

pair was allowed 1 week to adapt to the plastic chamber. At the end of this time, an unpaired male was removed from stock and directed into the plastic chamber through a side port. Twelve tests were made. In cases of conflict, the male that was successful in battle, and which afterward maintained close contact with the female roach (sharing the interior or immediate area of the mating chamber), was judged successful. Observed results are summarized.

1) No evidence indicates that a mating pair prefers or even recognizes its own mating chamber. In some cases occupancy was first established in the chamber originally constructed by the pair being tested, but, in each test, moves between original and alternate chambers occurred at least once during the week of adjustment.

2) In 10 of the 12 tests the resident male of each pair fought the intruder male. The resident male in each of seven mating pairs fought its intruder successfully. In three tests the resident was defeated by the intruder. The resident female was never observed in combat.

3) In 2 of the 12 tests conflict failed to occur, and resident and intruder roaches remained indifferent to one another for the period of confinement.

4) Defeated males remained indifferent to the resident pairs, and no repeat encounters of force were observed during the week after the conflict.

Behavior of the male intruder and mating pair individuals in the early stages of each test generally follows a pattern. The intruder runs two or three circuits of the plastic chamber after it is introduced by way of the chamber port farthest from the pair. Both male and female roaches of the mating pair instantly sense the intruder. Antennae become hyperactive, the usual almost motionless stance is broken by change in position, and rapid, jerky antero-caudad movements, which Cleveland (1) likened to those of termites, always occur in the resident male. First contact between the intruder and either individual of the pair is usually made in accidental collision. The intruder male continues running the circuit if this contact is made before hyperexcitability from handling has abated. Eventual behavior depends upon the sex of the member of the pair contacted by the intruder male. If female, the intruder male begins careful anterior and caudal end inspection. Rapid side-to-side female abdominal movements interrupt the inspection ritual whenever body

contact is made by the male. On occasion, the female displays great curiosity, even to the point of following the intruder as it wanders away after inspection. Meanwhile the resident male actively seeks the intruder, and inspection is brief when contact is made. Fighting begins with the resident male taking the offensive. The duration of contest is sometimes brief, and, as reported for two cases, battle failed to occur in tests in which the resident male remained apathetic.

Further tests with the same pairs were designed to determine whether a female roach selected from stock and offered as the intruder would battle either member of a chamber-adapted mating pair. Negative results were obtained in each of five tests.

In additional experiments it was found that isolated but chamber-adapted females do not battle male intruders, and that isolated and chamber-

adapted females do not battle female intruders. All stock roaches used as intruders in these tests were found paired in logs at collection time.

The behavior described takes on unusual significance when one considers the primitive status of *Cryptocercus*, a genus of a group possibly more primitive than termites (1, 2). Further investigations into other aspects of behavior and the phenomenon of possible pheromone release are warranted (3).

HOPE RITTER, JR.

Department of Biology, State University of New York, Buffalo

#### References and Notes

1. L. R. Cleveland, S. R. Hall, E. P. Sanders, J. Collier, *Mem. Am. Acad. Arts Sci.* 17, 185 (1934).
2. L. R. Cleveland, *J. Morphol.* 85, 197 (1949).
3. Supported by NIH grant E-4708 and NSF grant GB-988. I thank Dr. William L. Brown, Jr., Dr. Thomas Eisner, and Dr. Carl Gans for advice during experiment and manuscript preparation.

3 February 1964

## Lower Limit of Water Availability to Plants

*Abstract. Measurements of transpiration, cell division, and cell enlargement show that no single lower limit of available water can be defined for these three plant processes. The soil-water content at which permanent wilting is exhibited does not represent a true lower limit for any of these.*

The soil-water content at which plants appear permanently wilted has been found to correlate well with the soil-water content at 15 bars' suction. This water content has often been considered as the lower limit of available water in the soil. The exact interpretation of the permanent-wilting percentage and the relative availability of water above this water content have been subjects of interest to, and of some controversy among, soil scientists and plant physiologists (1). Some of the confusion has arisen from the frequent failure to distinguish between at least two different aspects of a plant's requirement for water, transpiration and growth. This report summarizes results of several studies showing that water availability affects these two processes differently.

The water in the plant is seldom in equilibrium with the water in the soil. In order for the plant to extract water from the soil, the potential energy of the water in the plant must be lower than that of the water in the soil. The difference in energy between plant water and soil water depends upon the rate

of uptake of water from the soil and the water-conducting properties of the soil and plant. Results of an experiment designed to measure this energy difference are shown in Fig. 1. The energy of the water is plotted as soil matric suction (soil-moisture tension) and leaf suction (diffusion pressure deficit), both measured as energy per unit volume of water expressed in bars. The suction in a leaf of a pepper plant and the average matric suction of the soil in which the plant was growing are plotted as a function of time. The experiment was conducted in a growth chamber with 12-hour alternate light and dark periods at a temperature of 25°C. The dark periods are indicated by the solid bars at the bottom of the figure. The plant was grown in a 3-gallon crock filled with a clay loam soil. The leaf suction was obtained by continuous monitoring of the  $\beta$ -ray transmission by a single leaf. The procedure was the same as that used by Mederski (2), except that  $Tc^{99}$  was used instead of  $C^{14}$  for the  $\beta$ -ray source. The transmitted radiation was detected with a thin-window Geiger counter, and the result

was recorded with a count-rate meter and a strip-chart recorder. At the conclusion of the experiment, the leaf was detached from the plant, rehydrated, and used to calibrate the counting system. A previously established relation between the water content and the suction was then used to estimate the suction. An absolute error in leaf suction as high as 2 bars is possible in this calibration procedure, but changes in suction can be estimated with much greater precision.

The soil suction was measured with a tensiometer when the suction was less than 0.8 bar and with a gypsum electrical resistance block for higher suctions. Samples were also taken for the determination of water content at high suctions, and the results were checked against a curve relating matric suction to water content. Since the soil suction was recorded once each day, the small variations due to the diurnal fluctuation in transpiration were not recorded.

The leaf suction shows the diurnal fluctuation postulated by Slatyer (1). The data for the first 5 days show very little dependence of leaf suction upon soil suction. Not until the soil suction exceeds about 2.5 bars does the leaf suction begin to be affected. This is approximately the suction at which the unsaturated conductivity of the soil has become low enough to restrict water movement significantly (3). The leaf suction reaches progressively higher peaks during the light period as the soil suction increases and then drops back to a value approximately equal to the soil suction at night. Because of the possible errors in each measurement, one cannot be certain that equilibrium was actually attained at night. The agreement may be merely fortuitous.

During the 8th day, the plant showed definite symptoms of wilting but recovered during the night. On the 9th day, the plant again wilted and had not recovered turgor during the succeeding night after which the experiment was concluded. The average daily transpiration rate remained virtually constant until the 8th day when it decreased appreciably. It decreased still further on the 9th day. For pepper plants, the reduction in transpiration coincides with very definite wilting symptoms which appear at a leaf suction of about 11 bars. We have found that a large number of plants exhibit marked evidence of wilting at approximately this value of leaf suction. The closing of the stomata and the consequent reduc-

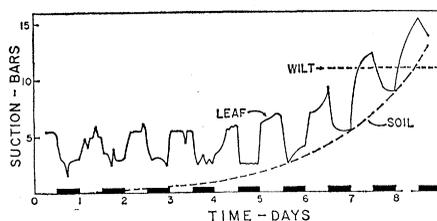


Fig. 1. Diurnal fluctuation of the suction (diffusion pressure deficit) of a pepper leaf (solid line) and average soil suction in root zone (dashed line). Solid bars along the abscissa indicate twelve-hour dark periods. The horizontal dashed line indicates leaf suction at which wilting symptoms appear. The plant was grown in a 3-gallon jar containing clay loam soil.

tion in transpiration rate seem to occur at approximately the same value of leaf suction at which very definite wilt symptoms appear.

In work reported elsewhere, the relationship of transpiration rate to soil suction has been investigated (4). The actual transpiration rate is reduced below the potential rate at soil suctions much less than 15 bars. So long as the leaf suction is less than the critical value at which the stomata close, soil suction has little effect upon transpiration. Above this critical leaf suction, increasing soil suction reduces the transpiration rate but does not eliminate transpiration completely. The critical leaf suction cannot be universally related to any particular soil suction since it is a function of both plant and soil properties.

It is virtually impossible to grow plants under conditions of constant soil suction. In order to gain insight into the relation between soil suction, leaf suction, and plant growth, a series of

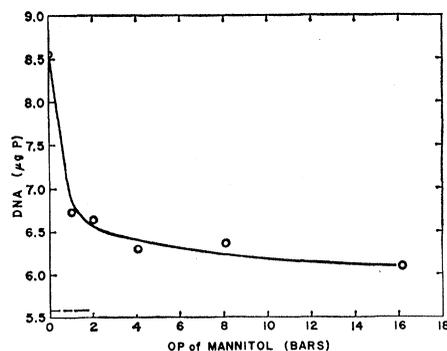


Fig. 2. DNA content (expressed as micrograms of phosphorus) per 20 cotyledonary leaves of radish as a function of the osmotic pressure (OP) of mannitol in the incubation medium. Each point is the average of two groups of 20 leaves. The dashed line indicates DNA content prior to incubation.

experiments was conducted with isolated cotyledonary leaves of radish (*Raphanus sativus* L.) subjected to an osmotic stress in place of soil suction. These leaves grow readily in isolation on a simple medium and, in the presence of white light, synthesize appreciable amounts of DNA, RNA, and protein (5). Cell counts have shown that the increase in DNA is linearly related to the increase in the number of cells.

Radish seeds were germinated in washed, moist, vermiculite at 25°C with 1.1 lu/cm<sup>2</sup> constant white light. Uniform fully turgid cotyledonary leaves were excised from 5-day-old seedlings and handled in groups of 20. Two groups were oven-dried (70°C) for the determination of moisture. The rest were washed with a 2 percent aqueous solution of neutral detergent and rinsed with sterile, distilled water. Six groups were frozen for the later analysis (6) of DNA content. Three groups were incubated at each of six different osmotic pressures (OP), one group for each 9-cm petri dish, with the leaves placed right side up on a pad (filter paper and cheese cloth) saturated with 10 ml of medium. The composition of the base medium (control) was as follows: sucrose, 1 percent; KNO<sub>3</sub>, 60mM; MgSO<sub>4</sub>, 10mM; phosphate (KH<sub>2</sub>PO<sub>4</sub> + K<sub>2</sub>HPO<sub>4</sub>), 5mM. These concentrations yielded optimum growth. This medium had a pH of 6.5 and an osmotic pressure of 4 bars. However, the solutes in this medium are taken up so rapidly by the radish leaf cells that they promote rather than suppress the uptake of water by the cells. A graded osmotic pressure series was prepared by incorporating mannitol in the medium to produce pressures of 1, 2, 4, 8, and 16 bars. Mannitol does not enter the radish leaf cells to any appreciable extent, and so the decrease in water potential of the medium is considered equivalent to a reduction due to soil suction. The leaves were incubated for 28 hours at 25°C, while exposed to 1.1 lu/cm<sup>2</sup> of white light.

After incubation, the leaves were blotted and weighed. One group from each treatment was dried for moisture determination; two groups were washed and analyzed for DNA content. Figure 2 shows a plot of DNA, expressed in micrograms of phosphorus, as a function of the osmotic pressure, or of the concentration of mannitol in the medium. The DNA replication, and hence, cell division, was reduced about

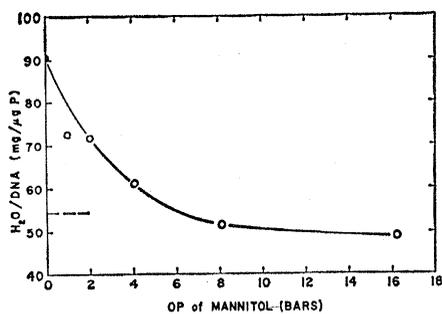


Fig. 3. Ratio of water content to DNA for radish cotyledonary leaves as a function of the osmotic pressure (OP) of mannitol in the incubation medium. Each point is the average of two groups of 20 leaves. The dashed line indicates the value of the ratio prior to incubation.

60 percent by just 1 or 2 bars, with a slight additional decrease as the osmotic pressure increased to 16 bars. The uptake of water also declined as the osmotic pressure increased. One bar of osmotic pressure suppressed the uptake by 60 percent, 8 bars, by 95 percent. At 16 bars, the leaf-water content dropped about 2 percent below the amount present before incubation. The nearly complete suppression of water uptake at 8 bars is in agreement with the estimate, based on plasmolysis, of about 8 bars for the osmotic pressure of the cell sap before incubation. This agreement supports the premise that it is primarily the osmotic pressure of the added mannitol rather than the total osmotic pressure of the medium (including the contribution made by the other solutes) that limits the uptake of water. The ratio of leaf-water content to DNA gives a measure of the cell size, and hence, of cell enlargement. Figure 3 shows a graph of this ratio plotted against the osmotic pressure of mannitol in the medium. Cell enlargement at first decreased more gradually than cell division with increasing osmotic pressure. But at 8 bars, enlargement was almost completely prevented whereas DNA was increased by about 14 percent. As a result, the ratio of water to DNA actually fell below the ratio before incubation. Total growth—the product of the two curves (Figs. 2 and 3)—virtually stopped above 8 bars.

It is usually observed that the highest rate of leaf growth occurs during the night when the suction is at a minimum. Thus, one might expect the growth rate to be more closely correlated with the minimum leaf suction than with the average leaf suction. The opposite is the case with transpiration, which is

more strongly influenced by the maximum suction near midday when wilting is more probable. The minimum leaf suction, in turn, seems closely related to the average soil suction so that a good correlation between leaf growth rate and average soil suction might be expected.

One reason for much of the confusion concerning water availability is well illustrated by Fig. 1. The leaf suction is relatively unaffected by the soil suction for several days after a soil is irrigated. During this time while the soil suction is low one can reasonably assert that the soil water is, for practical purposes, equally available for growth and transpiration. From day 7 through day 9 in Fig. 1, the increase in soil suction resulted in an increase in leaf suction which reduced growth and, eventually, transpiration. In the case of many soils, the range of water content represented by the range of soil suction from 1 to 15 bars is relatively small compared with the range from 0.2 to 1 bar. In the field, the time during which the soil suction is sufficiently high to reduce growth or transpiration may be short compared with the time during which the soil suction is negligibly low. This fact, coupled with the inherent inhomogeneity of soils and the nonuniformity of the water extraction pattern in the plant root zone, often tends to obscure the very definite effect of soil suction upon transpiration and growth which is observed in the laboratory.

While the percentage at 15 bars may continue to serve a useful purpose in many practical situations, it is increasingly clear that the permanent wilting point does not represent the absolute lower limit of available water. Indeed, no single limit for all plant processes can be defined in any precise way.

W. R. GARDNER

R. H. NIEMAN

United States Salinity Laboratory,  
Agricultural Research Service,  
Riverside, California

#### References and Notes

1. For a general review of this subject see R. O. Slatyer, *Bot. Rev.* **23**, No. 10 (1957).
2. H. J. Mederski, *Soil Sci.* **92**, 143 (1961).
3. W. R. Gardner, *ibid.* **89**, 63 (1960); — and C. F. Ehlig, *Science* **138**, 522 (1962).
4. W. R. Gardner and C. F. Ehlig, *J. Geophys. Res.* **68**, 5719 (1963).
5. R. H. Nieman and L. L. Poulsen, *Plant Physiol.* **37** (Suppl.), xxi, (1962).
6. —, *ibid.* **38**, 31 (1963).
7. Supported in part by the Meteorology Department, U.S. Army Electronic Research and Development Activity, Fort Huachuca, Ariz. 20 January 1964

## Radiation-Induced Aversion to Alcohol

Abstract. Mice genetically susceptible to alcohol orientation were allowed to develop a preference for 10 percent ethanol over tap water. A low dose (12 roentgens per hour for 4 hours) of whole-body gamma radiation was used as an unconditional stimulus to produce alcohol-avoidance behavior. A marked aversion to the alcohol solution occurred but was extinguished within 6 days, owing probably to the very high motivation of the animals to drink alcohol. The study extends the technique of radiation-produced avoidance conditioning to include alcohol consumption as a measure of response in genetically susceptible mice.

The use of ionizing radiation as an unconditional stimulus in avoidance learning experiments has been shown by Garcia, Kimeldorf, and others (1) to be effective with a variety of response measures in several different species. The intake of normally preferred solutions of saccharin by rats and mice or of chocolate-flavored milk by cats can be reduced if the ingestion of these substances is paired in an appropriate temporal arrangement with whole- or part-body exposure to ionizing radiation. That such low-dose radiation effects are not peculiar to the ingestion of foodstuffs or to the activation of taste receptors is revealed by a study in which spatial avoidance behavior was established with the use of x-rays as the aversive stimulus (1). Our study represents an attempt to extend the response generality of radiation-produced aversive conditioning by using ingestion of alcohol as a response measure in alcohol-oriented mice. Although the sensory mechanisms through which radiation acts as an unconditional stimulus to produce avoidance behavior are obscure (2), the potency of the avoidance response can be demonstrated if it results in the rejection of alcohol solutions by animals whose genetic susceptibility to (3) and experiential preference for alcohol has been shown.

Twenty-four male mice of the C57BL/Cum inbred strain were provided only 10 percent ethanol to drink for 14 days during which they were allowed free access to dry food. The ethanol consumption during this pe-