrunken in the region of cell elongation and turned off color. At first they turned various shades of pink, yellow and light purple, but darker discolorations followed a few days later. Injury was more extensive and more severe on avocado roots than on citrus roots, which did not show constricted areas but the latter became very soft, then slowly turned orange-yellow, and finally sloughed off.

The constriction of the tips of avocado roots and the soft pliable tips of citrus roots took place during the first day of exposure to low oxygen levels of 0.7 p.p.m. or less and became progressively worse as restriction of oxygen supply continued. Both avocado and citrus roots grew, though slowly, without injury at an oxygen level of 1.0 p.p.m. Apparently the critical oxygen level is somewhere between 0.7 and 1.0 p.p.m.

Fig. 2. *Normal avocado roots (left) and Injured Roots (right). The dark, shrunken root tips are a result of low oxygen supply in the nutrient solution.*

At the lowest oxygen level 0.05 to 0.06 p.p.m.) avocado roots failed to grow both during and after a 10-day treatment and the seedlings subsequently died. With a slightly higher oxygen supply (0.4 to 0.7 p.p.m.), the roots failed to grow during the 10-day treatment but resumed growth although rather poorly after treatment. At still higher oxygen levels root growth improved both during and after treatment until the normal supply of about 8.0 p.p.m. was reached. At oxygen levels near 10.0 p.p.m., growth was somewhat retarded and at levels near 30.0 p.p.m., where the cultures were aerated with pure oxygen gas the roots became very short and stubby with frequent branching, but the plants were not killed.

DISCUSSION

The results of these studies reveal the extreme sensitivity of avocados to oxygen supply, and indicate that one reason why over-moist soil conditions are detrimental to avocados is that the roots of this plant are injured in a few hours when the oxygen supply in the soil solution is reduced to a low level. If oxygen starvation continues for any length of time, recovery is very slow, and in some cases where the deprivation has been acute the plant may never fully recover. Under similar conditions citrus seedlings are also adversely affected but not to the same degree, and recovery is more certain.

The solubility of oxygen in the soil solution is very low, being at ordinary soil temperatures not over 8.0 parts per million. When the soil becomes waterlogged or even in a wet condition short of waterlogging, the oxygen actually dissolved in the soil solution is quickly used up by plant roots and microorganisms. With the pore spaces of the soil largely or completely filled with moisture the normal diffusion of air into and out of the soil is greatly impeded or almost completely cut off so that such oxygen as is present, both in the soil solution and in the trapped soil air, is rapidly exhausted and falls below the concentration necessary to support vital root activities. As shown by the experiments reported herein, a few hours of such conditions are enough to cause visible deterioration of avocado root tips and if the condition continues the uninjured portions of the roots fail to initiate new growth.

When one considers that in some avocado soils where drainage is poor, waterlogged conditions may prevail in at least certain portions of the soil profile for weeks, it is small wonder that avocado trees fail.

All of this is not to say that other conditions are not important. The evidence on hand indicates clearly that the fungus *Phytophthora cinnamomi* Rands, where present in an avocado soil, is a serious threat. However, the point of importance to be stressed at this point is that, apart from these other factors, *an over-moist soil condition and the oxygen starvation which may accompany it, is of itself an extremely important factor in the health of avocado roots.* Growers are therefore urged to do everything possible to avoid irrigation practices which will lead to subsurface saturation or a prolonged over-moist condition. Frequent use of the auger or soil tube to check on moisture conditions in the root zone should be made. Many articles have been written relative to irrigation practice and growers are urged to review this information. For the convenience of the reader, a list of publications is appended, many of which deal with soil moisture and irrigation practices and problems as relating to avocado culture.

II. NITRITE INJURY OF AVOCADO ROOTS

During the study of oxygen requirements of avocado roots described in the foregoing section, small amounts of nitrite nitrogen (up to 4.0 p.p.m. N as nitrite) were found in some of the cultures that were kept at low oxygen levels. Since earlier workers have reported injury due to nitrites on various plants including the avocado, it was considered advisable to study further the toxic effects of nitrites on avocado plants.

It was found that nitrites did not accumulate in the nutrient solution unless the oxygen supply was deficient and then only when the nitrogen in the nutrient medium was supplied as nitrate. When nitrogen was supplied in ammonium forms there was no appreciable accumulation of nitrites even at the lowest oxygen level. On the other hand, small additions of organic matter to water cultures containing NO_3 caused a rapid increase in the accumulation of nitrites wherever the oxygen supply was limited. A summary of the findings relating to nitrite toxicity is reported in the following paragraphs.

COMPARISON OF NITRITE INJURY ON AVOCADO AND CITRUS

A uniform selection of avocado and citrus seedlings was treated in well-aerated water cultures by adding different amounts of nitrite nitrogen to the nutrient solution. After an observation period of 10 days the culture solution was replaced with new solution free of nitrites and observations were continued as shown in table 1.

Root Injury.—At nitrite concentrations of 5.0 p.p.m. or less there was no injury either to avocado or citrus roots and the seedlings grew with good to excellent vigor; but at 10 p.p.m., dead root tips were found on both avocado and citrus after the first day of treatment. At higher nitrite concentrations injury was more pronounced, and injury increased as the time of exposure was increased through a period of about 6 days, after which no further differences could be detected. In all cultures, injury appeared to be more severe on avocado seedlings than on citrus.

Avocado injury was characterized by dark brown discolorations which appeared first at the root tips and then spread in uneven patterns up along the fleshy white feeder roots. The light shades of yellow, pink, and purple characterized by oxygen deficiency were lacking as were also the root constrictions. Injured citrus roots became very soft and pliable but kept their normal color for a few days, then turned orange-yellow and finally began to slough off. Hence, the appearance of nitrite injury on roots of citrus seedlings was not essentially different from the injury described in previous studies as caused by oxygen deficiency; but in the case of avocado roots, the injury caused by nitrite was not the same as that brought on by oxygen starvation.

Leaf Wilt.—Avocado leaves became slightly wilted 1 day after exposure to nitrite concentrations of 20, 50, and 100 p.p.m (table 1), but at 20 p.p.m., the leaves recovered as soon as new roots appeared. At 50 and 100 p.p.m., new root growth was lacking, wilting increased in severity, and the seedlings finally died.

Leaves of citrus seedlings were slightly wilted after 1 day's exposure to nitrite concentrations of 50 and 100 p.p.m., but they regained their normal turgor during the next few days and continued to thrive without further wilting.

TABLE 1
Comparison of Nitrite Injury on Avocado and Citrus Seedlings Grown in Water Cultures (10-day treatment at pH 5.5)

Culture number	Nitrite nitrogen added to culture p.p.m.	Avocado			Citrus		
		Root injury	Leaf wilt	New root growth (recovery) after treatment[1]	Root injury	Leaf wilt	New root growth (recovery) after treatment
1	0	none	none	excellent	none	none	excellent
2	1	none	none	excellent	none	none	excellent
3	2	none	none	excellent	none	none	excellent
4	5	none	none	good	none	none	good
5	10	slight	none	fair	slight	none	fair
6	20	moderate	slight	poor	slight	none	poor
7	50	severe	severe	none	moderate	slight	poor
8	100	severe	severe	none	severe	slight	poor

[1] *After the 10-day treatment with nitrite nitrogen, the culture solution was replaced with fresh solution free of nitrite and observations were continued.*

Recovery After Treatment.—After the 10-day treatment at different nitrite concentrations the culture solution was renewed with a solution that was free of nitrites, and observations were continued. Subsequent root growth in the first three cultures of both avocado and citrus (table 1) in which the nitrite concentration did not exceed 2.0 p.p.m. continued to be excellent. Growth was good but not excellent after exposure at 5.0 p.p.m., fair at 10.0 p.p.m., and poor at 20.0 p.p.m. (Fig. 3). After treatment at 50.0 and 100 p.p.m., avocado roots failed to grow, and the seedlings finally died. New roots of citrus seedlings grew after treatment at all nitrite concentrations including 100 p.p.m.

Nitrites More Injurious at Low pH.—Additional treatments at various pH levels demonstrated that nitrite injury becomes more severe as the pH is decreased. For example: 5.0 p.p.m NO_2 nitrogen caused injury at pH 5.5 but not at pH 6.0; *20* p.p.m. caused injury at pH 6.0 but not at pH 7.0. Avocado seedlings were more severely injured than citrus seedlings in all cases and showed less ability to recover from injury and produce new root growth after treatment.

Fig. 3. *Roots of avocado seedlings grown in culture solutions at pH 5.5. Left to right: solutions containing 0, 5.0, 10.0, and 20.0 p.p.m. nitrite nitrogen. Photographed 10 days after treatment.*

DISCUSSION

The investigations reported in this and the preceding paper show that lack of oxygen is detrimental to both citrus and avocado roots not only because of the direct effects of oxygen starvation on the vital activities of the plant roots, but also because under these same conditions small amounts of nitrite nitrogen may be formed. This constituent, if it attains high enough concentrations, is toxic. The conditions necessary for the formation of this product in nutrient solution cultures are a supply of nitrate, low oxygen levels, and organic matter. All of these conditions occur in soils from time to time and hence it is reasonable to suppose that when a soil becomes waterlogged avocado roots may suffer injury through lack of oxygen on the one hand and a gradual build up of nitrite on the other.

The type of injury caused by nitrite is somewhat different from that caused by oxygen lack, and the susceptibility of roots to nitrite injury is dependent, to some degree, upon ihe pH of the soil. Under acid conditions nitrite is more toxic than under alkaline conditions and probably other factors enter in also.

On the basis of present information, our view is that oxygen starvation will cause root injury of itself and also create conditions favorable for nitrite formation ; this product may thus build up to a point where it also exerts a toxic effect and thus add to the initial effects of a lack of oxygen.

III. EFFECT OF PHYTOPHTHORA CINNAMOMI ON AVOCADO SEEDLINGS GROWN UNDER VARYING CONDITIONS OF OXYGEN SUPPLY

In connection with the oxygen and nitrite investigations described in the preceding sections, it seemed desirable to study the interrelations of oxygen supply to the activity of the fungus *Phytophthora cinnamomi* since it has been considered that this organism affects the avocado more adversely under wet, poorly drained conditions, than under well-aerated conditions.

The technique employed in this work was similar to that used in the oxygen study. Avocado seedlings were grown in nutrient solutions aerated with mixtures of air and nitrogen so that the oxygen supply maintained in the culture solutions varied from 0.05 to 7.20 p.p.m. oxygen. Half of the seedlings at each oxygen level were inoculated with *P. cinnamomi* and the other half left untreated. Three different sets of trials were carried out. In one series the differential oxygen levels were maintained for only two days and were followed by restoration of normal aeration with air; in another set the variable oxygen regime was maintained 4 days, followed by normal aeration; in the other series, the variable oxygen levels were maintained 16 days, followed by normal aeration.

The results of this study were contrary to expectation. The fungus invaded the roots of the well-aerated cultures more rapidly and brought on quicker decline than in the cultures of low oxygen content. Affected roots first showed a brownish discoloration which appeared along the fleshy white feeder roots and then spread in uneven patterns throughout the entire root system. At the highest oxygen levels browning occurred in two days following inoculation with the fungus. At intermediate oxygen levels (2.30 to 0.9 p.p.m. O_2) the discoloration did not show up for 4 days, and at the lowest oxygen level the brown color did not show up for many days. However the effects of oxygen deficiency, as described in the former paper, showed up in both the inoculated and uninoculated cultures at concentrations of 0.5 p.p.m. oxygen and less. Tests for nitrite were made in all of the cultures but the maximum quantities found under the conditions of these experiments were less than 1.0 p.p.m. Since in the former experiments described no injury was noted to roots where nitrite was less than 5.0 p.p.m., it seems safe to assume that, under conditions of these experiments, nitrite was of no importance so far as the behavior of the avocado seedlings was concerned.

Although *P. cinnamomi* was slow to attack the plants grown under low oxygen conditions, these plants were eventually killed by the fungus as were the plants grown at higher oxygen levels. In the uninoculated series, the plants maintained at low oxygen levels (0.5 to 0.05 p.p.m.) for only 2 days, eventually recovered when normal aeration was restored. However those plants maintained at these levels for 4 days or longer did not recover when normal aeration was restored.

DISCUSSION

The results of experiments described in this and the preceding two sections indicate that *both* soil aeration and *Phytophthora cinnamomi* are factors of importance with respect to avocado root rot. In the presence of the fungus the outlook for an avocado orchard is not good.

Poor aeration on the other hand is harmful because if avocado roots are deprived of oxygen or rather if the oxygen level in the soil solution falls much below 1.0 p.p.m., root injury quickly takes place and if the condition of oxygen starvation continues, recovery is slow or may not occur at all when good aeration is restored. Roots thus injured may be killed more quickly by the fungus. Under conditions of poor aeration, nitrite may also build up to toxic levels and no doubt other toxic products form, due to the changed chemistry and biology of the soil.

Hence, waterlogged or over-moist soil conditions should be avoided because of the possibility of root injury from low oxygen or high nitrite concentration, and because of the activity of *P. cinnamomi* under wet, but not completely waterlogged, soil conditions.

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