at this time that there are many small hot spots rather than one or two large spots. Two possible geological interpretations of the hot spots are presented. (i) They are the vents of the active volcanoes (*11*) (this is supported by the Voyager 1 observation of the small spot in the region of P1). (ii) They are cracks caused by convection in the crust of the numerous black calderas seen on Io (*12*). Such cracks are frequently seen glowing red in the lava lakes that sometimes fill terrestrial calderas. However, if the surface of the black calderas is quenched amorphous sulfur, as has been suggested (*13*), it may not form cracks.

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7. The effect of the hot spots on the spectrum of Io is discussed by D. L. Matson, G. A. Ransford, and T. V. Johnson (in preparation) and W. M. Sinton (in preparation).
8. Most of the data from our monitoring program fall into these ranges. Earlier data [for instance, O. L. Hansen, in (*3*)] also fall into this range when reduced with the solar magnitudes from H. Johnson [*Commun. Lunar Planet. Lab.* **3**, 73 (1965)] that we used in our reductions.
9. The orbital phase angle is the angle along Io's orbit from superior geocentric conjunction with Jupiter. Since Io has a nearly circular orbit and keeps the same face toward Jupiter, the angle is also nearly the west longitude of the central meridian.
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14. This eclipse occurred in full daylight. By using a Quantex TV and digital image processing system and deep red filter, we could readily see Io at visual magnitude 5.6 before the eclipse. A single-frame background image obtained with Io slightly displaced was stored in the system memory. A running weighted average of 32 TV frames with this background subtracted was then displayed with greatly enhanced contrast.
15. W.M.S. was Visiting Astronomer at the Infrared Telescope Facility, which is operated by the University of Hawaii under contract from the National Aeronautics and Space Administration. E.E.B. and C.J. were visiting astronomers at the United Kingdom Infrared Telescope facility. T.J.L. is the astronomer-in-charge of the UKIRT, which is operated by the Royal Observatory, Edinburgh, on behalf of the Science Research Council. We are grateful to J. Pearl of NASA/Goddard Space Flight Center for discussions about the existence of high-temperature spots. This research was supported in part by NASA grant NGL 12-001-057.

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Internal Winds in Water Lilies: An Adaptation for Life in Anaerobic Sediments

Abstract. *The network of internal gas spaces in the yellow water lily constitutes a pressurized flow-through system which forces oxygen to the roots and rhizome buried in the anaerobic sediment. By the purely physical processes of thermal transpiration and hygrometric pressure, several liters of air per day enter the young, newly emerged leaves of* Nuphar luteum *against a small pressure gradient. This air moves en masse down the petioles of the young leaves (at rates up to 50 centimeters per minute) to the rhizome, forcing a simultaneous flow of gas (rich in carbon dioxide) from the rhizome up the petioles of the older emergent leaves to the atmosphere. The ventilation system has important physiological and ecological consequences.*

Plant roots growing in flooded soils must withstand long periods of anaerobiosis and the presence of soluble phytotoxins. The capacity of higher plants to survive these conditions is largely dependent on the rate of oxygen supply to the buried tissue, since oxygen supports root respiration and contributes to the detoxification of the rhizosphere (*1*). This transport of oxygen is generally achieved within the plant through an extensive system of gas spaces or lacunae.

Until now, models of gas transport in plants have held that the gas phase in the lacunae is essentially stationary, and that the individual gases simply diffuse along concentration gradients (*1, 2*). In his widely cited study, Laing (*3*) described the gas dynamics in the lacunae of the yellow water lily as the product of diffusion in a static gas phase along concentration gradients generated by plant photosynthesis and respiration. My research has shown that the gases in the lacunae are not static: they flow en masse at linear rates up to 50 cm/min.

Nuphar luteum, the yellow water lily, grows in lakes by means of a horizontal creeping rhizome that usually lies buried in the sediment. During summer growth new leaves continually develop and rise to the lake surface, supported by petioles up to 2 m long. I measured gas flows in the petioles of young emergent leaves by injecting a small volume (usually 0.1 ml, standard temperature and pressure) of ethane gas tracer into the upper end of the petiole. The tracer passed quickly down the petiole past the sampling point at the lower end (Fig. 1). This technique showed that gas flowed in the opposite direction in the petioles of older emergent leaves.

This pattern of bulk flow from the young leaves toward the older leaves was confirmed by another tracer experiment. All the leaves on an isolated shoot apex were enclosed in gas-impermeable Saran bags and an ethane tracer was injected into the upper portion of the petiole of the youngest emergent leaf. Results of a typical experiment are shown in Table 1, where more than 60 percent of the tracer had left the plant within several hours. None of the tracer escaped through the youngest leaf; most escaped through the oldest.

The flows originate in the lacunae of the youngest emergent leaves where gas pressures slightly greater than ambient were measured by a manometer. The pressures were highest during midday (up to 0.002 atm above ambient), and declined to ambient at night. The rate of gas flow down the petioles of these leaves was a linear function of the observed pressure gradient, in accordance with Darcy's law for flow through porous media (*4*).

The capacity of the young emergent leaves to draw air from the atmosphere into their lacunae against a pressure gradient was confirmed by inverting a 4-liter beaker over an influx leaf. With a healthy undamaged leaf exposed to sunlight inside the beaker, the water level in the beaker rose as much as 2 cm above the lake surface. This means that the leaf tended to draw a vacuum in the process of "pumping" air into its lacunae. There was no measurable selectivity by the pump for any particular component of the atmosphere. Except for the higher water vapor (humidity) in the gas of the midrib of these influx leaves, there was no measurable difference between the compositions of the lacunar gas and the atmosphere (*5*).

The diurnal pattern of pressurization measured in the lacunae of the young leaves suggests that the sun supplies the energy required by this pump. Rather than a photosynthetic basis for the pump, the pump's dependence on the sun was for its heat, not its shorter wave radiation. Rapid and sustained changes in lacunar gas pressure could be initiated in darkness by holding a source of radiant heat near the leaf. These experiments show that the temperature differential between the leaf and the atmosphere drives the pressurization (*5*).

In 1841, Raffineau-Delile (*6*) reported pressurization in leaves of *Nelumbo*, the lotus. The purely physical nature of the pump was demonstrated three decades later, when Merget reported the pressurization of the gases in a dead leaf (*6*). There have been few subsequent references to this work (*7*) since the role of the pressure differential in driving mass flows was not recognized.

Table 1. All the emergent leaves of a shoot apex were enclosed in gas-impermeable Saran bags, and 5 ml of ethane (C_2H_6) was injected into the upper end of the youngest emergent petiole (1). The amount of ethane flux from the leaves was determined by sampling the ethane concentration in the bags after several hours. The distribution of ethane throughout the plant at the end of the tracer experiment confirms the flow pattern. Even though the tracer was injected into the upper end of petiole 1, its concentration is lower there than in each of the other petioles. Such tracer behavior cannot be explained on the basis of diffusion.

Petiole rank	Ethane	
	Efflux from leaf (ml)	In petiole (ppm)
1 (youngest leaf)	0.00	80
2	0.03	270
3	0.79	420
4	1.01	1150
5 (oldest leaf)	1.26	1240

The flow of gases occurs along either gradients in partial pressure (diffusion) or gradients in total pressure (mass flow). The character of flow through a porous partition depends on the size of its pores (*8*). The porosity of young *Nuphar* leaves is such that diffusive flux dominates, allowing the net transport of gas from the atmosphere against a gradient in total pressure. The pressurization is accomplished by two independent diffusive processes well-known in physical theory as thermal transpiration (*9*) and hygrometric pressure (*10*). These processes are dynamic and operate as long as the temperature or vapor gradients (or both) are maintained. In the case of leaves in sunlight, they combine to increase the total gas pressure inside the lacunae of the influx leaves. The interior of the leaves is warmer than ambient, leading to pressurization due to thermal transpiration. This temperature gradient also increases the vapor pressure of water in the leaf relative to ambient, generating a hygrometric pressure gradient.

The significance of the elevated pressure lies in the fact that it drives an important bulk flow of gas through the entire plant. As the leaves of *Nuphar* grow and mature at the lake surface, their porosity increases, and they lose their capacity to support pressure gradients: gases can flow through them en masse (*11*). Since the lacunae of these older leaves are continuous with the lacunae of the younger leaves, the older leaves act to vent the pressure generated in the youngest emergent leaves. As a result, the lacunae of the entire plant behave as a pressurized flow-through system.

This flow-through system has the important physiological effect of transporting large volumes of oxygen to the rhizome buried in the anaerobic sediments. Integration of the flow rates observed in a single petiole throughout a day shows that 22 liters of air (4.6 liters of O_2, under ambient conditions) moved

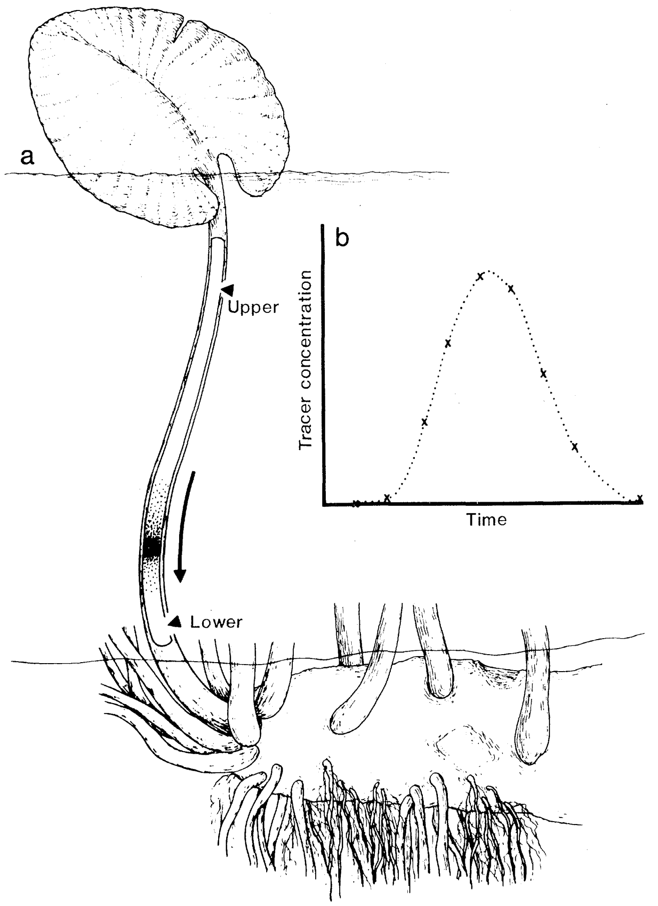

Fig. 1. Tracer movement down the petiole of a young, emergent (influx) leaf. (a) Tracer (represented by stippling in the cut-away view of the petiole) was injected into the upper end of the petiole and sampled at the lower end. (b) The plot of the tracer concentration as a function of time at the sampling point took the form of the typical elution curve seen in gas chromatography, with the size and shape of the curve depending on the volume of tracer injected and the linear rate of flow along the petiole. The volume rate of gas flow was calculated and the elution curve was integrated to show that virtually all the injected tracer passed the sampling point. The direction of gas flow was reversed in the older emergent (efflux) petioles. Tracer was injected into the base of these petioles, and it passed up the petioles in the same manner. No significant amounts of tracer were detectable upstream in either type of petiole when the tracer injection was made into the downstream end of the petiole. The volume of gas withdrawn for tracer analysis was very small, and did not affect the gas flow. Ethane was used as a tracer because it is physiologically inert and easily detectable.

down that petiole; the maximum rate observed for air was 60 ml/min (5). The significance of this diurnal pattern of oxygen transport is reflected in the pattern of oxygen abundance in the rhizome, often varying from less than 10 percent O_2 at night, to ambient (21 percent O_2) during daylight. Isotope experiments have shown that most of this O_2 originates in the atmosphere, not in photosynthesis (*12*).

The efflux of gas through the petioles of the older emergent leaves also has physiological and ecological significance. It carries CO_2 from the rhizome (often exceeding 3 percent during daylight) to those leaves. Experiments with $^{14}CO_2$ have shown that most of this CO_2 is fixed by photosynthesis (*12*). This flow also carries ecologically significant quantities of methane from the lake sediment to the atmosphere (*13*), and explains the diurnal pattern of CH_4 efflux previously reported (*13*).

Further studies are necessary to determine how widespread this circulation phenomenon is among plants. Earlier data on pressurization in other plant leaves (*6*) suggest that similar flow patterns may occur in them. The purely physical basis of the pump suggests that such pressure differentials may be common in other plants, although the magnitude and significance of the phenomenon is certain to be highly variable. Such a ventilation system would be most advantageous wherever plant parts are buried in anaerobic soils.

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8. When the pores are small (less than about 0.1 μm at 1 atm total pressure), the flow of gas is predominantly diffusive [L. B. Loeb, *The Kinetic Theory of Gases* (Dover, New York, 1934); E. H. Kennard, *Kinetic Theory of Gases* (McGraw-Hill, New York, 1938)]. Mass flows of the gas mixture do not occur until the pore size increases. As the pore size enlarges, any tendency for diffusive processes to generate gradients in total pressure is offset by mass flows that dissipate those gradients. The pressure gradients sustained by the young leaves of *Nuphar* denies the importance of mass flows between these leaves and the atmosphere: their exchanges must be essentially diffusive. On the other hand, the loss of resistance to mass flow in the older leaves demonstrates their increased porosity.
9. A temperature differential across such a diffusive partition leads to a pressure gradient due to differences in diffusion rates on the two sides, so that $p_1/p_2 = (T_1/T_2)^{1/2}$ [see references in (*8*)].
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11. Gas exchanges between the lacunae of *Nuphar* and the atmosphere occur through the epidermis and palisade parenchyma of the upper surfaces of the leaves [C. D. Sculthorpe, *The Biology of Aquatic Vascular Plants* (Arnold, London, 1967)]. My study of stomatal apertures in young leaves suggests that they are too large to present the limiting porosity. The flow-restricting porosity must therefore lie between the cells of the palisade tissue. A general feature of leaf growth in dicotyledons may be that during the later stages of development, the palisade cells cease dividing and enlarging before the overlying epidermal cells cease enlarging. This results in an expansion of the intercellular spaces in the palisade tissue [K. Esau, *Plant Anatomy* (Wiley, New York, 1953)]. This is the most probable explanation of the changing porosity of *Nuphar* leaves, since the declining ability of individual *Nuphar* leaves to sustain pressures was accompanied by expansion of the leaf blade area.
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14. I thank M. J. Klug for his support of this research under NSF grant DEB-78-05321; and K. Hogg Dacey for her help in all phases of this research. This is publication 380 of the W. K. Kellogg Biological Station; and publication 9065 of the Michigan Agricultural Experiment Station. The final manuscript was prepared at Woods Hole Oceanographic Institution.

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Opposite Polarity of Filipin-Induced Deformations in the Membrane of Condensing Vacuoles and Zymogen Granules

Abstract. *Filipin binding to membrane sterols induces deformations of the membrane that are detected by freeze-fracture either as 20- to 25-nanometer protuberances or as pits on the fracture faces. By using the filipin probe in pancreatic acinar cells, it was found that the polarity of filipin-induced deformations in the membrane limiting the Golgi condensing vacuoles is opposite that in the membrane limiting the mature zymogen granules. This asymmetry could be due to unequal partitioning of cholesterol between the membrane leaflets in these two compartments during the transformation of the condensing vacuole into the zymogen granule.*

Lipid asymmetries have been predicted in biological membranes (*1*) and have been found in a number of systems [(*2*); for reviews, see (*3*)]. Such asymmetries have been detected by biochemical and biophysical techniques; nonpenetrating reagents were used that label the outside leaflet of the membrane when the membrane is sealed, and both leaflets when the membrane is unsealed. Biochemical and biophysical approaches average information about the inside and outside leaflets of all the membrane within a preparation. If a membrane preparation is heterogeneous, differences between the components go undetected. To obtain information about the heterogeneity of a membrane preparation, morphological approaches must be used. Some attempts along these lines have been made with freeze-fracture followed by autoradiography, but this technique is laborious and fraught with technical problems (*4*). Morphological labels are needed that are capable of marking different lipid components between the leaflets. Such markers exist for charges (for example, cationized ferritin), antigenic components (the peroxidase-antiperoxidase and protein A-gold techniques), and carbohydrates (lectins). To date, however, no such marker has been claimed to label

Table 1. Quantitative evaluation (*8*) of the polarity of filipin-induced deformations (protuberances or pits) in the fracture faces (P and E leaflets) of zymogen granule and condensing vacuole membranes. Values are numbers of protuberances or pits ± standard errors of the mean per 1.0 μm^2 of membrane face; *N* is the number of exposed faces studied.

	P face			E face		
	Protuberances	Pits	*N*	Protuberances	Pits	*N*
Zymogen granules	0.24 ± 0.17	195.6 ± 14.6	25	182.8 ± 14.8	2.0 ± 1.1	43
Condensing vacuoles	153.4 ± 19.3	14.5 ± 3.2	29	11.8 ± 5.2	144.9 ± 19.3	16

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