

Response of *Spirodela oligorrhiza* to Phosphorus Deficiency

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ABSTRACT

The duckweed *Spirodela oligorrhiza*, growing in sterile defined nutrient media, was used to study some responses of plants to phosphorus deficiency. On a phosphate-deficient medium, growth of *Spirodela* soon slowed and eventually ceased. Older leaves became chlorotic, but newly formed leaves were dark green and contained much anthocyanin. The photosynthesis rate fell gradually, roots elongated, and chloroplasts became filled with starch.

Nitrogen metabolism was not markedly affected: the total protein content changed only slightly, and, although levels of glutamine and asparagine increased, the concentrations of the other amino acids remained constant. The effects of phosphorus deficiency on *Spirodela* are discussed in relation to those found in other higher plants.

The importance of phosphorus in plant nutrition has been long known, and the effects of phosphorus deficiency in plants have been frequently studied. Morphological and biochemical symptoms of phosphorus deficiency in crop plants have been reported (14): a number of workers have explained these symptoms in terms of changes in cell metabolism. Much of this past work on the biochemistry of phosphorus deficiency has suffered from the relatively insensitive techniques then available, and it is open to criticism on the grounds that plants were grown in nonsterile and usually undefined culture media.

Recently, *Spirodela oligorrhiza*, a small free-floating monocotyledonous water plant, has been used for detailed examination of plant nutrient responses (1, 2, 4, 5). The present study compares the response of this plant to phosphorus deficiency with those reported for other higher plants.

MATERIALS AND METHODS

Culture of Plants. *S. oligorrhiza* (Kurz) Hegelm. was obtained from Dr. K. V. Thimann in 1960 and has since been maintained in axenic culture at Fruit Research Division, D.S.I.R., Auckland (4). Normal plants (control plants) were grown on a complete mineral nutrient medium, and phosphorus-deficient plants were grown on medium from which phosphate had been omitted (phosphate-deficient medium) (1, 2). Nitrogen-deficient plants were grown on medium lacking $(\text{NH}_4)_2\text{SO}_4$ (4, 5). The pH of media was kept at 7.2 by including about 50 mg of solid sterile CaCO_3 in each flask. Plants were grown in 50-ml conical flasks on 20 ml of medium, at 24 C in continuous "daylight fluorescent" light, about 200 ft-c. To increase the growth rate and make it more uniform, glucose (1%) was routinely added to mineral nutrient media. The doubling time was then 1.9 days instead of

2.4 to 3.2 days in the absence of glucose. Further details of inoculation and culture are given in References 1 and 4.

Measurement of Growth. Three convenient criteria were used to measure growth. The frond number per flask was counted; all fronds extending beyond the margin of each parent frond were scored. The fresh weight per flask was measured: flasks were harvested, and plants were washed, blotted lightly, and weighed. The dry weight per flask was obtained, by drying each harvested sample for 24 hr at 110 C and then weighing. In all cases, the day of inoculation is referred to as day 0, and each successive day as day 1, day 2, etc.

Photosynthesis. Photosynthesis rates were measured manometrically (13). To minimize possible shock effects, plants, 80 mg fresh weight, were put into Warburg flasks in 16 ml of growth medium, and then 1.2 ml of $\text{KHCO}_3/\text{K}_2\text{CO}_3$ buffer (0.4 M, pH 8.8) were added to provide an adequate CO_2 reserve (final pH 8.2). Rates were measured for 1 hr at 25 C under daylight fluorescent light, approximately 200 ft-c.

Starch Content. *Spirodela* was harvested, washed, and freeze-dried. Starch content of the tissue was measured by an extraction, solubilization, and anthrone measurement procedure similar to that described in Reference 10.

Protein Pattern. Protein patterns of extracts from *Spirodela* were studied by acrylamide gel electrophoresis (12).

Amino Acid Levels. Soluble amino acids in *Spirodela* were extracted, separated by thin layer electrophoresis and chromatography, and then estimated colorimetrically with ninhydrin (3).

RESULTS

Growth of Phosphorus-deficient *Spirodela*. Growth decreased when plants were transferred to a phosphorus-deficient medium (Fig. 1a), and finally stopped by day 25, when the frond number had risen from 20 to between 200 and 250. The dry weight per frond, after decreasing slightly, increased progressively up to day 30 (Fig. 1b). By counting a large number of fronds at short time intervals, it was shown that this growth response to phosphorus deficiency was almost immediate (Fig. 2). Less growth was found in deficient plants within 6 hr of the transfer, and by 24 hr, the difference was significant at the 99% level.

Visible Symptoms of Phosphorus Deficiency in *Spirodela*. *Spirodela* grown on control medium (Fig. 3A) had fronds of uniform size, 3 to 5 mm long, joined together in colonies of three to eight fronds. Each frond had three short green roots with conspicuous root caps. When *Spirodela* was transferred to phosphate-deficient medium, there was a consistent sequence of changes. From day 0 to day 10, there was little external change in the fronds. By day 15, the older fronds were starting to become yellow, and the roots had elongated markedly. By day 25, the oldest fronds were completely yellow, and newly formed fronds were dark green, with heavy development of anthocyanin on the lower surface and margins. By day 35 (Fig. 3B) the oldest fronds

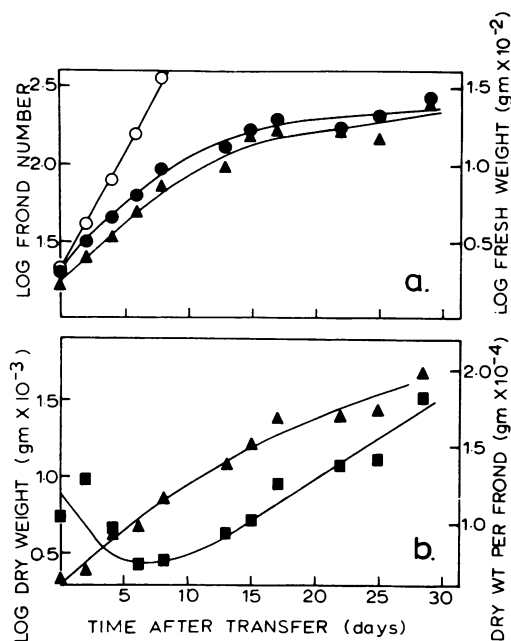


FIG. 1. Growth of phosphorus-deficient *Spirodela*. Flasks of phosphate-deficient medium were inoculated with control plants. a: O: Frond number of control cultures; ●: frond number of deficient cultures; ▲: fresh weight of deficient cultures, per flask; b: Dry weight of deficient cultures, ▲: per flask; and ■: per frond.

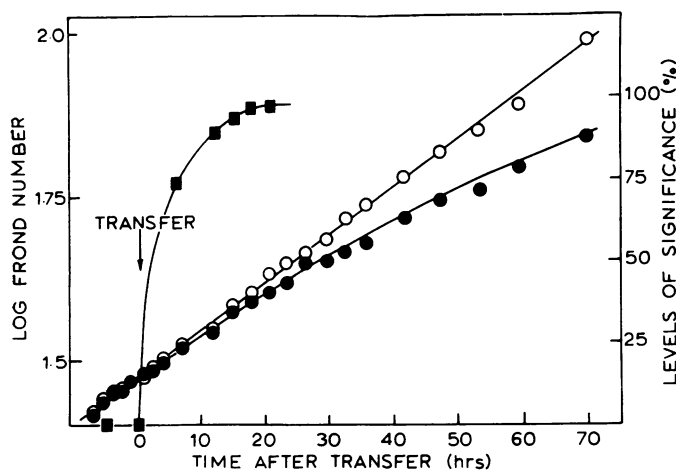


FIG. 2. Growth response of *Spirodela* to phosphorus deficiency. Large numbers of plants were transferred to phosphate-deficient medium and counted at short time intervals to determine growth rate (●). Control plants (○) were transferred to new control medium at the same time. The significance of the difference in frond numbers (■) was determined. Frond number per flask plotted: 20 flasks per treatment.

in the culture had died, and most fronds were yellowed. The youngest (and smallest) fronds were still dark green. If glucose was omitted from the culture medium, the onset of the symptoms of phosphorus deficiency was slower, in keeping with the slower growth rate, but the symptoms were essentially the same.

Chloroplasts of Phosphorus-deficient *Spirodela*. Chloroplast morphology changed markedly during growth of *Spirodela* on phosphate-deficient medium (Fig. 4). Control fronds had normal discoid chloroplasts, each containing one to three small starch grains (Fig. 4A). Partially deficient plants had chloroplasts that contained enlarged starch grains which gave a "lumpy" ap-

pearance to the chloroplast (Fig. 4B). The dark green fronds of fully deficient plants had large ovoid, starch-filled chloroplasts (Fig. 4C). In yellowed fronds, the chloroplasts had become disorganized and aggregated into a yellow amorphous mass (Fig. 4D) while many starch grains were free in the cells. Marinos (9) noted that the formation of starch grains in chloroplasts was a specific symptom of phosphorus deficiency in barley: nitrogen, potassium, sulfur, and magnesium deficiencies, though affecting chloroplast structure, did not cause a starch accumulation.

Photosynthesis Rate of Phosphorus-deficient *Spirodela*. Though plants were grown in a relatively low light intensity, with glucose present in the medium, the photosynthesis rate shown by control *Spirodela* was comparable with that of other leaf tissues (*Spirodela*, 2.8 mg of CO_2 fixed per g fresh wt per hr, corresponding to about $0.6 \text{ g/m}^2 \cdot \text{hr}$; compare sunflower, apple, sugarcane, corn, *Cucurbita pepo*, in high CO_2 concentration and high light intensity, $0.3\text{--}5 \text{ g/m}^2 \cdot \text{hr}$ [11]). During growth on phosphate-deficient medium, the photosynthesis rate of *Spirodela* fell only slowly (Fig. 5). By day 10, phosphorus-deficient plants still had a photosynthesis rate 80% that of control plants. In contrast, nitrogen deficiency reduced the photosynthesis rate to less than 50% the control value.

Starch Content of Phosphorus-deficient *Spirodela*. After an initial drop, day 0 to day 5, the starch content of *Spirodela* increased, until by day 30 75% of the tissue dry weight was due to starch (Fig. 6). If glucose was not included in the culture medium, starch content of phosphorus-deficient plants increased more slowly, to 30% of the tissue dry weight.

Amino Acids in Phosphorus-deficient *Spirodela*. Phosphorus-deficient *Spirodela* plants always had a high proportion of "storage" amino acids present in the soluble amino acid pool (glutamine, 10.1; arginine, 1.5; and asparagine, 4.0 $\mu\text{moles/g}$ fresh wt). These levels were almost identical to those (glutamine, 9.4; arginine, 1.6; and asparagine, 2.0 $\mu\text{moles/g}$ fresh wt) found in control plants which had been supplied with extra inorganic nitrogen. Control plants which had had the normal nitrogen supply (4) contained much less storage amino acid (glutamine, 0.1; arginine, 0.4; and asparagine, 0.3 $\mu\text{mole/g}$ fresh wt), even though the growth rate was not affected by the lower nitrogen supply. Most other soluble amino acids were unaffected by the various growth conditions (e.g., glutamic acid, 0.6–0.8; aspartic acid, 0.8–1.4 $\mu\text{moles/g}$ fresh wt).

Protein Patterns from Phosphorus-deficient *Spirodela*. The protein pattern, as revealed by acrylamide gel electrophoresis, was virtually unaffected by phosphorus deficiency (Fig. 7). Differences were in levels, rather than in the presence or absence of protein bands. Though the yellow, and oldest, phosphorus-deficient fronds contained much less protein than normal fronds, the total protein content of phosphorus-deficient plants was similar to that of control plants.

DISCUSSION

The earliest and most striking symptom of phosphorus deficiency in *Spirodela* was slowing of the growth rate. It was consistently found that a significant decrease could be detected within 6 hr of transferring plants to phosphate-deficient medium. It has been suggested elsewhere (2) that this response is a rapid one because most of the phosphate in the cell is segregated in the vacuole and is not readily accessible for growth. This limiting of growth rate could be responsible for several of the observed phenomena of phosphorus deficiency. For example, starch accumulation could result from a restriction of growth (i.e., utilization) without a concomitant decrease in photosynthesis (i.e., production). The small decrease in photosynthesis rate (to 80%) is consistent with the small decrease in phosphate ester turnover (to 75% [2]), as a result of phosphorus deficiency. It can be argued that in these ex-

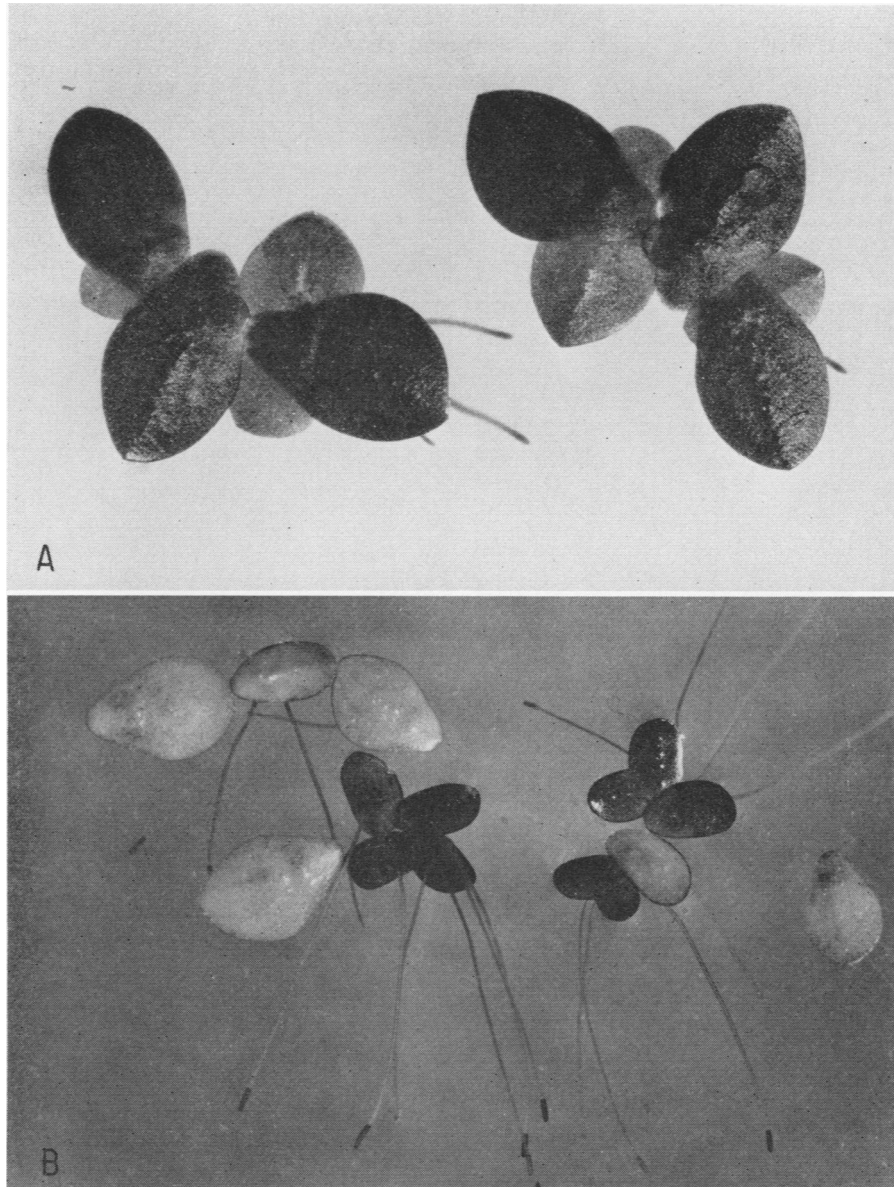


FIG. 3. Visual symptoms of phosphorus deficiency in *Spirodela*. A: Control plants; B: plants grown for 35 days on phosphate-deficient medium.

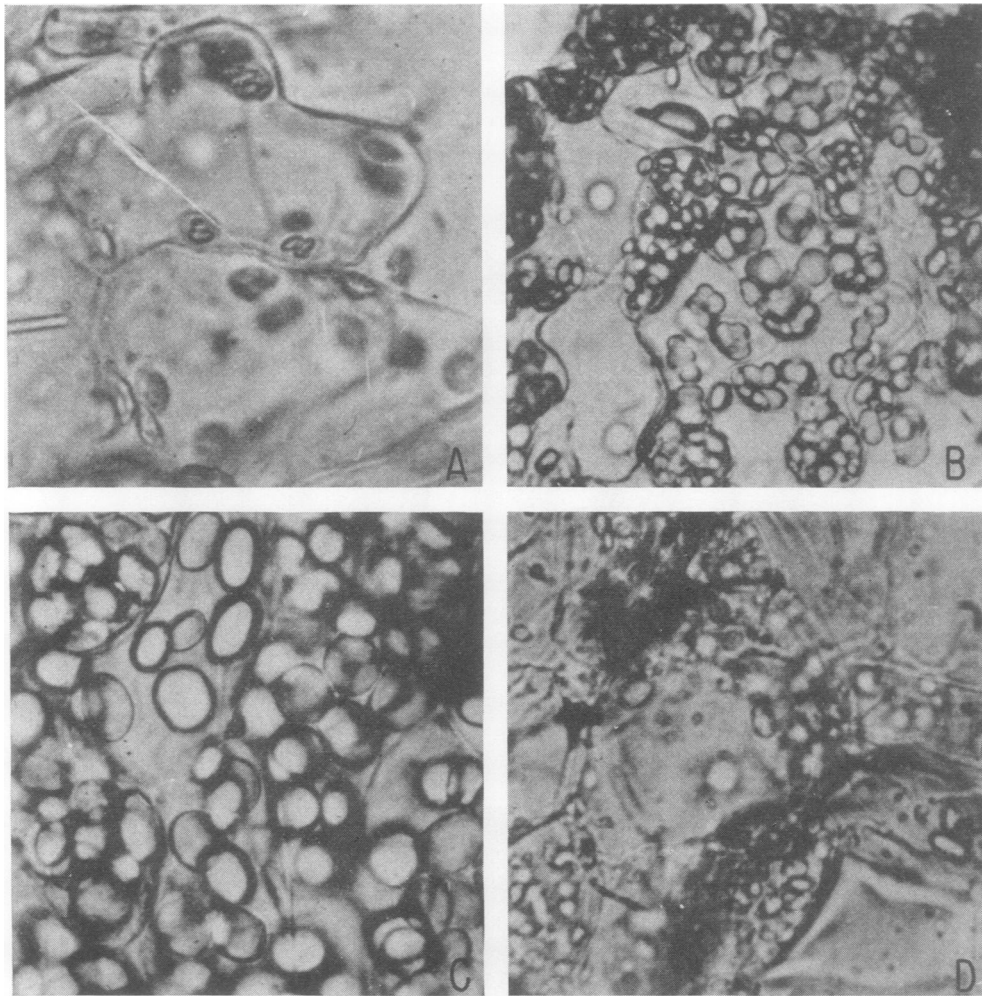


FIG. 4. Chloroplasts of phosphorus-deficient *Spirodela*. Fresh, hand-cut sections were prepared from the aerenchyma of *Spirodela* at different stages of phosphorus deficiency. Photographs were made of representative cells. $\times 500$. A: Control; B: deficient (day 15); C: cells from young, dark-green fronds of a deficient plant (day 25); D: cells from old, yellowed fronds of a deficient plant (day 25).

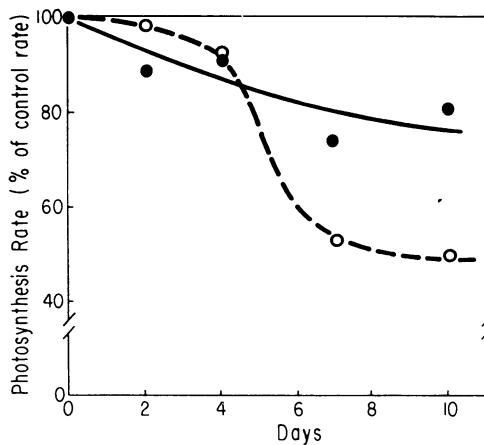


FIG. 5. Photosynthesis rates of phosphorus-deficient and nitrogen-deficient *Spirodela*, as a percentage of control rates. \bullet : Phosphorus-deficient; \circ : nitrogen-deficient. Values are means of three determinations. Absolute control rates, 1.1 to 1.4 ml O_2 /g fresh weight per hr, corresponding to about 0.6 g of CO_2 fixed/ $m^2 \cdot hr$.

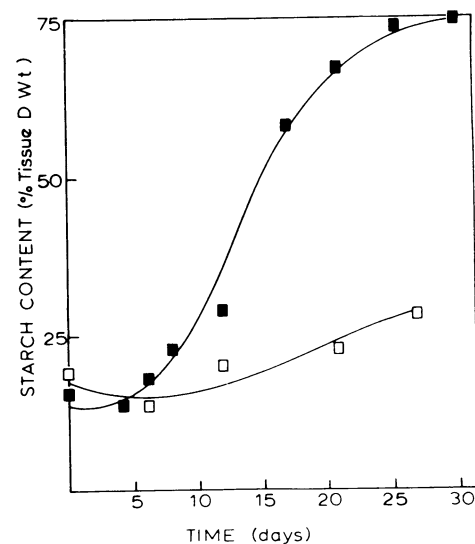


FIG. 6. Starch content of phosphorus-deficient *Spirodela*. The starch content of *Spirodela* grown in phosphate-deficient medium in the presence (\blacksquare) and absence (\square) of glucose was determined at intervals.

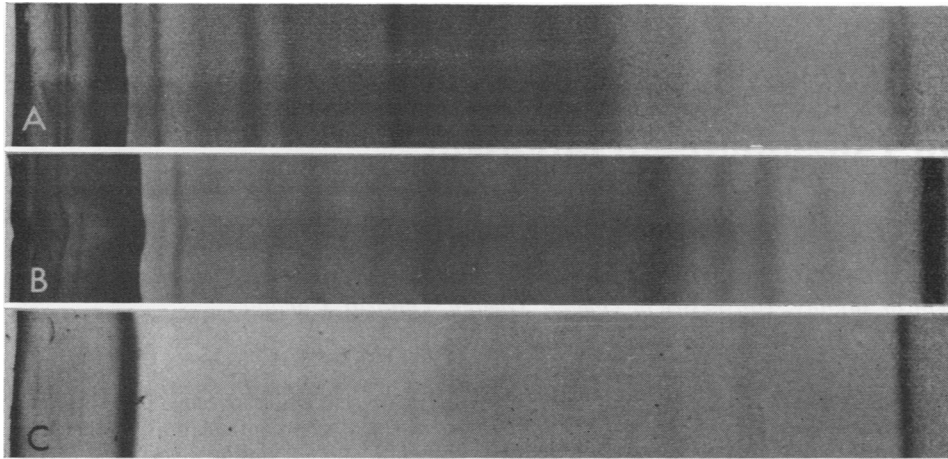


FIG. 7. Protein patterns from phosphorus-deficient *Spirodela*. Proteins in 0.02-ml aliquots of homogenates of *Spirodela* were separated by polyacrylamide gel electrophoresis. A: Control plants; B: deficient plants (day 30); C: yellowed fronds of deficient plants (day 30).

periments, since light intensity was low and glucose was supplied, photosynthesis was unaffected by phosphorus deficiency because it was already limited or inhibited in control plants. However, under the same conditions, nitrogen deficiency did inhibit photosynthesis. Also, the absolute rates of photosynthesis were high, particularly considering the low light intensity, and were concordant with rates of growth. By day 10, phosphorus-deficient plants were increasing in weight 1.3 mg dry weight per g fresh weight per hr: the measured photosynthesis rate corresponded to 1.4 mg of carbohydrate per g fresh weight per hr. Thus, photosynthesis alone is sufficient to account for the increase in dry weight of the deficient fronds, even when grown in glucose. It would be of interest to study the enzymes of starch synthesis, and perhaps to use ^{14}C -glucose in the external medium to look for changed paths of utilization. The results of Marinos (9) and the behavior of phosphorus-deficient *Spirodela* in absence of added glucose (Fig. 6) make it clear that starch accumulation may be a characteristic feature of phosphorus deficiency.

In the same way, another characteristic feature of phosphorus deficiency, accumulation of soluble nitrogen reserves, might occur through restricted utilization (*i.e.*, protein synthesis) without a corresponding decrease in uptake of nitrogen from the external medium. Several of the earlier workers (*e.g.*, Refs. 6, 8) have reported that soluble nitrogen compounds, particularly amides and nitrate, accumulate during phosphorus deficiency. It is suggestive that in *Spirodela* the amide composition of phosphorus-deficient plants is exactly like that of control plants which had been supplied with a high concentration of nitrogen in the external medium. As the nitrogen concentration of the control medium was lowered, the proportion of nitrogen in amides decreased. In rapidly growing large plants with a rather low nitrogen supply, uptake of nitrogen from the external environment could normally be limiting. By imposing a slower growth rate on the plant, phosphorus deficiency could result in the nitrogen supply becoming nonlimiting, resulting in increased soluble nitrogen in the plant.

The longer term effects of phosphorus deficiency in *Spirodela* were very like those normally associated with senescence. The old fronds became prematurely yellowed, the chloroplasts degen-

erated, and protein levels fell, probably as a result of export of reserves (7). The youngest fronds were dark green, though the chloroplasts were distorted by the large starch grains, and the amount and pattern of their proteins were like those of normal fronds. It could be that while many of the early effects of phosphorus deficiency in *Spirodela* are associated with rapid decrease in growth rate of the deficient plants, the longer term effects may be associated with an accelerated senescence. The morphological changes in the chloroplasts recorded here and elsewhere (9) can be interpreted as a type of senescent breakdown. Kinins, which are known to retard senescence in leaves, also markedly retard the frond yellowing caused by phosphorus deficiency in *Spirodela*.

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