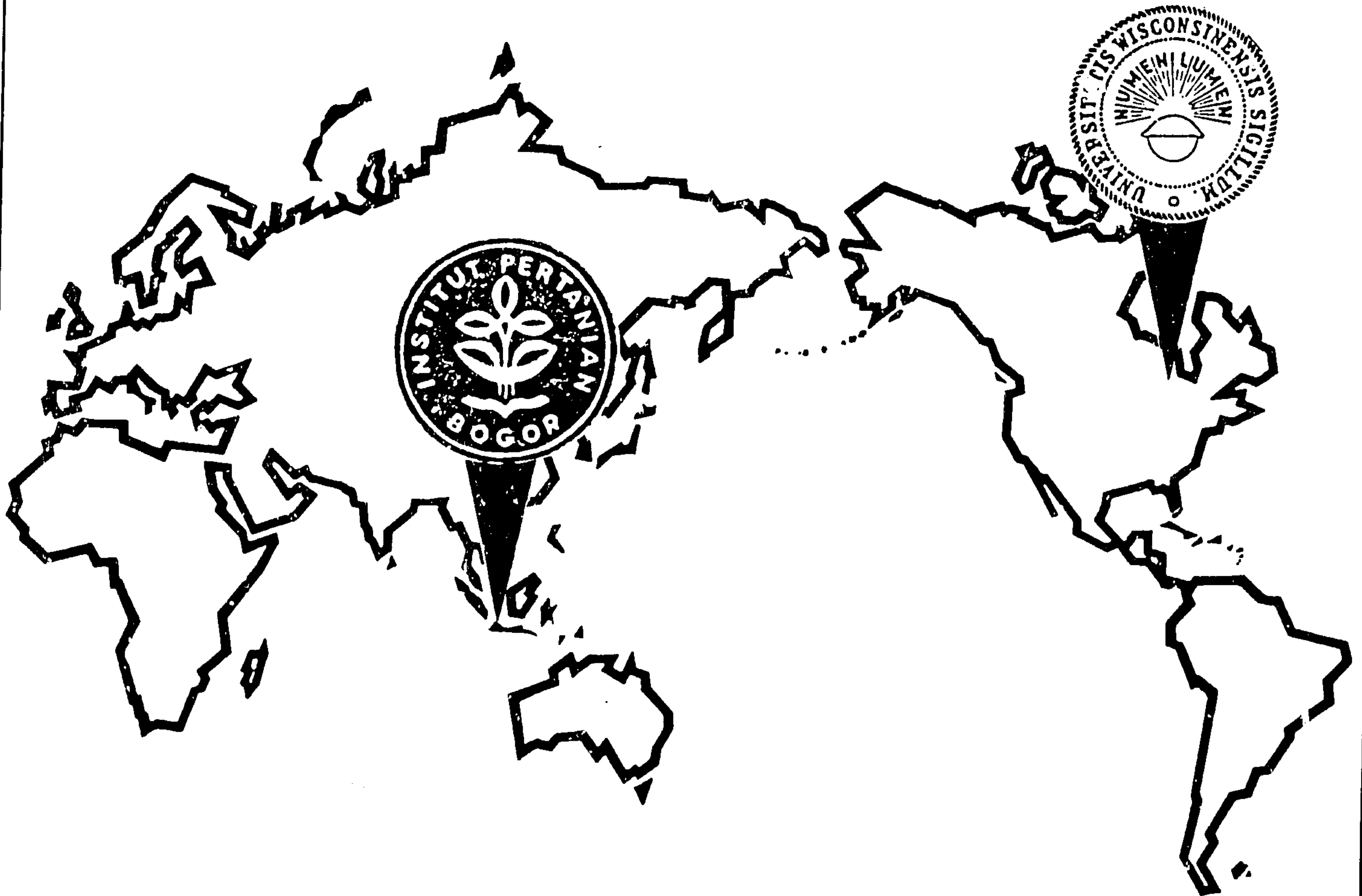
PN-AAU-165

Institut Pertanian Bogor * University of Wisconsin

GRADUATE EDUCATION PROJECT



Aid Project 497-0290

Report No. 23

SCHRADER

REPORT OF SHORT-TERM CONSULTANT

on

CROP PHYSIOLOGY

to

INSTITUT PERTANIAN BOGOR (IPB)
Bogor, Indonesia

by

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September 11 - October 6, 1983

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Duration of Consultant's Visit

Dr. L. E. Schrader, Professor of Plant Physiology in the Department of Agronomy at the University of Wisconsin-Madison, arrived in Bogor on Sunday, September 11, 1983, and departed on Thursday, October 6, 1983.

Objectives of Visit and Plan of Work

A major purpose of this visit was to serve as a visiting professor to teach part of Agronomy 535, entitled Fisiologi Tanaman, a graduate level course. Twelve students enrolled in the course, and several other students and faculty audited the course.

A second objective was to consult with both students and faculty about research and to visit several experimental fields and stations to learn more about crop production and problems facing agriculture in Indonesia.

A third objective was to present research seminars at several institutions throughout Indonesia and to discuss research topics of interest with those groups.

The fourth objective was to evaluate and make suggestions for improvement of graduate training in Crop Physiology at IPB. These recommendations include new courses, facilities and equipment, procedures and regulations used in graduate training, as well as some suggestions concerning the training sessions to be held at IPB in a few months to upgrade the capabilities of teachers in crop physiology at other institutions in Indonesia.

Summary of Accomplishments and Findings

A summary of the consultant's itinerary is presented in Appendix A.

Objective 1

Twelve students enrolled in Agronomi 535, Fisiologi Tanaman, and several students and faculty audited the lectures informally. Seven lectures of approximately two and one-half hours' duration were presented, and two examinations were given. An outline of the lectures is presented in Appendix B.

Objective 2

A partial listing of the students and faculty with whom the consultant visited about research is included in Appendix A. Several others visited briefly. The experimental farms visited are listed in the itinerary also.

Objective 3

A formal seminar concerning soybean and maize production in the U. S., physiological limitations on soybean production, and use of *Rhizobium japonicum* mutants to increase N_2 fixation in soybeans was presented at Gadjah Mada University, BATAN in Jakarta, Central Research Institute for Food Crops in Bogor, Padjadjaran University in Bandung, and at Udayana University in Denpasar. One to two hours of discussion followed each seminar.

Objective 4

With respect to the graduate program at IPB, the S_2 ("Magister" program equivalent to M.S.) program was started in 1975 and the S_3 ("Doktor" program or Ph.D.) program was started in 1978. Although Indonesia has 45 state universities, only 23 have programs in agriculture. About 12 percent of the undergraduate students in agriculture are trained at IPB, but 82 percent of the graduate students attend IPB.

Because IPB has the major responsibility for graduate education, the Administration needs to continue to be innovative and aggressive in attracting the top students from each province for graduate training. Furthermore, it is recommended that the faculty continue to upgrade their own training so that quality and breadth of training offered to students will continue to improve. It is noted that 71 of 256 staff in the Faculty of Agriculture had the equivalent of the S_3 degree in 1982. Additional personnel should be encouraged to complete requirements for the S_3 degree or Ph.D., and/or additional staff with a doctorate should be added as resources become available.

At the request of Dr. Edi Guhardja, Dean of the Graduate School, several aspects of graduate training at IPB have been examined. The following suggestions are submitted for consideration in order to further strengthen graduate training at IPB. Many of this consultant's comments are based on limited observations and discussions with personnel in Agronomy and may not accurately reflect the situation in all departments.

Agronomy currently requires S_2 and S_3 students to submit a thesis proposal to their committee. The candidate defends the proposal before his/her committee and then submits a revised proposal for approval by the committee and the Graduate School. This procedure should be adopted by all departments to insure that the candidate has chosen a suitable research problem and that he/she has outlined adequate procedures to accomplish his/her objectives. Submission of a budget will aid in assessing whether adequate resources are available for completion of the proposed research. Defense of the proposal by the candidate is an excellent learning experience for the candidate. It provides the committee members with an opportunity to examine and criticize the objectives and approach and make constructive suggestions for improving the research before the candidate proceeds too far.

This consultant strongly recommends that S_3 candidates be required to pass a preliminary examination after completion of the required course work. This examination should be used to assess whether the candidate has learned the important principles of his/her discipline and whether he/she is able to integrate the knowledge and apply it in solving research problems and/or answering questions. Currently, there seems to be considerable variation in the type of examination required; and in some cases, no preliminary examination is required of S_3 candidates. This requirement for a preliminary examination should be standardized for all graduate students in S_3 programs. This consultant recommends that an oral examination, rather than a written examination, be administered by the candidate's committee. The committee should be comprised of at

least five faculty members with at least two from outside the department in which the candidate is majoring.

There are several advantages of an oral examination as compared to a written examination. Oral examinations are more flexible and can be tailored to fit each student individually; whereas, written examinations will, of necessity, be more general if administered to several candidates at a time. Written exams require a great deal more faculty time for preparation and grading of the exam. In an oral exam, committee members often perceive a weakness of the candidate and can pursue that perceived area of weakness with additional questions; whereas, a written examination offers no opportunity to ask additional questions or seek clarification of a vague answer. One advantage of the written examination is that all candidates taking the exam at a particular time are asked the same questions, so uniformity is assured. In my opinion, written examinations of a general nature could be used as a qualifying examination early in a candidate's program to ascertain if the candidate has the necessary background to become an S_3 degree candidate. However, if high standards for admittance are maintained by each department, this qualifying exam does not seem essential.

A further recommendation is that all S_3 candidates be required to present a progress report on their research to their committee at the time they have completed about one-half of their thesis research. This informal session requires the candidate to assemble his/her research results and to present them. It permits the candidate to discuss problems encountered and to solicit counsel from committee members about

techniques and other aspects of the research. The progress report permits committee members to assess the candidate's progress and the quality and quantity of research accomplishments. All too often, committee members are not aware of problems until the thesis has been drafted and submitted for review. By then, it is too late for the candidate to do much to overcome any problems or deficiencies; whereas, earlier consultation may have permitted additional research or a better approach.

It is recommended that all S_3 candidates be required to present an open seminar to faculty and students upon completion of their dissertations. This open seminar could immediately precede the final examination by the committee or could be scheduled separately. This presentation provides another opportunity for the candidate to gain experience in making presentations before a group and also informs faculty and students of the research results of the candidate. It is further recommended that S_3 candidates write their dissertations in the form of research manuscripts, and that they be strongly encouraged to publish their results in journals or bulletins. Writing manuscripts rather than a more conventional, formal thesis will provide the candidates with valuable experience and training in writing for publication. Prompt publication of thesis results will make the information available to colleagues and scientists at other locations.

With respect to approval of new courses, a plan should be implemented by the Graduate School to insure that course offerings are coordinated throughout IPB to avoid unnecessary duplication and to insure that adequate courses are available to provide students with strong training

in each discipline. It is my understanding that an Academic Committee (with a representative from each department) exists for the purpose of discussing new courses and making recommendations to the Associate Dean I. The Associate Deans present such recommendations to an Academic Committee of IPB headed by Vice Rector I. Although this plan has been proposed, it needs implementation to assure that course offerings are fulfilling the needs of students, that minimal overlap or duplication occurs in various courses, and that a maximal number of courses are offered with the limited resources that are available. Some additional comments on coordination of Plant Science courses with those in Biology appear later in this report. Consideration should also be given to better coordination between courses for S_1 and S_2 candidates. That is, some advanced S_1 courses may be suitable for graduate credit for students transferring to IPB from other institutions or for other S_2 students who did not acquire training in a certain area during their S_1 training.

It came to my attention that some instructors do _not_ return examinations to students promptly after an examination. This situation should be corrected as students need to see the corrected examinations promptly so that they can ascertain what they missed or did not understand of the material presented. A key listing correct answers should be posted by the instructor after an exam, or the instructor should discuss the exam in class. Examinations should be a part of students' learning processes and, therefore, need to be returned promptly for students' use.

This consultant recommends that all courses be evaluated by students near the end of each course. An example of a short evaluation form is appended as Appendix C. The evaluations should be collected by a person other than the instructor and submitted to the department head who will retain the evaluations until after student grades are posted. The department head should examine the evaluations before returning them to the faculty member. Although all evaluation systems have weaknesses, student evaluations have been shown to be one of the most effective means of evaluating teaching effectiveness and quality of instruction. These evaluations provide the administration with a means of evaluating faculty teaching performance and should help teachers improve their course content and the presentation of lectures. It is suggested that outstanding teaching be recognized by presentation of a certificate and/or cash award to a limited number of outstanding instructors each year.

This consultant attempted to assess the extent to which important aspects of crop or plant physiology and biochemistry are covered in courses presently offered students at IPB. This task was difficult because course outlines or syllabi were not available for my examination. From discussions with Drs. Hari Suseno, Soleh Solahuddin, and others, I perceived that additional courses are needed to provide more advanced training to S_3 students.

The following is a summary of courses presently offered that are relevant to plant physiology. Currently BOT 531, Metabolisme Tumbuhan, is the basic plant metabolism course. BOT 534, Fisiologi Tumbuhan Lanjutan, deals with host-parasite relations, growth and development,

mineral nutrition, and post-harvest physiology. AGR 535, Fisiologi Tanaman, is a crop physiology course covering special physiological problems in major crops, ecophysiology of crops, stress physiology, source-sink relations, and xylem and phloem translocation. Another course (AGR 539) on plant-nutrient-water relations is being offered this year for the first time. Factors limiting and enhancing nutrient flow to plants are emphasized. Another course on micro-propagation of plants is being planned by Drs. Winata and Wattimena in Agronomy. A course on plant growth regulators is also being considered.

A visit with Dr. Anwar Nur revealed that he presently teaches a course emphasizing chromatographic techniques. His laboratory is well equipped with a gas chromatograph, amino acid analyzer, atomic absorption spectrometer, a high performance liquid chromatography, spectrophotometer, and a power supply for electrophoresis. An excellent series of laboratory experiments are offered in this course, but additional techniques should be taught to S_3 students. Additional experience should be provided in immunochemical techniques, as antibodies provide a powerful tool for several lines of Plant Science research. Dr. Nur has a power supply for electrophoresis already, so not much would be required to set up an immunochemical experiment. Other important areas include enzymology (purification and assay), photosynthesis and respiration measurements, protoplast and/or cell isolation from plants, centrifugation, fluorimetry, and tissue or cell culture.

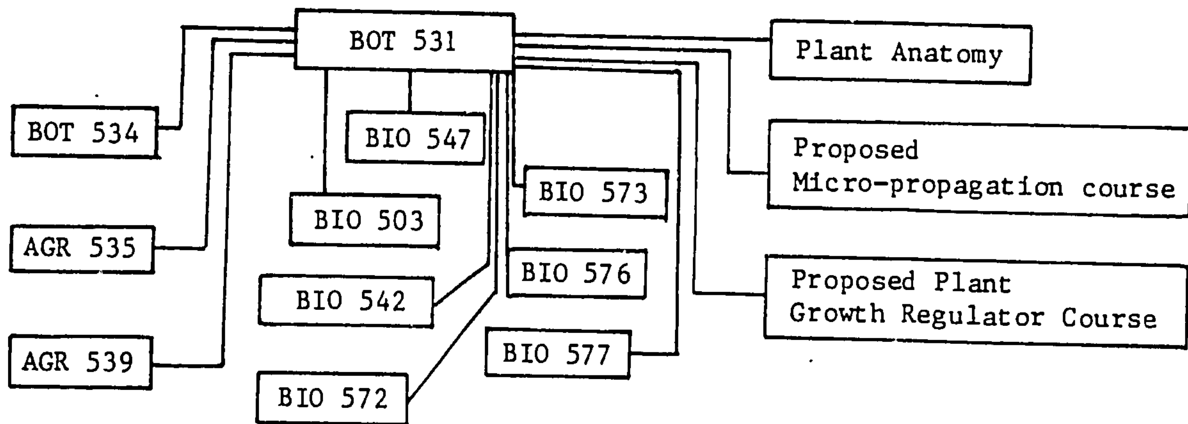
There are a large number of courses listed in Biology that could be useful to S_3 students in Plant Science. However, it appears as if these

courses have been developed and approved without adequate consultation between Biology and Plant Science. Thus, the courses are not presently oriented toward plants; and few, if any, graduate students from Plant Science are enrolling in the Biology courses. Consequently, the enrollment in some of the Biology courses has been too limited at times to make it feasible to offer the courses. This consultant readily acknowledges that many of the basic principles of animal and microbial biochemistry and metabolism apply to plants, so current courses in Biology should be useful to Plant Science students; and these students should be encouraged, if not required, to enroll in some of these courses. However, the teachers in Biology need to recognize that certain processes are unique to plants. For example, photosynthesis, nitrate reduction, sulfate reduction, and biological nitrogen fixation do not occur in animals. Growth hormones for plants are quite different from those in animals. Thus, present Biology courses should be revised to include these topics, or an additional course(s) should be added to adequately cover these important topics in Plant Science in more depth.

Based on course descriptions in the Graduate Catalog, the following courses appear to be of importance to students majoring in plant physiology or biochemistry.

BIO 503, Metabolism Intermedier; BOT 542, Teknik Radioisotop; BIO 577, Teknik Penelitian Biokimia; BIO 572, Bioenergetika; BIO 573, Regulasi Metabolisme; BIO 576, Metabolisme Sekunder; BIO 547, Fisiologi Sel; and possibly BIO 773, Biologi Molekuler and BIO 502, Bio-teknik Laboratorium.

To summarize these course offerings, the following schematic is shown to emphasize that BOT 531 should serve as a core course for all students in physiology to obtain the basics of plant physiology and metabolism. The other courses should serve as elective courses to provide students with more advanced training.



The present coverage of mineral nutrition in BIO 534 and AGR 535 could be expanded in a separate course on mineral nutrition and soil fertility. Plant biochemistry needs strengthening, as noted above; but it should be done in cooperation with Biology faculty to avoid overlap and duplication of effort. A graduate level course in plant anatomy should be added as soon as possible, as a solid understanding of plant anatomy is required to understand structure-function relationships.

Teaching laboratories are generally inadequate, with the exception of Dr. Nur's laboratory. Hopefully, the situation will improve with the move to Darmaga, but high priority should be given to providing better laboratories equipped with sufficient instrumentation to teach essential laboratory techniques to students.

High priority should be given to strengthening the basic natural and physical sciences at IPB. Based on my limited exposure to S_2 and S_3 students in AGR 535 and to graduate students sent to UW-Madison for graduate training, it is perceived that most of the students are inadequately trained in principles of inorganic and organic chemistry, biochemistry, and physics. These courses are so basic to graduate training in plant physiology and many other disciplines that the highest priority should be given by the Administration to strengthening these areas. With limited resources, some reallocation of resources may be required to effect these recommendations.

Another concern of this consultant is the failure to have new equipment and instrumentation installed upon arrival at IPB. Additional instrumentation is needed in several instances and should be added as

resources become available. However, equipment should not be purchased unless funds are also available to provide for installation and maintenance of that equipment. During visits to various laboratories at IPB, this consultant observed several expensive instruments that appear to have been sitting for several months awaiting installation. The warranties may have already lapsed in some cases before installation occurs. More importantly, the instrument is not available for student training and use of research scientists and students. Scientific instruments should be purchased for use, not display. It is imperative that future purchases of instruments include installation or set-up by the manufacturer and training of personnel. These requirements should be expressly stated in the specifications for future purchases, and a signed contract should be obtained from the manufacturer or supplier to assure that installation and training are provided before payment is made. For expensive instruments, it may be necessary to impose a fee for each sample analyzed. Unfortunately, this policy will discourage use by some students and faculty; but unless funds can be allocated for maintenance, it may be the only mechanism for maintaining expensive instruments.

It is recommended that researchers in Agronomy and other departments with limited equipment work out arrangements with Dr. Nur and others to use equipment such as the amino acid analyzer and high performance liquid chromatograph to support research of their students. Again arrangements should be made to share maintenance costs, but this arrangement will be more efficient than duplicating the equipment in several departments. In discussion with Dr. Nur, he indicated his willingness to cooperate with other faculty.

During a tour of facilities at BATAN in Jakarta, this consultant observed that several expensive instruments are already in use there by researchers. Some collaborative efforts between IPB faculty and students and the staff at BATAN were cited. These collaborative efforts are to be commended and encouraged, for the collaboration should be mutually beneficial. For example, Ir. Susanto, an S_2 student of Dr. Soleh Solahuddin, will analyze samples from his research for ^{15}N on the ^{15}N analyzer at BATAN. This isotope was also provided by BATAN. In turn, Dr. Soleh Solahuddin is advising Elsge Sisworo, a researcher at BATAN, as she works on the S_3 degree. This cooperation is commendable and should be continued and expanded to include others.

Dr. Edi Guhardja requested that this consultant make suggestions concerning the training sessions to be held in December at IPB for faculty from several other institutions in Western Java, Sumatra, and Kalimantan. The Directorate General of Higher Education in Jakarta has agreed to support this training program financially and requested that IPB manage the program and develop the curriculum for the sessions. The objective is to standardize the level of training in plant physiology at the S_1 level at these institutions so that S_1 graduates can enter the S_2 and S_3 programs at IPB with a relatively comparable level of training. Although the objective is meritorious, extensive planning and coordination will be required to successfully achieve the objective. There may be a considerable difference of opinion among the staff from the various institutions as to what the S_1 curriculum should include. Therefore, the first suggestion is that the participants from each

institution be contacted immediately and asked to submit a detailed outline of what they currently present to their students in lectures and laboratories. This information will provide the organizers with some insight into how diverse the present training is at the different institutions. The second recommendation is to solicit suggestions from each participant on what he/she believes would be most useful to him/her in improving or upgrading lectures and laboratories. Even though all recommendations may not be used, at least participants would feel that they have been involved in determining the content of instruction; and the recommendation should be useful in reaching a consensus at IPB on how to best use the time and resources available for the training session. A third suggestion is to ask what laboratory facilities and instrumentation are available for the teaching of plant physiology techniques. There is little point in teaching staff techniques for which they lack the instruments, etc., at their home institutions. Fourthly, ask what textbook(s) they presently use. If a consensus could be reached on using the same text at each institution, considerable progress should be made rather quickly in standardizing the content and level of instruction. Persons in charge of organizing this training should obtain an examination copy of those plant physiology texts not currently available at IPB, so that these texts can be on display for examination by staff.

It is recommended that training for laboratory techniques concentrate on teaching basic principles, rather than use of sophisticated instruments. For example, principles of pH can be taught with pH paper or with an inexpensive pH meter. Principles of spectrophotometry can be

demonstrated with an inexpensive colorimeter. Ion-exchange chromatography could be demonstrated by binding a colored substance (e.g., chlorophyll) to the resin in a column and then eluting it. Likewise the principles of gel filtration with Sephadex or Bio-Rad resins can be demonstrated nicely by mixing colored compounds of different molecular weight and then separating them on a column containing the resin. For example, blue dextrin, chlorophyll, and cytochrome C could be separated. A more sophisticated experiment would be extraction and assay of nitrate reductase activity. Roots and leaves could be compared. Students could learn several important principles from such an experiment as the assay is relatively simple, and a pink color develops as the product of the enzyme reaction. Other suggestions include determination of nitrate and total N in plant tissues. Students could grow plants under different conditions (e.g., different levels of nitrate-N or different light levels) and then assay for nitrate and total N.

Summary of Recommendations

The following recommendations are submitted for consideration to strengthen graduate training at IPB. Some recommendations are general, but others are specific to the plant physiology curriculum.

1. Upgrading of IPB faculty should continue by encouraging present faculty to obtain a Ph.D. or S_3 degree and by hiring additional faculty at the S_3 level as resources permit.
2. The requirements for the S_3 degree should be more standardized at IPB so that all S_3 candidates:

(a) submit a thesis proposal to a faculty committee comprised of at least five faculty and orally defend the thesis proposal before that committee;

(b) pass a preliminary examination for S_3 degree after or near completion of course work. An oral examination is recommended by this consultant;

(c) present a progress report to their committee on their thesis research when about one-half of the research is completed;

(d) present an open seminar upon completion of the research;

(e) successfully defend the thesis before their committee; and

(f) publish their results.

3. Graduate course offerings should be better coordinated across departments and faculties to use limited resources more efficiently. For example, more coordination between Animal and Plant Sciences is needed and between advanced S_1 courses and graduate courses.
4. All courses should be evaluated at the end of each term by students enrolled in the classes.
5. Teaching laboratories and instrumentation in Plant Science courses should be upgraded as soon as possible.
6. Basic natural and physical sciences should continue to be strengthened so that S_1 graduates are more adequately prepared for S_2 and S_3 candidacy.
7. New instruments should be installed promptly, and a mechanism for providing for maintenance and repair must be devised. Either

money needs to be allocated for this purpose or a user fee should be imposed for each sample analyzed on the instrument.

8. More cooperation among staff in different departments in sharing equipment for graduate training and research is encouraged, and more collaboration with institutes such as BATAN in Jakarta is recommended to make more research equipment and facilities available to students.

Appendix A

Itinerary for Professor L. Schrader - 1983

Sunday, September 11	Arrival at Halim Perdanakusumah, Jakarta
Monday, September 12	Orientation with several persons including Rector Andi Hakim Nasoetion; Acting Project Director, M. White; Head of IPB's Planning Board, Ikin Mansjoer; and several others. Also went to the Embassy in Jakarta to obtain identification card, etc.
Tuesday, September 13	Lectured to AGR 535, Crop Physiology class from 0730 to 1000. Also lectured to S_1 seminar and answered questions about careers and job opportunities in Plant Physiology and Agronomy.
Wednesday, September 14	Consultation with faculty and graduate students. Consulted with Edi Guhardja, Dean of the Graduate School; Rector Andi H. Nasoetion; and Jajah Koswara, Associate Dean of the Graduate School, about graduate education at IPB.
Thursday, September 15	Lectured to AGR 535 from 0730 to 1000. Consultation with Dr. Ismu Suwelo, plant breeder.
Friday, September 16	Consultation with Dr. Anwar Nur about biochemical techniques courses and with Dr. Hari Suseno and Dr. Soleh Solahuddin about crop and Plant Physiology courses.

Saturday, September 17	Visited Pasir Madang clove plantation, villages, and surrounding area with Dean Oetit Koswara, Dr. J. Koswara, Dr. F. Rumawas, Dr. S. Solahuddin, and Ir. Muchtar.
Sunday, September 18	Visited villages, farms, experimental farms, and campuses in Bogor and Darmaga areas with Graduate School Dean, Dr. Edi Guhardja. Also visited about graduate education.
Monday, September 19	Consultation with students and faculty. Ir. Nurita Toruan, S_3 candidate, presented her thesis proposal and we discussed it.
Tuesday, September 20	Lectured to AGR 535 from 0730 to 1005. Observed Agronomy facilities and experimental plots at Darmaga campus with Dr. S. Solahuddin.
Wednesday, September 21	Visited Tajur Experiental Farm and conferred with Dr. Amris Makmur and two S_3 candidates (Ir. Sunarto, M.S., and Ir. Asril Samad, M.S.) as well as other colleagues. Also visited Pasir Sarongge Experimental Farm with Ir. Suryono and Dr. S. Solahuddin.
Thursday, September 22	Lectured to AGR 535 from 0730 to 1000.
Friday, September 23	Consulted with Dr. J. Koswara about graduate student training at IPB.
Saturday, September 24	Lectured to AGR 535 from 0730 to 0930. Departed for Yogyakarta.

<u>Sunday, September 25</u>	Toured Gadjah Mada University campus, the main university experimental farm, and observed agriculture in the surrounding areas. Also conferred with Ms. Lilik Kusdiarti, former student at UW-Madison.
<u>Monday, September 26</u>	Presented an invited seminar on corn and soybeans, followed by discussion, to about 60 faculty and students at Gadjah Mada University. Conferred with the Dean of Agriculture, Joedera Soedarsono; Agronomy Department Head, Eddy Mitoyat; and several research scientists.
<u>Tuesday, September 27</u>	Presented seminar and discussion to approximately 30 scientists at BATAN in Jakarta, visited facilities, and conferred with scientists about research techniques and instrumentation.
<u>Wednesday, September 28</u>	Conferred with Dr. Sjamsoe'oed Sadjad about his teaching and research in seed technology. Observed a class of 40 working on lab experiments. Also visited the Botanical Gardens in Bogor with Dr. G. A. Wattimena. Graded examination given to AGR 535 on September 27.

Thursday, September 29	Lectured to AGR 535 from 0730 to 1000. Consulted with Elsge Sisworo about her S_3 thesis research to be conducted at BATAN. Also conferred with Dr. Livy Winata about research problems, lab techniques, and lab facilities and instrumention. Worked on this report.
Friday, September 30	Wrote letter to support Dr. Livy Winata's application for an AMBO Fellowship to attend a course in Japan. Presented seminar to 75 scientists and answered questions for two hours at the Central Research Institute for Food Crops in Bogor. Worked on this report.
Saturday, October 1	Presented seminar to 180 faculty and students in the Faculty of Agriculture at Padjadjaran University in Bandung. Conferred with Dr. Hasbi Tirtapradja, Dean of Agriculture; the Dean of the Graduate School; and others.
Sunday, October 2	Traveled to Denpasar and toured surrounding regions observing agriculture in the area. Conferred with Dr. S. Solahuddin about several issues.
Monday, October 3	Presented seminar to 48 students and faculty at Udayana University in Denpasar. Answered questions and discussed research for two hours follcwing seminar.

Tuesday, October 4	Returned from Denpasar and worked on this report.
Wednesday, October 5	Lectured to AGR 535 from 1100 to 1300. Prepared an examination to be given later. Finished draft of this report.
Thursday, October 6	Departure from Jakarta for Madison.

Appendix B

Outline of Subject Matter Covered in Lectures in Agronomi 535

Lecture 1

A. Basic Requirements for Dry Matter Accumulation and Growth of Plants

B. Maximum Potential Crop Productivity - An Estimate

1. Based on available solar radiation
2. Based on observed rates of CO_2 fixation
3. Influence of composition of seeds from different crop species on net productivity.

Lecture 2

A. Compare estimated maximum crop productivity to actual reported yields for several crops

B. Photosynthesis - A review

1. Light and dark reactions
2. Three phases of photosynthesis
    a. Reductive phase
    b. Carboxylative phase
    c. Regenerative phase
3. Quantum requirement for CO_2 fixation

C. Photorespiration

1. Review pathways
2. Impact of photorespiration on photosynthesis, crop productivity, and physiological quantum requirement

Lecture 3

A. Use of mutants to elucidate pathway of photorespiration and attempts to eliminate photorespiration through mutation of plants

B. Photorespiratory Nitrogen Cycle

C. New method for measuring photosynthesis and photorespiration concurrently

D. Species variation in photorespiratory losses.

Lecture 4

A. Metabolite transport by chloroplasts in light and dark

   1. Phosphate translocator

   2. Dicarboxylate translocator

B. Review Carbohydrate Biosynthesis

C. Review Carbohydrate Degradation

D. Compare Carbohydrate metabolism in green and non-green tissues

E. Regulation of starch synthesis in chloroplasts

F. Importance of phosphorous nutrition in regulating photosynthesis and partitioning of photosynthate

Lecture 5

A. N Cycle

   1. Six processes that contribute N to cycle

   2. N cycle

      a. Ammonification of organic matter

      b. Nitrification

      c. Immobilization of N in organic matter

3. Losses from Cycle
    a. Denitrification
    b. Leaching
    c. Plant uptake and harvesting of plants or seeds

B. Discussion of ways to minimize losses of N

C. Nitrate uptake
    1. Induction of uptake system
    2. Genotypic differences in rates of uptake and potential for selecting more efficient genotypes
    3. Effect of plant age on uptake and discussion of when to apply N for most efficient use

Lecture 6

A. Nitrate Accumulation in Maize

B. Nitrate Reduction
    1. Enzymes involved in localization in leaf
    2. Requirements for reductant and involvement of photosynthesis
    3. Roots versus leaves as site of nitrate reduction
        a. Comparison of activities
        b. Comparison of energy requirements

C. Biological N_2 Fixation
    1. Reactions, enzymes, and reductants
    2. Role of hydrogenase
    3. Role of photosynthesis in N_2 fixation
    4. Energy costs of N_2 fixation

5. Compounds formed during N_2 fixation
    a. Ureides
    b. Amides
6. Importance of ureides in N_2 fixation by soybeans, cowpeas, and <u>Phaseolus</u>

Lecture 7

A. Sulfur metabolism in plants

B. Effects of marginal S deficiency
    1. N metabolism
    2. Chlorophyll
    3. Plant growth

C. The use of unusual genetic resources in physiological and biochemical research
    1. Use of isogenic alfalfa polyploids as a model system to understand the consequences of polyploidization in plants on photosynthesis and other physiological processes. Many research techniques were presented as a part of this discussion.
    2. Use of corn genotypes differing by only one recessive gene to study source-sink relations and to determine whether "source" or "sink" limits productivity of maize. Students and faculty have expressed much interest in partitioning of photosynthate and source-sink relations, so this lecture will discuss this research as a model system for studying and understanding the mechanisms involved.

Appendix C

Course Evaluation Form

This evaluation form is designed to provide student reaction to Agronomy. It is important that all students participate. The completed forms will be placed in a sealed envelope, held in the Agronomy office until after the final grades are turned in, and then returned to the instructor. Do not sign your name.

I. For each of the statements below, circle the appropriate number that best describes your rating. Ratings are as follows: 5 - EXCELLENT, 4 - GOOD, 3 - AVERAGE, 2 - FAIR, 1 - POOR. Check the NA column for those statements that do not apply to your course.

		E	G	A	F	P	NA
1.	Professor's preparation for class	5	4	3	2	1	___
2.	Professor's knowledge of subject	5	4	3	2	1	___
3.	Professor's ability to explain subject in an interesting way	5	4	3	2	1	___
4.	Tying of information together	5	4	3	2	1	___
5.	Currentness of information	5	4	3	2	1	___
6.	Professor's enthusiasm for teaching	5	4	3	2	1	___
7.	Professor's willingness to entertain questions during class	5	4	3	2	1	___
8.	Professor's presentations are well delivered	5	4	3	2	1	___
9.	Good illustrations are used	5	4	3	2	1	___
10.	Books and references relate to material presented in class	5	4	3	2	1	___
11.	Grading procedures used in the course	5	4	3	2	1	___
12.	Audio-visual aids used in the course	5	4	3	2	1	___
13.	This course compares with other courses I have taken	5	4	3	2	1	___

II. For each of the following statements, check the appropriate column which best describes your response. The responses are as follows: TOO GREAT, ABOUT RIGHT, and TOO LITTLE. Check the NA column for those statements that do not apply to your course.

		Too Great	About Right	Too Little	NA
1.	The course coverage by examination .	____	____	____	___
2.	The amount of outside reading is . .	____	____	____	___

	Too Great	About Right	Too Little	NA
3. The frequency of exams is				
4. The length of exams is				
5. The use of audio-visual aids is . .				
6. The rate of presentation is				

III. For each of the following statements, check either YES or NO to indicate your evaluation. Check the NA column for those statements that do not apply to your course.

	Yes	No	NA
1. The textbook is useful			
2. Other books and references are useful			
3. Too much material in the course is covered in other courses			
4. Too much prior knowledge is assumed			
5. Some topics are not stressed sufficiently. . .			
6. Some topics should be cut shorter			
7. You would recommend this course to others . .			

IV. Please use the space below to make suggestions for improving this course. List any strengths and weaknesses and any topics understressed or overstressed.

CHAPTER 3

NITRATE UPTAKE, REDUCTION AND TRANSPORT IN THE WHOLE PLANT

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In: J.D. Bewley (Ed.) Nitrogen and Carbon Metabolism. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague (1981), pp. 49-91.

INTRODUCTION

The major form of inorganic nitrogen available to higher plants in most soils is nitrate because fertilizer N and ammonia from ammonification of organic matter are readily oxidized to nitrate-N through nitrification by Nitrosomonas and Nitrobacter. It is estimated that only about 50% of the fertilizer N that is applied to crops is normally recovered by the crop during the year of application (1-4).

Utilization of nitrate by higher plants includes several processes, any one of which could limit nitrate assimilation (5-7). The first control point for nitrate assimilation is the uptake system (8). Nitrate absorbed by roots may be reduced by nitrate reductase (NR) in the roots, accumulated in storage sites (e.g., vacuoles), or transported via the xylem to the shoot. Nitrate accumulated or stored in vacuoles or other sites in which NR does not occur may be translocated at a later time to a site of reduction (9,10) and thereby serve as a reserve pool of nitrate; or nitrate may remain in the storage sites until harvest (4,7,11). The most limiting enzyme involved in assimilating nitrate into amino acids is thought to be NR (7,12,13). Some species reduce considerable amounts of nitrate-N in their roots whereas others reduce most of it in the shoots (13). Ammonia formed during reduction of nitrate in roots or shoots must be combined with carbon before it can be transported to other plant parts or cells (14). The formation of amino acids and other forms of reduced N is regulated (15) and thus may be a control point. The loading and transport of

these nitrogenous compounds may also be controlled or limited in some species.

Each process in the uptake, distribution, reduction, and incorporation of N into organic forms is under genetic influence (16); hence one might expect to find genetic variability for each of these traits or processes. Identification of this genetic variability and knowledge of the inheritance of these traits should permit breeders to select genotypes possessing an increased capacity to convert soil N to plant protein. A better understanding of the physiology and biochemistry of nitrate assimilation in plants is needed, however, to help identify the process(es) that is most limiting.

Because of space limitations, comprehensive coverage of even the most recent literature in all these areas will be impossible. Several recent review articles will be cited for the reader who desires a more extensive coverage of these topics.

NITRATE UPTAKE

The uptake of nitrate from the environment has been studied in several important crop plants, and many reviews deal with the characteristics of the uptake system involved (5-7,17,18). Here we will briefly mention the salient points of the physiological capacity of plants to absorb nitrate from the environment and indicate areas where progress may be made in improving the efficiency of nitrate uptake by crop plants.

Some crop plants previously grown without nitrate show a lag or induction phase when given nitrate. The rate of uptake is initially slow, but increases steadily until an "accelerated" rate is attained (6). This pattern of uptake is in contrast to that of other ions which generally exhibit linear rates during the initial stages of

uptake. The phenomenon of an apparent "induction" phase may not be of general occurrence as liquid cultures of perennial ryegrass (Lolium perenne) when given nitrate after a period of N starvation did not show a lag phase in nitrate uptake (19).

Uptake appears to require energy (5,20) although an anion ATPase has not been conclusively demonstrated for nitrate uptake in higher plants. Inhibitors of RNA and protein synthesis have been shown to decrease nitrate uptake suggesting a dependence on the continual synthesis of a protein or postulated "permease" (6). The observed enhancement of nitrate uptake by light may be partly the result of an increased supply of energy (20) or an increased supply of assimilates from the shoot. Ben Zioni et al. (21,22) proposed a model in which nitrate uptake is regulated by nitrate reduction and malate production in the shoot. Malate is postulated to be transported along with K^+ to the root where malate is oxidized and decarboxylated to form bicarbonate which is then exchanged for external nitrate. While evidence has been published to support this scheme (e.g., 22-24), there is still no direct evidence that K^+malate is involved in the control of nitrate uptake. Total organic acids, rather than malate, have usually been measured. Deane-Drummond et al. (25) observed a decrease in root malate concentrations after decapitation of barley shoots indicating that the increase in malate (and presumably nitrate uptake) in root tissue during the day was dependent on the downward translocation of malate from the shoot. It would be of interest to know if the observed diurnal rhythm of nitrate uptake (26) is accompanied by similar fluctuations in malate transport. The transfer of the negative charge from malate to the external solution via bicarbonate in exchange for nitrate is thought to be related to changes

in internal pH (27) but further work is required to substantiate this model. It is doubtful that the Ben Zioni model (21) is applicable to all crop plants as, for example, some C_3 plants such as tomato appear to retain malate in the shoot rather than transport it to the root (28). In C_4 plants such as maize, difficulties can be expected in differentiating between the effects of nitrate metabolism on leaf malate content from those associated with malate production during C_4 metabolism. The model is further complicated by the observation that malate may be utilized rather than synthesized during nitrate reduction in maize leaves, via malate oxidation which has been shown to provide NADH for nitrate reduction (29).

The decrease in nitrate uptake following decapitation of the shoot and a partial restoration of the uptake rate to that of the intact plant by glucose feeding indicates the close dependence of the uptake process on a continual supply of assimilates from the shoot (6,30). We require more information on the role of assimilates in nitrate uptake and ideally in systems where the effects on uptake and reduction can be separated. Doddema & Otten (31), using a mutant of Arabidopsis which absorbs nitrate but has no NR, have shown that there is a correlation between nitrate uptake and root malate content. Further use of similar mutants in other crop species will provide an insight into the role of the shoot in the regulation of nitrate uptake.

Ambient ammonium and high internal concentrations of nitrate have been shown to decrease nitrate uptake (5,18,32) although the precise mechanisms are not known. Generally there is a parallel "induction" of nitrate uptake and reduction but again little is known about the exact nature of the relationships between the two (5,7,17).

From kinetic data of nitrate uptake there appear to be at least two mechanisms involved. Nitrate uptake rates

measured over a range of low nitrate concentrations can be described by a simple rectangular hyperbola. At higher concentrations the rate continues to increase above the apparent saturation rate observed at lower concentrations (5). Work with mutants of Arabidopsis also indicates that there are two or more independent mechanisms which presumably are genetically determined (33). These complex kinetic data for nitrate uptake are similar to those reported for other ions showing bi- or multiphasic kinetics (34,35). It is difficult to assess what these kinetic data mean with respect to the field situation as the concentrations of available nitrate in the soil depend on various environmental conditions, but it is likely that uptake usually occurs over a range of low nitrate concentrations. Clement et al. (36) showed little change in nitrate uptake rates of ryegrass (Lolium) over a wide range of nitrate concentrations likely to be found in the field. These results suggest that plants may have a mechanism to maintain uptake rates at low ambient nitrate concentrations.

One of the important goals for the future improvement of nitrate uptake by crop plants is to determine the extent of the genetic variability in a species' capacity to efficiently utilize the available nitrate in the soil. Selection for variants which have higher uptake rates could result in more rapid recovery of soil N before losses to the environment occur. This could improve the current figure of a 50% efficiency of fertilizer utilization (4). Genotypic differences in nitrate uptake have been reported in maize (37) and wheat (5). An increase in uptake rate per se may not be beneficial in terms of crop productivity if the increase is a result of increased root growth at the expense of shoot growth. Ideally a lowering of the apparent K_m for uptake (increased affinity) is desired as this would be unlikely to alter the root:shoot ratio. There is little evidence for intraspecific dif-

ferences in the apparent K_m (or half-saturation constant) for nitrate uptake amongst higher plants although interspecific differences have been noted (e.g., 5,38). In algae intraspecific differences have been observed. Clones of the same species of marine phytoplankton had different K_m values for nitrate uptake (39). Those isolated from nutrient-poor waters had higher affinities (lower K_m's) than clones of the same species isolated from nutrient-rich waters.

Research to improve nitrate uptake has been hampered by the lack of a suitable isotope of N. Currently the use of $^{36}ClO_3$ as an analogue of nitrate in uptake studies is being tested as a tool for the screening of plants for increased uptake efficiencies (Rhodes, Zabala & Filner, personal communication). A disadvantage of this technique is that it is likely to be destructive due to chlorate toxicity.

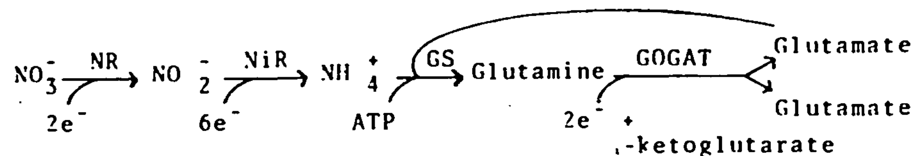
A problem which is likely to be encountered with any improved capacity for nitrate uptake is that of nitrate accumulation (7). Friedrich et al. (9) showed that the accumulated nitrate in roots and stems of maize is available for assimilation especially if the supply of ambient nitrate decreases during grain filling. This taken together with the finding that most of the N in the ears of maize is absorbed by the plant prior to silking (10) indicates that, in these plants, the most N-efficient will be those which can absorb most of their N early during vegetative growth and efficiently remobilize any accumulated nitrate. In plants which absorb nitrate during reproductive growth (40), it will be important to determine if this N is remobilized efficiently into the harvested plant parts.

Physiologists need to identify the limiting and controlling factors involved in nitrate accumulation and remobilization so that any genetic variations in these processes, such as those noted for nitrate accumulation

(37,41) can be exploited via breeding programs.

NITRATE ASSIMILATION

Nitrate assimilation includes both reductive and non-reductive processes in converting nitrate-N to amino-N. The reactions of this pathway have been discussed in several recent reviews (40,42-46). The four enzymes involved are NR, nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT). Three of these reactions are reductive, and a total of 10 electrons must be provided, as shown below, for the assimilation of a nitrate ion to glutamate. The provision of these electrons will be discussed later.



Nitrate Assimilation in Roots versus Shoots of Plants.

As noted by Lee (13), nitrate assimilation has been examined in non-photosynthetic tissues of several species with most emphasis on the roots. Plant species differ widely in the proportion of incoming nitrate that is reduced in the roots as compared to the shoots (6,47). Relative activities of NR in different plant parts vary with species as does the relative distribution of nitrate and reduced N in exuding xylem sap. It is not clear why differences in the relative proportions of nitrate reduced in roots exist among genotyes and species. Jackson (6) discussed the potential competition between reduction and xylem deposition components of the nitrate assimilation pathway in roots. He suggested that NR in the roots appears to have priority over xylem deposition for incoming nitrate. He reported that the rate of deposition of nitrate into the xylem differed significantly in three maize

hybrids. These hybrids differed little in the amount of nitrate absorbed during the course of the experiment, but the amount of nitrate translocated out of the root system appeared to be inversely related to the amount of nitrate reduced in the roots.

In vitro NR activity has been assayed in the root tissue of a number of plant species (6,13). The activity is generally low compared with NR activity in leaves. Wallace (48) found that in maize seedlings, less than 20% of the total NR was found in the roots. Robin *et al*. (49) observed that NR activity in maize roots was only 20% of that in shoots. Solahuddin and Schrader (unpublished) recently compared *in vitro* root and shoot NR activity in 16 maize inbreds. Although casein was added to stabilize the NR activity (50), root NR activity per g fresh weight was generally only about 10% of the shoot NR activity in each genotype. Significant genotype differences in NR activity were observed in both root and shoot extracts. Maize genotypes with higher levels of shoot NR activity also had higher levels of root NR activity as indicated by a highly significant correlation (r=0.786**) when root and shoot NR activity per gram of tissue for the 16 genotypes were compared. When total NR per shoot was compared to total NR per root, the correlation was also highly significant (r=0.787**). Brunetti and Hageman (51) compared levels of extractable NR with the *in vivo* assay in leaves and roots of wheat plants, and found that although much higher levels of extractable NR were present in shoots versus roots, the *in vivo* assays indicated approximately equal levels of activity in the two tissues. Hence the low activities of NR observed in extracts from roots may need to be examined further due to the presence of an inactivating enzyme in root extracts (52-55).

The other enzymes involved in nitrate assimilation have not been studied as extensively in non-green tissues as has NR, but Lee (13) has recently reviewed the status

of work in that area. Major differences may exist between green and non-green tissues in the processes that provide the reductant needed for nitrate assimilation.

Intracellular Localization of Enzymes.

With respect to subcellular localization of the enzymes of nitrate assimilation, most evidence suggests that NR is a soluble enzyme localized in the cytoplasm of both leaf and root tissue (13). A particle-bound NR was prepared by Miflin (56-58) from non-sterile barley roots, but Blevins et al. (59) later showed that the particle-bound form was probably of microbial origin. Butz and Jackson (17) proposed the existence of a membrane-bound enzyme with nitrate transporting, nitrate reducing, and ATPase activities. This proposal would account for the frequently observed correlation between nitrate absorption and nitrate reduction, but more evidence is needed before this proposal can be accepted. In leaves, Rathnam and Das (60) and Rathnam and Edwards (61) reported that NR is localized on the outer envelope of the chloroplast and thus is easily solubilized during extraction.

NiR is localized within the chloroplasts in leaf tissue (62-64). Evidence has been presented (65,66) that NiR in non-green tissue is associated with proplastids.

Lee (13) recently reviewed the evidence that GS is localized both within the plastids and cytosol in green and non-green tissues. Hence the enzyme appears to be present to detoxify ammonia formed by nitrite reduction in chloroplasts and proplastids, and ammonia released during photorespiration and other deamination reactions (67).

GOGAT is localized in the chloroplasts (64,68-70) and appears to be associated with proplastids in non-green tissues (44,66).

Support for the findings cited above is provided by studies (71,72) in which nitrite was reduced to ammonia and amino acids by isolated chloroplasts in a light-de-

pendent, CO_2-independent reaction, without other additives. Anderson and Done (69,70) have shown that isolated chloroplasts catalyze nitrite-dependent and glutamine plus α-ketoglutarate-dependent oxygen evolution. These results indicate that nitrite reduction and glutamate synthesis are dependent on reductants generated by the chloroplast electron transport chain.

Intercellular Localization of Enzymes.

Rathnam and Edwards (61) studied the intercellular localization of nitrate-assimilating enzymes in three C_4 groups of plants. NR, NiR, GS and GOGAT were predominantly localized in mesophyll cells of the species studied. Harel et al. (73) found more GS and GOGAT in maize bundle sheath cells than did Rathnam and Edwards (61) and attributed the differences to improved extraction procedures. Neyra and Hageman (74) found 95% of the NR and NADP-malic dehydrogenase in mesophyll cells of maize. They reported that the nitrate concentration was 16 times higher in mesophyll cells than in bundle sheath cells, suggesting the direct transport of nitrate to mesophyll cells from the xylem (not through bundle sheath cells). Attempts to induce NR with nitrate in bundle sheath strands failed. NAD-malate dehydrogenase and NAD-glyceraldehyde-3-P dehydrogenase were about equally distributed between the two cell types, but NAD-malate dehydrogenase was about 10 times higher in activity than was glyceraldehyde-3-P dehydrogenase in mesophyll cells. On the basis of enzyme activities and the fact that malate is formed during CO_2 fixation in the mesophyll cells of C_4 species, it seems likely that malate oxidation is an important and perhaps the major source of NADH for NR in C_4 species. Glyceraldehyde-3-P is formed in bundle sheath chloroplasts by the Calvin cycle and must be transported from the bundle sheath cells to the mesophyll cells to provide reductant for reactions in the mesophyll cells. Moore and Black (75) reported similar

results for localization of these enzymes in crabgrass. Mesophyll protoplasts contained most of the NR and NiR, but GS was equally distributed between mesophyll and bundle sheath cells, and GOGAT was higher in bundle sheath cells. They suggest that nitrate assimilation in mesophyll cells results in a "division of labor" between mesophyll and bundle sheath cells, and that a spatial separation of the oxygen evolution accompanying nitrite reduction (in mesophyll cells) from carbon dioxide fixation (in bundle sheath cells) helps keep the oxygen away from the ribulose bisphosphate carboxylase/oxygenase. This may reduce the level of photorespiration.

Electron Donors for Nitrate Assimilation.

Nitrate reductase from a variety of green and non-green tissues has been shown to use NADH as the electron donor (13,76), although NR from some tissues has the ability to use NADPH as well (77-81). The NAD(P)H NR does not appear to be highly specific for NADPH, whereas NADH-NR from many tissues is specific in its requirement for NADH (13).

Ferredoxin, a natural one-electron carrier in plants, provides the reductant for NiR in illuminated chloroplasts (82). The reductant for non-green tissues has not been identified. Reduced pyridine nucleotides do not donate electrons directly to NiR although there is some evidence that a reductase exists to transfer electrons from pyridine nucleotides through ferredoxin to NiR (83). Because the properties of NiR from green and non-green tissues are so similar (84-86), it seems likely that a compound similar to ferredoxin also serves as the reductant in non-green tissue. However, ferredoxin has not yet been isolated from non-green plant tissue, but perhaps a one-electron carrier similar to ferredoxin exists in these tissues.

Ferredoxin also serves as the immediate electron donor for GOGAT purified from green leaves, but pyridine

nucleotides are ineffective donors (64,87). In non-green tissues, GOGAT generally uses NADH as a reductant more effectively than NADPH (88,89).

Relationship of Nitrate Assimilation and Photosynthesis.

Nitrate assimilation in green tissue is dependent on photochemical reactions of the chloroplast to provide the reductant and ATP used to convert nitrate to amino acids (Fig. 1). Nitrate reduction in the cytosol can be provided indirectly with reductant (NADH) from photosynthesis by oxidation of substrates (e.g., dihydroxyacetone phosphate [DHAP] or malate) that are transported from the chloroplasts to the cytosol by the phosphate translocator or dicarboxylate translocator (90,91). Inside the chloroplasts, the reduction of nitrite to ammonia can be coupled directly to light reactions through reduced ferredoxin as the electron donor. ATP from photophosphorylation can be used for the GS reaction and reduced ferredoxin can be used directly for the GOGAT reaction. As noted in Fig. 1, the quantum requirement for reduction of nitrite to ammonia is 12. The conversion of glutamine to two glutamates requires four photons. If a triose phosphate (e.g., DHAP) is translocated from the chloroplast to the cytosol to provide the NADH for nitrate reduction, the reduction of the PGA to glyceraldehyde-3-P in the chloroplast will require four photons (Fig. 1). Therefore in illuminated green tissues, the total quantum requirement for nitrate reduction to ammonia is 16 photons per nitrate ion, and for conversion of nitrate to glutamate is 20.

For non-chlorophyllous tissue or dark reduction of nitrate in green tissues, the reductant for nitrate assimilation must come from oxidation of carbohydrates or organic acids. On the assumption that most of the carbon is provided by sucrose transported to the site of reduction, each hexose can provide 12 pairs of electrons if totally oxidized to CO_2 and H_2O in glycolysis and the Krebs' tri-

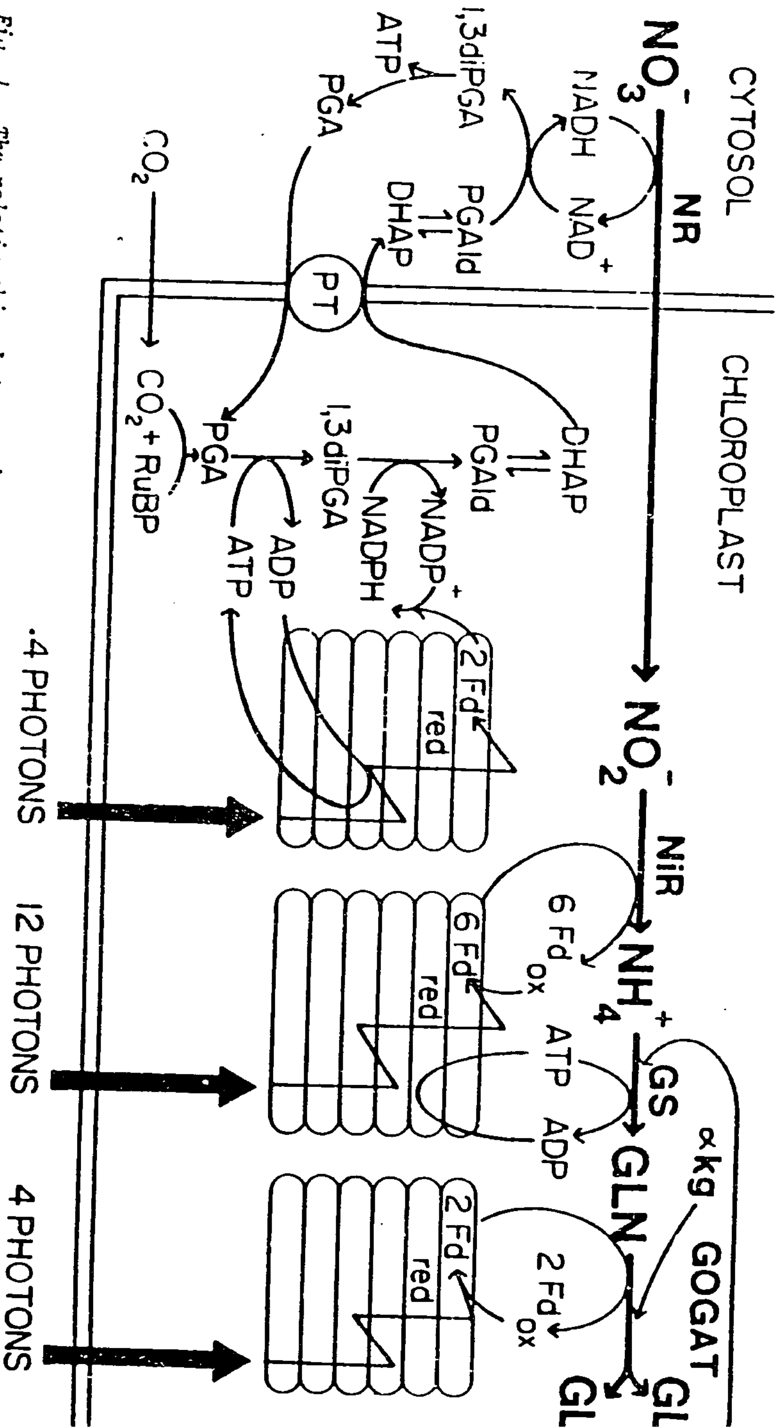


Fig. 1. The relationships between nitrate assimilation and photochemical reactions in the chloroplast. PT = phosphate translocator; NR = Nitrate reductase; NiR = nitrite reductase; GS = glutamine synthetase; GOGAT = glutamate synthase; GLN = glutamine; GLU = glutamate; Fd_{red} = reduced ferredoxin; Fd_{ox} = oxidized ferredoxin; DHAP = dihydroxyacetone phosphate; PGAld = 3-phosphoglyceraldehyde; 1,3 diPGA = 1,3 bisphosphoglycerate; PGA = 3-phosphoglycerate; RuBP = ribulose 1,5-bisphosphate; αkg = α-ketoglutarate

carboxylic acid cycle (92). Therefore each carbon of the hexose can provide two pairs of electrons. To obtain the 10 electrons required for conversion of each nitrate ion to glutamate, the equivalent of 2.5 fixed carbons must be oxidized. If one accounts for the photorespiratory losses normally associated with CO_2 fixation, the quantum requirement for fixation of carbon in plants is at least 14 to 15 (93). Therefore the quantum requirement for nitrate reduction in non-green tissue or green tissue in the dark appears to be almost double that in green tissue in the light.

If excess reductant is present in chloroplasts under high-light conditions (94), nitrate reduction in green leaves in light may be essentially free to plants. As noted above, reduction of nitrate in the dark or in non-green tissue is expensive. Nitrate reduction in roots and other non-green tissues may be important in providing a continual supply of reduced nitrogen to other plant parts during the night. However, from an energetic point of view and from the standpoint of maximizing productivity, it seems desirable to reduce a large portion of the nitrate in the light in green tissues.

Regulation of Nitrate Reductase.

The regulation of NR is complex and appears to differ from species to species as well as in different plant parts. This topic has ben reviewed by several authors (6,7,13,40,42,95-97) and the interested reader is referred to these. The various regulatory factors can be divided into those that affect (a) the amount of enzyme and (b) the activity of existing enzyme.

a) Regulation of amount of enzyme

Nitrate has been shown to induce the de novo synthesis of NR (42,98). Light has also been shown to be involved in the induction process (95,99) although its requirement is not absolute (100). The effects of light on the formation

of new enzyme are numerous and include (a) increasing the uptake of nitrate into leaves (101), (b) maintaining polyribosome levels and hence the capacity for protein synthesis (100), (c) acting via a phytochrome system which affects the movement of nitrate across membranes, e.g., from a "storage" pool to a "metabolic" pool (102,103), and (d) inactivating an inhibitor of NR which is formed in the dark (104).

Much of the work on the "induction" of NR, while showing that protein synthesis is necessary, does not distinguish between de novo synthesis of the enzyme and assembly of pre-existing polypeptides. Moreover, decreased rates of inactivation or degradation of NR could result in higher levels of extractable NR activity (97,105). There is some evidence that NR may be a constitutive enzyme (97). Ammonium-grown Chlorella cells with almost no NR activity were shown to contain cross-reacting material which binds to NR antibody suggesting the presence of a NR precursor protein (106). These results together with inhibitor and labelling studies (107,108) suggest that NR mRNA and precursor protein are present in cells which have not been subjected to inducing conditions (presence of nitrate). The so called "induction" of NR by nitrate may thus involve an assembly of pre-existing enzyme components and not strictly a de novo synthesis (see 97 for detailed discussion of enzyme component assembly). Further serological work is required to test this suggestion in higher plants which generally contain low NR activity when grown with ammonium.

b) Regulation of nitrate reductase activity

Numerous factors affect NR activity. The availability of substrate (nitrate) and reductant (usually NADH) are key regulatory factors, but other factors that inactivate or degrade the enzyme are involved in regulation too.

Perhaps the most important factor in the regulation of NR in leaf tissue is the flux of nitrate into the leaves from the roots or storage tissues. Following the

work of Meeker et al. (109) who showed that leaf NR activity was more closely correlated with nitrate concentration of the midrib of maize leaves than with the concentration in the lamina, Shaner and Boyer (110) showed that the nitrate flux into the leaves was more important in the regulation of NR than was leaf nitrate content. The results can be interpreted in terms of there being two pools of nitrate in the leaf tissue: (1) a storage pool (possibly in the vacuole), and (b) a smaller rapidly metabolized pool in the cytoplasm (111). According to this proposal (111), NR is regulated by the flux of nitrate entering the metabolic pool via the transpiration stream. The storage pool would contribute largely to the total leaf nitrate content but little towards the metabolic pool because of sequestration in the vacuole (110). In this way, changes in NR activity can occur without significant changes in total leaf nitrate content.

Availability of NADH for NR in leaf tissue provides another regulatory system. The reduction of ^{15}N-nitrate and -nitrite in leaves has been shown to cease in the dark (112). However, nitrate reduction in the dark can be promoted by anaerobic conditions (113), or by inhibiting the respiratory electron transport chain with CO (114) or antimycin A, but not amytal or rotenone (113). This promotion of nitrate reduction in the dark has been attributed to availability of extramitochondrial NADH at times when the external NADH dehydrogenase of the mitochondria is inhibited (113). In the light, the respiratory electron transport chain is thought to be inhibited by a high ATP:ADP ratio that prevails in leaves (115). Further support for these proposals was provided recently by Woo et al. (116) with a reconstituted system from spinach leaves containing supernatant, mitochondria, NAD^+, oxaloacetate and an oxidizable substrate. They showed that the transfer of reducing power from the mitochondria to the soluble phase via the oxaloacetate/malate shuttle can pro-

vide NADH for the cytoplasmic reduction of nitrate. This rather elegant regulatory system in leaves serves at least two important roles: (a) it prevents the accumulation of nitrite in leaf tissue during the night when photochemical reductants (i.e., ferredoxin) are not being produced in the chloroplast, and (b) it requires the leaf to use light-dependent reductants rather than fixed carbon sources as a source of reductant. In contrast to the results cited above, Aslam et al. (117) reported that dark reduction of nitrate in barley seedlings was about 50% of that observed in the light. These conflicting results cannot be explained at this time. Roots and other non-photosynthetic tissues apparently depend on factors other than NADH availability for regulation of NR, as oxidative phosphorylation in mitochondria in those tissues presumably competes for NADH thoughout the photoperiod.

Light is thought to be involved in activation of the enzyme via several direct and indirect effects (42,95,97,118) including a direct activation of the enzyme (119), an increase in accessibility of the enzyme to nitrate via phytochrome-mediated membrane changes and/or other phytochrome effects (120), and provision of reductant via photosynthesis (121,122).

The enzyme can be converted from an active to an inactive form by various chemical changes including an "over-reduced" state with NAD(P)H or dithionite (123). These effects can be enhanced by the presence of cyanide (123). Further evidence that cyanide is involved in the regulation of NR in algae has been published (124)and a model linking the regulation of CO_2 fixation and nitrate assimilation and involving the production of cyanide has been proposed (118). The NAD(P)H- and cyanide-inactivated form of the enzyme can be reactivated by ferricyanide which is thought to reoxidize the enzyme (123). These results suggest that NR activity may be controlled by the redox state of the cells (125). Hewitt et al. (97) have

obtained evidence that a similar regulatory effect of cyanide exists in higher plants but further work is required before this suggestion can be generally accepted as a major regulatory factor of NR in vivo. Reversible inactivation also occurs with ADP and other adenine nucleotides (97).

In green algae and simple aquatic plants such as Lemna, ammonium has been shown to repress or prevent the increase in extractable NR activity in the presence of nitrate (126-128). There is still controversy over whether or not the effect of ammonia is on NR itself or if it first inhibits nitrate uptake into algal cells (126). In most higher plants studied, ammonium does not repress NR (7,40,42). Furthermore although Orebamjo and Stewart (129) observed an inactivation of NR by addition of ammonium to nitrate-induced Lemna, similar experiments using in vitro assays of NR in maize revealed that monovalent cations (NH_4^+, Na^+ and K^+) at equal concentrations had similar inhibitory effects on NR (7). These results suggest that ionic strength rather than a specific effect of ammonium ions is the critical factor.

In general amino acids, as eventual products of nitrate reduction, do not inhibit leaf NR activity or induction (7,40,96). There is some evidence tnat amino acids may regulate the induction of NR in root tissue (130,131) and plant tissue cultures (132). These results suggest that there may be different regulatory mechanisms operating in the root and shoot.

NR-inactivating proteins have been isolated from corn roots (52), rice cell cultures (133) and seedlings (134), soybean leaves (104), wheat (135,136) and cucumber leaves (137). These proteins may be responsible for the loss of NR activity following removal of nitrate from the medium and for the frequently observed diurnal rhythms in NR activity (135). The proteins isolated from the different tissues studied appear to have different characteristics

(135,138). For example, the protein isolated from corn roots has proteolytic activity (52) whereas that isolated from rice plants and soybean leaves has no proteolytic activity (104,138,139). Thus there appear to be at least two types of protein, one which is a NR-degrading protein (52) and the other a NR-specific binding protein (140). In addition to these inactivating factors, there is some evidence from studies with wheat and soybean leaves for the presence of activating factors (104,135). Sherrard _et al._ (136) have suggested that because of the presence of these activating factors, it may be premature to describe the inactivating proteins as being specific for NR, as a negative result of an inactivating factor on any enzyme may be due to the presence of contaminating protective factors.

The effects of CO_2 on nitrate reduction are complex (7), but generally CO_2 in the presence of light enhances nitrate reduction probably by increasing the amount of fixed carbon available to provide reductant. Increasing concentrations of CO_2 may decrease leaf NR activity by decreasing stomatal opening and hence nitrate flux into the leaves (29,109).

The level of NR activity in plant tissues is dependent to some extent on other nutrient elements. Molybdenum is a constituent of the enzyme and must be present for synthesis of active NR (42). Because the enzyme is sulfhydryl-dependent (141), adequate sulfur must be present to induce and maintain high levels of NR activity (142). Friedrich and Schrader (142) observed that S-deprived maize seedlings lost NR activity more quickly than other enzymes involved in nitrate assimilation, and prior to any decrease in soluble protein. Furthermore, NR activity had decreased to 50% of the control before any visual symptoms of S deficiency began to appear. With stricter pollution control of our atmosphere, the removal of more SO_2 from the atmosphere may cause marginal S deficiency to become

more prevalent in crops. It will be important to watch carefully for this condition, as nitrate assimilation may be severely limited by even marginal deficiencies of sulfur.

Temperature also influences NR activity (96). Increasing temperatures may increase NR activity for short periods of time, but prolonged periods of high temperature result in lower NR activity, although the magnitude of inactivation varies according to species (96). In some cases, lower than optimal temperatures afford some protection to the inactivation of NR activity (143). Onwueme _et al._ (143) reported a protective effect of low temperature against dark inactivation of leaf NR in barley. Alofe _et al._ (144) studied the effects of high day temperatures (35°C) and different night temperatures (7,16, and 24°C) on NR activity of 16 genotypes of maize under controlled environment conditions. They observed that NR activity was much lower at the high night temperature. Deane-Drummond _et al._ (25) observed that both _in vitro_ and _in vivo_ NR activity in roots of barley were higher when roots were grown at 10°C with the shoot at 20°C compared with whole plants grown at 20°C. This apparent adaptive response may be relevant to field situations in which soil temperatures are generally lower than air temperatures. The contribution of the root system to nitrate assimilation of the whole plant may need to be reassessed.

In many cases, high temperature stresses may be associated with water stress (145), so that it is difficult to ascertain whether temperature or water stress is responsible for the observed decreases in NR activity. Decreases in water potential below -4 to -2 bars causes NR activity to decrease in barley (145) and maize (147,148).

Relationship of NR to Grain Protein and Yield.

Because NR is thought to be the rate-limiting enzyme in nitrate assimilation (76), Hageman and others have studied

the relationship between NR activity and grain yield and protein in cereals. Hageman (146) recently reviewed several experiments conducted in his laboratory (149-152) in which NR activity was shown to be correlated with either yield or protein in wheat or maize. In other laboratories (153-157) a positive relationship has also been demonstrated. Less successful results have been obtained by others (158-160).

It is not surprising that some of the results have been variable when one considers the complexity of the regulation of NR. Several environmental factors, nitrate availability, and the supply of reductant (NADH) in leaf tissue are some of the factors which participate in the complex regulation of NR in leaf tissue. A highly positive correlation between NR activity and yield will not likely be observed except in those environments in which nitrogen is the factor most limiting yield.

TRANSPORT OF REDUCED NITROGEN

Much of the reduced N in a plant must be transported to other cells at least once during the life cycle of a plant. As we noted earlier, nitrate may be reduced in roots or other non-green tissues and in leaves. The site of nitrate reduction influences the transport of reduced N. Although the xylem is the principal path for long-distance transport of nitrogenous solutes and roots to organs that transpire (47,161-164), there are reported exceptions. In Phaseolus vulgaris upward transport of ^{15}N has been reported in the xylem and phloem (165). Joy and Antcliff (166) found that readily soluble organic N solutes may be transported upward in the phloem of Beta vulgaris. The principal path for export of reduced N from leaves is the phloem (161,162,167,168).

Leaves and other vegetative tissues do not export all the reduced N produced during nitrate reduction. Instead they store considerable amounts of proteins (169) which

become important sources of reduced N during later stages of grain-fill in many species (169-173). Remobilization of these proteins becomes important because nitrate reduction is usually insufficient to meet the demands of the developing seeds for reduced N during this period (146). One of the reasons for this deficit of reduced N is the inadequate supply of nitrate in leaves to provide saturating levels of substrate (nitrate) for leaf NR (149,152).

Composition of Xylem and Phloem Saps in Plants Grown on Nitrate.

According to Pate (162,174), N solutes frequently comprise the major component of dry matter in xylem sap, and are second only to carbohydrates in the phloem sap. The C:N weight ratio in xylem sap ranges from 1.5 to 6, whereas the ratio in phloem sap ranges from 15 to 200.

The composition of xylem sap varies considerably from species to species (47,162,174). The ratio of organic N:nitrate-N also varies widely because species differ in their ability to assimilate nitrate in the roots (47,162,164). Over 95% of the xylem N may consist of nitrate in some species that have low NR activity in their roots (e.g., Cucumis, Xanthium and Gossypium) whereas other species with high root NR activity (e.g., Pisum, Raphanus and Lupinus) have less than 20% of the xylem N as nitrate-N (47,162). Reduced N in roots is incorporated into a limited number of amino acids, amides and other solutes for transport to the shoot, but each plant species seems to have a characteristic spectrum of these N compounds (47,164,174). The most common N solutes in plants grown on nitrate are aspartate and glutamate and their amides (47,164,174). Ureides and other solutes play an important role in xylem transport in certain species grown on nitrate (175), but ureides are most important as a transport solute in certain tropical legumes that are dependent on nitrogen fixation for much of their reduced N

(176,177). Ureide assimilation will therefore not be discussed here.

The types of N solutes found in the phloem sap also vary from species to species, but because of the difficulties of collecting phloem sap from many species, the contents of only a limited number of species have been examined (162,174). In contrast to the xylem, N solutes in the phloem are organic solutes (162,174). Nitrate is usually absent from, or present in only trace amounts in the phloem (161,178,179). Asparagine was highest in the phloem of the legume Lupinus (179). Frequently the glutamine/glutamate and asparagine/aspartate fractions are both high, as observed in Ricinus phloem exudate (180). Serine, however, was found to be the amino acid exported from young soybean leaves in the greatest quantity (181). Later studies showed that aspartate, glutamate, alanine, and gamma-aminobutyric acid were also transported in significant amounts in soybean petioles (182,183). Amides also appear in the phloem sap of some species at the time of senescence (184,185) and are considered to result from ammonium production often accompanying protein hydrolysis (186).

Di- and oligopeptides comprise an additional class of nitrogenous compounds that may be important in transport. Duke et al. (187) reported large quantities of peptides in the axes of developing soybean seedlings, suggesting that peptide transport may be occurring. Higgins and Payne (188,189) have demonstrated active transport of dipeptides and oligopeptides up to five amino acids in length into the scutellum of germinating barley embryos. They suggest that in view of the energetics of membrane transport, a plant can conceivably transport a smaller number of peptide molecules more efficiently than an equivalent quantity of free amino acids. If the phloem of leaf tissue is also capable of loading and translocating peptides, these compounds may be an important form of

N transport in the mature plant. This mechanism may be particularly important during senescence when high rates of protein hydrolysis occur in the leaves.

Phloem loading and Transport of Nitrogenous Solutes.

Housley et al. (168) verified that organic N is exported via the phloem from soybean leaves. A petiole was heat girdled, disrupting phloem but not xylem functioning. The attached leaf was exposed to $^{14}CO_2$ for 2 hours, but no radioactive sugars or amino acids moved out of the fed leaf, thus demonstrating the importance of the phloem in exporting amino-N from leaves. These results were consistent with earlier studies (190,191).

Subsequent studies of amino acid export from soybean leaves followed the transport and conversions of photosynthetically-derived ^{14}C-amino acids (182). Only five of the amino acids in the transport pathway contained substantial amounts of ^{14}C after a 2-hour exposure of a source leaf to $^{14}CO_2$. The distribution of ^{14}C in aspartate and gamma-aminobutyrate was much higher in the path than in the source leaf, whereas serine, alanine and glutamate were substantially labeled in both the source leaf and the path. The total percentage of recovered ^{14}C in amino acids also differed, with 8 to 17% in leaf amino acids compared to only 2 to 6% in the petiole amino acids. These findings suggest some selectivity in the efflux processes from mesophyll cells into the apoplast, and/or in the loading processes. In contrast, Housley et al. (168) applied several amino acids individually to an abraded spot on a mature leaf, and found that amino acids not typically occurring in the phloem (lysine and leucine) were loaded as readily as those such as serine which are common in the phloem. Transport velocities for these amino acids also were similar to those obtained for ^{14}C-photosynthates produced by feeding a similar leaf with $^{14}CO_2$ (168). Servaites et al. (192) found that the rates of

transport of ^{14}C-amino acids applied to an abraded spot on the leaf were similar to those of sucrose, and were not dependent on the nature of the functional group of the amino acid (i.e., leucine [neutral] vs. glutamate [dicarboxylic] vs. gamma-aminobutyrate [lacking alpha-amino group]). Sucrose neither inhibited nor enhanced the loading of leucine when applied with leucine to the abraded spot (192) suggesting separate and independent carriers. On the basis of these studies, the observed selectivity noted earlier (182) could be effected in the process by which amino acids are transferred from the mesophyll cells into the apoplast for loading.

Little is known about the mechanism of phloem loading of amino acids. For example, what is the phloem's capacity for transporting N solutes? Is active transport involved? Are specific carriers involved in loading each amino acid, or does a less specific carrier exist for a group of related amino acids?

The possible involvement of carriers for amino acid uptake has been implicated in studies of tissues and cells other than those of the phloem. Amar and Reinhold (193) reported that amino acid uptake by bean leaves was severely reduced by osmotic shock or EDTA, suggesting that a protein involved in transport was released from the membranes. Absorption studies also suggested the existence of amino acid carriers since uptake rates could be saturated with increased concentrations in the surrounding medium (194). This uptake pattern had two saturation phases, as did those of five other amino acids in similar experiments using cultured tobacco cells (195). These biphasic responses indicate multiple carriers, which were examined by Berlin and Mutert (195). Three separate carriers, for neutral, basic and acidic amino acids, were proposed on the basis of competition studies. Further evidence indicated that neutral amino acid loading took place via all three carriers, that acidic amino acids

could be loaded on both the acidic and basic carriers, and that only basic amino acids were specifically transported by their own carrier (195). This theory is also supported by earlier work (196) on cultured soybean root cells. Similar competition studies, again between neutral, basic, and acidic amino acids, suggested at least three amino acid uptake carriers. The properties of such amino acid carriers have been examined in non-plant tissues (197), but evidence for such systems is scarce in plants, and especially for the phloem.

The involvement of a carrier, or carriers, in phloem loading of amino acids and sucrose in soybean leaves was suggested by the concentration dependence of sucrose and leucine loading and transport (192). The amount of leucine or sucrose loaded and transported increased as the concentration applied to the abraded spot was increased, until saturation levels were reached. The uptake of ^{14}C-sucrose from the free space of an abraded spot followed a biphasic saturation pattern and was similar to that observed in several other tissues (198). Although the amino acid leucine also showed saturation responses typical of carrier-mediated transport, the uptake pattern was triphasic rather than biphasic (192). The apparent K_m's for the leucine uptake phases were 3, 21, and 52 mM, respectively, compared to apparent K_m's of 35 and 100 mM for sucrose. The existence of distinct carrier systems for leucine and sucrose was further demonstrated by competition studies. When ^{3}H-leucine and ^{14}C-sucrose were supplied in a 1:4 concentration ratio to an abraded spot, the transport characteristics of leucine remained unchanged. Sucrose at 100 mM (about five times more concentrated than would be expected in the apoplast) also had no effect on the loading of 10 mM leucine (192). The significance of the three phases for leucine absorption must yet be determined, but they do indicate different carrier systems for sucrose and amino acid loading.

Furthermore, these data suggest that the carrier for leucine has a higher affinity than the one for sucrose.

In soybean leaves, both amino acid and sucrose loading were inhibited by the presence of the uncouplers DNP (2,4, dinitrophenol) and CCCP (carbonylcyanide-M-chlorophenyl hydrazone) (192). This suggested that phloem loading or transport of amino acids is metabolically dependent. The involvement of sulfhydryl groups in phloem loading of amino acids was also shown by using PCMBS (p-chloromercuribenzene sulfonic acid) which complexes with sulfhydryl groups (199). Applications of high concentrations of KCl inhibited both amino acid and sucrose loading by approximately 50% in soybeans. These effects of potassium concentration and the inhibition of amino acid loading by uncouplers are consistent with the model proposed for K^+- and H^+-mediated transfer of sucrose (200) and amino acids (201). According to this model, solute movement into the phloem is mediated by specific carriers and occurs with simultaneous co-transport of a proton which partially neutralizes the proton gradient across the sieve tube plasmalemma (200). To re-establish the protein gradient, ATP is hydrolyzed by a plasmalemma ATPase and protons are extruded from the sieve tube into the free space. High concentrations of KCl and uncouplers could dissipate the proton gradient, and thus inhibit loading.

Transport of Nitrogen as a Possible Limiting Factor for Productivity.

In some crop species, such as soybeans, the demand for reduced N in developing seeds is extremely high (202). Soybean seeds contain 6 to 8% N at maturity. At 7% N in the seeds, a seed yield of 3,360 kg hectare^{-1} (50 bushels acre^{-1}) requires 235 kg N hectare^{-1} (210 lb N acre^{-1}) for seed alone. If the nitrogen harvest index is 0.67, 353 kg N hectare^{-1} (315 lb N acre^{-1}) are required for the production of aboveground (seed + stover) plant parts. Most of

the N in the seed is in protein. Because protein is about 16% N, protein constitutes from 37.5 to 50% of the dry weight of seeds containing 6 to 8% N, respectively. To meet the heavy demand for N during seed development, Sinclair and deWit (203) estimated that up to 9 kg N hectare^{-1} day^{-1} must be transported to the seeds. This demand exceeds the capacity of nitrogen fixation to provide reduced N, and may exceed the capacity of the plant's transport system to the seeds. Thus more information is needed on the properties of the carriers and mechanisms by which amino acids and other nitrogenous assimilates are transported to the seeds.

Although sucrose is the predominant solute in the phloem of most plant species, the following calculations will emphasize the importance of N transport during seed development of crops such as soybeans. Penning deVries (204) estimated that 1 gram of photosynthate (glucose-equivalent) can produce 0.83 g of carbohydrate, 0.33 g of lipid, 0.46 g of lignin, or 0.62 g of nitrogenous compounds (when ammonia-N is provided). Less protein can be produced per unit of photosynthate when nitrate is provided, but the value for ammonia-N will be used here because reduced N is transported to seeds. On the basis of these estimates, 1.74 grams of photosynthate will be required to produce a gram of soybean seed of the composition shown (Table 1). On the assumption that sucrose provides the carbon for energy production, and for carbohydrate, lignin, and lipid synthesis; and that amino acids provide the carbon skeletons for protein synthesis, amino acid transport would provide 0.42 g of dry matter (42% protein by dry weight) and sucrose transport would provide the balance (1.74 g minus 0.42 g = 1.32 g sucrose) of the photosynthate used for production of dry matter and energy. Therefore about three times as much carbon would be provided by sucrose as compared to amino acids. However, sucrose has 12 carbons per molecule whereas the

Table 1. Photosynthate requirements for production of soybean seeds.

Composition of soybean seed*	g photosynthate/g of seed
42% protein	0.677
20% lipid	0.606
31% carbohydrate	0.373
4% lignin	0.087
3% ash	----
100%	1.743

* Based on values used in (203).

average amino acid has about four carbon atoms. Hence one would expect one amino acid molecule for every sucrose molecule in the phloem during seed development. This would provide for a C:N ratio of 16:1 in the phloem of the transport pathway between vegetative tissues and developing fruits. These estimates would be modified somewhat for those species in which amides or ureides play an important role in transporting reduced N to the seeds.

Based on these assumptions and calculations, it seems conceivable that transport of reduced N to seeds could limit productivity. The frequently observed failure of foliar-applied fertilizers to increase soybean yields (205,206) could be due to a limited capacity of the plants to transport N to the seeds from leaves and other vegetative tissues. The concentration of N, P, and K increased in response to foliar applications of these elements (206,207), but yields were not increased (206).

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SUMMARY

Nitrate uptake, assimilation and transport are extremely important processses for most crop species. Nitrogen frequently limits productivity, and therefore any improvements in the efficiency with which nitrate is absorbed and assimilated may result in higher protein and/or higher yields of these crops. These processes are complex and there are several control points at which regulation could be effected: nitrate uptake; transport of nitrate; accumulation of nitrate; remobilization of stored nitrate; reduction of the nitrate; transport of reduced N; and remobilization of stored N. Many, if not all, of these processes or phenomena are under genetic control. Hence one should be able to select for variability for these traits and then incorporate the desirable traits into new or existing genotypes to produce genotypes that are more efficient in utilization of nitrate-N.

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1

Table 1. Calculation of the potential daily productivity by a crop surface receiving 500 cal/cm^2 day of insolation. From *Loomis* and Williams (36).

Total solar radiation per day	500 cal/cm^2
Visible radiation, 400-700 mμ	222 cal/cm^2
Total quanta, 400-700 mμ (8.64 Einsteins/cal)	4320 μ Einsteins/cm^2
Albedo loss	-360 μ Einsteins/cm^2
Inactive absorption loss	-432 μ Einsteins/cm^2
Total quanta, 400-700 mμ, available for photosynthesis	3528 μ Einsteins/cm^2
Amount of carbohydrate (CH_2O) produced (ϕ = 10)	353 μ moles/cm^2
Respiration loss	-116 μ moles/cm^2
Net production of carbohydrate (CH_2O)	237 μ moles/cm^2
Net production (30 g/mole (CH_2O))	71 g/m^2 (14μg/cal)

It is assumed that: Moon's average sky condition represents the spectral composition of sunlight; albedo losses equal 6, 12, and 6% of the 400-510, 510-610, and 610-700 mμ spectral regions, respectively; inactive absorption is nonspecific and equals 10% of the incident visible light 400-700 mμ; net light transmission is zero; 10 quanta are required to reduce each molecule of CO_2, and respiration equals 33% of photosynthesis.

If inorganic nutrients comprise 8% of the dry weight, then 71/0.92 = 77 g dry matter/m^2 day (15 μ g/cal) is the potential net productivity. This corresponds to: 770 kg/ha day (690 lb/A day, or about 34 T/A in 100 days).

POTENTIAL PRODUCTIVITY BASED ON CO_2 FIXATION

ASSUME CROP FIXES CO_2 AT 45 MG CO_2 DM^{-2} HR^{-1}

IF 1/3 GOES TO RESPIRATION, NCE =

30 MG CO_2 DM^{-2} HR^{-1}

FOR A 12 HR. DAY, 30 X 12 = 360 MG CO_2 DM^{-2} DAY^{-1}

$$\frac{CH_2O}{CO_2} = \frac{30 \text{ G MOLE}^{-1}}{44 \text{ G MOLE}^{-1}} \times 360 \text{ MG } CO_2 = 246 \text{ MG } CH_2O \text{ DAY}^{-1}$$

IF INORGANIC NUTRIENTS COMPRISE 8% OF DRY WT,

THEN 246/0.92 = 267 MG DRY WT DM^{-2} DAY^{-1}

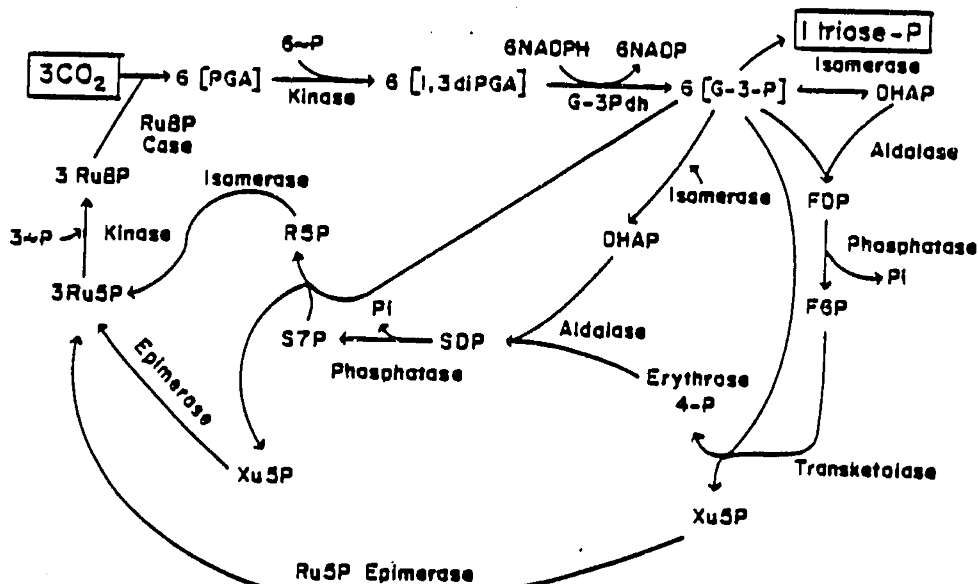
FOR 50 DAYS GRAIN-FILL PERIOD:

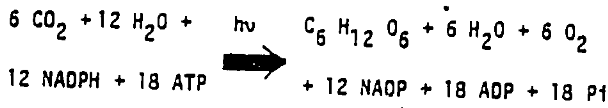
267 X 50 = 13,350 MG DRY WT DM^{-2}

= 13,350 KG DRY WT HA^{-1}

IF LAI = 3, 13,350 X 3 = 40,050 KG DRY WT HA^{-1}

IF LAI = 4, 13,350 X 4 = 53,400 KG DRY WT HA^{-1}





So 2 NADPH + 3 ATP per CO_2 fixed

2

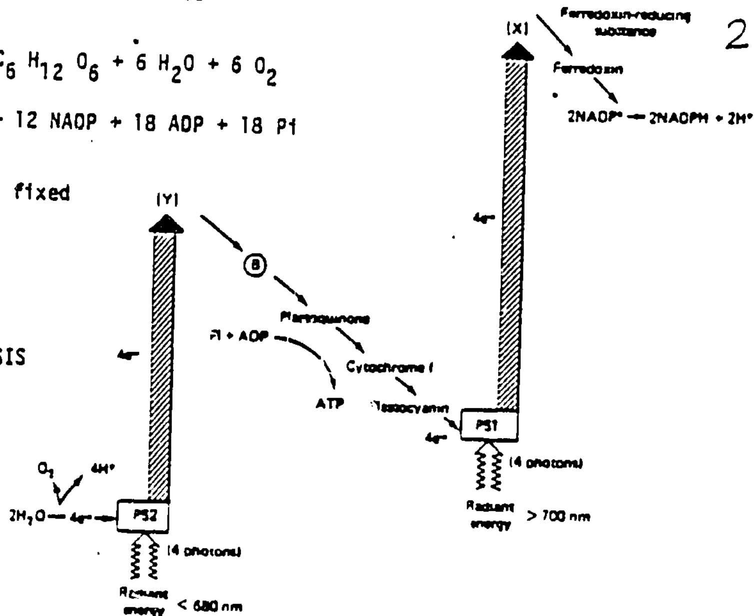
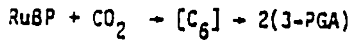


Figure 11. Reaction scheme for the formation of ATP and reduced NADP during photosynthesis. Two pigment systems, photosystem 1 (PS1) and photosystem 2 (PS2), play a role in photosynthetic energy transport, photophosphorylation, and the formation of reduced NADP. Photosystem 1 is composed of several forms of chlorophyll with the major component a special aggregation of chlorophyll-a and protein known as chlorophyll P700. Radiant energy with wavelengths of 700 nm or longer excite PS1. Photosystem 2 also contains several forms of chlorophyll. Chlorophyll-b appears to be present as well as some chlorophyll-a, both in close association with protein. Photosystem 2 is excited by radiant energy of wavelengths less than 680 nm. Electrons e^- extracted from water pass through a series of intermediates identified in the figure as (Y), plastoquinone, cytochrome-b_6, cytochrome-f, plastocyanin, (X), ferredoxin, and ferredoxin-NADP reductase. The identities of (Y) and (X) are uncertain at this time. Some of the energy of the excited electrons in passing through the above intermediates generates ATP.

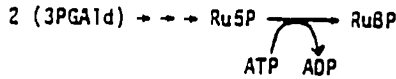
THREE PHASES OF PHOTOSYNTHESIS

1. CARBOXYLATIVE PHASE
2. REDUCTIVE PHASE
3. REGENERATIVE PHASE

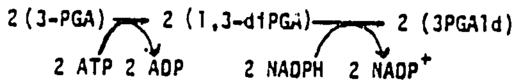
CARBOXYLATIVE PHASE:

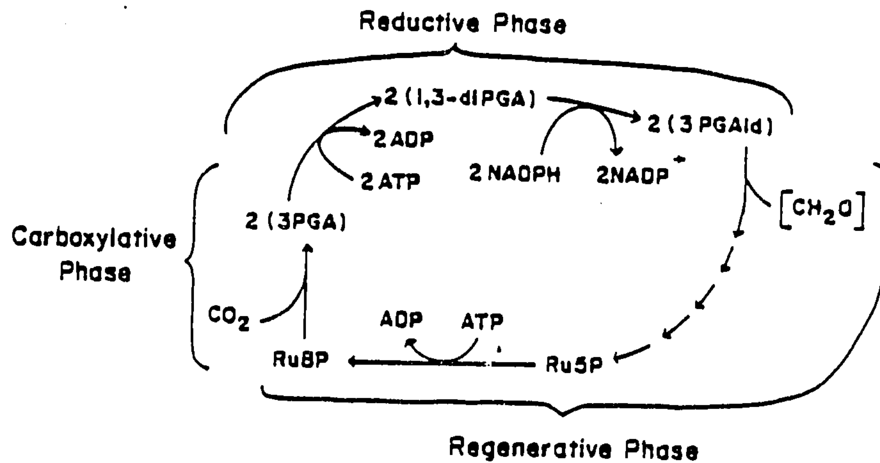


REGENERATIVE PHASE:



REDUCTIVE PHASE:





Per CO_2 fixed:	ATP	NADPH
Reductive Phase	2	2
Regenerative Phase	1	0
Total	3	2

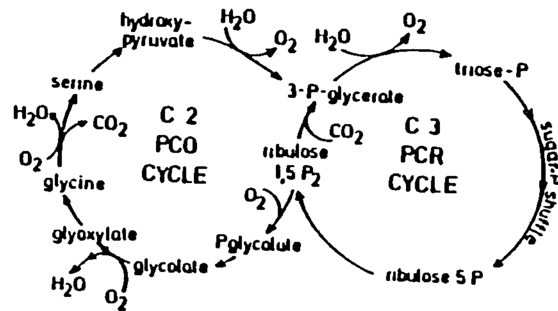


Figure 1 Integration of the C-3 PCR and C-2 PCO cycles is accomplished by the carboxylation or oxygenation of RuBP. The C-3 PCR cycle is capable of independent operation. However, the C-2 PCO cycle is parasitic, being dependent upon the C-3 PCR cycle to regenerate RuBP from 3-P-glycerate. At the CO_2 compensation point this system constitutes what may be the first recorded instance of a futile bicycle. Under natural atmospheres, it more closely resembles a penny-farthing.

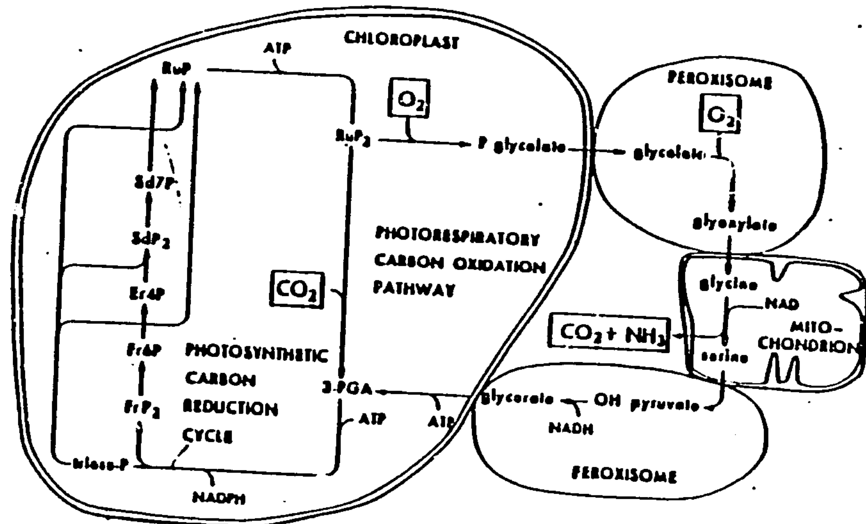


Fig. 1. Nonstoichiometric reaction sequence in the integrated photosynthetic carbon-reduction cycle and photorespiratory carbon-oxidation pathway, showing location of reactions in chloroplasts, peroxisomes and leaf mitochondria. 3-PGA, 3-phosphoglycerate; RuP_2, ribulose 1,5-bisphosphate; RuP, ribulose 5-phosphate; Fr6P, fructose 6-phosphate; FrP_2, fructose 1,6-bisphosphate; triose-P, triose phosphate; P glycolate, phosphoglycolate; Er4P, erythrose 4-phosphate; Sd7P, sedoheptulose 7-phosphate; SdP_2, sedoheptulose 1,7-bisphosphate.

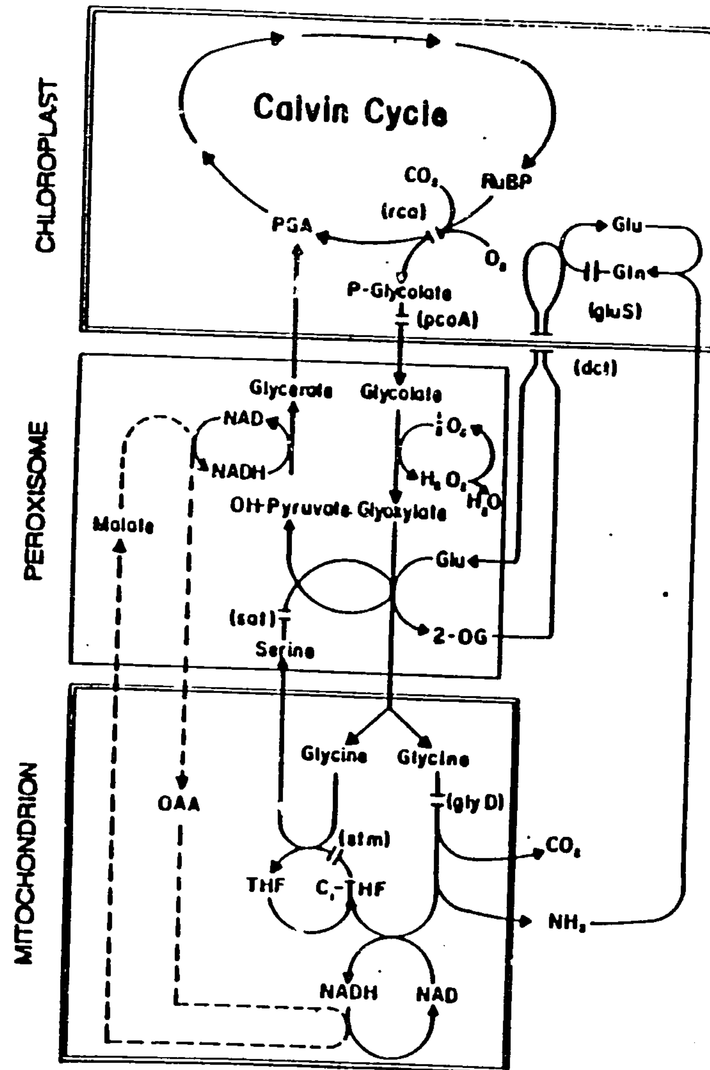


Fig. 1. C3 photosynthesis and the photorespiratory cycle. Mutants with defects in the following enzymes have been characterized: P-glycolate phosphatase (pcoA), glycine decarboxylase (glyD), serine transhydroxymethylase (stm), serine-glyoxylate aminotransferase (sat), glutamine synthase (gluS), chloroplast dicarboxylic acid transport (dct), and Rubi[illegible] activation (rca).

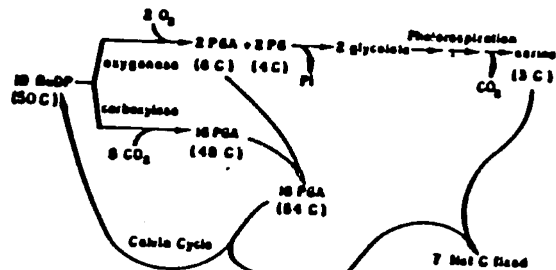


Figure 1. Influence of atmospheric conditions (approximately 240 μM O_2 and 10 μM CO_2 in solution at 25°C) on photosynthesis and photorespiration (modified from Laing et al., 1974). The following abbreviations are used in Figures 1 to 4: *RuDP*, ribulose 1,5-diphosphate; *PGA*, 3-phosphoglycerate; *PG*, phosphoglycolate; *Pi*, inorganic phosphate.

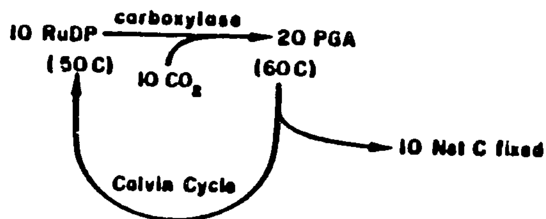


Figure 2. Influence of oxygen-free air and 10 μM CO_2 on photosynthesis and photorespiration (modified from Laing et al., 1974).

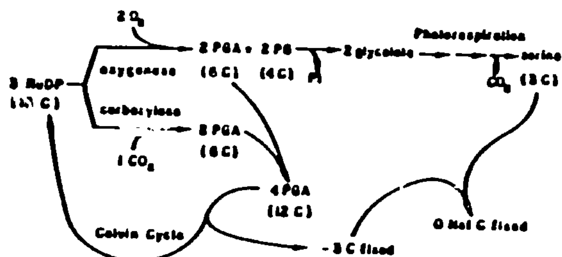


Figure 3. Influence of CO_2 compensation point conditions on photosynthesis and photorespiration.

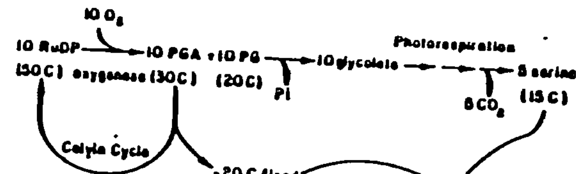


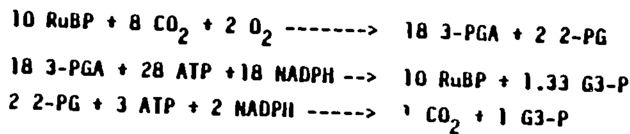
Figure 4. Influence of CO_2-free air and 240 μM O_2 on photosynthesis and photorespiration.

Table 1. Influence of O_2 and CO_2 concentration on RuDP carboxylase to oxygenase ratio, net fixation of carbon, and CO_2 evolved per RuDP

Treatment	Ratio of carboxylase to oxygenase	Net C fixed per RuDP utilized	CO_2 evolved per RuDP
Air (control)	4:1	0.7	0.1
No O_2; normal CO_2	5:0	1.0	0
CO_2 compensation pt.	1:2	0	0.33
No CO_2; normal O_2	0:5	−0.5	0.5

Figures & Table 1 are from Schrader, L.E. 1978. CO_2 Metabolism and Productivity in C_3 Plants: An Assessment. IN: R.H. Burris & C.C. Black. CO_2 Metabolism and Plant Productivity. Univ. Park Press, Baltimore

Under normal atmospheric conditions,


10 RuBP + 8 CO_2 + 2 O_2 -------> 18 3-PGA + 2 2-PG

18 3-PGA + 28 ATP + 18 NADPH --> 10 RuBP + 1.33 G3-P

2 2-PG + 3 ATP + 2 NADPH -----> 1 CO_2 + 1 G3-P

---

7 CO_2 + 31 ATP + 20 NADPH ----> 2.33 G3-P

1 CO_2:4.4 ATP:2.9 NADPH

C_3 photosynthesis requires 1 CO_2:3 ATP:2 NADPH

So photorespiration requires 1.4 ATP & 0.9 NADPH per CO_2 fixed.

Reprinted from Nature, Vol. 280, No. 5725, pp. 833-836, August 30 1979

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A phosphoglycolate phosphatase-deficient mutant of *Arabidopsis*

C. R. Somerville & W. L. Ogren*

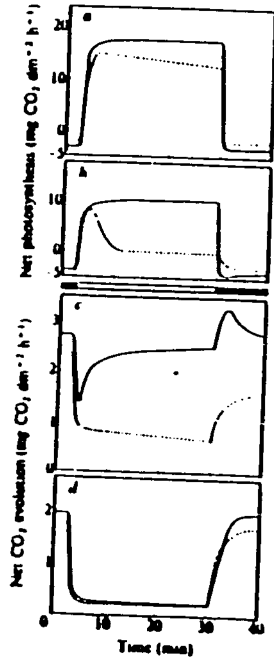

Fig. 1 Net CO_2 exchange of wild-type and mutant *Arabidopsis* in various gas regimes. Plants were grown in 1% CO_2, 21% O_2 and bal. N_2 at 25 °C and 75% RH under continuous fluorescent illumination (170 μ einsteins $m^{-2} s^{-1}$) on vermiculite irrigated with half-strength Hoagland's solution. Gas exchange was monitored on intact plants (at floral initiation stage) from which the lower leaves had been removed. The plants were placed in a glass chamber of 20 ml volume and continually flushed with a humidified (75% RH) gas stream at a flow rate (100–150 ml min^{-1}) sufficient to maintain a ΔCO_2 of less than 15 μl l^{-1}. The CO_2 concentration of the exiting gas was continuously monitored with an IR gas analyser. The total volume of the system, including measuring cuvette, was 46 ml. Light (300 μeinsteins $m^{-2} s^{-1}$) was provided by a 300 W tungsten lamp filtered through 15 cm of water. The plant chamber was maintained at 25 °C by immersion in circulating water. The composition of gas entering the chamber was: *a*, 355 μl l^{-1} CO_2, 2.0% O_2, bal. N_2; *b*, 352 μl l^{-1} CO_2, 21% O_2, bal. N_2; *c*, 50% O_2, bal. N_2; *d*, 2% O_2, bal. N_2. The response of the wild-type plants is indicated by the solid line, and that of the mutant by the broken line. The open or closed bar inserted in the figure represents light or dark conditions, respectively.

Table 1 Products of $^{14}CO_2$ assimilation by wild type and mutant *Arabidopsis*

	Wild-type	CS119	Wild-type HBA-treated*	CS119 HBA-treated*
Basic fraction	21.4†	11.0	3.2	7.6
Glycine	10.5	0.5	0.3	0.4
Serine	8.4	0.9	0.3	0.5
Alanine	2.0	9.0	1.4	5.9
Neutral fraction	6.1	14.8	3.6	9.3
Acid-1 fraction	36.7	23.3	61.7	23.9
Glycolate	2.7	0.9	28.8	1.8
Acid-2 fraction	8.8	29.1	6.2	28.1
Phosphoglycerate	6.3	5.6	3.8	7.8
Phosphoglycolate	0.7	19.6	0.7	19.6
Acid-3 fraction	24.4	17.6	23.7	27.4
Recovery	97.4	95.8	98.4	96.3

Intact plants, grown as described in Fig. 1, were removed to a photosynthesis chamber and flushed with 400 μl l^{-1} CO_2, 21% O_2 and bal. N_2 at 25 °C, 75% relative humidity (RH), at 300 μeinsteins $m^{-2} s^{-1}$ for 2 min. At this time the system was closed and $^{14}CO_2$ introduced. After 2 min of $^{14}CO_2$ incorporation, the plants were quickly (3–5 s) removed to liquid nitrogen, then extracted by grinding in 4 M formic acid. The CO_2 concentration in the chamber remained at 350–400 μl l^{-1} during the period of incorporation. The extract was lyophilised and the residual water-soluble compounds were fractionated by ion exchange and paper chromatography as described previously[8] except that the acid fractions were recovered from a Dowex-1 (formate) column by successive elution with 10 ml 4 M formic acid (acid-1 fraction), 10 ml 8 M formic acid (acid-2 fraction) and 6 ml 4 M HCl (acid-3 fraction). Phosphoglycolate was identified by co-migration with authentic samples in several solvent systems, before and after phosphate ester hydrolysis.

* Inhibition of in vivo glycolate oxidase activity was accomplished by submerging the roots of intact plants in 5 mM 2-hydroxy-3-butynoic acid (HBA)[16] for 20 min in the elevated CO_2 regime described in Fig. 1. Following treatment, the plants were labelled, extracted and fractionated as above. Control experiments, in which identically treated wild-type and mutant plants were assayed for glycolate oxidase activity, indicated that the HBA treatment resulted in >95% inhibition of enzyme activity.

† Values indicate % of total water-soluble counts recovered, and represent the mean of two independent experiments.

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Inhibition of photosynthesis in *Arabidopsis* mutants lacking leaf glutamate synthase activity

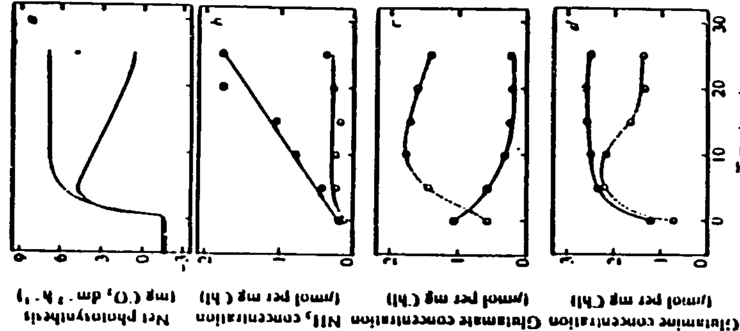

Fig. 1 Changes in *a*, photosynthesis rate and levels of *b*, NH_3, *c*, glutamate; and *d*, glutamine during illumination of wild-type and mutant *Arabidopsis* in photorespiratory conditions. Plants were grown in a 1% CO_2 atmosphere[10] on an artificial medium irrigated with a solution containing 2.5 mM KH_2PO_4 (pH 6.5), 5 mM KNO_3, 2 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$ and micronutrients. The plants were placed in darkness in standard atmospheric conditions several hours before use to allow equilibration of metabolite pools. The plants were then placed in a photosynthesis chamber at 25 °C and continuously flushed with a gas stream (357 μl l^{-1} CO_2, 50% O_2, balance N_2) which exited through an infrared gas analyser. At time zero, the plants were illuminated at 300 [illegible] $m^{-2} s^{-1}$. At the indicated intervals, plants were removed from the chamber and the leaf material ground in 2 ml chloroform/methanol/water (5:12:1). An additional 1.5 ml of chloroform and 1 ml of water was added, and the phases separated by centrifugation. Chlorophyll was determined on an aliquot of the organic phase. NH_3 was assayed in an aliquot of the aqueous phase by a colorimetric procedure[11]. Amino acid concentrations were determined with a Beckman (Model-119CL) amino acid analyser. Mutant and wild-type averaged 1.8 mg chlorophyll per g fresh weight. The response of the *gluS* mutant strain CS113 is indicated by the solid lines and symbols (●) and that of the wild type by broken lines and open symbols (○). Chl, chlorophyll.

Nature Vol. 291 513-515, 11 June 1981

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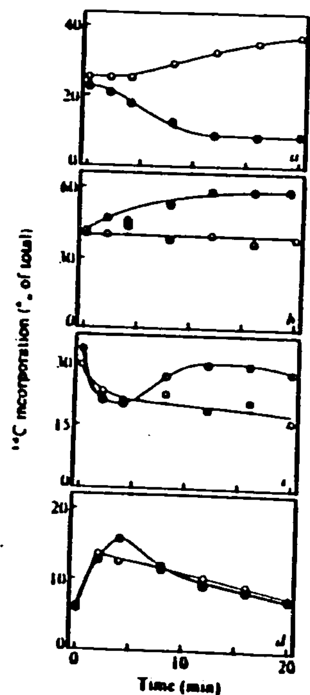


Fig. 2 Products of $^{14}CO_2$ assimilation by wild-type and mutant *Arabidopsis*. Intact plants were placed in a photosynthesis chamber and flushed with a humidified gas stream containing 350 μl l^{-1} CO_2, 21% O_2, balance N_2. At time zero the plants were illuminated (300 μeinstein m^{-2} s^{-1}). At the indicated intervals the system was closed and $^{14}CO_2$ introduced. After 2 min the plants were quickly (3-5 s) removed to liquid nitrogen. The labelled products were extracted and separated as described previously[16]. The CO_2 concentration in the chamber remained between 300 and 350 μl l^{-1} during the period of incorporation. *a*, Basic fraction (amino acids); *b*, acid-1 fraction (organic acids and sugar monophosphates); *c*, combined acid-2 and acid-3 fractions (mostly sugar diphosphates); *d*, neutral fraction (sugars). The response of the mutant strain CS113 is indicated by solid symbols and that of the wild type by open symbols.

Table 1 Specificity factor and component kinetic constants for RuBP carboxylase/oxygenase purified from several species

Species	Specificity factor ($V_c K_o / V_o K_c$)	K_c (μM)	K_o (μM)	V_c/V_o (ratio)
C_3 plants				
Glycine max	82 ± 5	9	430	1.7
Tetragonium expansa	81 ± 1	13	600	1.8
Spinacea oleracea	80 ± 1	14	480	2.3
Lolium perenne	80 ± 1	16	500	2.6
Nicotiana tabacum	77 ± 1	11	650	1.3
C_4 plants				
Amaranthus hybridus	82 ± 4	16	640	2.0
Zea mays	78 ± 3	34	810	3.3
Green algae				
Scenedesmus obliquus	63 ± 2	38	660	3.6
Chlamydomonas reinhardii	61 ± 5	29	480	3.7
Euglena gracilis	54 ± 2	25	410	3.3
Cyanobacteria				
Aphanizomenon flos-aquae	48 ± 2	105	990	5.1
Coccochloris peniocystis	47 ± 2	121	1,220	4.7
Photosynthetic bacteria				
Rhodospirillum rubrum	15 ± 1	89	406	3.3
Rhodopseudomonas sphaeroides II	9 ± 1	80	220	3.3
Rhodopseudomonas sphaeroides I	62 ± 4	36	840	2.7

Enzymes were purified and specificity factors were determined as described in Fig. 1 legend. For K_c and K_o determinations, the proteins were preincubated at 4 °C for 4 h in 10 mM $NaH^{14}CO_3$ (2 Ci mol^{-1}), 10 mM $MgCl_2$ and 50 mM Bicine at pH 8.5. Assays (30 s) were initiated by adding 100 μg or less of activated protein in 20 μl to reaction mixtures at 25 °C containing 0.40 mM RuBP, 10 mM $MgCl_2$ and 50 mM Bicine at pH 8.22 or 8.30 in a total volume of 1.0 ml. $NaH^{14}CO_3$ concentrations varied between 0.7 and 15 mM, and O_2 concentrations were 0, 0.62 and 1.24 mM. The reactions were stopped by adding 0.5 ml of 3M formic acid in methanol. After drying the samples, $^{14}CO_2$ incorporation was determined by scintillation spectroscopy. K_c was estimated from a Scatchard plot; K_o, determined as the $K_i(O_2)$, was estimated from Dixon plots and from secondary plots of $K_c(O_2)$ (apparent) as a function of CO_2 concentration. Lines were fitted by least-squares analysis. V_c/V_o was calculated from the relationship: V_c/V_o = specificity factor × (K_c/K_o). All experiments included *S. oleracea* enzyme as a control. Standard errors in the specificity factor determinations are listed. The mean standard error for K_c was ±7% of the value, and ±12% for K_o.

7

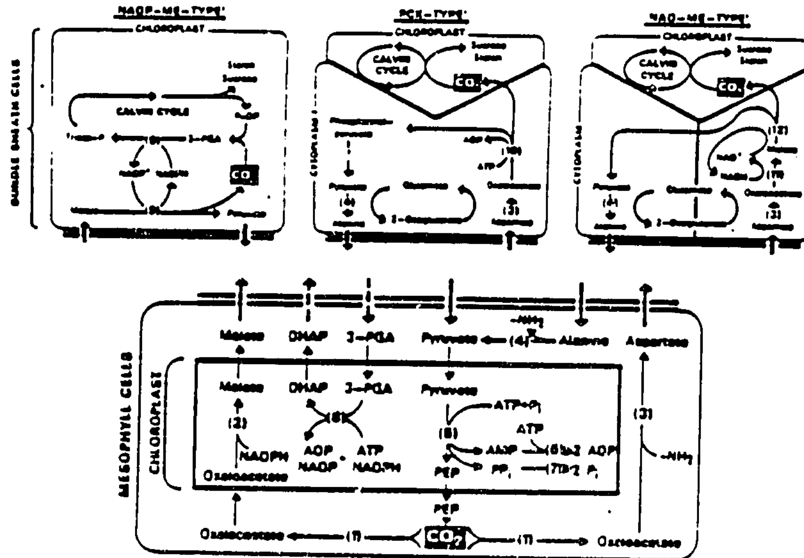


Figure 4. Models for the mechanism of C_4 pathway photosynthesis in mesophyll (*lower*) and bundle sheath cells (*upper*). Enzymes are: (*1*) PEP carboxylase; (*2*) NADP malate dehydrogenase; (*3*) aspartate aminotransferase; (*4*) alanine aminotransferase; (*5*) pyruvate P_i dikinase; (*6*) adenylate kinase; (*7*) pyrophosphatase; (*8*) 3-PGA kinase, NADP glyceraldehyde-3-P dehydrogenase and triose-P isomerase; (*9*) NADP malic enzyme; (*10*) PEP carboxykinase; (*11*) NAD malate dehydrogenase; and (*12*) NAD malic enzyme.

metabolite transport in spinach chloroplasts

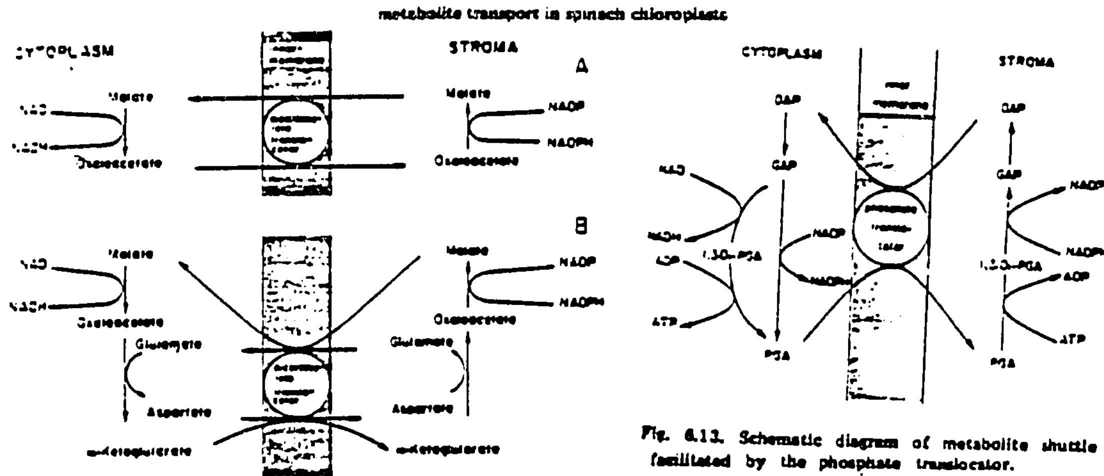


Fig. 6.13. Schematic diagram of metabolite shuttle facilitated by the phosphate translocator.

Fig. 6.12. Schematic diagram of metabolite shuttle facilitated by the dicarboxylate translocator.

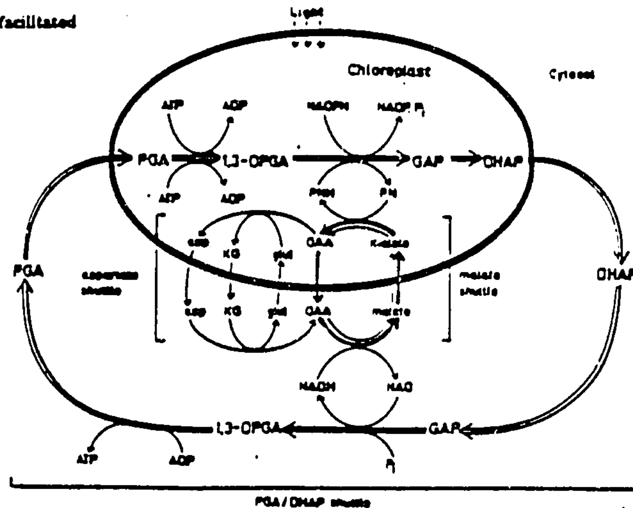


Fig. 5.6. Schematic representation of metabolite transfer between chloroplasts and cytosol designed to export ATP from chloroplasts in the light. Shuttle transfer of dihydroxyacetone phosphate and phosphoglycerate mediates indirect transport of NADH and ATP. Back transfer of NADH is possible by cyclic transfer of malate and oxaloacetate or of malate, glutamate, α-ketoglutarate and aspartate.

8

CARBOHYDRATE BIOSYNTHESIS

1) UDPG Pyrophosphorylase

   G-1-P + UTP → UDPG + PPi

2) Sucrose-P Synthetase

   UDPG + F-6-P → Sucrose-P

3) ADPG Pyrophosphorylase

   G-1-P + ATP → ADPG + PPi

4) Starch Synthase

   ADPG + $(Glucose)_n$ → $(Glucose)_{n+1}$ + ADP

5) Q Enzyme (Branching Enzyme)

   α-1,4-glucan + (Glucose) → Amylopectin
   (Amylose)

CARBOHYDRATE BREAKDOWN

1) Beta-amylase

   Starch → maltose units from non-reducing end of amylose or amylopectin

2) Alpha-amylase

   Amylose → $(glucose)_n$

3) R-enzyme (Debranching enzyme)

4) D-enzyme (transglycosylase)

   maltotriose → glucose + maltose

5) Phosphorylase I - germinating seeds

   Starch + Pi → G-1-P

   Phosphorylase II and III - may be important in chloroplast

6) Maltase

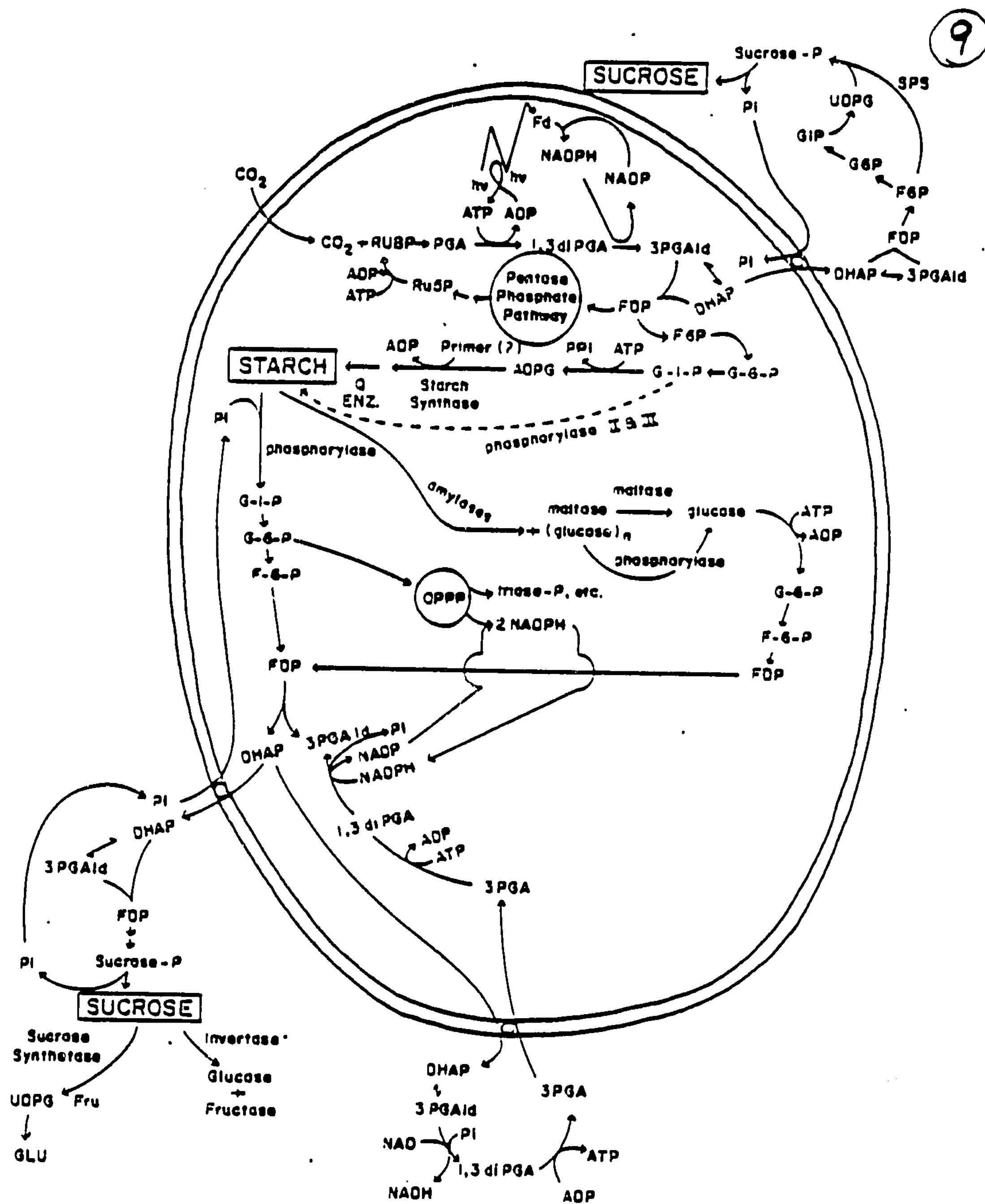
   Maltose → 2 glucose

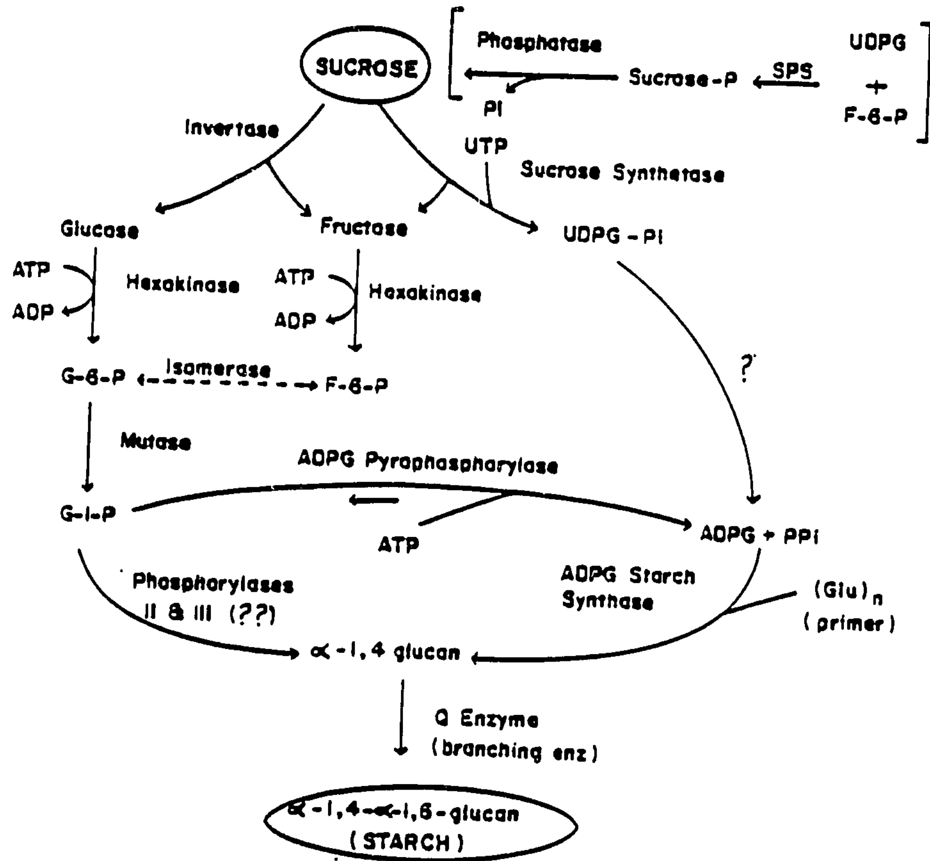
7) Invertase

   Sucrose → Fructose + Glucose

8) Sucrose Synthetase

   UDP + Sucrose ⇄ UDPG + Fructose


9
SUCROSE
Sucrose - P
Pi
SPS
UDPG
G1P
G6P
F6P
FDP
DHAP
3 PGAld
Fd
NADPH
NADP
hv
ATP
ADP
CO_2
CO_2 + RUBP → PGA
1,3 di PGA
3PGAld
Pi
DHAP
Pentose Phosphate Pathway
RuSP
ADP
ATP
FDP
F6P
STARCH
ADP
Primer (?)
PPi
ATP
ADPG
G-1-P
G-6-P
Q ENZ.
Starch Synthase
phosphorylase I & II
Pi
phosphorylase
amylases
maltase
maltose
glucose
ATP
ADP
(glucose)n
phosphorylase
G-1-P
G-6-P
F-6-P
OPPP
triose-P, etc.
2 NADPH
G-6-P
F-6-P
FDP
FDP
DHAP
3PGAld
Pi
NADP
NADPH
1,3 di PGA
ADP
ATP
3PGA
Pi
DHAP
3PGAld
FDP
Pi
Sucrose - P
SUCROSE
Sucrose Synthetase
Invertase
UDPG
Fru
Glucose
Fructose
GLU
DHAP
3 PGAld
3PGA
NAD
Pi
1,3 di PGA
ATP
NADH
ADP



CARBOHYDRATE SYNTHESIS IN NON-GREEN TISSUE
10
SUCROSE
Phosphatase
Sucrose-P
SPS
UDPG
+
F-6-P
Pi
Invertase
UTP
Sucrose Synthetase
Glucose
Fructose
UDPG - Pi
ATP
ADP
Hexokinase
ATP
ADP
Hexokinase
?
G-6-P
Isomerase
F-6-P
Mutase
ADPG Pyrophosphorylase
G-1-P
ATP
ADPG + PPi
Phosphorylases
II & III (??)
ADPG Starch
Synthase
(Glu)n
(primer)
α-1,4 glucan
Q Enzyme
(branching enz)
α-1,4-α-1,6-glucan
(STARCH)

(11)

Ghosh and Preiss, J. Biol. Chem. [illegible] (1966)

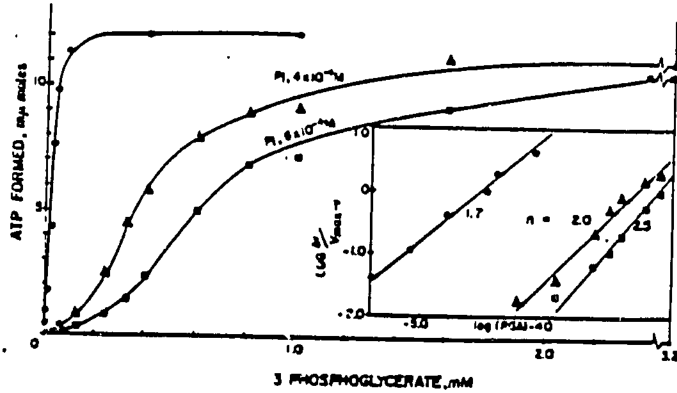


FIG. 4. Effect of phosphate on the 3-phosphoglycerate saturation curve. Assay conditions were the same as in Assay B except that the pH was 8.5, the 3-phosphoglycerate (PGA) concentration was varied, and phosphate was present as indicated. V_{max} was determined as described in Fig. 1. Δv is the amount of ATP formed in the presence of 3-phosphoglycerate minus the amount of ATP formed in the absence of 3-phosphoglycerate.

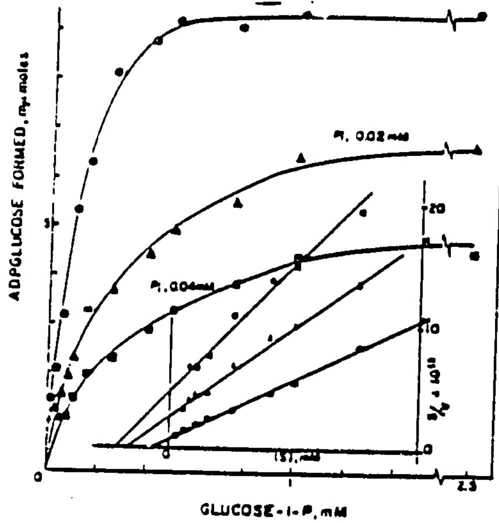


FIG. 5. Effect of P_i on the kinetics of spinach ADP-glucose pyrophosphorylase in the absence of activator. Assay C was used, except that glucose-1-P concentration was varied and P_i was present as indicated.

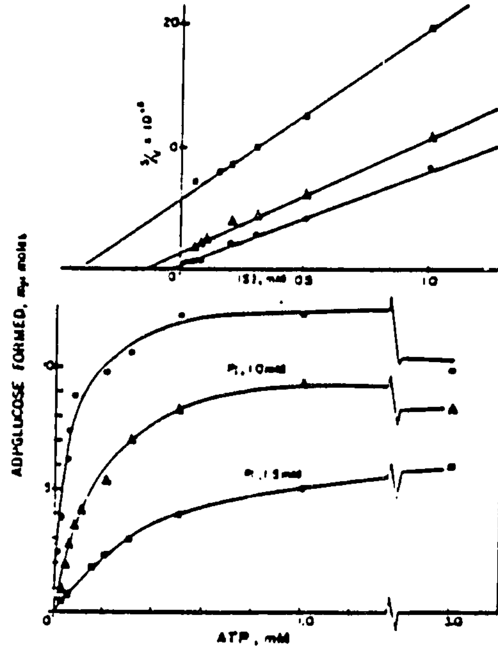


FIG. 8. Effect of P_i on the kinetics of spinach ADP-glucose pyrophosphorylase in the presence of activator. Assay D was used, except that inorganic pyrophosphatase was omitted. ATP concentration was varied, and P_i was present as indicated.

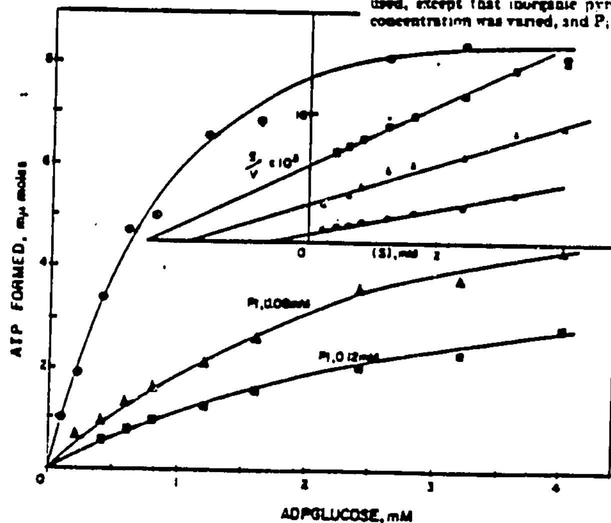


FIG. 9. Effect of P_i on the kinetics of spinach ADP-glucose pyrophosphorylase in the absence of activator. Assay A was used, except that ADP-glucose concentration was varied and P_i was present as indicated.

Okita et al.
Plant Physiol. Vol. 64: 187-192 (1979) ENZYMES OF SPINACH LEAF STARCH METABOLISM (12)

Table I. Distribution of Starch Biosynthetic Enzymes in Spinach Leaf Homogenates

Chloroplasts were isolated from spinach leaves (30 g) by the aqueous method (28, 40) as modified in the text. The crude chloroplast pellet was collected by centrifugation and washed once; the resulting wash was combined with the original supernatant fluid and designated the soluble protein fraction. The ratio of RuBPCase recovered in the chloroplast pellet relative to the total activity observed in both the chloroplast pellet and soluble protein fraction reflects the percentage of intact chloroplasts recovered. The corrected percentage of enzyme activity present in the chloroplasts was obtained by dividing the percentage of each enzyme activity by the percentage of RuBPCase which are recovered in the chloroplast pellet. Each value reported is the average obtained from three different homogenates.

Enzyme	Units in Chloroplast Pellet	Total Units Recovered	% in Chloroplast Pellet	Units/mg Chl	Corrected % in Chloroplast Fraction
RuBPCase	1.68	9.4	18.4 ± 2.4[1]	1.23	100
ADP-Glc pyrophosphorylase	1.80	12.1	18.3 ± 2.8	0.70	98.8 ± 1.2
Starch synthase	0.36	2.2	18.4 ± 1.7	0.16	97.7 ± 3.4
Starch branching enzyme	6.63	48.6	18.6 ± 3.3	2.25	94.8 ± 6.0

[1] ± SE.

Table II. Activities of Starch Degradative Enzymes in Chloroplasts Obtained from Spinach Leaf Homogenates

For details see the legend to Table I. Values reported are the average of the number of experiments shown in parentheses.

Enzyme	% of Total Activity Present in Chloroplast Pellet	Units/mg Chl	% RuBPCase Chloroplast Pellet	Corrected % in Chloroplasts
Amylase (9)	3.0 ± 0.2[1]	0.170	18.9 ± 1.7	16.4 ± 1.2
R-enzyme (5)	6.9 ± 0.7	0.122	21.2 ± 2.6	34.1 ± 1.0
Phosphorylase (3)	6.8 ± 0.4	0.123	20.6 ± 1.7	33.7 ± 2.8
D-enzyme (3)	3.9 ± 0.6	0.039	20.5 ± 1.7	19.8 ± 2.9
Maltase (3)	<1.0	<0.003		

[1] ± SE.

Table III. Activities of Starch Biosynthetic and Degradative Enzymes in Chloroplasts Isolated by Sucrose Density Gradient Centrifugation of Protoplast Lysates

Twice-washed protoplasts of hydroponically grown spinach were lysed and the organelles resolved on a 30 to 58% (w/w) sucrose density gradient as described in the text. Representative examples of the distribution of enzyme activities, Chl, and organelles on sucrose density gradients are illustrated in Figures 1 and 2. The total activity of each enzyme associated with intact chloroplasts was obtained by summing the activity of each fraction in the area where intact chloroplasts sedimented. Each value reported is the average of the number of experiments shown in parentheses.

Enzyme	Units in Intact Chloroplasts	Units/mg Chl	% of Total Activity Observed in the Sucrose Density Gradient	Corrected % in Chloroplasts
Chl (4)	1.31		78.5	
RuBPCase (4)	0.82	0.68	77.6	100
ADP-Glc pyrophosphorylase (2)	0.68	0.48	77.5	98.9
Starch synthase (2)	0.04	0.03	78.1	98.7
Starch branching enzyme (2)	1.61	0.82	70.8	90.3
Amylase (3)	0.33	0.24	15.9	20.6
R-enzyme (3)	0.31	0.22	40.0	52.0
Phosphorylase (2)	0.08	0.07	30.7	38.4
D-enzyme (2)	0.02	0.02	29.8	37.2

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Plant Physiol. Vol. 59, 1146, 1977

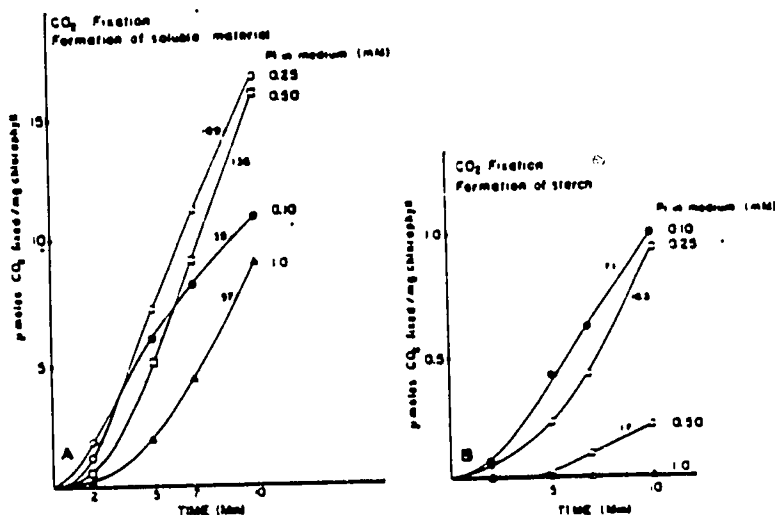

Fig. 2. Formation of soluble (A) and insoluble (B) products during CO_2 fixation by spinach chloroplasts (0.025 mg Chl/ml) in the presence of 10 mM ^{14}C-$NaHCO_3$ (0.2 Ci/mol) and phosphate as indicated. Temperature was 20 C. At the times indicated, samples (200 µl) were withdrawn and deproteinized with 100 µl 3 N HCl. After centrifugation 200 µl of the supernatant was evaporated on a heating plate, and the radioactivity of the residue was measured by liquid scintillation counting (Fig. 2A). The pellet was washed once. Three hundred µl 1 N HCl and about 80 mg quartz sand were added and the sample was vigorously shaken. Two hundred µl of the fine suspension was withdrawn, after heating radioactivity was measured (Fig. 2B). Numbers are the rates of ^{14}C incorporation/mg chl·hr after about 8 min photosynthesis.

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(13)

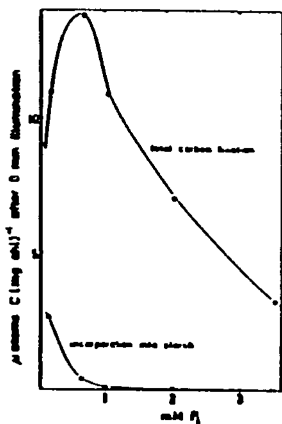

FIG. 3. Carbon fixation and carbon incorporation into starch as affected by the concentration of Pi in the medium after 8 min illumination in the presence of 2 mM HCO_3^-. Other additions were catalase (1,400 unit ml^{-1}), 50 µM PGA as a primer to reduce the lag time after the beginning of illumination. The intensity of red light was 120 w m^{-2}; pH of the reaction mixture was 7.6.

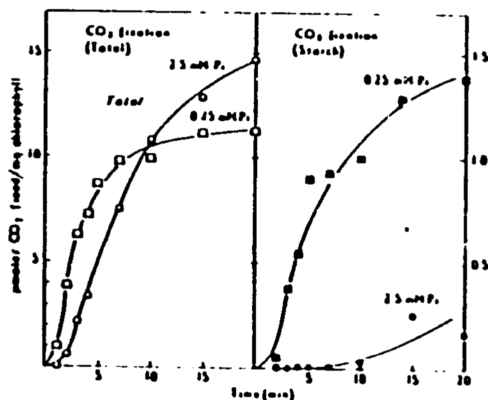

FIG. 4. Formation of total (left) and insoluble products (right) during photosynthesis by spinach chloroplasts in the presence of 10 mM ^{14}C-$NaHCO_3$ and Pi as indicated. Chl: 100 µg/ml. Temperature was 20 C. At the times indicated, samples were removed, deproteinized, and subjected to paper chromatography.

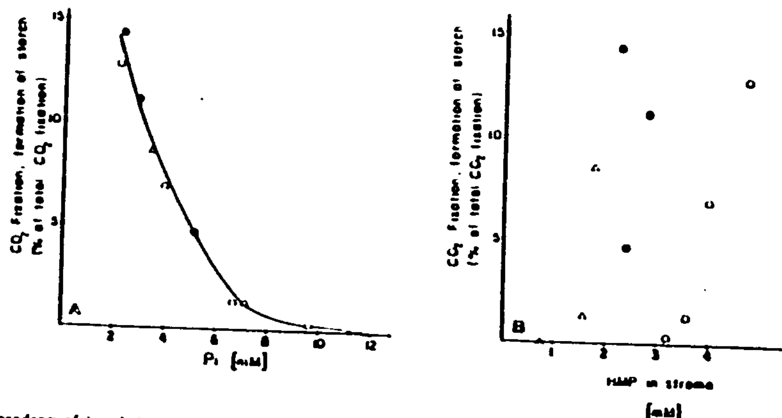

FIG. 6. Dependency of the relative rate of starch fixation on the metabolite concentrations measured in the chloroplasts. Data from Table III and two other experiments carried out under identical conditions. A: dependency on Pi; B: dependency on the sum of heptose- and hexosephosphates.

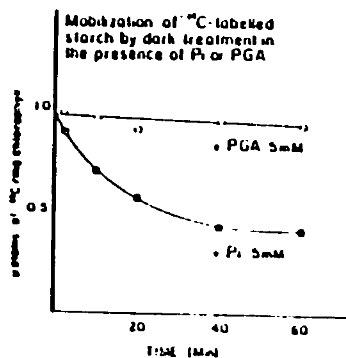

FIG. 7. Mobilization of ^{14}C-labeled starch by dark treatment in the presence of Pi or PGA. For synthesizing ^{14}C labeled starch, spinach chloroplasts (0.1 mg Chl/ml) were illuminated in the presence of 8 mM ^{14}C-$NaHCO_3$ (0.5 Ci/mol) and 0.25 mM Pi for 25 min at 20 C. The sample was then cooled to 0 C, centrifuged (2,000 rpm, Sorvall SS 34 rotor, 1 min), the pellet resuspended, centrifuged again, and resuspended. The sample was then placed in the dark in a water bath (20 C), 5 mM Pi or PGA were added, and samples taken for the determination of ^{14}C-labeled insoluble material (see Fig. 2 legend).

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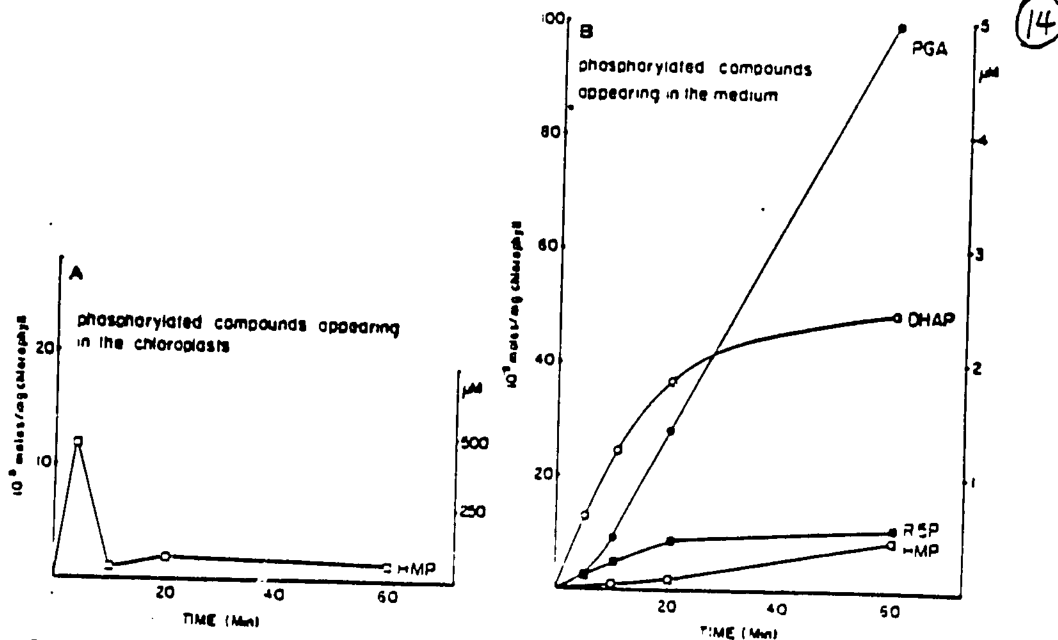

FIG. 8. Starch mobilization in the dark. Chloroplasts were allowed to synthesize starch and washed afterwards, according to the legend of Figure 7. For the mobilization of starch, 1 mM ^{32}P-orthophosphate (30 Ci/mol) was added. At the times indicated, samples (200 µl) were taken and immediately subjected to silicone layer filtering centrifugation. The resulting pellet and supernatant fractions were analyzed for ^{32}P-labeled compounds by ion exchange chromatography.

Table IV. Relationship between the concentration of inorganic phosphate in the medium and the formation of starch during CO_2-fixation

Simultaneous measurement of metabolite concentration in the stroma and in the medium. The rates of CO_2-fixation were measured between 5 - 10 min, the samples for metabolite assay were taken 7 min after the start of illumination. Chloroplast concentration 0.025 mg Chl/ml, stroma space 24 µl/mg Chl.

Experiment 101	A	B	C	D
Phosphate in the medium at beginning of experiment (mM)	1.0	0.50	0.25	0.10
CO_2 fixation (µmol/mg Chl/hr) - soluble material	31	107	108	52
starch	0.3	1.4	7.3	7.7
total	31	108	114	60
starch as % of total	0.3	1.3	6.3	12.6
Metabolite concentrations (mM)				
a) in stroma				
inorganic phosphate	9.6	7.0	4.0	2.2
phosphoglycerate	2.3	6.0	5.3	8.3
triosephosphates	0.17	0.33	0.40	0.25
hexosemonophosphates	3.2	3.7	4.1	4.9
ADP-glucose	<0.01	<0.01	0.08	0.07
phosphoglycerate/phosphate	0.30	0.86	1.7	4.0
b) in the medium (mM)				
inorganic phosphate	0.38	0.43	0.19	0.077
phosphoglycerate	0.004	0.009	0.016	0.024
triosephosphate	0.028	0.048	0.041	0.021

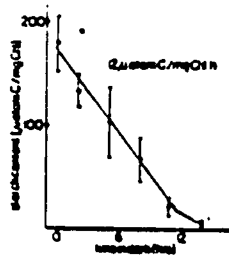

FIG. 1. Starch breakdown in spinach leaves. Each point represents mean ± SE of five separate samples. Leaf discs were taken from two separate matched leaves for each sample.

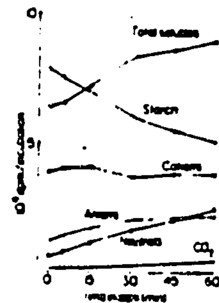

FIG. 3. Breakdown of ^{14}C labeled starch in spinach protoplasts. Protoplasts were prelabeled with [^{14}C]CO_2 and passed three times through a nylon net, and the remaining chloroplasts were isolated by an isoosmotic Percoll gradient, incubated in 5 mM Pi in the dark, and quenched in $HClO_4$ after various time periods. CO_2 driven off after acidification was absorbed in KOH. The insolubles were then separated by centrifugation, and the resulting supernatant (combined with CO_2 to give total solubles) was separated into anions, cations, and neutrals using ion exchange columns.

Table I. *Calculation of the Rate of Starch Breakdown in Starch-Loaded Spinach Chloroplasts*

Protoplasts from starch-loaded leaves were used to isolate chloroplasts, either directly or after prelabeling with [^{14}C]HCO_3. In seven separate experiments, the redistribution of ^{14}C out of starch into glucose, maltose, anions, and CO_2 was determined (Fig. 3), providing an estimate of the relative fluxes from starch into these products. In four experiments with unlabeled chloroplasts, the rate of accumulation of glucose and of HMP, fructose bisP, triose-P, and PGA (combined as anions) was measured by enzymic substrate analysis (Table II). The rates of CO_2 and maltose formation were extrapolated from the measured rate of glucose accumulation and the relative distribution of ^{14}C between glucose, CO_2, and maltose.

	Increase in Labeling as Percentage of ^{14}C Lost from Starch	Rate of Accumulation	
		Measured by enzymic analysis	Extrapolated
		μmol/mg Chl·h	
Total Products	106		10.4
Anions	38 ± 12	4.6 ± 2.8	
CO_2	9 ± 2		0.8
Neutral	59 ± 13		
Glucose	39 ± 10	3.3 ± 0.7	
Maltose	19 ± 5		1.7

Table V. *[^{14}C]CO_2 Release from Specifically Labeled Glucose Supplied to Intact Starch-Loaded Spinach Chloroplasts*

Chloroplasts were isolated and incubated, as in Figure 4, for 30 min, with 0.5 mM [1-^{14}C]glucose, [2-^{14}C]glucose, or [6-^{14}C]glucose and Pi, if indicated. The results are the means of 2 separate experiments, each with 3 replicate samples.

Glucose Labeled in Carbon	CO_2 Release	
	Pi, 5 mM	nil Pi
	nmol CO_2/mg Chl·h	
1	10.4	8.5
2	2.4	2.6
6	1.9	4.9

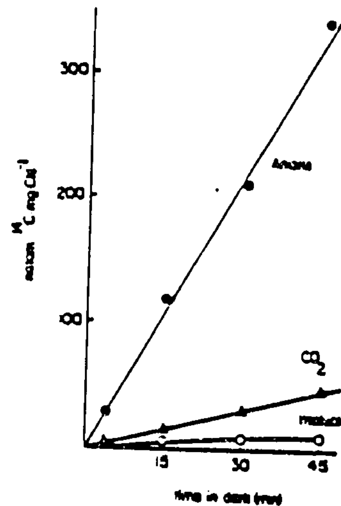

FIG. 5. Metabolism of 2 mM [U-^{14}C]glucose by starch-loaded intact spinach chloroplasts. Starch-loaded chloroplasts were prepared (by using a Percoll gradient) from protoplasts and incubated at 100 μg Chl/ml in 5 mM Pi with 2 mM [U-^{14}C]glucose in the dark. Label in anionic compounds (●——●), CO_2 (▲——▲), and insolubles (○——○) was determined. The results are the means of three separate samples.

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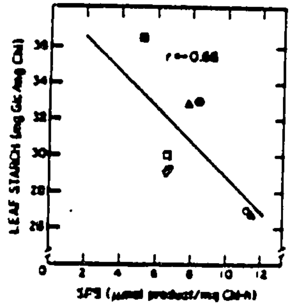

FIG. 1. Leaf starch content at 1100 h (mg glucose · mg Chl) compared with activity of SPS (μmol product formed/mg Chl·h) in soybean leaf extracts. Open and closed symbols represent nodulated and nonnodulated plants, respectively: (○, ●), Tracy; (□, ■), Arksoy; (△, ▲), Lincoln; (▽, ▼), Williams. The correlation was significant at the 10% level.

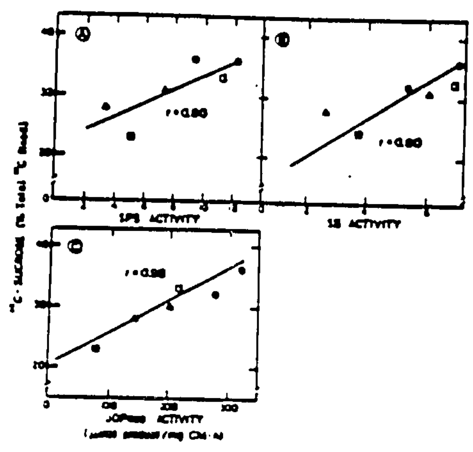

FIG. 2. Sucrose formation (percentage of total $^{14}CO_2$ fixed) in isolated soybean Ransom cells compared with activity in leaf extracts of SPS (A), SS (B), and UDPase (C), as affected by photoperiod. Plants were grown and maintained on a 14-h photoperiod (control, open symbols) or transferred to a 7-h photoperiod (closed symbols). Samples were taken on day 0 (○, ●), day 2 (△, ▲), and day 4 (□, ■).

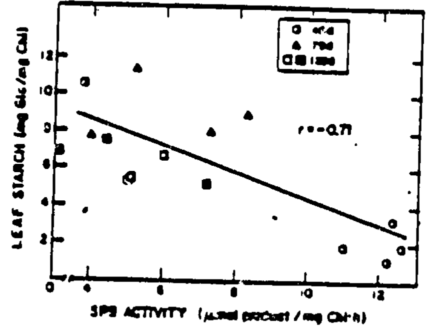

FIG. 4. Leaf starch content (mg glucose/mg Chl) compared with activity of SPS in leaf extracts of four soybean varieties at three stages of development.

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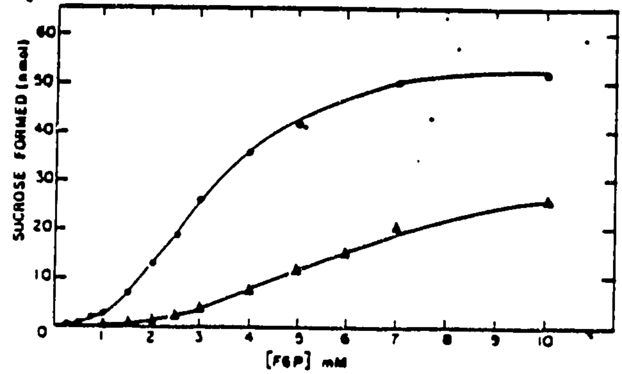

Figure 1 Synthesis of sucrose-P catalyzed by spinach leaf sucrose-P synthase in the absence (●) or presence of 5 mM P_i (▲). The data was obtained from Amir & Preiss (3). The reaction mixture contained 40 mM HEPES buffer, pH 7.5, fructose-6-P as indicated, 10 mM UDP-glucose, 4 mM $MgCl_2$, and 1.6 mg/ml of bovine serum albumin.

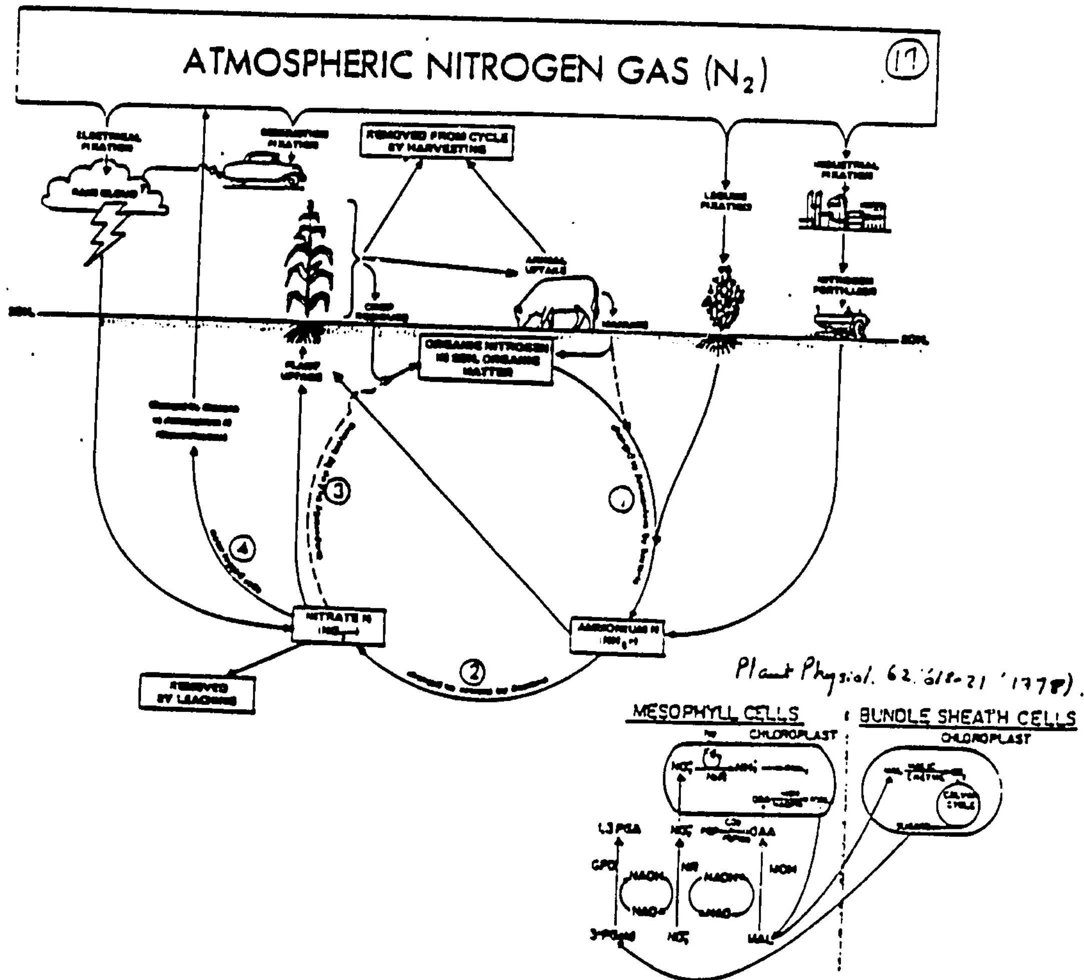

FIG. 1. Proposed pathway for nitrate assimilation in corn leaves.

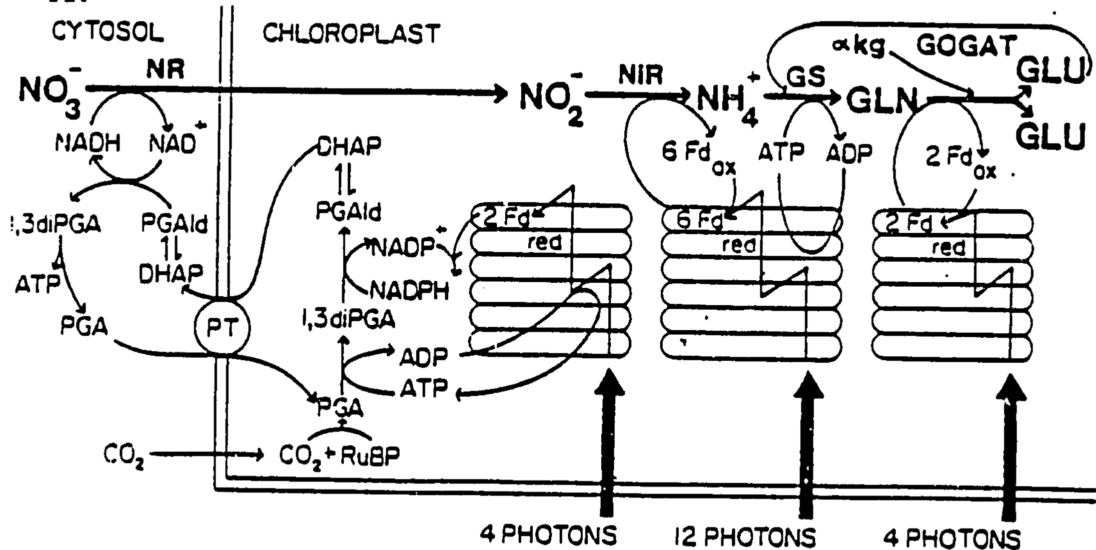

Fig. 1. The relationships between nitrate assimilation and photochemical reactions in the chloroplast. PT = phosphate translocator; NR = Nitrate reductase; NiR = nitrite reductase; GS = glutamine synthetase; GOGAT = glutamate synthase; GLN = glutamine; GLU = glutamate; Fd_{red} = reduced ferredoxin; Fd_{ox} = oxidized ferredoxin; DHAP = dihydroxyacetone phosphate; PGAld = 3-phosphoglyceraldehyde; 1,3 diPGA = 1,3 bisphosphoglycerate; PGA = 3-phosphoglycerate; RuBP = ribulose, 1,5-bisphosphate; αkg = α-ketoglutarate (From Schrader + Thomas. 1981. Chap. 3. In: J.D. Bewley (ed.) Nitrogen and Carbon Metabolism. Martinus Nijhoff/Dr W. Junk, The Hague pp. 49-93.

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Plant Physiol. 51:120-127 (1973)

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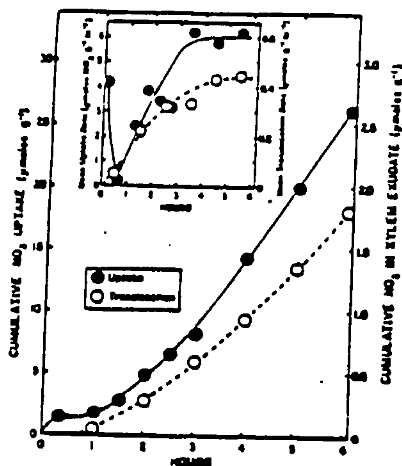

FIG. 1. Nitrate uptake and translocation of nitrate to exuding xylem sap of dark-grown corn seedlings. Shoots were excised immediately prior to placing the solutions in the uptake solution. Initial nitrate concentration of the aerated uptake solution was 0.5 mM as $Ca(NO_3)_2$ and also contained 1.0 mM Na 2-(N-morpholino)ethanesulfonate, pH 6, 5 μg Mo per liter and 50 μg chloramphenicol ml^{-1}. The solution was not changed during the experiment so the nitrate concentration progressively declined. Rates of uptake and translocation shown in the insert are plotted at the midpoint of each measurement period. Temperature was 30 C throughout.

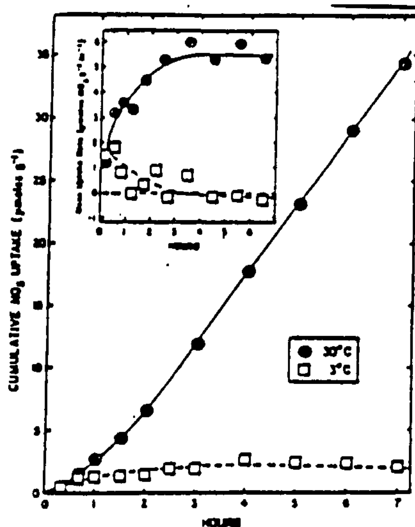

FIG. 2. Influence of temperature on nitrate uptake. Other conditions were as in Figure 1.

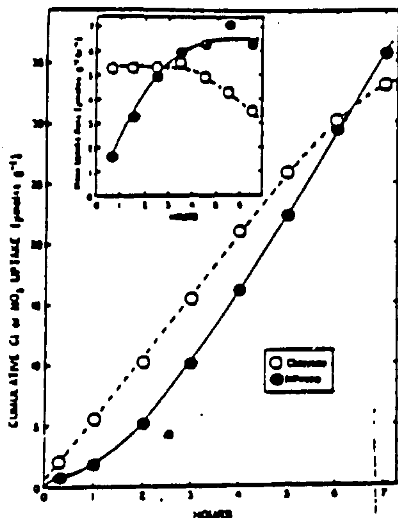

FIG. 4. Comparison of chloride and nitrate uptake (from 0.5 mM solutions replaced hourly. Other conditions were as in Figure 1.

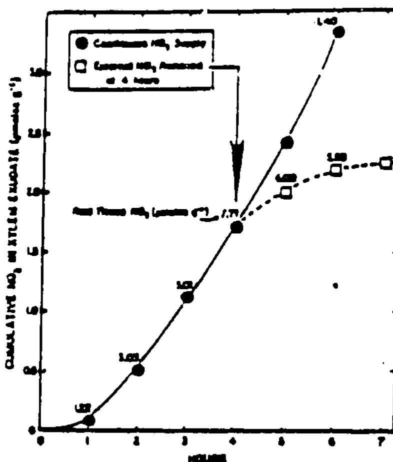

FIG. 3. Influence of presence of external nitrate on deposition of nitrate in exuding xylem sap. Values shown beside the plotted points are the nitrate concentrations of the total root tissue. Conditions were as in Figure 1.

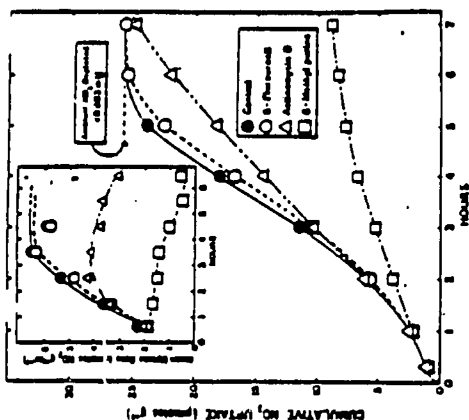

FIG. 10. Influence of 5-fluorouracil (2.5 mM), actinomycin D (40 μg ml^{-1}) and 6-methylpurine (0.5 mM) on nitrate uptake. Other conditions were as in Figure 1.

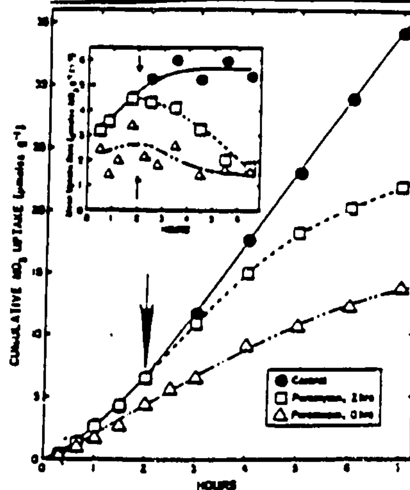

FIG. 13. Influence on nitrate uptake of puromycin (400 μg ml^{-1}) applied at the outset and after 2-hr exposure to nitrate. Other conditions were as in Figure 1.