temp reduced root respiration and carbohydrate utilization and high temp decreased carbohydrate translocation. Domanski et al. (4) reported that temp induced effects on rooting result from changes in levels of endogenous growth regulators and possible co-factors. Our research showed geranium and poinsettia root initiation and elongation were reduced during 1 to 5-day periods at high or low temp, and the inhibition continued for 5 days after treatment. Since high temp induced root inhibition was found after 1 day treatment and the change from favorable to inhibitory temp occurred within the 5°C interval from 30 to 35°C, the adverse effects are probably not related to reduced carbohydrate levels. Studies in progress have shown a measurable reduction in root elongation for both plant species after 12-hr treatments at 5 and 35°C.

Geranium and poinsettia roots were white and appeared normal after 5 days of treatment at 5 to 25°C. Slight browning of poinsettia roots was noted following 5 days at 30°C, but geranium roots remained white. Root browning of both species resulted from treatment at 35°C. The browning became more extensive as the duration of treatment increased from 1 to 5 days, and some roots after 5 days appeared to have highly suberized root tips. The distance from the root tip to the first xylem element progressively increased for temp treatments from 5 to 25°C, and declined with treatments from 25 to 35°C. Poinsettia roots at 25°C had more cortical cells in the meristematic region than roots grown at lower (5 to 20°C) or higher (30 to 35°C) temp. Roots in the 35°C treatment had a very short region of cell elongation and a rapid increase in cortical cell size within 0.2 to 0.3 mm from the root tip.

Greenhouse medium temp decline below the optimum for root growth when cold irrigation water is applied (3) and rises above the optimum range when sunlight warms the soil or container surface. Further studies are required to determine the extent to which adverse medium temp affect root growth and hence crop production in the greenhouse.

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Levels of Hydrogen Sulfide Toxic to Citrus Roots[1]

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Abstract. The toxicity level for H_2S, generated under waterlogged conditions, is a concn-time relationship associated with the dissolved molecular H_2S concn in the root rhizosphere. A molecular concn of 2.8 ppm for 5 days is one toxicity threshold for significant root injury in controlled tests with rough lemon (*Citrus jambhiri* Lush.) seedlings. Rapid bacterial generation of H_2S in the root rhizosphere was indicated by a doubling of the concn every 24 hr. Sulfides were present in root tissues that died.

Hydrogen sulfide can usually be detected under O_2 deficient conditions when the citrus root environment becomes waterlogged. The presence of H_2S has been associated with citrus root injury in the poorly drained flatwoods areas of Florida (4). Citrus roots can tolerate O_2 deficiency *per se* longer than when H_2S is present (2). From a practical standpoint, the presence of H_2S is being used to evaluate the need for additional drainage in problem areas. A handful of disturbed soil and roots from the waterlogged zone is smelled to detect H_2S (4). The determination of toxic levels for H_2S and the interaction with time of exposure has been difficult to establish because of the many complex reactions occurring in the root rhizosphere under anaerobic conditions.

Materials and Methods

A multicelled apparatus capable of being monitored and controlled under anaerobic conditions (2) was used to measure the rate of sulfide production in the root rhizosphere and to establish a toxicity threshold for citrus seedlings at a root temp of 23°C.

The solution circulating apparatus handled simultaneously 6 plants for each of 2 solution treatments or a single treatment of 12 plants (Fig. 1). Rough lemon (*Citrus jambhiri* Lush.) seedlings, uniform in size and root density, and grown under conditions where the watering source contained sulfate reducing organisms, were planted in the apparatus using a medium of washed, graded, sterile white sand. Solution sampling tubes, terminating within 2 mm of a feeder root were installed in each cell to sample the solution abutting the root system. Four hundred ml of sterile solution containing 3 ppm total sulfides

[1]Received for publication August 23, 1972. Contribution from the Soil-Water-Atmosphere-Plant (SWAP) Project cooperative between the Florida Agricultural Experiment Stations and the Soil and Water Conservation Research Division, Agricultural Research Service, U. S. Department of Agriculture, Gainesville, Florida, Journal Series No. 4552.

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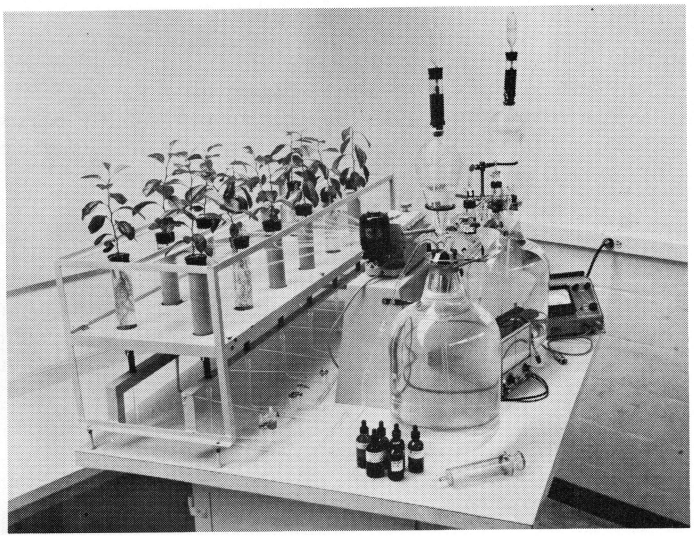

Fig. 1. The solution circulating apparatus.

were flushed through each cell in order to lower the O_2 content. The sulfide content of the flushing solution usually dropped to zero during this treatment. Three ppm of H_2S was then added to the distilled water in each carboy and circulated over the roots of the plants for 8 hr. This was followed by an additional 2 ppm for 16 hr. By this method, > 0.1 ppm O_2 was established throughout the circulated solutions within 24 hr for all experiments reported here. Hydrogen sulfide, rather than N, was used to remove O_2 in order to establish an initial redox level below +50 mv Eh. A low redox value was necessary to stimulate the development of the sulfate reducing bacteria. Once the desired anaerobic environment (zero O_2, zero H_2S and low redox) was established, circulation was either stopped to measure the rate of bacterial generation of sulfides in the root rhizosphere over a period of 6 days or adjusted to preselected sulfide and pH levels with concd H_2S and 0.05 N HCl or NaOH and circulated for 4 to 6 days. The amounts of H_2S, HCl, and NaOH required to obtain specific changes in solutions were obtained by calculation and from prior studies (2). One hundred ppm K_2SO_4 was added to those solutions involving bacterial generation of sulfides, otherwise, the only source of sulfate for the bacteria was the root system of the plants transplanted from the greenhouse. Added sulfate was necessary to promote rapid and prolonged bacterial generation of H_2S.

Oxygen and redox were measured at sampling ports with a Yellow Springs Instrument Co. membrane type O_2 electrode and a platinum-calomel combination electrode. Solutions of H_2S, as prepared in carboys attached to the apparatus (2), were measured colorimetrically by the p-amino dimethylaniline method (8).

Bound or sorbed sulfides were determined by using an Orion silver-silver sulfide ion activity electrode for sulfide ion levels and the p-aminodimethylaniline color test for total sulfides. Sulfide ion activities and total sulfides were determined after adjusting to pH 7. An H_2S solution was prepared in O_2-free water containing the same total sulfide content and pH level as the sample. A lower ion activity in the test solution compared to that in distilled water indicated the percent bound or sorbed sulfides. The test was necessary in order to establish total concn of dissolved molecular H_2S in the circulating system and in the root rhizosphere. It was assumed that chemically bound, precipitated, or sorbed sulfides in the root rhizosphere would not be toxic to citrus roots.

A longitudinal section of the tap root was removed from selected seedlings prior to transplanting into aerated sand and after completion of flooding tests. The section of tap root removed contained phloem, cambium, and xylem. The segment of tap root was stained with 0.1% solution p-aminodimethylaniline plus 10% $FeCl_3$ by the method of Takagi (9) to note the presence of sulfides in tissues. Stained tap root segments showed sulfide concn as areas of methylene blue. The darker blue areas, most prevalent in phloem and xylem, were associated with regions of the tap roots that were dead at the end of 10 days in aerated sand.

Results

Preliminary studies were conducted with circulating solutions of varying total sulfide concn from 1 ppm to 6 ppm for 5 days at pH 6.0 and 23°C. Three ppm total sulfides killed all feeder roots and 25% ± 10% of tap roots of rough lemon seedlings. Root killing was based on the regions of new root growth after the plants had been held 10 days in aerated soil. No root injury was noted with 1 ppm total sulfides. Two ppm resulted in the destruction of some feeder roots whereas 6 ppm killed the entire seedling. Roots were more readily damaged at pH 6 than at pH 7 under the same levels of total sulfides.

Toxicity of dissolved molecular H_2S. There was no difference in apparent toxicity to roots of plants in contact with 2.8 ppm of dissolved molecular H_2S at pH 5.5 and pH 7 for 5 days. Total sulfides were initially 8.5 ppm at pH 7 and 3.0 ppm at pH 5.5. After 3.5 days at pH 7, the total sulfide level increased to 9 ppm apparently due to activity of sulfate reducing bacteria. To correct for this interfering factor, the pH of the circulated solution was increased to 7.2 in order to maintain the dissolved molecular concn of H_2S at 2.8 ppm. After 3 days in the pH 5.5 treatment, sulfides were being sorbed within the circulating system. To adjust, the total sulfide level was increased to 3.5 ppm to maintain the dissolved molecular H_2S concn at 2.8 ppm. All plants and root systems looked similar after 5 days of anaerobiosis. The translocation of H_2S within the tap root and feeder roots, as indicated by staining, was similar for all plants in the 2 treatments. The growth of new roots from tap roots and noninjured secondary roots following 10 days in aerated soil indicated that the extent of root injury was similar for all plants.

Generation of sulfides in the root rhizosphere. The rate of sulfide production increased rapidly with time (solution circulation stopped), after establishing an anaerobic environment at 23°C and pH 6.0 (Fig. 2). No sulfides were produced in a similar study at 14°C. The concn of total sulfides as measured within 2 to 3 mm of feeder roots doubled every 24 hr during a 6-day study. The pH in the rhizosphere varied between 5.8 and 6.2. Plants usually collapsed showing severe

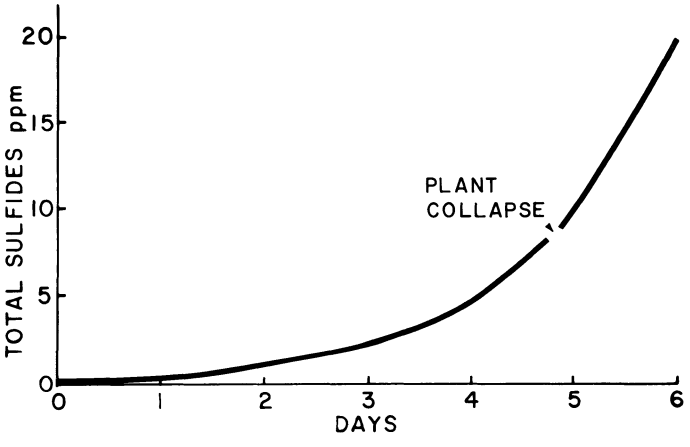

Fig. 2. Bacterial generation of sulfides in citrus root rhizosphere pH 5.8 to 6.2.

symptoms of epinasty when the total sulfide level reached 9 to 11 ppm in the root rhizosphere. The concn of sulfides in the rhizosphere was always 5 to 14 times higher than sulfide levels measured 2 cm from the roots in all experiments set up to make such a dual measurement. Plants that collapsed, even in tests of only 5 days duration, invariably died when transplanted to aerated sandy soil for 10 days. The tap roots of all collapsed seedlings that were checked had an excess of sulfides extending to the crown, as indicated by dark blue stained deposits of methylene blue in phloem and xylem. Sulfide levels appeared to be excessive in distal areas of the root before there was active transport toward the crown.

Discussion

Toxicity of H_2S to citrus roots under waterlogged conditions appeared to be associated with the molecular concn of dissolved H_2S in the root rhizosphere. The generation of H_2S, presumably by sulfate reducing bacteria in the root rhizosphere, was logarithmic as indicated by a doubling of the H_2S concn every 24 hr. The importance of the root rhizosphere as a source of H_2S under anaerobic conditions was demonstrated by the generation of sulfides in the circulating solution in controlled experiments. The root rhizosphere is known as a zone of high bacterial activity. Sulfate reducing bacteria function best during anaerobiosis on an energy source of lactic and citric acids (3), compounds reported to be exudates from plant roots (7).

Hydrogen sulfide toxicity has a concn-time relationship. There was more root damage at 2.3 ppm molecular H_2S in 7-day tests (2) than 2.8 ppm in 5-day tests. The killing of citrus root tissue by H_2S as indicated by stained dark methylene blue areas suggests that sulfides must be in excess, presumably after overcoming all oxidative systems, before the tissues are beyond the point of regenerating a functional root system.

Differential permeability to the H_2S molecule rather than the more active sulfide ion can probably be explained by the fact that the molecule is small, fat soluble, and readily penetrates plant roots (1). It is suggested that the bisulfide and sulfide ion may not diffuse into roots because of the possibility of a high hydration number. Differential permeability occurs with certain 0.5 mil PVC membranes (5) which are permeable only to the H_2S molecule.

Citrus apparently is reasonably tolerant to O_2 deficiency *per se* in the rhizosphere. Seven-day exposures to O_2 deficiency in the multicelled apparatus did not seriously injure roots (2). It has been suggested that citrus may have an internal downward O_2 transport system as indicated by analyses for air spaces in citrus roots (6). The presence of O_2 in air spaces could contribute to a delay in the transport of toxic concn of H_2S in citrus roots.

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Pecan Nutlet Set and Carbohydrate Level of Various Tissues in the Spring as Affected by Fungicide Sprays[1]

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Abstract. Fentin hydroxide and benomyl increased nutlet set in 1969 but not in 1970. Fungicide sprays had little effect on starch content of wood tissue in late March of the year following application. Starch and sugar content of 6 wood tissues in March and nutlet set was not associated with leaf scorch index the previous fall. Starch content of wood tissues in late March ranked as follows from greatest to least: Roots > 2.5 cm diam, trunk, scaffold limbs, new wood, 1-year-old wood, and roots < 1.3 cm diam. Soluble sugar content ranked: Roots < 1.3 cm diam, 1-year-old wood, new wood, roots > 2.5 cm diam, trunk, and scaffold limbs.

Annual production is a goal of most pecan growers, however, trees must be sprayed if this goal is to be met (18). Spraying to control insects and diseases aids in leaf retention (3) and consequently increases photosynthesis (10, 11). Previous reports (18) have shown that spraying with fungicides that control the scab fungus also increases nutlet set the following year. The report herein gives additional results of the effects of the same and additional fungicides on nutlet set in a year when nutlet set was very heavy (1969) and medium (1970).

Spraying to control the scab fungus also reduces incidence of leaf scorch, a malady responsible for early defoliation of pecans (6, 20). Early defoliation also reduces nutlet set the next year (8, 17). It appears logical then that these sprays should increase the reserve carbohydrate (CHO) content of the tree which perhaps controls nutlet set. Smith and Waugh (15) studied CHO content of small roots of pecan and found that starch appeared to be the most important reserve material, and high levels of starch in the fall were associated with large crops the following year. Further evidence that starch is an important reserve CHO

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