

# **The experimental manipulation of ovule tissues**

---

Edited by G.P. Chapman, S.H. Mantell  
and R.W. Daniels

---

## Preface

The ovule is a receptive tissue whether of photo-assimilate or male gametes. Interpretation of its role as a megasporangium is both a fundamental concept and a recurrent stimulus to enquiry. For many biologists however, the preoccupation has recently been not with the female but the male part of the plant.

Since the papers of Guha and Maheshwari, nearly 20 years ago, the anther or microsporangium and especially its product, pollen grains or microspores have occupied experimental morphologists and plant breeders as a source of haploid plants. More recently, attempts to modify pollen DNA have shifted the emphasis of interest toward the tissue that is receptive to such modified pollen.

It is readily apparent that the ovule is a more complex tissue. Even its normal function is diversified throughout the Angiosperms and has generated an immense literature. The responses of the ovule to modified pollen, or in the absence of pollen, to other stimuli are now matters of great interest both theoretically and practically for plant improvement.

This volume brings together contributions some as reviews, some as experimental papers, from workers in tissue culture, genetics, plant breeding and biochemistry and relates to events before, during and after fertilization. The arrangement we have adopted is one that seeks, as far as possible, to integrate our understanding of how the ovule can be made to function. In so doing one aspect to emerge clearly is how the more ambitious objectives require complementary skills to accomplish them. Another aspect is the awareness of considerable potential in several directions such as the range of genetic expression that can be imposed, the scope for genetic analysis and the prospect of raising haploids where pollen has proved refractory and the extent to which wide hybridization can be extended.

Manipulation of ovule tissues is clearly an emerging area of technology, that is essential to increasing our understanding of the fundamental biological processes involved in meiosis, pollen

**viii Preface**

development, fertilization and embryogenesis. This volume provides a guide to what has already been accomplished and we hope a stimulus to further enquiry.

*Wye College.*  
*November 1984*

**G. P. Chapman**  
**S. H. Mantell**  
**R. W. Daniels**

---

## Acknowledgements

We would like to thank the various contributors to this publication for the readiness with which they have met our various requests.

It is also a pleasure to acknowledge the help of various colleagues at Wye, especially Mrs Kate Daniels who meticulously corrected the original typescripts, Miss Pippa Allington for arranging and mounting the photographs and Mrs Sue Briant for secretarial help.

Unilever Research kindly contributed to the cost of the colour plates and its help is greatly appreciated.

Finally, our thanks are due to Pitman Publishing Ltd. for its help in the production of the present volume.

G.P.C.  
S.H.M.  
R.W.D.

---

## List of contributors

**B. S. Ahloowalia**

*Plant Breeding Department, Agricultural Institute, Oak Park  
Research Centre, Carlow, Ireland.*

**P. M. Allington**

*Department of Biological Sciences, Wye College, Ashford, Kent  
TN25 5AH, England.*

**C. A. Beasley**

*National Weather Service, University of California, Riverside,  
CA 92521, U.S.A.*

**R. R. Bergquist**

*Pfister Hybrid Corn Company, P.O. Box 187, El Paso, IL 61738,  
U.S.A.*

**D. Bossoutrot**

*Laboratoire d'Amélioration des Plantes, Bât. 360, Université  
Paris-Sud, 91405 Orsay Cedex, France.*

**D. Boulter**

*Department of Botany, University of Durham, Science Laboratories,  
South Road, Durham DH1 3LE, England.*

**D. E. Brink**

*Crop Evolution Laboratory, Department of Agronomy, University of  
Illinois, 1102 South Goodwin Avenue, Urbana, IL 61801, U.S.A.*

**G. P. Chapman**

*Wye College, University of London, Ashford, Kent TN25 5AH,  
England.*

**C. E. Cohen**

*Martin Marietta Laboratories, 1450 South Rolling Road, Baltimore,  
MD 21227, U.S.A.*

**A. E. de Wet**

*Crop Evolution Laboratory, Department of Agronomy, University of Illinois, 1102 South Goodwin Avenue, Urbana, IL 61801, U.S.A.*

**J. M. J. de Wet**

*Crop Evolution Laboratory, Department of Agronomy, University of Illinois, 1102 South Goodwin Avenue, Urbana, IL 61801, U.S.A.*

**K. Dressler**

*Lehrstuhl für Botanische Entwicklungsphysiologie, University of Hohenheim, D-7000 Stuttgart 70, Federal Republic of Germany.*

**D. Ferdinando**

*Unilever Research Laboratory, Colworth House, Bedford England.*

**P. Gates**

*Department of Botany, University of Durham, Science Laboratories, South Road, Durham DH1 3LE, England.*

**G. K. Goldwin**

*Department of Horticulture, Wye College, Ashford, Kent TN25 5AH, England.*

**J. R. Harlan**

*Crop Evolution Laboratory, Department of Agronomy, University of Illinois, 1102 South Goodwin Avenue, Urbana, IL 61801, U.S.A.*

**A. Hopher**

*Department of Botany, University of Durham, Science Laboratories, South Road, Durham DH1 3LE, England.*

**D. Hess**

*Lehrstuhl für Botanische Entwicklungsphysiologie, University of Hohenheim, D-7000 Stuttgart 70, Federal Republic of Germany.*

**R. D. Hirtz**

*Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.*

**D. Hosemans**

*Laboratoire d'Amélioration des Plantes, Bât. 360, Université Paris-Sud, 91405 Orsay Cedex, France.*

**xii List of contributors**

**W. A. Hughes**

*Unilever Research Laboratory, Colworth House, Bedford  
England.*

**J. Hulme**

*Unilever Research Laboratory, Colworth House, Bedford  
England.*

**N. Inomata**

*Department of Biology, College of Liberal Arts and Sciences,  
Okayama University, Okayama 700, Japan.*

**D. J. James**

*Crop Production and Plant Science Division, East Malling Research  
Station, East Malling, Maidstone, Kent ME19 6BJ, England.*

**W. A. Jensen**

*Department of Biological Sciences, Ohio State University, 484 West  
12th Avenue, Columbus, OH 43210, U.S.A.*

**C. J. Keijzer**

*Department of Plant Cytology and Morphology, Agricultural  
University, Arboretumlaan 4, 6703 BD Wageningen, The  
Netherlands.*

**S. Konle**

*Lehrstuhl für Botanische Entwicklungsphysiologie, University of  
Hohenheim, D-7000 Stuttgart 70, Federal Republic of Germany.*

**K. A. Kumari**

*Department of Botany, Andhra University, Waltair 530 003, India.*

**J. Lagriffol**

*Laboratoire d'Histophysiologie Vegetale, 12 rue Cuvier, F-75005  
Paris, France.*

**R. E. Litz**

*Tropical Research and Education Center, University of Florida,  
Institute of Food and Agricultural Sciences, Homestead, FL 33031,  
U.S.A.*

**K. A. D. Mackenzie**

*Crop Production and Plant Science Division, East Malling Research  
Station, East Malling, Maidstone, Kent ME19 6BJ, England.*

- E. C. Menhinick**  
*Crop Production and Plant Science Division, East Malling Research Station, East Malling, Maidstone, Kent ME19 6BJ, England.*
- M. Monnier**  
*Laboratoire d'Histophysiologie Vegetale, 12 rue Cuvier, F-75005 Paris, France.*
- C. A. Newell**  
*Advanced Genetic Sciences Inc., 6701 San Pablo Avenue, Oakland, CA 94608, U. S.A.*
- A. J. Passey**  
*Crop Production and Plant Science Division, East Malling Research Station, East Malling, Maidstone, Kent ME19 6BJ, England.*
- M. K. Rao**  
*Department of Botany, Andhra University, Waltair 530 003, India.*
- J. Schell**  
*Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.*
- A. Sherman**  
*Department of Botany, University of Durham, Science Laboratories, South Road, Durham DH1 3LE, England.*
- P. Stabel**  
*Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.*
- H.-H. Steinbiss**  
*Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.*
- S. A. Tarawali**  
*Department of Biological Sciences, Wye College, Ashford, Kent TN25 5AH, England.*
- R. Töpfer**  
*Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.*



**H. J. Wilms**

*Department of Plant Cytology and Morphology, Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands.*

**G.-Y. Zhou**

*Shanghai Institute of Biochemistry, Academia Sinica, China.*

---

## Colour plates

Plate 1 (see p. 144). Distribution of ions within and around the oil palm embryo from Fig. 5 of Chapter 11, as visualized by X-ray Digimap analysis.

Plate 2 (see p. 146). Paired phase contrast (left) and fluorescence (right) micrographs of mature oil palm zygotic embryo sections stained with completed antisera raised against oil palm zygotic embryos (methods are described in 'Materials and methods', p. 136 of Chapter 11). Magnification  $\times 125$

Plate 3 (see p. 146). Fluorescence micrographs of oil palm callus (left) and somatic embryo (right) stained with completed antisera raised against oil palm zygotic embryos (methods are described in 'Materials and methods', p. 136 of Chapter 11). Magnification  $\times 300$

Plate 4 (see p. 45). Unfixed wheat embryo stained with DAPI to show the nuclei.

Plate 5 (see p. 189). Polytene nucleus from cultured *Pisum fulvum* cotyledon tissue squashed and stained with DAPI. Note the intensely stained foci. (Zeiss photomicroscope with fluorescence attachment using filter set number 2) (Bar =  $10\ \mu\text{m}$ )

Plate 6 (see p. 45). Triticale embryo sac isolated from an ovule three days after fertilization. After staining with the DNA specific stain DAPI the nuclei are clearly visible. The large antipodal nuclei have begun to degenerate and endosperm cellularization has commenced at the micropylar end. a = antipodal nuclei; ce = cellular endosperm; e = embryo; fe = free endosperm nuclei; p = pollen grain. Bar represents  $100\ \mu\text{m}$

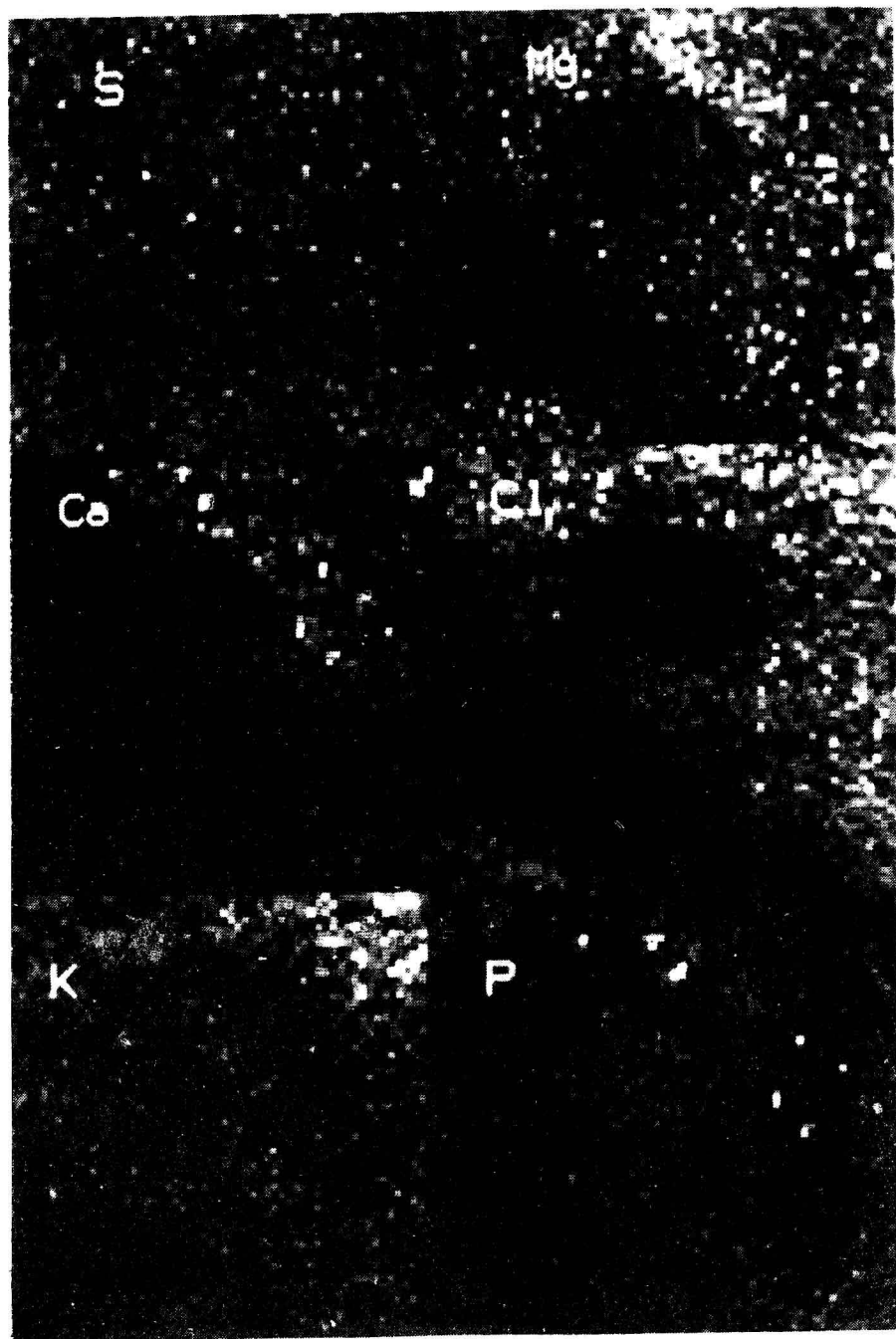


Plate 1

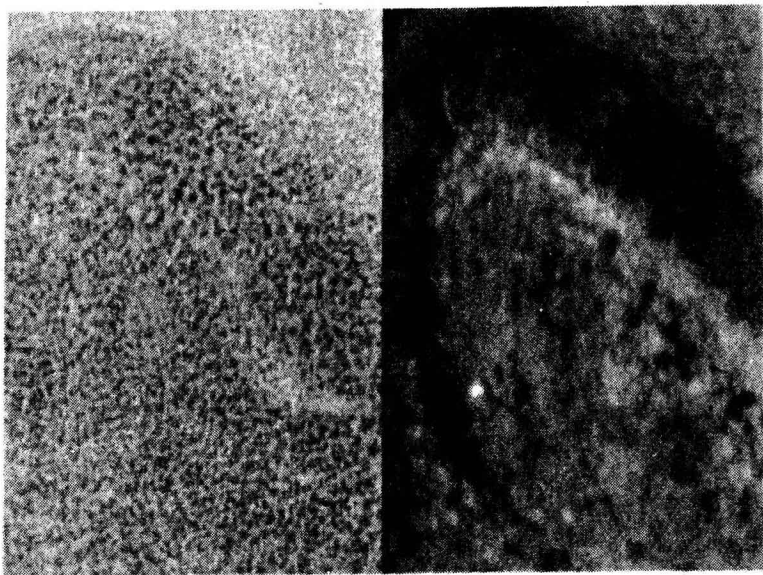


Plate 2

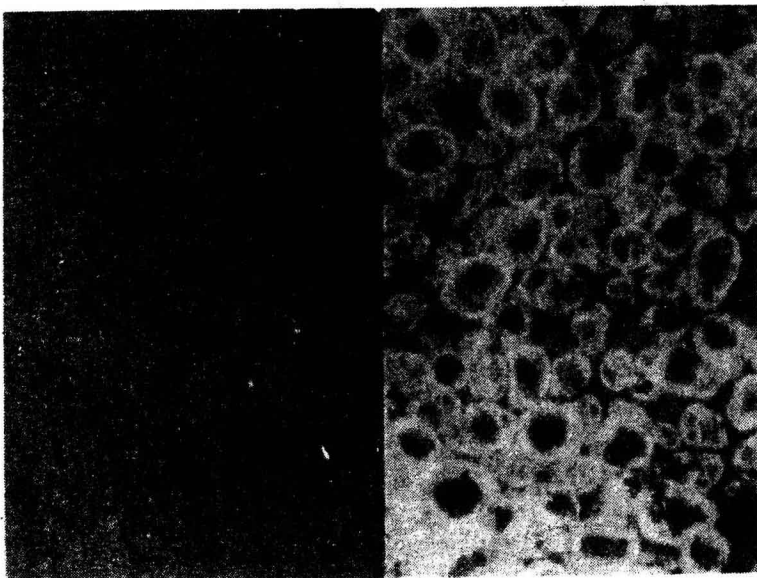


Plate 3

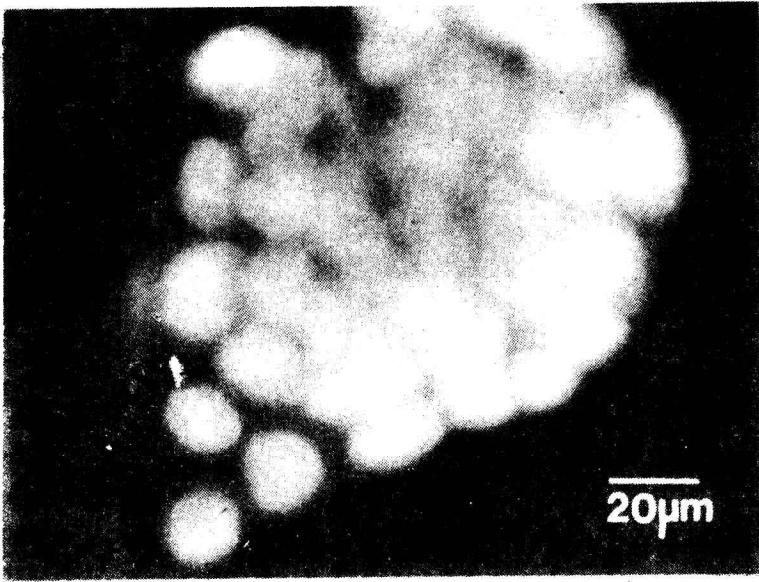


Plate 4

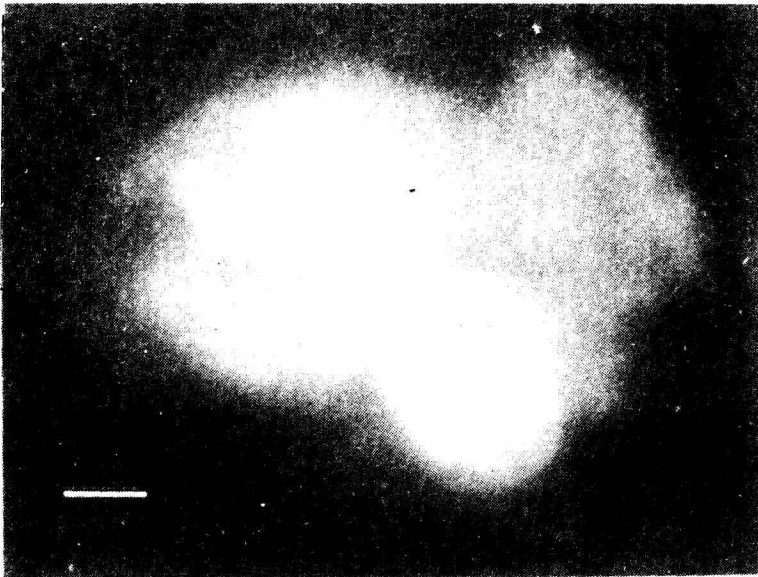


Plate 5

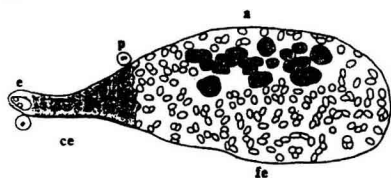
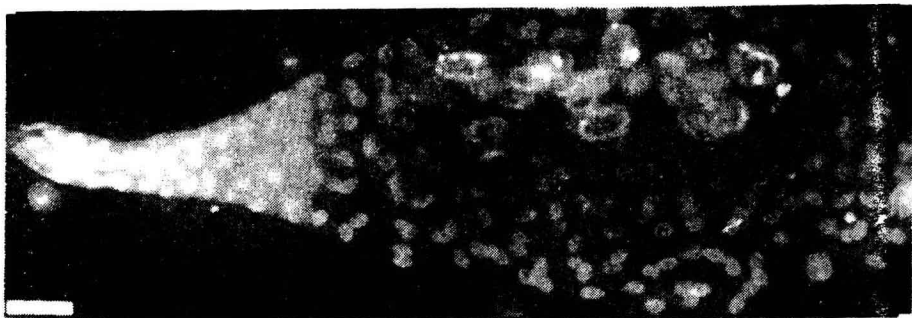


Plate 6

---

# Contents

Preface vii

Acknowledgements ix

List of contributors x

## The ovule tissue complex

1. Nuclear events and the angiosperm megagametophyte: A perspective. 3  
*G. P. Chapman.*
2. Cotton ovule culture: Hormonal effects and role of synergids in fertilization. 15  
*C. A. Beasley and W. A. Jensen.*
3. Cytology of pollen tube and embryo sac development as possible tools for *in vitro* plant (re)production. 24  
*H. J. Wilms and C. J. Keijzer.*

## Micromanipulation

4. Micromanipulation of the unfixed cereal embryo sac. 39  
*P. M. Allington.*
5. Microinjection of gene vectors into pollen and ovaries as a potential means of transforming whole plants. 52  
*A. Hopher, A. Sherman, P. Gates and D. Boulter.*
6. Transformation of plant cells by microinjection of DNA. 64  
*H.-H. Steinbiss, P. Stabel, R. Töpfer, R. D. Hirtz and J. Schell.*

## Developments using unpollinated ovules

7. *In vitro* culture of unpollinated beet (*Beta vulgaris* L.) ovules of male sterile and male fertile plants and induction of haploid plants. 79  
*D. Hosemans and D. Bossoutrot.*
8. The role of ovules in the survival of hormone-induced, parthenocarpic apple flowers. 89  
*G. K. Goldwin.*

## vi Contents

9. Callus induction from ovary culture in wheat, *Triticum aestivum* L. 109  
*B. S. Ahloowalia.*

### Developments using pollinated ovules

10. Development of embryos in *Capsella* ovules cultured *in vitro*. 117  
*M. Monnier and J. Lagriffol.*
11. Oil palm embryogenesis: A biochemical and morphological study. 135  
*D. Ferdinando, J. Hulme and W. A. Hughes.*
12. Cell development during early embryo and endosperm development in eight inbred lines of pearl millet — *Pennisetum americanum* (L.) Leeke. 151  
*M. K. Rao and K. A. Kumari.*
13. A revised medium for *in vitro* culture of *Brassica* ovaries. 164  
*N. Inomata.*
14. Regeneration of tropical fruit trees by culture of ovule tissues. 177  
*R. E. Litz.*
15. Polyteny in cotyledon nuclei. 189  
*S. A. Tarawali.*

### The modification of syngamy

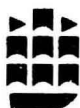
16. Exogenous gene transfer in maize (*Zea mays*) using DNA-treated pollen. 197  
*J. M. J. de Wet, R. R. Bergquist, J. R. Harlan, D. E. Brink, C. E. Cohen, C. A. Newell and A. E. de Wet*
17. The effects of pollen irradiation on the development of the post-fertilization ovule of apple (*Malus pumila* Mill.). 210  
*D. J. James, A. J. Passey, K. A. D. Mackenzie and E. C. Menhinick.*
18. Gene transfer in higher plants using pollen as vectors: Bacterial transferase activity expressed in *Petunia* progenies. 224  
*D. Hess, K. Dressler and S. Konle.*
19. Genetic manipulation of the ovule after pollination. 240  
*G.-Y. Zhou.*

---

# **The experimental manipulation of ovule tissues**

---

Edited by G.P. Chapman, S.H. Mantell  
and R.W. Daniels



**Longman**

**NEW YORK • LONDON**

---




Longman Group Limited  
Longman House, Burnt Hill, Harlow  
Essex CM20 2JE, England  
Associated companies throughout the world

Published in the United States of America  
by Longman Inc., New York

© G P Chapman, S H Mantell, R W Daniels  
and the individual contributors, 1985

All rights reserved; no part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the Publishers.

ISBN 0 582 07801 6

Set in IBM 11/13pt Press Roman by  Tek-Art Croydon, Surrey  
Printed and bound in Great Britain at the Bath Press, Avon.