maximum contribution of sapropelic material to a potential source bed may simplify the search for oil by ruling out parts of a formation and pointing to the potential source beds in a basin of deposition.

Inasmuch as the Chattanooga and Pierre shales contain kerogen that is primarily of humic (terrestrial) origin, they should, in spite of the fact that they are marine, be considered coaly shales (*11*).

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Chemical Control of Water Loss in Growing Plants

Abstract. Immersing the roots of growing strawberry plants in aqueous solutions of 8-hydroxyquinoline sulfate closes the stomata, reduces water loss, and increases the time before complete wilting under drought conditions. Under such drought conditions plant survival and vigor are increased. Prolonged closing of the stomata seems to be the principal mode of action of the chemical.

This study verifies observations made over a period of years that 8-hydroxyquinoline sulfate retards wilting in several species of plants. Zelitch (*1*) has shown that 8-hydroxyquinoline sulfate prevents closed stomata of tobacco leaves from opening under conditions favorable for the opening of stomata (*1*). Ferri and Levy have shown that an aqueous solution of β-naphthoxyacetic acid watered on the soil closes the stomata of nasturtium leaves (*2*).

Because most of the water loss from a plant is through the stomata, it was postulated that the reduction of water loss in plants treated with 8-hydroxyquinoline sulfate is due to the closing of the stomata by this material. Our experiment shows that this material closes open stomata and keeps them closed under conditions favorable for opening.

The roots of five growing strawberry plants with open stomata were immersed in tap water, and the roots of five others were immersed in aqueous solution of 8-hydroxyquinoline sulfate (2000 ppm) for ½ hour. At the end of this period of time the stomata in a sample of five leaves from the plants immersed in water were still open. In a sample of five leaves from the plants immersed in the test solution the stomata were closed. A duplicate experiment in which the plant roots were immersed for 3 minutes gave identical results. The "open" or "closed" position of the stomata was determined by silicone-rubber impressions of the lower epidermis (*1*). The almost immediate closing of the stomata after immersion of the roots indicates a direct effect of 8-hydroxyquinoline sulfate on the stomata and not an indirect effect by action on the roots. We have observed immediate closing of the stomata of excised leaves of chrysanthemum where the cut end of the petiole was immersed in a test solution, indicating that the stomata are closed by direct action and that roots are not essential to the action.

We suggest that closed stomata are the chief factor for the reduced water loss shown in the following experiment. Young growing strawberry plants were removed from the soil, and their roots were washed clean with tap water. The roots of five plants were then immersed in tap water, and five each in 1000-ppm and 2000-ppm aqueous solutions of 8-hydroxyquinoline sulfate. After immersion, the roots were again washed thoroughly in tap water, and the plants were set in waterproof pots containing 350 g of dry sand. After planting, 150 ml of water was added to each pot, and the pots were weighed. No more water was added during the experiment. The pots were reweighed 4, 6, 10, and 14 days later to determine water loss (Fig. 1). Fertility of the sand was low, and there was no growth

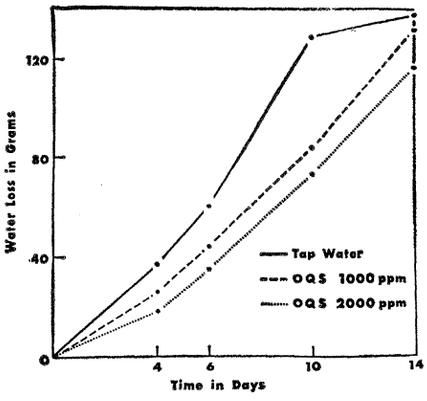

Fig. 1. Chemical reduction of water loss by treatment of roots for 30 minutes before planting. Water is lost from both pot and plant.

of plants to offset loss in weight by transpiration and by evaporation of water from the uncovered surface of the sand.

Up to the 14th day the plants soaked in water showed a greater water loss than those soaked in 8-hydroxyquinoline sulfate, and the plants soaked in 1000 ppm of the test solution showed a greater loss than those soaked in 2000 ppm. The greatest difference between treatments in water loss was during the first four days. On the 14th day four of the five control plants had completely wilted, with a consequent reduction in water loss. The treated plants continued to lose water at the former rate. The higher loss at the lower concentration can be accounted for by differential action of the two concentrations on the stomata, with the lower concentration allowing the opening of more stomata. The time to complete wilting was as follows: water, 11 days; 1000-ppm solution, 15.6 days; and 2000-ppm solution, 19.2 days. Similar experiments showed that these results were reproducible.

To determine whether the preplanting treatment would influence the survival of the plants once they had wilted, all the plants were rewatered 1 day after the last plant had wilted or 21 days after the start of the experiment. Within 2 days all five plants treated at a concentration of 2000 ppm had revived; 3 of the plants looked as if they had never wilted. Four plants treated at a concentration of 1000 ppm survived but were in poorer condition than those treated at a concentration of 2000 ppm, and one was barely alive. Only one plant that had been soaked in water survived, and it was barely alive. Thus

reducing water loss permitted plants to survive for 21 days without rewatering, but lack of water for the same time was fatal to most untreated plants. Results were similar in other tests.

It was found in two tests that, after treated plants wilt, they require much longer to regain turgidity when placed in water than untreated wilted plants. Similar results were obtained with detached chrysanthemum leaves. Several investigators have shown that with fully open stomata the transpiration of water by plants can be affected by several factors but when 50 percent or more of the stomata are closed the stomata are the principal factor controlling transpiration. To determine stomatal closure in wilted leaves and how this was effected by treatment, detached chrysanthemum leaves were placed in water or 1000 ppm of 8-hydroxyquinoline sulfate for 20 minutes. Then they were removed and allowed to wilt. The percentages of stomata open before soaking, after soaking but before wilting occurred, and after wilting occurred, respectively, were as follows: in 1000-ppm 8-hydroxyquinoline sulfate for 20 minutes, 58, 30, and 10; in water for 20 minutes, 64, 88, and 82.

Stomata on leaves kept continuously in water were 61 percent open at the start and 80 percent open at the end, almost the same as leaves kept in water only 20 minutes and then allowed to wilt.

After wilting occurred most stomata on treated leaves were closed. However, most stomata remained open on untreated leaves, whether wilted or left continuously in water. The closed or partially closed stomata of treated plants were mainly responsible for decreased transpiration, creating less tension on the water in the xylem and less tendency to pull water into the plants. Thus treated plants could be expected to regain turgidity more slowly than untreated plants.

Zelitch (*1*) has shown that many materials affect stomata in plants with a consequent reduction of water loss. Ferri and Levy (*2*) have shown that β-naphthoxyacetic acid, when applied to the soil in which plants are growing, closes the stomata; and Odom (*3*) reported that 8-hydroxyquinoline sulfate prevents wilting of cut flowers. Stalfelt (*4*) caused opening of stomata and prevented closure with sodium azide on excised leaves and leaf sections of *Vicia faba*. The work described in this report demonstrated that immersing the roots of strawberry plants in an aqueous solution of 8-hydroxyquinoline sulfate closes the stomata and reduces water loss, thus enabling the plants to withstand prolonged drought. For high value crops, field use of chemical reduction of water loss would be a possibility in areas of infrequent rainfall.

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26 March 1962

Chloroplasts of Euglena gracilis Affected by Furadantin

Abstract. Treatment of *Euglena gracilis* with furadantin results in colorless cells which produce only white descendants. The treatment also prevents the greening of dark-grown euglena which are exposed to light. This finding suggests that furadantin acts upon some stage of chloroplast synthesis subsequent to that blocked by darkness.

The purpose of this report is to add the antibiotic, furadantin [N-(5-nitro-2-furfurylidine)-1-aminohydantoin] to the growing list of physical (*1*) and chemical (*2*) agents which produce permanently bleached but viable euglena.

Table 1. Effect of furadantin on growth of euglena.

Time (days)	10^5 Cells/ml at furadantin concn. in μg/ml		
	0	65	130
0	0.37	0.37	0.37
2	0.87	0.97	0.62
5	7.0	7.0	3.7
7	21	13	6.1

Table 2. Effect of furadantin on colony color of euglena.

Time (days)	Percentage of green colonies of furadantin concn. in μg/ml		
	0	65	130
0	100	100	100
2	100	78	5
5	100	50	1*

* Some of these colonies were variegated with white margins and green centers.

Euglena gracilis strain Z was grown on the peptone, yeast extract, and acetate medium of Brawerman and Chargaff (*3*) at about 20°C under fluorescent light of intensity 500 ft-ca. (*4*). Cell counts were made with a hemocytometer. To test the ability of treated euglena to produce green colonies, cells were plated on the above medium (solidified with 1.5 percent agar). Both the green and the white colonies could be counted after 1 week.

The results show that furadantin slows the growth of euglena (Table 1) and leads to the production of individuals which are no longer capable of forming green colonies (Table 2). Continued exposure to furadantin (130 μg/ml) results in completely bleached cultures, the cells of which produce only white descendants upon further growth in furadantin-free liquid medium.

When dark-grown euglena are suspended in "resting medium" and exposed to light, they become fully green in a matter of 3 days (*3*). Treatment with furadantin (120 μg/ml) prevents greening, suggesting that this drug affects some step in chloroplast formation subsequent to that blocked by darkness. It is of interest that streptomycin (*5*) and high temperatures (*6*) appear to block a process (or processes) prior to the dark block.

Preliminary results, obtained with sensitivity disks, indicate that another member of this group of antibiotics, furacin (semicarbazide of 5-nitrofuraldehyde) also bleaches euglena cells, but that somewhat higher concentrations are required for equal effect.

Euglena chloroplasts appear to be considerably more sensitive to furadantin than those of some other green organisms. Tchan and Gould have recently reported that green algae are not affected by this compound (*7*), and Owens has used a variety of 5-nitrofuran derivatives, including furadantin, to control fungal diseases of beans and tomatoes (*8*). He has not reported any bleaching of the green plants.

The mechanism by which furadantin and other agents interfere with chloroplast formation is at present obscure. Since furadantin appears to act at a stage other than the stages which are affected by streptomycin and high temperature, it may prove to be a useful tool in the future study of chloroplast development.

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