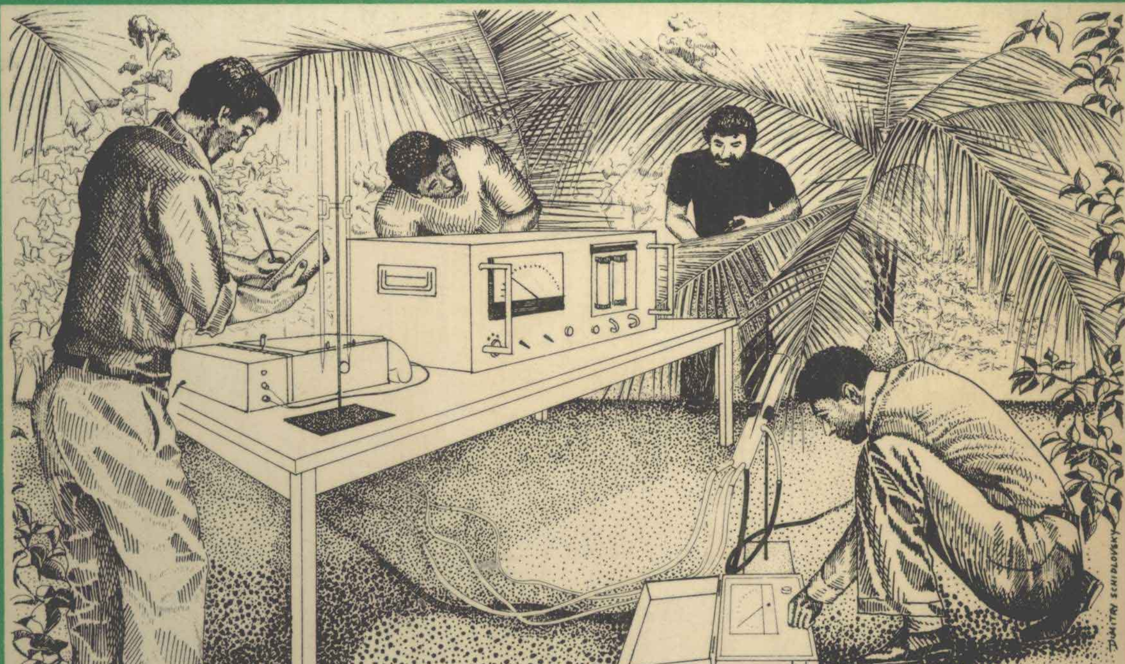
Techniques in Bioproductivity & Photosynthesis



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J Coombs & D O Hall

TECHNIQUES IN BIOPRODUCTIVITY AND PHOTOSYNTHESIS

Edited by

J. COOMBS and D. O. HALL

Sponsored by the United Nations Environment Programme

PERGAMON PRESS

OXFORD · NEW YORK · TORONTO · SYDNEY · PARIS · FRANKFURT

U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
U.S.A.	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
CANADA	Pergamon Press Canada Ltd., Suite 104, 150 Consumers Road, Willowdale, Ontario M2J 1P9, Canada
AUSTRALIA	Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W. 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, 6242 Kronberg-Taunus, Hammerweg 6, Federal Republic of Germany

First edition 1982

British Library Cataloging in Publication Data

Techniques in bioproductivity and
photosynthesis.—(Pergamon international
library)
1. Plant physiology
2. Field crops
3. Photosynthesis
I. Coombs, J. II. Hall, D. O.
631.5 SB185.5 80-42235

ISBN 0 08 027382 3 hardcover
0 08 027383 1 flexicover

Printed in Great Britain by A. Wheaton & Co., Ltd., Exeter

This manual has been prepared from materials used in training courses and workshops under a project (FP/1303-78-02) of the United Nations Environment Programme and King's College, University of London with additional financial support and participation from the following organizations: United Nations Development Programme; United Nations Educational, Scientific and Cultural Organization; Commission of the European Communities; British Council; Commonwealth Foundation; Bundesministerium fur Wissenschaft und Forschung (Austria); Tate and Lyle Ltd (UK); National Science Foundation (U.S.A.); Madurai Kamaraj University (India); University of Nairobi (Kenya); Maize Research Institute (Yugoslavia).

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PREFACE

This manual has evolved as a by-product of a scheme sponsored by the United Nations Environment Programme to provide training in the field and laboratory techniques associated with the measurement of plant productivity, with particular emphasis on photosynthesis. So far three training courses have been held, one in India, one in Kenya and one in Yugoslavia. Each course ran for 2 weeks and was attended by about twenty-five students from developing countries. The aim of the courses has been to train students to apply the best available and most relevant techniques to their own problems. Hence, they have been conducted in an environment similar to that which they will find in their own countries relying on local facilities, often using equipment manufactured or adapted from that available in the host institution. This publication is based on the content of the courses. It does not set out to be all-inclusive, or to be a lecturer's handbook, but rather reflects the interactions between the students and the lecturers on the courses, covering the areas of study in which the students have had the greatest interest or desire to learn.

The courses are obviously aimed at meeting a need, that of increasing knowledge of productivity of plant communities in warmer regions. The importance of this reflects the major problem facing many such countries—the rising cost of energy and its relevance to productivity in agriculture and forestry and to providing local sources of energy. This is particularly true of those areas which lack both fossil-fuel reserves and the industrial base to earn money to pay the ever-increasing oil bill. For such countries the only answer may be a greater reliance on indigenous energy sources such as geothermal, hydroelectric and solar. As far as many warmer countries are concerned the best, or in some cases the only, option appears to be the use of solar energy trapped by growing plants, i.e. biomass. The use of biomass as a fuel presents one major problem at present—the conflict between the production of food or the production of an energy crop in areas where land, water or other resources may be finite. Ironically, many of the countries which appear to offer the best opportunities for a biomass programme are those facing the greatest food shortages. This is, of course, the result of interaction between many factors, some agricultural, some social, some economic. However, in theory it should be possible to define the potential of any given area, crop or ecosystem to produce biomass, and to attach a numerical value to the amount of plant material which can be produced. In other words, it should be possible to **predict the potential productivity of agricultural, forestry or aquatic systems, in terms of the photosynthetic capacity of both existing crops and possible new plant species currently not being fully exploited. To do this requires that the plants are studied *in situ*,** rather than extrapolating from studies carried out in temperate conditions. For example, following the elucidation of the photosynthetic carbon-reduction cycle, many photosynthesis researchers considered that there was little left to discover about photosynthetic carbon metabolism. No one could have predicted the existence of C_4 plants which are adapted to sunnier and/or drier areas. In the same way the biology of nitrogen fixation in symbiotic associations in tropical legumes and grasses differs from that in many of the standard temperate crops used as experimental material for much of the work currently reported in the literature.

Without adequate training the scientist experimenting with new species, or in a new ecosystem, and obtaining results which *differ* from those in the textbook, may discard what could be a new discovery as important as that of C_4 photosynthesis. At a more practical level the worker using instruments in the field, to measure CO_2 fixation, light intensity or moisture stress or even carrying out analysis of crop growth by simple gravimetric means, may be faced with problems of calibration, technique or interpretation which lie well outside the scope of his experience. The aim of these courses has been to enable a carefully chosen group of young people entering this area of work to interact with experienced researchers so that they can gain the skills to solve such problems as they encounter in their own work—and perhaps of even greater importance spread the knowledge that they have gained amongst their fellow countrymen.

J. Coombs
D. O. Hall

INTRODUCTION

It is often possible to use plant material as a source of fuel and fibre and at the same time provide enough food in many of the warmer countries. Types of processes which might be used are summarized in Section 10, which also shows that the **products of photosynthesis** are about ten times the world's present total energy use. Furthermore, the standing biomass is comparable with proven reserves of fossil fuel. As far as land plants are concerned the major production occurs in the warmer regions. It is also in these regions that species with the highest rates of production are found.

In order to use the potential of the **biomass** to the full value considerable scientific and technological input will be required. The aims of any system designed for sustained use of plant material are:

(a) High yields.
(b) Low inputs.
(c) Use of all plant material.
(d) Use of process wastes.
(e) Maximum use of land, water, fertilizer, etc.
(f) Selection of plants for non-food as well as for food biomass.

In general, much **agriculture** in warmer countries is at the subsistence level. This is characterized by:

(a) Absence of cash inputs.
(b) Low crop yields.
(c) Decreasing soil nutrient content.
(d) Shifting cultivation, often with destruction of forest, followed by destruction of the soil.
(e) Production increases resulting from an expansion of the cultivated area with yields per hectare remaining static or falling.

In contrast agriculture in the developed countries has many inputs such as:

(a) Inorganic fertilizers.
(b) (Chemical) pest, disease and weed control.
(c) Mechanical cultivation and harvesting.
(d) Storage and process facilities.

If these inputs are available and put into subsistence-level farming, an exponential increase in production can be obtained. However, this is correlated with a decrease in **energy input/output ratios** (i.e. a higher energy requirement) and mechanization (i.e. a lower use of manpower). An alternative approach is to increase the scientific inputs into the system so that the natural resources of biological nitrogen fixation and recycling of organic material lead to increased soil fertility and higher yields. Other factors such as extremes of temperature, deficiencies of water, nutrients or light, disease and pests, also decrease yields, often through their effect on photosynthesis or on the plant's ability to carry out photosynthesis.

In theory about 6% of the solar energy falling on a given area could be converted to plant organic material. However, in practice, yields are consistent with conversion efficiencies well below this figure. In order to reach towards the theoretical value, and possibly to improve it, an understanding of photosynthesis and the techniques which have been developed in order to investigate it are of paramount importance.

So far the term **yield** has been used without definition. Yields can be expressed in terms of amount of plant material produced in a given time (usually one year or one crop period) on the basis of land area. On the other hand, in consideration of plant material as an energy source, yields expressed in terms of amount produced per energy unit input, or per person, may be of equal importance.

The total photosynthate produced may be termed true biological yield. This will differ from the useful or economic yield which will be of smaller magnitude. The fraction used is known as the harvest index:

$$\text{Harvest index} = \frac{\text{economic yield}}{\text{biological yield}} \times 100$$

The growth rate and hence productivity of a crop is limited by the size of the assimilatory system, i.e. the leaf area of the crop at any time, and dependent on the net assimilation rate, i.e. the ability of this leaf area to fix CO_2 by photosynthesis. Therefore in simple terms:

$$CO_2 + H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} (CH_2O)_n + O_2$$
$$\text{(Leaf area)}$$

Since light and CO_2 assimilation rates are important limiting factors, accurate measurement is essential. This is covered in Part I. In the above equation the dry matter produced is represented by $(CH_2O)_n$ or carbohydrate. Of course in the plant carbohydrate is metabolised further to proteins, lipids, lignin, etc. The direct determination of dry matter measured at the end of an experimental period (described in Section1) gives a value which can be misleading as it represents only the difference between what has been produced and what has been lost. It also gives little information about the underlying growth process. To understand such processes, an understanding of both the physiology and biochemistry of photosynthesis is required. Some aspects of these subjects are dealt with here particularly on laboratory techniques used for isolation of chloroplasts and enzymes from plant tissue. In addition to methods applicable to photosynthetic CO_2 assimilation, methods for investigating nitrogen fixation are detailed—the importance of the nitrogen status of plants in relation to photosynthetic productivity is often ignored.

Experimentation

In order to obtain meaningful information accurate observations are required which in turn means experimentation, i.e.

(a) Design.
(b) Perform.
(c) Observe.
(d) Record.
(e) Conclude.

The importance of these elements are stressed throughout the course. In particular the following aspects should be noted:

(a) *Design*	(i)	Define question to be answered.
	(ii)	Choose adequate healthy material—identify it.
	(iii)	If using material over a period of time be systematic, record all inputs, note any disease or pests or treatments.
	(iv)	Have adequate controls.
	(v)	Be simple—answer one point at a time.
(*b*) *Perform*	(i)	Be accurate.
	(ii)	Avoid contamination.
	(iii)	Avoid stresses or artifacts.
(c) *Observe*	(i)	Be critical.
	(ii)	Be honest.
	(iii)	Be accurate.
	(iv)	Be objective.
(d) *Record*	(i)	Be accurate.
	(ii)	Be systematic.
	(iii)	Be thorough.
(e) *Conclude*		Be objective. Is your conclusion justified or have you been biased towards a preconceived idea?

Recording and presenting your results

It is essential that a clear and concise account is kept of all your experimental results and conclusions. It is suggested that you keep two notebooks, a small one for recording observations as they are made, for calculating, for example, how much of a given chemical to weigh out when making solutions, and a larger notebook (about 21×30 cm, A4 size) in which all experiments should be fully written up. The experimental reports are examined at the end of the course and taken into account when final evaluations are made.

Experiments should be set out under the following headings: *title* (complete with a date and experiment code number which should also be used on each page of the small notebook when primary data is being recorded); a description of the *aims* of the experiment; the plant *material* used; the *methods* used; the *results* (numerical) plus a note of any unusual delays or occurrences which might have contributed to spurious results; *calculations* of results derived from the primary data (e.g. leaf area, rates of reaction, concentration of chlorophyll); *conclusions*. In the conclusions it should be noted whether these have answered the questions as detailed in the aims of the experiment, suggestions for further or better experiments should also be given.

In recording results care should be taken in respect to the following:

(a) *Replication*, a single weighing may be sufficient but most other measurements should be taken at least twice. At least two replicate determinations, using material from a similar source, should be made. In general, agreement should be such that they do not vary by more than 5%. If the variation is larger, sampling must be repeated; if continued variation is observed, check the method being used.

(b) *Significance*, do not record your results to a greater accuracy than the variation between replicates justify, e.g. two weighings of 11.349 and 12.016 would be recorded as 11.7 rather than 3 places of decimals.

(c) *Statistics*, do not give a mean and standard error when you only have a few results; your calculator may give a result but it is not very meaningful unless you are dealing with samples of 30 or more. However, if you find your results appear to fall on a straight line do use a calculator to obtain the "best fit" (least squares), slope and intercept.

It is essential that all experimental measurements are completed and that all data is treated as fully as possible. For instance, results of measurements of rates of CO_2 assimilation should be combined with determinations of leaf area, fresh weight, dry weight and chlorophyll content to give rates of photosynthesis in terms of leaf area, weight or chlorophyll content for a given light intensity.

Again, in biochemical experiments results should be treated in terms of weight, chlorophyll or protein content. For enzyme experiments results should be expressed in terms of the amount of substrate converted in a given time by a given amount of protein.

Please enjoy the work and derive maximum benefit from it!

CONTENTS

PART I

WHOLE PLANT PHOTOSYNTHESIS