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.

E. M. STODDARD
P. M. MILLER
Department of Plant Pathology, Connecticut Agricultural Experiment Station, New Haven, Connecticut

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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.

D. R. MCCALLA
Department of Chemistry, McMaster University, Hamilton, Ontario

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12 February 1962

Excitable Cells in Mimosa

Abstract. By inserting microelectrodes into cells of various tissues, it was shown that elongated parenchyma cells in the phloem and protoxylem, which have larger membrane potential than inexcitable cells of other types, generate action potentials with conduction. The electrical features of these cells are essentially similar to those of nerve and muscle cells.

Electrophysiological studies on excitatory conduction in the sensitive plant, *Mimosa pudica* L., have been performed (*1*), but no conclusive evidence has yet been obtained about which kind of cell is excitable and generates the action potential. Several workers (*2*) have surgically removed various tissues from the petiole and have concluded that excitatory conduction takes place in the phloem. However, the experiment by Bose (*3*), in which an electric probe was inserted into the petiole at various depths, showed that excitation is conducted not only in the phloem but also in the protoxylem located in the inner part of the xylem. The protoxylem was called the "internal phloem" by Bose, but the tissue consists only of elongated parenchyma cells and contains no sieve tubes. In my experiment, I inserted a microelectrode into intact cells, and found excitable cells in both the phloem and the protoxylem.

Table 1. Membrane potentials of cells in various tissues. Values are means and standard deviations of several observations in each of eight separate leaves.

Tissues	Membrane potential (mv)	
	Resting	Action*
Epidermis	−44 ± 6	
Cortex	−52 ± 5	
Sclerenchyma sheath	−52 ± 8	
Phloem:		
small cells	−161 ± 15	−22 ± 15
large cells	−61 ± 1	
Protoxylem	−154 ± 12	−19 ± 13
Pith	−58 ± 4	

* Figures showed potential values at the peak of action potential. Values of spike height were 139 ± 12 mv in phloem and 141 ± 15 mv in protoxylem.

At the middle of the petiole of a leaf detached at its base, a part on the lateral side about 3 mm in length was cut off with a razor under water. The cut surface was parallel to the median plane of the petiole. After several hours the prepared leaf was placed on the stage of a microscope; its petiole was kept horizontal and held by a Plexiglas assembly so that the cut part was immersed in a small pool of dilute saline solution (*4*) centered under the objective lens. The plane of the cut was inclined almost at 45° to perpendicular, and the cut faced upwards. All tissues exposed at the cut surface could be seen under the microscope by reflected light. The base of the petiole dipped into a water-filled vessel. Microcapillary electrodes were prepared by the ordinary method and filled with 3*M* KCl. A reference electrode was placed in the pool of saline solution. When an electrode was inserted into a cell at the cut surface, the membrane resting potential between cell interior and the external medium in the pool could be recorded on an oscillograph with a d-c amplifier of high input impedance. The cells can be divided into two groups on the basis of their resting potentials. Some cells in the phloem and all cells in the protoxylem have a resting potential of about −160 mv, all other cells about −50 mv (Table 1).

When the petiole was stimulated electrically at its apex and an excitatory conduction was generated basipetally, a membrane action potential was always elicited in the cells of larger resting potential (Fig. 1*A*). In cells of smaller resting potential there was only a slight change in potential (Fig. 1*B*). This small change is probably an electrotonic change due to the action current of the surrounding excitable cells.

The protoxylem is localized just inside the vessels, and is composed only of elongated parenchyma cells which are nearly of uniform size, 10 μ in diameter and 120 μ long. They form a number of cell rows along the longitudinal axis. All of these cells showed the larger resting potential and generated an action potential upon stimulus.

The phloem consists of several kinds of cells. Cells of large diameter (17 to 28 μ), probably the sieve tubes or

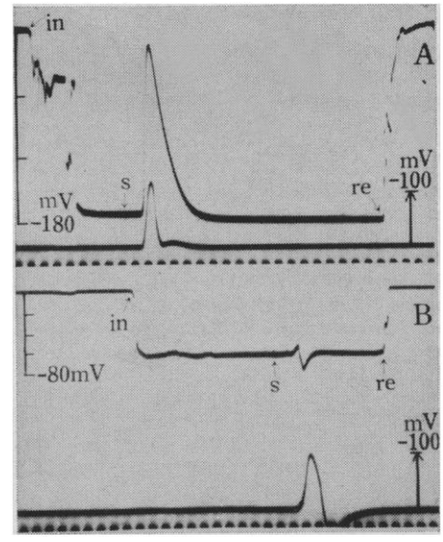

Fig. 1. Membrane potentials (upper trace) in cells of protoxylem (*A*) and pith (*B*). Microelectrodes inserted into cells at *in* and removed from cells at *re*. Petiole is stimulated at *s*. Diphasic action potentials (lower trace) led externally between the pool and the basal cut end are simultaneously recorded. Time marks, 1 sec.

tube cells, showed the smaller resting potential and no action potential. Some of the elongated parenchyma cells, on the other hand, showed the larger resting potential and an action potential, but the cells tested could not be positively identified under the microscope. Microscopic observations of longitudinal sections of the petiole revealed cells in the phloem similar to but somewhat shorter than those in the protoxylem.

The results clearly showed the site of the excitable cells, and the same cells are probably the pathway of conduction. As shown in Fig. 1*A*, the membrane of the excitable cells is polarized; the cell interior is about 160 mv negative to the exterior, and during activity the potential changes toward the direction of depolarization. This feature is essentially similar to that in the axon, muscle fiber, and characeous internode.

TAKAO SIBAOKA

Faculty of Science,
Tohoku University, Sendai, Japan

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