form the imaginal epidermis, thereby blocking the subsequent cycle of cuticular deposition, and it inhibits chitin synthesis by established epidermal cells. Whether this cytostatic or antimitotic activity is a direct or indirect effect of diflubenzuron treatment remains to be determined. The effect of diflubenzuron on the formation of the imaginal epidermis does not appear to be the result of a moribund condition. The pupae appear to be healthy for at least the first 48 hours after treatment with diflubenzuron. During this time, the larval tissues not programmed for degeneration have intact plasma and nuclear membranes and an absence of autolytic vacuoles and pyknotic nuclei. That physiological processes are proceeding in the treated pupae in synchrony with those of the control groups is evident in the secretion of the pupal cuticle by the larval epidermis, continued selective phagocytosis of specific larval muscles, and cellular proliferation of other organ systems through the first 48 hours after diflubenzuron treatment. An interesting effect of diflubenzuron was that this compound retarded the programmed cell death of the larval epidermis. Although phagocytosis of other larval tissues proceeded at the same rate as in the control groups, degeneration of the larval epidermis was still occurring at 48 hours whereas phagocytosis of this tissue had been completed by 48 hours in the control groups.

The effect of diflubenzuron on the proliferation of imaginal epidermis has not been previously reported, probably because few studies on the mode of action have depended on histological observations for corroboration and because most studies have been made on insects in which the larval epidermis does not undergo metamorphosis (*3*). These studies have included hemimetabolous organisms such as the Orthoptera and Homoptera and also two orders of holometabolous insects, Coleoptera and Lepidoptera (*5*).

Although the effect of diflubenzuron on the stable fly may be limited to cyclorrhaphous Diptera, it should be noted that Hymenoptera, which include several economically important species, also form an imaginal epidermis. Therefore studies concerning the effect of diflubenzuron on this order should be made.

SHIRLEE M. MEOLA
RICHARD T. MAYER
Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research, Science and Education Administration, College Station, Texas 77840

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Phosphorus Sources for Aquatic Weeds: Water or Sediments?

Abstract. *Nine common species of aquatic macrophytes took all their phosphorus from the sediments when grown in situ in both a mesotrophic and a mildly eutrophic bay. Even under hypertrophic conditions, the sediments contributed an average of 72 percent of all the phosphorus taken up during growth. These experiments unambiguously demonstrate for the first time that submergent macrophytes in nature overwhelmingly depend on the sediments for their phosphorus supply and characterize them as potential nutrient pumps to the open water.*

Whether aquatic macrophytes (*1*) take their nutrients from the sediments or from the open water has been a long discussed but unresolved question. A quantification of the relative contribution of water and sediments in nutrient uptake would identify macrophytes as nutrient pumps or sinks and would contribute to weed control strategies in areas where excessive growth is a problem.

Several investigators have attempted to establish the relative importance of roots and shoots in the phosphorus (P) nutrition of macrophytes under artificial conditions (*2*). These studies have qualitatively shown the ability of macrophytes to take up and translocate some P by way of the root system. However, they were not designed to resolve the question of whether macrophytes in nature obtain their nutrients from the water or the sediments, or both sources.

Our approach to the question was to grow in situ various species rooted in ^{32}P-labeled sediments of known available P-specific activity (*3*), with the shoot in free contact with the unlabeled open water P. If during growth the plants obtain P exclusively from the water, they should not show any ^{32}P activity; conversely, if the sediment is the only source of P, the plants should show a specific activity equal to the specific activity of the available sediment P. If P is assimilated both from the sediments and the water, the ratio of plant P to available sediment P-specific activities should provide a direct measure of the relative importance of each source. As the relative contribution of water and sediments in P uptake is probably related to the relative P availability of sediments and water, the study was carried out in three locations representing a broad range of relative water P and sediment P availabilities (Table 1).

At each site, the sediments were collected with a Petersen dredge and pooled as large single batches (100 kg). They were then labeled with ^{32}P as $H_3{}^{32}PO_4$ (*4*), carefully homogenized, and allowed to equilibrate for 4 weeks, after which growth experiments were initiated. In early June 1977 and 1978, small sprouting plants were collected, weighed, and potted in closed 1.5-liter polyethylene

Table 1. Water P and sediment P characteristics for the three southern Quebec sites investigated. The interstitial soluble reactive P (SRP) was sampled by dialysis; TP designates total P.

Site	Water		Sediment		
	Mean TP (μg/liter)	Mean SRP (μg/liter)	Mean TP (μg/g)	Mean available P (μg/g)	Mean interstitial SRP (μg/liter)
Central Lake Memphremagog	9.7	0.5	799	66.7	169
Southern Lake Memphremagog	29.8	1.9	785	195	1200
Rivière-du-Sud	290	167	1199	228	1490

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Table 2. Mean percentage uptake of P (± standard error) from the sediments for six macrophyte species grown in central Lake Memphremagog. The number of replicates is given in parentheses.

Species	Percentage uptake from sediments
Myriophyllum alterniflorum	104.4 ± 1.0 (4)
Potamogeton zosteriformis	107.4 ± 2.3 (3)
Potamogeton foliosus	98.6 ± 2.1 (6)
Callitriche hermaphroditica	94.2 ± 2.4 (3)
Elodea canadensis	99.0 ± 2.5 (2)
Najas flexilis	100.8 (1)

jars filled with labeled sediments (Fig. 1). The plants and control jars were then returned to their original locations and allowed to grow for 6 to 10 weeks. We sampled control jars at intervals for measurement of available sediment P-specific activity, using an ion-exchange method (*5*). The available sediment P-specific activity did not remain constant with time but decreased at a rate of 2 to 4 percent per week during the growth period.

Upon harvesting (*6*), the plants were vigorously shaken with filtered lake water to dislodge as much periphyton as possible. The shoots and roots were then dried and analyzed separately for total P (*7*) and total ^{32}P activity (*8*). For each plant, the amount of P derived from the sediments was calculated from the following formula

$$\%S = \frac{\Sigma A \cdot 100}{P \cdot SA}$$

where $\%S$ is the percentage of total plant P coming from the sediments, ΣA is the total amount of ^{32}P activity found in the plant, P is the total plant P less the estimated initial P present in the young sprout (*9*), and SA is the mean available sediment P-specific activity during the growth period (*10*).

Tables 2 and 3 show that macrophytes growing in the mesotrophic part of Lake Memphremagog (Quebec-Vermont) obtained all their P from the sediments. The plants grown in the eutrophic part of the lake showed a mean uptake of 91.6 percent from the sediments. This last value underestimates the real sediment contribution since the plants growing at this particular site harbored a thick coating of periphyton that could not be completely removed upon harvesting, thus producing an unlabeled P contamination. The results obtained from Rivière-du-Sud are of particular interest since this site represents a natural upper extreme of water P availability. With a mean summer soluble reactive P (SRP) concentration of 167 μg/liter in the water, the sediments still provided at least 71.5 percent of the P requirement of the macrophytes.

These results show that in both oligotrophic and mildly eutrophic lakes, characterized by relatively high interstitial P concentrations in their sediments, the sediments constitute the only significant source of P to rooted macrophytes. Only in rarely encountered hypereutrophic waters is there significant P uptake from the water. Therefore, the relative contribution of water and sediments appears to be a function of their relative P availability.

The sediment P uptake values observed for the three species investigated in Rivière-du-Sud (Table 3) are very similar. This is surprising in view of the large morphological differences (such as the ratio of leaf area to biomass) between these species. This interspecific uniformity suggests that a simple predictive model of relative water P and sediment P contribution in P uptake for macrophytes could be readily constructed.

The overwhelming importance of root uptake in nature shows the importance of sediments in determining the extent of rooted macrophyte development. Long-

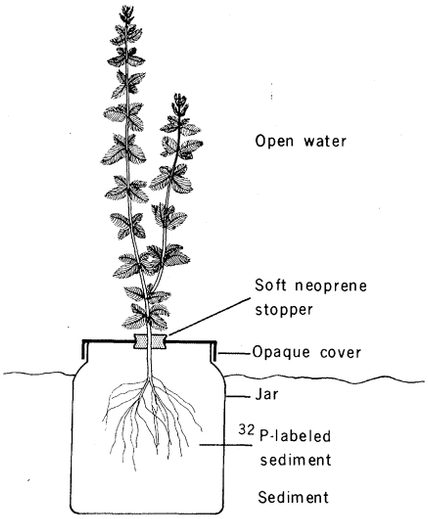

Fig. 1. Experimental apparatus used for growing macrophytes in situ.

term macrophyte control programs will thus need to focus primarily on the sediment compartment. Submergent macrophytes are very active sediment P recyclers and should be viewed as potential P pumps. However, the importance of these plants as pumps to their epiphytic cover and to the overlying waters will remain unclear until information on P excretion rates and on the fate of macrophyte P upon senescence in nature becomes available (*11*).

R. CARIGNAN*
J. KALFF

Department of Biology,
McGill University,
Montréal, Québec, Canada H3A 1B1

Table 3. Mean percentage uptake of P (± standard error) from the sediments for three macrophyte species grown under various conditions of water and sediment P availabilities. The number of replicates is given in parentheses.

Site	Percentage uptake from sediments		
	Myriophyllum spicatum	*Heteranthera dubia*	*Vallisneria americana*
Central Lake Memphremagog	99.4 ± 0.5 (13)	95.2 ± 3.2 (8)	103.1 ± 5.6 (9)
Southern Lake Memphremagog	93.2 (1)	95.7 ± 0.1 (2)	86.0 ± 2.0 (5)
Rivière-du-Sud			
Shoot	66.7 ± 1.3 (4)	68.7 ± 1.3 (6)	69.4 ± 2.8 (5)
Root	85.4 ± 2.1 (4)	84.8 ± 1.7 (6)	81.1 ± 2.7 (5)
Whole plant	70.0 ± 0.5 (4)	70.3 ± 1.2 (6)	74.2 ± 2.7 (5)

References and Notes

1. The term "macrophyte" refers here to the larger aquatic plants as distinct from the microscopic planktonic and benthic plants [C. D. Sculthorpe, *The Biology of Aquatic Vascular Plants* (Arnold, London, 1967)].
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3. The "specific activity" of plant or sediment P refers to the $^{32}P/^{31}P$ ratio expressed as ^{32}P activity (in counts per minute) per unit weight of ^{31}P (in micrograms).
4. The amount of ^{32}P added was adjusted to give an initial specific activity of approximately 1 μCi per milligram of available sediment P.
5. R. Carignan and J. Kalff, *J. Fish. Res. Board Can.* **36**, 1002 (1979). The available sediment P-specific activities measured in jars with and without plant were not significantly different. The specific activity of the available sediment P as measured by the ion-exchange method was identical to the specific activity of the interstitial SRP sampled by dialysis.
6. All species were harvested at or shortly after flowering time, except *Elodea canadensis* and *Myriophyllum spicatum* which did not flower at the study sites.
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8. The samples were counted in Aquasol (New England Nuclear) or by Cerenkov counting in water with a Beckman liquid scintillation system; the counts were corrected for the efficiency of Cerenkov counting by an internal standard method.

9. We estimated the initial P present in the young plants by regressing total P on fresh weight for a dozen similar plants collected in the same area; the resulting correlation coefficients (r^2) were greater than .90.
10. The mean available sediment P specific activity was calculated by integration, within the growth period, of the regression best fitting the observed specific activity versus time values. The best-fitting regressions were of the form: $y = \ln(t) + c$, where t is time and c is a constant; r^2 was always greater than .96. This method assumes a constant uptake rate of P with time.
11. R. Carignan and J. Kalff, in preparation. Our results for *Myriophyllum* show that, although macrophyte-excreted P is readily available to epiphytes and algae, excretion rates are very low.
12. Supported by grants from the Québec Department of Education, the Inland Water Directorate of Environment Canada to the Memphremagog Project, and predoctoral scholarships from the Direction Générale de l'Enseignement Supérieur du Québec and the National Sciences and Engineering Research Council of Canada to R.C. We thank Dr. D. Planas for providing SRP data for Rivière-du-Sud.
* Present address: Canada Center for Inland Waters, Burlington, Ontario, Canada L7R 4A6.

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Carbon Dioxide Sensitivity in Mosquitoes Infected with Sigma, Vesicular Stomatitis, and Other Rhabdoviruses

Abstract. *Carbon dioxide, usually an innocuous narcotic for insects, kills mosquitoes infected with rhabdoviruses. Such toxicity was originally observed in* Drosophila *harboring a hereditary virus, sigma, and has been considered unique to* Drosophila. *The new findings support the possibility that insects with piercing and sucking mouthparts harbor similar hereditary viruses and transmit some of them to vertebrates or plants.*

Approximately 40 years ago it was discovered that a certain laboratory strain of the fruitfly *Drosophila melanogaster* failed to recover as expected from the narcotic effect of CO_2 when returned to air (*1*). It soon was demonstrated that this "sensitivity" to CO_2 in the stock was inherited but, surprisingly, not in a Mendelian manner (*2*). Later it was shown that the CO_2 sensitivity could be transferred to normally "resistant" *D. melanogaster* by transplantation of organs or injection of hemolymph, but was not contagious in the usual sense (*3*). Eventually it was shown by filtration and other techniques that the CO_2 sensitivity was conferred by a replicating particle in the size range of a virus, which was named sigma. Electron microscopy revealed that sigma virus had the bullet-shaped morphology characteristic of rhabdoviruses (*4*). Sigma virus is not cytopathogenic for cell cultures, and viral particles are difficult to demonstrate by electron microscopy. Consequently, the only practical way to detect the virus is by assay in *D. melanogaster*. Transmissible CO_2 sensitivity has been found in wild *D. melanogaster* (*5*) as well as in other species of *Drosophila* (*6*), but never in any other kind of insect. The physiology of the CO_2 toxicity has not been explained, although it has been linked to replication of virus in the thoracic ganglia (*7*).

The recent demonstration of generation to generation (vertical) transmission by mosquitoes (*8*) and sandflies (*9*) of viruses affecting man and domestic animals has directed the attention of epidemiologists to the well-studied sigma-*Drosophila* relationship. Two aspects are of particular interest. The first is the mechanism that assures the survival of sigma virus in *D. melanogaster*. Since the virus is not always transmitted to 100 percent of the next generation, its persistence in nature (and in laboratory colonies) suggests that it confers some advantage on its insect host. The second is the ultra-rho form of sigma virus that is transmitted from generation to generation in *D. melanogaster* in an inapparent form (*10*). It is important to know if such masked forms occur among the viruses transmitted to vertebrates and plants by insects when evaluating the possible role of vertical transmission in their epidemiology.

When injected into certain species of mosquitoes, sigma virus was found to replicate but CO_2 sensitivity was not induced nor was vertical transmission of the virus observed (*11*). (Replication in mosquitoes was demonstrated by assay of their triturated bodies in *D. melanogaster*.) In the work reported here the objective was to compare the vertical transmission of sigma virus with that of viruses transmitted by insects to vertebrates in a common insect host. In the course of the work it was found that sigma virus not only replicated in the mosquito species chosen for study but also rendered them lethally sensitive to CO_2. Mosquitoes then were infected with vesicular stomatitis and other rhabdoviruses since such viruses were known to confer CO_2 sensitivity on *D. melanogaster* (*12*).

The sigma virus was obtained from a laboratory colony of *D. melanogaster* started from a single fertilized female captured in nature in Honolulu, Hawaii, in 1978. The descendants of this fly were uniformly sensitive to CO_2 (and not to N_2) in each generation tested. Their longevity and fertility were not different from those of the CO_2-insensitive *D. melanogaster* captured at the same time. Their CO_2 sensitivity could be transmitted to insensitive *D. melanogaster* by injection. Thus they met the criteria of a line "stabilized" for sigma virus (*6*). A virus-containing suspension was prepared by triturating 1.96 g of pooled flies from the sensitive colony in 10.0 ml of heated (56°C for 30 minutes) bovine serum. The suspension was centrifuged to remove large particulate matter, filtered through a Millipore filter with an average pore diameter of 450 nm, and stored in equal portions at −70°C. Filtered material was inoculated intrathoracically (*13*) in 0.17-μl amounts into *Toxorhynchites amboinensis* mosquitoes from a laboratory colony. These mosquitoes were held at 20°C and different groups were tested at 7, 14, 21, and 28 days after inoculation for CO_2 sensitivity. The mosquitoes were exposed to pure CO_2 for 15 minutes at a temperature of 13° to 15°C and then returned to air at room temperature. The number of mosquitoes that failed to recover was determined several hours later and again the next day. In this experiment the numbers of mosquitoes that were CO_2-sensitive at the four time intervals after inoculation were 3 of 12, 23 of 26, 22 of 24, and 18 of 18, respectively. All of the uninoculated control mosquitoes for the same time periods recovered normally. The sigma virus suspension could be diluted as much as 1000-fold and still induce CO_2 sensitivity in at least one-half of the *T. amboinensis* inoculated. Sensitivity to CO_2 could also be induced by sigma in *Culex quinquefasciatus* mosquitoes from a laboratory colony, but not in colonized *Aedes albopictus* mosquitoes. Both *T. amboinensis* and *C. quinquefasciatus*, when inoculated with the same amount of sigma virus, recovered normally from narcosis with N_2.

The same three species of mosquitoes were inoculated with a variety of other rhabdoviruses and three other mosquito-borne viruses to determine whether CO_2 sensitivity would ensue. Table 1 shows that all rhabdoviruses tested, with the exception of VSV-Indiana (vesicular stomatitis virus), induced such sensitivity in one or more mosquito species. For the tests summarized in Table 1 the mosquitoes were held for 7 or 8 days at 32°C after inoculation. This temperature is

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