Lichens On Galápagos Giant Tortoises

Abstract. *The association of* Physcia picta *with the giant Galápagos tortoise is believed to be the first reported occurrence of lichens on land animals. The habitat is restricted to specific sites on the carapace of male tortoises.*

The giant land tortoise, *Geochelone elephantopus* (Harlan) has been found to serve as a host for the lichen, *Physcia picta* (Sw.) Nyl. Tortoises with epizoic colonies of lichens were discovered in February 1964, in the interior of western Santa Cruz (Indefatigable) Island by a field party of the Galápagos International Scientific Project. The occurrence is noteworthy because it represents possibly the first recorded instance of a lichen growing on a living land animal. *Physcia picta* is one of the most ubiquitous of Galápagos lichens, growing on many different substrates, including lava rock, dry wood, bark of living trees, and evergreen leaves.

The potential area for lichen colonization is restricted by the habits of the tortoise. The lower part of the carapace is unavailable because of the fact that the tortoises spend long periods of time partially submerged in shallow freshwater pools when these are (seasonally) available. The front, top, and sides are unavailable because the animal is constantly pushing its way through dense underbrush, thereby scouring the carapace. This leaves only a small crescent-shaped area on the upper rear of the carapace which is not vulnerable to one or the other deterrent to colonization; upon this area the lichens were found. Only males are so colonized; in the case of females the area in question is heavily abraded by ventral surfaces of males attempting copulation.

Fragments of the outer, horny layer of the carapace were removed with the attached lichens and are preserved in the lichen herbarium of the University of Colorado Museum.

JOHN R. HENDRICKSON
University of Hawaii, Honolulu
WILLIAM A. WEBER
University of Colorado, Boulder

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Osmotic Stress: Effects of Its Application to a Portion of Wheat Root Systems

Abstract. *Application of a slight osmotic stress to only a portion of young wheat roots significantly affected certain metabolic fractions throughout the entire plant. These components change rapidly with time and therefore time studies must be used in experiments of this type concerned with an evaluation of changes in metabolic fractions in plants that are induced by environmental factors.*

Studies of moisture stress on plants are often confounded with an insufficient definition of conditions. Whether nutrient uptake is affected by soil-moisture tension per se or only through its indirect effects on soil characteristics has been of interest to, and of some controversy among, soil scientists and plant physiologists. Mederski and Wilson (*1*) used a split-root technique in which the top portion of corn roots developed in sand culture while the remaining portion developed in soil adjusted to known moisture contents in the range of wilting point to field capacity. The readily available water in the sand compartment and the high humidity of the atmosphere precluded the development of a growth-limiting water stress in the plant tops. The results of their technique—decreased growth with increasing stress—must have been due to reduced uptake of ions or translocation of ions rather than to a loss of turgescence in the tissue with subsequent effect on physiological processes. They indicated that soil factors relating to moisture films might be the regulating influence on ion uptake.

Our study was conducted with solution cultures, and thus precluded soil factors that reduce uptake of ions. Similar results were obtained with an osmotic stress in the presence of ample nutrients and water. Alterations in the physiology of the root are probably responsible for the reduced concentrations of phosphorus, substantiating the conclusions of Dean and Gledhill (*2*).

Nine-day-old wheat plants, with their roots initially trimmed to two roots, were grown in a divided container so that each root could be placed under separate treatment. Osmotic solutions of "Carbowax 6000" (*3*) in nutrient solutions were used as stress treatments. Plants were harvested 24, 48, 96, and 120 hours after treatment.

The conditions maintained were a temperature of 18°C, a 16-hour light period with an intensity of approximately 3.3 lu/cm^2, and 55 to 65 percent relative humidity. These conditions indicate a low evaporative demand and should preclude internal water deficits being set up in the plant because of limited water movement.

All results are reported on plants with one root in nutrient solution and the other in an osmotic solution at a pressure of 1 bar. The control had both roots in nutrient solution.

Relative turgidity measurements indicated no difference between the control and those plants with one root under 1-bar stress. Tops of the stressed plants did not have a significantly higher percentage of dry matter than control plants until 120 hours after application of the stress. The stressed root had a significantly higher percentage of dry matter than either the nonstressed root of the same plant or the roots of the control plants at all harvests. The nitrogen content of the plant tops, with a root under stress, was not significantly different from the control throughout the experiment. The stressed root had a significantly lower nitrogen content than the control roots, whereas, the nonstressed root had the same nitrogen content as the control. This would indicate a decreased uptake of nitrogen. The phosphorus contents of the tops and of the stressed roots were significantly lower than those of the control. This would indicate an immediate reduction in phosphorus uptake under stress. Why stress on only one root should result in such a reduction throughout the plant is unknown but suggests an initial physiological shock.

Nitrate percentage in the tops was not affected significantly by applying a stress of 1 bar to one root. The nitrate percentage of the stressed root was significantly lower than that of the control, indicating that uptake was probably reduced and that internal translocation of nitrogen was not suf-

ficiently rapid to equalize the concentrations. Soluble carbohydrate content of the tops was not affected significantly by the partial stress, but the initial (24- and 48-hour) carbohydrate content of both roots of the partially stressed plant was increased over that of the control. Subsequently it was the same as the controls. These results suggest that utilization of carbohydrate was reduced by a stress on any part of the root system before translocation from the top was affected.

A gross measurement of all soluble ninhydrin-positive material (α-amino groups, primarily amino acids and peptides) indicated that this fraction was greatly reduced in the shoots subjected to partial stress at 24 and 48 hours, but this fraction subsequently increased. The content of this fraction in the roots was also reduced below that of the control. This would indicate that syntheses of these compounds may be reduced by a stress on any portion of the plant and could be a reflection of the reduced nitrogen uptake indicated earlier.

The results obtained are likely due to some specific effect occurring at the root, resulting in subsequent disturbance of metabolism throughout the plant. That the results were not due to changes in the turgidity of the plant or in the energy status of the water in the plant was indicated by relative turgidity measurements and the appearance of the plants.

RAYMOND E. MEYER
Boll Weevil Research Laboratory, State College, Mississippi
JOE R. GINGRICH
Department of Agronomy, Oklahoma State University, Stillwater

References and Notes

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Chromic Oxide Indicator Method for Measuring Food Utilization in a Plant-Feeding Insect

Abstract. *The chromic oxide indicator method was used to determine percentage utilization of three diets prepared from lyophilized plant tissues for fifth-instar larvae of the pale western cutworm. Because of the simplicity of the method and uniformity of results within each of the diets, the procedure can be used routinely in feeding trials with this insect, and perhaps others with biting and chewing mouth parts.*

The quantitative aspects of food consumption and utilization have frequently been neglected in previous studies of insect nutrition (*1*). Many phytophagous insects feed readily only on fresh material, making it difficult to determine food consumption accurately. Moreover, determination of food utilization by the classical gravimetric procedure requires that excreta must be quantitatively collected and weighed. The indicator technique originally described by Bergeim (*2*) does not require quantitative measurements of consumption and excretion and has been used extensively with laboratory and farm animals, Cr_2O_3 being used as the index compound. In this report we describe the application of this method to a plant-feeding insect.

This method requires that the indicator compound incorporated in the food be neither absorbed from the gut nor toxic at the concentrations used; under these circumstances, percentage utilization is given by [1 − (concn of indicator in dry matter of food/concn of indicator in dry matter of excreta)] × 100. The method has not previously been applied to insects, probably because of difficulty in distributing the index compound uniformly in their diets. Larvae of the pale western cutworm, *Agrotis orthogonia* Morr., can be reared on plant tissues that have been lyophilized and ground (*3*), and therefore the indicator method was practical. Moreover, availability of Cr_2O_3 paper (*4*), which can be more easily distributed in the diet than Cr_2O_3 itself, contributed to the feasibility of this approach.

Table 1. Utilization of dry matter in diets by fifth-instar larvae of the pale western cutworm as determined by the Cr_2O_3 indicator method.

Diet	Mean wt. of larvae (mg)		Percentage utilization*
	Initial wt.	Gain in wt.	
Sprouts	65 ± 21	159	41 ± 2
Sprouts and cellulose	62 ± 21	155	21 ± 2
Pith	66 ± 23	46	16 ± 1

* $\left(1 - \frac{\mu g\ Cr_2O_3/mg\ dry\ matter\ in\ food}{\mu g\ Cr_2O_3/mg\ dry\ matter\ in\ excreta}\right) \times 100$

Three diets known to vary in nutritional value were prepared from (i) sprouts of Thatcher wheat; (ii) equal parts of sprouts and cellulose powder (wt/wt); (iii) pith from stems of solid-stemmed Rescue wheat. The first two diets are excellent foods for the cutworm, whereas pith is nutritionally unsatisfactory (*5*). The wheat plant tissues were grown as described (*6*), lyophilized, and ground to pass a 40-mesh screen. Chromic oxide paper (*4*), also ground to pass a 40-mesh screen, was added to each of the dry plant materials to give a concentration of Cr_2O_3 near 4 percent. This concentration was not toxic to the cutworm, had no apparent effect on consumption, and was not absorbed from the gut. Uniform distribution of Cr_2O_3 was accomplished by tumble mixing for 48 hours. Distilled water was added to each dry meal in quantities sufficient to provide a medium that was acceptable to the cutworm. The diets were packaged in approximately 1-g portions in aluminum foil and stored at −30°C until fed.

Percentage utilization of each diet was determined with 10 newly molted fifth-instar larvae of the cutworm. Individual larvae were maintained in 60 × 15-mm petri dishes and had unrestricted access to a single diet in a humid atmosphere in the dark. The larvae were transferred to clean dishes and provided with fresh food each day. Excreta were separated daily from residual food and were combined into a single collection per insect. Larvae that were fed the diets containing sprouts were maintained on these foods until they molted into sixth instar, about 5 days; those fed the pith diet were retained on test for 7 days, but none molted. The excreta and samples of the diets were dried at 120°C for 2 hours. After the dry material was pulverized and mixed in a mortar, samples were analyzed in triplicate for Cr_2O_3 by a micro-method in which perchloric acid digestion was followed by colorimetric determination of chromium with diphenylcarbazide (*7*).

The percentages of dry matter utilized by individual larvae within a diet were similar (Table 1) but the percent-