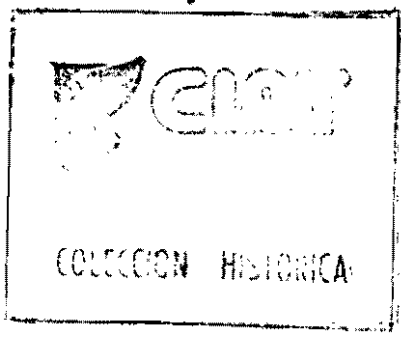


68
122
•E4
P76



13302
BIBLIOTECA

~~PROCEEDINGS~~ OF A WORKING GROUP MEETING
ON VIRUS DISEASES OF BEANS AND COWPEAS IN AFRICA.
KAMPALA, UGANDA

17th-21st January, 1990

CIAT Africa Workshop Series, No. 13

Editor : D.J. Allen

Workshop Sponsors : The regional programmes in Africa of the
Centro Internacional de Agricultura Tropical
(CIAT) and the International Institute of
Tropical Agriculture (IITA)

CIAT

IITA

PREFACE

This volume, recording the proceedings of a meeting convened jointly between CIAT and IITA on legume virus diseases in Africa, is one of a series that documents the findings of researchers on bean (*Phaseolus vulgaris*) in Africa. The objectives of this meeting were to bring together scientists concerned with virus diseases of legumes in eastern and southern Africa, so as to exchange information, establish research priorities and develop a network for collaborative research in the region. The meeting was run in a fully informal manner so as to foster free discussion: these proceedings in some places have been edited to reflect that informal style. These proceedings form part of the activities of the Pan African bean research network which serves to stimulate, focus and co-ordinate research efforts on the crop.

Publications in this series include the proceedings of workshops held to assess the status, future needs and methodological issues of research in selected topics that constrain production or productivity of beans in Africa. Publications in this series are listed on the last page of these proceedings.

The network is organised by the Centro Internacional de Agricultura Tropical (CIAT) through three interdependent regional projects, for the Great Lakes region of Central Africa, for Eastern Africa and, in conjunction with SADCC, for the Southern Africa Region.

Support for the regional bean projects and for this publication comes from the Canadian International Development Agency (CIDA), the Swiss Development Co-operation (SDC) and the United States Agency for International Development (USAID).

Further information on regional research activities on beans in Africa is available from:

Regional Co-ordinator, SADCC/CIAT Regional Programme on Beans in Southern Africa, P.O. Box 2704, Arusha, Tanzania.

Regional Co-ordinator, CIAT Regional Programme on Beans in Eastern Africa, P.O. Box 41541, Addis Ababa, Ethiopia.

Coordinateur Regional, CIAT, Programme Regional pour l'Amelioration du Haricot dans la Region des Grands Lacs, B.P. 259, Butare, Rwanda.

Information on regional research activities on cowpeas in Africa is available from:

Director, Grain Legume Improvement Programme, IITA, PMB 5320, Ibadan, Nigeria.

Regional Coordinator, SADCC/IITA Cowpea Research Project, INIA, CP 2100, Maputo, Mozambique.

The organizers of this working group meeting would like to acknowledge the contribution of the following individuals and organizations that made the meeting a success, and these proceedings possible :

Professor Joseph Mukiibi, Secretary for Research,
Ministry of Agriculture, Uganda

Dr. Samson Owera, Lecturer,
Faculty of Agriculture, Makerere University

Staff of the Nile Hotel and International Conference Centre,
Kampala

Miss Eva Ngalo, for secretarial services

The Canadian International Development Agency

The United States Agency for International Development, and

The Swiss Development Cooperation.

TABLE OF CONTENTS

SESSION I: INTRODUCTION: PARTICIPANTS, PROGRAMME AND OBJECTIVES	1
SESSION II: VIRUS DETECTION IN PLANT AND SEED	
An evaluation of a virus/bacterial slide agglutination test for rapid virus identification D.G.A. Walkey, N.F. Lyons and J.D. Taylor	3 ✓
Two immunocytochemical methods for <u>in situ</u> detection of antigens with the light microscope H. Lohuis and Jeanne Dijkstra	6 ✓
Discussion Gaylord Mink (Femi Lana, Rapporteur)	13
SESSION III: VIRUS IDENTIFICATION AND GEOGRAPHICAL DISTRIBUTION	
Recent progress in the identification of viruses of <i>Phaseolus vulgaris</i> in Africa H.J. Vetten and D.J. Allen	18 F
Discussion H.J. Vetten (Nicola Spence, Rapporteur)	22 F
SESSION IV: VIRUS STRAINS : ORIGINS AND IMPLICATIONS	
Studies into the distribution and identity of African strains of bean common mosaic virus (BCMV) Nicola J. Spence.	27 F
Discussion Francisco Morales (David Allen, Rapporteur)	29 F
SESSION V: VIRUS ECOLOGY AND ECONOMIC IMPORTANCE	
Possible dependence of geographical distribution of virus diseases of cowpea in Africa on agro-ecological parameters H.W. Rossel and G. Thottappilly	33
Discussion Hennie Rossel (Howard Gridley, Rapporteur)	37

SESSION VI: VIRUS EPIDEMIOLOGY : VECTORS, SEED AND WEEDS

Epidemiology of bean common mosaic virus
disease t
A.F. Lana 39

Discussion
Femi Lana and Mike Thresh (J. Dijkstra,
Rapporteur) 42

SESSION VII: DEVELOPMENT OF AN ACTION PLAN

Discussion of virologists' perspective
David Allen (Gaylord Mink, Rapporteur) 51

Discussion of breeders' perspective
Howard Gridley (John Taylor, Rapporteur) 56

LIST OF PARTICIPANTS 57

ANNOUNCEMENTS 60

CIAT AFRICAN WORKSHOP SERIES 61

SESSION I - INTRODUCTION

PARTICIPANTS, PROGRAMME AND OBJECTIVES.

D.J. Allen, H.W. Rossel and S. Owera

The objectives of the meeting were to bring together virologists, and breeder/pathologists concerned with virus diseases, from within eastern and southern Africa to focus attention on recent advances made in the identification of viruses naturally infecting legumes in Africa, with particular reference to beans and cowpeas; to establish priorities and a programme for future research; and to develop a network for collaborative research in the region.

Dr. Samson Owera opened the meeting by welcoming participants to Uganda where, he said, protocol had been essentially dispensed with. Clearance for the meeting had been obtained from Government and the Secretary for Research, Dr. Joseph Mukiibi, had been informed. Owera concluded with the remark that bean common mosaic virus (BCMV) was a priority problem in Uganda and his links with Dr. H.J. Vetten in Braunschweig had proved most valuable.

David Allen then described the background to this Legume Virus Working Group Meeting which was one in a series of CIAT Pan African workshops and working groups, priorities for which were set at regular meetings of the steering committees governing CIAT's regional programmes in Africa. Such meetings are discipline-specific, and are designed to bring together national scientists to form a network, and to set research priorities for the region. This meeting was being run jointly by CIAT and IITA, so as to avoid undue commodity crop bias, to encourage a better understanding of relationships between viruses naturally infecting beans and cowpeas in Africa, and to foster links between the few plant virologists in eastern and southern Africa. This time, national scientists were outnumbered by virologists from outside the region. Allen stressed that this working group had not been designed as an international workshop and for this reason would be run in a fully informal but structured manner so as to maximise opportunities for free discussion. Practical outcomes from this legume virus working group meeting might be expected to be : the exchange of information on recent research progress on the identification of viruses in the region, so as to assess their economic importance; to assess the degree of pathogenic variation within the principal legume viruses and appraise what implications strains may have for crop improvement programmes; and to provide a solid basis for updating legislation underlying local plant quarantine services.

Allen drew attention to certain projects concerned with bean viruses in Africa, proposing that appropriate linkages be developed between them. Such projects included the following :

- (i) The bean/cowpea collaborative research support programme (CRSP) between Washington State University and Sokoine University of Agriculture in Tanzania (represented by Dr. Gaylord Mink).
- (ii) The Braunschweig/BMZ/CIAT project designed to identify viruses other than BCMV in beans in Africa (represented by Dr. H.J. Vetten).
- (iii) The Institute of Horticultural Research/ODA/CIAT project to identify BCMV strains in Africa (represented by Dr. David Walkey and Nicola Spence).
- (iv) The CIAT regional collaborative research sub-projects on BCMV (led by Dr. Samson Owera and Prof. Femi Lana).
- (v) And a PhD thesis study at Cambridge University with field work in Zimbabwe (supported and co-supervised by CIAT) of Olivia Mukoko.

Hennie Rossel stressed the possible evolutionary links between legume viruses, suggesting that this meeting provided opportunities for valuable discussion, and to devise means of aiding breeders, including virus strain identification. He suggested that we should hope to attain a new level of understanding of the technical problems as well as of one another.

Femi Lana took the opportunity of congratulating the organizers in this important initiative.

SESSION II - VIRUS DETECTION IN PLANT AND SEED

AN EVALUATION OF A VIRUS/BACTERIAL SLIDE
AGGLUTINATION TEST FOR RAPID VIRUS IDENTIFICATION

D.G.A. Walkey, N.F. Lyons and J.D. Taylor

INTRODUCTION

The use of a virobacterial slide agglutination (VBA) test for rapid diagnosis of plant viruses was first reported by Chirkov et al (1984). The technique involves the agglutination of the bacterium, *Staphylococcus aureus*, on a glass slide. *S. aureus* is first killed by heat and formaldehyde treatment and then conjugated with the specific antiserum required. The large amount of protein A that occurs naturally on the surface of the bacterium conjugates readily and immediately with immunoglobulins, particularly IgG, when they are mixed. This working conjugate is then mixed with crude plant sap infected with the virus to be tested. The virus links with its specific antibody and causes the bacterial particles to be linked together, and agglutination occurs.

REAGENTS AND PROTOCOL FOR THE TEST.

Reagents:

- i. Phosphate buffered saline (PBS), for one litre:

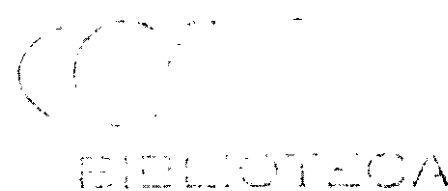
2.9g Na₂HPO₄ 12H₂O
 0.2g KH₂PO₄
 8.0g NaCl
 0.2g KCl

at pH 7.2. Add sodium azide (NaN₃) at 2mg/ml

- ii. Formalin treated suspension of *Staphylococcus aureus* (available from HRI Wellesbourne).

METHOD

- i. Dilute virus antiserum with PBS buffer in the ratio of one vol. antiserum (50:50 antiserum/glycerol mixture) to 24 vols. of PBS buffer.
- ii. Prepare conjugate by mixing *Staphylococcus aureus* suspension with diluted antiserum in the ratio of one vol. bacterium : five vols. of diluted antiserum.
- iii. This conjugation may then be coloured by adding several drops of saturated alcoholic basic fuchsin stain. (The colour makes the bacterial agglutination easier to see).



- vi. Approximately 4 μ l of this conjugate is then mixed with 2 μ l of antigen (infected crude sap) on a wetted, blue, multitest slide (available from Flow Laboratories Limited). The mixture should be quickly stirred with a thin glass rod.
- v. It is essential that a comparable negative control test, using healthy crude sap mixed with the conjugate, is prepared at the same time.
- vi. A positive reaction is indicated by agglutination of the bacterial particles within 0.5 to 3 mins of mixing. This is best observed with a hand-lens with the slide held over a black background lit by diffuse light.
- vii. Continual observation of the control reaction is important, for agglutination in this mixture will also occur over a longer period of time (particularly if the antiserum contains antibodies to healthy plant sap), but the positive virus-test reaction should have occurred long before the control agglutinates.
- viii. The bacterial/antiserum conjugate may be used after storage at 4°C for some months, but should be checked with a hand-lens for clumping before use. If clumping has occurred the clumps may be broken down by vigorous shaking (vortex stirrer) or mild sonication.

RESULTS AND DISCUSSION

The present evaluation has shown that using a reasonably good titred antiserum, agglutination occurs 30 seconds to 3 minutes after mixing. It is essential to use a comparative control test in which healthy plant sap is mixed with the conjugate as plant antibodies in an impure antiserum can also cause agglutination.

The test has given good results with all viruses so far tested, including alfalfa mosaic, bean common mosaic, bean yellow mosaic, beet western yellows, cauliflower mosaic, cucumber mosaic, clover yellow vein, leek yellow stripe, potato virus X, potato virus Y, tobacco mosaic and turnip mosaic viruses. No cross reaction was observed in homologous and heterologous tests between the following potyviruses: turnip mosaic, bean common mosaic, potato virus Y, lettuce mosaic, papaya ringspot, zucchini yellow mosaic and sugarcane mosaic.

Homologous and heterologous tests between five strains of bean yellow mosaic (BYMV) and clover yellow vein viruses gave identical results indicating that the test does not distinguish between individual BYMV strains. The test may be readily used, however, to distinguish BYMV from bean common mosaic virus.

The sensitivity of the VBA test was compared with electron microscope serology (ISEM) and host assay, for the detection of alfalfa mosaic, (AlfMV) and potato X (PVX) viruses in crude sap extracts. Both AlfMV and PVX were detected in VBA tests at dilutions of 10^{-3} , but not at 10^{-4} . In the ISEM tests AlfMV was detected at 10^{-3} but not at 10^{-4} , and PVX was detected at 10^{-2} but not at 10^{-3} . In the host bioassay tests the mean number of local lesions produced in half-leaf replicates of *Chenopodium quinoa* was as shown in Table 1.

The dilution of antiserum used (one vol. of antiserum : 24 vols of phosphate buffered saline) and the ratio of *S. aureus* to antiserum (one vol. of bacterium : five vols of antiserum) proved to be suitable for all antisera and viruses tested, but these ratios are flexible. Good results have also been obtained with antiserum/PBS dilutions of 1:12 and 1:36, and *S. aureus*/antiserum ratios of 1:2.5 and 1:10.

In conclusion, the VBA test provides a very rapid, simple and sensitive test for the detection of virus in crude sap samples. Provided a reasonably high-titred antiserum, free of healthy plant antibodies, is used, the test appears to be applicable to all viruses. The test is particularly suitable for laboratories where limited equipment is available, and could also be used as a field test.

REFERENCE

Chirkov, S.N., Olovnikov, A.M., Surgachyova, N.A. and Atabekov, J.G. (1984). Immunodiagnosis of plant viruses by a virobacterial agglutination test. Annals of applied Biology 104, 477-483.

Table 1. Mean number of local lesions produced in half-leaf replicates of *Chenopodium quinoa* in host bioassay tests with alfalfa mosaic virus (AlfMV) and potato virus X (PVX).

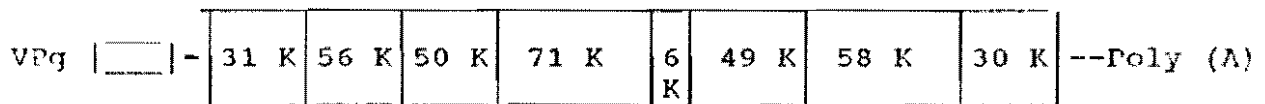
Virus	Dilution					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
AlfMV	51	44	7	4	2	1
PVX	123	76	31	4	0.5	0.8

TWO IMMUNOCYTOCHEMICAL METHODS FOR *IN SITU* DETECTION OF
ANTIGENS WITH THE LIGHT MICROSCOPE

H. Lohuis and Jeanne Dijkstra

INTRODUCTION

The RNA of potyviruses is translated into one big precursor polyprotein of about 346,000 D. The functional (mature) proteins are formed by proteolytic processing catalysed by virus-encoded proteinases of 49,000 D and the 20,000 D segment of the 56,000 D protein which also contains the so-called helper component necessary for aphid transmission. At the moment, eight mature proteins are known as cleavage products of the precursor polyprotein. These are shown in the following genetic map of a potyvirus (tobacco etch virus).



Transport protein (?) (31 K)

Helper component - proteinase (56 K) (amorphous inclusion protein)

Unknown function (50 K)

Cylindrical inclusion protein (71 K)

Viral protein genome - linked (VPg, 6 K)

Proteinase (49 K) }

} (nuclear inclusion proteins)

Polymerase (58 K) }

Capsid protein (30 K)

All these mature proteins, formed in equimolar amounts in infected plants, may essentially be used for detection.

To detect potyviral proteins in plants, ELISA is useful for quantitative assay and immunofluorescence for the intracellular localization of potyviral gene products in the plant and for the assessment of the number of infected cells. However, the drawback of these assay methods is the necessity for rather specialized equipment (ELISA plates and ELISA reader, fluorescence attachment to microscopes).

The methods we describe, namely immunostaining and immunogold-silverstaining, combine the specificity and sensitivity of ELISA with the ease of light microscopy. The

immunostaining method is a modification of the one described by Luciano et al. (1989), the immunogold-silverstaining is a modification of the one published by Van Lent and Verduin (1987). The schedules of each method are summarised in Table 1 and 2, respectively.

Originally, we developed these modifications to be able to demonstrate the presence of iris severe mosaic virus, a potyvirus, in scales of iris bulbs, immediately after lifting. As bulb scale material contains a large number of amyloplasts with starch grains which might obscure positive staining reactions, we tested the methods out on leaves and stems of *Nicotiana benthamiana* and cowpea infected with blackeye cowpea mosaic virus (BlCMV).

IMMUNOSTAINING

Sections of leaves and stems, approximately 1/2 mm thick, were cut with a razor blade. The sections were transferred to a 5% solution of Triton X-100 for better contrast due to dissolution of plastids, and they were then evacuated to approximately 55 mbar (30 min).

Thereafter, the specimens were decolourized in 96% ethanol for c. 1 hour and subsequently rinsed in phosphate-buffered saline with Tween (PBS-Tween). They were then incubated with gammaglobulin from primary antibodies to either the whole virus or cylindrical inclusion protein isolated from BlCMV-infected plants. This incubation can be done overnight.

After rinsing in PBS-Tween, the sections were incubated with secondary antibodies conjugated to alkaline phosphatase (goat-anti rabbit) (dilution 1:1000). Following incubation, sections were rinsed in PBS-Tween and in AP 9.5 (= 0.1 M Tris/HCl, 0.1 M NaCl, 5 mM MgCl₂, pH 9.5) and subsequently incubated in a substrate, for instance a mixture of nitro blue tetrazolium and 5-bromo-4-chloro-3 indolyl phosphate p-toluidine salt.

On incubation for up to one hour at room temperature in the dark, the reaction was stopped by replacing the substrate with a solution containing Tris/HCl and EDTA, as soon as a violet colour became visible in tissues of diseased plants. The sections were then mounted in a drop of deionized water and viewed in a Wild light microscope with brightfield or darkfield.

The presence of coat protein or cylindrical inclusion protein could readily be established by accumulation of dark violet colour in epidermis and mesophyll of leaves, and in the epidermis and cortex of stems. Sections of virus-free control material showed no such accumulation of colour under the light microscope although sometimes the sections looked slightly violet when observed with the naked eye. However, the violet colour was so diffuse that it could never be traced to accumulations in cells when viewed in the light microscope. We found that, in the

case of such an aspecific reaction, the host plant played an important role. In contrast to *N. benthamiana*, such aspecific colour reaction was never observed in cowpea leaves. When, however, bulb scales were screened for the presence of capsid protein or cylindrical inclusion protein of iris severe mosaic virus, there were strong aspecific colour reactions in virus-free bulb scales. Probably, the presence of certain phosphatases in healthy plants gave rise to such colour reactions.

Besides potyviruses, other viruses such as cowpea mosaic virus (CPMV) and *Crotalaria* mosaic virus could be detected readily by this method.

In those cases where aspecific colour reactions occur, the other immunocytochemical method we describe may be more suitable.

IMMUNOGOLD-SILVERSTAINING (IGSS)

Sections of leaves or stems, this time approximately 1mm thick, were cut with a razor blade in cold *Karnovsky fixative* (3% glutaraldehyde, 2% paraformaldehyde and 1.5 mM CaCl_2 in phosphate/citrate buffer (PC), pH 7.2).

During fixation, the solution was evacuated over a period of 30 minutes for better infiltration. The specimens were then washed in PC-buffer and in deionized water, whereafter they were decolourized in a graded series of ethanol and subsequently rinsed with PBS to replace the ethanol. This can be done over night. The sections were then incubated with gammaglobulin purified from antiserum to either BICMV or cylindrical inclusion protein. After washing in PBS, the specimens were incubated in drops of suspension of 7-nm protein A-gold (pAg) particles diluted to $A_{520 \text{ nm}} = 0.1$ in PBS, subsequently washed in PBS, fixed in glutaraldehyde and washed again, first in PBS and then in double distilled water. Silver staining was carried out as described by Van Lent and Verduin (1987), using a mixture of silver lactate and hydroquinone in citrate buffer. Silver enhancement was stopped by washing the specimens in double distilled water. In the light microscope with brightfield illumination, the stain was visible as black precipitate. Just as in the case of immunostaining, with IGSS staining of pieces of leaves, stain may be found in all the cells of the cut surface, but not in those below it, probably due to lack of penetration of the gammaglobulins. A clear difference in distribution of the stain could be observed, depending on the antiserum used. In the case of antibodies to whole virus, the stain was distributed all over the cell, whereas with antiserum to cylindrical inclusion proteins the precipitate was less dispersed and accumulated in the centre of the cells.

An advantage of the IGSS method is that infected tissues positively reacting in IGSS are suitable for subsequent electron microscopical studies on the exact localization of antigen.

We are now trying to apply this method to bulb scales of iris infected with iris severe mosaic virus, in order to determine the distribution of viral antigen in bulb tissue shortly after lifting when it is usually impossible to demonstrate the presence of the virus by ELISA.

REFERENCES

- Luciano, C.S., Gibb, K.S. and Berger, P.H. (1989). A general method for the detection of potyviral gene products in plant protoplasts and tissue. Journal of Virological Methods 24: 347-356.
- Van Lent, J.M.W. and Verduin, B.J.M. (1987). Detection of viral antigen in semi-thin sections of plant tissue by immunogold-silverstaining and light microscopy. Netherlands Journal of Plant Pathology 93: 261-272.

Table 1. The process of immunostaining.

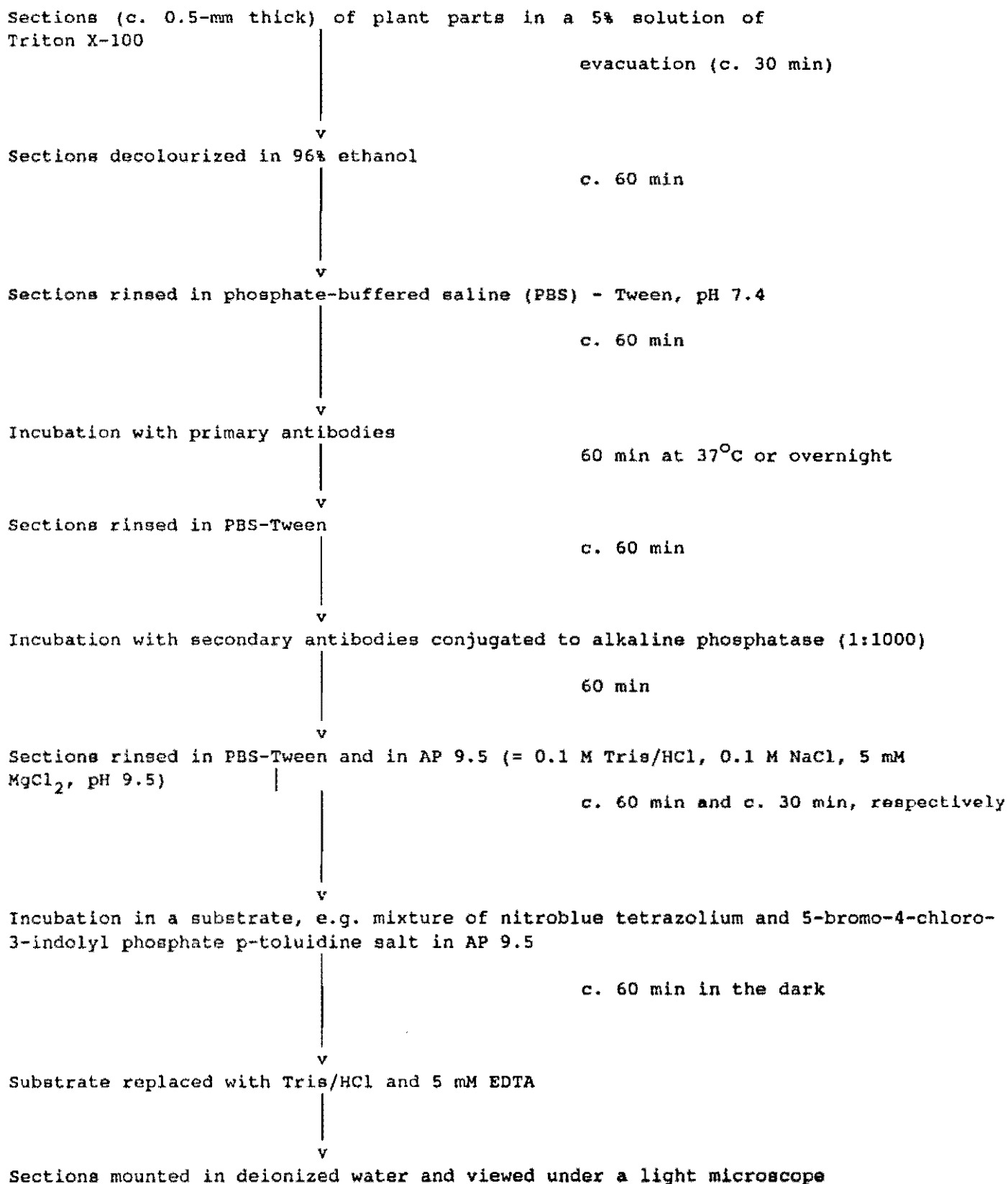


Table 2. Immunogold-silver staining (IGSS)

Sections (1-mm thick) of plant parts in Karnovsky fixative (= 3% glutaraldehyde, 2% paraformaldehyde, 1.5 mM CaCl₂ in phosphate/citrate (PC) buffer, pH 7.2)

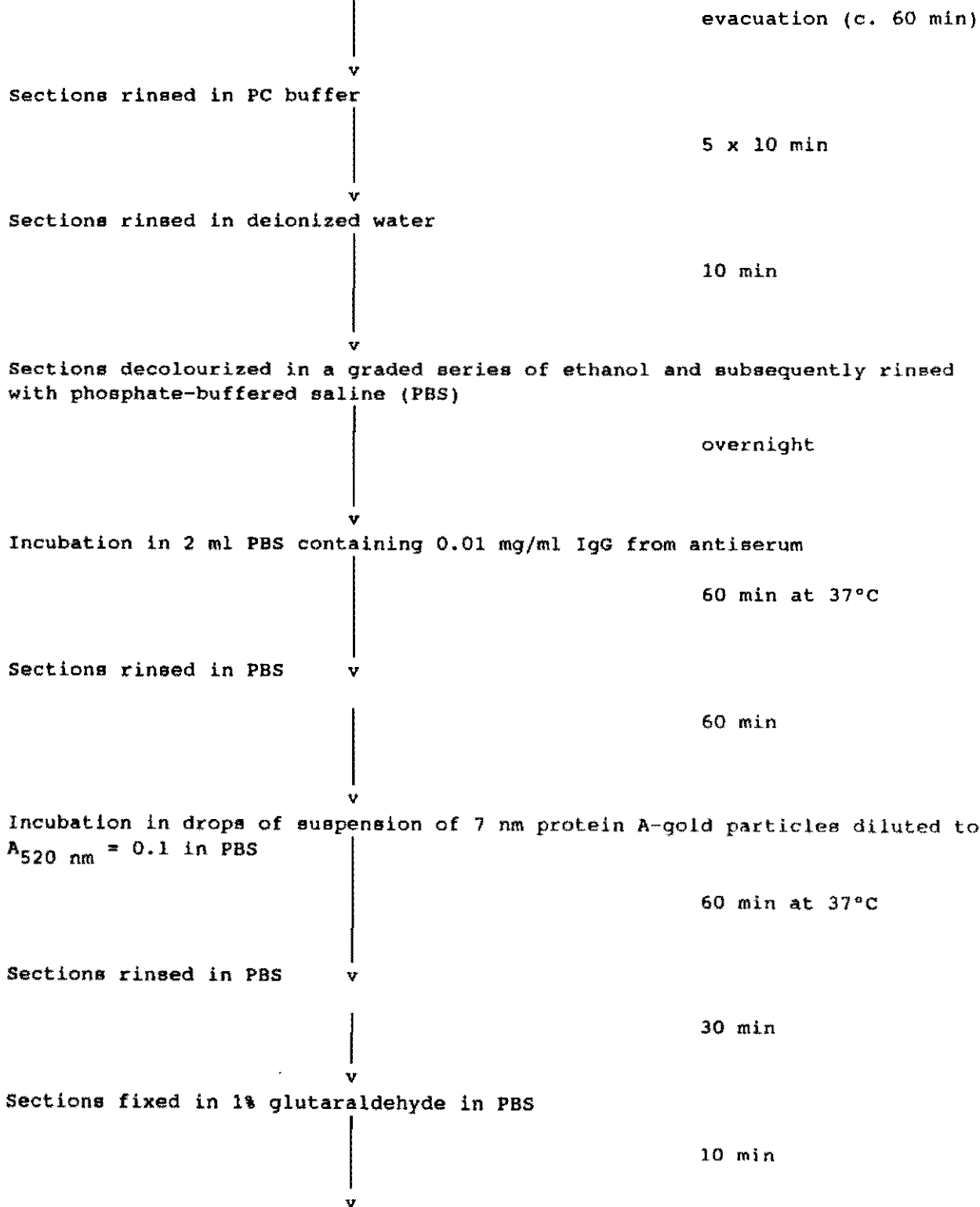


Table 2. Immunogold-silver staining (IGSS) (cont..)

Sections rinsed in PBS and in double distilled water

1x10 min and 2x10 min, respectively

v

Silver staining with mixture of silver lactate and hydroquinone in citrate buffer

c. 10 min

v

Sections rinsed in double distilled water

3x5 min

v

Sections mounted in double distilled water and viewed under a light microscope

VIRUS DETECTION IN PLANT AND SEED : DISCUSSION

Gaylord Mink : Lead discussant
Femi Lana : Rapporteur

Mink : Many methods are available with which to detect viruses. Essentially, there are six different categories : General ones : Light microscope; EM; ds RNA. And specific ones : Bioassay; serology and cDNA probes. Some require expensive equipments; some not. Some require specialized training; some not.

There are disadvantages and advantages of each method. With the light microscope, the materials needed are easily available and the procedure is simple. The work carried out by Christie over fifteen years is centrally important.

Thresh : How useful is this method? Christie has spread the 'gospel' but do his trainees continue to use it after they get home to their labs?

Mink : We are not in a position to answer this yet. A workshop by Christie is being organized in Prosser. This type of work requires long term experience but it is quick, once experience is acquired.

Allen : How critical or accurate are these methods, e.g. in terms of the number of cuticles needed?

Mink : This is erratic, it can be difficult to determine. Each scientist must define specific accumulation with each virus.

Morales : I agree, but this method is a long process which takes a long time to detect inclusion bodies (IB) and this detection can only be acquired with long interaction with Christie himself through training. The other limitation is that the stains are not easily available. Though an excellent technique, it involves a lot of inconvenience. It is very practical but it requires full-time devotion to become familiar with the technique.

Omunyin : What happens in cases of mixed infections?

Mink : The clue is that you would see many inclusion bodies.

Allen : What is the effect of the host on the value of these techniques?

Mink : The effect will be consistent, in that the same virus will induce the same bodies in different hosts.

Turning now to the electron microscope (EM) technique, this gives a special usage that gives a clue as to where to go. If you are to monitor them, you would easily know what to look for. However, accessibility to EM in Africa is difficult. It is possible however to put some samples on grids and send them to developed labs overseas.

The limitation also includes problems with spherical viruses. Although this becomes easier to distinguish once you acquire the experience. The other limitation is that it is non-specific.

Walkey : It is expensive, too.

Mink : I agree with you entirely.

Morales : But that notwithstanding, it is still a useful diagnostic tool.

Thresh : How long can you keep these grids and still use them?

Mink : Experience shows you can use them for a period of 5 years before they begin to deteriorate.

Rossel : They are difficult to keep for long in the lowland humid tropics.

Mink : ds DNA is a fad technique which is used for double-stranded RNA viruses; its limitation is that it takes a lot of effort, is complex and time consuming, and requires enormous supplies of liquid N.

Dijkstra: You need a lot of suitable herbaceous host material too.

Mink : Yes.

Morales : Is it difficult to produce a good antiserum to these by this method?

Mink : Yes, we have not been able to get the viruses in pure form. We used this specifically because we were able to transmit a woody virus to herbaceous hosts.

Morales : Similar problems exist with cassava.

Mink : Let's now consider some specific techniques in identification. cDNA probes have the advantage of high specificity and the disadvantage of a high amount of expertise.

Owera : Are there probes specific to viruses or to any group or range of viruses?

Mink : You can use them for both. Maxwell of Wisconsin will be interested in samples from Africa, especially golden mosaic.

Allen : I have just sent some calcium chloride samples of suspected geminiviruses to Dr. Maxwell.

Owera : Who supplies nylon or membranes?

Morales : We can send you these from CIAT.

Mink : Bioassays are specific in detection. Their success depends on how much you know about the virus.

Omunyin : Is there any difference between SGR and Dubelle Witte?

Mink : No, it's just a question of choice.

Thresh : How great is the effect of temperature?

Mink : Many of the BCMV isolates will cause necrosis above 35°C under experimental conditions.

Walkey : Are you considering combining the 'I' gene with other types of resistance?

Mink : I cannot speak for the breeders.

Mink : The dotblot method

Atcham : Has anyone used dotblot ELISA in detecting NL3?

Mink : Yes, we have - in 1984. The test was okay for mosaic but anytime you have any form of necrosis - whether from virus, bacteria or fungi, a vigorous reaction is triggered off.

Walkey : We have not looked at disease resistance mechanisms of these systems.

Mink : A group in Texas is working with Matt Silbernagel in attempting to clone the genes.

Mink : Serological techniques include ID, ISEM, dotblot and ELISA. The limitation with ISEM is the need for access to EM, and the limitations of dotblot and ELISA are that soaking seed allows leakage of virus, and that damage to seeds affects germination.

Omunyin : How sensitive is this method?

Mink : I don't know how to react to this but we know that viruses can be confined to the embryo or to the cotyledon, or to both. This depends on the variety and

the way you use the test depends on what information you want e.g. for seed transmission you must cut the seed not to affect the embryo. Powdered seeds can also be used.

In the clinical use of ELISA, the method caters both for detection and diagnosis. As a means for testing seeds, ELISA enables collection of quantitative data, it satisfies certification or quarantine requirements and provides information for management decisions. Certain precautions are necessary in research tests : samples must be carefully selected, and the test must be done by one person. Relatively few samples are needed, tests can be repeated if necessary and results can be combined and analysed.

Features of clinical tests are that sampling is usually done by non-researchers; tests are performed by several people; hundreds of samples are often processed in a day. Deadlines often prevent re-testing and the test is required to give yes/no answers.

Dijkstra: Limitations of the two immunocytochemical methods I have described are :

1. uneven distribution of inclusion bodies (IB) in different plant parts.
2. depends on stage of infection - when late, you don't find any inclusion bodies.
3. Not in all cases do you find IB in epidermal tissues.
4. It is possible not to detect IB's with all viruses e.g. tobacco rattle virus.

The limitation of other detection method is that they need a lot of equipment.

An advantage of the methods I have described is that you can even use the section for EM studies and no further staining is needed.

Rossel : What about latent infections?

Dijkstra: We can demonstrate this even with low concentrations of virus in tissue.

Rossel : EM of cowpea aphidborne mosaic virus sections is extremely difficult.

Dijkstra: Yes, that is our experience too.

Sengooba: Are potyviruses always with pin-wheel inclusions?

Dijkstra: These are general terms but they are specifically inclusion bodies.

Walkey : Advantages of the slide agglutination test are that it is rapid, easy to use and it is sensitive. It involves economic use of the reagents.

Rosel : Can the *Staphylococcus* be produced locally?

Taylor : Yes.

SESSION III - VIRUS IDENTIFICATION AND GEOGRAPHICAL DISTRIBUTION

RECENT PROGRESS IN THE IDENTIFICATION OF VIRUSES OF *Phaseolus vulgaris* in AFRICA¹

H.J. Vetten and D.J. Allen

There are at least 18 well-characterized and formally described viruses which naturally infect *Phaseolus vulgaris* (Allen, 1983). Although bean common mosaic virus (BCMV) is common and widespread in Africa (Kulkarni, 1973; Silbernagel et al., 1986; Edington & Whitlock, 1988), most of the other viruses are not known to occur in beans in Africa. With the advent of regional programmes on the improvement of beans in Africa and with the concomitant need for germplasm exchange between CIAT and its regional bases, it appeared essential to characterize hitherto unrecognized viruses in natural infections of *Phaseolus* spp. in Africa. Characterization of these viruses and a more complete understanding of virus distribution would ensure that the quarantine legislation governing the movement of bean seed remains firmly based, and moreover would assist in determining to what extent viruses other than BCMV need to be taken into account by bean improvement programmes in Africa.

In November/December 1987, February/March 1988, August 1988, and December 1989 four collecting trips were conducted covering Burundi, Ethiopia, Kenya, Malawi, Rwanda, Sudan, Tanzania, Uganda, Zambia and Zimbabwe. Samples were collected in vials in which they were preserved by dessication over CaCl₂ for later processing in Braunschweig. In addition, many samples collected in Cameroon, Ethiopia, Lesotho, Mozambique, Uganda and Zaire were sent by various collaborators to Braunschweig for future analysis. In Braunschweig, dried samples were ground to a fine powder, a portion of which was tested in a double-antibody sandwich ELISA (DAS-ELISA), using up to 25 different antisera to viruses known to infect legumes in Africa and/or beans in other parts of the world. For serotyping of BCMV isolates, we used the method of Wang et al. (1982).

From a total of about 750 bean samples analysed, approximately one third was found to be infected with BCMV (Table 1). Since most of the BCMV-infected samples gave strong reactions only with antiserum to BCMV strain NL5, the vast majority of BCMV isolates from Burundi, Rwanda, Tanzania, Uganda and Zambia are serotype A isolates and are likely to be necrotic strains that can cause systemic necrosis ("black root") in bean cultivars possessing the dominant "1" gene. In marked contrast, however, in Ethiopia and perhaps also in Zimbabwe, non-necrotic strains predominate over the necrotic types. Collections from most other countries indicate the presence of necrotic strains

¹An abridged version of this paper has been published in the Annual Report of the Bean Improvement Cooperative 34, 3-4 (1991).

elsewhere, but because of the limited numbers of samples collected their relative prevalence remains unclear. The high proportion of samples from Zimbabwe that gave strong reactions with antiserum to BCMV-NL5 as well as that to BCMV-NY15 is puzzling and suggests either that many samples are infected with mixtures of serotype A and serotype B isolates or that intermediate serotypes of BCMV occur in Zimbabwe. These preliminary results confirm previous findings (Mink, 1985; Silbernagel et al., 1986) that necrotic strains of BCMV predominate over most parts of eastern and southern Africa. Further work is required to collect more samples, to survey other areas, and to identify the specific strains involved.

As shown in Table 2, the incidence of other viruses was generally low. However, an appreciable number of samples from Zambia and Zimbabwe were infected with cucumber mosaic (CMV) and peanut mottle (PnMV) viruses. CMV, which we detected frequently in mixed infections with PnMV or BCMV, like these two potyviruses, is non-persistently transmitted by aphids and may possibly warrant some attention in some areas. Since cowpea mild mottle virus (CMMV) was found in one to two samples each from Uganda, Tanzania, Malawi and Mozambique, it appears to be widespread and associated with a leaf curling and a plant stunting on bean in Africa. The high incidence of CMMV in bean samples from the Sudan, where CMMV was associated with a severe leaf curl disease, indicates CMMV may be of economic importance particularly in bean-growing areas of lower altitudes where *Bemisia tabaci* Gen., the whitefly vector of CMMV, is abundant. Bean yellow mosaic virus (BYMV), which is among the most important legume viruses in Europe, is apparently rare in beans in Africa. It was only detected in three of 21 samples collected in Njoro, Kenya confirming a previous report of BYMV in Kenya (C.L.A. Leakey, cited by Allen, 1983). A few samples from plants which showed conspicuous virus symptoms in the field in Burundi and Uganda gave ELISA-negative reactions. Subsequent infectivity tests led to the isolation of potyviruses which were serologically related to blackeye cowpea mosaic virus (BlCMV) but strikingly distinct from cowpea isolates of BlCMV in their pathogenicity to a range of cowpea lines. Further characterization of these potyvirus isolates is underway in Braunschweig.

REFERENCES

- Allen, D.J. 1983. The pathology of tropical food legumes: disease resistance in crop improvement. John Wiley & Sons, 413 pp.
- Edington, B.R. and Whitlock, V.H. (1988). Identification of an isolate of bean common mosaic virus from the Transvaal and Natal. Annals of Applied Biology 113, 645-648.

- Kulkarni, H.Y. (1973). Notes on East African plant virus diseases. 1. Bean common mosaic virus. East African Agriculture and Forestry Journal 39, 72-76.
- Mink, G. 1985. Bean virus serological survey, Kenya & Tanzania. Washington State Univ., Prosser, (unpublished report).
- Silbernagel, M.J., Mills, L.J. and Wang, W.J. 1986. Tanzanian strain of bean common mosaic virus. Plant Disease 70, 839-841.
- Wang, W.Y., Mink, G. and Silbernagel, M.J. 1982. Comparison of direct and indirect enzyme-linked immunosorbent assay (ELISA) in the detection of bean common mosaic virus. Phytopathology 72, 954. (Abstr.).

Table 1. SEROTYPING OF BEAN SAMPLES FROM VARIOUS AFRICAN COUNTRIES USING ANTISERA TO BCMV-NL5 (SEROTYPE A) AND BCMV-NY 15 (SEROTYPE B).

Country (location)	Total number of samples tested	Serotype		
		A*	B*	A + B*
Burundi	48	17	5	0
Ethiopia	184	1	27	0
Kenya (Njoro)	21	2	2	0
Lesotho	10	2	1	0
Malawi (Bunda)	7	6	0	1
Rwanda	105	23	0	0
Sudan	20	0	0	0
Tanzania	60	23	5	2
Uganda	127	46	1	0
Zambia	91	30	3	6
Zimbabwe	62	2	9	12

* Number of samples which gave strong reactions with either antiserum or with both antisera in DAS-ELISA.

Table 2. VIRUSES OTHER THAN BCMV IN AFRICA.

Virus	Incidence	Location
Cucumber mosaic virus (CMV)	27 of 91 24 of 62	Msekera, ZAMBIA Harare and Shamva, ZIMBABWE
Peanut mottle virus (PnMV)	19 of 91	Lusaka and Msekera, ZAMBIA
Cowpea mild mottle virus (CMMV)	1 - 2 plants only 14 of 20	Lushoto, TANZANIA, Bunda, MALAWI, Umbeluzi, MOZAMBIQUE Kabanyolo, UGANDA Ed-Damer, SUDAN
Bean yellow mosaic virus (BYMV)	3 of 21	Njoro, Nakuru, KENYA
Unidentified potyvirus (BlCMV ?)	2 of 48 1 of 94	Cibitoke, BURUNDI Kabanyolo, UGANDA

VIRUS IDENTIFICATION AND GEOGRAPHICAL DISTRIBUTION : DISCUSSION

Jupp Vetten : Lead discussant
Nicola Spence : Rapporteur

- Walkey : Question about selection of differential cultivars, could the number be reduced ?
- Morales : Drijfhout's results were published as an "act of faith" : you never get the same reactions. The genotypes of cultivars used by different people may vary. You should use as many differentials as possible.
- Rossel : Approach of variability of strains. Major problem is each cultivar may be homogeneous with some strains and not others. There is a need to approach the problem with as wide a range of cultivars as possible from breeders material; germplasm collections have insufficient seed. Need to determine degree of differential reaction for each cultivar and strain. Relevant differences are biological differences. Need to relate differences in strains with extent to which strains occur in new geographical distribution. Need to keep relevance to the breeding programme.
- Thresh : Do host-strain interactions occur within a host differential group ?
- Rossel : Cannot apply statistical analysis to strain - host differentiation because of the numbers of plants involved. Arbitrary at moment when deciding if a strain is new or different.
- Morales : Bean genotypes within a differential cv. group differ in their response to infection. Do not use cultivars to score but obtain pure lines of differentials. Black Turtle Soup can revert back to susceptible stage: need to maintain purity. Drijfhout's lines represent most genotypes available.
- Rossel : Germplasm collections should be homogeneous but typically they are segregating populations.
- Walkey : It is important to multiply germplasm in glasshouses to maintain purity.
- Morales : You must tolerate minor variability in host differential reactions.
- Rossel : Scoring systems for host reactions (on a 1-5 scale) to decide if symptoms are different between plants. Restricted numbers of plants can cause difficulty in interpreting results.

Vetten : There are interesting differences in particle lengths of BCMV strains :

non-necrotic	850-870 nm
necrotic	810-830 nm
NY15 (non-necrotic ?)	820-831 nm

NL3 and NY15 a/s in direct ELISA to find differences

Sero A	NL3, NL5, NL8
Sero B	all the others

Direct vs. indirect ELISA with NL5 and NY15 a/s

Direct : NY15 a/s reaction with NL1, NL4, NL7, NY15,
Fla a/s : good reaction NL6

Values of indirect ELISA higher than those of direct ELISA

Direct NL5 a/s good reactions with NL 3, 5 and 8; no or weak reactions with rest

Using monoclonal antibody B-I-3 produces identical reaction to NL5 a/s with all serotype A isolates but no reaction with serotype B isolates

Electrophoresis - proteolytic degradation of virus coat protein can occur. Other viruses have undegraded capsid protein. Some strains were more stable than others. Sequence data of BCMV differences must have some reflection in biological/serological differences.

Morales : Nothing similar to "black root" in non-I-gene cultivars in farmers fields. Distinguish between black root and non-black root

Black root - vein necrosis only

Non-black root - systemic necrosis and vein necrosis

Black root caused by BCMV only I gene material

Soybean mosaic and peanut mottle viruses may look similar. Systemic necrosis in non-I-gene material is not the same as black root in I gene materials. Top necrosis can occur in non-I-gene material

NL3 can cause superficial necrosis without spread to vascular system. I gene can occur in land races naturally, therefore possible to have necrosis in wild populations.

Vetten : Incidence of BCMV generally lower at altitudes above 1800m. Dilution of virus in seed due to altitude (no aphids) ?

Rossel : Cowpea virus epidemiology is largely dependent on seed transmission.

- Walkey : At high altitude there are no aphids, and so no spread of virus between plants and weeds.
- Rossel : Relevance of lower disease incidence at altitude questioned.
- Thresh : Altitude: can get aphids (e.g. Costa Rica) but may be different species.
- Allen : Could it be that aphids are better colonizers at lower altitudes ?
- Omunyin : The change in number of aphids above 2000m altitude has been studied in Kenya but the species have not been determined
- Rossel : Reliable seed transmission rates need large numbers of plants. CMV transmission is a nuisance and can be high in many cultivars - should be eradicated from lines.
- Vetten : CMV not important in terms of damage but may be important in mixed infection with other viruses.
- Morales : Severe CMV symptoms have been reported in beans in Spain and elsewhere in Europe
- Rossel : No CMV seen in cowpeas except in very early stages.
- Morales : Occurrence of CMV very widespread and may be very important.
- Thresh : CMV with satellite RNA may present a problem
- Vetten : CMV isolate from legumes does not infect cucumber
- Rossel : CMV important issue for quarantine - can be eliminated if you go back to germplasm.
- Vetten : PnMV inoc. onto differentials, virus could be detected in plants but only systemic necrosis in few cultivars. In Africa, PnMV may not cause systemic necrosis but it does in other areas.
Cowpea mild mottle virus (CMMV). Verified by ISEM (Brunt a/s)
Leaf deformation, leaf curling
Sudan: severe curl (also insect feeding damage by whitefly?) would expect recovery but not seen.
- Rossel : CMMV can be transmitted by whitefly
- Vetten : CMMV can be transmitted readily by sap inoculation
- Morales : Reaction related to genotype in field. If different cv used for transmission experiments may not get the same symptoms. Do not get same symptoms by whitefly

transmission. Important to know cultivar.

Rossel : In field surveys, the cultivar should be known. Cannot discuss symptoms in this way.

Allen : Example of similar symptoms in cowpea sample but not beans.

Walkey : Best cultivar for CMMV ?

Vetten : BT II

Allen : Storey recorded CMMV as "Ngomeni Mottle" in lowland Tanzania.

Rossel : CMMV is very common in legumes in Nigeria

Vetten : CMMV appears to be a low altitude problem, perhaps related to the vector.

Black eye cowpea mosaic (BlCMV) found in Burundi. Causes vein-banding and green blisters on leaves.

Ring spots on *C. quinoa*, similar to BCMV. An isolate of BlCMV from Uganda causes chlorotic local lesions on *C. quinoa* and necrotic lesions on *C. amaranticolor*. BlCMV on beans causes interveinal chlorotic spots, vein banding and severe leaf malformation. Bean isolates of BlCMV produce similar results on differentials.

Antisera to serotype B isolates of BCMV give good reactions with BlCMV isolates
ISEM cross decoration of BCMV NY15 and BlCMV

One Burundi isolate did not infect any cowpea differentials, other isolates infected some differential cowpea lines.

Morales : Nomenclature based on serological properties - why call it BlCMV if it doesn't affect cowpeas ?

Rossel : Ecological significance important not serological properties for characterization. Need 10 years surveying to get impressions.

Vetten : I don't know the significance of virus but should do some characterization to classify. Criteria for classification depend on different areas of interest.

Thresh : No incompatibility between detailed surveys of lesser known viruses (HJV) and important large scale economic ones (HR).

Allen : Need surveys for solid decision making - need to know which viruses exist.

Morales : If CpMV is reported in a region then opinion will deter farmers from growing cowpeas.

Omunyin : Advances in nomenclature should be understood for complete picture.

Rossel : Strain concept important to discuss.

Vetten : Unknown isometric (30nm) virus on 3 plants from Harar area in Ethiopia. Low incidence of virus in bean production.

Causes vein chlorosis, slight deformation, chlorotic spotting on inoculated leaves and systemic.

Purified prep - electrophoresis 66,000 kd capsid.
- Nepovirus?

Particles disrupted on CsCl gradients not CsSO₄
RNA disrupted in virions by RNase when sucrose gradients were used.

Thresh : What is the importance of nematode transmitted viruses?

Vetten : No serological relationships with nepoviruses
No aphid transmission
Capsid 55-60 kda
Ds RNA - three bands
One sedimenting component in sucrose gradients

Possible geminiviruses - not identified
- chlorosis similar to Mn deficiency.

BYMV detected by ELISA, from the Nakuru area of Kenya. It may be important in peas in Kenya. BYMV has wide host range - may occur in a wide range of plants. ISEM positives. 3/20 samples with BYMV in farmers' fields. Sent to V. Lisa in Turin.

Morales : Reaction of BYMV depends on source of isolate and reaction on bean differentials. GN 31 resistant to all strains of BYMV. Generally a temperate virus may be due to temperate alternative hosts (e.g. *Gladiolus*) should be checked for BYMV - important and widespread virus.

SESSION IV - VIRUS STRAINS

STUDIES INTO THE DISTRIBUTION AND IDENTITY OF AFRICAN STRAINS OF BEAN COMMON MOSAIC VIRUS (BCMV)

Nicola J. Spence

INTRODUCTION

The most important method for control of BCMV is resistance. The dominant I gene has conferred stable resistance in CIAT lines for 25-30 years and is used in all lines produced at CIAT. However, in the last 12-13 years I gene material in Africa has been severely affected by strains of BCMV which cause the hypersensitive resistant reaction called black root. The most predominant strain of BCMV detected in Africa is the necrosis-inducing NL3 strain and resistance to this strain is achieved by combining the I gene with the protecting recessive bc-3 gene. We need to know the distribution and importance of necrotic strains of BCMV so that breeders can introduce material with the protected I gene where necessary.

The origin of necrotic strains of BCMV is not known but is the subject of much speculation. Information about the distribution of necrotic strains in Africa and the possible occurrence of BCMV in wild legumes may help to improve our understanding of the origins of these strains.

A collaborative project between IHR, Wellesbourne and CIAT started in July 1989 and this paper outlines our results to date and proposes areas of further research.

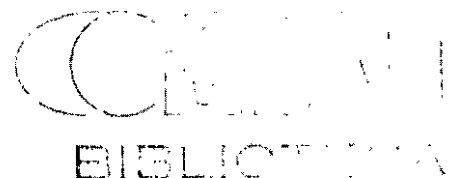
OBJECTIVES

1. Identification of African strains of BCMV
2. Geographical distribution of African strains of BCMV
3. Seed transmission studies
4. African varietal mixtures
5. Alternative legume hosts

IDENTIFICATION OF AFRICAN STRAINS OF BCMV

Strains of BCMV have been isolated from seed or leaf material collected in Africa. BCMV is detected using Electron Microscopy and Immunoassays then strains are identified as closely as possible using host differential studies. Using seed of differential hosts multiplied in 1988 and 1989, strain identification began immediately with BCMV isolates collected from Dr. J.D. Taylor's CIAT/Africa Bean Quarantine Programme and from material collected by CIAT personnel in Africa. Results indicate that the NL3 strain predominates in many areas though another necrotic strain, NL6 has also been detected in Rwanda. Several non-necrotic strains have also been detected. A survey of Southern Africa (Swaziland, Lesotho, Malawi and Zimbabwe) in

13306



Jan-Feb 1990 by Dr. D.G.A. Walkey should provide a large number of isolates for identification.

Virus strains are being purified and antisera produced in order to study the serological identification of strains.

GEOGRAPHICAL DISTRIBUTION OF AFRICAN STRAINS OF BCMV

Results of the strain identification will be used to construct a map to show the geographical distribution of the different strains. This will be used by breeders in order to determine where the I gene can be used on its own and where it has to be protected by the recessive gene.

SEED TRANSMISSION

Necrotic strains are not seed transmitted in material carrying the I gene but are transmitted in seed of cultivars of certain host groups. As seed transmission is the primary source of infection, the rate of transmission of local strains in local cultivars may provide important information about the epidemiology of these strains and will therefore be investigated.

AFRICAN VARIETAL MIXTURES

In Central Africa and parts of East Africa many farmers plant traditional varietal mixtures in preference to a single cultivar and it is unlikely that they will change this practice even if a good resistant single cultivar were available. An investigation into the genes for resistance to BCMV present in the components of these mixtures will be carried out to study the epidemiology of the disease in mixtures and the potential for adding resistant varieties to the mixtures.

ALTERNATIVE LEGUME HOSTS

In nature BCMV predominantly infects *Phaseolus* species, especially *P. vulgaris*. The virus has also been found naturally occurring in *Rhynchosia minima* and *Lupinus luteus* and can be artificially inoculated onto various other hosts. The presence of alternative legume hosts in African could have important implications as a source of spread of the virus to *P. vulgaris* and a possible source of new strains of BCMV. An attempt will be made to isolate virus from wild legumes with virus symptoms found near farmers' fields in order to determine if BCMV is present, and if so which strains.

VIRUS STRAINS: ORIGINS AND IMPLICATIONS

Francisco Morales : Lead discussant
David Allen : Rapporteur

Morales : I will focus on BCMV. Typical symptoms of mosaic are green vein-banding. Necrotic reactions result from an interaction of the dominant I gene and necrosis inducing strains. There are also recessive resistance genes. All strains induce mosaic in cultivars without resistance genes.

Black root is a resistant reaction, a hypersensitivity that prevents systemic infection. It is a defence mechanism, effective in Latin America where the I gene has been widely used for 20-30 years over which it has proved stable and effective. As CIAT became involved in Africa, it became clear that black root posed a problem. What was the reason? It was found that the NL3 strain predominates in Africa. It also occurs in Latin America, but only at low incidence. Why? Aphid species differences occur but vectors may not be the cause. The strategy at CIAT now is to protect the 'I' gene, with the recessive bc2² gene. Genotypes possessing these genes show only pin-point local lesions. Restricted vein necrosis has also been observed in genotypes possessing the I-gene protected by recessive genes other than bc2², and occurs only on leaves directly inoculated. The bc3 gene affords complete protection, but this gene was not available in tropically adapted backgrounds. Now it has been put into adapted cultivars. CIAT now has materials with resistance to both the mosaic and necrotic strains. The problem now is to protect otherwise valuable germplasm against necrotic strains, so there is a need to determine the geographical distribution and economic importance of necrotic strains.

Opio : What does a national programme do if it identifies promising material that is susceptible to black root?

Morales : You can deploy the I gene, based on a knowledge of the distribution of strains. If that is not possible, use only the protected I gene materials.

Rossel : Is black root a problem on farmers' fields ?

Opio : It can be, yes.

Thresh : Why then are unprotected materials being evaluated ?

Morales : Because a much wider range of material is being evaluated in Africa than is yet available from CIAT with the protected I gene.

Omunyin : Also, there is urgency here. There is a need to release varieties of acceptable seed types, and the development of networks are important.

Gridley : One of the things we are doing in Uganda is to screen protected I gene germplasm.

Lana : Shouldn't we be screening these materials in 'hot spots'?

Thresh : Unprotected I gene materials would tend to allow the situation to get worse, surely ?

Morales : If we could grow only I gene materials, BCMV would die out as a disease, because of the elimination of seedborne inoculum. Now, regarding the possible origin of the necrosis-inducing strains of BCMV. NL3 is highly seedborne in Dubbele Witte and also in Group 4 cultivars, particularly in navy beans (Sanilac, Michelite). NY15 is also highly seed-borne in navy bean cultivars.

Bean-production areas where black root and NL3 are particularly important are areas where navy beans have been exchanged with Europe. This is true of Chile, in Latin America, and also of parts of Africa. Thus, it would be interesting to know the incidence of NL3 - like strains in Europe. However, one point of concern is that other legume potyviruses can also cause systemic necrosis in I gene cultivars but without typical common mosaic symptoms.

On the other hand, NL3 and NY15, and Florida and NL6 are very similar in pathogenicity spectra, except on I gene cultivars, and both NL2 and NL6 cover the pathogenicity range of NL5 in I gene cultivars. It could be that NY15 was introduced into Africa where it mutated into a necrotic strain.

Lana : Are you saying that this is the basis of the NL3 in Africa ? Are you saying that NL3 is a product of mutation from NY15 ? It could be.

Thresh : Has anyone done any sequencing to evaluate the substance of this theory ?

Vetten : Such work is in progress.

Walkey : But are the navy beans you mention (Sanilac etc.) cultivated in Europe ? They are American cultivars which found their way into Europe.

Rossel : Why shouldn't the necrotic strains have originated in Latin America, where you say they do exist ?

- Morales : Because of their low incidence in Latin America, except in places where beans have been exchanged with Europe or America.
- Thresh : There is perhaps a case for a survey from seed samples, giving an opportunity for much larger samples.
- Rossel : Evidence suggests that necrotic strains are not at a selective disadvantage over 'normal' strains in susceptible cultivars. This is alarming.
- Morales : If it is true that necrotic strains do not predominate in Ethiopia, then this could be used as a multiplication site.
- Thresh : There is an evolutionary balance between strain "virulence" and seed transmission rates. Highly virulent strains can lead to very little seed being set: there may be a "symbiosis" between moderate virulence, seed set and transmission rates.
- Spence : The IHR project is focussing on BCMV strain identification in Africa. The project is in 3 parts : host differential studies, serological studies; and pathogenicity studies.
- A knowledge of the geographical distribution of African strains of BCMV will enable us to construct a map. We will also investigate methods of storage. All strain typing to-date has been from seed samples. Liquid N may be preferable for longer term storage; CaCl₂ is useful, simple but infectivity is low.
- Allen : How successful has calcium chloride proved as an initial collection technique ?
- Vetten : We have obtained low infection from CaCl₂.
- Rossel : Vital that the initial work is done in National Programmes locally. (Much discussion followed, endorsing this but also pointing to value of work done overseas).
- Spence : Our project is looking also at varietal mixtures.
- Allen : Yes, this is an approach that has proved valuable in fungal and perhaps bacterial diseases. Component breeding is an approach gaining ground in improving mixtures.
- Thresh : But there is apparently no evidence of the protective effect of mixtures from viruses.
- Taylor : With halo blight, we have found some useful resistance lurking within varietal mixtures in Burundi.

- Spence : What about alternative legume hosts ?
- Morales : *Rhynchosia minima* is a common BCMV host in Latin America.
- Mink : Silbernagel believes there must be a wild host in eastern Africa of BCMV.
- Rossel : I believe this is little more than of academic interest. We have found the wild *Oryza* host of rice yellow mottle virus, showing it is a rice virus. But this has no practical relevance to the management of the disease.
- Lana : I disagree. What about the wild host of lettuce mosaic, the strain proving lethal to lettuce ?
- Thresh : Swollen shoot comes from wild hosts in West Africa, and they are an important source of variability. Nicola has identified NL1, 4, 6 and particularly NL3 from seed from the Great Lakes.
- Allen : Lets' conclude discussion on strain variation in BCMV. Would Rossel like to brief us on the position in CAMV/BlCMV ?
- Rossel : One striking contrast is that *Vigna* is native to Africa, unlike *Phaseolus*. The incidence of CAMV is not as high as that of BCMV on farm. Wild hosts probably don't play a role in either. In terms of virus nomenclature, we prefer use of CAMV, as a possible complex of related potyviruses in *Vigna*. Use of a standard isolate is vital; we are using advanced breeding materials to pick up 'new' isolates. The reversed ranking of host responses to cowpea (yellow) mosaic (CYMV) and cowpea severe mosaic (CSMV) viruses provided strong evidence that these are distinct viruses.
- Dijkstra: Why are you using the name of a virus (CYMV) that no longer exists ! Neither the virus nor the antiserum is available now. Why don't you use the name Blackeye cowpea mosaic virus ?
- Rossel : BlCMV is American, the type of CAMV is Italian and likely to be more relevant.
- Vetten : Isn't it likely that CAMV is a Mediterranean virus ?
- Rossel : I agree that CAMV is not as described by Bock and Conti.

V. ...

SESSION V - VIRUS ECOLOGY AND ECONOMIC IMPORTANCE

POSSIBLE DEPENDENCE OF GEOGRAPHICAL DISTRIBUTION OF VIRUS DISEASES OF COWPEA IN AFRICA ON AGRO-ECOLOGICAL PARAMETERS

H.W. Rossel and G. Thottappilly

INTRODUCTION

Eight viruses are reported from cowpea in Africa. Three are beetle-transmitted (cowpea yellow mosaic, cowpea mottle and southern bean mosaic viruses), two are aphid-borne (cowpea aphid-borne mosaic and cucumber mosaic viruses), two are whitefly-transmitted (cowpea golden mosaic and cowpea mild mottle viruses), and for one (sunn-hemp mosaic virus) the vector remains unknown. Cowpea viruses are described both from lowland, humid and sub-humid West Africa, as well as from mid-altitude ecological zones found mainly in eastern and southern Africa. However, further information on their distribution seldom is given relative to agro-ecological zone nor has any such restricted distribution been explained in terms of agro-ecological parameters.

This paper reports results of studies of the geographical distribution of cowpea aphid-borne mosaic virus and the three beetle-transmitted viruses so far described from cowpea in Africa. These data have been obtained during surveys in various ecological zones of West and South-eastern Africa, as well as from the available literature on the occurrence of such viruses in Africa. Finally, geographical distribution is discussed against an ecological background.

MATERIALS AND METHODS

An extensive survey throughout much of West Africa, ranging from Ghana to Cameroon, and covering all major agro-ecological zones in this region, was conducted in September, 1988. Another survey in south-eastern Africa, in February/March, 1989, covered Mozambique, Swaziland, Botswana and Zambia. The surveys were primarily aimed at confirming our observations concerning zonal distribution of cowpea viruses in Nigeria, as well as what had been reported from other parts of Africa. During our surveys, we monitored specially the performance of elite, IITA cowpea breeding materials, focusing on their susceptibility to, and their rate of infection with locally occurring viruses, or virus strains. Such assessments took place at testing sites used by national programmes for evaluating materials for performance and adaptation to specific environments.

A 'virus disease nursery' containing multiple virus resistant genotypes was inspected and evaluated at a number of the testing locations including sites in Cameroon, Niger, Burkina Faso, Togo, Zambia and Botswana. Accessions in this nursery had been selected for specific susceptibilities to certain viruses,



or virus stains. The nursery so constituted functioned as a field indicator allowing, with a reasonable degree of reliability, for tentative identification of virus(es) and virus strains prevalent at a particular location.

Also during our surveys, we took samples from suspected virus-infected leguminous weeds and tested them for presence of the three beetle-transmitted viruses of cowpea, as well as for cowpea aphidborne mosaic virus (CABMV).

Samples collected during our surveys were tested for presence of cowpea yellow mosaic virus (CYMV), cowpea mottle virus (CMeV) and southern bean mosaic virus (SBMV) by means of agar-gel diffusion tests. Representative samples from sites at which all samples taken tested negative for these three viruses were retested, by ELISA, for possible presence of CABMV or cucumber mosaic virus (CuMV).

RESULTS AND DISCUSSION

It was found that, whereas the three beetle-transmitted viruses occurred in the humid Guinea savanna and transitional forest zones of the lowland West African region, they appear to be absent from the dry, Sudan and Sahel savanna zones. In the latter agro-ecological zones where most of the continent's cowpea is grown, CABMV seems to be the only virus occurring both in farmers' fields and on research stations, albeit at very low incidence in the former. CABMV has so far only been found in cowpea, including its close wild relative, *Vigna unguiculata* subsp. *dekindtiana* var. *dekindtiana*.

During one of our surveys in 1989, a type of CYMV was found in the leguminous weed species, *Vigna reticulata*, at Mokwa in the southern Guinea savanna region of Nigeria. However, this particular strain seems to be of no relevance to cowpea: we found that only very few normally susceptible accessions showed susceptibility to this strain. CYMV. A comparable observation had earlier been made with an aberrant strain of CYMV obtained from cowpea in northern Nigeria (IITA, 1986; IITA, 1988; Rossel, unpublished; Thottappilly, unpublished). It seems that there is a high degree of host specialization within CYMV.

Although very little is known about alternate wild host reservoirs of these viruses, it appears that the occurrence of such plant species and their ability to survive a long dry season is the determining factor for the restricted distribution of the three beetle-transmitted viruses of cowpea in Africa. Conversely, CABMV and CuMV (cowpea strain), which appear to be the two viruses most commonly encountered in the dry Sudan and Sahel savanna zones, are also commonly found in other ecological zones, presumably because of their effective seed transmissibility.

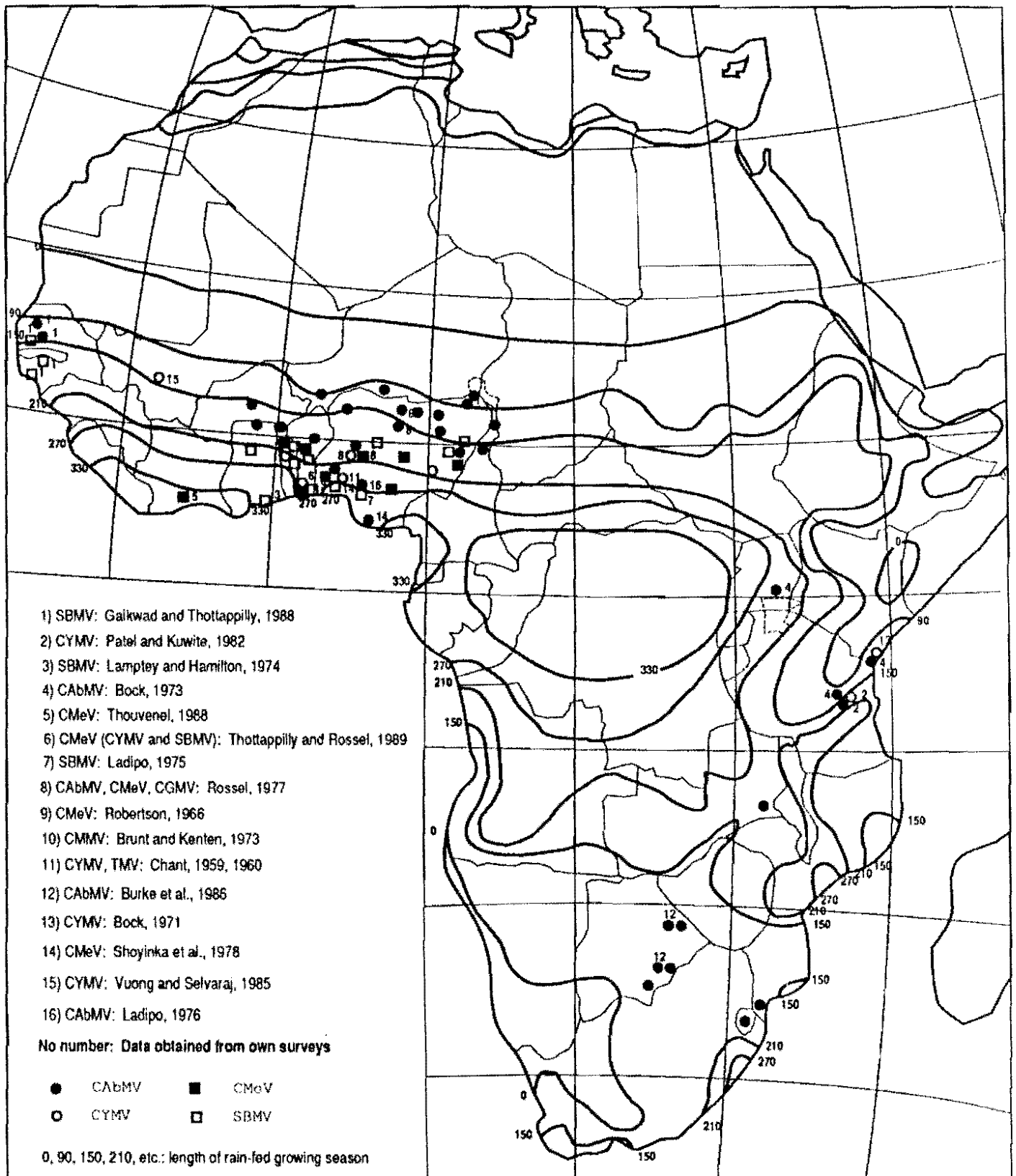
Results of our surveys, supplemented by data in the literature, are presented in Figure 1, which illustrates the agro-ecological zonation of Africa in terms of length of the main, rain-fed growing season to emphasise the ecologically dependent distribution pattern of the viruses we discuss.

REFERENCES

- Bock, K.R. (1971). Notes on East African plant virus diseases. I cowpea mosaic virus. E. Afr. agr. For. J. 37: 60-62.
- Bock, K.R. (1973). East African strains of cowpea aphid-borne mosaic virus. Ann. appl. Biol., 74: 75 - 83.
- Burke, D.W., Ditshipi, P. and DeMooy, C.J. (1986). Virus diseases of cowpeas in dryland and irrigated plots in Botswana. Plant Disease 70: 801.
- Chant, S.R. (1959). Viruses of cowpea, *Vigna unguiculata* L. (Walp.) in Nigeria. Ann. appl. Biol. 47: 565-572.
- Gaikwad, D.G. and Thottappilly, G. (1988). Occurrence of Southern Bean Mosaic Virus on Cowpea in Senegal. J. Phytopathology 121: 366-369.
- Givord, L. (1981). Southern bean mosaic virus located from cowpea (*Vigna unguiculata*) in the Ivory Coast. Plant Disease 65: 755 - 756.
- IITA, (1978). In: Annual Report for 1977, International Institute of Tropical Agriculture, Ibadan, Nigeria. p. 89.
- IITA, (1980). In: Annual Report for 1979, International Institute of Tropical Agriculture, Ibadan, Nigeria. p. 136.
- IITA, (1981). In: Annual Report for 1980, International Institute of Tropical Agriculture, Ibadan, Nigeria. p. 169-170.
- IITA, (1982). In: Annual Report for 1981, International Institute of Tropical Agriculture, Ibadan, Nigeria. p. 138.
- IITA, (1985). In: Annual Report for 1984, International Institute of Tropical Agriculture, Ibadan, Nigeria. p. 86.
- Ladipo, J.L. (1975). Southern bean mosaic virus (cowpea strain) in Nigeria. Nigerian Agric. J. 12: 25 - 28.
- Ladipo, J.L. (1976). A vein banding strain of cowpea aphid-borne mosaic virus in Nigeria. Nigerian J. Sci. 10: 77 - 78.
- Lamptey, P.N.L. and Hamilton, R.I. (1974). A new cowpea strain of southern bean mosaic virus from Ghana. Phytopathology 64: 1100 - 1104.

- Patel, P.N. and Kuwite, C. (1982). Prevalence of cowpea aphid-borne mosaic virus and two strains of cowpea mosaic virus in Tanzania. Indian Phytopath. 35: 399 - 403.
- Robertson, D.G. (1966). Seed-borne viruses of cowpea in Nigeria. B.Sc. thesis, University of Oxford, 111 p.
- Rossel, H.W. (1977). Preliminary investigations on the identity and ecology of legume virus diseases in Northern Nigeria. Trop. Grain Leg. Bull. 8: 41 - 46.
- Rossel, H.W. and Thottappilly, G. (1985). Virus Diseases of Important Food Crops in Tropical Africa. pp. 21 - 39 (61p), International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Shoyinka, S.A., Bozarth, R.F. Reese, J. and Rossel, H.W. (1978). Cowpea mottle virus: a seed borne virus with distinctive properties infecting cowpea in Nigeria. Phytopathology 68: 693 - 699.
- Thouvenel, J.C. (1988). A serious disease caused by cowpea mottle virus in the Ivory Coast. Plant Disease 72: 363.
- Thottappilly, G. and Rossel, H.W. (1989). Occurrence of cowpea mottle virus and other viruses (cowpea yellow mosaic virus, southern bean mosaic virus) in the Republic of Benin. FAO Plant Protection Bulletin 36: 184 - 185.

Fig. 1 : Distribution of CABMV and the three beetle-transmitted viruses of cowpea (CYMV, CMeV and SBMV) with respect to agro-ecological zones in Africa



Map after FAO/AGLS (Dec. 77), Agro-climatic suitability assessment for rain-fed crop production

VIRUS ECOLOGY AND ECONOMIC IMPORTANCE : DISCUSSION

Hennie Rossel : Lead discussant
Howard Gridley : Rapporteur

Rossel : Eighty per cent of the world's cowpea crop is grown in Nigeria in the Sudan, Sahel and Guinea savanna zones. The crop is often grown in association with millet in a relay cropping system and is important as human food and fodder for livestock.

A number of viruses attack cowpea of which the most important are cowpea aphidborne mosaic and cowpea (yellow) mosaic. Both are seed transmitted; their vectors are aphids and beetles (*Oothea*), respectively.

Surveys of virus distribution in Nigeria are not comprehensive, being derived from IITA testing sites, but distribution is related to the presence of vectors. However, frequency and distribution is also allied to geographic zones defined by the number of rain days on a North/South transect, and this pattern would appear to be similar in other parts of Africa. A knowledge of frequency and distribution is essential to formulate effective breeding strategies. A 1 to 5 (1=no symptoms, 5= heavily infected) scale is used at IITA to rate virus symptoms, and these grades correlate with yield reduction. Grade 2 represents an adequate level of resistance for breeding purposes, although problems arise in rating some germplasm accessions where considerable between-plant variation occurs.

Walkey : I have noticed similar variation in UK for some apparently homozygous vegetable lines being assessed for virus reaction.

Gridley : Such variation in germplasm accessions may perhaps reflect genuine genetic variation if such accessions are land races.

Allen : In my experience at IITA, it was often easy to find resistance in germplasm to certain cowpea viruses. These should be selected and further evaluated.

Rossel : Different strains of cowpea viruses in Nigeria do exist but their differences are not great. However, detailed work on strain identification, such as undertaken on BCMV, has not yet been done for cowpea aphidborne mosaic.

May I now invite representatives of national programmes to give brief reports on the legume virus situation in their respective countries ?

Omunyin : The National Horticultural Research Station at Thika is the main station for work on legumes including beans, cowpeas, soya bean, green gram, peas, faba bean and chickpea; Katumani and other stations also conduct legume trials. Ken Bock did the original work in Kenya on cowpea aphidborne mosaic virus. I have found the same virus and a yellow mosaic in lines from IITA. There is a need to ascertain the yield depression caused by these viruses to evaluate whether cowpea resistance breeding should be undertaken. Work is also required on the distribution of the viruses in Kenya. There is also need to survey the viruses present in other legume crops, as large acreage increases have occurred in the last 10 years for a number of legumes, particularly green beans and soya bean; the area under irrigation has also increased dramatically, and this may increase virus infection.

Atcham : Cowpeas are mainly grown in the north and West of Cameroon where they are infected by several viruses. The little work undertaken has identified cucumber mosaic and southern bean mosaic viruses and a virus strain related to BCMV. Viruses endemic to Nigeria also probably exist in the crop. A project to develop monoclonal antibodies is underway for strain identification in a range of crops. The cooperation between IITA and Cameroon is important for the future work on virus identification.

Lana : In Tanzania, beans are the major legume followed by cowpeas, both being usually intercropped with cereals. Other legumes grown are soya bean, groundnut and green gram. BCMV is the most serious virus problem; both serotype A and B are present with NL 3 the predominant strain, although its exact distribution is not known. Other viruses identified are soya bean mosaic, groundnut rosette, tomato spotted wilt, cowpea mild mottle and cowpea aphidborne mosaic.

I should like to add that CIAT has liaised very effectively with the Tanzania national programmes, and perhaps such a cooperative model could be followed with IITA.

SESSION VI - VIRUS EPIDEMIOLOGY

EPIDEMIOLOGY OF BEAN COMMON MOSAIC VIRUS DISEASE

Allan Femi Lana

INTRODUCTION

Considerable information has accumulated over the years on legume viruses and the diseases that these induce. Most of these are well known. For instance, we all know that Bean Common Mosaic Virus (BCMV) is readily transmitted by sap; has a limited host range; has several aphid vectors which transmit the virus in a non-persistent manner, and it is seedborne - between 0.5% to 60%, depending on host cultivar. Many BCMV strains also exist in different locations that affect cultivars differently and which necessitate different resistance. The strategy for BCMV control calls for weakening of the epidemiological cycle -- but can we? Certainly so, by making the appropriate genetic changes in the host; by breaking the cycle during the pre-epidemic stage when the factors responsible for the disease are often most vulnerable.

The epidemiology of BCMV strains is determined by interactions between hosts, vectors, virus and the effect of environmental conditions on them. Effects of environment on vector population and activity are also involved, as are the geographical range over which these favourable environmental factors prevail. I strongly believe that there is much to be gained from a detailed study of virus spread at specific sites, or regions, particularly with regard to the spread of different strains of BCMV. As in the case of soybean mosaic, evidence is accumulating that the best approach to BCMV control may be through a better understanding of the ecology of the vectors as well as of the virus itself.

SEED TRANSMISSION

Seed transmission of BCMV is epidemiologically important for several reasons: it helps to carry over and perpetuate the pathogen during unfavourable environmental conditions; it provides early, random infection foci within a crop from which virus spreads to other parts of the field. Seed transmission also helps in the dispersal, introduction and establishment of the virus (or its strain) into new and distant localities, regions and countries and may, in fact, be present in germplasm collections leading to various complications in breeding programmes.

From experience, we know something about the spatial movement of BCMV between related and unrelated individuals in the same locality. Mink and Oweru have each identified BCMV strains in different legumes besides the bean in the same localities. In a survey carried out in 1985 in Kenya and Tanzania, Mink reported a serotype B of BCMV in green gram, and serotype A in beans at

13308

the same location. Mink also suggested that serotype B on green gram (*Vigna radiata*) was introduced with seed from other areas. BCMV has also been isolated from non-bean plants, suggesting horizontal seed transmission. In some cases, seed transmission is perpetuated from one generation to another, down the pedigree line, parent to progeny for several generations, which is a vertical transmission. There is also ample evidence of areas where BCMV has never been reported but in which it now prevails. This can only be due to seed exchange, or vertical seed transmission.

In general, we know that viruses are carried in the seed's embryo and, in the case of BCMV, it can be at a rate from 1.2% to 60%, the success and amount of seed transmission depending both on the host species and the virus strain. We also know that the earlier the infection of the host plant, the greater the number of seeds transmitting the virus.

What then is the best way to control BCMV in seeds, other than the use of certified virus free seed stocks? The alternative is to breed for non-seed transmissibility. This, in my own opinion, becomes the central and logical target. My limited knowledge of bean virology suggests to me that this area has been neglected. This may be due to the perpetual problem with necrotic gene I. Under the circumstances, the incorporation of the I gene in commercial cultivars becomes a must. Unfortunately, as Morales has wisely indicated, the monoculture of I gene cultivars is seldom possible due to the persistent cultivation of the traditional common mosaic cultivars by small scale farmers. Morales has again offered a solution: it may be possible to produce bean genotypes which are resistant to BCMV and black root. To this end, he said that the dominant necrosis gene I has to be protected by a recessive gene which is not attacked by a known necrotic strain of BCMV.

VECTOR TRANSMISSION: Several aphid species transmit BCMV. We know that environmental conditions may influence populations of vectors vis-a-vis virus spread. There is a need to intensify studies of BCMV epidemiology, including the monitoring of vectors, to allow us to interpret the pattern and sequence of BCMV spread. How do we monitor these? Are some vector species specific to a particular host cultivar? Are there ways we can use weather experience before beans are planted to predict vector abundance and/or their behaviour, and hence subsequent virus spread? Do we have any indication of what type of changing pattern exists in these BCMV vector populations? How are these vectors distributed on non-bean hosts? What is the nature of their build-up on weed hosts?

WEEDS

Besides serving as reservoirs or alternative hosts, weeds act as a stabilising "screen" that would block survival of any new strains selected by new resistance genes incorporated into the crop. This is of considerable importance to bean.

Consideration of alternative weed hosts and development of resistance on non-hosts should then take account of the strains and vectors surrounding the weeds. For instance, if BCMV is transmitted by a particular vector to its progeny and no weeds occur, then resistance to infection should become primary targets of resistance breeding. What I would like to know is whether or not infections in weeds can assist the development of strains of BCMV, or indeed of any other virus of legumes.

CONCLUSION

We all know that genes for resistance or tolerance to infection are usually found in cultivars grown in areas where plants have been continuously or intensively cultivated and exposed to virus infection for long periods. We should discuss what "resistance" means in an epidemiological sense, with reference to legume viruses. I consider this especially important for our discussion since virus incidence in a field will involve all components, the host genotype, the ability to restrict transmission by resistance to vectors, and the ease with which virus is acquired from and transmitted to hosts. In situations where plant tolerance has been detected, field virus incidence during an epidemic can be reduced considerably. In other words, changing a host's tolerance of a virus alone can bring about a significant reduction in virus spread. Again we must bear in mind that the importance of legume virus disease depends on the genotypes being grown. The severity of disease and the cause of epidemics are a reflection of interactions between the particular legume cultivar, the environment, the virus and the vector. I believe that legume species have so much genetic variability that, if wisely manipulated, new cultivars should and can become poor hosts of legume viruses and their vectors, so that losses due to virus diseases may be considerably reduced.

DISCUSSION ON VIRUS EPIDEMIOLOGY :
VECTORS, SEED AND WEEDS

Femi Lana and Mike Thresh : Lead discussants
Jeanne Dijkstra : Rapporteur

- Lana : For epidemiology, we should bring together all the factors we have been discussing before, using BCMV as a case study. Different symptoms, seed transmission: What is the best strategy to control this disease and avoid epidemic situations? Introduction of BCMV into new areas : in 1984, there was no evidence of BCMV in Tanzania, but in 1985 serotype B of BCMV was found in green gram and serotype A in beans in the same location. Is it not desirable to breed for seed non-transmissibility ?
- Morales : Nobody is breeding for resistance to seed transmission. We have found cultivars which have very low seed transmission (< 1%, Immune, Great Northern UI 31 and 123 and Pinto 114). They are highly resistant to infection of the embryo by introduction of the I-gene. We take the short-cut. Resistance to mosaic is important. The ratio of resistant : susceptible plants is 1:15.
- Rossel : There is no straight forward answer. The introduction of a new crop (e.g. soybean) should be done in such a way as to avoid seed transmission. In cases of an established crop (cowpea, bean) it won't be so effective. One important point is that, while breeding for resistance, you are avoiding seed transmission, although the correlation is not established. There is practically no seed transmission in the lines which have been selected for resistance. You can get away with something which has got a low susceptibility; immunity is not necessary. There is a slow rate of spread in tolerant cultivars.
- Mink : How do we set standards for crops in Idaho where beans for seed are grown? There are very stringent regulations. When at seed inspection one plant is found diseased, the whole plot is discarded. Even with that regulation, seed transmitted viruses have been found in certified material. Visual observations were not adequate. We are going to apply routine seed testing regulations. In the pea seed industry, companies submit a seed sample for testing. Although the samples are small for monitoring, in practice it has worked very well in reducing seed-borne transmission in peas. Now there is a proposal to the bean industry in Idaho and Washington to adopt these regulations, but not mandatorily.

- Allen : In the case of green beans produced in Kenya, seeds have been shipped from Europe to Africa.
- Morales : They have resistance genes, but not the dominant I-gene. What was the origin of the original shipment ?
- Mink : In 1985, no cultivar had I-gene resistance. Nobody knows the route these seed lots take. Bean seed lines are trans-shipped to the Netherlands, and then Dutch seed trading companies are able to move seeds from Idaho to Africa ! At the moment there is a quarantine regulation for shipment of seeds from Idaho to Africa. The origin of these necrotic types has not been verified.
- Thresh : The soybean example given by Rossel is a model for BCMV spread. It is important to remember that soybean mosaic virus is not a problem in Illinois. There is low incidence of infection in the first crop from which seed should be taken. The second crop is highly infected.
- Rossel : This is comparable to the situation in Nigeria. The crop was 100% infected, but we cleaned it up. After one or two seasons roguing, it was finished. It behaved like a 'circus animal' ! That is what happens when there is a very low seed transmission rate.
- Mink : In Idaho, they don't have completely clean virus-free seed, but it is not a problem.
- Walkey : What are the long-term control measures ? How do we put resistance in cultivars ?
- Mink : All cultivars released in the USA contain the I-gene which makes them susceptible to the necrotic strains. Non I-gene lines are grown in Europe. When they are introduced into the USA, they may cause a disaster.
- Walkey : The European people should also breed with the I-gene.
- Mink : In the last 10 years, disasters in Idaho and Washington have occurred.
- Thresh : If BCMV is important for Europe, then there is impetus.
- Mink : In Europe, cultivation is small farm oriented. The impetus is for replacing seed lines frequently, more than trying to develop resistant lines.
- Thresh : There is a precedent for it, in the hop, with non-persistent transmission by aphids, mosaic tolerance and introduction into certification programme.

- Mink : I see it as an African origin problem.
- Thresh : What is the isolation requirement ?
- Omunyin : Black root and mosaic in French bean with dominant I-gene ? May not be true. We have quarantine, but the seed companies introduce illegally. Seeds from neighbouring countries. What is the role of the farms ? Farms get the seeds, they plant them, they propagate their own seed lots, and this will build up the virus. In the case of maize, every time there is new seed, we have to identify the really important viruses. How does the rate of transmission vary? Beans in Kenya, small kind of enterprise, along with other crops.
- Morales : Back to the USA: We believe in protecting the I-gene.
- Lana : We should focus on relevance for national programmes.
- Gridley : Three cultivars recommended for release. Some of the seeds were 90% infected with BCMV. Extremely difficult. Off-season multiplication in irrigated fields.
- Walkey : Origin source at the moment is seed transmission.
- Morales : The situation in Colombia is far more complicated than in Africa, as people are very particular about grain type. Introduction of I-gene must be easier in Africa.
- Thresh : Only in Chile: protected lines. Chileans have given it up. Black root is not a limiting factor under farm conditions. What proportion has got the I-gene? Farmers always retain their landraces. Most of the national programmes have been releasing I-gene lines.
- Omunyin : The question is whether the I-gene is present in the seed of lines having other desirable traits. In Kenya, there are lots of susceptible material, as landraces. Now we are collecting landraces and find accumulation of virus in it.
- Rossel : Is the issue testing through nurseries ?
- Morales : Yes. There are some nurseries with lines without I-gene. Only done in screenhouses. Seeds are tested for presence of virus, bacteria and so on.
- Omunyin : Trying to multiply in Wellesbourne. Standards should be met. Homogeneity should be maintained. In our fields multiplication of BCMV, although field is clean. But later the seed shows virus. We stopped it.

- Thresh : Let's shift the discussion. What is the critical level of seed transmission to give rise to an epidemic? Californian work on lettuce mosaic is relevant.
- Morales : 50% transmission is high. They say "I" can bring it down to 1%. But 1% = 2500 infected plants per ha. Grown under field conditions, but some under controlled conditions. Results: we could clean up these seeds by roguing, as soon as we were looking at primary leaves. Within a month 100% infected in fields surrounded by sugarcane fields! Never advocate roguing!
- Opio : No study has been done on epidemiology, only identification.
- Rossel : Substantial evidence. Testing in ecological zones : cowpea testing for the past 7-8 years. Natural infection pressure is low. In certain places 100% infected, due to local varieties. We look at artificial situations at testing locations long-term stable equilibrium situated is important. High transmission rate in local varieties. I am not too worried, because cowpea is an indigenous crop. But bean is an introduced crop. In the main growing areas, the infection is rather low. Long-term prediction is different.
- Lana : Turning now to consider vectors, there is a fundamental question to answer. In the case of BCMV, we don't know much about the pattern of spread by vectors. How high can the vector go? How should we monitor the vectors? There is a BCMV outbreak in one year and not in the next.
- Allen : Is there aphid specificity in necrotic and non-necrotic lines? What effect has vector resistance? And the relationship between virus and vector?
- Lana : Are there vectors in specific lines?
- Morales : Breeding for resistance to insects? No.
- Rossel : Vectors are not the limiting factor.
- Morales : I agree with Rossel. One thing is clear: the incidence of BCMV is higher in lowlands than in highlands. This is most likely due to aphid activity. Interesting to study. Beans without resistance were virus free in highlands.
- Omunyin : We need to rank the importance of various factors. Vectors are secondary to seed transmission. More work is needed in combination with entomologists.

- Morales : BCMV: we should concentrate on determining the factors in the incidence in the highlands. Are necrotic strains present there ?
- Vetten : BCMV occurs in low incidence at higher altitude.
- Opio : In the Ugandan highlands, also incidence of BCMV.
- Allen : We cannot separate seed transmission from aphid transmission. Vector resistance may not be important in non-persistent virus transmission. But this is not true for beetle or whitefly - transmitted viruses. In these, vector resistance may be useful.
- Thresh : Site and elevation effect: a simple experiment is needed. Use seeds with established seed transmission rates. Put them at high and low elevation. Then we know what the ground rules are. We need these data.
- Morales : I fully agree. We are researchers, not observers. BCMV at high altitude in high incidence. Under field conditions it is not quite what you expect. Those experiments can be conducted under farmers' conditions.
- Thresh : Perhaps there is also seasonal influence. In N. India, potato crops are free from virus in January, February and March. Seed potatoes grown in that part of India in that period.
- Rossel : The most susceptible varieties are grown at high altitude.
- Thresh : Is anybody prepared to do some aphid monitoring work ? Interesting to see the relation between potato zones and bean zones.
- Omunyin : We are trying to do that in Kenya. In some parts of Kenya, there was some correlation.
- Rossel : Is crop density in the highlands different from that in lowlands ?
- Morales : No.
- Allen : We held a training course recently on aphid identification in Malawi, with emphasis on the epidemiology of BCMV.
- Morales : In Ethiopia, monitoring aphids is important, especially at high altitudes.
- Lana : Because of importance of necrotic strains, do we have alternate weed hosts ? Is it possible that there is a reservoir. Not only BCMV, but other viruses. From

Cassia a virus to bean.

- Allen : Seed transmission first, then vectors. Reservoir is primarily the seed. For whitefly - transmitted virus, weeds are important. Whitefly is breeding on wide range of weeds.
- Walkey : Could other crops be important besides weeds ?
- Rossel : Cowpea golden mosaic virus : Incidence of wild sources is low. There is wild cowpea.
- Vetten : Geminiviruses have a narrow host range in general.
- Mink : Necrotic strains occur at detectable levels in weeds.
- Thresh : If you have high levels of seed transmission in cowpea then you don't have to worry about other factors.
- Morales : 2000 different viruses from 2000 different weeds !
- Vetten : Sample from *Cassia occidentalis*.
- Allen : An idea of the origin of epidemics is important. Whereas cowpeas are African, beans are exotic, introduced into Africa 3-400 years ago. Could black root be a new encounter between American host and Africa virus ? It could have epidemiological importance.
- Mink : In Tanzania, all serotypes of BCMV seem to be scattered over the area.
- Morales : All legume potyviruses come from weeds, giving necrosis. 'In a war one does not care about malaria when so many bullets are flying around' !
- Thresh : Looking at different virus problems and crops, it is clear that the breeding approach for resistance has overwhelming priority. It is not as easy at that : there is a consumer preference for certain grains, as Morales mentioned. It is also true of rice. Resistance may be linked with some undesirable characteristics. Why do we stick to certain varieties? The breeder is interested in overall improvement. Releasing a variety and developing it is governed by other factors. If we have a virus disease that is present in one year and not in another year, or in another place, then resistance is not that important. That is why we still have disease problems.

The pathogen comes up with a resistance - breaking approach. In the developed situation, a strategy can be developed in manipulating planting dates. These

things are not easy to do under African conditions; dependent on the rainy season. Also it is not easy to shift to another area. These problems necessitate an epidemiological approach. An obvious example comes from Idaho. We heard a lot about virus identification and so on, but not much on epidemiology. No doubt that for BCMV healthy seed is important. We did not hear anything about losses, nor about distance of spread. What is the degree of movement from a high level infected crop to a low level infected one? An important experiment was once conducted in Zambia. Infested cowpea provided a focus of *Aphis craccivora*. This point source was surrounded by a BCMV infected bean crop, surrounded by I-gene cultivars; and the gradient of disease was measured. This gave an idea of the distance of primary spread. We don't have to go that far for forecasting.

Walkey : Seed transmission is not the major cause of crop losses.

Morales : It depends on the cultivar. Some may suffer tremendously when grown from infected seed. I once conducted a study with clean seeds of a susceptible variety. I sent seed to Chile to be increased there under dry conditions, from where the seed was shipped back to Colombia. The result was that the farmers' seed yielded more! The incidence of BCMV in the seeds from Chile was 31%, but when the plants were in the field they looked so clean, and only lower leaves showed symptoms. All the plots had 50% loss. This shows that in certain genotypes you may get more losses.

Mink : You may have minor effects on susceptible varieties. Other varieties (non I-gene resistant) may be more severely affected, especially when spread is early in the season. Are there any quality effects caused by BCMV in beans? In peas (pea seed-borne virus) there is a quality effect. The other aspect, I-gene varieties grown next to non-I-gene varieties give great losses. In beans, it is more complicated than in soybean or cowpea.

Rossel : Studies are important. The point is who is going to do it? For us it is important to get material which does not give symptoms. I believe the crop loss aspect is not that important.

Thresh : What is the economic importance of BCMV?

Allen : Breeders have to set programmes based on perceived economic importance of virus disease. We have to get more data on crop loss.

- Gridley : It is possible we have over-rated the importance of certain diseases among constraints. BCMV proved to be an important factor in yield losses, with about 250 kg/ha reduction in on-farm trials.
- Walkey : BCMV losses have been estimated at US\$ 150 x 10⁶ a year, but are these figures reliable?
- Gridley : We have resistance to BCMV.
- Thresh : Is there a high level of seed transmission in bean landraces ?
- Morales : In landraces seed transmission is very high. Variety Calima is grown in Africa, but you cannot see symptoms. It yields well, it is tolerant. We found there was not a reduction in size of the seed, but there was 56% yield loss over non-infected plants, because the pods did not have many seeds in them.
- Walkey : Thresh said that the likelihood of breakdown of resistance is appreciable. In CMV, perhaps, but not with BCMV. The I-gene has stood up for 50-70 years.
- Rossel : I have not seen evidence of any breakdown, at least in cassava.
- Morales : The I-gene is not specific resistance, but recessive genes are mostly strain specific. They have broken down. Furthermore, black root is not a disease; it is a resistance reaction.
- Walkey : Perhaps there is evolution of new strains by exposing plants to strains which were already present.
- Morales : The introduction of strain-specific genes gave rise to different strains.
- Omunyin : What is the proportion of varieties having necrosis genes? In landraces, there may be types of resistance that operate in different ways. If there is a 55-67% reduction in yield after inoculation of susceptible plants, in the field it will vary, in part because of vector pressure.
- Mink : Inoculum pressure changes with the source of seeds. With the international trade in bean seeds, the picture in Africa may change.
- Thresh : The international institutes may not be able to tackle the problem.
- Rossel : We can.

- Thresh : Who is going to do the work in National Programmes which have special objectives ?
- Rossel : The main constraint is man-power resources in getting things organised.
- Allen : Regional cooperation has paid off handsomely in other parts of the world.
- Mink : Regional research projects have been successful. In the USA, we have made regional progress with scientists from different states.
- Allen : Regional cooperation is not new to Africa. For example, Ken Bock's virus unit in Kenya served East Africa. A virus laboratory could be connected with a quarantine station, or with germplasm banks such as that being establishment in Lusaka. I strongly support the idea of cooperation.
- Thresh : We are getting far away from epidemiology ! What about crop loss ?
- Allen : Field surveys are very important. Data should be subjected to critical assessments.
- Thresh : What protection do varietal mixtures provide ?
- Allen : With regard to BCMV, we don't know. Certainly bean mixtures can be very diverse, often particularly in highland areas, but landraces of a single seed type may also possess important genetic diversity.
- Allen : Training should be given in response to needs.
- Thresh : Are we talking about short term courses ?
- Rossel : We have to set priorities, with high degree of specialisation, not necessarily commodity-specific.
- Thresh : Who is giving training in general virology ?
- Dijkstra : At Wageningen in the Netherlands, there are crop protection courses including Virology.
- Allen : Both short-term and long-term training may be required. CIAT runs specific training courses which technicians can attend.
- Walkey : We prefer 'sandwich' training at the Ph.D level. Training programmes are very important.
- Thresh : Ways in which virology is progressing is remote from real problems. We should start an M.Sc. course in Africa.

SESSION VII - DEVELOPMENT OF AN ACTION PLAN

DISCUSSION OF VIROLOGISTS' PERSPECTIVE

David Allen : Lead Discussant
Gaylord Mink : Rapporteur

INTRODUCTION

David Allen established the framework for the discussion by listing a series of questions to be addressed by the group, as follows :

1. What viruses are present in the region, and which are absent?
2. What is their geographical distribution ? What is their ecological distribution ?
3. How important are they (occurrence, crop loss) ?
4. How variable are they (strains) ?
5. How do they survive and spread (reservoirs, seed, vectors) ?
6. How may they be managed (host resistance, cropping systems, exclusion) ?

These six questions may be considered under two main headings.

Distribution and importance

- Literature surveys.
Sparse; biassed; inaccurate ?
- Field surveys.
In progress (Braunschweig, IHR/CIAT; IITA).
Gaps ? Risks ?
'Outside' v. 'inside' laboratories
A regional facility ? SADCC ?
Association with a germplasm bank ?
Across commodities ?
- Economic importance.

Crop loss assessments ?

BCMV = # 1. where ?
Which strains ? Aphid x strain interactions ?

CAMV/B1CMV = # 2. Strains in eastern and southern
Africa ?

Local/seasonal importance
CYMV; CMMV (in beans);

CGMV - characterize.
Others ?

Risks. Known and 'new'.

Epidemiology and management

- Seed transmission

Key to aphid transmission of Potyviruses.
Guidelines for safe exchange (FAO/IBPGR).

Opportunities for clean seed production (e.g. Ethiopian highlands) ? But on-farm seed production more appropriate ?

Define critical thresholds ?

- Vector transmission

Key to beetle/whitefly transmission
Viruses + not seedborne.
Information on rate of spread, disease gradients ?
Differential transmission of strains (e.g. BCMV) ?

- Reservoirs.

Wild relatives/volunteers more important than weeds ?
Vectors not important in carry-over of bean/cowpea viruses ?

- Crop management.

- Host plant research is No.1 strategy.

DISCUSSION

Allen : Do we need to establish regional cooperation and facilities ? Femi Lana and Samson Owera's cooperative project is one example of within-area cooperation, but we need others.

Morales : How many more surveys have to be made before we are satisfied ?

Vetten : The German project will end this year. We will publish some of the results, even though not all activities are finished yet. We think we have a good idea of which viruses are important and now have some idea of their distribution.

Morales : We need to get the results of all these surveys published, or at least distributed to others. It would be very valuable also to have a comprehensive review of literature of legume viruses in East Africa; possibly one like a CIAT publication.

- Allen : Yes, this can be done.
- Dijkstra : Can additional surveys be made by national programme people on their own ?
- Allen : Yes. Some will be done, like the Lana Owera collaborative sub-project on BCMV.
- Mink : During our 1984/85 surveys, we photographed all samples. Many of these samples were never identified. Could we publish through CIAT or elsewhere the symptoms of these virus or virus-like diseases that were not identified ? This would assist national programme people in selecting unidentified virus samples.
- Allen : Where are the gaps for cowpeas ?
- Rossel : My surveys always involve international programmes. We must try to promote more involvement by local people. I see no urgent need to continue surveys of cowpea, but we do need to maintain communication with local scientists. We may require follow-up work in some areas.
- Mink : I want to outline a concept for a regional virus testing facility that would perform service tests for several eastern African countries. Such a facility could be established in one location with one or two technicians and a very limited amount of equipment and materials. It would supplement and expand activities at the national level. In one place we could test for many different viruses of many different crops. If successful, activities could continue year-round and thus be a more efficient use of facilities and people, and could be used to train skilled people.
- Rossel : I don't see a need for regional testing. It should be done at the national level.
- Wangai : How will a regional laboratory affect national quarantine organizations ?
- Mink : At Prosser, our "regional" facility conducts tests for several state and national regulatory agencies. These organizations find it cheaper and more efficient to have an on-going laboratory such as ours to do their testing for a fee than to invest in ELISA equipment for a limited number of tests per year. Furthermore, one must have someone who is well-trained in the techniques, otherwise an untrained person often runs poor tests. Once the regional facility is known to do good work for reasonable fees, they receive many more samples than was ever expected.

- Thresh : Most national programmes will not be able to set up virus testing, if the past is any measure. There is a strong case for a central facility that can specialize in pathogen detection. It is likely that each country will not be able to do so for decades, if ever.
- Morales : It will not be possible to set up something in East Africa because of quarantine problems in sending samples; and also because of the specialized training involved.
- Allen : The regional facility could be expanded to include training for national scientists and technicians. There are moves afoot to make exchanges of materials among countries easier.
- Owera : It may be difficult now but it should get easier in future. I support the regional centre idea.
- Thresh : I also support the regional centre idea. The success of a regional programme will depend heavily upon the degree of cooperation among national scientists and countries. We would need to define what the regional centre should do.
- Taylor : There would be a need to test germplasm for pathogens other than viruses. We should not limit thinking to viruses, but include bacteria.
- Lana : I support a regional centre, especially if testing is broader than viruses.
- Morales : What would be the function of a regional centre ? If you think about testing germplasm, the centre should not undermine the capacity of the national programme to do its work.
- Owera : Most national programmes are too poor to do testing themselves. At the moment, the national programmes need much more help than they can get.
- Thresh : This concept is trying to provide a pragmatic solution to a practical problem. The international donors and programmes have been trying for more than 50 years to develop programmes at national levels. We are no closer to success now than at the beginning.
- Mink : A regional testing laboratory would handle a much wider array of samples than just germplasm.
- Allen : There is need to start regional activity in a moderate way. "Regional" means sharing of activities.

- Owera : Mink's procedures of mailing strips to Prosser for testing give more flexibility to national programmes. An African facility might do the same.
- Walkey : The concept of a regional laboratory goes beyond simply running tests and training.
- Allen : In summary, national people appear supportive. We recognize there will be difficulties, but we have to start somewhere. But there are still some differences of opinion on what a regional laboratory should do.
- Thresh : The technical gap between Africa and other parts of the world is widening instead of getting smaller.
- Allen : I suggest Mink makes his pre-proposal available to scientists from Tanzania, Kenya and Uganda for their input.
- Walkey : Eventually such a testing facility will need expensive equipment such as EM, high-speed centrifuge, and so on.
- Mink : One can develop a test facility very modestly, using ELISA technology at first, and expanding later, perhaps.
- Morales : I agree with the need for a service laboratory in Africa.
- Allen : There remains some disagreement over what and how a regional laboratory should be developed. Let's start with Mink's proposal. The three African countries represented here can then review it and make recommendations.

FUTURE LINKS BETWEEN IARCS AND NATIONAL PROGRAMMES

Lana suggested that IARCs could assist National Agricultural Research Systems (NARS) in surveys. Rossel said that surveys are expensive to conduct and should only be undertaken if viruses are considered a major problem to be tackled by resistance breeding. He preferred to train national scientists at IITA who could then undertake national surveys. Lana also stated that germplasm exchange between countries in Africa is becoming more and more difficult, threatening crop improvement programmes which depend on such exchanges. As such, IARCs should try and alleviate this problem. Rossel replied that the IARCs are not that powerful to influence national policy on plant import, but they had organised some meetings to address this problem.

DISCUSSION OF BREEDERS' PERSPECTIVE

Howard Gridley : Lead Discussant
John Taylor : Rapporteur

Gridley : BCMV is important and causes yield reduction, but by how much ? There is a build-up of virus in seed stocks held by small farmers. Some farmer-seed producers may be made expert in roguing. There is priority to incorporate BCMV resistance including the use of CIAT materials. National Programmes (NPs) should use the protected I gene in adapted genotypes, using a backcross programme. At the moment, NPs are dependent on CIAT for materials like the VEF nursery which is a good source of variation. In Uganda, we found that 125 lines out of 500 out-yielded local cultivars by as much as 100 per cent. A local virologist is needed to screen segregating populations in the backcross programme. The protected I gene is being incorporated at CIAT into Rubona 5, G 13671 and the local White Haricot.

Access to the required cultivars is currently restricted to the international centres. This capability must be developed locally. The I gene is said to be very stable but work should continue on alternative sources of resistance. There is variation in rates of seed transmission, and cultivars with a low transmission rate could perhaps be used as a means of control.

Mink : Alternatives to breeding to obtain new resistance include genetic engineering, limited by the failure to regenerate *Phaseolus* from tissue culture.

Morales : Landraces in Latin America are susceptible but some black-seeded types have the I gene.

Gridley : There is a problem with yield improvement, especially in determinate genotypes of growth habit type I.

Omunyin : There is a barrier to bean breeding in Kenya because of the one year delays from quarantining introductions. Meanwhile, seed multiplication in Kenya is improving.

Morales : Would you take I gene material to farmers' trials ? Black root incidence can be shocking !

Lana : Would you receive lines from Tanzania for trial in Uganda ?

Gridley : Materials from Tanzania will be included in regional trials, but probably not including TMO 216.

LIST OF PARTICIPANTS

Dr. David J. Allen,
SADCC/CIAT Regional Programme
on Beans in Southern Africa,
P.O. Box 2704,
Arusha,
Tanzania.

Telex: 42106 CANWHT TZ/42119 LIONS TZ
Fax : 057.7864
Tel. : 057.2268

Dr. Theresa Atcham - Agneroh,
Centre Universitaire de Dschang,
P.O. Box 96,
Dschang,
Cameroun.

Telex: 7013 KN

Dr. Jeanne Dijkstra,
Department of Virology,
Wageningen Agricultural University,
P.O. Box 8045,
Wageningen 6700 EM,
The Netherlands.

Dr. Howard Gridley,
Centro Internacional de Agricultura
Tropical (CIAT),
Kawanda Research Station,
P.O. Box 6247,
Kampala,
Uganda.

Telex: 61406 RAYMA UG
Fax : 256.41.245597/567625 (Attn. CIAT)

Prof. A. Femi Lana,
Department of Crop Science & Production,
Sokoine University of Agriculture,
P.O. Box 3005,
Morogoro,
Tanzania.

Telex: 55308 UNIVMOG TZ

Dr. Gaylord Mink,
Irrigated Agricultural Research &
Extension Center,
Washington State University,
Prosser,
P.O. Box 30,
Washington WA 99350,
U.S.A.

Telex: 5107741099 COLL AG WASH
Fax : 509.7863.454

Dr. Francisco Morales,
Centro Internacional de Agricultura
Tropical (CIAT),
A.A. 6713,
Cali,
Colombia.

Telex: 05769 CIAT CO
Fax : 57.23.647243

Mr. Michael Ogunyin,
Kenya Agricultural Research Institute,
National Horticultural Research Station,
P.O. Box 220,
Thika,
Kenya.

Mrs. Fina Opio,
Kawanda Research Station,
P.O. Box 6247,
Kampala,
Uganda.

Telex: 61406 RAYMA UG
Fax : 256.41.567635/245597

Dr. Samson Odera,
Department of Crop Science,
Makerere University,
P.O. Box 7062,
Kampala,
Uganda.

Ir H.W. Rossel,
International Institute of Tropical
Agriculture (IITA),
PMB 5320,
Ibadan,
Nigeria.

Telex: 31417 TROPIC NG

Miss Nicola Spence,
Institute of Horticultural Research,
Wellesbourne,
Warwick CV35 9EF,
U.K.

Telex: 957251 EMRS G
Fax : 0789.470552
Tel : 0789 470382

Dr. John Taylor,
Institute of Horticultural Research,
Wellesbourne,
Warwick CV35 9EF,
U.K.

Telex: 957251 EMRS G
Fax : 0789.470552
Tel : 0789 470382

Dr. J.M. Thresh,
Institute of Horticultural Research,
East Malling,
Maidstone, Telex: 957251 EMRS G
Kent ME19 6BJ, Fax : 0732 - 849067
U.K. Tel. : 0732 - 843833

Now at
Natural Resources Institute,
Central Avenue, Telex: 263907/8 LDN G
Chatham Maritime, Fax : 0634.880066/77
Kent ME4 4TB,
U.K.

Dr. H.J. Vetten,
Institute of Plant Viruses,
Federal Research Centre for Agriculture
& Forestry,
Messeweg 11-12,
D-300 Braunschweig, Telex: 17.5318300 BRABS
West Germany. Fax : 0531.399239

Dr. David G.A. Walkey,
Institute of Horticultural Research,
Wellesbourne,
Warwick CV35 9EF, Telex: 957251 EMRS G
U.K. Fax : 0789.470552
Tel : 0789 470382

Mrs. Anne Wangai,
National Plant Breeding Research
Institute,
P.O. Njoro,
Kenya.

ANNOUNCEMENTS

29 June - 10 July 1992.

British Council Course - PLANT VIRUS EPIDEMIOLOGY AND CONTROL, directed by Dr. J.M. Thresh, National Resources Institute of the United Kingdom. Contact: Courses Department, The British Council, 10 Spring Gardens, London SW14 2BN. Tel. no: (0) 71.389.4264 or 4406; Fax no. (0) 71.289.4154.

4th International Symposium: PLANT VIRUS EPIDEMIOLOGY, Bari, Italy 27-30 July, 1992 "Plant viruses and the environment" Contact: Professor G.P. Martelli, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy.

CIAT AFRICAN WORKSHOP SERIES

Other publications in this series are :

- No. 1. Beanfly Workshop, Arusha, Tanzania, November 16-20, 1986.
- No. 2. Bean Research in Eastern Africa, Mukono, Uganda, June 22-25, 1986.
- No. 3. Soil Fertility Research for Bean Cropping Systems in Africa, Addis Ababa, Ethiopia, September 5-9, 1988.
- No. 4. Bean Varietal Improvement in Africa, Maseru, Lesotho, January 30 - February 2, 1989.
- No. 5. Troisieme Seminaire Regional sur l'Amelioration du Haricot dans la Region des Grands Lacs, Kigali, Rwanda, 18-21 Novembre 1987.
- No. 6. First SADCC/CIAT Regional Bean Research Workshop, Mbabane, Swaziland, October 4-7, 1989.
- No. 7. Second Regional Workshop on Bean Research in Eastern Africa, Nairobi, Kenya, March 5-8, 1990.
- No. 8. Atelier sur la Fixation Biologique d'Azote du Haricot en Afrique, Rubona, Rwanda, Octobre 27-29, 1988.
- No. 9. Actes du Quatrieme Seminaire Regional sur l'Amelioration du Haricot dans la Region des Grands Lacs, Bukavu, Zaire, 21-25 Novembre, 1988.
- No. 10. National Research Planning for Bean Production in Uganda, Makerere University, Kampala, Uganda, January 28 - February 1, 1991.
- No. 11. Proceedings of the First Meeting of the Pan African Working Group on Bean Entomology, Nairobi, Kenya, 6-9 August, 1989.
- No. 12. Ninth SUA/CRSP Bean Research Workshop, and Second SADCC/CIAT Regional Bean Research Workshop. Progress in Improvement of Common Beans in Eastern and Southern Africa, Sokoine University of Agriculture, Morogoro, Tanzania 17-22 September, 1990.