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Reconsideration of the distinction between the severe and yellow strains of cowpea mosaic virus

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Abstract

The properties of two isolates of cowpea mosaic virus (CPMV), previously considered representing the yellow and the severe strains of CPMV are compared. The two isolates characteristically differ in host range, type of symptoms produced, serology, the ratio of the virus components as shown by the sedimentation pattern, and in thermal inactivation.

Based on these differences, and earlier experiences on the genetic relationship among the two types of virus isolates, the severe and yellow strain isolates of CPMV, the authors tend to distinguish these as two different viruses, both members of the cowpea mosaic virus group.

In view of taxonomical complications however, the two viruses may be kept together for the time being as two considerably differing strains of one virus.

Introduction

Agrawal (1964) examined three isolates of cowpea mosaic virus (CPMV), R/1:1.5/24 + 2.5/33:S/S:S/Cl, designated Vu, Vs and Sb, originating from Surinam and compared these with isolates of cowpea mosaic virus from Trinidad (Dale, 1949) and cowpea yellow mosaic virus from Nigeria (Chant, 1959). It was concluded that all isolates belonged to cowpea mosaic virus (CPMV). Two strains were distinguished: (1) the severe or 'S' strain comprising the Vu, Vs and Trinidad isolates, and (2) the yellow or 'Y' strain, including the Sb and the Nigerian isolates. Purified infectious preparations of CPMV contain two nucleoprotein particles similar in size but differing in RNA content; both are necessary for virus infectivity. Further research on CPMV has shown that the genetic properties of CPMV are distributed among both nucleoprotein components (see Van Kammen, 1972, for review).

In previous studies on the 'S' and 'Y' strain a close genetic relationship between both strains was not found in experiments on heterologous mixing of the two nucleoprotein components of both strains to obtain hybrid strains (Van Kammen, 1968) and in experiments to compare the nucleotide sequences of the viral RNA's of each strain by molecular hybridization (Van Kammen and Rezelman, 1972). The present authors have doubted whether it is justified to consider the isolates as strains of one virus and have therefore reexamined symptoms and host ranges, serology and some physical properties of the two virus 'strains'.

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Table 1. Host range and symptoms of Cowpea mosaic virus isolates Nig and Vs

Plant species	Isolate Nig from Nigeria	Isolate Vs from Surinam
1. *Vigna unguiculata* 'Blackeye Early Ramshorn'	Loc.[1] chlorotic spots turning into yellow mosaic Syst. bright yellow mosaic, reduction in size of leaves, leaf distortion	Loc. chlorotic and necrotic spots, veinal necrosis Syst. stem necrosis, leaf distortion, plants collapse
2. *V. unguiculata* 'Going'	Loc. chlorotic, later necrotic spots Syst. light green mosaic	Loc. diffuse chlorotic spots Syst. arrested development
3. *V. unguiculata* 'Early Red'	Loc. small white LL Syst. no symptoms[2]	Loc. diffuse chlorotic spots Syst. severe leaf deformation, vein yellowing
4. *V. unguiculata* 'PI 221731'	Loc. chlorotic spots, veinal necrosis Syst. stem necrosis, plants collapse	Loc. as for isolate Nig Syst. as for isolate Nig
5. *Phaseolus vulgaris* 'Beka'	Loc. diffuse, light green LL, spreading along the veins Syst. vein clearing, leaf deformation, yellow starlike spots	Loc. diffuse LL Syst. severe deformation and apical necrosis
6. *Ph. vulgaris* 'Scotia'	Loc. brown necrotic LL ⌀ 1–2 mm Syst. no symptoms	Loc. dark brown necrotic LL ⌀ ± 0,5 mm Syst. no symptoms
7. *Chenopodium amaranticolor*	Loc. necrotic lesions ⌀ 0,5–1 mm Syst. chlorotic spots, severe apical deformation	Loc. necrotic lesions ⌀ 0,5–1 mm Syst. no symptoms
8. *Chenopodium quinoa*	Loc. chlorotic lesions Syst. leaf distortion, partial leaf yellowing	Loc. chlorotic, later necrotic lesions Syst. no symptoms
9. *Nicotiana tabacum* 'Samsun'	no symptoms	Loc. white necrotic ring formation[3] Syst. no symptoms
10. *Nicotiana tabacum* 'Samsun NN'	no symptoms	as for 'Samsun'
11. *Nicotiana glutinosa*	no symptoms	Loc. white necrotic ring formation Syst. sporadic white necrotic ring formation
12. *Nicotiana clevelandii*	Loc. no symptoms Syst. plant stunting[4]	Loc. white necrotic spots Syst. chlorotic spots
13. *Trifolium incarnatum*	Loc. light green mosaic (3 of 8 plants) Syst. light green mosaic (3 of 8 plants)	Loc. as for isolate Nig (1 of 8 plants) Syst. as for isolate Nig (1 of 8 plants)
14. *Petunia hybrida* 'Pink Beauty'	no symptoms	no symptoms[5]
15. *Pisum sativum* 'Zoete Eminent'	Loc. no symptoms Syst. apical vein yellowing, small brown spots on leaves and veins (2 of 8 plants)	Loc. no symptoms Syst. apical chlorotic spots, getting necrotic (1 of 8 plants)

Tabel 1. Waardplantenreeks en symptomen van de isolaten Nig en Vs van 'cowpea'-mozaïekvirus.

Materials and methods

The yellow strain isolate of CPMV from Nigeria, designated Nig, and the severe strain isolate from Surinam, designated Vs, both described by Agrawal (1964), were used in the experiments. The viruses were propagated in cowpea *Vigna unguiculata* (L.) Walp 'Blackeye Early Ramshorn'. Purified virus was used as inoculum for host range determinations. Virus was purified by the polyethylene glycol - NaCl method (Van Kammen, 1967). Purified virus was suspended in 0.01 M phosphate buffer, pH 7.0 and preserved on ice. Virus concentrations were determined spectrofotometrically using an extinction coefficient (1 mg/ml; 1 cm light path) $E_{260} = 8.1$.

Local lesion assays were made on *Phaseolus vulgaris* 'Scotia'. If tested on opposite half leaves the virus concentration in the inoculum had to be four times higher with Vs than with Nig to obtain equal numbers of lesions. The concentration of Nig in the inoculum used in host range studies was 210 μg/ml and that of Vs 840 μg/ml. The differential hosts are listed in Table 1. With beans and cowpeas the primary leaves were inoculated just before the trifoliate leaves unfolded. Other species were inoculated in a fit young stage of development, varying from 30 to 60 days after sowing. Plants were grown in the greenhouse at 22 °C.

For inclusion bodies epidermal strips were taken from the lower side of leaves or from stems, five days after inoculation and stained in a solution of 1 % phloxin in cellosolve for 30 min. The strips were washed twice in distilled water for 10 min and mounted in water for immediate observation with a Wild microscope. Photographs were taken at a magnification of times 400.

Antisera against Nig and Vs were prepared by giving rabbits two intravenous injections of 4 mg of virus in 1 ml two days apart and two weeks later one intramuscular injection of 12 mg of virus mixed with an equal volume of Difco incomplete Freund's adjuvant. The rabbits were bled from the ear after 6 weeks. Absorbed antisera were made by adding 2 mg of Nig to 1 ml of Vs antiserum and vice versa. The flocculent precipitate was separated after two hours by centrifugation at 1000 *g* for 15 min. The titers of the antisera were determined by the agar gel diffusion test. A virus concentration of 1 mg/ml was used in all tests.

Sedimentation patterns of the isolates were examined in a Spinco model E analytical ultracentrifuge using Schlieren optics. For determining the thermal inactivation points of the viruses, samples of purified CPMV-Nig (210 μg/ml) and Vs (840 μg/ml) were heated for 10 minutes at temperatures between 55 °C and 80 °C with 5 °C intervals. The samples were cooled in tap water and inoculated immediately on cowpea, 'Blackeye Early Ramshorn'.

←———

[1] Loc. = local symptoms; Syst. = systemic symptoms; LL = local lesions.

[2] 20 % of the plants showed a deformation of trifoliate leaves. Back inoculation to *V. unguiculata* 'Blackeye Early Ramshorn' proved the presence of virus. Trifoliate leaves without symptoms did not contain any virus.

[3] These local symptoms appeared on the youngest inoculated leaf.

[4] Virus was shown by testing on *V. unguiculata* 'Blackeye Early Ramshorn'.

[5] The presence of CPMV-Vs and CPMV-Nig in the non-inoculated leaves was shown by testing on *V. unguiculata* 'Blackeye Early Ramshorn'.

Samples of both viruses were dialysed against 0.1 M phosphate buffer solutions of pH 5.0, 7.0 and 9.0 for 24 h in the cold room to determine the effect of the pH on infectivity.

Results

Host range and symptoms. In Table 1 the symptoms of Nig and Vs on fifteen plant species and cultivars are compared. The symptoms of both isolates differ markedly on most species tested. Cowpea 'PI 221731' reacted with similar local and systemic symptoms to infection with both isolates. Likewise the two isolates could not be distinguished by symptoms on *Trifolium incarnatum*. *Petunia hybrida* did not react with symptoms, but both isolates could be recovered from noninoculated leaves. On all other species tested, both isolates could be easily distinguished. Nig did not produce symptoms on any of the four *Nicotiana* spp. tested but could be recovered. There was, however, some reduction in growth of *N. clevelandii*. In contrast, Vs produced clear symptoms on the inoculated leaves of the four *N.* spp. Reaction to Vs was more severe than to Nig on the *Vigna*, *Phaseolus* and *Nicotiana* spp. listed in Table 1. The opposite held for the *Chenopodium* spp., which reacted more severely to infection with Nig.

Cowpea 'Blackeye Early Ramshorn', French bean 'Beka' and *Chenopodium amaranticolor* are good differentials for both isolates. 'Pinto' and 'Scotia' are the most suitable bean cultivars of *Phaseolus vulgaris* for local lesion assay of both isolates.

Inclusion bodies. Inclusion bodies were observed in a number of plant species after staining with phloxin as a red amorphous mass near or surrounding the nucleus, but never in healthy plants. The type of inclusion did not differ for Nig and Vs. The inclusion bodies were observed in epidermal strips from the inoculated primary leaves of 'Blackeye Early Ramshorn', 'Going' and 'PI 221731', and in strips from the stem of 'PI 221731', all infected with either Nig or Vs. They were also found in strips from the stem of 'Blackeye Early Ramshorn' and in strips from inoculated leaves of *Pisum sativum* with Vs but not with Nig.

Serology. The Nig antiserum had a titer of 128 if tested with Nig and of 8 if tested with Vs. The Vs antiserum had a homologous titer of 256 and a heterologous titer of 16.

When the Nig antiserum was absorbed with Vs antigen, the heterologous reaction disappeared completely whereas the homologous reaction with isolate Nig was virtually unchanged. The same held for the Vs antiserum absorbed with Nig. The Nig antiserum kept a homologous titer of 96 and the Vs antiserum had a homologous titer of 512 after absorption. Thus the two isolates have a rather weak antigenic determinant in common, but each possesses a specific antigenic group. This is further illustrated by the agar gel diffusion patterns presented in Fig. 1. The patterns 1 and 2 show the strong reaction of the two antisera with the homologous virus and a weak precipitation line in the heterologous reaction. The latter line fuses with the precipitation line of the homologous reaction indicating the relationship of the antigenic groups involved. The absorbed antisera (pattern 3 and 4) produce only precipitation lines between the wells with antiserum and the homologous virus and the heterologous reaction is absent. A mixture of absorbed antisera (pattern 5) produces crossing precipitation lines de-

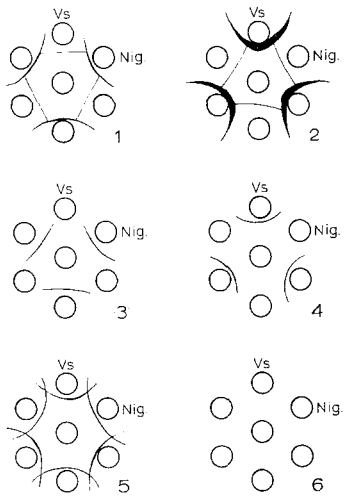

Fig. 1. Serological comparison of the isolates Nig and Vs by the agar-gel diffusion test. The central wells contain the antisera: 1) Nig; 2) Vs; 3) Nig absorbed with Vs; 4) Vs absorbed with Nig; 5) a 1:1 mixture of Nig and Vs absorbed; 6) normal serum. In all patterns, the surrounding wells alternatingly contain isolates Vs and Nig, both in a concentration of 1 mg/ml.

Fig. 1. Serologische vergelijking van de isolaten Nig en Vs met behulp van de agar-gel diffusie test. De middelste putjes bevatten de antisera, respectievelijk: 1) Nig; 2) Vs; 3) Nig verzadigd met Vs; 4) Vs verzadigd met Nig; 5) een 1:1 mengsel van Nig verzadigd met Vs en Vs verzadigd met Nig; 6) normaalserum. In alle patronen bevatten de omringende putjes afwisselend de isolaten Vs en Nig, beide in een concentratie van 1 mg/ml.

monstrating the lack of relationship between the principal antigenic groups of both antigens. Pattern 6 is a control with normal serum.

Sedimentation. Fig. 2 shows the Schlieren diagrams of purified Nig and Vs. Both preparations consist of three centrifugal components, with sedimentation coefficients of about 58 (top), 95 (middle) and 115 S (bottom). There is a large difference between the two isolates in the ratio between the relative amounts of the 95 S and the 115 S components, being about 2 for Nig and 10 for Vs.

Purified preparations of Vs from cowpea 'Blackeye Early Ramshorn', 'Early Red' and 'Going', and from *Nicotiana clevelandii* all showed similar sedimentation patterns. Likewise Nig produced similar relative amounts of centrifugal components if grown in 'Blackeye Early Ramshorn' and 'Going'. The ratios were apparantly characteristic of the virus isolate and did not depend on the host species.

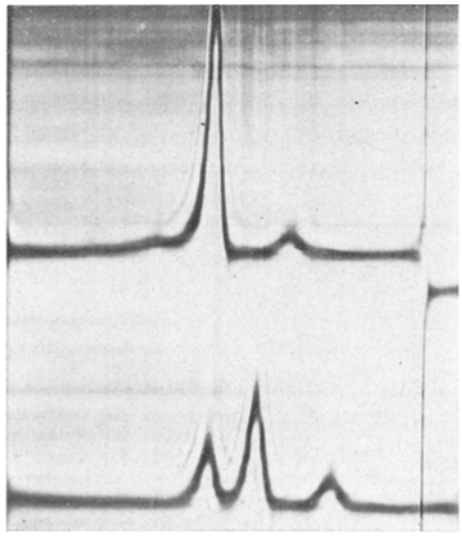

Fig. 2. Schlieren pattern of the purified isolates Vs (above), and Nig (below), both in a concentration of 3.0 mg/ml in 0.01 M phosphate buffer, pH 7.0. Sedimentation is from right to left. Ultracentrifuge run at 20 °C and 31,410 rev/min.

Fig. 2. Schlieren patroon van de gezuiverde isolaten Vs (boven) en Nig (onder), beide in een concentratie van 3,0 mg/ml in 0,01 M fosfaatbuffer pH 7,0. Sedimentatie is van rechts naar links. Ultracentrifugering bij 20 °C en 31.410 rpm.

Fig. 3. Inclusion body near the nucleus in the cell of an epidermal strip from a leaf of Vigna 'PI 221371', 5 days after inoculation with the isolate Nig. Magnification × 400.

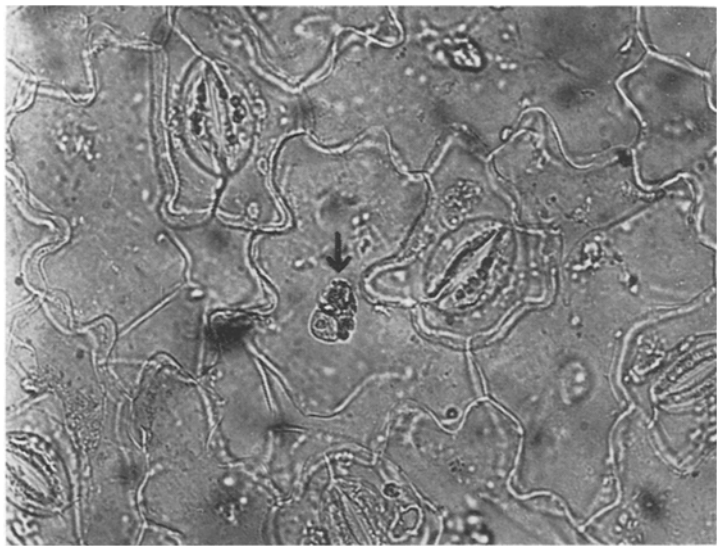

Fig 3. Insluitsel bij de kern in een epidermiscel van een Vigna blad 'PI 221371', 5 dagen na inoculatie met het isolaat Nig. Vergroting 400 ×.

Thermal inactivation points. The thermal inactivation points of the two isolates were compared for preparations of equal infectivity. Vs lost its infectivity completely at 75 °C for 10 minutes. Nig still infected all inoculated plants if heated for 10 minutes even at 80 °C. The thermal inactivation points of both isolates have not been compared by Agrawal (1964).

Discussion

From these investigations it appears that both virus isolates differ considerably. Our results present a more detailed comparison of the symptoms and host range of the two isolates than that of Agrawal (1964). As far as we examined the same plant species our results are in agreement with those of Agrawal except for the symptoms produced by Vs on *Nicotiana glutinosa.*

The symptoms of Nig and Vs are different on most of the hosts tested. Although they have many hosts in common Vs produced symptoms on four *Nicotiana* spp. but Nig did not. In cells of plants infected with each isolate inclusion bodies frequently occurred. The type of inclusion bodies did not differ from those described by Agrawal (1964). No distinction could be made between the two virus isolates on the basis of the type of inclusion bodies. It appears of interest to investigate if all viruses of the cow-pea mosaic virus group give rise to similar inclusion bodies.

There is only a weak serological relationship. Our observations correspond in this respect with those of Agrawal and Maat (1964). Both isolates have strong antigenic

groups, not showing any relationship. The weak relationship appears to be due to a second antigenic determinant.

The two virus isolates clearly differ in thermal inactivation. Both isolates have three centrifugal components with a ratio middle to bottom component characteristic for each isolate and different among themselves.

The viruses of the cowpea mosaic virus group have a genome divided among two nucleoprotein components. Both middle and bottom components are necessary for virus multiplication. It was earlier reported (Van Kammen, 1968) that heterologous mixtures of middle component of the isolate Trinidad (similar to the Vs isolate) and bottom component of Nig, or the reverse, are non-infectious. Apparently, the components of the two isolates do not complement each other for infectivity.

Van Kammen and Rezelman (1972) showed by molecular hybridization experiments that there is no homology in nucleotide sequences between the RNA's of the isolates Nig and Vs. This further indicates that there is no close genetic relationship between the two isolates.

Thus there are considerable phenotypic and genetic differences between the two virus isolates. On the basis of such observations it seems hardly justified to consider the two groups of isolates of cowpea mosaic virus as strains of one virus. However, splitting cowpea mosaic virus into two different viruses, might lead to some nomenclatural problems. Previously Dale (1949) named the virus isolate from Trinidad, cowpea mosaic virus and the isolate from Nigeria was indicated as cowpea yellow mosaic virus by Chant (1959). These were perfectly good names and, in case of splitting, it would only be fair to return to these names. However, this would mean that much of the recent work on the properties of cowpea mosaic virus has then to be referred to cowpea yellow mosaic virus, with consequent confusion. For practical reasons the isolates may be therefore kept together.

The importance of molecular hybridization for the classification of plant viruses deserves further serious consideration. This also holds for the importance of genetic crossing experiments in case of plant viruses with a divided genome, like the viruses of the cowpea mosaic virus group.

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Samenvatting

Herbeschouwing van het onderscheid tussen de 'severe' en de gele stam van 'cowpea'-mozaïekvirus

Agrawal (1964) heeft de eigenschappen van vijf verschillende isolaten van het 'cowpea'-mozaïekvirus (CPMV) vergeleken en onderscheidde twee stammen: een gele, waartoe de isolaten Nig en Sb behoren en een 'severe', waartoe de isolaten Vu, Vs en Trinidad behoren.

Nader onderzoek is verricht over het isolaat Nigeria (Nig) en het isolaat Vs, af-

komstig uit Suriname, als vertegenwoordigers van respectievelijk de gele en de 'severe' stam.

Het onderzoek toonde aan dat beide isolaten in waardplantenreeks, op een enkel karakteristiek verschil na sterk overeenkomen, maar dat de aard van symptomen op de meeste plantesoorten verschilt (Tabel 1). Onderzoek op de aanwezigheid van insluitsels in epidermisstrips van geïnfecteerde bladeren bracht géén verschil aan het licht tussen de twee isolaten. Bij iedere positieve reactie werd voor het isolaat Nig zowel als voor het isolaat Vs dezelfde amorfe structuren gevonden, die steeds tegen of rondom de kern lagen (Fig. 3).

De serologische verwantschap tussen Nig en Vs is erg zwak. Beide hebben een sterke eigen, geheel verschillende, virus-specifieke antigene structuur. Een tweede antigene structuur is verantwoordelijk voor de geringe verwantschap (Fig. 1). Bij het bekijken van de sedimentatiepatronen in de analytische ultracentrifuge bleek dat de verhouding van midden- tot bodemcomponent voor Vs veel groter is dan voor Nig (Fig. 2).

Het isolaat Vs bleek na 10 minuten verhitting bij 75 °C niet meer tot infectie in staat te zijn, terwijl Nig bij 80 °C nog een goede infectie geeft.

Op grond van deze verschillen en op grond van eerder gedaan onderzoek waarbij de genetische verwantschap van de twee isolaten is bestudeerd aan de hand van een vergelijking van de nucleotidenvolgorde van de beide RNA's met behulp van moleculaire hybridisatie (Van Kammen en Rezelman, 1972), en met infectiositeitsproeven met mengsels van componenten van beide typen isolaten (Van Kammen, 1968), lijkt het nauwelijks verantwoord de twee groepen isolaten van cowpea mozaïekvirus te blijven beschouwen als stammen van één virus.

Wanneer echter op dit moment van de twee groepen virusisolaten twee verschillende virussen gemaakt worden, zou dat zeer vervelende nomenclatuur-problemen met zich meebrengen. Om zuiver praktische redenen kunnen de isolaten voorlopig beter nog als twee (zij het zeer verschillende) stammen van één virus beschouwd worden.

Het lijkt echter van groot belang om de betekenis die moleculaire hybridisatie kan hebben voor de classificatie van plantevirussen, nader uit te werken. Hetzelfde geldt voor de betekenis van kruisingsexperimenten, die gedaan kunnen worden met plantevirussen met een verdeeld genoom, waarvan de virussen van de cowpea mozaïekvirusgroep een voorbeeld zijn.

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Book review

Gregory, P. H.: Microbiology of the atmosphere. 2nd edition. 377 pp., 8 plates. Leonhard Hill, Aylesbury (Bucks.) 1973.

'Microbiology of the atmosphere' written by the former Head of the Plant Pathology Department at Rothamsted Experimental Station, and first published in 1960, was the first and only treatise on this topic; this year an updated and expanded second edition has been published. The most important publications of the last decade have been incorporated in the text and bibliography (38 pages). New chapters have been added on rain-splash dispersal, inhaled microbes in relation to respiratory infection and allergy, and on survival in the atmosphere.

After a historical introduction and descriptions of the general behaviour of spores in still air and the structures of the atmosphere, the path of the constituents of the air spora is followed: spore liberation (in particular in splash droplets), horizontal diffusion and deposition processes. Gregory gives a modern review of sampling techniques and the constituents of the air spora in lower and upper layers with a discussion of survival. Following topics are effects of deposition in rain, snow and hail, and deposition gradients and long-distance dispersal. These two chapters are of the greatest importance to plant pathologists, since the factors determining dispersal of epidemic air-borne plant pathogens such as *Puccinia* spp., *Tilletia*, *Phytophthora* and *Peronospora* are fully discussed. Professor Gregory has contributed much to research by his wind-tunnel studies on spore translocation and sedimentation and, together with his wife, Margaret F. Gergory, to the subsequent mathematical formulation of the processes. From the deposition gradients predictions on the spread of some plant diseases are possible; the long-distance transport of certain species, however, largely escapes exact experimental study. For a more detailed account the reader is referred to Gregory's contribution on interpretation of plant disease dispersal gradients in Annual Review of Phytopathology Vol. 6 (1968). The aerobiological approach not only affects understanding of general spread of a pathogen but also various other aspects of plant pathology, e.g. the interpretation of spraying trials on plots surrounded by untreated ones. While the book as a whole offers a concise but highly readable text, the mathematical treatment is so condensed that it is often difficult to follow; a list of symbols used would have been helpful.

The two chapters on air-spora of enclosed spaces and on inhaled microbes are of importance to hygienists and allergologists. Two coloured plates (1000 times enlarged) with fungal and other microbial spores, and one in black and white with pollen grains illustrate the subject nicely, but should not be used with too much confidence for identification. In a concluding chapter entitled 'Aerobiology', the author reviews the various aspects, pointing out some uncertainties and suggesting that a fresh study of aerobiology would need simultaneous work in different parts of the world by similar methods.

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