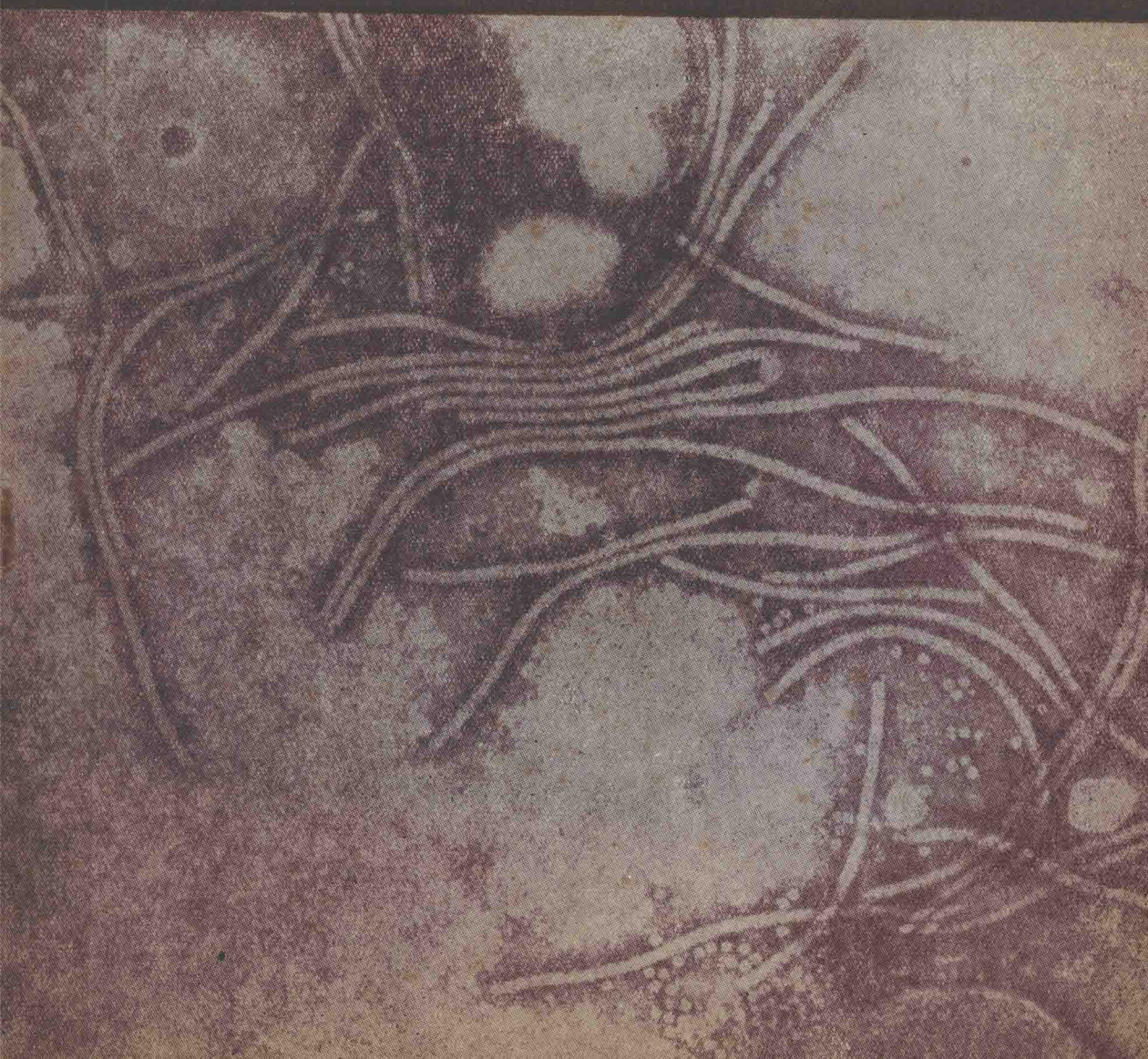
Identification of plant viruses

Methods & experiments

D. Noordam



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With 101 figures, 33 colour plates and indexes

by D. Noordam

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ERRATA

page	line		
29	1–2	insert: Bawden, F. C., 1964. Plant viruses and virus diseases, 4th ed. Ronald Press, New York, 361 pp.	
	26-27	insert: Hertzsch, W., 1928. Beiträge zur infektiösen Chlorose. Z. Bot. 20:65-85.	
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31	34	rose bengal = bengal rose	
33		Fig. 4 line 2: rose bengal = bengal rose	
34		Fig. 5 line 1: crystals; (C) = crystals (C);	
37		Fig. 10 line 3: (C) = (◡)	
40	11	not all = not at all	
89	5	minimum at 260 nm = minimum at 250 nm	
93	8	(0.756/3.1) = (0.765/3.1)	
100	17	against (abscissa) = against λ (abscissa)	
103	10	Högland = Höglund	
103		Fig. 32 line 3: Högland = Höglund	
108	25	tated = tates	
117	7	40 % ammonium = 40 % saturation ammonium	
119		Fig. 52 line 7: ts = its	
123	24	100 ml = 75 ml	
162	15	see Fig. 18.1 to 18.4 = see Fig. 97 to 100	
169	7	insert: Teakle, D. S., 1969. Fungi as vectors and hosts of viruses. In: K. Maramorosch (ed.). Viruses and vegetation: 23-54. Interscience, New York.	
187	20	Bawden, F. C., 13, **16,** 24,	insert: **29,**
188	13	Hagedoorn – Hagedorn	
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Dr D. Noordam, phytopathologist, teaches identification of plant viruses at the Agricultural University of Wageningen, the Netherlands

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Contents

*Experiments with practical instructions

Preface

This book has grown out of a practical course on the identification of plant viruses that I gave with help of Prof. Dr. J. Kochman and his staff at the Agricultural University of Warsaw, Poland, in September 1966. Some of the experiments for this course were borrowed from a stencilled guide composed by Dr J. Dijkstra for use at the Laboratory of Virology at Wageningen. A Dutch version of the Warsaw manuscript was prepared in 1968 with the help of Dr E. R. 't Mannetje-Baruch. This edition is the first one to be published, and is new in many respects.

The layout and content of this book make it suitable for a students course. However the methods, background and procedures are treated in such a way that the book is also of use in research. The author and subject indexes assist the reader to find the particular topic in which he is interested.

The references are only a selection from the literature that could be cited. Especially, in sciences other than virology, I have only referred to a few publications which I know treat the point in question adequately. Many other books, however, may provide the same information.

During the years many people, perhaps unknowingly, have contributed to this book. Those people who are mentioned here contributed to the last draft of the book. Nearly all figures were drawn by Mr K. Boekhorst and some by Mr W. C. T. Middelplaats. The colour photographs were taken by Dr E. R. 't Mannetje-Baruch (3, 13, 17, 23, 24, 25), Dr L. Bos and Dr M. A. Nour (8), Mr G. Eimers (28, 32); the others by students and by myself. The electron micrographs were taken by Mr J. Groenewegen. Mr G. Eimers and Miss M. Usmany prepared the photographs. Mrs J. D. Saayer-Riep performed many experiments underlying the methods described in this book. Some pieces of apparatus were machined out by Mr G. van Surksum and his colleagues; and Mr G. Looyen took care of test plants. Dr B. J. M. Verduin commented on the chapters on purification and spectrophotometry. Serology was discussed with Dr D. H. M. van Slogteren and Miss N. P. de Vos. Dr H. A. van Hoof and Dr J. Seinhorst contributed to the chapter on nematode transmission, while some matters were discussed with Dr F. Quak. Mrs R. A. Esveldt-Elshof applied the card-index which made it easy to find the literature, and she typed the manuscript. Mrs E. M. Brouns-Murray of Pudoc helped with the English, and Mr T. Goedewaagen, Dr E. Meijer Drees and Mr R. J. P. Aalpol of Pudoc prepared the manuscript for publication.

I wish to express my appreciation to all of them in the conviction that without their help this book would never have been published.

Wageningen, April 1973 D. Noordam

Symbols and abbreviations

Use was made of the index by Irvine. In: Netter (1969), Theoretical Biochemistry 870–882; Donbrow (1967, see Chapter 12) and Trautman (1964, see Chapter 11).

A	absorbance, lg I_0/I. In spectrophotometry A is linearly related to concentration
A_{min}	absorbance minimum in a absorption spectrum
A_{max}	absorbance maximum in a absorption spectrum
$A_{max/min}$	value of A_{max} divided by the value of A_{min}
$A_{280/260}$	value of A_{280} divided by the value of A_{260}
av. or av	abbreviation of average
cP	centipoise (a unit symbol for viscosity)
D	an alternative symbol for A, absorbance (spectrophotometry). Not recommended
d	an alternative symbol for A. Not recommended
E	an alternative symbol for A
$E^{0.1\%}_{1cm,\ 260\ nm}$	specific extinction coefficient = the absorbance value of a 0.1 % solution in a cuvette with an optical path length of the light of 1 cm, and measured at a wavelength of 260 nm
$E^{0.1\%}_{1cm,\ 280\ nm}$	Idem, but at a wavelength of 280 nm
$E^{0.1\%}_{1cm,\ 260\text{-}290\ nm}$	Idem, but the value of absorbance at 260 nm diminished by the value of absorbance at 290 nm
g	gram (a unit symbol of mass)
g	initial acceleration in free fall. Values in ultracentrifugation are given in this gravity unit
I	intensity of the light transmitted by a solution of e.g. a virus in a cuvette
I_0	intensity of the light transmitted by the solvent of the virus solution
lg	the common logarithm, to base 10. Alternative log or $\log_{10}$
M	molar concentration in g/l of solution. For instance: 0.2 M-soln Na_2HPO_4. H_2O contains $0.2 \times 178 = 35.6$ g in 1000 ml (molecular weight of $Na_2PO_4.H_2O = 178$)
max. or max	abbreviation of maximum
min. or min	abbreviation of minimum
mg	milligram (a unit symbol for mass) = 0.001 gram
ml	millilitre (a unit symbol for volume) = 0.001 litre
mol wt	molecular weight
mμ	millimicron, an alternative for nm. Not recommended

N.A.	numerical aperture (microscopy), a measure for the angle of the cone of light emerging from the condensor or entering the objective
n	exponent in λ^n, used in equations on light scattering (the value of n varies dependent on the size of molecules which cause light scattering)
nm	nanometre = 10^{-9}m (formerly mμ was used)
OD	alternative symbol for A
Pi	performance index, accounts for the longest distance which particles have to cover to sediment at the bottom of tubes in centrifugation
pH	hydrogen ion exponent (a unit symbol for the degree of alkalinity or acidity)
pK_a	the negative lg of K_a, the dissociation constant of an acid
R	radius, e.g. distance of the axis of rotation to meniscus of fluid, R_{min}; to bottom of tube, R_{max}; or in the middle between R_{min} and R_{max}: R_{av}
RNA	abbreviation of ribonucleic acid
rev/min	revolutions per minute (centrifugation)
rpm	revolutions per minute (centrifugation)
S	Svedberg unit, 10^{-13}sec. Values of sedimentation coefficients
s	sedimentation, e.g. s rate
s	sedimentation coefficient or constant
$s_{20,w}$	sedimentation coefficient at 20 °C in water. Values are in Svedberg units
UV	ultraviolet
ε	the molecular extinction coefficient
λ	the wavelength
μ	micro = 10^{-6}, e.g. μm = 10^{-6}m
′ ″	degree and minute, respectively (unit symbols for angles)

Abbreviations of viruses

BCMV	bean common mosaic virus
BYMV	bean yellow mosaic virus
CMV	cucumber mosaic virus
CaLV	carnation latent virus
CaMV	carnation mottle virus
CaRSV	carnation ringspot virus
PLRV	potato leafroll virus
PVX	potato virus X
PVY	potato virus Y
TEV	tobacco etch virus
TMV	tobacco mosaic virus
TNV	tobacco necrosis virus
TRV	tobacco rattle virus
TRSV	tobacco ringspot virus
TmSWV	tomato spotted wilt virus

1 Introduction

The exercises of this course are intended to acquaint the student with methods which are in use for the identification of viruses, dealing mainly with the phytopathological aspects of plant virology. This manual contains instructions for the identification of several viruses. The design of it makes it possible to divide a class of students in pairs. Each pair works with a different virus, in so far as it fits in with a course of about 4 weeks. To terminate the course in a shorter time is inefficient because it will take 4 weeks before some results appear; so a course of 4 weeks of half days is more useful than one of 14 days. If students see results of pairs working with other viruses, they can form an idea of the properties which distinguish one virus from the other.

To begin research on a disease, suspected to be caused by a virus, in general, first symptoms are observed and then conjectures on the outbreak and spread of the diseases are made. Dependent on results, an investigation will be started by trying to transmit the 'unknown' by grafting, by vectors, with soil, or by sap inoculation. In a students course, however, a special chronological order for the experiments is preferred to prevent waste of time. An example of such a scheme for one pair of students is presented here. It is evident that the order in Section 1.1 and in the manual is quite different.

To follow this course successfully some basic knowledge of plant virology is necessary. The books of Smith (1968), and Köhler (1964) in particular are recommended. Also certain chapters in Corbett & Sisler (1964), Bawden (1964), Matthews (1970), and Klinkowski (1968) are useful. In composing this course the Chapter 'the identification of plant viruses' of Ross in Corbett & Sisler (1964) has been rifled.

1.1 Chronological order of the work for a 4-week course

The following scheme is for one pair of students working with carnation ringspot virus (CaRSV), but also performing several experiments with other viruses.

Date	Number of the experiment	
1	3.1	Mechanical transmission of CaRSV to a host range. Read Chapter 2.
	18.3.1	Item 19: 10 seedlings of *N. tabacum* 'White Burley' are planted in sterilized soil

2	21.1	Stem-graft method
	21.2	Tuber grafting.
	7.1	Dilution end-point determination with CaRSV.
3	18.3.1	Isolation of nematodes (*Trichodorus* species).
	3.1	Observe the plants. Describe symptoms, if any, and make colour pictures.
	20.1	Seeds of bean *(Phaseolus vulgaris)* gathered from plants infected with bean common mosaic virus are sown, e.g. 5 pots each with 3 seeds.
4	8.1	Thermal inactivation point determination with CaRSV.
	16.1	Rearing virus-free aphids. Fifty wingless parthenogenetic viviparous females are put on a leaf of *Brassica pekinensis* in a Petri dish.
	3.1	Describe symptoms.
5	16.2A	Item 1–3. Transfer of aphids to diseased plants.
	10.1	Cross-protection test. Inoculate 6 of the 9 *N. glutinosa* plants with cucumber mosaic virus.
	16.1	Transfer freshly born aphids to a healthy plant of chinese cabbage.
	7.1	Count lesions if clearly visible. If not, wait to another date.
8	9.1	Longevity in vitro (aging) determination.
	16.2	Items 4 and 5. See working schedule of Section 16.2, 4th day.
	10.2.2	Inoculate 6 out of 12 plants with tobacco mosaic virus.
	3, 7, 8	Record results if necessary.
9	16.2	Item 6. See working schedule of Section 16.2, 5th day.
	21.1	Item 6. Inoculate with tobacco mosaic virus.
	4	Check internal symptoms, Sections 4.1–4.4. On date 9 and on the following days if time is left.
10	11.7.2	Purification of CaRSV. Follow procedure 2. If desired, stop after item 12 and put the suspension in a refrigerator. Continue on date 11.
	16.2	Item 8. Schedule 6th day.
11	11.7.2	Continue with the purification of CaRSV. Finish procedure 2, and continue up till procedure 1 item 13.
	4.5	Starch accumulation in chloroplasts. Inoculate the cotyledons of 2 *Cucumis sativus* seedlings.
	21.1	Record results: local lesions on the inoculated leaves of *N. glutinosa*.
	9.1	Count lesions if clearly visible.
12	11.7.2	Continue with the purification item 14 of procedure 1. Continue:
	11.3	Density-gradient centrifugation with the virus suspension of CaRSV.
	10.1	Cross-protection test. Inoculate with the yellow strain of cu-