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Bulletin 63

BEAN COMMON MOSAIC VIRUS AND RELATED VIRUSES IN AFRICA

Overseas
Development
Administration

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Nicola J. Spence and D. G. A. Walkey

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Contents

Foreword

Phaseolus beans are widely grown in many African countries and recent FAO estimates indicate a total production throughout the continent of 1.8 million tonnes from an area of 2.6 million hectares. The crop provides a particularly important part of the diet and one of the main sources of protein in Sub-Saharan Africa. In several countries there is also a thriving export trade in dried or fresh produce to European markets and some production of seed for sale by international companies.

Yields are usually low and there are many reasons for this including the impact of pests and diseases. These are known to cause serious losses and bean common mosaic virus (BCMV) is prevalent in many countries. This virus is not usually a problem in developed countries because of the availability of resistant varieties and the attention given to raising certified seed stocks of good health status. There is a long history of research on BCMV in the USA and in several European countries including the Netherlands and the United Kingdom where scientists at Horticultural Research International, Wellesbourne have played a particularly prominent role.

Comparable studies have been lacking in Africa although the Centro Internacional de Agricultura Tropical (CIAT) has in recent years supported a bean improvement programme with scientists based in Tanzania, Uganda and Ethiopia, which are three of the African countries in which beans are of particular importance. One of the main aims of the CIAT project and of collaborators in several National Programmes has been to develop varieties that are resistant to strains of BCMV that are prevalent in Africa. To meet these objectives detailed information is required on the virus strains encountered and in particular on the distribution of the 'necrotic' strains that can infect varieties that are resistant to other strains. The Wellesbourne virologists were well qualified to undertake a preliminary study of the problem from their previous studies on BCMV in UK and by drawing on the experience of collaborators in the US and the Netherlands. Funding for a 3-year project was provided by the Natural Resources and Environment Division of the Overseas Development Administration and this Report provides details of their findings.

It is a pleasure to write the foreword to this publication and to acknowledge the contribution made by Dr David Walkey and Dr Nicola Spence in their indefatigable travels in many countries of eastern Africa and subsequent studies at Wellesbourne. The project has in many ways been an exemplary one in demonstrating how UK expertise can be utilized to assist developing countries. Much new information has been obtained of immediate benefit to CIAT and National Breeding programmes. Exciting scientific issues have also been raised concerning the role of weeds and wild legumes in the epidemiology of at least some strains of BCMV. This emphasizes the need for further studies that are likely to provide biological information of great importance in the continuing debate on the origin and status of the necrotic strains. As discussed in the bulletin, these strains are considered by some to be of a separate virus that differs from BCMV *sensu stricto* which seems to be of New World origin.

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GLOSSARY AND ABBREVIATIONS

The definitions of the terms used in this glossary relate specifically to the meaning in which they are used by the authors in this manuscript. In addition to the abbreviations listed below, abbreviations relating specifically to Table A6–A14 are shown in Appendix 1.

Glossary

Gene-for-gene relationships: the hypothesis that corresponding and matching alleles for resistance and virulence occur in host and pathogen respectively.

Hypersensitivity: a reaction in a host plant which results from virus infection and involves rapid death of infected tissues. The dead cells may be restricted to discrete local lesions or necrosis which may spread rapidly through the vascular system and can be lethal. Hypersensitivity is considered to be a form of resistance to virus spread.

Isolate: a single collection of a virus that has been obtained from a single infected plant.

Novel isolate: an isolate that has a phenotypic expression in the differential host cultivars that is distinct from any previously, well characterized isolate (in this study the phenotype of an isolate is considered distinct if it differs from the standard strain by failing to infect all cultivars of a particular differential host group). Novel isolates which induce identical differential host cultivars responses are considered to belong to the same novel strain.

Pathogenicity group: a grouping of virus isolates that have the same pathogenicity genes.

Pathogenicity phenotype: a description of the responses that a virus isolate induces in a range of differential host cultivars.

Resistance: is normally defined as the ability to suppress or retard virus replication and spread, but for the purposes of the differential host reactions described for each BCMV isolate in the tables in this bulletin, host cultivars are considered resistant if the virus isolate is unable to cause systemic infection in the bean plant. The isolate may, however, infect the primary leaves (Drijfhout, 1978).

Resistance group: a grouping of differential host cultivars having the same resistance genes.

Serotype: a grouping of virus isolates that share antigenic similarity with one another but which are distinct from other groupings of isolates with respect to most antigens. The BCMV strains are divided into the 'A' serotype (necrotic strains) and the 'B' serotype (non-necrotic strains).

Standard strain: a BCMV isolate which is a standard representative of a particular pathogenicity group as described previously by Drijfhout (1978).

Strain: a grouping of virus isolates that are serologically and pathogenically similar.

Strain-type: an isolate that has a phenotype which is serologically and pathogenically similar to a previously well characterized strain, but which may have small phenotypic characteristics which are different from the standard strain isolate (i.e. its differential host cultivar responses may differ from those of the standard strain in that it may fail to infect an individual host cultivar of a host group, but it will infect other cultivars of that particular host group).

Susceptible: for the differential host reactions induced by the BCMV isolates described in this bulletin, the term is only used if the isolate infects the plant systemically. If the isolate only infects the primary leaf the plant is considered resistant (Drijfhout, 1978).

Tolerance: has been used for the purposes of this bulletin to describe a host response to virus infection that results in negligible or mild symptom expression, but where virus can be detected by ELISA or back-inoculation to a susceptible host.

Abbreviations

Viruses

AlfMV	Alfalfa mosaic virus
AZMV	Adzuki bean mosaic virus
BCMV	Bean common mosaic virus
BLCMV	Blackeye cowpea mosaic virus
BNMV	Bean necrosis mosaic virus
BYMV	Bean yellow mosaic virus
CAbMV	Cowpea aphid-borne mosaic virus
CSMV	*Cassia* severe mosaic virus
CYSV	*Cassia* yellow spot virus
CMV	Cucumber mosaic virus
PGV	Peanut greening virus
PnMoV	Peanut mottle virus
PnSV	Peanut stunt virus
PnStV	Peanut stripe virus
PWV	*Passiflora* woodiness virus
SBMV	Southern bean mosaic virus
SoMV	Sowbane mosaic virus
SoyMV	Soybean mosaic virus
TeSV	*Tephrosia* symptomless virus
WMV-2	Watermelon mosaic virus 2

Symptoms

Chl	Chlorosis
CVB	Chlorotic vein banding (MCVB, mild; SCVB, severe)
GVB	Green vein banding (MGVB, mild; SGVB, severe)
LD	Leaf distortion (MLD, mild; SLD, severe)
Mos	Mosaic (MMos, mild; SMos, severe)
Mot	Mottle (MMot, mild; SMot, severe)
Nec	Necrosis (LNec, local)
St	Stunting (MSt, mild; SSt, severe)

General

ATCC	American Type Culture Collection
BCIP	Bromo-chloro-indolyl phosphate
BSA	Bovine serum albumin
CIAT	Centro Internacional de Agricultura Tropical
cv	Cultivar
cvs	Cultivars
DAS-ELISA	Direct double antibody sandwich ELISA
EDTA	Ethylenediaminetetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
HRI	Horticulture Research International
IgG	Immunoglobulin G
ISABU	Institute des Sciences Agronomiques du Burundi
ISAR	Institut des Sciences Agronomiques de Rwanda
ISEM	Immunosorbent electron microscopy
M	Metres above sea level
NBT	Nitro blue tetrazolium
NI	No virus isolated
NRI	Natural Resources Institute
NT	Not tested

PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PBS-T	Phosphate-buffered saline 'Tween
Poly A	Polyadenylic acid
PTA-ELISA	Indirect plate-trapped antigen ELISA
PVP	Polyvinyl pyrrolidone
SDS	Sodium dodecylsulphate
TBS-T	Tris-buffered saline 'Tween
Tris	Tris(hydroxymethyl)amino methane

Summaries

SUMMARY

This bulletin reviews the current status of research knowledge on bean common mosaic potyvirus (BCMV) and reports the results of a survey of the occurrence of BCMV in the main bean-growing areas of Africa. The strain of each BCMV isolate collected has been identified by its reactions on a standard set of differential bean cultivars and distribution maps of strain occurrence have been prepared for each country surveyed. BCMV isolates were collected from *Phaseolus vulgaris* bean crops and from wild species of legumes and other non-*Phaseolus* legume crops.

Section 1: An introduction to BCMV

The importance of the bean (*P. vulgaris*) crop in Africa is discussed together with the main diseases affecting beans in Africa. A review of the existing literature on BCMV is presented.

Section 2: The occurrence of BCMV in *P. vulgaris*

The methods of identifying BCMV strains are described and the details of the strains identified in Burundi, Ethiopia, Kenya, Lesotho, Malawi, Rwanda, Swaziland, Tanzania, Uganda, Zambia, Zaire, and Zimbabwe are discussed. Distribution maps of strain occurrence are given for each country and the pathogenicity details used for the identification of each isolate are shown in table form.

Section 3: Alternative hosts of BCMV in Africa

The occurrence and identification of BCMV strains in wild legume and other non-*Phaseolus* species is reported with details of the isolates found in Uganda, Rwanda, Malawi, Burundi and Kenya. The susceptibility of various wild legume species to laboratory infection by the standard strains (NL1, NL3, NL4, NL6 and NL8) of BCMV is described and wild legume isolates obtained from *Crotalaria incana*, *Rhynchosia* sp., *Macroptilium atropurpureum* and *Cassia occidentalis* were aphid-transmitted to their original host species and to *P. vulgaris* beans. The isolates were also transmitted in the seed of several of the wild legume species and bean.

Section 4: Occurrence and identification of other legume viruses

Although BCMV was the prevalent legume virus found in the survey, peanut mottle potyvirus was occasionally found in Uganda, Ethiopia and Kenya, and alfalfa mosaic virus was very common in bean crops in Morocco. Cucumber mosaic cucumovirus was found once in *P. lunatus* in Ethiopia and a new potyvirus, named cassia severe mosaic virus, is described from an isolate found in *Cassia occidentalis* in Ethiopia.

Section 5: Differentiation of BCMV strains by serology and physical characteristics

ELISA and Western blots, molecular weight of the coat protein and virus particle lengths have been used to demonstrate differences between BCMV strains. The standard strains of BCMV could be distinguished into 'A' or 'B' serological groups on the basis of their reactions with monoclonal antibodies and polyclonal antisera in ELISA and Western blots. Also, the 'A' and 'B' serotypes of the standard BCMV strains were distinguished according to the molecular weight of their coat protein and their mean particle length. The NL6 strain and some of the novel isolates found in the survey have characteristics that are intermediate between the 'A' and 'B' serotypes.

Section 6: General discussion and conclusions

The implication of the results of the survey are discussed. Strain identification, based on phenotype reactions of the differential host cultivars, has shown that the necrotic 'A' serotype strains of BCMV are predominant in central, eastern and southern Africa in both beans and wild species of legumes. Strain-types similar to the NL3 strain were prevalent in much of the area, but NL8 strain-types was also widespread in Burundi, Rwanda and Tanzania. Isolates belonging to the temperature-dependent NL6 strain-type, although not as prevalent as NL3, were also common in most of the countries surveyed. The widespread occurrence of the necrosis-inducing isolates of the NL3 and NL8 strain-type and other novel 'A' serotypes, emphasizes the importance of protecting new bean cultivar introductions against these potentially devastating necrotic strains. The exception appears to be Ethiopia, where NL3 and NL8 strain-types were absent.

The importance of the occurrence of BCMV in wild species of legumes in relation to the ecology, epidemiology and possible evolution of the necrotic 'A' serotypes in Africa is discussed, together with the significance and possible origins of the isolates found in the survey that had novel phenotype reactions in the differential host cultivars. It is proposed that two genes may be responsible for the necrotic reactions. The gene inducing the temperature-independent necrosis is designated *Px* and the gene inducing the temperature-dependent necrosis Px^2.

Appendix 1

Details of the methods used to collect and preserve the virus isolates found in the survey are described, together with details of the various biological, physical and serological methods used to identify and differentiate the BCMV strains. Information on the site location, original field symptoms, serological and differential host reactions of each isolate collected is presented in table form, together with recipes for the preparation of the various chemical solutions used in the study.

RÉSUMÉ

Ce bulletin passe en revue l'état actuel des connaissances en matière de recherches concernant le potyvirus mosaïque commun du haricot (BCMV) et les résultats d'une étude de l'occurence du BCMV dans les principales régions productrices de haricots en Afrique. La souche de chaque isolat BCMV a été identifiée grâce à ses réactions sur un ensemble normalisé de différents cultivars de haricots et il a été établi, pour chaque pays étudié, des cartes de répartition des occurences des souches. On a rassemblé des isolats de BCMV des cultures de haricots *Phaseolus vulgaris*, ainsi que des espèces sauvages de gousses (légumineuses) et cultures de gousses autres que *Phaseolus*.

Section 1: Présentation de BCMV

Il est examiné l'importance de la culture du haricot (*P. vulgaris*) en Afrique, ainsi que les principales maladies affectant les haricots en Afrique. Les ouvrages existants traitant du BCMV sont passés en revue.

Section 2: Occurence du BCMV dans *P. vulgaris*

Les méthodes permettant l'identification des souches de BCMV sont décrites tout autant que les détails des souches ayant été identifiées au Burundi, en Ethiopie, au Kenya, au Lesotho, au Malawi, au Rwanda, en Swaziland, en Tanzanie, en Ouganda, en Zambie au Zaïre et au Zimbabwe. Il est fourni pour chaque pays des cartes de répartition des occurences des souches et les détails de pathogénicité utilisés pour l'identification de chaque isolat sont indiqués sous forme de tableaux.

Section 3: Variantes d'hôtes du BCMV en Afrique

L'occurence et l'identification des souches de BCMV dans les légumineuses sauvages et espèces autres que *Phaseolus* sont signalées ainsi que les détails des isolats observés en Ouganda, au Rwanda, au Malawi, au Burundi et au Kenya. La susceptibilité de diverses espèces de légumineuses sauvages à l'infection en laboratoire par les souches standards (NL1, NL3, NL4, NL6 et NL8) de BCMV est décrite et les isolats de légumineuses sauvages obtenus des espèces *Crotalaria incana*, *Rhynchosia* sp., *Macroptilium atropurpureum* et *Cassia occidentalis* étaient transmis par les pucerons à leurs espèces hôtes d'origine et aux haricots *P. vulgaris*. Les isolats étaient aussi transmis dans la graine de plusieurs espèces de légumineuses sauvages et de haricot.

Section 4: Occurence et identification d'autres virus de légumineuses

Bien que BCMV constitue le principal virus de légumineuse observé dans l'étude, on a parfois observé des potyvirus tachetés de l'arachide en Ouganda, en Ethiopie et au Kenya, et le virus mosaïque de la luzerne était très fréquent dans les cultures de haricots au Maroc. Le cucumovirus mosaïque du concombre a été observé une fois sur *P. lunatus* en Ethiopie et un nouveau potyvirus, appelé virus mosaïque sévère de la casse est décrit provenant d'un isolat observé sur *Cassia occidentalis* en Ethiopie.

Section 5: Différentiation des souches de BCMV par les caractéristiques physiques et la sérologie

Il a été fait appel aux tests ELISA et Western blot, au poids moléculaire des anticorps globuliniques recouvrant les globules rouges et les longueurs des particules des virus pour faire la démonstration des différences entre les souches de BCMV. On peut distinguer les souches standards de BCMV en groupes sérologiques 'A' ou 'B' sur la base de leurs réactions aux anticorps monoclones et aux antisérums polyclones dans les tests ELISA et Western blot. Par ailleurs, on a distingué les sérotypes 'A' et 'B' selon le poids moléculaire de leurs anticorps globuliniques recouvrant les globules rouges et leur longueur moyenne de particules. La souche NL6 et quelques-uns des nouveaux isolats observés dans l'étude présentent des caractéristiques qui sont intermédiaires entre les sérotypes 'A' et 'B'.

Section 6: Discussion générale et conclusions

Les implications des résultats de l'étude sont examinées. L'identification des souches, basée sur les réactions phénotypes des différents cultivars hôtes, a montré que les souches sérotypes nécrotiques 'A' de BCMV sont prédominantes en Afrique centrale, de l'Est et du Sud, aussi bien dans les haricots que les espèces sauvages de légumineuses. Des types de souches similaires à la souche NL3 étaient répandus dans une grande partie de la région, toutefois les types de souches NL8 étaient aussi largement répandus au Burundi, au Rwanda et en Tanzanie. Bien que moins fréquents que NL3, des isolats appartenant au type de souche NL6 tributaires de la température, étaient aussi courants dans la plupart des pays étudiés. L'occurence générale des isolats provoquant la nécrose des types de souches NL3 et NL8 et autres nouveaux sérotypes 'A' fait ressortir l'importance de la protection des nouvelles introductions de cultivars de haricots contre ces souches nécrotiques dont le potentiel est ravageur. Il semble que l'exception soit l'Ethiopie, se caractérisant par l'absence des types de souches NL3 et NL8.

L'importance de l'occurence de BCMV dans les espèces sauvages de légumineuses, relativement à l'écologie, l'épidémiologie et l'évolution possible des sérotypes nécrotiques 'A' en Afrique est examinée, ainsi que l'importance et les origines possibles des isolats observés dans l'étude qui ont des réactions phénotypes nouvelles sur les divers cultivars hôtes. Le gène provoquant la nécrose autonome de la température est nommé *Px* et le gène provoquant la nécrose tributaire de la température est nommé Px^2.

Annexe 1

Il est décrit les méthodes mises en oeuvre pour rassembler et conserver les isolats de virus observés dans l'étude, ainsi que les détails des diverses méthodes biologiques, physiques et sérologiques employées afin d'assurer l'identification et la différentiation des souches BCMV. Il est présenté, sous forme de tableaux, des renseignements concernant l'implantation géographique des sites, les symptômes de terrain d'origine, ainsi que des formules pour la préparation des diverses solutions chimiques utilisées dans l'étude.

RESUMEN

Este boletín pasa revista a la situación actual de la labor de investigación sobre el potivirus mosaico común de la judía (BCMV) y presenta los resultados de un estudio sobre la presencia del BCMV en las principales zonas de Africa donde se cultiva la judía. Se ha llevado a cabo la identificación de la cepa de cada aislado de BCMV recogido por sus reacciones a una serie estándar de cultivares de judía diferenciales y se han preparado mapas de su distribución para cada país estudiado. Se recogieron aislados de BCMV de la judía *Phaseolus vulgaris* y de especies silvestres de legumbres y de otras leguminosas no pertenecientes a dicha familia.

Sección 1: Introducción al BCMV

Se examina la importancia del cultivo de la judía (*P. vulgaris*) en Africa, junto con un estudio de las principales enfermedades de la judía en Africa. También se pasa revista a la bibliografía actual sobre el BCMV.

Sección 2: Presencia del BCMV en *P. vulgaris*

Tras haberse descrito los métodos de identificación de las cepas del BCMV, se presenta información sobre las cepas identificadas en Burundi, Etiopía, Kenya, Lesotho, Malawi, Rwanda, Swazilandia, Tanzania, Uganda, Zambia, Zaire y Zimbabwe. También se presentan para cada país mapas de distribución de la cepa, junto con información tabulada sobre datos de patogenicidad utilizados para la identificación de cada aislado.

Sección 3: Huéspedes alternativos del BCMV en Africa

Se describe la presencia e identificación de cepas de BCMV en leguminosas silvestres y en otras especies no-*Phaseolus*, así como información sobre los aislados encontrados en Uganda, Rwanda, Malawi, Burundi y Kenya. En esta sección se presenta la susceptibilidad de diversas leguminosas silvestres a infección en laboratorio con cepas estándar (NL1, NL3, NL4, NL6 y NL8) de BCMV. También se llevó a cabo la obtención de aislados de las leguminosas silvestres *Crotalaria incana, Rhynchosia* sp., *Macroptilium atropurpureum* y *Cassia occidentalis*, que fueron transmitidos mediante áfidos a sus especies huésped originales y a las judías *P. vulgaris*. Los aislados fueron asimismo transmitidos a las semillas de diversas leguminosas silvestres y de judías.

Sección 4: Presencia e identificación de otros virus de las leguminosas

Si bien el BCMV fue el virus prevalente en las leguminosas durante el estudio, también se encontró ocasionalmente en Uganda, Etiopía y Kenya el potivirus de la mancha del cacahuete, mientras que, en Marruecos, el virus mosaico de la alfalfa se encontraba muy generalizado en las judías. En

una ocasión, se encontró en Etiopía el cucumovirus mosaico del pepino en *P. lunatus*. También se describe un nuevo potivirus—conocido con el nombre de virus mosaico grave de la *cassia*—de un aislado encontrado en la *Cassia occidentalis*, en Etiopía.

Sección 5: Diferenciación de las cepas de BCMV mediante serología y características físicas

Se han utilizado ELISA (ensayo inmunosorbente enzimático) y Western electroblots, peso molecular de la proteína del cápside y longitud de las partículas virales para demostrar las diferencias existentes entre distintas cepas de BCMV. Las cepas estándar pueden diferenciarse en grupos serológicos A y B sobre la base de sus reacciones con anticuerpos monoclonales y antisueros policlonales en ELISA y Western electroblots. También se llevó a cabo la distinción de serotipos 'A' y 'B' de las cepas de BCMV estándar, de acuerdo con el peso molecular de la proteína del cápside y longitud media de las partículas. La estirpe NL6 y algunos nuevos aislados encontrados en el estudio poseen características intermedias entre los serotipos 'A' y 'B'.

Sección 6: Discusión general y conclusiones

En esta sección se examinan las consecuencias de los resultados del estudio. La identificación de capas sobre la base de las reacciones fenotípicas de los cultivares huésped diferenciales ha puesto en claro que las cepas serotipo 'A' necróticas de BCMV predominan en las zonas central, oriental y meridional de Africa, tanto en la judía como en las leguminosas silvestres. Si bien cepas similares a la NL3 presentaron prevalencia en gran parte de esta zona, también se encontraron de manera generalizada en Burundi, Rwanda y Tanzania tipos de cepa similares al NL8. Aunque no con la prevalencia del NL3, fue común encontrar en todos los países estudiados aislados pertenecientes al tipo de cepa NL6 termodependiente. La presencia generalizada de aislados inductores de necrosis de los tipos de capa NL3 y NL8, junto con otros nuevos serotipos 'A', viene a subrayar la importancia de proteger cualquier nueva introducción de cultivares de judías contra estas cepas necróticas potencialmente devastadoras. La excepción parece ser Etiopía, en donde no se observó la presencia de NL3 o NL8.

El boletín estudia asimismo la importancia de la presencia del BCMV en leguminosas silvestres, en relación con la ecología, epidemiología y posible evolución de serotipos 'A' necróticos en Africa, junto con la importancia y posible origen de los aislados encontrados en el estudio con nuevas reacciones fenotípicas en los cultivares huéspedes diferenciales. Se ha sugerido la posibilidad de dos genes como causa de las reacciones necróticas. El gene inductor de la necrosis no termodependiente ha sido denominado *Px*, mientras que el gene inductor de la necrosis termodependiente ha recibido el nombre de Px^2.

Apéndice 1

Se proporcionan aquí datos sobre los métodos utilizados en la recolección y preservación de los aislados virales encontrados en el estudio, junto con información relativa a los diversos métodos biológicos, físicos y serológicos utilizados para identificar y diferenciar las cepas de BCMV. También se presenta, en forma tabular, información sobre la ubicación, síntomas originales sobre el terreno y reacciones serológicas y diferenciales del huésped para cada aislado recogido, junto con sugerencias para la preparación de las diversas soluciones químicas utilizadas en el estudio.

RESUMO

Este boletim faz uma revisão da posição atual do conhecimento obtido com a pesquisa sobre o potivirus do mosáico comum do feijão (BCMV) e relata os resultados de um levantamento da ocorrência do potivirus do mosáico nas principais áreas de cultivo de feijão na África. A raça de cada potivirus do mosáico isolado colhido foi identificada pelas suas reações sobre um conjunto padrão de cultivares diferentes sendo que foram preparados mapas de distribuição das raças para cada país investigado. Os virus BCMV isolados foram colhidos em culturas de feijão *Phaseolus vulgaris* e em espécies silvestres de legumes e outras culturas leguminosas não pertencentes ao gênero *Phaseolus*.

Seção 1: Introdução ao BCMV

A importância da cultura do feijão (*P. vulgaris*) na África é discutida junto com as principais doenças que afetam o feijão na Africa. Apresentamos uma revisão da literatura existente sobre BCMV.

Seção 2: Ocorrência da *P. vulgaris*

São descritos os métodos de identificação das raças BCMV e discutidos os pormenores das raças identificadas em Burundi, Etiópia, Quênia, Lesoto, Malawi, Ruanda, Suazilandia, Tanzania, Uganda, Zambia, Zaire e Zimbabue. São apresentados mapas da distribuição da ocorrência das raças por país e dados os pormenores patogênicos empregados na identificação de cada virus isolado em forma de quadros.

Seção 3: Hospedeiros alternativos do BCMV na África

A ocorrência e identificação das raças BCMV em legumes silvestres e outras espécies não *Phaseolus* está relatada com detalhes dos virus isolados encontrados na Uganda, Ruanda, Malawi, Burundi e Quênia. A suscetibilidade de várias espécies de legumes silvestres à infecção de laboratório pelas raças padrões (NL1, NL3, NL4, NL6 e NL8) do BCMV está descrita e os virus isolados de legumes silvestres obtidos da *Crotalaria incana*, *Rhynchosia* sp., *Macroptilium atropurpureum* e *Cassia occidentalis* foram transmitidos por afídeos às suas espécies hospedeiras originais e ao feijão *P. vulgaris*. Os virus isolados foram ainda transmitidos através da semente de diversas espécies de legumes silvestres e feijão.

Seção 4: Ocorrência e identificação de outros virus de legumes

Embora o BCMV fosse o virus predominante de legumes encontrado no levantamento, encontraram-se de vez em quando manchas do potivirus no amendoim na Uganda, Etiópia e Quênia e o virus do mosáico na alfafa era muito comum nas lavouras de feijão de Marrocos. Encontrou-se o cucumovirus do mosáico do pepino uma vez em *P. lunatus* na Etiópia e um novo potivirus denominado virus grave do mosáico da cássia é descrito através de um virus isolado encontrado na *Cássia occidentalis* na Etiópia.

Seção 5: Diferenciação das raças BCMV por serologia e características físicas

O ensaio ELISA e manchas descoloridas, peso molecular da camada de proteína e comprimentos das partículas do virus foram usados para demonstrar as diferenças entre as raças BCMV. As raças padrões de BCMV podem ser distinguidas em grupos serológicos 'A' ou 'B' na base das suas reações com anticorpos monoclonais e antisoros policlonais no ensaio ELISA e manchas descoloridas. Também os serótipos 'A' e 'B' das raças padrões BCMV foram distinguidos de acordo com o peso molecular da sua camada de proteína e do comprimento da sua partícula principal. A raça NL6 e alguns dos novos virus isolados encontrados no levantamento têm características que são intermediárias entre os serótipos 'A' e 'B'.

Seção 6: Discussão geral e conclusões

São discutidos as implicações dos resultados do levantamento. A identificação das raças baseada nas reações fenótipos das diferentes cultivares hospedeiras tem demonstrado que as raças serótipos necróticas 'A' de BCMV predominam no África central, oriental e do sul tanto no feijão como nas espécies silvestres de legumes. Os tipos de raças similares à raça NL3 predominavam na maior parte da região mas os tipos NL8 também disseminavam em Burundi, Ruanda e Tanzânia. Os virus isolados pertencentes ao tipo NL6 que é dependente da temperatura, embora não fossem tão predominantes como os NL3 eram também comuns na maioria dos países investigados. A disseminação da ocorrência dos virus isolados que induzem a necroses do tipo NL3 e NL8 e outros novos serótipos 'A', enfatizam a importância de se proteger a introdução de novas cultivares de feijão contra essas raças necróticas potencialmente devastadoras. A exceção parece ser a Etiópia onde os tipos NL3 e NL8 estavam ausentes.

A importância da ocorrência do BCMV em espécies silvestres de legumes em relação à ecologia, epidemiologia e provável evolução dos serótipos 'A' necróticos na África á discutido junto com a significância e prováveis origens dos virus isolados encontrados no levantamento que tiveram novas reações fenótipos nas diferentes cultivares hospedeiras. A opinião é que dois genes possam ser responsáveis pelas reações necróticas. O genes que induzem à necrose independente da temperatura é designado por *Px* e o genes que induzem à necrose dependente da temperatura Px^2.

Apêndice 1

Os pormenores dos métodos usados para colher e preservar os virus isolados encontrados no levantamento estão descritos juntamente com os pormenores dos vários métodos biológicos, físicos e serológicos usados para identificarem e diferenciarem as raças BCMV. A informação sobre o local, sintomas de campo originais, reações dos hospedeiros diferentes e serológicos de cada virus isolado colhido está apresentada em forma de quadro, junto com receitas para o preparo de várias soluções químicas usadas no estudo.

Section 1

Introduction

BEANS (*PHASEOLUS VULGARIS*) IN AFRICA

Bean production and cultivation

Legumes are grown throughout the world as a source of food for humans and animals. The estimated production of dry beans (*Phaseolus vulgaris*) worldwide in 1990 was over 16 million tonnes. Soybeans are the only legume crop to exceed this level of production. Beans originated in Latin America and were introduced to the rest of the world by traders. West European traders introduced beans to Africa, and currently Africa is the third largest producer after Latin America and North America, respectively (FAO, 1990).

Twenty-four African countries are reported to produce beans, and seven (Angola, Burundi, Cameroun, Rwanda, Tanzania, Uganda and Zaire) devote large areas to the crop (Adams *et al.*, 1985). Total bean production in Africa has increased over the last decade with over 1.7 million tonnes produced in 1990. However, this increase has largely been achieved by an expansion in the production area rather than through greater yields. It has also been observed that in Africa a large part of the total bean production is consumed locally which is not reflected in published FAO estimates (Kirkby, 1987). The average bean production in Africa is approximately 660 kg/ha, although it may be as low as 214 kg/ha, (Lesotho). This compares with average yields of 1500 kg/ha in North America and 2–3000 kg/ha in parts of Europe (FAO, 1990).

In Africa, beans are normally cultivated on small farms using traditional methods under rainfed conditions (Kirkby, 1987) (Plate 1). Most beans are grown in cropping systems with maize, sorghum or bananas (Plate 2). In parts of eastern Africa beans are often grown as traditional mixtures, where cultivars present in the mixture are selected for taste, seed colour, speed of cooking and disease resistance. Bean production is limited by low soil fertility, periodic water deficits, pests and diseases.

Beans are very important as a source of dietary protein in eastern Africa. The highest world percentage of total protein provided by beans to the human diet is in Rwanda and Burundi (45%). In Kenya and Uganda the comparable figure is over 10% (Kirkby, 1987). Because beans provide a cheap form of protein, they are particularly important to low-income families.

Diseases of beans in Africa

The most important fungal diseases of beans are rust (*Uromyces appendiculatus*) (Plate 3), anthracnose (*Colletotrichum lindemuthianum*) (Plate 4), ascochyta (*Phoma exigua* var. *diversispora*) (Plate 5) and angular leaf spot (*Phaeoisariopsis griseola*) (Plate 6). Bacterial diseases include common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*) (Plate 7) and halo blight (*Pseudomonas syringae* pv. *phaseolicola*) (Plate 8) (Thurston, 1984; Allen, 1987). Bean common mosaic virus (BCMV) is by far the most important virus disease of beans in Africa,

although other viruses have also been isolated from beans in Africa. They include bean yellow mosaic potyvirus, cucumber mosaic cucumovirus, peanut mottle potyvirus, cowpea mild mottle carlavirus and blackeye cowpea mosaic potyvirus (Vetten and Allen, 1991).

BEAN COMMON MOSAIC VIRUS

Geographical distribution and economic importance

BCMV is worldwide in its distribution, occurring wherever *Phaseolus* beans are grown and is economically important throughout Africa, Europe, North, Central and South America. The virus can cause severe crop losses, although the disease is often sporadic in its occurrence and its severity is dependent on the prevalence of its aphid vector. BCMV may be the limiting factor controlling bean yields in some areas and infection levels may reach 100% with estimated yield losses ranging from 35–98% (Galvez, 1980). In bean crops in Oregon yield reductions due to BCMV were calculated as 53–68% depending on severity (Hampton, 1975). In 1972 and 1974 there were severe outbreaks of BCMV in Morocco in which 50% of all bean plantings exhibited symptoms. This disease outbreak started from a seed-borne infection and was spread by aphid transmission (Lockhart and Fischer, 1974). Yield losses were estimated at 50% and 34% of the harvested seed was infected as a result of the epidemic.

In tropical bean-growing areas such as Africa, the occurrence of BCMV is less frequent at high altitudes (about 2500 m) near the upper limit of bean cultivation, than at lower altitudes (N.J. Spence and D.G.A. Walkey, unpublished data). The low level or absence of the virus in such crops is probably associated with the infrequent occurrence of the aphid vectors at high altitude. When occasional plants infected with BCMV do occur, they probably originate from infected seed.

Symptoms of BCMV

BCMV causes two types of symptom in *P. vulgaris*, common mosaic and black root. The type of symptom is determined by the strain of BCMV, temperature and the host genotype. Symptoms associated with common mosaic include: leaf rolling or blistering, light and dark green patches on the leaf (green mosaic), chlorotic vein banding, yellow mosaic and growth reduction (Plates 9 and 10). Mottling and malformation of the primary leaves is an indication that the primary infection occurred through seed (Galvez, 1980). Cultivars which develop common mosaic may have distinct chlorotic or necrotic local lesions which do not extend into the vascular system. In contrast, the black root symptom is characterized by local necrotic lesions which extend into the veins causing a systemic necrosis in the vascular system. Blackroot only occurs in cultivars possessing a dominant resistance gene, referred to as *I* (Plates 11 and 12) (see page 86). This necrosis can extend into the roots, stem and meristem and may result in plant death if the plant is infected at an early stage. If infection occurs later in development, the plant may survive but some tissues or organs may die. Pods may also become discoloured and become unmarketable as a consequence (Drijfhout, 1978; Morales and Bos, 1988).

Physical and chemical characteristics of the virus

BCMV is a member of the potato virus Y (potyvirus) group. This is the largest group of plant viruses and collectively causes the greatest agricultural losses of any virus group (Zettler, 1990). Potyviruses have a worldwide distribution, but are particularly prevalent in tropical and sub-tropical countries. The particles are

typically flexuous rod-shaped filaments 720–900 nm long and about 11 nm wide and thin sections viewed in the electron microscope show that the cytoplasm of infected plant cells develops characteristic inclusion bodies. The serological relationships between potyviruses are complex, most being related to at least one if not several other members of the group (Hollings and Brunt, 1981).

BCMV has flexuous rod-shaped particles 720–770 nm long and 12–15 nm wide (Plate 13a). The particles are composed of 95% protein, usually of one main polypeptide species of mol. wt 32–35 000 Da (as determined by electrophoresis). A component of mol. wt 29 000 Da may also be found in virus preparations which have undergone limited proteolysis. The remaining 5% of the particle is made up of single-stranded RNA, mol. wt 3 500 000 Da. The particles form a single sedimenting and buoyant density component and have a sedimentation coefficient ($s_{20,w}$) of 154–158 S (measured in US1 and US5 strains) and a buoyant density in caesium chloride of 1.31–1.32 g/cm^3. The absorption coeffecient (A_{260}/A_{280}) is 1.12–1.27 (uncorrected for light scattering), depending on the strain (Morales and Bos, 1988). The extinction coefficient (the absorbance of a 1 mg/ml preparation in a 1 cm light path at 260 nm) of potyviruses is 2.4–2.9 (Hollings and Brunt, 1981). Plant cells infected with BCMV develop characteristic cytoplasmic inclusion bodies of the pinwheel-associated scroll type, typical of the Group 1 potyviruses described by Edwardson and Christie (1978) (Plate 13b).

The stability of BCMV in sap is dependent on the strain, source of virus and other physical and environmental conditions. Thermal inactivation point ranges from 50–60°C, dilution end point is between 10^{-3} and 10^{-4}, and the virus normally retains its infectivity in sap for 1–4 days at room temperature.

Host range of BCMV

Natural hosts of BCMV are mainly restricted to *Phaseolus* spp., especially *P. vulgaris* (Drijfhout, 1978). However, BCMV has been isolated naturally from other leguminous hosts. Zaumeyer and Thomas (1957) described the isolation of BCMV from *Vigna unguiculata* (cowpea) and Kaiser and Mossahebi (1974) isolated a strain of BCMV from *Vigna radiata* (mungbean). Singh and Singh (1977) isolated a strain of BCMV from *Crotalaria juncea* (sunhemp) in India and Sarkar and Kulshreshtha (1978) reported the isolation of BCMV from *Crotalaria striata*. Meiners *et al.* (1978) isolated BCMV from the leguminous weed *Rhynchosia minima* in Colombia. BCMV has also been isolated from *Lupinus luteus* (yellow lupin) in Poland, where it was also shown to be seed-transmitted in this host (Frencel and Pospieszny, 1979).

There are many reports of the isolation of potyviruses which resemble BCMV or have a very close serological relationship with it, from various hosts. Sharare and Raychaudhuri (1963) described a virus infecting *Vigna mungo* (Urdbean) which was similar to BCMV. Potyviruses with a close serological relationship to BCMV have been isolated from mungbean and soybean (Green *et al.*, 1986, 1988). The occurrence of BCMV-like viruses in alternative hosts to bean could have important implications in the epidemiology of the virus (see Section 6 p.91).

BCMV may also be artificially inoculated to a number of experimental host plants. The botanical range of hosts is fairly narrow compared with other potyviruses such as BYMV, and consists mainly of legumes. These experimental leguminous hosts (and non-hosts) are described by Galvez (1980); Boswell and Gibbs (1983) and Morales and Bos (1988). Non-leguminous hosts include *Chenopodium album* var. *centrorubrum*, *C. quinoa* and *C. amaranticolour* which develop local lesions only (Horvath, 1986) and *Nicotiana clevelandii*, which develops systemic infection (Drijfhout and Bos, 1977). Christie and Crawford (1978) infected *N. benthamiana* with the ATCC strain pv25 of BCMV.

Transmission and epidemiology of BCMV

Seed transmission

Seed is usually the primary source of BCMV inoculum and is probably the most important factor determining initial levels of infection in crops (Morales and Bos, 1988). Seed transmission of BCMV was first demonstrated in bean by Reddick and Stewart (1919) and the virus has also been shown to be transmitted in pollen by Reddick (1931). The virus is located mainly in the embryo (Quantz, 1961; Provvidenti and Cobb, 1975) although virus particles can be detected in pollen grains and ovules of infected plants (Galvez, 1980).

The level of seed transmission varies according to the cultivar and virus strain used. The necrosis-inducing strain NL3 (see p.12 and 16) has low transmissibility in most cultivar groups but in the navy bean cvs Michelite and Sanilac, which are members of host group 4 (Table 2), the NL3 strain is highly seed-transmissible.

Maximum seed transmission is achieved when plants are inoculated at the primary leaf stage and few cultivars will transmit virus in seed when plants are inoculated 30 days after sowing (Morales and Castaño, 1987). Seed transmission is also much reduced when plants become infected after flowering (Nelson, 1922; Schippers, 1963). Morales and Castaño (1987) suggested that the differences observed in seed transmission rates may be due to a mechanisn providing resistance to seed transmission in genotypes that exhibit a characteristic mosaic symptom. BCMV particles are reported to remain viable in bean seed for 30 years (Zaumeyer and Thomas, 1957).

There are reports of BCMV being transmitted in the seed of several other *Phaseolus* species. Chamberlain (1939) demonstrated transmission in *P. coccineus* (scarlet runner bean) and Provvidenti and Cobb (1975) observed transmission in *P. acutifolium* var. *lactifolius* (tepary bean). BCMV was shown to be transmitted in the seed of a number of other *Phaseolus* species from the USDA (United States Department of Agriculture) collection (Klein *et al.*, 1988). BCMV has also been transmitted in the seed of some accessions of *Macroptilium lathyroides* (phasemy bean) (Provvidenti and Bravermen, 1976) and *Vigna mungo* (Agarwal *et al.*, 1979a, b).

Vector transmission

Aphid vectors provide the means of introducing BCMV to a healthy crop or of spreading virus introduced in the seed. The virus is transmitted in a non-persistent manner by several aphid species which normally do not colonize *P. vulgaris* but transmit BCMV as winged migrants. *Acyrthosiphon pisum, Aphis fabae* and *Myzus persicae* are important vectors (Kennedy *et al.*, 1962; Zettler and Wilkinson, 1966), but others include: *Aphis gossypii, A. medicaginis, A. rumicus, Hyalopterus atriplicis, Macrosiphum ambrosiae, M. pisi* and *M. solanifolii* (Zaumeyer and Thomas, 1957).

Zettler and Wilkinson (1966) investigated the pre- and post-feeding behaviour of *Myzus persicae* and found it determined the efficiency of transmission of BCMV. Zettler (1969) demonstrated that the availability of virus to aphids was associated with symptom expression and chlorotic areas were better sources of virus for transmission. He also showed that leaves formed soon after inoculation were better sources of virus than older leaves. In Colombia, large apterous aphid populations were found to result in 100% secondary spread when only 15–25% of seed were infected (CIAT, 1973; 1974). Experiments in India reported by Yash Gupta and Chowfla (1988) demonstrated that younger bean plants were more susceptible than older ones to BCMV transmitted by *Myzus persicae* and that nymphs and apterous aphids were more efficient vectors than winged adults. Sohati *et al.* (1992) observed that, in Zambia, *A. fabae* occurred commonly on

beans but did not infest cowpea, pigeonpea, groundnut or bambara groundnut even if beans were in their vicinity. Winged adults of *Toxoptera citricidus*, *Brevicoryne brassicae* and *Tetraneura nigriabdominalis* were occasionally found in bean crops but their ability to transmit BCMV has not been demonstrated.

The distribution of BCMV and history of strain identification

History of BCMV strain identification

BCMV was first described in the United States (Stewart and Reddick, 1917) and was called bean mosaic virus, although a mosaic disease of beans had previously been described in the Soviet Union (Iwanowski, 1899). In a review of viruses of beans in 1934, Pierce (1934a) called the common bean mosaic virus described by Stewart and Reddick (1917) bean virus 1, in order to distinguish it from bean yellow mosaic virus which he called bean virus 2. By this time several other viruses had been isolated from bean and found to be distinct from BCMV, they included tobacco ring-spot nepovirus (Wingard, 1928), tobacco mosaic tobamovirus (Price, 1930) and alfalfa mosaic virus (Weimer, 1931).

Jenkins (1940, 1941) described a new virus of snap beans called 'black root' which caused vascular necrosis. Grogan and Walker (1948) proved that BCMV and the new virus were the same, however, the black root symptom was found to occur only in cultivars with resistance derived from cv Corbett Refugee. Three strains of BCMV (the common mosaic, greasy pod and Burkholder's strains) and one of BYMV were used to inoculate beans of various cultivars by sap and approach-graft inoculation. It was found that necrotic symptoms were never induced by BYMV but could be induced by all three strains of BCMV in certain cultivars. The necrotic symptoms caused by the first two BCMV strains were not dependent on temperature, but necrosis caused by the Burkholder strain was temperature-dependent.

The first strain of BCMV to be described by Stewart and Reddick (1917) came to be known as the Type strain. Richards and Burkholder (1943) described a variant strain which was isolated from the cvs Michelite and Robust which were resistant to the Type strain. This variant was called 'New York' 15 (NY15) as it was isolated in New York State. GN UI 1 (the U.S.A. Great Northern cultivar) was susceptible to NY15, but resistance to this strain was found in cvs GN UI 81 and GN UI 59. Dean and Hungerford (1946) found NY15 in Idaho where cv Red Mexican (RM) 24 was susceptible, but cvs GN UI 56, GN UI 81 and GN UI 123 were resistant. Zaumeyer and Thomas (1947) described the 'shiny or greasy pod' strain of BCMV, but later Zaumeyer and Thomas (1948) concluded that it was not a new variant because cultivars susceptible to this isolate were also susceptible to the Type strain.

Van der Want (1954) described the 'Westlandia' strain from Holland, isolated from the bean cultivar of that name, cvs Beka and Double White (Dubelle Witte) were susceptible and this strain was later renamed NL1 for convenience (Drijfhout, 1978). Dean and Wilson (1959) described a strain from Idaho and compared its pathogenicity on different cultivars with the Type and NY15 strains. They found cvs GN UI 31 and GN UI 123 to be susceptible to this strain which they called the 'Idaho' strain. The cv Improved Tendergreen (with the dominant resistance referred to as the *I* gene, see pages 8 and 16) developed symptoms when infected with the Type and 'Idaho' strains, but was resistant to the NY15 strain. Later the 'Idaho' strain was designated US3 (Drijfhout, 1978). Zaumeyer and Goth (1962, 1964) described the 'Florida' strain ('FLA'), which produced more severe symptoms than any other strain previously described. Cultivars Pinto 111, Sanilac, RM 34, GN 123 and GN UI 31 were resistant to 'FLA', but cv Stringless Green Refugee was susceptible. Unlike the Type and NY 15 strains, the 'FLA' strain did not cause local necrosis in cv Top Crop at 32 °C.

Hubbeling (1963) described three new strains from crops in Holland: 'Imuna', 'Michelite' and 'Great Northern', these were isolated from cvs: Imuna, Michelite and GN 123 respectively and could be differentiated from the NL1 strain which only produced symptoms in cv Double White. The 'Imuna' and 'Michelite' strains could induce symptoms in cvs Imuna and Michelite but not in cv GN UI 123, which was, however, susceptible to the 'Great Northern' strain. These three cultivars could be used to differentiate the three BCMV strains. In addition, the 'Michelite' strain produced systemic necrosis in cv Widusa and other cultivars carrying the dominant *I* gene for resistance at a temperature of about 20 °C. The 'Imuna' strain could only induce systemic necrosis in cultivars carrying the dominant *I* gene at higher temperatures (> 30 °C). The 'Michelite' strain was the first example of a temperature-insensitive strain because it induced necrosis at 20 °C, strains which only induced necrosis at temperatures above 30 °C were accordingly described as temperature-sensitive. Later the strains were renamed NL2 ('Imuna'), NL3 ('Michelite') and NL4 ('Great Northern') (Drijfhout, 1978).

A strain isolated in Costa Rica by Moreno *et al.* (1968) was described as new but no difference in pathogenicity to the Type and 'FLA' strains was demonstrated. Silbernagel (1969) isolated a strain from a Mexican accession which was similar to the Type and NY 15 strains in inducing necrosis in cv Topcrop at 32 °C, but which failed to produce symptoms in cv Improved Tendergreen. He called it the 'Mexican' strain and it is now known as US6 (Drijfhout, 1978). Hubbeling

Table 1 Gene-for-gene relationship between host resistance groups of *Phaseolus vulgaris* and pathogenicity groups of BCMV (Drijfhout, 1978)

	Pathogenicity genes	Pathogenicity group of the virus									
		I	II	III	IVa	IVb	Va	Vb	VIa	VIb	VII
	P0	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
	P1		*P1*		*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*
	$P1^2$				$P1^2$	$P1^2$			$P1^2$	$P1^2$	$P1^2$
	P2			*P2*			*P2*	*P2*	*P2*	*P2*	
	$P2^2$										$P2^2$
Host group	Resistance genes	Reaction of virus on host group									
1	(*bc*-u)	+	+	+	+	+	+	+	+	+	+
2	*bc*-u *bc*-1	-	+t	-	+	+	+t	+	+t	+	+
3	*bc*-u $bc\text{-}1^2$	-	-	-	+	+	-	-	+t	+t	+
4	*bc*-u *bc*-2	-	-	+	-	-	+	+	+	+	-
5	*bc*-u *bc*-1 *bc*-2	-	-	-	-	-	+	+	+	+	-
6	*bc*-u $bc\text{-}1^2$ $bc\text{-}2^2$	-	-	-	-	-	-	-	-	-	+
7	*bc*-u *bc*-2 *bc*-3	-	-	-	-	-	-	-	-	-	-
8	(*bc*-u) *I*	-	-	N	-	n	-	-	N	N	-
9a	(*bc*-u) *bc*-1 *I*	-	-	-	-	N	-	n	N	N	-
9b	(*bc*-u) *bc*-1 *I*	-	-	-	-	n	-	n	N	N	-
10	(*bc*-u) $bc\text{-}1^2$ *I*	-	-	-	-	-	-	-	-	N	-
11	(*bc*-u) $bc\text{-}1^2bc\text{-}2^2$ *I*	-	-	-	-	-	-	-	-	-	-

Notes:

+	host susceptible to systemic infection
-	host resistant to systemic infection
+t	symptomless host but systemic infection detected by ELISA
N	systemic necrosis at 26 °C and 32 °C
n	systemic necrosis at 32 °C, but not at 26 °C
(*bc*-u)	in parenthesis indicates gene may or may not be present in different cultivars

(1972) described a further two strains isolated from the cvs Jolanda and Colana. The 'Jolanda' strain gave rapid necrosis in the same cultivars as the 'Michelite' strain (NL3) at 20 °C except that it induced systemic necrosis in the cv Jubila in which the 'Michelite' strain induced only local necrosis. The 'Colana' strain induced necrosis at a lower temperature (20 °C) but did not induce symptoms in cvs Michelite and Sanilac. The 'Jolanda' and 'Colana' strains were later designated NL5 and NL6 respectively (Drijfhout, 1978). Drijfhout and Bos (1977) recorded two further strains in the Netherlands which they called NL7 and NL8. The cvs Michelite and Sanilac were resistant to NL7 but susceptible to NL8. The NL8 strain induced systemic necrosis in some cultivars at 20 °C but only local necrosis in others, in contrast the NL7 strain did not induce necrosis in any cultivars. These strains make up the 10 pathogenicity groups of BCMV as described by Drijfhout (1978) (Tables 1 and 2). More recently, strains have been described which have small differences from existing strains in their pathogenicity. For example, Silbernagel *et al.* (1986) described a seedborne isolate from Tanzania which was similar serologically and in pathogenicity to the severe

Table 2 Differentiation and grouping of BCMV strains (Drijfhout, 1978)

Differential hosts		Pathogenicity group of the virus									
Group	Cultivar	I NL1 US1	II NL7	III NL8	IVa US5 FLA NVRS	IVb NL6 US3 US4	Va US2 NY15	Vb NL2	VIa NL3	VIb NL5	VII NL4 US6
1	Double White The Prince Stringless Green Refugee Common Red Mexican	+	+	+	+	+	+	+	+	+	+
2	Redlands Greenleaf C Puregold wax Imuna	-	+t	-	+	+	+t	+	+t	+	+
3	Redlands Greenleaf B GN UI 59 GN UI 123	-	-	-	+	+	-	-	+t	+t	+
4	Michelite Sanilac Pinto III Red Mexican 34	-	-	+	-	-	+	+	+	+	-
5	Pinto 114	-	-	-	-	-	+	+	+	+	-
6	Monroe Red Mexican 35 GN UI 31	-	-	-	-	-	-	-	-	-	+
7	IVT 7214	-	-	-	-	-	-	-	-	-	-
8	Black Turtle Soup Widusa	-	-	N	-	n	-	-	N	N	-
9a	Jubila	-	-	-	-	N	-	n	N	N	-
9b	Improved Tendergreen Topcrop	-	-	-	-	n	-	n	N	N	-
10	Amanda	-	-	-	-	-	-	-	-	N	-
11	IVT 7233	-	-	-	-	-	-	-	-	-	-

Notes:
+ host susceptible to systemic infection
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, but not at 26 °C

necrotic strain (NL3) found in Europe and Michigan. This isolate differed from the NL3 strain, however, in being more pathogenic to group 2 cultivars.

Re-classification of strains of BCMV

Results have recently been published which suggested the need for radical changes in the classification of BCMV strains. Vetten *et al.* (1992) described differences between necrotic and non-necrotic strains of BCMV in terms of: host symptoms, serology, virus particle length, molecular weight of coat proteins and cytology. These differences, together with differences in the coat protein sequences at the N-terminus between NL8 (necrotic) and NL4 (non-necrotic), led them to suggest that BCMV should be re-classified into two distinct potyviruses. They concluded that the necrotic strains should be classified as strains of a distinct virus for which they suggest the name bean necrosis mosaic virus (BNMV) and that the non-necrotic strains should remain classified as BCMV. McKern *et al.* (1992a) published the results of high-performance liquid chromatographic peptide profiles of coat protein digests of 22 BCMV strains and strains of blackeye cowpea mosaic virus (BLCMV) and peanut stripe potyvirus (PnStV). The peptide profiles of the BCMV strains also indicated two distinct potyviruses with the necrotic strains: NL3, NL5, NL8 and TN-1 having 90% homology in one group (the 'A' group) and the remaining (non-necrotic) BCMV strains in another group (the 'B' group) sharing between 60 and 80% homology. The non-necrotic strains NL4 and NL6 had only 35–40% homology with the necrotic strains. Peptide profile data indicated that BLCMV, PnStV, azuki bean mosaic potyvirus (AZMV) and three potyvirus isolates from soybean, described by McKern *et al.* (1992b) were strains of the same virus and should be considered strains of the 'B' group of BCMV. On this basis they proposed that BCMV should be re-classified as two distinct viruses, as suggested by Vetten *et al.* (1992), with the 'A' group of strains being called BNMV and the 'B' group of BCMV strains, together with BLCMV, PStMV, AZMV and the three potyvirus isolates from soybean, being called BCMV.

The current guidelines for the use of peptide profiling for potyvirus classification are that strains of the same virus should have homology of more than 90% whereas distinct viruses may have a homology of between 35–75% (Frenckel *et al.*, 1989; Shukla and Ward, 1989); the data for the new BCMV classification falls within these guidelines. These findings will be discussed at the International Working Group on Legume Viruses, July, 1993 and recommendations will then be forwarded for consideration to the International Committee on the Taxonomy of Viruses (ICTV) through the ICTV Plant Virus Subcommittee. Until the new system of classification has been agreed and for the purposes of this bulletin all strains of BCMV, necrotic and non-necrotic, are still referred to as members of the bean common mosaic virus group, and BLCMV, PnStV, and AZMV are each referred to as distinct viruses. However, the proposed new system of classification will be discussed later in relation to the results of this study (see Section 6).

Distribution of BCMV strains

Synonyms of BCMV include bean mosaic virus, common bean mosaic virus, bean virus 1 and *Phaseolus* virus 1 (Martyn, 1968). BCMV is found throughout the World wherever beans are grown. In the USA BCMV was thought to be of minor importance after the introduction of seed certification and cultivars carrying the *I* gene (Provvidenti, 1990). However, in recent years, severe epidemics of necrotic strains have occurred in the north-western area of the USA. In Michigan, only the NY15 and Type strains were present until 1982, when a severe necrotic strain was found (Kelly *et al.*, 1982). Further investigation revealed that it resembled the NL3 strain (Kelly *et al.*, 1984). Strain NL8 has been

isolated from severely affected bean crops of the cv Sanilac in Western New York (Provvidenti *et al.*, 1984), Idaho (Myers *et al.*, 1990) and Southwestern Ontario (Tu, 1986). In each case, the strains were identified using differential host cultivars.

Schmidt *et al.* (1987) reported a survey of BCMV in bean crops in the then German Democratic Republic (GDR), Hungary and the USSR. Isolates were identified serologically as BCMV and differential host cultivars were used to identify the strains. Strains belonging to all 10 known pathogenicity groups (see Tables 1 and 2) were found in GDR and USSR and strains belonging to groups III, IV, VI and VII were found in Hungary. A survey of snap beans in Bulgaria (Kostova and Poryazov, 1989) indicated the presence of group VIa isolates, similar to NL3 which were identified using differential host cultivars. Mosaic and necrosis-inducing strains of BCMV have been isolated in Turkey (V. Lisa, 1989, personal communication), but the strains have not yet been characterized.

BCMV occurs in Latin America but necrotic strains are not common (F. Morales, 1990, personal communication). In Peru, Mattos and Fernandez-Northcote (1987) identified four isolates of BCMV using differential host cultivars. The strains were similar to pathogenicity group VI, but they produced slightly different reactions to those previously reported and were therefore designated VIc. In Brazil, Trinidade *et al.* (1987) obtained 16 BCMV isolates from seed which were identified using differential host cultivars as belonging to pathogenicity groups I, II and IV.

Information about the distribution of BCMV in Africa is incomplete. Buruchara and Gathuru (1979) isolated a severe strain of BCMV from cv Canadian Wonder in Kenya. The strain was designated K-BCMV and was shown to be similar but not identical to a known strain (NL3), according to the reactions of differential host cultivars (Drijfhout, 1978). However, the isolate caused mosaic symptoms in cultivars carrying the dominant *I* gene which is atypical. A survey of beans in Kenya (Omunyin, 1980) identified BCMV isolates with similar pathogenicity on differential host cultivars as NL3 and NL8. There were, however, differences in the reactions to the isolates of cultivars carrying the *I* gene. Bock *et al.* (1980) isolated BCMV from beans in several important bean-growing areas in Kenya: Thika, Naivasha, Kakamega and Muguga. The isolates were tested on the range of cultivars used by Drijifhout (1978) to differentiate strains and were found to induce reactions similar to NL3. They also isolated BCMV from *Vigna mungo* and *P. acutifolius*. Silbernagel *et al.* (1986) described a BCMV strain from Tanzania (TN-1) which was pathogenically and serologically similar to the severe necrotic strain (NL3) found in Europe and Michigan. Vetten and Allen (1991) and Mink (1988, personal communication) have conducted surveys of virus isolates from beans in various African countries identifying isolates mainly by serology. Their results indicated that temperature-insensitive, necrosis-inducing strains (NL3 and NL8) were widespread throughout Africa, except in Ethiopia. In Morocco, the first report of a serious outbreak of BCMV was by Lockhart and Fischer (1974). Edington and Whitlock (1988) identified isolates of BCMV from Transvaal and Natal as group V strains using differential host cultivars.

Resistance of beans to BCMV

History of resistance to BCMV in beans

The first bean cultivar with BCMV resistance was developed by Spragg in Michigan in 1915 (Spragg and Down, 1921), the cultivar was called Robust and was a white pea-bean variety. Pierce and Hungerford introduced the resistant cv Great Northern UI 1 in 1929 (Pierce, 1934b). This cultivar was developed from a

plant with BCMV resistance selected from a population of the susceptible cv Common Great Northern. In 1934, Wisconsin Refugee and Idaho Refugee were introduced as BCMV-resistant snap bean cultivars. Their resistance was derived from cv Corbett Refugee, a variety developed in 1931 by Ralph Corbett which was the progeny of a resistant plant found in a field of otherwise BCMV-infected plants of the cv Stringless Green Refugee (Pierce and Walker, 1933). Other resistant cultivars derived from Corbett Refugee which followed included cvs Sensation Refugee 1066, Sensation Refugee 1017 and U.S. no. 5 Refugee. These cultivars were resistant only to the Type strain of BCMV.

Pierce (1935) showed that resistance derived from Corbett Refugee was distinct from resistance derived from Robust or GN UI 1, this was the first indication that different genes for resistance existed. Results from crosses showed that resistance derived from Corbett Refugee was dominant, whilst that derived from Robust and GN UI 1 was recessive. Parker (1936) performed crosses between Stringless Green Refugee and Robust, the results of which suggested a cytoplasmic influence on the inheritance of resistance. Wade and Andrus (1941) crossed a resistant cultivar with cv US 5 Refugee, and their results indicated that resistance in cv Refugee was controlled by a single dominant gene. Approach-graft experiments (Grogan and Walker, 1948) showed that cultivars with resistance derived from Corbett Refugee could develop systemic necrosis and die when inoculated with BCMV, but this necrosis only developed at elevated temperatures (>30 °C) and never occurred in cultivars with Robust resistance.

Ali (1950) used Grogan and Walker's approach-graft system to determine the genetic basis of BCMV resistance. He concluded that cultivars with Robust resistance were immune to the Type strain because of a recessive resistance gene designated *a*, while derivatives of Corbett Refugee possessed a dominant inhibitor gene designated *I* which conferred a hypersensitive resistance and prevented the development of the mosaic symptom. The *I* gene was temperature-sensitive as the symptom was localized at <30 °C but systemic at >30 °C. Ali postulated that a gene designated *A* was necessary for virus infection, rendering plant tissues susceptible, but in the presence of the *I* gene the *A* gene was ineffective and the development of mosaic was inhibited.

However, necrosis can develop in a genotype with the *I* and the *A* genes. Ali suggested that the *I* and *A* gene interact to cause a response in the plant which results in a hypersensitive reaction that causes cell necrosis which inhibits further virus spread. In the field, cultivars with the *I* gene would have 'field resistance' as the transmission of BCMV by aphids would only result in the introduction of small amounts of virus. This would induce a small area of necrosis, inhibiting further spread of the virus and the plant would not be severely affected.

Ali suggested that cv Stringless Green Refugee had the genotype *AAii* (fully susceptible), cv Corbett Refugee had the genotype *AAII* (necrotic but resistant to mosaic) and cv Robust had the genotype *aaii* (resistant to Type strain). It was suggested that the theoretical genotype *aaII* would develop neither mosaic nor necrosis. Peterson (1958) postulated the existence of a third gene *S*, thought to be involved in symptom development. However, Hubbeling (1972) could not reconcile all the observed phenotypes with theoretical combinations of the *S*, *I* and *A* genes; the possible occurrence of the *S* gene is not referred to in any subsequent literature.

Resistance derived from cv Robust inherited as a recessive trait, is strain-specific and effective against the Type strain but not the NY15 strain (Zaumeyer and Meiners, 1975). Many dry navy bean types carry resistance from cv Robust and cv GN UI 1, and some Michigan cultivars carry resistance to strain NY15 derived from cv Corbett Refugee. However, the discovery of temperature-insensitive BCMV strains which were isolated in the Netherlands and caused

systemic necrosis at 15–20 °C (Hubbeling, 1963, 1972), caused new sources of resistance to be sought.

Genetic basis of resistance

A study of the genetic interaction between BCMV and *P. vulgaris* by Drijfhout (1978) showed that there was a gene-for-gene relationship between the pathogen and its host (Flor, 1971). Strains of BCMV were categorized into 10 pathogenicity groups and cultivars were selected to represent 11 host resistance groups which formed a standard set of differentials (Table 2).

Drijfhout (1978) described four strain-specific resistance genes, where the notation *bc* refers to bean common mosaic virus and replaces *A* and *S* as the standard gene nomenclature in accordance with Camacho *et al.* (1977). Genes *bc*-1 and *bc*-1^2 are allelic as are *bc*-2, *bc*-2^2. These genes are rendered ineffective by corresponding pathogenicity genes designated *P*1, *P*1^2, *P*2 and *P*2^2. Pathogenicity genes *P*1 and *P*1^2 are apparently not allelic in the virus but the allelism of *P*2 and *P*2^2 is not excluded. A fifth resistance gene (*bc*-3) has been found in accession IVT 7214, but no corresponding pathogenicity gene has yet been found in any BCMV strain. A strain non-specific gene (*bc*-u) has also been identified which confers no resistance on its own but its presence is required for strain-specific genes to be expressed. The dominant allele of this gene can therefore be considered to be a suppressor (or inhibitor) of resistance. Table 1 summarizes the genetics of the relationship between host resistance groups in *P. vulgaris* and pathogenicity groups of BCMV and Table 2 summarizes the differentiation of strains of BCMV using host resistance groups.

The existence of temperature-insensitive strains which cause systemic necrosis in *I* gene cultivars is a problem for bean breeders in Africa, Europe, U.S.A. and parts of Latin America such as Chile. Cultivars carrying the dominant *I* gene are susceptible to the 'black root' symptom when infected by these strains unless they possess the recessive *bc* resistance genes (Davis *et al.*, 1987). Sources of such resistance are available for example in IVT 7214 (*bc*-3) and IVT 7233 (*bc*-2^2) (Drijfhout, 1978) and the cvs Turkish Brown, Valja and 1750–73 were also found to be resistant to all known strains of BCMV (Innes and Walkey, 1980). At CIAT cultivars have been bred which combine the *I* gene and various *bc* genes. These are currently being evaluated in areas where temperature-insensitive strains of BCMV are known to occur (Davis *et al.*, 1987).

Sources of resistance

The *I* gene occurs extensively in *P. vulgaris* germplasm and landraces which have not been developed through genetic improvement programmes, for example, in the many tropical black beans (Kelly, 1988). Central American black bean cultivars were initially used in the CIAT breeding programme to introduce the *I* gene into new cultivars. However, the *I* gene and colour-intensifying genes which resulted in undesirable dark seed coat types are tightly linked and the association was never broken (Temple and Morales, 1986). No such linkage was observed in the Corbett Refugee type beans of Andean origin, and CIAT used components of the land race Pompadour which carried the *I* gene and were a red mottle colour. Pompadour was from the same Andean centre as the original sources of the *I* gene which formed the basis of the CIAT programme for its incorporation into small Central American seed types. There is therefore, a strong possibility of two independent sources of the *I* gene with two different centres of domestication, with the Andean source remaining independent of the colour-intensifying genes (Kelly, 1988).

There is also evidence that the hypersensitive, temperature-insensitive resistance conferred by the *I* gene is associated with resistance genes to other potyviruses. Provvidenti (1983) showed that the resistant response in *I* gene cultivars to cowpea aphid-borne mosaic virus (CAbMV), blackeye cowpea mosaic virus (BLCMV) and watermelon mosaic virus 2 (WMV-2) was very similar to that induced by BCMV. Further work resulted in the identification of two linked genes *Bcm* (dominant resistance to BLCMV) and *Cam* (dominant resistance to CAbMV) (Provvidenti *et al.*, 1983). Kyle *et al.* (1986) showed a tight linkage between the genes *I*, *Bcm*, *Cam* and *Wmv* (dominant resistance to WMV-2) (Kyle and Provvidenti, 1987). Later a temperature-insensitive gene for hypersensitivity, *Hss* was found to soybean mosaic virus (SoyMV). This gene was also closely linked to the four others described above (Kyle and Dickson, 1988). The indications are that there are several tightly linked genes for resistance to several viruses at, or close to, the *I* locus in *P. vulgaris*. Other studies indicate that the gene conferring hypersensitive resistance to Race 3 of the halo blight pathogen (*Pseudomonas syringae* pv. *phaseolicola*) is also linked to the *I* locus (Teverson, 1991).

Control of BCMV by cultural practices and vector control

Although host resistance is the most effective method of controlling BCMV, modification of cultural practices, clean seed certification schemes and vector control have also been used.

Modification of planting date can reduce the incidence of BCMV in susceptible cultivars. Burke (1964) found that early planting of susceptible cultivars, at a time when the incidence of the aphid vectors of legume viruses was low, gave yields as good as or better than similar plantings of resistant cultivars. However, in Africa, planting time is determined by the rains so it would be difficult to adjust planting date to avoid insect vectors in the absence of irrigation.

In many countries seed certification schemes now operate successfully to reduce the introduction of seed-borne infections. However, in Africa, this is often limited by the resources and technology available for effective processing of healthy seed. Also a large proportion of seed sown is kept by farmers from the preceding year (Kirkby, 1987) which would make seed certification more difficult.

Chemicals cannot be used to eliminate BCMV in infected plants but have been used in attempts to control the virus vector and consequently reduce the spread of infection. Experiments at CIAT demonstrated that spraying with aphicide could reduce transmission of BCMV (CIAT, 1975), but other studies have shown that the use of aphicides to control the spread of non-persistently aphid-transmitted viruses, such as BCMV may be ineffective (Walkey, 1991). Walkey and Dance (1979) used diluted mineral oils successfully to reduce aphid transmission of BCMV by *Myzus persicae*, *Brevicoryne brassicae* and *Aphis fabae* in the glasshouse. However, the mineral oils had a phytotoxic effect at higher concentrations and in the field, concentrations which did not cause phytotoxicity failed to control the aphids. In Africa, the use of chemicals to control aphids, even if effective, would not be financially practicable. The most effective and economic method for long-term disease control is to provide farmers with cultivars with host resistance to BCMV.

Interplanting and varietal mixtures

The physical environment surrounding bean plants is changed by interplanting with other crops. This may affect the incidence of some diseases (van Rheenen *et*

al., 1981). There are numerous examples of mixed cropping systems which can reduce the incidence of disease (Allen, 1983). Maize/bean mixtures have been shown for instance, to reduce the incidence of anthracnose (Msuku and Edje, 1982) and common bacterial blight (van Rheenen *et al.*, 1981). In Zambia, Sithanantham *et al.* (1992) observed a more rapid increase in BCMV incidence in bean crops that were sown later and attributed this to vector activity. Intercropping beans with maize reduced aphid infestation and also BCMV infection.

Varietal mixtures may also influence disease incidence and epidemiology, such mixtures dominate many important bean-growing areas in Africa such as Rwanda, Burundi, south-eastern Tanzania, Malawi, north-eastern Zambia, south-western Uganda and north-western Cameroun (Plate 14). Mixtures are also found in subsistence farming in Kenya and Ethiopia (Mukishi and Trutmann, 1988). A 1984 survey in Rwanda indicated that 96% of farmers preferred to plant mixtures (Voss, 1988) because they resulted in higher and more predictable yields. In central and eastern Africa up to 15 cultivars can be used in mixtures (Dessert, 1987), and the average number of cultivars in a mixture in Rwanda in 1984 was 19.8 (Voss, 1988). The number of components of a mixture and their identity may change year to year (Dessert, 1987). In 1988 the average number in a mixture in Rwanda was found to be 11 (Hardman and Lamb, 1988).

Mixtures can restrict the spread of disease if components differ in susceptibility (Wolfe, 1985), but little is known about the relative proportions of resistant and susceptible components in mixtures that are cultivated at present. Neither is there information on the optimum mixture to provide suitable control. However, theoretical studies with mixtures of barley cultivars have shown that those with three components were the most effective in reducing powdery mildew caused by *Erysiphe graminis* (Wolfe *et al.*, 1981). If new cultivars are to be incorporated into a mixture several desirable characteristics need to be taken into consideration. These include: taste, colour preference, speed of cooking, disease resistance and yield. However, when mixtures are grown it may not be necessary to incorporate all these characteristics into a single cultivar, which is the difficult task normally facing plant breeders producing a single cultivar for growing in a monoculture. Several components of the mixture, each possessing different characteristics, could be introduced to improve overall disease resistance, yield, and farmer and consumer acceptability (Voss, 1988).

PROJECT OBJECTIVES

Identification of BCMV strains occurring in Africa

The principal objective of this study was to survey and collect BCMV isolates from the main *Phaseolus vulgaris* bean-growing areas of Africa and, using host differentials and serology, to identify the virus strains to which the isolates were related. From this diagnosis, detailed maps of BCMV strain distribution in Africa could be prepared and this information could be used in national bean-breeding programmes in each African region to enable plant breeders to deploy the appropriate resistance genes to control the disease. Each isolate collected has been stored at Wellesbourne and will be returned to African national breeding programmes as required.

Development of BCMV diagnosis

The second project objective was to develop procedures for the improved identification of BCMV and its strains. This included studying strain relationships by serological, electrophoretic and morphological comparisons.

Ecology of BCMV in wild species of legumes

It became apparent early in the project that wild species of legumes and other non-*Phaseolus* hosts could be important in the ecology of BCMV in Africa. The main objectives of the project were extended, therefore, to study the role of these hosts in the ecology and epidemiology of BCMV and related viruses.

Section 2

The Occurrence of BCMV in *Phaseolus vulgaris*

INTRODUCTION

The details of all samples collected during the various field surveys in Africa are listed in Appendix 2. Each sample collected in the field showed suspected virus symptoms and upon return to Wellesbourne they were tested by ELISA to determine if they contained BCMV. These serological tests identified the serotype of any BCMV present, but did not identify the virus strain. If BCMV was isolated

Table 3 Reactions of differential hosts to standard strains of BCMV

Differential hosts		Symptom score of differential hosts inoculated with standard BCMV strains						
Group	Cultivar	NL1	NL3	NL4	NL5	NL6	NL8	NY15
Reactions at 26 °C								
1	The Prince	4*	4	4	4	4	0	4
	SGR	4	4	nt†	4	4	0	4
	Double White	4	4	4	4	4	4	4
	Sutter Pink	4	4	4	4	4	4	4
	CRM	4	4	nt	4	4	4	4
2	PGW	0	4	4	4	4	1	+t
	RGC	0	4	3	4	4	0	4
3	RGB	0	4	4	4	4	0	0
	GN 59	0	+t	nt	+t	4	0	1
	GN 123	0	nt	nt	nt	nt	nt	nt
4	Michelite	0	4	1	4	1	4	4
	Sanilac	0	4	1	4	1	4	4
5	Pinto 114	0	4	0	4	0	0	3
6	Monroe	0	1n	4	1n	1n	1n	1n
	RM 35	0	1n	4	0	1n	0	1n
	GN 31	0	1n	4	nt	nt	nt	nt
8	BTS	0	5n	0	5n	0	5n	0
	Widusa	0	5n	0	5n	0	5n	0
9a	Jubila	0	4n	0	5n	0	1n	0
9b	ITG	0	5n	0	5n	0	1n	0
	TC	0	5n	0	5n	0	1n	0
10	Amanda	0	1n	0	2n	0	1n	0
Reactions at 32 °C								
8	BTS	0	5n	0	5n	5n	5n	0
	Widusa	0	5n	0	5n	5n	5n	0
9a	Jubila	0	5n	0	5n	5n	1n	0
9b	ITG	0	5n	0	5n	5n	1n	0
	TC	0	5n	0	5n	5n	1n	0
10	Amanda	0	1n/2n‡	0	5n	1n	1n	0

Notes: * symptom scores as for Table A5, 1 or 1n denotes a local symptom only
† not tested
‡ in 50% of tests symptom scores of Amanda were 2n.

from any of the original field samples by sap transmission to glasshouse test plants, its identify was confirmed by electron microscope serology (see Appendix 1, p. 111). The serotype was confirmed by a further ELISA test and the virus strain identified by inoculating the isolate to a set of bean differential host cultivars (Drijfhout, 1978) (see p. 103). The results of the studies with differential host cultivars to identify BCMV strains are divided into two sections: the first details the reactions obtained for the differential hosts grown at 26 °C (Table A7) and the second for those grown at 32 °C (Table A8).

A preliminary experiment was carried out to ensure that the standard BCMV strains, reacted as described by Drijfhout (1978) in the diagnostic differential hosts. The standard strains: NL1, NL3, NL4, NL5, NL6, NL8 and NY15 all produced symptoms in the differential hosts that were typical of the strain reactions described by Drijfhout, with the occasional exception of NL3, NL8 and NL6 (Tables 3 and 4). Drijfhout (1978) reported that NL3 did not normally induce systemic necrosis in the cv Amanda at either 26 °C or 32 °C, although he qualified this statement by saying that NL3 might occasionally induce systemic necrosis in cv Amanda at 32 °C, with its occurrence being dependent on local differences in temperature, light and physiological conditions. In our study NL3

Table 4 Pathogenicity phenotype of standard BCMV strains on differential hosts

Differential hosts		Infectibility of differential hosts inoculated with standard BCMV strains						
Group	Cultivar	NL1	NL3	NL4	NL5	NL6	NL8	NY15
1	The Prince	+	+	+	+	+	-	+
	SGR	+	+	nt	+	+	-	+
	Double White	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	+	+
	CRM	+	+	nt	+	+	+	+
2	PGW	-	+	+	+	+	-	+t
	RGC	-	+	+	+	+	-	+
3	RGB	-	+	+	+	+	-	-
	GN 59	-	+t	nt	+t	+	-	-
	GN 123	-	nt	nt	nt	nt	nt	nt
4	Michelite	-	+	-	+	-	+	+
	Sanilac	-	+	-	+	-	+	+
5	Pinto 114	-	+	-	+	-	-	+
6	Monroe	-	-	+	-	-	-	-
	RM 35	-	-	+	-	-	-	-
	GN 31	-	-	+	nt	nt	nt	nt
8	BTS	-	N	-	N	n	N	-
	Widusa	-	N	-	N	n	N	-
9a	Jubila	-	N	-	N	n	-	-
9b	ITG	-	N	-	N	n	-	-
	TC	-	N	-	N	n	-	-
10	Amanda	-	-	-	N	-	-	-
Pathogenicity genes		*P0*	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P1*[2] *P2*[2]	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P1*[2]	*P0* *P2*	*P0* *P1* *P2*

Notes:
+ host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, not at 26 °C

regularly induced necrosis in cv Amanda at 32 °C but never at 26 °C. NL3 was used as a comparative control strain in the series of differential host cultivars on 30 occasions and in half of these tests cv Amanda developed mild systemic necrosis (usually with symptom score 2n) at 32 °C (see Table 3).

In contrast to NL3, NL5 is the only strain that always induces systemic necrosis in the cv Amanda and this reaction was expected to occur at both 26 °C and 30 °C. In our study, therefore, isolates that induced mild systemic necrosis in cv Amanda at 32 °C but not at 26 °C, were designated NL3 rather than NL5 strain-types. However, because of the variability in the reactions of cv Amanda it was not considered to be a reliable differential and NL3 and NL5 could therefore not be differentiated in this study.

Drijfhout (1978) also reported that NL3 induced questionable or very weak symptoms in the cvs Redlands Greenleaf C (RGC) and Puregold Wax (PGW), but the standard NL3 strain used in our study produced distinct symptoms in these cultivars, although the symptoms were often slow to develop (Table 3).

The standard strain of NL8 did not induce systemic symptoms in cvs The Prince and Stringless Green Refugee (SGR) in our tests, although Drijfhout (1978) had reported that both cultivars were susceptible to strain NL8. Finally, the standard strain NL6 used in our studies only induced systemic necrosis in cultivars carrying the *I* gene at 32 °C, whereas Drijfhout (1978) reported that this strain induced necrosis in these cultivars at 26 °C. However, the NL6-induced necrotic reaction is known to be temperature-dependent, so small changes in local temperatures may suffice to induce the necrosis which may account for these differences in reactions caused by NL6.

IDENTIFICATION AND DISTRIBUTION OF BCMV STRAINS IN AFRICA

BCMV strains were isolated and identified by differential host reactions from 149 of the 647 samples collected from *Phaseolus vulgaris* in Africa (Table 5). From the remaining 498 samples either no virus was detected, or BCMV was detected by ELISA but not isolated (Table A13). Viruses other than BCMV were isolated from some samples and these are described in Section 4.

Table 5 Summary of BCMV strain-types isolated from *P. vulgaris* and identified by their reaction in differential host cultivars

Country	Number of BCMV strains-types identified							
	NL1	NL3	NL6	NL8	NY15	US5	Novel*	Total
Burundi	1	10	2	8	0	0	5	26
Ethiopia	1	0	1	0	0	0	3	5
Kenya	0	7	0	0	1	0	7	15
Lesotho	0	2	0	0	0	0	1	3
Malawi	0	7	1	0	0	0	6	14
Rwanda	0	22	2	4	0	0	3	31
Swaziland	0	1	1	0	0	0	1	3
Tanzania	1	6	0	2	0	0	6	15
Uganda	1	11	2	0	0	0	0	14
Zaire	0	1	0	0	0	0	3	4
Zambia	0	4	1	0	0	0	1	6
Zimbabwe	0	8	2	0	0	1	2	13
Totals	4	79	12	14	1	1	38	149
%	2.7	53.0	8.1	9.4	0.6	0.6	25.5	

* novel isolates of BCMV

Approximately 53% of all isolates identified were classified as NL3 strain-types based on their phenotypic reactions on the differential host cultivars which were similar to those of the standard NL3 strain. They occurred widely in all the areas surveyed except Ethiopia (see Figure 1). Isolates categorized as NL8 strain-type had a more limited distribution, being found in *P. vulgaris* only in Rwanda, Burundi and Tanzania. Isolates categorized as NL1 strain-type were only found occasionally and an isolate similar to the US5 strain was found only in one sample from Zimbabwe. The distribution of isolates conforming to NL6 strain-

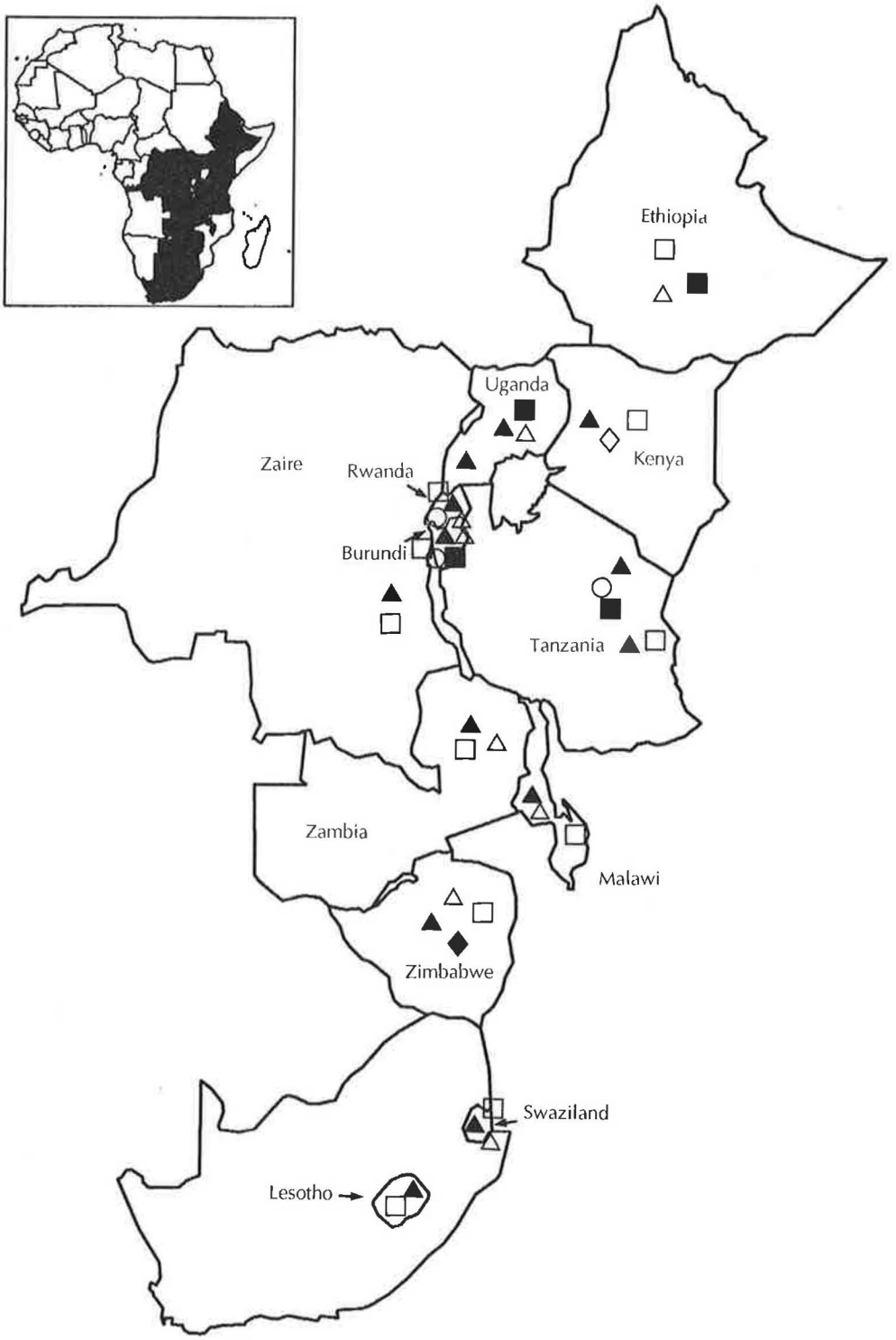


Figure 1 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Central, Eastern and Southern Africa: NL1, ■; NL3 ▲; NL6, △; NL8, ○; NY15, ◇; US5, ◆; Novel, □.

type was more widespread and they were frequently isolated from infected seed. The distribution of isolates similar to the NY15 strain was restricted to western Kenya. The occurrence of isolates similar to NY15 strain in western Kenya was in marked contrast to the rest of the country where all other isolates conformed to the NL3 strain-type.

In Ethiopia, there was a much lower incidence of BCMV than in the other main bean-growing areas of Africa surveyed and the absence of necrotic 'A' serotype strains is highly significant. When infection by BCMV was found, the symptoms were generally mild. Only two confirmed strain identifications were made and these were both non-necrotic 'B' serotypes conforming to strain-types NL1 (226) and NL6 (289). The ELISA results on Ethiopian samples also indicated the widespread distribution of the 'B' serotypes. In addition, three novel isolates (277, 286 and 289) were found at different locations in the Rift Valley which were confirmed to be BCMV 'B' serotypes by serology, but which had pathogenicity phenotypes distinct from any previously described strain of BCMV.

In most countries, there was little difference in the strains found at or near research stations compared with those found in farmers' fields. In Malawi, however, isolates conforming to the NL3 strain-type were only found near the Bunda College and Makapwa research stations and isolates categorized as the NL6 strain-type were only found in farmers' fields. The isolation of an isolate conforming to the US5 strain-type in Zimbabwe at the Harare research station may be associated with imported virus-infected seed.

Details of the BCMV isolations made in individual countries are discussed below in alphabetical order.

Burundi

One field survey was made in November, 1991 (Figure 2). Several samples, collected south of the Rwandan border on the road to Bujumbura, were infected with isolates of the 'A' serotype and isolates conforming to the NL8 strain-type were obtained from two samples (838 and 842) collected in the villages of Banga and Kayanza at altitudes of 2000 m. These isolates were similar to standard NL8 in carrying only the *P*2 pathogenicity gene, except that 838 induced systemic necrosis in cv Jubila at 32 °C (Table 6). In the area north-east of Bujumbura, towards and in the vicinity of Gitega, at altitudes of 1750–2000 m, the symptoms observed in beans were generally mild and no virus was isolated from any of the samples collected, although potyviruses which might have been BCMV were detected in two samples (855 and 858) by ELISA.

In the area immediately north of Bujumbura as far as the Zaire/Rwanda/Burundi border, at altitudes of 800–1100 m, many plants showed severe mosaic symptoms. Necrotic 'A' serotypes were detected by ELISA in all samples from this area and subsequently isolates carrying the *P*1, *P*1^2 and *P*2 pathogenicity genes conforming to the NL3 strain-type were isolated from most samples (869, 872, 873, 875, 876 and 877); one isolate (878) conforming to the NL8 strain-type was also obtained. All the isolates categorized as the NL3 strain-type (see Table 7) were typical of the standard NL3 strain. Isolate 878 differed from the standard NL8 strain in the same way as isolate 838, in inducing systemic necrosis in cv Jubila at 32 °C (Table 6).

Isolate 874, however, could be a novel strain as its pathogenicity phenotype precludes it from being a NL3 or NL8 strain-type. It behaved as an 'A' serotype in inducing necrosis in cultivars carrying the *I* gene, but differed from NL8 by infecting cv Pinto 114 (a group 5 host) and from NL3 in not infecting group 2 and 3 cultivars.

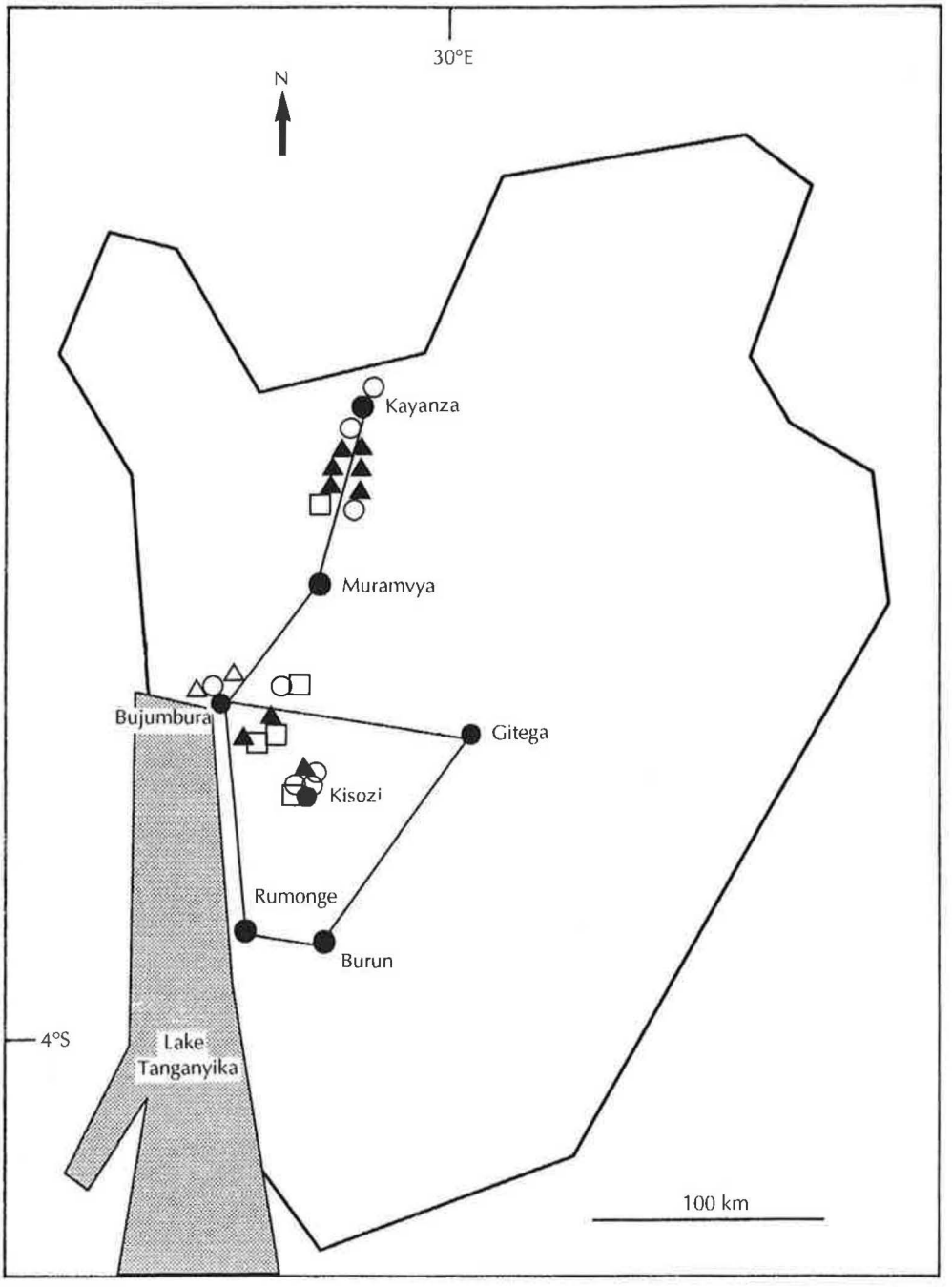


Figure 2 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Burundi. A number of isolates were identified including one NL1 and one NL3 strain-type from unknown locations: NL3, ▲; NL6, △; NL8, ○; Novel, □.

In farmers' fields near the ISABU Research Centre in Bujumbura, most samples were infected with the 'A' serotype. Isolates conforming to the NL3 strain-type were isolated from two samples (879 and 880), and both induced responses identical to the standard NL3 strain (Table 7). Isolates conforming to the NL8 strain-type were obtained from another two samples (881 and 884). Isolate 881 induced reactions typical of the standard NL8 strain but 884 differed in the same way as sample 838 mentioned above (Table 6). Elsewhere in the area three samples were found infected with the 'B' serotype and a novel isolate (887) was found in one of these samples (Table 8). Isolate 887 is probably a novel strain, it caused symptoms only in group 1 cultivars indicating that it carries only the *P*0 gene as if it were an NL1 strain-type, but it also induced a temperature-

Table 6 The reactions of differential host cultivars to various BCMV isolates from Burundi compared with the standard NL8 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL8 strain and unidentified isolates									
Group	Cultivar	NL8	583	584	586	838	842	878	881	884	587
1	The Prince	-	+	-	+	-	-	-	-	+	+
	SGR	-	+	nt	-	nt	nt	nt	nt	nt	nt
	Double White	+	+	+	+	+	+	+	+	+	+
	Sutter Pink	+	nt	+	nt	+	+	+	+	+	nt
	CRM	+	+	nt	+	nt	nt	nt	nt	nt	nt
2	PGW	-	-	-	-	-	-	-	-	-	+
	RGC	-	-	-	-	-	-	-	-	-	+
3	RGB	-	-	-	-	-	-	-	-	-	+
4	Michelite	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	-	-	-	-	-	-	-	-	-	+
6	Monroe	-	-	-	-	nt	nt	nt	nt	nt	nt
	RM 35	-	-	-	-	-	-	-	-	-	-
	GN 31	nt	nt	nt	nt	-	-	-	-	-	nt
8	BTS	N	N	N	N	N	N	N	N	N	N
	Widusa	N	N	n	N	N	N	N	N	N	N
9a	Jubila	-	-	n	-	n	-	n	-	n	-
9b	ITG	-	-	-	-	-	-	-	-	-	-
	TC	-	-	-	-	-	-	-	-	-	-
10	Amanda	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0* *P1* *P1*[2]
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*

Notes: + host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, but not at 26 °C

dependent, necrotic reaction in group 8 and 9 cultivars carrying the *I* gene, behaving like an NL6 strain-type. The significance and implications of this and other novel strains of BCMV found in the survey are further discussed in Section 6, p. 94. In the area immediately south of Bujumbura at Ruzibe on the shore of Lake Tanganyika, two samples were isolated (852 and 854) which conformed to the NL6 strain-type (see Table 8). Sample 852 was typical of the standard NL6 strain but 854 did not induce symptoms in cv Puregold Wax (PGW). A third isolate (853) was also found at this site which induced pathogenicity phenotype reactions which suggested that it may be a novel strain (see Table 8). The isolate induced temperature-dependent necrotic reactions in group 8 and 9 cultivars, but failed to infect group 2 cultivars. A further possible novel 'B' serotype isolate (847) was also found in this area, which only infected group 1 and 4 cultivars but which also caused an atypical necrotic reaction in cv Red Mexican 35. It did not, however, cause a necrotic reaction in cultivars carrying the *I* gene (Table 8).

In addition to the above samples, several dried leaf samples were sent to Wellesbourne from CIAT colleagues. These had been collected from research station trials at Parambo and Gisozi in the south-east of Burundi. From three of the samples (583, 584, and 586) isolates conforming to the NL8 strain-type were obtained and an NL3 strain-type was isolated from another (578). Isolates 583, 584 and 586 were all typical of standard NL8, except 584 did not induce

Table 7 The reactions of differential host cultivars to various BCMV isolates from Burundi compared with the standard NL3 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates											
Group	Cultivar	NL3	578	869	872	873	875	876	877	879	880	2046	874
1	The Prince	+	+	+	+	+	+	+	+	+	+	+	+
	Double White	+	+	+	+	+	+	+	+	+	+	nt	+
	Sutter Pink	+	nt	+	+	+	+	+	+	+	+	+	+
	CRM	+	nt	nt	nt	nt	nt	nt	nt	nt	nt	+	nt
2	PGW	+	+	+	+	+	+	+	+	+	+	+	-
	RGC	+	+	+	+	+	+	+	+	+	+	+	-
3	RGB	+	+	+	+	+	+t	+	+	+	+	+	-
	GN 59	+t	nt	nt	nt	nt	nt	nt	nt	nt	nt	-	nt
4	Michelite	+	+	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+	+	+	+	+
6	Monroe	-	-	nt	nt	nt	nt	nt	nt	nt	nt	-	nt
	RM 35	-	-	-	-	-	-	-	-	-	-	-	-
	GN 31	-	nt	-	-	-	-	-	-	-	-	nt	-
8	BTS	N	N	N	N	N	N	N	N	N	N	N	N
	Widusa	N	N	N	N	N	N	N	N	N	N	N	N
9a	Jubila	N	nt	nf	N	n	N	N	n	-	n	N	n
9b	ITG	N	N	N	N	N	N	N	N	N	N	N	N
	TC	N	N	N	N	N	N	N	N	N	N	N	N
10	Amanda	-	-	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
		P1	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*
		$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*

Notes:
+ host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis only at 32 °C

systemic necrosis in cv Widusa at 26 °C and induced necrosis in cv Jubila at 32 °C (Table 6). A further isolate (587) was quite different, in that it induced symptoms in group 2 and 3 cultivars and cv P114, as well as necrosis in group 8 cultivars. This phenotype cannot be explained by it being a mixture of previously described strain-types and it must be assumed that it is a novel strain.

Isolates conforming to the NL1 (2433) and NL3 (2046) strain-types were also identified from samples of CIAT seed received from Burundi. Unlike the standard isolate of NL3, isolate 2046 failed to induce symptoms in cv Great Northern 59 (GN59), but infection of this cultivar by the standard strain of NL3 can be latent and it is possible that latent infection of this cultivar by isolate 2046 may not have been detected (Table 7).

The necrotic 'A' serotype strains of BCMV appear to be widespread in farmers' fields in Burundi, with isolates conforming to the NL3 and NL8 strain-types being equally prevalent. The occurrence of the non-necrotic 'B' serotype strain NL6 and other possible novel strains appeared to be much more restricted and the only isolate conforming to the NL1 strain-type was from a seed sample, although it seems probable that this seed was from a farmer's local variety rather than from imported seed stock.

Table 8 The reactions of differential host cultivars to various BCMV isolates from Ethiopia, Burundi and Zambia compared with the standard NL6 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL6 strain and unidentified isolates								
Group	Cultivar	NL6	289	852	854	1003	853	887	1008	847
1	The Prince	+	+	+	+	+	+	+	+	+
	Double White	+	+	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	+	+	+	+
2	PGW	+	+	+	-	+	-	-	+	-
	RGC	+	+	+	+	+	-	-	+	-
3	RGB	+	+	+	+	+	-	-	-	-
4	Michelite	-	-	-	-	-	-	-	-	+
	Sanilac	-	-	-	-	-	-	-	-	+
5	Pinto 114	-	-	-	-	-	+	-	-	-
6	RM 35	-	-	-	-	-	-	-	-	n
	GN 31	nt	-	-	-	-	-	-	-	-
8	BTS	n	n	n	N	n	n	n	n	-
	Widusa	n	n	n	n	n	n	n	n	-
9a	Jubila	n	-	n	-	N	n	-	-	-
9b	ITG	n	N	n	N	N	n	n	n	-
	TC	n	n	n	n	n	n	n	n	-
10	Amanda	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0* *P1* *P1*[2]	*P0* *P1* *P1*[2]	*P0* *P1* *P1*[2]	*P0* *P1* *P1*[2]	*P0* *P1* *P1*[2]	*P0* *P1* *P2*	*P0*	*P0* *P1*	*P0* *P2*

Notes: + host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis only at 32 °C, but not at 26 °C

Ethiopia

The main bean-growing areas of Ethiopia were surveyed in August, 1990 (Figure 3). In the western area around Metu at 1500 m, many climbing beans were found but virus symptoms were very mild and only observed in a few plants. A possible 'B' serotype of BCMV was detected by ELISA tests in three samples, but no virus was isolated. Further south near Jima, a NL1 strain-type was isolated from a climbing bean sample (226) with mild virus symptoms and between Jima and Bonga, a possible 'B' serotype was detected in a number of samples but again, no virus was isolated. Possible 'B' serotypes were also detected in several samples from farmers' fields in Sekka, Ataro and Sebaka on route to Bonga at altitudes of 1600–1700 m. At Bonga, possible 'B' serotypes were detected in a further two samples. Between Addis Ababa and Mojo, at about 1550 m, several areas were surveyed but either no virus symptoms were observed or symptoms were very mild.

Along the Rift Valley between Mojo and Awasa many sites were surveyed and possible 'B' serotypes were detected by ELISA in many samples from this region, but virus was only isolated from one sample (277) with severe mosaic symptoms collected at Zway. This isolate produced reactions on the differential host cultivars that were atypical of any previously described BCMV strain (Table 9). It

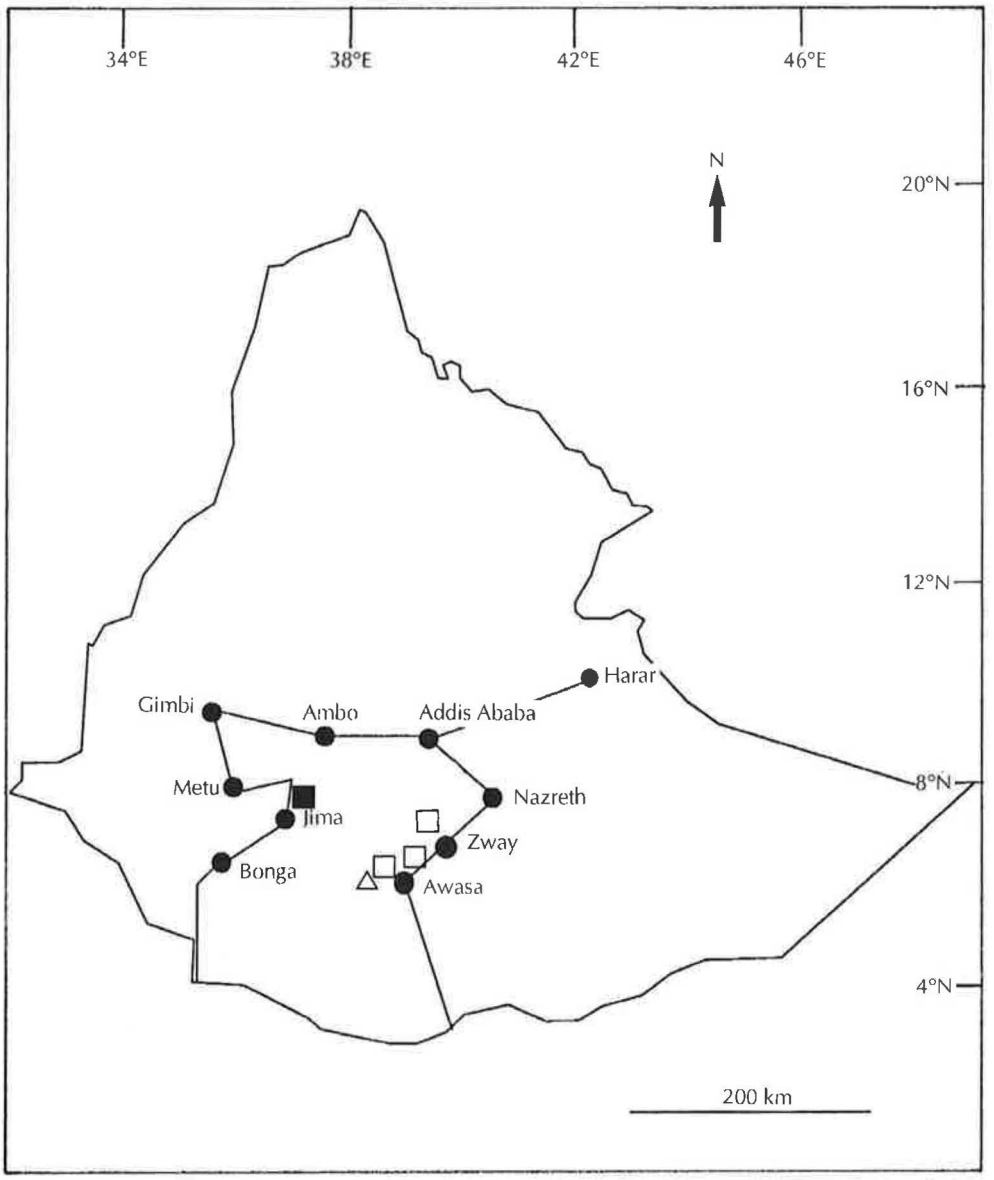


Figure 3 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Ethiopia. A number of isolates were identified: NL1, ■; NL6, △; Novel, □.

induced symptoms only in group 1 and 2 cultivars and a temperature-independent necrosis in group 8 cultivars, which indicated that it was a novel strain. A further possible novel strain (286) was isolated from a plant showing mild mosaic symptoms at a site approximately 40 km nearer Awasa (Table 9). This isolate induced symptoms in group 1 and 3 cultivars and also caused a temperature-independent necrosis in group 8 cultivars. The phenotype reactions of this isolate were identical to those of an isolate (319) obtained east of Nazreth on the Awasa road. (Table 9). The three novel isolates react in ELISA as 'B' serotypes and their phenotypic reactions in the differential hosts cannot be explained by their being a mixture of strain-types. In Awasa a number of samples were infected with possible 'B' serotypes and an isolate (289) conforming to a NL6 strain-type was obtained from a plant with severe mosaic symptoms. This isolate was similar to the standard NL6 strain except that it induced no systemic necrosis in cv Jubila at 32 °C (Table 8).

South of Shashmene towards Soddo at an altitude of 1600 m, possible 'B' serotypes were detected by ELISA in two plants (295 and 296) with severe mosaic

Table 9 The reactions of differential host cultivars to unidentified BCMV isolates from Ethiopia and Lesotho compared with the standard NL8 strain

Differential hosts		Infectibility of differential hosts inoculated with standard NL8 strain and unidentified isolates				
Group	Cultivar	NL8	50	277	286	319
Reactions at 26 °C						
1	The Prince	-	+	+	+	+
	Double White	+	nt	+	+	+
	Sutter Pink	+	nt	+	+	+
	CRM	+	+	nt	nt	nt
2	PGW	-	-	+	-	-
	RGC	-	-	+	-	-
3	RGB	-	-	-	+t	+t
	GN 59	-	-	nt	nt	nt
4	Michelite	+	-	-	-	-
	Sanilac	+	-	-	-	-
5	Pinto 114	-	-	-	-	-
6	Monroe	-	-	nt	nt	nt
	RM 35	-	-	-	-	-
	GN 31	nt	nt	-	-	-
8	BTS	N	n	N	N	N
	Widusa	N	n	N	N	N
9a	Jubila	-	-	-	-	-
9b	ITG	-	-	-	-	-
	TC	-	-	-	-	-
10	Amanda	-	-	-	-	-
Pathogenicity genes		*P0* *P2*	*P0*	*P0* *P1*	*P0* *P1*^{2}	*P0* *P1*^{2}

Notes:
+ host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, but not at 26 °C

symptoms but no virus was detected in other plants with mild symptoms. East of Nazreth on the Awasa road, two possible 'B' serotypes were detected in samples with severe mosaic and the possible novel isolate (319) mentioned above was isolated (Table 9)

In the farmers' fields around Nazreth, beans showed very mild symptoms but no virus was detected. Two possible 'B' serotypes (329 and 330) were detected by ELISA in samples collected in the Chercher Highlands near Kibo at an altitude of 2000 m, but no virus was isolated.

The absence of necrosis-inducing 'A' serotype strains from Ethiopia has already been highlighted in the introduction to this section. In addition, these results also indicated that the overall occurrence of BCMV in Ethiopia was very low.

Kenya

In November, 1991 samples were collected in the Kiambu district, west of Nairobi at an altitude of 2000 m (Figure 4). In farmers' fields three samples were found by ELISA to be infected with the 'A' serotype, and isolates conforming to the NL3 strain-type were isolated from two of these samples (896 and 897). These isolates conformed with the phenotype of the standard NL3 strain except for their failure to induce systemic necrosis in cv. Jubila at 26 °C (Table 10). South-east of Nairobi, in the Machakos drylands region at 1600 m, samples were collected near Katumani but virus was not detected in any of the bean samples, although the 'A' serotype was detected by ELISA in two samples from pigeon pea (see Section 3).

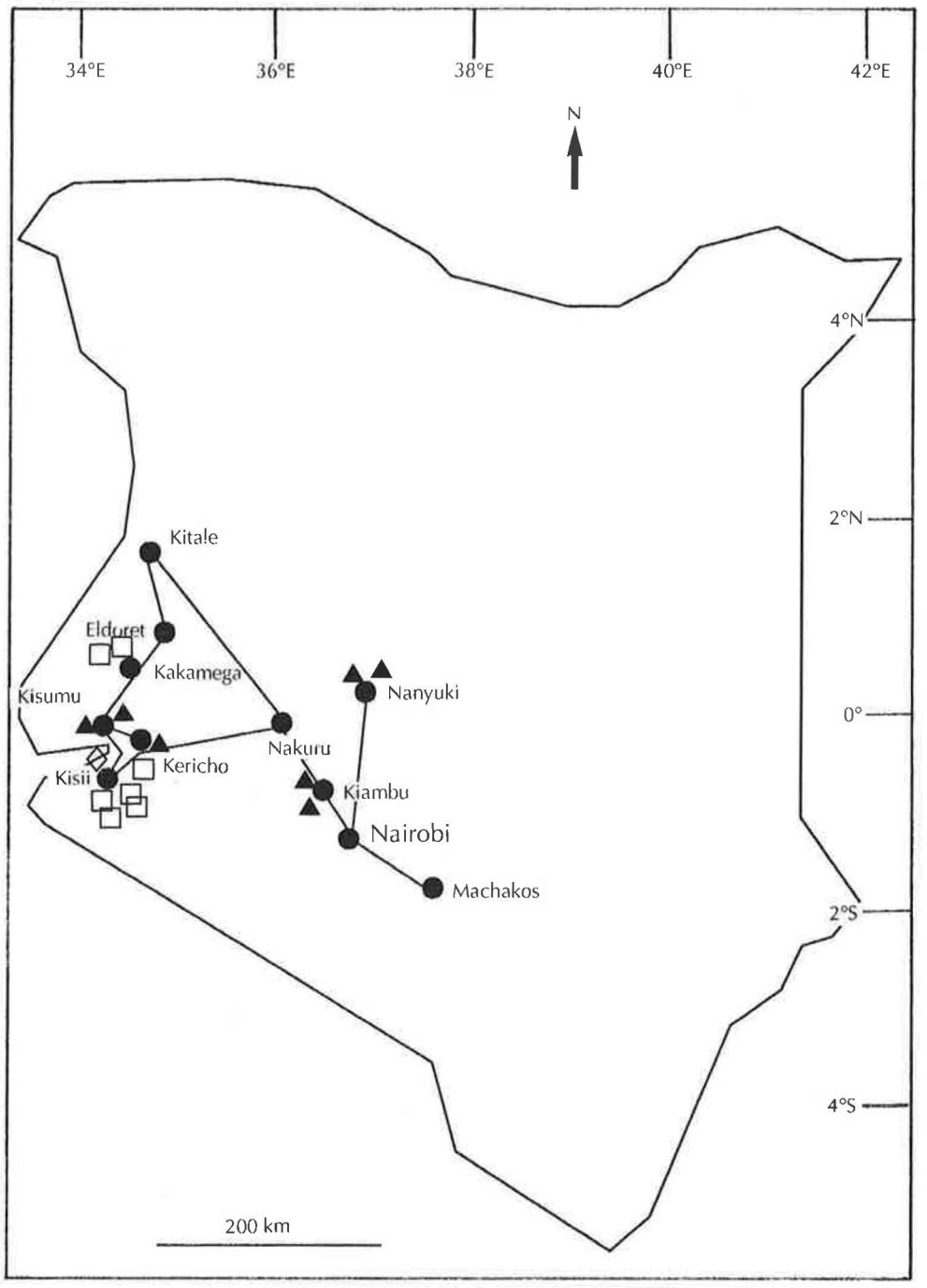


Figure 4 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Kenya. A number of isolates were identified: NL3, ▲; NY15, ◇; Novel, □.

Table 10 The reactions of differential host cultivars to various BCMV isolates from Kenya compared with the standard NL3 strain

Differential Hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates							
Group	Cultivar	NL3	896	897	946	947	950	951	952
1	The Prince	+	+	+	+	+	+	+	+
	Double White	+	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	+	+	+
2	PGW	+	+	+	+	+	+	+	+
	RGC	+	+	+	+	+	+	+	+
3	RGB	+	+	+	+	+	+	+	+
4	Michelite	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+
6	RM 35	-	-	-	-	-	-	-	-
	GN 31	-	-	-	-	-	-	-	-
8	BTS	N	N	N	N	N	N	N	N
	Widusa	N	N	N	N	N	N	N	N
9a	Jubila	N	n	n	n	N	N	N	n
9b	ITG	N	N	N	N	N	N	N	N
	TC	N	N	N	N	N	N	N	N
10	Amanda	-	-	-	-	-	-	-	-
Pathogenicity genes		*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0
		*P*1	*P*1	*P*1	*P*1	*P*1	*P*1	*P*1	*P*1
		$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$
		*P*2	*P*2	*P*2	*P*2	*P*2	*P*2	*P*2	*P*2

Notes: + host susceptible to systemic infection
- host resistant to systemic infection
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis only at 32 °C

In the eastern dryland areas between Mutomo and Meru many samples had leaf distortion symptoms; no BCMV was detected, although one sample (917) had a possible potyvirus infection. On the northern slopes of Mount Kenya near Embu, Meru, and north towards Nanyuki some plants had leaf distortion and chlorotic spot symptoms although again no virus was isolated. At Tharaka an 'A' serotype was detected by ELISA in one sample (935) and an unidentified potyvirus in another (937), but no BCMV was isolated. At a higher altitude of 2200 m at Timau, two isolates conforming to the NL3 strain-type were obtained from bean samples (946 and 947) with differential host reactions similar to the standard NL3 strain except that isolate 946 failed to induce systemic necrosis in cv Jubila at 26 °C (Table 10). In western Kenya at Kericho (1950 m), many beans had strong mosaic symptoms and isolates conforming to the NL3 strain-type were isolated from three samples (950, 951 and 952) (Table 10).

Further west in the Kisii district at Nyangusu and in Kisii itself, the pattern of BCMV distribution was different with only the 'B' serotype being isolated. An isolate (955) conforming in its differential host reactions to the NY15 strain-type was isolated from beans in a farmer's field at Nyangusu and an isolate (956) with identical phenotype reactions was obtained from a legume weed (*Vigna vexillata*) growing closeby. Their differential host reactions indicated that both isolates carried the *P*1 and *P*2 pathogenicity genes (Table 25). A further isolate (953) was also obtained in the same area, and although its differential host reactions resembled those of the NY15 strain-type, they were distinct in that the isolate failed to infect group 2 cultivars. An isolate (973) with similar differential host reactions to 953 was found in the Kakamega area. These two isolates are considered to be novel belonging to a previously undescribed pathogenicity group.

Four isolates (961, 963, 964 and 967) were obtained which differed in their differential host reactions from the NY15 strain-type by infecting group 3, in addition to group 2, 4 and 5 cultivars. Their infection of group 3 cultivars showed that they carried the $P1^2$, in addition to the *P*1 and *P*2 genes and these isolates are possibly of the same novel strain-type (Table 25). Isolates 961 and 963 were isolated from bean and a legume weed (*Crotalaria comanestiana*), respectively, on the same holding at Nyangina, near Kisii and isolates 964 and 967 from beans growing near Kisii and Oyagis.

Isolate 959 which was also found in the Nyangusu area of the Kisii district and isolate 970 obtained from near Kakamega were shown from their differential host reactions to be carrying the pathogenicity genes *P*1, $P1^2$ and *P*2 by infecting host groups 3,4 and 5, but they differed from the four above isolates by not infecting group 2 cultivars (Table 25). These are also considered to be novel isolates.

ELISA tests showed that 'B' serotypes of BCMV were present in various other samples collected in the Kisii, Oyugis and Kakamega areas, but no virus was isolated from these samples. The variation observed in isolates conforming to the NY15 strain-type and other 'B' serotypes isolated from beans and wild legumes is discussed further in Section 6.

Our results indicated that isolates conforming to the NL3 strain-type were prevalent in several areas around and to the east of the Rift Valley. These results are in agreement with those of Buruchara and Gathuru (1979), Bock *et al.* (1980) and Omunyin (1980) who also reported the occurrence of BCMV isolates related to the standard NL3 strain occurring in Kenya. Omunyin also reported the isolation of an isolate resembling the standard NL8 strain, but no NL8 strain-type was found in Kenya in our survey. To the west of the Rift valley only isolates conforming to the NY15 strain-type and other novel 'B' serotypes were found in our survey. The significance of the occurrence of the NY15 strain-type in western Kenya is discussed further in Section 6.

Lesotho

Collections were made in January 1990, at field trials at Maseru Research Station (Figure 5) and although, visually, there appeared to be a high level of infection in different plots (5–30%), only two isolations of BCMV were made. The differential host cultivar reactions of one of these isolates (57) conformed with the NL3 strain except that it induced systemic necrosis in cv Amanda at 32 °C but not in cv Jubila at 26 °C (Table 11). The differential host reactions of the other isolate (50), indicated that it was a novel strain distinct from the isolates (277, 286 and 319) found in Ethiopia. In common with the Ethiopian isolates it induced a necrotic reaction in group 8 cultivars at 32 °C, but otherwise it only infected group 1 cultivars (Table 9).

At a higher elevation near Roma, only a very few isolated plants were observed with mild BCMV-like symptoms. The scattered distribution of BCMV at these higher elevations suggested that infection resulted from seed transmission and there was no evidence of any secondary aphid spread. An isolate conforming to the NL3 strain-type was isolated from one sample (65) at this site which caused no infection in cv Pure Gold Wax (PGW) and no systemic necrosis in cv Jubila at 26 °C (Table 11).

As only a few BCMV isolates were identified in Lesotho, it is not possible to draw firm conclusions on the BCMV strains that were present. It is notable, however, that the two strains positively identified there, both conformed to the NL3 strain-type, a pattern similar to that observed in central Africa.

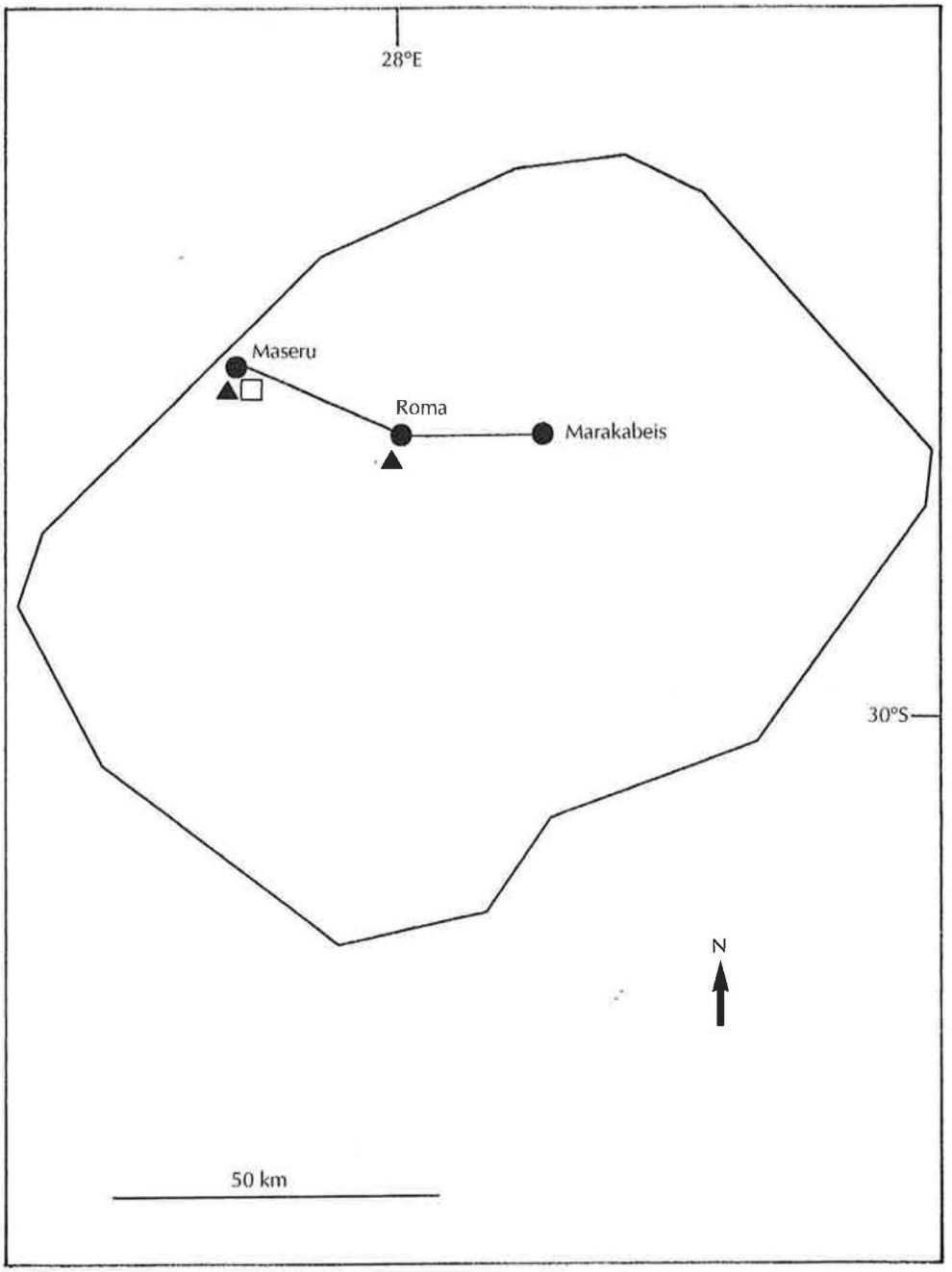


Figure 5 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Lesotho. A number of isolates were identified: NL3, ▲; Novel, □.

Table 11 The reactions of differential host cultivars to various BCMV isolates from Lesotho, Tanzania and Swaziland compared with the standard NL3 strain

Differential Hosts		Infectivity of differential hosts inoculated with the standard NL3 strain and unidentified isolates										
Group	Cultivar	NL3	57	65	88	400	402	405	406	451	454	100
1	The Prince	+	+	+	+	+	+	+	+	+	+	+
	SGR	+	+	nt	nt	nt	nt	+	+	nt	nt	nt
	Double White	+	nt	nt	nt	+	+	+	+	+	+	nt
	Sutter Pink	+	nt	nt	nt	+	+	nt	nt	nt	nt	nt
	CRM	+	+	+	+	nt	nt	+	+	nt	nt	+
2	PGW	+	+	-	-	+	+	+	-	+	+	-
	RGC	+	+	+	+	+	+	+	-	-	+	+t
3	RGB	+	+	+	+	+	+	+	+	+	+	-
	GN 59	+t	nt	+t	-	nt	nt	nt	-	nt	nt	-
4	Michelite	+	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	-	+	+	-	+
6	Monroe	-	-	-	-	-	-	-	-	-	-	-
	RM 35	-	-	-	-	-	-	-	-	-	-	-
	GN 31	-	nt	nt	nt	nt	nt	nt	-	nt	nt	nt
8	BTS	N	N	N	N	N	N	N	N	N	N	N
	Widusa	N	N	N	N	N	N	N	N	n	N	N
9a	Jubila	N	n	n	n	n	n	N	N	n	n	n
9b	ITG	N	N	n	N	N	N	N	N	N	n	N
	TC	N	N	N	N	n	n	n	N	n	-	n
10	Amanda	-	n	n	-	-	-	n	-	n	n	n
Pathogenicity genes		*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* *P2*

Notes:

+	host susceptible to systemic infection
nt	not tested
-	host resistant to systemic infection
+t	symptomless host but systemic infection detected by ELISA.
N	systemic necrosis at 26 °C and 32 °C
n	systemic necrosis at 32 °C, but not at 26 °C

Malawi

The survey was made in February, 1990 (Figure 6). Samples were collected in the northern district of Mzimba at Bunda College's field trial site at Champhira (1440 m). Very few plants with virus symptoms were observed although the vector *Aphis fabae* was present. Two bean samples (139 and 140) were found to be infected with isolates whose differential host reactions conformed with the reactions of the standard NL3 strain, except both isolates induced systemic necrosis in cv Amanda at 32 °C, and cv Jubila failed to develop systemic necrosis at either temperature when inoculated with isolate 140 (see Table 12). Further south at Linthipe, north of Dedza, two isolates (149 and 151) were obtained that could be novel strains. They induced a temperature-sensitive necrotic reaction similar to the standard NL6 strain in host groups 8 and 9, but did not conform with the NL6 strain-type reaction in group 2 and 3 cultivars (see Table 24). Isolate 149 failed to infect both group 2 and 3 cultivars and 151 failed to infect group 3 cultivars. In southern Malawi near Thyolo, two further isolates (178 and 179) were obtained that induced a temperature-sensitive necrotic reaction in group 8 and 9 cultivars. The differential host reactions of isolate 178 suggested that it was

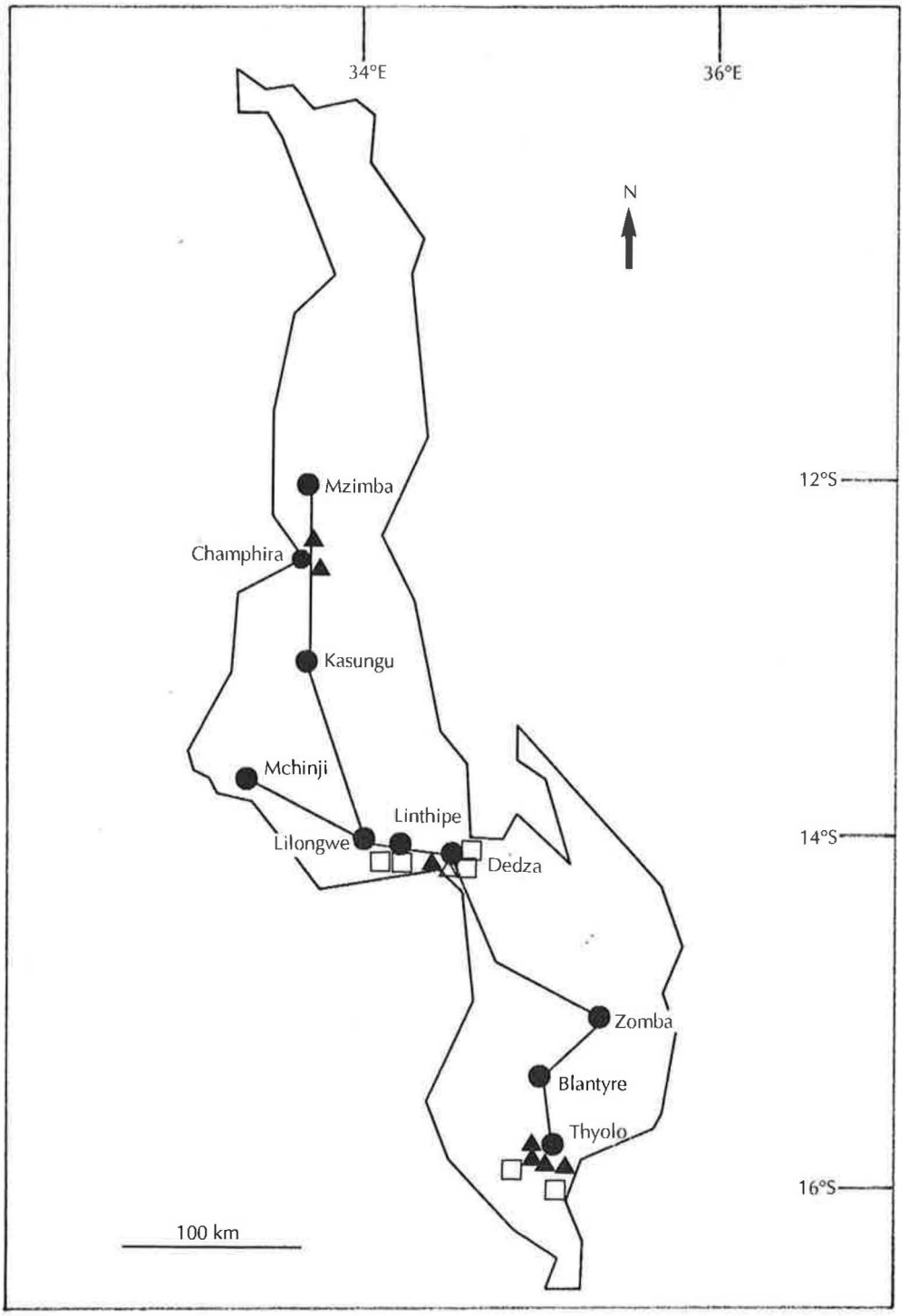


Figure 6 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Malawi. A number isolates were identified: NL3, ▲; NL6, △; Novel, □.

the same novel strain-type as isolate 149, and the reactions of isolate 179 were similar to those of isolate 151. In addition, a seed sample collected from a market near Dedza was infected with an isolate (197) that conformed to the NL6 strain-type (see Table 24).

At another site near Dedza at 1350 m, some farmers' fields showed beans with mild virus-like symptoms but no virus was isolated. Three isolates (161, 162 and 164) which were found in climbing beans at the Bunda College field trial site had differential host reactions that conformed with the NL3 strain-type (see Table 12). The reactions deviated only in that 161 failed to induce symptoms in cvs

Table 12 The reactions of differential host cultivars to various BCMV isolates from Malawi compared with the standard NL3 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates									
Group	Cultivar	NL3	139	140	161	162	164	166	167	168	169
1	The Prince	+	+	+	+	+	+	+	+	+	+
	CRM	+	-	+	+	+	+	+	+	+	+
2	PGW	+	+t	+	-	+	-	+t	+t	+	+
	RGC	+	-	+	+	+	+	+t	-	+	+
3	RGB	+	+t	+	+	+	+	+t	+t	+	+
	GN 59	+t	-	+	-	-	+t	-	-	+	+
4	Michelite	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+	+	+
6	Monroe	-	-	-	-	-	-	-	-	-	-
	RM 35	-	-	-	-	-	-	-	-	-	-
8	BTS	N	N	N	N	N	N	N	N	N	N
	Widusa	N	N	N	n	N	N	N	N	N	N
9a	Jubila	N	N	-	n	n	n	n	N	n	n
9b	ITG	N	N	N	n	n	N	N	n	N	N
	TC	N	N	N	-	n	N	N	N	n	N
10	Amanda	-	n	n	-	-	-	-	n	-	-
Pathogenicity genes		*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0
		*P*1	*P*1	*P*1	*P*1	*P*1	*P*1	*P*1	*P*1	*P*1	*P*1
		$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$
		*P*2	*P*2	*P*2	*P*2	*P*2	*P*2	*P*2	*P*2	*P*2	*P*2

Notes:
+ host susceptible to systemic infection
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA, tolerant reaction
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, but not at 26 °C

PGW and GN59 and 162 failed to induce symptoms in cv GN59. All three isolates failed to induce systemic necrosis in cv Jubila at 26 °C and 164 did not induce it at 32 °C. Also, 161 failed to induce systemic necrosis in cvs ITG and TC at 26 °C and TC at 32 °C and 162 failed to induce systemic necrosis in cv TC at 26 °C (see Table 12). Despite these small variations the three isolates clearly carried the *P*1, $P1^2$ and *P*2 pathogenicity genes characteristic of the NL3 strain-type.

In southern Malawi, at the Ministry of Agriculture Research Station at Matapwa (800 m) there was little virus infection but NL3 strain-types were isolated from four climbing bean samples (166, 167, 168 and 169). These isolates conformed with the NL3 strain-type (Table 12), although their differential host reactions deviated slightly from the standard NL3 strain. Isolate 167 failed to induce symptoms in cvs RGC and GN59, and 166 did not induce symptoms in GN59. Also 166 and 169 failed to induce systemic necrosis in cv. Jubila at 26 °C, 167 failed to induce systemic necrosis in cv. ITG at 26 °C although it did induce systemic necrosis in cv. Amanda at 32 °C and 168 failed to induce systemic necrosis in cv. TC at 26 °C.

In Malawi, as in Zimbabwe and Uganda, BCMV strain-types strains similar to NL3 were prevalent and the NL6 strain-type was also common. It is notable that the NL3 strain-type isolations were made at research station field trial sites and the NL6 strain-types were isolated from farmers' fields and seed. The significance of the novel isolates found is discussed further in Section 6.

Table 13 The reactions of differential host cultivars to various BCMV isolates from Uganda, Tanzania, Swaziland, Rwanda and Zimbabwe compared with the standard NL6 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL6 strain and unidentified isolates												
Group	Cultivar	NL6	133	136	193	194	195	75	802	127	403	404	407	575
1	The Prince	+	+	+	+	+	+	+	+	+	+	+	+	+
	SGR	+	nt	nt	+	+	nt	+	nt	nt	nt	+	nt	+
	Double White	+	nt	nt	nt	nt	nt	+	+	nt	+	+	+	+
	Sutter Pink	+	nt	nt	nt	nt	nt	nt	+	nt	+	nt	+	nt
	CRM	+	+	+	+	+	+	nt	nt	+	nt	+	nt	nt
2	PGW	+	-	+	+	+t	+	+	+	-	-	-	+	+
	RGC	+	+	+	+	-	+	+	+	-	-	-	+	+
3	RGB	+	-	+	+t	+t	+	+	+	-	-	-	-	+
	GN 59	+	+	+t	+t	+t	+	nt	nt	-	nt	-	nt	nt
4	Michelite	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sanilac	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Pinto 114	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Monroe	-	-	-	-	-	-	-	nt	-	-	-	-	-
	RM 35	-	-	-	-	-	-	-	-	-	-	-	-	-
	GN 31	nt	nt	nt	nt	nt	nt	nt	-	nt	nt	-	nt	nt
8	BTS	n	N	n	n	n	N	N	n	N	n	n	N	n
	Widusa	n	N	n	-	n	-	N	n	N	n	n	N	n
9a	Jubila	n	-	-		-	-	n	-	n	-	n	-	n
9b	ITG	n	n	n	n	n	n	n	n	n	n	n	n	n
	TC	n	n	n	n	n	-	N	n	n	n	n	-	n
10	Amanda	-	-	-	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0*	*P0*	*P0*	*P0* *P1*	*P0* *P1* *P1*^{2}

Notes:
+ host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA.
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, but not at 26 °C

Morocco

A limited survey was carried out in bean-growing areas in November, 1990. Samples were collected from seven sites in the Beni Mellal area and at one site east of Fes. At the first site 35 km west of Beni Mellal, the 'A' serotype of BCMV was detected by ELISA in three samples and BCMV was isolated from one of these samples (347). However, this sample was also infected with alfalfa mosaic virus (AlfMV). The remaining samples from this site were possibly infected with the 'B' serotype of BCMV, but virus was only isolated from three (344, 347 and 348) and these were also infected with AlfMV (see Section 4). Attempts were made to separate the two viruses, but AlfMV has a much wider host range than BCMV and all hosts of BCMV tested were also hosts of AlfMV. Consequently, AlfMV was isolated on its own but not BCMV. Seed harvested from bean plants with the mixed infection was also found to be infected with both viruses, so the BCMV strain-types of these isolates could not be determined. Both 'A' and possible 'B' serotypes of BCMV were also detected in samples from the Fes site, however no isolations of BCMV were made and one sample (366) was also infected with AlfMV. The widespread distribution of alfalfa as a forage crop in Morocco and its known susceptibility to AlfMV (Walkey *et al.*, 1990), is reflected in the number of isolations of AlfMV from beans there.

Rwanda

Field surveys were made in May and November 1991 (see Figure 7). In May, at the ISAR Lowland Research Station at Karama (1400 m), the incidence of BCMV was high. The two isolates (510 and 511) collected there belonged to the 'A' serotype and conformed to the NL3 strain-type, although neither isolate induced systemic necrosis in cv Jubila at 26 °C (Table 14). In several farmers' fields nearby, the incidence of BCMV was much less than at the research station and mainly at the periphery of the fields. All the samples collected in these fields were found in ELISA tests to be infected with the 'A' serotype and the two isolates (512 and 513) isolated were NL3 strain-types Both these isolates induced systemic necrosis in cv Amanda at 32 °C (Table 14). Between Karama and Butare in the Kanzenze sector, samples from two fields were all infected with the 'A' serotype. Two samples (519 and 520) from one field of climbing beans were shown to be infected with NL3 strain-types. Isolate 520 failed to induce systemic necrosis in cvs Widusa, Jubila and Top Crop (TC) at 26 °C, but did so at 32 °C (see Table 14). An 'A' serotype isolate (521) found in the same field of climbing beans as isolates 519 and 520, induced reactions in the differential hosts that suggested it may be a novel strain. It infected host groups 1, 2 and 4 indicating that it carried the *P*1 and *P*2 pathogenicity genes and also caused necrosis in group 9a cultivars (Table 15).

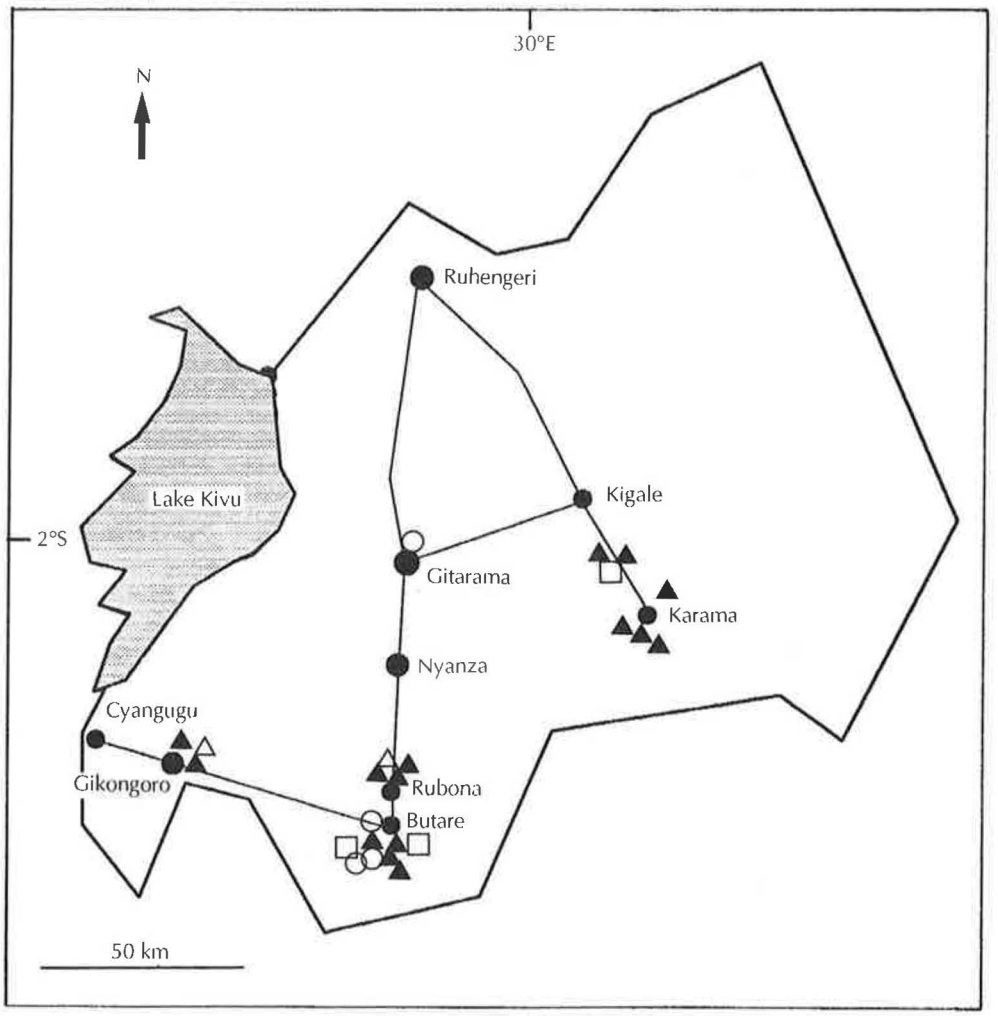


Figure 7 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Rwanda. A number of isolates were identified, including seven NL3 strain-types from seed samples of unknown origin: NL3, ▲; NL6, △; NL8, o; Novel, □.

Table 14 The reactions of differential host cultivars to various BCMV isolates from Rwanda compared with the standard NL3 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates															
Group	Cultivar	NL3	510	511	512	513	519	520	564	565	567	568	569	526	530	535	537
1	The Prince	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	SGR	+	+	nt	+	nt	nt	nt	+	nt	+	+	+	nt	nt	nt	+
	Double White	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Sutter Pink	+	nt	+	nt	+	nt	+	nt	nt	nt	nt	nt	nt	nt	nt	nt
	CRM	+	+	nt	+	nt	nt	nt	+	nt	+	+	nt	nt	nt	nt	+
2	PGW	+	+	+t	+	+	+	+	+	+	+	-	-	+	+	+	+
	RGC	+	+	+t	+t	+	+	+	+	+	+	-	-	+	+	+	+
3	RGB	+	+	+t	+t	+	+	+	+	+	+	+t	+t	+	+	+	+
4	Michelite	+	+	+	+	+	+	+	+	+	+	nt	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+
6	Monroe	-	-	-	-	nt	-	-	-	-	-	-	-	-	-	-	-
	RM 35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GN 31	-	nt	nt	nt	-	nt	-	nt	nt	nt	nt	nt	nt	nt	nt	nt
8	BTS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	Widusa	N	N	N	N	N	N	n	N	N	N	N	N	N	N	N	N
9a	Jubila	N	n	n	n	N	n	n	n	n	n	N	-	N	-	n	n
9b	ITG	N	N	N	N	N	N	N	N	n	N	n	N	N	n	N	N
	TC	N	N	N	N	N	N	n	N	N	N	N	nt	N	-	n	N
10	Amanda	-	-	-	n	n	-	-	n	N	-	-	-	-	-	-	n
Pathogenicity genes		*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*	*P0* *P1* $P1^2$ *P2*

Notes:

+	host susceptible to systemic infection
nt	not tested
-	host resistant to systemic infection
+t	symptomless host but systemic infection detected by ELISA.
N	systemic necrosis at 26 °C and 32 °C
n	systemic necrosis at 32 °C, but not at 26 °C

Table 15 The reactions of differential host cultivars to various BCMV isolates from Rwanda, Tanzania and Zaire compared with the standard NL8 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL8 strain and unidentified isolates												
Group	Cultivar	NL8	442	456	523	524	529	437	444	450	521	528	532	550
1	The Prince	-	+	+	-	+	-	-	+	-	+	+	-	+
	SGR	-	-	nt	nt	nt	nt	+	nt	nt	nt	nt	nt	+
	Double White	+	+	+	+	+	+	+	+	+	+	+	+	+
	Sutter Pink	+	nt	+	+	+	+	nt	nt	+	+	+	+	nt
	CRM	+	+	nt	nt	nt	nt	+	nt	nt	nt	nt	nt	nt
2	PGW	-	-	-	-	-	-	+	+	-	+t	+t	-	-
	RGC	-	-	-	-	-	-	-	+	-	-	-	-	-
3	RGB	-	-	-	-	-	-	-	-	-	-	-	-	+
	GN 59	-	-	nt	nt	nt	nt	-	nt	nt	nt	nt	nt	nt
	GN 123	nt	-	nt	nt	nt	nt	-	nt	nt	nt	nt	nt	nt
4	Michelite	+	+	+	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	-	-	-	-	-	-	-	-	+	-	-	+	+
6	Monroe	-	-	-	nt	-	-	-	-	-	nt	nt	-	-
	RM 35	-	-	-	-	-	-	-	-	-	-	-	-	-
	GN 31	nt	-	nt	-	nt	nt	nt	nt	nt	-	-	nt	nt
8	BTS	N	N	N	N	N	N	N	N	N	N	N	N	N
	Widusa	N	N	N	N	N	N	N	N	N	N	N	N	N
9a	Jubila	-	N	n	-	-	-	-	-	-	-	n	-	-
9b	ITG	-	-	-	-	-	-	-	-	-	-	-	-	-
	TC	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Amanda	-	-	-	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
								P1	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*
														P1[2]
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*

Notes:
+ host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
nt symptomless host but systemic infection detected by ELISA,
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C but not at 26 °C

In the survey of farmers' fields around Butare in the Tambwe, Runinya, Ngone and Mbazi regions,at 1700–1900 m, there was a high incidence of BCMV-like symptoms, particularly in climbing beans. Most samples were infected with the 'A' serotype, and isolates conforming to the NL3 strain-type were isolated from four samples (526, 530, 535 and 537) (Table 14). Some of the NL3 strain-types isolated, although carrying the *P*1, *P*1[2] and *P*2 pathogenicity genes typical of the standard NL3 strain, did induce some variant necrotic reactions in host groups 8, 9, and 10 cultivars. Isolate 530 failed to induce systemic necrosis in cvs Jubila and Top Crop at both 26 °C and 32 °C or in cv ITG at 26 °C, although 537 did induce systemic necrosis in cv Amanda at 32 °C. In contrast, isolate 526 isolated from bean on another farm in the same region induced reactions on the differential hosts that were typical of the standard NL3 strain. In addition to the NL3 strain-type isolated from bean at the Runinya site, an isolate (531) conforming to the NL3 strain-type was also isolated from *Vigna vexillata*. The details of this isolate are discussed in Section 3.3.

Besides the NL3 strain-type isolates obtained at these sites, a number of other 'A' serotype isolates were also isolated. Of these, isolates 523, 524 and 529 had

differential host reactions that conformed with those of the standard NL8 strain (Table 15), but isolates 528 and 532 were distinct and could be novel strains. The latter two isolates in addition to having pathogenicity gene *P*2, also carried pathogenicity gene *P*1 which resulted in their infecting the group 2 cultivar PGW.

In the high altitude area of Gikongoro at 2000 m, many plants were severely affected by virus. Of three samples collected all were infected with the 'A' serotype and the NL3 strain-type was isolated from two, 564 and 565. These isolates induced differential host reactions that conformed with those of the standard NL3 strain except they induced systemic necrosis in cv Amanda at 32 °C (Table 14).

At the ISAR Research Station at Rubona, samples were taken from several field trials of climbing beans. Most samples were infected with the 'A' serotype and the NL3 strain-type was isolated from three samples (567, 568 and 569). The NL3 strain-type isolates at Rubona were typical of the standard NL3 strain except that they did not induce systemic necrosis in cv Jubila at 26 °C. Also isolates 568 and 569 failed to induce symptoms in cvs PGW and RGC and isolate 569 did not induce symptoms in cv P114 (Table 14). In addition to these 'A' serotypes, a 'B' serotype isolate (575) was found at Rubona which conformed with the NL6 strain-type in its differential host reactions (Table 13).

In the November survey, several samples were collected in farmers' fields west and south-west of Butare (1800–2000 m). Necrotic 'A' serotypes of BCMV were detected by ELISA from several samples of climbing beans but no virus was isolated.

At Kyanika, a 'B' serotype (802) was isolated that conformed with the standard NL6 strain, except that it induced no systemic necrosis in Jubila at 32 °C (Table 13). In the high-altitude area north of Kigali towards Ruhengeri (2000–2300 m), mild symptoms were observed in some plants but BCMV was only detected by ELISA in one sample, and this was identified as a possible 'B' serotype.

The pattern of strain occurrence in Rwanda was very similar to that in Burundi. Necrotic 'A' serotype strains were widespread throughout the country in farmers' fields. The NL3 strain-type was predominant, but the NL8 strain-type was also common. The NL6 strain-type was the only previously identified 'B' serotype strain isolated.

Swaziland

The High Veld, Middle Veld and Low Veld were surveyed in January 1990 (Figure 8). At a farmer's field in the High Veld at 1200 m, a NL3 strain-type was isolated from the only infected sample (88) collected. The differential host reactions of isolate 88 conformed with those of the standard NL3 strain except that it did not infect cvs PGW and GN59, and did not induce systemic necrosis in cv Jubila at 26 °C (Table 11) At Mbekelwen in the Middle Veld at about 700 m, a further 'A' serotype was isolated from another bean sample (100). This isolate failed to infect host group 3 cultivars indicating that it does not carry the $P1^2$ pathogenicity gene. It also differed from the NL3 strain-type in causing necrosis in cv Amanda at 32 °C (Table 11). These reactions suggest that it is a novel strain. No virus was isolated from samples collected in the Low Veld. In addition, a seed sample (195) collected in Manzini market was found to be infected with an isolate that conformed to the NL6 strain-type. Its differential host reaction differed from the standard NL6 strain only in that it did not induce systemic necrosis in cvs Widusa, Jubila or TC at 32 °C (Table 13).

As in Lesotho, virus symptoms were scarce in beans grown at high elevations in Swaziland. Only three positive isolations were made, of which one was identified as a NL6 strain-type. The other 'A' serotype was possibly a novel strain. The occurrence of the NL3 and NL6 strain-types in Swaziland is similar to the pattern of strain occurrence observed elsewhere in central Africa.

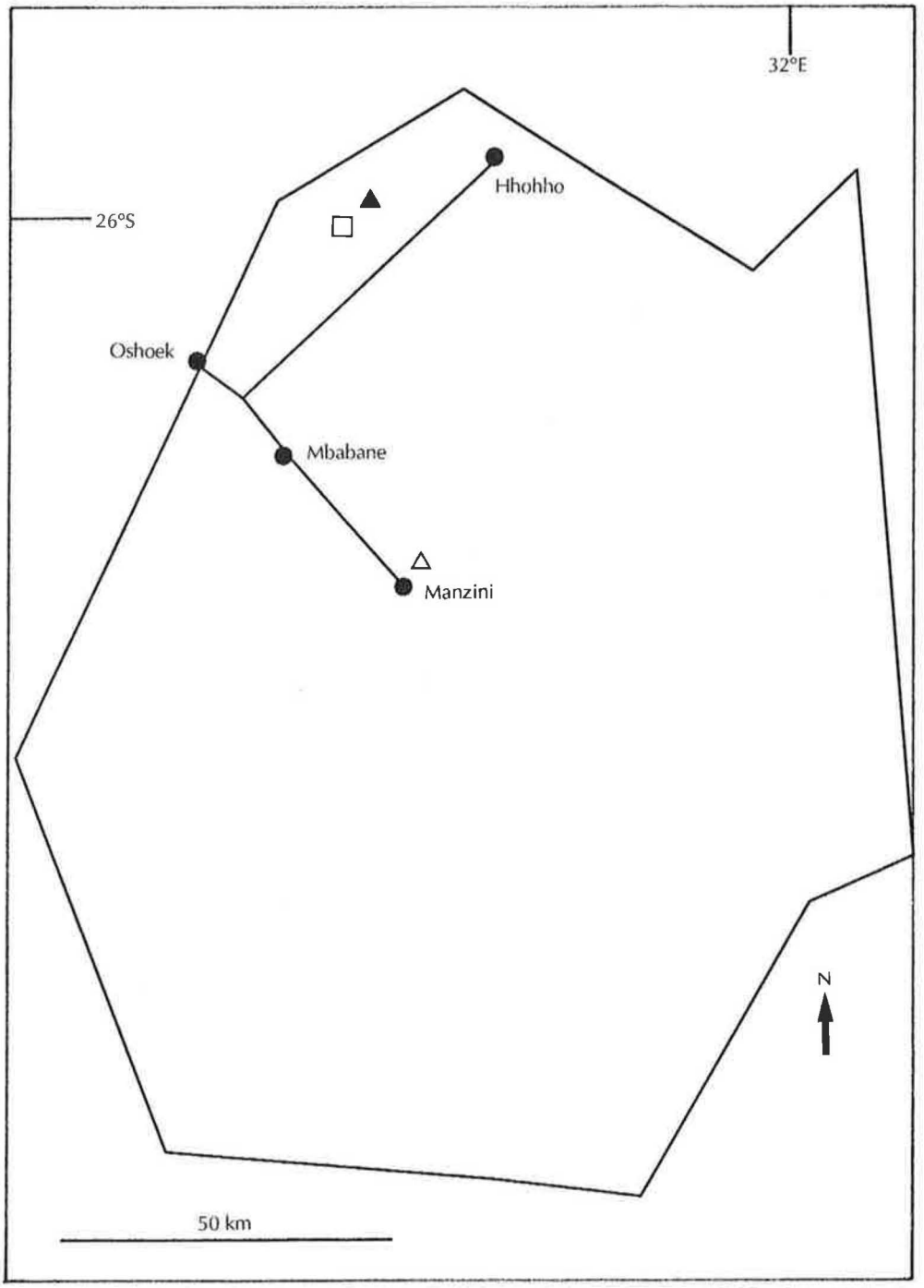


Figure 8 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Swaziland. A number of isolates were identified. The NL6 strain-type was from a seed sample from Manzini market: NL3, ▲; NL6, △; Novel, □.

Tanzania

A field survey was made in various bean-growing areas in May, 1991. Samples were collected at Sokoine University of Agriculture, Morogoro from field trials of bean breeding material showing BCMV infection (Figure 9). BCMV isolates from four samples (400, 402, and 406) induced reactions in the differential hosts that conformed with the NL3 strain-type. They only differed from the standard NL3 strain in that isolates 400 and 402 failed to induce systemic necrosis in cv Jubila at 26 °C (Table 11). Isolate 406 failed to induce symptoms in cvs PGW, RGC and

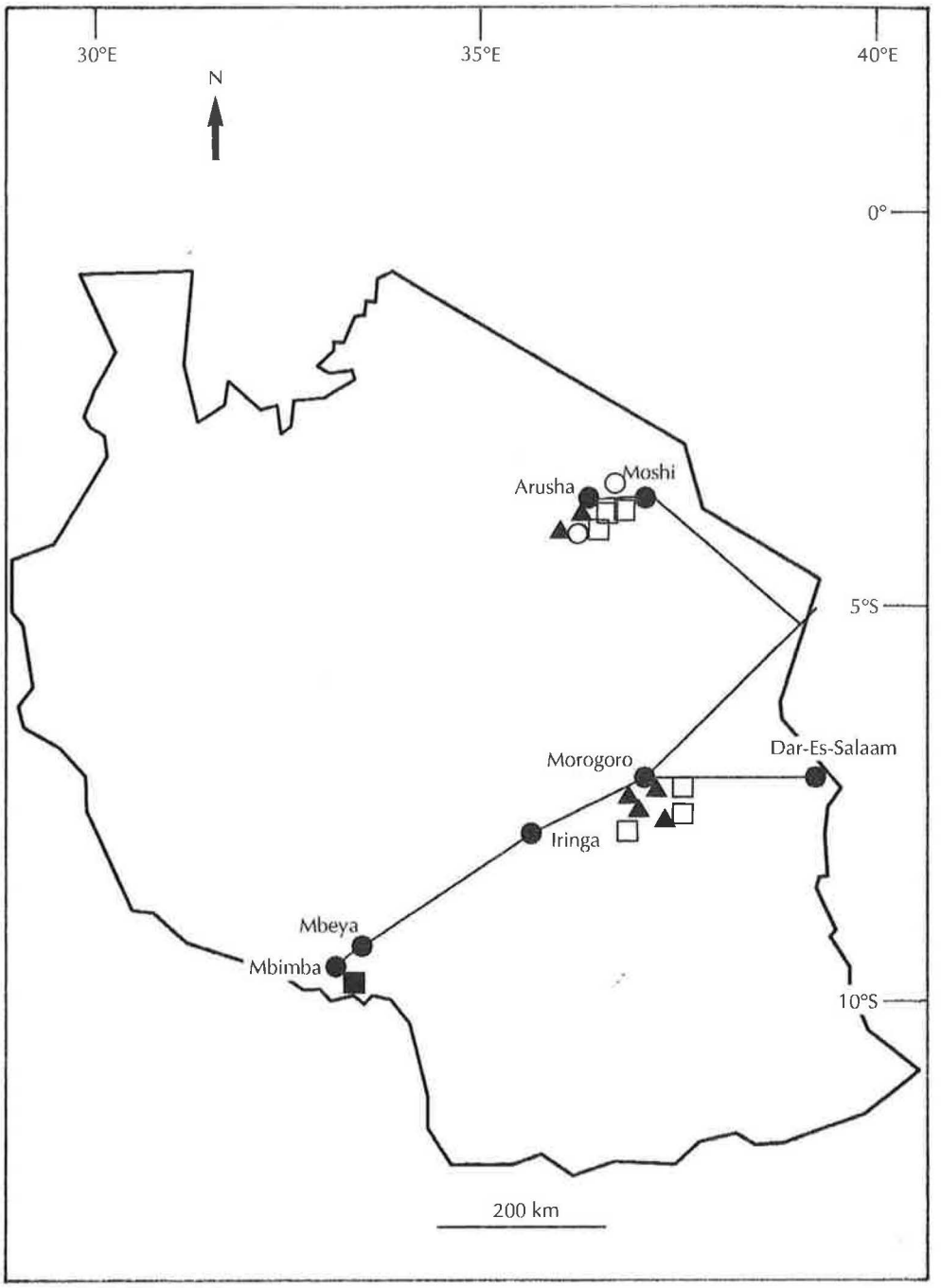


Figure 9 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Tanzania. A number of isolates were identified: NL1, ■; NL3, ▲; NL8, ○; Novel, □.

GN59. Isolate 405 differed from the NL3 strain-type by failing to infect the group 5 cultivar Pinto 114. Three possible novel isolates were obtained at this site which resembled the NL6 strain in causing a temperature-sensitive necrotic reaction in group 8 and 9 cultivars. Isolates 403 and 404 had differential host reactions that were very similar (Table 13) and they could be of the same novel strain type. They both differed from the NL6 strain type in failing to infect host group 2 and 3 cultivars. In contrast isolate 407 infected group 2 but not group 3 cultivars (Table 13).

Further south at Mbimba (1700 m), the bean plants were older and conditions were very dry so it was difficult to observe virus symptoms. Virus was detected by ELISA however, in two samples and a NL1 strain-type was isolated from one of these samples (412). South of Mbeya samples were taken from isolated plants with severe leaf distortion symptoms but no virus was detected.

In northern Tanzania, east of Moshi (1000 m), several samples were infected with an unidentified potyvirus not related to BCMV (see Section 4). Between Moshi and Arusha what seemed to be the same unidentified potyvirus was found, as well as two samples infected with the 'A' serotype of BCMV.

Around Arusha samples were taken from several farmers' fields where the incidence of BCMV was low. ELISA tests showed most samples to be infected with the 'A' serotype of BCMV and one isolate (451) had host differential reactions that conformed with the NL3 strain-type (Table 11)

A second isolate (454) differed from the NL3 strain-type in not infecting the group 5 cultivar Pinto 114 and in this respect it was very similar to the previously described isolate (405) found at Morogoro. In common with isolate 405 it induced a necrotic reaction in the group 10 cultivar Amanda at 32 °C (Table 11).

Four other 'A' serotype isolates (442,444,450 and 456) were also found in the Arusha area. Of these isolates, 442 and 456 caused differential host reactions that generally conformed with the NL8 strain-type indicating that they carried only the *P*2 pathogenicity gene (Table 15). They differed from the standard NL8 strain, however, in that 442 induced systemic necrosis in the cv Jubila at 26 °C and 32 °C. The other two isolates (444 and 450) had differential host reactions that suggested that they may be novel. Isolate 444 appears to carry both *P*1 and *P*2 pathogenicity genes, infecting host group 2 cultivars, but not Pinto 114 (Table 15), while isolate 450 also appears to carry the *P*1 and *P*2 genes, but infects cv Pinto 114 and not host group 2 cultivars.

In Tanzania NL3 and NL8 strain-types were again the predominant BCMV strains found. Of the 'B' serotypes found, isolates that caused a temperature-dependent necrosis in host group 8 and 9 cultivars were again prevalent, but although they generally caused similar symptoms to the standard NL6 strain, they were quite distinct.

Uganda

The main survey was carried out in May 1991, although some samples were collected during January 1990 in off-season multiplication trials (Figure 10). At Bukalasa Research Station, in off-season trials two isolates were found (4 and 5) which conformed to the NL3 strain-type except that they induced no systemic necrosis in cv Jubila and isolate 4 did not infect cvs RGC and GN59 (Table 16).

In the May survey, isolates (458, 459 and 460) from field trials at Kawanda Research Station were found to be similar to the standard NL3 strain except that they all failed to induce systemic necrosis in cv Jubila at 26 °C (Table 16), and at Bukalasa a bean sample (464) with severe mosaic symptoms was infected with an isolate that conformed to the NL1 strain-type. Sample 464 is discussed in Section 4 (Table 21), as it was collected near a wild legume isolate (465) of BCMV.

In collections from farmers' fields between Bukalasa and Kampala at 1300 m, an isolate (471) that conformed to the NL3 strain-type was found which induced systemic necrosis in cv Amanda at 32 °C. A potyvirus was detected in another sample but not isolated. South of Kampala, collections were made in several farmers' fields by the roadside towards Kabale at 1500 m. The necrotic 'A' serotype of BCMV was detected in several of these samples and isolates from two samples (481 and 482) were typical of the standard NL3 strain, except 482 induced systemic necrosis in cv Amanda at 32 °C (Table 16). Around Kabale samples were collected from fields at approximately 1900 m and the 'A' serotype was detected by ELISA in four samples although no virus was isolated. The Research Sub-Station for Highland Crops at Kalangyere had several bean trials at an altitude of 2500 m, which represents the upper altitude limit for bean cultivation in Africa. Severe virus symptoms were observed in a few of these plants and these probably resulted from seed-borne infection. An 'A' serotype strain was detected in one sample (494). At the Rubaare Agricultural Station (1700 m), there were several bean field trials. Three 'A' serotype infections were

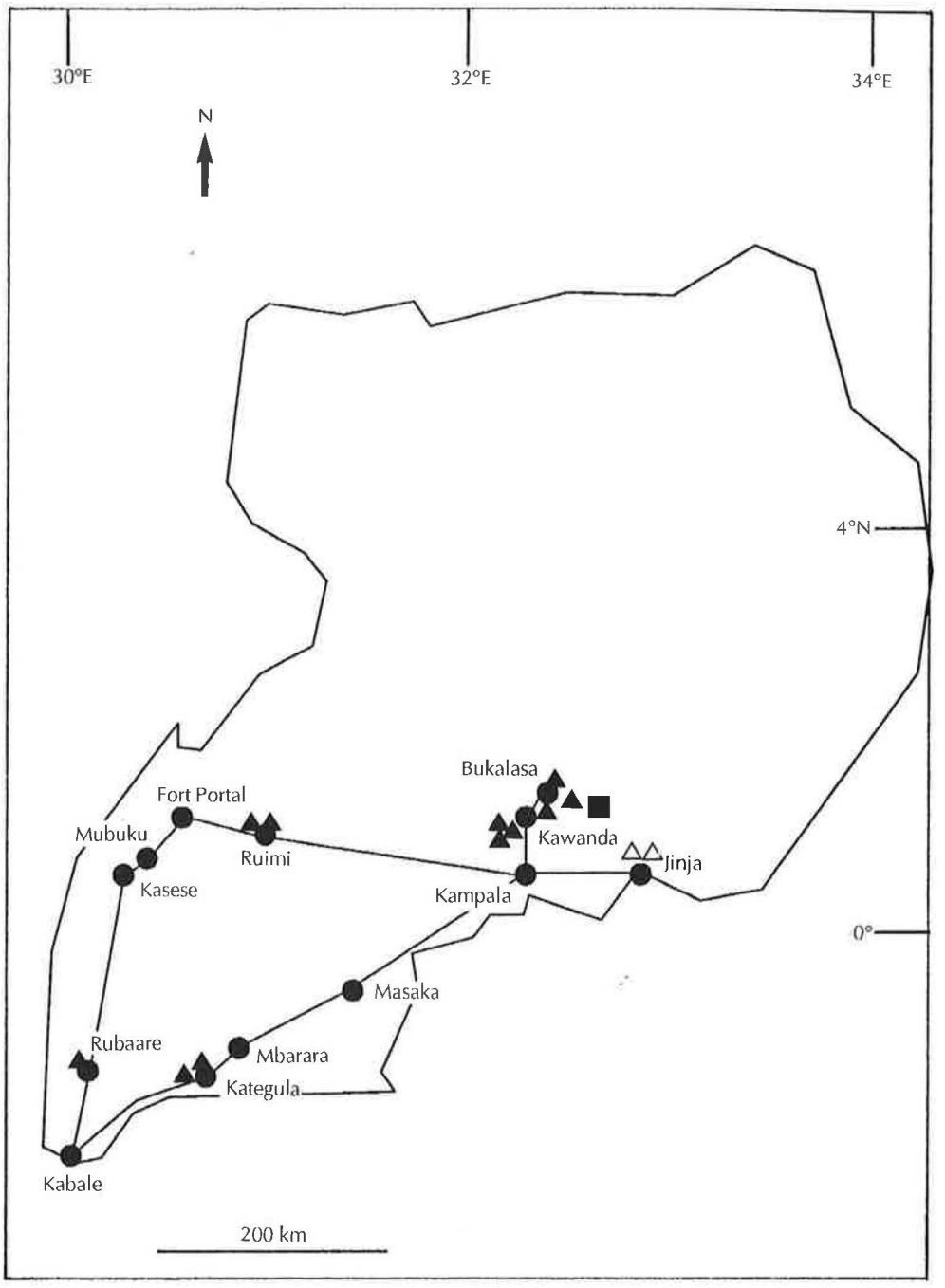


Figure 10 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Uganda. A number of isolates were identified. The NL6 strain-types were from seed samples from Jinja market: NL1, ■; NL3, ▲; NL6, △.

detected by ELISA from this site and an isolate from one of these samples (497) was distinct. It did not cause symptoms in host group 2 cvs PGW and RGC and it induced systemic necrosis in cv Amanda at 32 °C. (see Table 22). East of Kasese (1200 m), samples were taken from various farmers' fields and of five 'A' serotypes detected two (504 and 505) conformed to the NL3 strain-type. Samples 504 and 505 were similar to 497 in that they too induced systemic necrosis in cv Amanda at 32 °C (Table 22). The host differential reactions of these three isolates are summarized in Table 22 and discussed on p. 57 as a NL3 strain-type (499) was isolated from a wild legume nearby. In addition, isolates from seed samples collected in the market in Jinja, had differential host reactions that conformed to the standard NL6 strain, except neither induced systemic necrosis in cv Jubila at 32 °C and 194 failed to cause symptoms in RGC (Table 13).

Table 16 The reactions of differential host cultivars to various BCMV isolates from Uganda compared with the standard NL3 strain

Differential Hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates								
Group	Cultivar	NL3	4	5	458	459	460	471	481	482
1	The Prince	+	+	+	+	+	+	+	+	+
	SGR	+	+	nt	nt	nt	+	+	+	+
	Double White	+	nt	nt	+	+	+	+	+	+
	Sutter Pink	+	nt	nt	+	+	nt	nt	nt	nt
	CRM	+	+	+	nt	nt	+	+	+	+
2	PGW	+	+	+	+t	+	+t	+	+	+
	RGC	+	-	+	+t	+	+t	+	+t	+
3	RGB	+	+	+	+	+	+t	+	+t	+
	GN 59	+t	-	+t	nt	nt	nt	nt	nt	nt
4	Michelite	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+	+
6	Monroe	-	-	-	-	nt	-	-	-	-
	RM 35	-	-	-	-	-	-	-	-	-
	GN 31	-	nt	nt	nt	-	nt	nt	nt	nt
8	BTS	N	N	N	N	N	N	N	N	N
	Widusa	N	N	N	N	N	N	N	N	N
9a	Jubila	N	n	n	n	n	n	n	n	n
9b	ITG	N	N	N	N	N	N	N	N	N
	TC	N	n	N	N	N	N	N	N	N
10	Amanda	-	-	-	-	-	-	n	-	-
Pathogenicity genes		*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P2* *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*

Notes:
+ host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA.
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C but not at 26 °C

In Uganda the predominant strain found in beans in farmers' fields and at research stations was of the NL3 strain-type. The single occurrence of a NL1 strain-type at the Bukalasa trial site is probably explained by the fact that the experimental plots from which it was isolated may have been infected from seed-transmitted virus. Likewise, a *Cassia hirsuta* plant growing nearby was also infected with a NL1 strain-type (465) (Section 3), and this too was probably infected from the same source. The only NL6 strain-type isolates were found in seed samples. Although the NL8 strain-type was not isolated from *P. vulgaris* in Uganda, isolates very similar to NL8 were identified from *Crotalaria incana* and *Glycine max* growing at Nakabango, near Jinja (see Section 3).

Zaire

A very limited survey was made in Zaire in farmers' fields around the Mulungu area (Figure 1). Many plants showed severe leaf deformity with mosaic and chlorotic mottle symptoms. However, in this area it appeared that soil nutrient deficiencies could be responsible for these symptoms. Virus was only detected in two samples which were infected with the 'A' serotype. An isolate (550) was obtained from one of these samples that appeared to be novel, in that it differed from the NL3 strain in failing to infect host group 2 cultivars and from the NL8 strain by infecting group 3 and group 5 cultivars (see Table 15).

Table 17 The reactions of differential host cultivars to various BCMV isolates from Zaire and Zambia compared with the standard NL3 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates							
Group	Cultivar	NL3	1004	1007	1011	1014	3496	3507	3515
1	The Prince	+	+	+	+	+	+	+	+
	SGR	+	nt	nt	nt	nt	+	+	+
	Double White	+	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	nt	nt	nt
	CRM	+	nt	nt	nt	nt	+	+	+
2	PGW	+	+	+	-	+	-	-	-
	RGC	+	+	+	+t	+	-	-	-
3	RGB	+	+	+	+t	+	+	-	-
	GN 59	+t	nt	nt	nt	nt	+	-	-
4	Michelite	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	-	+
6	Monroe	-	nt	nt	nt	nt	-	-	-
	RM 35	-	-	-	-	-	-	-	-
	GN 31	-	-	-	-	-	-	-	-
8	BTS	N	N	N	N	N	N	n	N
	Widusa	N	N	N	N	N	N	n	N
9a	Jubila	N	N	N	N	N	N	n	n
9b	ITG	N	N	N	N	N	N	N	N
	TC	N	N	N	N	n	N	N	N
10	Amanda	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P2*	*P0* *P1* *P2*

Notes:
+ host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, but not at 26 °C

Three 'A' serotypes (3496, 3507 and 3515) were also isolated from Zaire germplasm received from the Royal Botanic Gardens, Kew, although the origins of this seed were not known. All three isolates had differential host reactions that were distinct from the NL3, NL5 or NL8 strain-type and they could be novel (Table 17). Isolate 3496 differed from the standard NL3 strain in failing to infect host group 2 cultivars and isolate 3515 differed in not infecting group 2 or 3 cultivars. Isolate 3507 resembled the NL8 strain in only infecting cultivars of host groups 1 and 4, but it caused a systemic necrosis in group 8, 9a and 9b cultivars (Table 17).

Zambia

The only samples tested from Zambia were preserved as dried leaves collected from several locations. Four samples isolated from Lutembwe (1004), Thanila (1007), Kalichero (1011) and Msekera Research Station (1014) (see Figure 1) were infected with isolates that induced reactions in the differential hosts that conformed to the NL3 strain-type (Table 17). They differed from the standard NL3 strain in that isolate 1007 did not induce systemic necrosis in cv Jubila at 32 °C and 1014 did not induce systemic necrosis in cv TC at 26 °C.

Two 'B' serotype isolates were also obtained from Zambia. One (1003) was collected at Msandile which had differential host reactions which conformed to the NL6 strain-type, and the other (1008) was found at Mangwe and although it induced temperature-sensitive necrosis in group 8 and 9 hosts, it differed from the NL6 strain-type in not infecting the group 3 cultivar RGB (Table 8). This indicated that the isolate was novel as it did not carry the *P1*^{2} pathogenicity gene.

The prevalence of NL3 and NL6 strain-types in Zambia was similar to the situation in Malawi, Uganda and Zimbabwe.

Zimbabwe

A survey was made in January 1990 (Figure 11). In field plots at the Crop Breeding Institute, Harare, the local cultivar Natal Sugar (non-*I* gene germplasm) showed strong mosaic symptoms and another adjacent plot containing *I* gene germplasm had typical blackroot symptoms. An isolate similar to the NL3 strain-type was isolated from a sample (114) of cv Natal Sugar with mosaic symptoms and it appeared that the virus had spread by aphid transmission from seed-borne infection in this non-*I* gene cultivar, to the adjacent *I* gene cultivars to produce the blackroot symptoms (see Plate 10). Isolate 114 differed from the standard strain of NL3 in that it failed to induce symptoms in cvs PGW and GN59 and it did not cause systemic necrosis in Jubila at 32 °C (Table 18). An isolate typical of the US5 strain was also isolated from a Canadian Wonder type cultivar (sample 115) and since this was the only incidence of this strain in Africa, it is likely that its occurrence in Harare may have resulted from the importation of infected seed.

Table 18 The reactions of differential host cultivars to various BCMV isolates from Zimbabwe compared with the standard NL3 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates									
Group	Cultivar	NL3	114	119	121	122	128	134	190	192	191
1	The Prince	+	+	+	+	+	+	+	+	+	+
	CRM	+	+	+	+	+	+	+	+	+	+
2	PGW	+	-	+	+t	+t	+	+t	-	+t	-
	RGC	+	+	+t	+	+	+	+t	+	+t	-
3	RGB	+	+	+	+	+t	+	+t	+	+t	-
	GN 59	+t	-	+t	+t	-	+t	-	-	-	-
4	Michelite	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+	+	+
6	Monroe	-	-	-	-	-	-	-	-	-	-
	RM 35	-	-	-	-	-	-	-	-	-	-
8	BTS	N	N	N	N	N	N	N	N	N	N
	Widusa	N	N	N	N	N	N	N	N	N	N
9a	Jubila	N	N	N	N	N	N	n	N	n	n
9b	ITG	N	N	N	n	N	N	N	n	N	N
	TC	N	N	N	n	N	n	N	N	N	N
10	Amanda	-	-	-	-	-	-	n	-	-	-
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
		P1	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*
		P1^2	*P1*^{2}	*P1*^{2}	*P1*^{2}	*P1*^{2}	*P1*^{2}	*P1*^{2}	*P1*^{2}	*P1*^{2}	
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*

Notes:
+ host susceptible to systemic infection
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA.
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis at 32 °C, but not at 26 °C

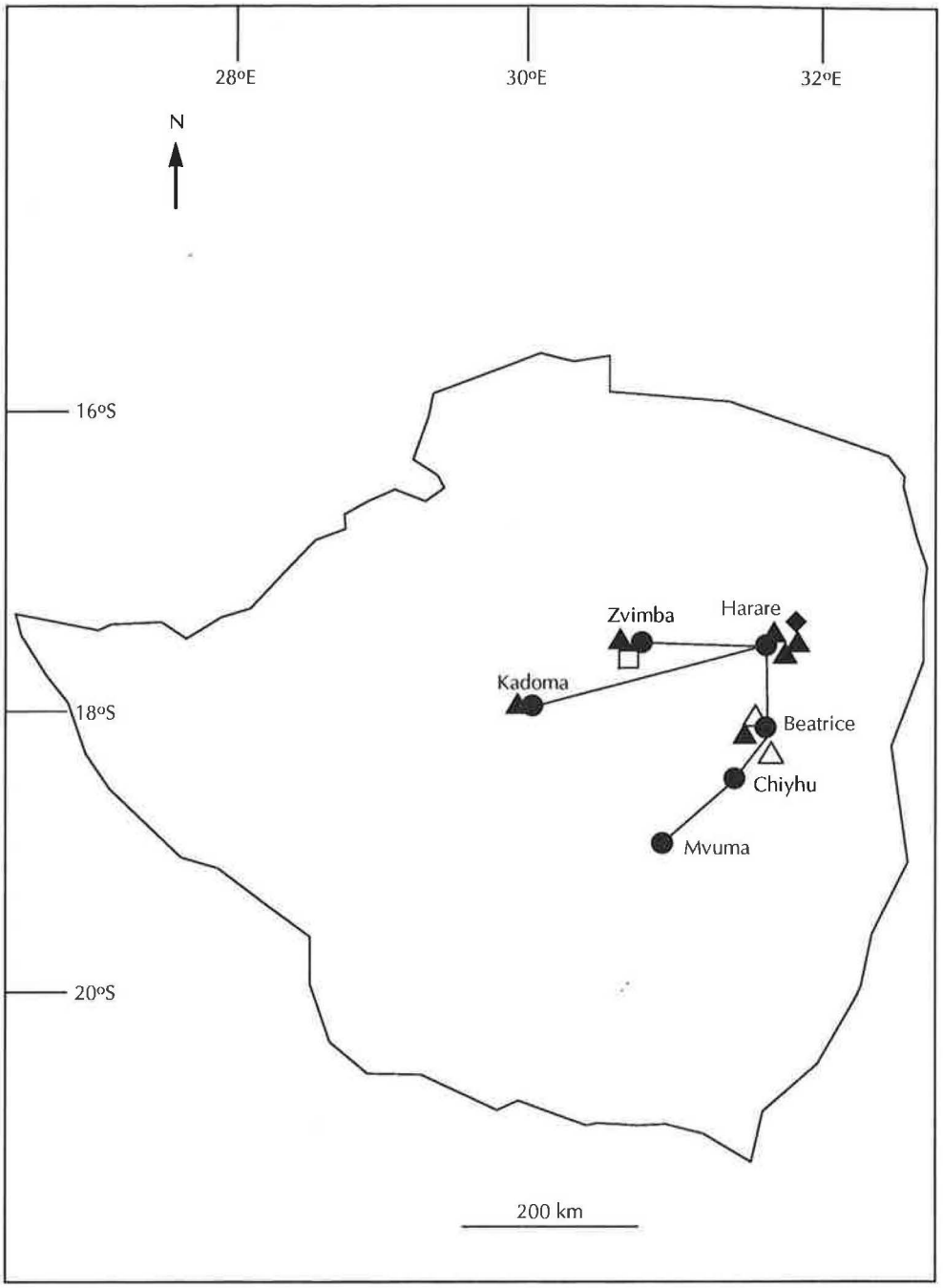


Figure 11 Distribution of BCMV strain-types and novel isolates from *P. vulgaris* in Zimbabwe. A number of isolates were identified including two NL3 strain-types from seed samples from seed samples from Charter and Bikita districts, and one novel isolate from Buhera district: NL3, ▲; NL6, △; US5, ◆; Novel, □.

At other trials at Gwebi College outside Harare, there was a similar situation with a large amount of secondary aphid-transmitted virus spreading from the cv Natal Sugar and causing blackroot in adjacent plots of cultivars with the *I*-gene. Subsequently, two isolations (119 and 121) of a NL3 strain-type were made from Natal Sugar. An NL3 strain-type (128) was also isolated from Natal Sugar at the Cotton Breeding Station at Kadoma. Isolate 119 had differential host reactions typical of the standard NL3 strain but isolates 121 and 128 both failed to induce systemic necrosis in cv TC at 26 °C and isolate 121 also failed to induce systemic necrosis in cv ITG at 26 °C and Jubila at 32 °C (Table 18).

In farmers' fields near Zvimba, a NL3 strain-type (122) was isolated from Natal Sugar and a temperature-dependant necrosis inducing 'B' serotype (127) from a mixture of cultivars. At Tavistock Farm, a site of commercial Natal Sugar production, one isolate of the NL3 strain-type (134) and two isolates (133 and 136) of the NL6 strain-type were found. The differential host reactions of isolate 122 differed from those of the standard NL3 strains in that the isolate did not infect cv GN59 and isolate 134 deviated in not inducing systemic necrosis in cv Jubila at 26 °C, although it did cause systemic necrosis in cv Amanda at 32 °C (Table 18). Isolates 133 and 136 had differential host reactions that were close to the standard NL6 strain, except that 133 induced systemic necrosis in group 8 cultivars at 26 °C as well as at 32 °C and did not infect cvs PGW and RGB (Table 13). In contrast, isolate 127 could be a novel strain. It caused necrosis in group 8 and 9 cultivars, but failed to infect group 2,3,4 and 5 cultivars. Isolates 191 and 192 also failed to induce systemic necrosis in cv Jubila at 26 °C (Table 18). In addition 'A' serotypes were isolated from seed samples collected in Buhera (190), Charter (191) and Bikita (192) districts. Isolate 190 and 191 had differential host reactions that conformed to their being NL3 strain-types, but isolate 191 was distinct and could be novel (Table 18). Isolate 191 failed to infect cultivars of host groups 2 and 3 and caused systemic necrosis in cv Jubila only at 32 °C.

These results indicated that necrotic NL3 strain-types were the prevalent strains in Zimbabwe and that NL6 strain-types were also relatively widespread. The distribution, variation and origins of the BCMV isolates and strains obtained from *P. vulgaris* in this survey are discussed in Section 6.

Section 3

Alternative hosts of BCMV in Africa

INTRODUCTION

Wild legume species are numerous in Africa and there are reports of BCMV and other closely related potyviruses infecting wild legumes in several other parts of the world (Section 1). A study of the ecology and epidemiology of these viruses in wild legume species in Africa is essential, for if such species are natural, indigenous sources of BCMV they could be an important factor to consider in any future resistance breeding strategy against the virus in the areas in which they occur. Such virus reservoirs could provide an inoculum potential for infection of bean crops and the potential for the evolution of new pathogenic strains of BCMV.

This section describes the isolation and identification of viruses from wild legume species and other non-*P. vulgaris* species which were found in areas of Africa where bean crops are grown. The susceptibility of some of the most prevalent wild legume species and a cultivated variety of cowpea (*Vigna unguiculata*) to the standard strains of BCMV is described, and the aphid- and seed-transmission of the BCMV isolates found in legume weeds is demonstrated.

OCCURRENCE OF BCMV IN ALTERNATIVE LEGUME SPECIES

Isolation and identification of virus isolates

Leaf samples were collected from legume species with virus symptoms which were growing close to bean plots and also from plants growing in areas where beans were absent, as described in Appendix 1. In addition, seed was collected from healthy plants and plants showing virus symptoms. The purpose of collecting such seed samples was to isolate virus from infected samples, investigate the susceptibility of healthy seedlings to a range of standard BCMV strains and to test experimentally the seed-transmissibility of BCMV in these species.

The correct identification of the species from which virus was isolated was essential in this study. Herbarium specimens were therefore collected, and photographs taken of all species found with virus symptoms. Legume species were identified with the aid of several keys (Hubbard and Milne-Redhead, 1959; Lock, 1989; Milne-Redhead and Polhill, 1967, 1971a, b; Polhill, 1982; Thulin, 1983).

The isolates from wild legume species and other non-*Phaseolus* species which were identified as BCMV strain-types are summarized in Tables 19 and A10. Many of the strains identified were of the necrotic, 'A' serotype of BCMV. Each of the original field samples of these isolates tested positive in ELISA for

Table 19 Summary of BCMV strain-types isolated from wild legume species identified using differential host cultivars

Country	BCMV strain-types identified					
	NL1	NL3	NL6	NY15	Novel	Total
Kenya	0	0	0	1	1	2
Malawi	0	0	1	0	0	1
Rwanda	0	1	0	0	3	4
Uganda	1	1	0	0	3	5
Total	1	2	1	1	7	12
%	8.3	16.6	8.3	8.3	58.3	

either an 'A' or 'B' serotype of BCMV. After propagation in glasshouse test plants, the isolates were screened in the standard set of BCMV bean host differential cultivars to identify the BCMV strain-type to which they were related (see Section 2, p. 21).

In addition to these isolates, BCMV-related viruses were detected by ELISA in a number of other wild legume samples collected during the survey. This method of detection only identified the serotype of BCMV to which the virus possibly belonged and since no virus was isolated from these samples, the strain of the virus could not be determined by host differential reactions. The BCMV serotypes and other viruses detected in wild legume species by ELISA are summarized in Table 34. A complete summary of the collection information for each wild legume BCMV strain-type is given in Table A10 in the appendix and a summary of virus isolation, ELISA and electron microscopy results is given in Table A11. In addition, Table A12 details the reactions of host differential plants of groups 1 to 6 at 26 °C and Table A13 details the reactions of host groups 8 to 10 at 26 °C and 32 °C. The BCMV strain-types identified are listed in Table 20.

In general, the survey indicated that wild herbaceous legume species that might be hosts of BCMV were most prevalent in the wetter, more humid bean-growing areas of Africa and were most numerous in Uganda, Rwanda, Malawi, Burundi and the wetter parts of Kenya and Ethiopia. Far fewer herbaceous legumes were observed in Lesotho, Zaire, Zimbabwe, Swaziland, Tanzania and the eastern parts of Kenya and Ethiopia and no samples from wild legumes were collected in Lesotho, Zaire, Zimbabwe or Swaziland as none was observed to have virus symptoms.

The individual virus isolations made in each country are described in the following sections.

Uganda

Isolates 28 and 30 were collected from mature *Crotalaria incana* plants showing distinct mosaic symptoms (see Plates 15 and 16) and isolate 38 was from a soybean (*Glycine max*) plant at the pod-stage which showed mosaic leaf symptoms. All three isolates were found within a few hundred metres at Nakabango, near Jinja, and were collected after an off-season bean multiplication trial had been harvested in January, 1990. Samples 29, 32, 34, 35, 36, 37, 39, 40, 41 and 42 were collected from several other wild legume species showing distinct virus symptoms growing along tracks within 200 to 500 m of this site, but no virus was isolated from them. Peanut mottle virus was however, detected by ELISA and subsequently isolated from groundnut (*Arachis hypogea*, sample 31) at the same site (see Section 4).

Table 20 BCMV isolates from wild legumes species in Africa

Isolate number	Host species	Location	ELISA results			Possible BCMV serotype	BCMV strain-type determined by differential hosts
			MAb* 197	PAb† I2	PAb† BLCMV		
28	*Crotalaria incana*	Uganda	4‡	4	0	A	Novel
30	*Crotalaria incana*	Uganda	4	4	0	A	Novel
38	*Glycine max*	Uganda	4	4	0	A	Novel
145	*Rhynchosia* sp.	Malawi	4	0	0	B	NL6
465	*Cassia hirsuta*	Uganda	3	0	0	B	NL1
499	*Macroptilum atropurpureum*	Uganda	4	4	0	A	NL3
531	*Vigna unguiculata*	Rwanda	4	4	0	A	NL3
820	*Cassia sophera*	Rwanda	2	3	2	A	Novel
830	*Cassia sophera*	Rwanda	4	4	2	A	Novel
836	*Cassia sophera*	Rwanda	4	4	3	A	Novel
956	*Vigna vexillata*	Kenya	4	0	2	B	NY15
963	*Crotalaria comanestiana*	Kenya	4	0	1	B	Novel
Standard control strains							
NL3			4	4	0	A	NL3
NL4			4	0	0	B	NL4
BLCMV-NR¶			1	0	4	B	
CAbMV§			1	0	2	B	

Notes:
* Monoclonal antibodies
† Polyclonal antisera to BLCMV-NR
‡ ELISA absorbance values at 405 nm:1 = <0.3, 2 = 0.3–0.7, 3 = 0.7–1.1, 4 = >1.1, 0 = negative result
¶ Blackeye cowpea mosaic virus, necrotic ringspot strain
§ Cowpea aphid-borne mosaic virus

The reactions of isolates 28, 30 and 38 on the bean differential hosts (Table 21) conformed closely with the standard NL8 strain, but differed in the type of systemic symptom they caused in cultivars of host resistance groups 1 and 4. Instead of a systemic mosaic symptom typical of BCMV in these non-*I* gene cultivars, the isolates caused a systemic veinal necrosis (Plate 17). However, typical necrotic lesions followed by systemic 'blackroot' symptoms were induced in cultivars of group 8 which carry the *I* gene (Table 21). Although these results indicated that the three isolates varied slightly from the standard NL8 strain, they showed that like the NL8 strain, isolates 20, 30 and 38 carried the *P2* pathogenicity gene. The ELISA results (Table 20) confirmed that these three isolates were the necrotic 'A' serotype of BCMV. In ISEM decoration tests, all three isolates were decorated by polyclonal antibodies to the standard 'A' serotype NL3 strain, but were not or only slightly decorated by antibodies to the standard 'B' serotype strains NL4, NY15, NVRS or BLCMV-NR. All three isolates produce local chlorotic spot lesions followed by systemic chlorotic spots and mottle in *Chenopodium quinoa* seedlings (Plate 18). In contrast, the standard NL8 strain did not infect *C. quinoa* systemically, although it did induce local chlorotic lesions. Isolates 28, 30 and 38 were propagated in *C. quinoa* and purified from this host for diagnostic studies. In addition, an antiserum was produced to isolate 30 for further diagnostic studies (see Section 5). The serological identification of these isolates as necrotic, 'A' serotypes and the similarities in their reactions in the host differential bean cultivars to those of the standard NL8 strain, demonstrated that these isolates were closely related, but not identical to NL8. It is proposed that they should be designated as novel strain-types.

Table 21 The reactions of differential host cultivars to various BCMV isolates from legume weeds and bean compared with the standard NL8 and NL1 strains.

Differential hosts		Infectibility of differential hosts inoculated with the standard NL8 and NL1 strains and unidentified isolates*						
Group	Cultivar	NL8	28	30	38	NL1	464	465
1	The Prince	-	-	-	-	+	+	+
	SGR	-	nt	nt	nt	+	nt	+
	Double White	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	+	+
	CRM	+	nt	+	+	+	nt	+
2	PGW	-	-	-	-	-	-	-
	RGC	-	-	-	-	-	-	-
3	RGB	-	-	-	-	-	-	-
4	Michelite	+	+	+	+	-	-	-
	Sanilac	+	+	+	+	-	-	-
5	Pinto 114	-	-	-	-	-	-	-
6	Monroe	-	-	-	-	-	nt	nt
	RM 35	-	-	-	-	-	-	-
	GN 31	nt	-	-	-	-	-	-
8	BTS	N	n	n	n	-	-	-
	Widusa	N	n	n	n	-	-	-
9a	Jubila	-	-	-	-	-	-	-
9b	ITG	-	-	-	-	-	-	-
	TC	-	-	-	-	-	-	-
10	Amanda	-	-	-	-	-	-	-
Pathogenicity genes		*P0* *P2*	*P0* *P2*	*P0* *P2*	*P0* *P2*	*P0*	*P0*	*P0*

Notes: * Isolates 28 and 30 were isolated from *Crotalaria incana*; 38 from *Glycine max*; 464 from *Phaseolus vulgaris* and 465 from *Cassia hirsuta*
+ Host susceptible to systemic infection
- Host resistant to systemic infection
nt Not tested
N Systemic necrosis at 26 °C and 32 °C
n Systemic necrosis only at 32 °C, but not at 26 °C

Several more samples were collected from wild legumes in Uganda in the May, 1991 survey. Sample 461 from *Crotalaria ochroleuca*, being grown as a green manure crop, was collected at Kawanda Research Station and shown by ELISA to be infected with a potyvirus, possibly a 'B' serotype of BCMV, but no virus was isolated. At Bukalasa Field Station sample 465 from *Cassia hirsuta* was infected with a potyvirus which reacted like a 'B' serotype in ELISA (Table 20) and produced symptoms on host differentials which conformed to the standard NL1 strain (Table 21). Sample 464 from a bean growing adjacent to this legume was similarly infected with a NL1 strain-type. At the same site, samples from *Crotalaria incana* and a *Desmodium species*, (possibly *D. cajanifolium*) were shown by ELISA to be infected with the 'A' serotype although no virus was isolated.

In western Uganda at Mubuku, east of Kasese, sample 499 from *Macroptilium atropurpureum* with vivid mosaic systems was infected with the 'A' serotype (Table 20) which produced differential host reactions that conformed to the NL3 strain-type. It only differed from the standard NL3 strain in that it did not infect the cv Jubila systemically at 26 °C (Table 22). In the same field a bean plant was infected with a possible 'A' serotype but no other virus symptoms were seen on beans. However, a few kilometres away at Kyanga three bean samples were

Table 22 The reactions of differential host cultivars to various BCMV isolate from legume weeds and bean compared with the standard NL3 strain.

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and unidentified isolates*					
Group	Cultivar	NL3	499	504	505	497	531
1	The Prince	+	+	+	+	+	+
	SGR	+	+	+	nt	+	nt
	Double White	+	+	+	+	+	+
	Sutter Pink	+	+	nt	+	nt	+
	CRM	+	+	+	nt	+	nt
2	PGW	+	+	+	+	-	-
	RGC	+	+	+	+	-	+t
3	RGB	+	+	+	+	+t	+t
4	Michelite	+	+	+	+	+	+
	Sanilac	+	nt	+	+	+	+
5	Pinto 114	+	+	+	+	+	+
6	Monroe	-	-	-	-	nt	-
	RM 35	-	-	-	-	-	-
	GN 31	-	-	nt	-	nt	nt
8	BTS	N	N	N	N	N	N
	Widusa	N	N	N	N	N	n
9a	Jubila	N	n	n	N	n	n
9b	ITG	N	N	N	N	N	n
	TC	N	N	N	N	N	N
10	Amanda	-	-	n	n	n	n
Pathogenicity genes		*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*

Notes:
* Isolate 499 was isolated from *Macroptilium atropurpureum*; 531 from *Vigna unguiculata* and 497, 504 and 505 from *Phaseolus vulgaris*
\+ Host susceptible to systemic infection
nt Not tested
\- Host resistant to systemic infection
+t Symptomless host but systemic infection detected by ELISA, tolerant reaction
N Systemic necrosis at 26 °C and 32 °C
n Systemic necrosis at 32 °C, but not at 26 °C

infected with 'A' serotype isolates and NL3 strain-types were isolated from two (504 and 505). The differential host reactions of isolate 504 from beans were similar to those of the *Macroptilium atropurpureum* isolate in that neither systemically infected the cv Jubila at 26 °C. In addition, and in contrast to isolate 499, isolates 504 and 505 induced systemic necrosis in cv Amanda at 32 °C (Table 22). The 'A' serotype (497), found at the same site as isolate 499, was quite distinct in that it did not infect group 2 host cultivars (Table 22). This could be a novel strain.

Rwanda

An 'A' serotype (531) of BCMV was isolated in the May, 1991 survey, from *Vigna unguiculata* (Table 20) growing as a weed in a bean plot on a farm at Runinya near Butare. This isolate produced reactions in the bean host differentials similar, but not identical to the standard NL3 strain. It induced no systemic symptoms in

cvs Widusa or Jubila at 26 °C, but systemic necrotic symptoms developed in cv Amanda at 32 °C and cv PGW failed to develop systemic mosaic symptoms (Table 22). In the same field most bean samples were infected with 'A' serotype isolates and a NL3 strain-type isolate (530) was isolated from one sample and NL8 strain-type isolates (528 and 529) from others. The details of these bean isolates are discussed in Section 2 and their host reactions shown in (Tables 14 and 15)

In November, 1991, Rwandan sample 820 from *Cassia sophera* (see Plate 19) reacted in ELISA with monoclonal antibodies 197 and I2 suggesting it was an 'A' serotype of BCMV (see Section 7.1), but it also reacted in ELISA with polyclonal antisera to BLCMV-NR (Table 20). Its differential host reactions showed that it was distinct from the standard NL8 strain in that it induced latent infection in host group 2 and 3 cultivars (Table 23). This isolate could therefore be a novel strain. Near Ngoma and Kibungo several kilometres from the site of sample 820, necrotic 'A' serotypes were detected in two samples (806 and 807) of *Tephrosia vogelii*, (Plate 20) and in several samples from beans (808–812), but virus was not isolated from any of these samples.

Table 23 The reactions of differential host cultivars to various BCMV isolates from *Cassia sophera* and bean in Rwanda compared with the standard NL8 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL8 strain and unidentified isolates*				
Group	Cultivar	NL8	830	835	820	836
1	The Prince	-	+	+	+	+t
	Double White	+	+	+	+	+
	Sutter Pink	+	+	+	+	-
2	PGW	-	-	-	-	-
	RGC	-	-	-	+t	-
3	RGB	-	-	-	+t	+t
4	Michelite	+	+	+	+	+
	Sanilac	+	+	+	+	+
5	Pinto 114	-	-	-	-	-
6	RM 35	-	-	-	-	-
	GN 31	nt	-	-	-	-
8	BTS	N	N	N	N	N
	Widusa	N	N	N	N	N
9a	Jubila	-	-	-	-	-
9b	ITG	-	n	-	-	-
	TC	-	N	-	-	-
10	Amanda	-	-	-	-	-
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*
					P1	
					$P1^2$	$P1^2$
		P2	*P2*	*P2*	*P2*	*P2*

Notes: * Isolates 820, 830 and 836 were isolated from *Cassia sophera* and 835 from *Phaseolus vulgaris*
+ Host susceptible to systemic infection
nt Not tested
- Host resistant to systemic infection
+t Symptomless host but systemic infection detected by ELISA, tolerant reaction
N Systemic necrosis at 26 °C and 32 °C
n Systemic necrosis at 32 °C, but not at 26 °C

At Nemba, north of Butare on the Kigale road (see Figure 1), sample 830 from *Cassia sophera* induced reactions on host differentials that were sufficiently different to those of the standard NL8 strain for it to be considered a novel strain. It differed from the standard NL8 strain by inducing necrotic symptoms in host group 9b cultivars (Table 23). An isolate (835) from bean at a nearby site induced differential host reactions that conformed closely to the NL8 strain-type (Table 23) At another site in the village of Musambira in the Gitarama district a further 'A' serotype was isolated from *Cassia sophera* (836) which produced reactions on host differentials which were distinct from those of the standard NL8 strain in that it caused a latent infection in cv RGB. This could be a novel strain. The three isolates from *Cassia sophera* (820, 830 and 836) were similar to each other in that all reacted in ELISA with BLCMV-NR. The significance of these novel isolates is discussed later in Section 6 p.94.

Malawi

Isolate 145 was collected from a non-flowering wild legume (a *Rhynchosia* species) showing mosaic symptoms at Chimphira in the northern district of Malawi. It induced reactions in the bean differential host cultivars that were similar to those caused by standard NL6 strain except that it did not induce a reaction in the cv RGC (see Table 24) and ELISA tests confirmed that 145 was a 'B' serotype of BCMV (see Table 20). No isolates of the NL6 strain-type were found in beans in the vicinity of where this sample was collected but in southern Malawi near Dedza, a NL6 strain-type was isolated from a bean seed sample (197)(see Table 24). Elsewhere in Malawi a number of isolates (149, 151, 178 and 179) were found which induced a temperature-dependent necrosis in group 8 and 9 cultivars, but whose differential host reactions were otherwise distinct from those of the NL6 strain-type (Table 24). The details of these possible novel strains are discussed in Section 6 p.94. Isolates 158 and 165 were collected from other legume species with virus symptoms in Malawi, but no virus was detected in them.

Burundi

Samples were collected from *Desmodium dichotomum* which showed virus symptoms at Ruzibe (848) and Bujumbura (886) and although 'A' serotypes were detected by ELISA in both samples no virus was isolated. Also at Ruzibe, a possible 'B' serotype was detected by ELISA in *Neonotonia wightii* (851) showing distinct mosaic symptoms and a few kilometres north of Bujumbura 'A' serotypes were detected in *Eriosema psoratoides* (868) showing leaf mottle symptoms and in an unidentified wild legume (871) showing mosaic symptoms (Plate 21). No virus was isolated from these samples.

Kenya

In January, 1990, several cowpeas and a legume species known locally as 'Omto' were sampled at the University of Nairobi but virus was not detected. In November, 1991, as part of the bean survey many more legume species suspected of being infected with virus were collected. Near the KARI Research Station at Katumani samples were collected of pigeon pea (*Cajanus cajan*) with chlorotic leaf blotch symptoms (Plate 22). The necrotic 'A' serotype was detected by ELISA in two of these samples (904 and 905) but no virus was isolated. Similarly, east of Meru, the 'A' serotype was detected in a further two pigeon pea samples (918 and 919) without isolation of virus.

Table 24 The reactions of differential host cultivars to various BCMV isolates from a wild legume species and beans in Malawi compared with the standard NL6 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL6 strain and unidentified isolates*						
Group	Cultivar	NL6	145	197	149	151	178	179
1	The Prince	+	+	+	+	+	+	+
	SGR	+	+	-	nt	nt	nt	nt
	Double White	+	+	nt	nt	nt	nt	nt
	Sutter Pink	+	+	nt	nt	nt	nt	nt
	CRM	+	+	+	+	+	+	+
2	PGW	+	+	+	-	-	-	-
	RGC	+	-	-	-	+	-	+t
3	RGB	+	+	+	-	-	-	-
	GN 59	+	+t	-	-	nt	-	-
4	Michelite	-	-	-	-	-	-	-
	Sanilac	-	-	-	-	-	-	-
5	Pinto 114	-	-	-	-	-	-	-
6	Monroe	-	nt	-	-	-	-	-
	RM 35	-	-	-	-	-	-	-
	GN 31	nt	-	nt	nt	nt	nt	nt
8	BTS	n	N	n	n	N	n	N
	Widusa	n	N	n	n	n	n	N
9a	Jubila	n	n	-	n	n	n	-
9b	ITG	n	n	n	n	N	n	n
	TC	n	n	n	n	N	n	n
10	Amanda	-	-	-	-	-	-	-
Pathogenicity genes		*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0*	*P0* *P1*	*P0*	*P0* *P1*

Notes: * Isolate 145 was isolated from a wild legume (possibly *Rhynchosia*) and 197 from *Phaseolus vulgaris* seed
+ Host susceptible to systemic infection
nt Not tested
- Host resistant to systemic infection
+t Symptomless host but systemic infection detected by ELISA
N Systemic necrosis at 26 °C and 32 °C
n Systemic necrosis at 32 °C, but not at 26 °C

In western Kenya, near Kisii, where a NY15 strain-type was found in bean, several wild legume species also had virus symptoms. At Nyangusu a 'B' serotype isolate (956) from *Vigna vexillata* produced differential host reactions that conformed to the standard NY15 strain, except no symptoms were produced in cv RGC (Table 25). However, as RGC is tolerant to the NY15 strain this could have been due to a failure to detect low concentrations of virus by ELISA. Isolates 953 and 955 from beans growing adjacent to the *Vigna* species produced similar reactions to 956 on the differential except that isolate 953 failed to infect cv PGW (see Table 25) and Section 2. At Nyangena, about 30 km from the Nyangusu site, isolates were obtained from *Crotalaria comanestiana* (963) and bean (961) in the same farmers' field. Their ELISA reactions showed them to be 'B' serotypes and both had similar differential host reactions (Table 25) that suggested that they were of a novel strain-type. The details of these isolates and other similar 'B' serotypes isolated in the Kisii area are described further in Section 2.

Table 25 The reactions of differential host cultivars to various BCMV isolates from wild legume species and bean in Kenya compared with the standard NY15 strain.

Differential hosts		Infectibility of differential hosts inoculated with the standard NY15 strain and unidentified isolates*										
Group	Cultivar	NY15	955	956	961	963	964	967	953	973	959	970
1	The Prince	+	+	+	+	+	+	+	+	+	+	+
	Double White	+	+	+	+	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	+	+	+	-	+	+
2	PGW	+t	+t	+	+	-	+	-	-	-	-	-
	RGC	+	-	-	+	+t	-	+t	-	-	-	-
3	RGB	-	-	-	+	+t	+	+t	-	-	+	+t
4	Michelite	+	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+	+	+	+
6	Monroe	-	nt	nt	-	-	nt	nt	nt	nt	-	nt
	RM 35	-	-	-	-	-	-	-	-	-	-	-
	GN 31	nt	-	-	-	-	-	-	-	-	-	-
8	BTS	-	-	-	-	-	-	-	-	-	-	-
	Widusa	-	-	-	-	-	-	-	-	-	-	-
9a	Jubila	-	-	-	-	-	-	-	-	-	-	-
9b	ITG	-	-	-	-	-	-	-	-	-	-	-
	TC	-	-	-	-	-	-	-	-	-	-	-
10	Amanda	-	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
		P1	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*
					P1^2	*P1*^{2}	*P1*^{2}	*P1*^{2}			*P1*^{2}	*P1*^{2}
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*

Notes: * Isolate 956 was isolated from *Vigna vexillata*, 963 from *Crotalaria comanestiana* and 953, 955, 959, 961, 964, 967, 970 and 973 from *Phaseolus vulgaris*
+ Host susceptible to systemic infection
nt Not tested
- Host resistant to systemic infection
+t Symptomless host but systemic infection detected by ELISA

Tanzania

Very few wild legume species were observed in the areas surveyed and no virus was detected in any samples which were collected. A dried sample of *Lablab purpureus* from Arusha and *Neonotonia wightii* from Nkundi with suspected virus symptoms were collected by Dr David Allen, but no virus was detected in either sample.

Zambia

Four dried samples of *Vigna unguiculata* were received from Msekera Research Station and ELISA results suggested that three were infected with BLCMV and one with BCMV, but no virus was isolated.

Ethiopia

Samples were collected from many wild legume species in Ethiopia and although several different viruses were isolated (see Section 4), BCMV was not detected.

THE SUSCEPTIBILITY OF WILD LEGUMES AND A COMMERCIAL COWPEA CULTIVAR TO STANDARD BCMV STRAINS

The twenty-nine wild legume species listed in Table 26 were sap-inoculated with standard BCMV strains NL1, NL3, NL4, NL6 and NL8 and isolates 30 and 316 from wild legumes. Five species (*Cassia didymobotrya, Crotalaria laburnifolia, Desmodium heterocarpon, D. triflorum* and *Rhynchosia sublobata*) of the twenty-nine tested were totally resistant to all the standard BCMV strains used. A further five species, *Centrosema pubescens, Crotalaria anagyroides, C. lanceolata, C. ochroleuca* and *Rhynchosia minima* were susceptible to all five BCMV strains (see Plate 23). Twenty-two species were resistant to the NL1 strain, but many species were susceptible to the other four standard BCMV strains, especially the NL3 strain. In many cases infection was latent and only detected by ELISA, which may reflect tolerance to infection by these wild species compared with cultivated hosts. The overall susceptibility of the legume genera tested to the five standard BCMV strains is summarized in Table 27. This data emphasizes that many wild legume species and genera are susceptible to the prevalent BCMV strain-types occurring in Africa. Only *C. anagyroides, C. incana* and *Vigna angularis* were susceptible to isolate 30, a novel strain-type from *C. incana*. Also only *C. anagyroides* and *R. edulis* were susceptible to isolate 316 (*Cassia* severe mosaic virus).

Table 26 Reactions of wild species of legumes to inoculation with BCMV strains

Legume species	Seed source	Standard BCMV strain					Legume isolates	
		NL1	NL3	NL4	NL6	NL8	30	316
Cassia didymobotrya	Ethiopia	0	0	nt	0	0	0	0
Cassia hirsuta	Uganda	mos	mos/E	E	mos/E	0	0	0
Cassia occidentalis	Ethiopia	0	mos/E	nt	0	mos/E	0	0
Cassia sophera	Ethiopia	0	0/E	nt	0/E	0/E	0	0
Cajanus cajan	Yemen	0	0/E	0	0/E	0	0	0
Centrosema plumieri	Colombia	0	0	0/E	0	0/E	0	0
Centrosema pubescens	Colombia	mos	mos	mos	mos	mos	0	0
Crotalaria anagyroides	Colombia	vc/E	vc/E	vc/E	vc	vc/E	vc	vc
Crotalaria goreensis	Colombia	0	0	0/E	0	0	0	0
Crotalaria incana	Colombia	0	vc/E	0	cl	vc/E	vc/E	0
Crotalaria juncea	Colombia	0	0/E	0	0	0	0	0
Crotalaria laburnifolia	Colombia	0	0	nt	0	0	0	0
Crotalaria lanceolata	Colombia	0/E	vc/E	0/E	0/E	0/E	0	0
Crotalaria ochroleuca	Colombia	mos	mos/E	0/E	0/E	0/E	0	0
Crotalaria retusa	Colombia	0	0	0/E	0	0	0	0
Crotalaria verrucosa	Colombia	0	0/E	0	0/E	0	0	0
Desmodium heterocarpon	Colombia	0	0	0	0	0	0	0
Desmodium triflorum	Colombia	0	0	0	0	0	0	0
Glycine max	USA	0	cl/E	0/E	0	0/E	0	0
Glycine tomentella	Colombia	0	0/E	0	0	0/E	0	0
Indigofera hirsuta	Uganda	0	0/E	0/E	0/E	0/E	0	0
Macroptilium atropurpureum	Colombia	0	0	0	0	mos	0	0
Rhynchosia diversifolia	Colombia	0	0/E	0	0	0/E	0	0
Rhynchosia edulis	Colombia	0	0/E	0	0	0	0	E
Rhynchosia minima	Colombia	mos	n/E	n/E	n/E	n/E	0	0
Rhynchosia sublobata	Colombia	0	0	0	0	0	0	0
Vigna angularis	Yemen	mot	0/E	0/E	mot/E	0	mot/E	0
Vigna radiata	Yemen	0	mot	0	mot/E	0	0	0
Vigna unguiculata	Yemen	0	0/E	0	0/E	0/E	0	0

Key to symptoms: 0, no symptoms; mos, systemic mosaic; vc, systemic vein clearing; cl, systemic chlorosis; mot, systemic mottle; n, systemic necrosis; /E, virus detected by ELISA using the monoclonal antibody 197; nt, not tested.

Table 27 Summary of susceptibility of wild African legume genera to strains of BCMV

Genus	No. species tested	No. susceptible to BCMV strains				
		NL1	NL3	NL4	NL6	NL8
Cassia	4	1	3	1	2	2
Cajanus	1	0	1	0	1	0
Centrosema	2	1	1	2	1	2
Crotalaria	9	3	6	5	4	4
Desmodium	2	0	0	0	0	0
Glycine	2	0	2	1	0	2
Indigofera	1	0	1	1	1	1
Macroptilium	1	0	0	0	0	1
Rhynchosia	4	1	3	1	1	2
Vigna	3	1	3	1	3	1
Total	29	7	20	12	13	15

Table 28 Susceptibility of *Vigna unguiculata* variety California Blackeye no. 5 to standard BCMV strains and isolates from wild species of legumes

Isolate	Symptom*	ELISA†	Overall susceptibility‡
NL1	st, mos, lvn, vn	+	+
NL3	st, mos, lvn, vn, ln	+	+
NL4	st, mos, lvn, vn	+	+
NL5	mos, lvn, vn	+	+
NL6	st, mos, lvn, vn	+	+
NL8	st, mos, lvn, vn, ln	+	+
NY15	st, mos, lvn, vn	+	+
28	mos, vn	+	+
30	mos, vn, ld	+	+
38	st, mos, lvn	nt	+
145	mot, vc	-	+
316	0	-	-
465	0	nt	?
499	0	-	-
820	0	-	-
821	0	+	+
830	mot, lvn	+	+
963	mot, ld	+	+
CAbMV	mot, ld	+	+
BlCMV	vc, ln	+	+

Notes: * Key: 0, no symptoms; st, stunting; mos, systemic mosaic; lvn, local vein necrosis; vn, systemic vein necrosis; ln, local necrotic ringspots; ld, leaf distortion; mot, systemic mottle; vc, vein clearing

† ELISA results with monoclonal antibody 197: +, positive; -, negative; nt, not tested.

‡ +, susceptible reaction; -, resistant reaction; ?, inconclusive reaction.

In order to test the susceptibility of cowpea (*Vigna unguiculata*) to infection by standard BCMV strains and other related virus isolates, seedlings of the commercial cv California Blackeye No. 5 were sap-inoculated with the viruses and virus isolates shown in Table 28. California Blackeye no. 5 was very susceptible to all

the standard BCMV strains and the standard strains of BLCMV and CAbMV, but only some of the wild legume isolates of BCMV were able to infect this cultivar. Virus was detected in many samples tested by PTA-ELISA using monoclonal antibody 197 and isolates 28, 30, 38, 830 (novel strain-types) and 963 (a novel 'B' serotype) induced leaf symptoms. Isolates 316, 465, 499 and 820 did not induce virus symptoms and no virus was detected by ELISA in the test cowpea seedlings. Isolate 145 (a NL6 strain-type) did induce virus symptoms but no virus was detected by ELISA, probably because of low virus content. These results indicated that the commercial cultivar California Blackeye no. 5 was susceptible to all standard BCMV strains and to some of the BCMV isolates obtained from wild legumes.

APHID TRANSMISSION OF VIRUS ISOLATES FROM WILD LEGUME SPECIES

Isolates 28, 30 and 38 (novel strain-types) from *Crotalaria incana* in Uganda (propagated in *Chenopodium quinoa*), isolate 145 (a NL6 strain-type) from a *Rhynchosia* species in Malawi (propagated in *P. vulgaris*), isolate 499 (a NL3 strain-type) from *Macroptilium atropurpureum* in Uganda (propagated in *P. vulgaris*) and isolate 316 (cassia severe mosaic virus) from *Cassia occidentalis* in Ethiopia (propogated in *Nicotiana clevelandii*) were used in aphid transmission studies. The transmission of these isolates using short acquisition and inoculation access feeding times was made to *Nicotiana clevelandii, Chenopodium quinoa, P. vulgaris* cvs The Prince and Double White, *Crotalaria incana, Cassia occidentalis* and *Cassia sophera* (Table 29).

Table 29 Aphid-transmission of virus isolates from wild species of legumes

Host species	Aphid transfer of virus isolates					
	28	30	38	145	316	499
Cassia occidentalis	2/2 m	2/2 m	1/2 m	nt	1/2 m	nt
Cassis sophera	1/2v	1/2v	0/2	nt	0/2	nt
Chenopodium quinoa	2/2c	2/2c	2/2c	nt	0/2	nt
Crotalaria incana	2/2v	2/2v	2/2v	nt	1/2c	nt
Nicotiana clevelandii	0/2	0/2	0/2	nt	2/2c	nt
P. vulgaris cv The Prince	0/2	0/2	0/2	nt	0/4	nt
P. vulgaris cv Double White	2/2n	2/2n	2/2n	1/4m	0/4	2/4

Key: Number of seedlings which developed symptoms out of the total number tested: m, mosaic; v, vein clearing; c, chlorosis; n, necrosis; nt, not tested.

The results showed that isolates 28, 30 and 38 were aphid-transmitted in a non-persistent manner to *Crotalaria incana* (the host from which they were isolated), *Cassia occidentalis,* bean cv Double White and *C. quinoa* (the host in which they were propagated). Also, isolates 28 and 30 were non-persistently aphid-transmitted to *Cassia sophera*. Isolate 316 (cassia severe mosaic virus) was aphid-transmitted non-persistently to *Cassia occidentalis* (the host from which it was isolated), *Crotalaria incana* and *N. clevelandii,* but was not transmitted to *Cassia sophera* (a species closely related to *C. occidentalis*) or either of the bean cultivars. Isolates 145 and 499 were only tested for aphid-transmission with cv Double White and they were both non-persistently aphid-transmitted to this cultivar.

SEED TRANSMISSION STUDIES

Seed transmission of standard strain BCMV in wild legume species

Of the twenty-nine species inoculated with the standard BCMV strains and other isolates (Table 26) only seven produced seed. Some plants failed to flower and others flowered but either fruits were not produced, or they contained no seed. Of the plants which did produce seed, some had a low percentage germination and produced relatively few healthy seedlings and others produced many. If a species produced many viable seedlings fifteen were tested for seed-transmitted virus by ELISA, but for species where fewer seedlings germinated all seedlings were tested. The only plants of the species *Crotalaria ochroleuca, Cassia occidentalis* and *C. sophera* to produce seed were those infected with NL3 and NL8, and in each case a proportion of the resultant seedlings were infected (Table 30). Infected plants of *Crotalaria incana* produced seed and a proportion of the resultant seedlings from plants infected with NL1, NL3, NL4, NL6, NL8 and the novel strain-type isolate 30 from *C. incana* in Uganda were infected.

Table 30 Seed transmission of standard BCMV strains in wild species of legumes

Legume species	Seed source	Number of seedlings infected with BCMV strains					
		NL1	NL3	NL4	NL6	NL8	30*
Cassia occidentalis	Ethiopia	nt	2/5	nt	nt	2/5	nt
Cassia sophera	Ethiopia	nt	3/5	nt	nt	2/5	nt
Crotalaria incana	Colombia	4/15†	2/15	1/15	1/15	3/15	5/15
Crotalaria lanceolata	Colombia	0/14	0/15	nt	0/6	0/6	nt
Crotalaria ochroleuca	Colombia	nt	2/5	nt	nt	2/5	nt
Macroptilium atropurpureum	Colombia	3/15	4/15	9/15	1/7	10/15	nt
Rhynchosia diversifolia	Colombia	5/5	2/2	2/2	nt	4/5	nt

Notes: * Isolate 30 from *Crotalaria incana* (Uganda)
† number of infected seedlings total number of seedlings tested
nt not tested as no seed produced

All the standard BCMV strains were seed-transmitted in *Macroptilium atropurpureum* in a proportion of seedlings, although in the host range studies only the NL8 strain was found to infect *M. atropurpureum* following sap inoculation (Table 26). This can only be explained by the concentration of virus in the parent plants of *M. atropurpureum* inoculated with NL1, NL3, NL4 and NL6 being too low to be detected by ELISA, even though some of the seed produced by these plants was infected.

These results have shown that standard isolates of the most prevalent BCMV strains occurring in Africa may be seed-transmitted in a range of wild legume species that commonly occur as weeds in Africa. It should be noted, however, that *R. diversifolia* has not been reported in Africa.

Seed transmission of wild legume virus isolates in *Phaseolus vulgaris*

In some cases the symptoms induced in the bean cvs Double White and Sutter Pink infected with BCMV isolates from wild legumes were so severe that no seed was produced. Alternatively, seed was produced by one cultivar but not by the other (Table 31). Virus isolates 28, 30, 38 (novel strain-types), 499 (a NL3 strain-type) and 963 a novel 'B' serotype prevented seed production in both cultivars.

Table 31 Results of seed transmission of BCMV isolates from wild species of legumes in *Phaseolus vulgaris* cultivars Double White and Sutter Pink

Isolate	Strain-type	Number of infected seedlings			
		Double White		Sutter Pink	
		Mosaic*	ELISA†	Mosaic	ELISA
145	NL6	nt	nt	4/8	4/8
465	NL1	0/7	0/7	nt	nt
820	Novel	3/11	3/11	0/15	0/15
830	Novel	1/10	1/10	0/10	0/10
956	NY15	0/13	0/13	nt	nt

Notes: * seedlings with mosaic symptoms
† seedlings which were positive for BCMV in ELISA
nt not tested as no seed was produced

In contrast, both cultivars produced seed when infected with either isolate 830 or 820 (novel 'A' serotypes). With the latter isolates the resultant seed from Sutter Pink was not infected but a proportion of the seedlings from Double White were. Virus was not transmitted in seed from plants of cv Double White infected with isolates 465 (a NL1 strain-type) or 956 (a NY15 strain-type). Isolate 145 (a NL6 strain-type) was seed-transmitted in cv Sutter Pink, but no seed was produced by plants of cv Double White infected with this isolate. Isolate 277 was an unidentified BCMV isolate from *Phaseolus vulgaris* in Ethiopia (see Section 2 p. 29) and was included as a novel strain-type, this isolate was seed-transmitted in cv Double White. These preliminary seed-transmission studies clearly demonstrated that BCMV strain-types isolates from wild legume species in Africa are seed-transmitted in some susceptible *P. vulgaris* cultivars.

The importance of the occurrence of BCMV isolates in wild legume species is discussed in Section 6.

Section 4

Occurrence and Identification of Other Legume Viruses

INTRODUCTION

Although BCMV was the most common virus isolated from *P. vulgaris* in Africa, a number of other viruses have also been isolated (see Section 1 p. 8). During the field surveys, samples were collected from *P. vulgaris* and a number of wild species of legumes and other non-*Phaseolus* hosts which appeared to have virus symptoms. The BCMV isolates obtained from the *P. vulgaris* samples are described in Section 2 and those from other legume hosts in Section 3. In a number of samples, viruses other than BCMV were detected and isolated, these viruses are described in this section.

RESULTS AND DISCUSSION

Collection details of each sample are given in the Appendix (Table A14). The methods used to identify the viruses were similar to those described in Section 2 and 3 to identify BCMV strains. All samples collected were tested by ELISA with monoclonal antibodies and a range of polyclonal antisera and many were examined with the electron microscope as described in Appendix 1. A summary of the results of these tests, and any subsequent virus isolations, is given in Table A14. The viruses isolated are summarized in Table 32.

In addition to the above viruses, virus infection was also detected in some samples by ELISA and EM tests, but no virus was isolated from them. Further identification of these isolates was therefore, not possible. These included samples which reacted in the initial ELISA test with either polyclonal antisera to blackeye cowpea mosaic (BLCMV) or alfalfa mosaic (AlfMV) viruses, on their own or in mixtures, or to monoclonal antibodies. If such samples reacted positively with either of the monoclonal antibodies BC3 or 197 it could have been due to the presence of a 'B' serotype isolate of BCMV or another potyvirus (see Appendix 1, p. 108). In contrast, the monoclonal antibodies BC1 and I2 reacted specifically only with the 'A' serotype of BCMV. A summary of the ELISA reactions of the viruses that were detected, but not isolated, are given in Tables 33 (viruses detected from *P. vulgaris* samples) and 34 (viruses detected from other hosts).

In Ethiopia, potyviruses were isolated from two samples (225 and 228) of *Cassia sophera* near Jima and one (242) at Bonga (see Plate 24 and Table 32). All three isolates systemically infected the bean cvs The Prince and Double White and also *C. quinoa* and *N. clevelandii*. Virus particles of all three isolates were attracted by polyclonal antiserum to peanut mottle virus (PnMoV), blackeye cowpea mosaic virus (BLCMV) and standard BCMV strains NL3 and NY15 in ISEM tests, but only PnMoV antiserum decorated the particles. Also, in ELISA, these isolates reacted strongly with PnMoV antiserum, and none reacted with the monoclonal antibodies BC1 and BC3 (see Appendix 1, p. 108). Potyviruses

Table 32 Summary of viruses isolated other than BCMV

Isolate	Country	Place	Host species	Virus isolated
31	Uganda	Nakabango	*Arachis hypogea*	Peanut mottle potyvirus
225	Ethiopia	Jima	*Cassia sophera*	Peanut mottle potyvirus
228	Ethiopia	Jima	*Cassia sophera*	Peanut mottle potyvirus
242	Ethiopia	Bonga	*Cassia sophera*	Peanut mottle potyvirus
250	Ethiopia	Diri	*Phaseolus lunatus*	Cucumber mosaic cucumovirus
313	Ethiopia	Lake Shalla	*Phaseolus vulgaris*	Peanut mottle potyvirus
314	Ethiopia	Lake Shalla	*Phaseolus vulgaris*	Peanut mottle potyvirus
316	Ethiopia	Nazreth	*Cassia occidentalis*	Cassia severe mosaic potyvirus*
336	Ethiopia	Melkassa	*Cassia laburnifolium*	Unidentified potyvirus
338	Ethiopia	Melkassa	*Cassia laburnifolium*	Unidentified potyvirus (as 336)
344	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
347	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
348	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
366	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
428	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
429	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
430	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
434	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
435	Tanzania	Masasani	*Phaseolus vulgaris*	Unidentified potyvirus
522	Rwanda	Musambira	*Vigna* sp.	Unidentified isometric virus
945	Kenya	Meru	*Cassia* sp.	Peanut mottle potyvirus

* proposed name

isolated from two *P. vulgaris* samples (313 and 314) at Lake Shalla in Ethiopia, had a similar host range and properties to isolates 225, 228 and 242. In ISEM and decoration tests the particles of isolates 313 and 314 were attracted by antiserum to various potyviruses (PnMoV, BLCMV, soybean mosaic virus (SoyMV) and BCMV strain NL3), but only PnMoV antibodies decorated the particles. In ELISA isolates 313 and 314 reacted with monoclonal antibody 197, but not I2. In a sample (945) from a *Cassia* species collected in Kenya at Meru, a potyvirus was isolated which systemically infected the two *P. vulgaris* cvs The Prince and Double White, *C. quinoa* and *N. clevelandii*. In ISEM tests, particles of this isolate were attracted by antisera to BLCMV, BYMV, NL3 and PnMoV, but were decorated only by PnMoV antiserum. Isolate 945 also reacted strongly in ELISA with PnMoV antiserum and with monoclonal antibody 197 but not I2. Similarly, an isolate from a sample (31) of *Arachis hypogea* from Nakabango in Uganda was infected with a potyvirus which had identical properties to isolates 225, 228, 242, 313, 314 and 945. These seven isolates were all designated as strains of peanut mottle virus (PnMoV). PnMoV is predominantly found infecting *A. hypogea* and although it has a limited host range, it has been isolated from other leguminous species, including *Cassia occidentalis, C. leptocarpa, C. tora* and *P. vulgaris* (Brunt *et al.*, 1990).

In Ethiopia, at Diri between Bonga and Abelti, an isometric virus was isolated from a sample (250) of *Phaseolus lunatus* (Plate 25). This isolate infected all the *P. vulgaris* cultivars used to differentiate pathotypes of BCMV, *C. quinoa* and *N. clevelandii*. In ISEM tests many particles were attracted by antisera to both cucumber mosaic cucumovirus (CMV) and peanut stunt cucumovirus (PnSV), but only the antiserum to CMV decorated the particles. This isolate was designated as a strain of CMV and was the only one which was found in the survey, which is surprising considering the very wide host range of this virus in many species, including *P. vulgaris*. Another isometric virus (522) was isolated from a sample from a *Vigna* species collected at Musambira in Rwanda. This virus was tested in ISEM and decoration tests with antisera to CMV, PnSV, tephrosia symptomless carmovirus (TeSV), southern bean sobemovirus (SBMV), cowpea mosaic comovirus (CpMV) and sowbane mosaic virus (SoMV). Antisera

Table 33 Number of samples of *P. vulgaris* in which virus was detected by ELISA but not isolated

Country	BCMV*			BLCMV?†	BCMV/ BLCMV?‡	AlfMV	AlfMV/ BCMV¶	Potyvirus	Potyvirus?§	Haloblight	Nothing detected	Total number tested
	A	B	?									
Burundi	6	0	2	0	0	0	0	10	2	0	12	32
Ethiopia	0	0	11	0	11	0	0	23	1	0	52	98
Kenya	4	0	0	0	0	0	0	13	0	0	37	54
Lesotho	0	0	0	0	0	0	0	0	0	0	28	28
Malawi	0	0	0	0	0	0	0	0	0	0	22	22
Morocco	5	7	7	0	1	1	3	2	0	0	6	32
Rwanda	14	0	7	0	0	0	0	7	0	1	18	47
Swaziland	1	0	0	0	0	0	0	0	0	0	32	33
Tanzania	5	0	9	0	1	0	0	1	1	0	25	42
Uganda	5	0	17	2	0	0	0	1	0	1	13	39
Zaire	1	0	3	0	0	0	0	1	0	0	16	21
Zambia	0	0	0	0	0	0	0	3	0	0	5	8
Zimbabwe	0	0	7	0	0	0	0	1	0	0	31	39
Total	41	7	63	2	13	1	3	62	4	2	297	495

Notes:
* BCMV A or B serotype detected or inconclusive serotype (?)
† Possible detection of blackeye cowpea mosaic virus (BLCMV)
‡ Possible detection of BCMV, BLCMV or a mixture
¶ Detection of AlfMV and BCMV (mixture)
§ Possible detection of a potyvirus (inconclusive)

Table 34 Number of samples from wild species of legumes in which virus was detected by ELISA but not isolated

Country	BCMV*			BLCMV?	BCMV/ BLCMV?†	CSMV	PnMoV	Potyvirus	Potyvirus?	CMV	Nothing detected	Total number tested
	A	B	?									
Burundi	4	0	0	0	0	0	0	1	0	0	3	8
Ethiopia	0	0	3	0	1	1	3	7	6	1	12	34
Kenya	0	0	5	0	0	0	1	3	0	0	14	23
Malawi	0	0	1	0	0	0	0	0	0	0	6	7
Rwanda	3	0	0	0	0	0	0	1	0	0	3	7
Tanzania	0	0	0	0	0	0	0	0	0	0	3	3
Uganda	0	0	0	0	0	0	1	0	0	0	21	22
Zambia	0	0	1	3	0	0	0	0	0	0	0	4
Total	7	0	10	3	1	1	5	12	6	1	62	108

Notes: * BCMV A or B serotype detected or inconclusive serotype (?)
† Possible detection of BCMV, BLCMV or a mixture

to SBMV, SoMV and CPMV attracted virus particles but none of the particles were decorated. Further studies are required to characterize and identify this virus.

In Ethiopia, near Nazreth, a potyvirus was isolated from a sample (316) of *C. occidentalis* with severe mosaic symptoms (Plate 26). This isolate infected *N. clevelandii* (Plate 27) but not *C. quinoa*, or any of the *P. vulgaris* cultivars used to differentiate BCMV pathotypes. In ISEM tests it was attracted by antisera to cowpea aphid-borne mosaic virus (CAbMV), BLCMV, PnMoV, peanut stripe virus (PnStV), peanut green mosaic virus (PGV), *Cassia* yellow spot virus (CYSV), bean yellow mosaic virus (BYMV), SbMV and BCMV standard strains NL3, NL4 and NY15, but all these antisera failed to decorate the particles. In ELISA the isolate reacted with monoclonal antibodies BC3 and 197, but not BC1 or I2 and did not react with any of the antisera to standard BCMV strains or any of the other standard antisera used. Isolate 316 is therefore, considered to be possibly a previously undescribed virus and has tentatively been named cassia severe mosaic potyvirus (CSMV).

CSMV has a very narrow host range. It was readily sap- and aphid-transmitted to *Cassia occidentalis, Cassia sophera, Crotalaria incana* and *N. clevelandii* but not to *C. quinoa* or the bean cvs The Prince or Double White (Table 29). Furthermore, none of the 22 bean cultivars used to differentiate BCMV pathotypes was susceptible to sap-inoculation by this virus. The physical, chemical and serological properties of CSMV are discussed in Section 5.

In Ethiopia, at Nazreth Research Station, Melkassa, potyviruses were isolated from two samples (336 and 338) of *Cassia laburnifolium*. Both isolates caused severe mosaic and inter-veinal necrosis in all of the bean cultivars used to differentiate pathotypes of BCMV, but no symptoms in *N. clevelandii* and *C. quinoa*. Potyvirus particles were seen in both samples in the electron microscope, but ISEM and decoration tests with antisera to BLCMV, standard BCMV strains NL3 and NY15, PnMoV, SbMV, BYMV, PnStV and passiflora woodiness virus (PWV) were negative for both isolates. Also in ELISA, both isolates failed to react with monoclonal antibodies BC1 and BC3, and polyclonal antisera to standard BCMV strains, BLCMV and PnMoV. Further work is necessary to characterize these isolates.

Particles characteristic of alfalfa mosaic virus (AlfMV) were seen with the electron microscope in four samples (344, 347, 348 and 366) of *P. vulgaris* collected near Beni Mellal in Morocco and AlfMV was isolated from them. In addition, samples 344, 347 and 348 were also infected with BCMV. Attempts were made to separate the two viruses but as AlfMV has a much wider host range than BCMV and all the hosts of BCMV tested were also hosts of AlfMV, AlfMV was isolated separately but BCMV was not. Seed harvested from bean plants with the mixed infections was also found to be infected with both viruses, so the BCMV pathotype of these isolates could not be determined although ELISA tests detected both the 'A' or 'B' serotypes of BCMV. Both A and possible B serotypes of BCMV were also detected in samples from the other collecting sites in Morocco, however, no isolations of BCMV were made. The widespread distribution of alfalfa as a forage crop in Morocco and its widespread infection with AlfMV, is reflected in the number of isolations of AlfMV from beans.

In Tanzania, samples (428, 429, 430 and 434) of *P. vulgaris* from a farmer's field 10 kilometres east of Moshi, and a sample (435) collected between Moshi and Arusha, at Masasani, were infected with potyviruses with similar properties. All the isolates produced severe mosaic and leaf distortion symptoms on many bean cultivars, but did not infect *C. quinoa* or *N. clevelandii*. In ELISA, none of the isolates reacted with monoclonal antibodies BC1 and BC3, except 434 which reacted with BC3, and only 430 and 435 reacted with the polyclonal

antiserum to standard NL3 strain. These isolates do not appear to be strains of BCMV and further work is required to characterize them.

In Ethiopia, between Ambo and Nekempte, four samples (201, 202, 203 and 205) of *Cassia occidentalis* were tested in ELISA and two contained potyviruses which were possible 'B' serotypes. Near Metu, from four samples (213, 214, 215 and 216) of *Cassia sophera*, a possible 'B' serotype was detected from one sample. Between Metu and Jima, from five legume samples (217, 218, 219, 221 and 222) possible 'B' serotypes were detected in two *C. occidentalis* (217 and 218) and one *Crotalaria* species (probably *C. incana*) (219). In Ethiopia, near Bako a possible 'B' serotype was detected in a *Cassia didymobotrya* sample (264).

Possibly the most striking feature of these results is that during the survey comparatively few non-BCMV virus isolates were found infecting *Phaseolus vulgaris* beans in Africa. The infrequent occurrence of viruses other than BCMV in beans was also reported by Vetten and Allen (1991), in a study to investigate the importance of all viruses infecting beans in Africa. They also found peanut mottle and cucumber mosaic viruses to be the most common viruses other than BCMV to infect beans. In addition, they occasionally found cowpea mild mottle carlavirus and bean yellow mosaic potyvirus infecting beans, but these viruses were not isolated from bean in the present survey.

Section 5

Differentiation of BCMV Strains by Serology and Physical Characteristics

INTRODUCTION

The use of host cultivars to differentiate strains of BCMV is effective, but time-consuming and requires considerable facilities to screen many plants. Although serological methods of identification, such as ELISA, can be used to detect BCMV in a sample, there is at present no serological method capable of distinguishing individual strains.

Studies by Wang *et al.* (1982) using direct ELISA, suggested that polyclonal antisera might have a potential for differentiating BCMV strains. Working with three polyclonal antisera and nineteen strains of BCMV they found that an antiserum to the NY15 strain was specific to its homologous virus and that the other two antisera had a narrow range of strain specificity, reacting with some strains but not others. In indirect ELISA, a less specific range of strain reactions was observed, which was more useful for general BCMV surveys than for the differentiation of strains, as each of the three antisera detected all BCMV strains, except NL3 and NL5.

Later, Wang *et al.* (1984) produced hybridoma lines secreting specific monoclonal antibodies to BCMV strains which could differentiate BCMV strains into serotypes, 'A' and 'B'. The 'A' serotype included the NL3, NL8, NL5 and TN-1strains, and the remaining strains formed the 'B' serotype. Another hybridoma line reacted with all strains from both serotypes, and also with isolates of certain other potyviruses (Wang *et al.*, 1985). Millar *et al.* (1988) developed hybridoma lines which produced monoclonal antibodies specific to the NL3 strain in ELISA and other lines which produced monoclonal antibodies which reacted with both NL3 and NL4 in ELISA. These monoclonal antibodies are valuable as diagnostic tools for the identification of BCMV as they are capable of distinguishing between the two distinct serotypes of BCMV.

This section describes studies to evaluate the use of ELISA and Western blotting in the identification and differentiation of standard BCMV strains and the relationship between standard BCMV strains and BCMV strain-types isolated from *P. vulgaris* and wild species of legume in Africa. Studies on strain differentiation by virus particle length and polyacrylamide gel electrophoresis are also described.

RESULTS

Serology

ELISA

The serological relationships between the standard BCMV strains, and between BCMV strain-types and wild legume isolates of BCMV were studied using homologous and heterologous combinations of each isolate with polyclonal antisera in direct double antibody sandwich ELISA (DAS–ELISA) and with monoclonal antibodies in indirect plate trapped antigen ELISA (PTA–ELISA).

The results of the reactions of the standard BCMV strains showed that the monoclonal antibodies 197 and I2, distinguished them into two distinct serotypes, 'A' (necrotic NL3, NL5 and NL8 strains) and 'B' (non-necrotic NL1, NL4, NL6 and NY15 strains). The 'A' serotype was detected by both antibodies and the 'B' serotype only by 197 (Table 35). The wild legume isolates were similarly differentiated by the monoclonal antibodies into the 'A' or 'B' serotype according to whether they were necrotic or non-necrotic strain-types (Table 36).

The polyclonal antisera raised against the standard BCMV strains showed considerable variation in their affinity for each of the standard BCMV strains. The antisera raised against NL1 and NL4 reacted with all strains at sap dilutions of 1/1. At 1/10 there was a greater degree of specificity with the antiserum against NL1 detecting all strains except NL6 and NY15, and the antiserum against NL4 detecting all strains except NL6, NY15, NL5 and NL8. In contrast, the NL6 antiserum raised against NL6 was highly specific in detecting only NL6 and no other BCMV strains. In general, the antisera raised against NL5 and NL8 and the antiserum raised against isolate 30 reacted strongly at both dilutions with only the necrotic 'A' serotypes strains NL3, NL5 and NL8. However, the antiserum raised against NL5 only detected NL8 at the 1/1 dilution (Table 35). The antiserum raised against NY15 detected all strains at the 1/1 dilution except NL8, but at the 1/10 dilution it only detected NL3 and NL5 strains. The antiserum raised against NL3 detected all strains except NY15, at the 1/1 dilution but at the 1/10 dilution it only detected NL1, NL3, NL5 and NL8 strains.

The results showed that the antiserum raised against NL5, NL8 and isolate 30 were capable of distinguishing the 'A' and 'B' serotypes in a similar way to the monoclonal antibody I2 and that the antiserum raised against NL6 had unique specificity to its homologous BCMV strain in a similar way to the antiserum raised against NY15 as described by Wang *et al.* (1982).

To evaluate the antisera further and to aid in the identification of isolates from wild species of legumes, the monoclonal antibodies and polyclonal antisera were also tested with isolates of virus from the wild legumes and viruses other than BCMV. The reactions of isolate 465 (a NL1 strain-type) were similar to those of the standard NL1 strain. It was detected by monoclonal 197 only and by the antisera raised against NL1, NL4 and NY15 strains but not by the antisera raised against NL5, NL8 and isolate 30. However, isolate 465 was not detected by the antiserum raised against NL3 but was detected strongly by the antiserum raised against NL6 (Table 36). This suggested that although 465 belonged to the 'B' serotype it was serologically distinct from the standard NL1 strain.

Isolate 145 (a NL6 strain-type) was detected by all antisera to 'B' serotype strains in a similar way to standard NL6 but differed in that it was also detected by the antiserum raised against NL5 and isolate 30 at the 1/1 but not the 1/10 sap dilution. Serologically, isolate 145 differed from the standard NL6 strain as it reacted with polyclonal antisera which otherwise only detected the 'A' serotype, although its reaction with monoclonal antibodies suggested it was of a 'B' serotype. Isolate 956 (a NY15 strain-type) reacted in a similar way to the standard

Table 35 Reactions of standard BCMV strains in PTA-ELISA with monoclonal antibodies and DAS-ELISA with polyclonal antisera*

Antigen	Monoclonals		Reactions of polyclonal antisera to standard BCMV strains and isolate 30 at two sap dilutions (1/1 and 1/10)															
			NL1		NL4		NL6		NY15		NL3		NL5		NL8		30	
	197	I2	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10
Standard BCMV strains																		
NL1	++++	-	++	+	+	+	-	-	++++	-	++	+	-	-	-	-	-	-
NL4	++++	-	+	+	+++	++	-	-	++++	-	+	-	-	-	-	-	-	-
NL6	++++	-	+	-	+	-	++++	++	++++	-	+	-	-	-	-	-	-	-
NY15	++++	-	+	-	+	-	-	-	++	-	-	-	-	-	-	-	-	-
NL3	++++	++++	++	+	++	++	-	-	++	+	+++	++	++++	++++	++++	++++	++++	++++
NL5	++++	++++	+	+	+	-	-	-	+++	+	++	+	++++	++++	++++	++++	++++	++++
NL8	++++	++++	+	+	+	-	-	-	-	-	+	+	++++	-	++++	++	++++	++++

*ELISA absorbence values read at 405 nm after 90 minutes; +, <0.3; ++, 0.3–0.7; +++, 0.7–1.1; ++++, >1.1; -, negative (i.e. value less than twice the mean healthy control)

Table 36 Reactions of isolates from wild species of legumes and viruses other than BCMV in PTA-ELISA with monoclonal antibodies and DAS-ELISA with polyclonal antisera

Antigen antisera*		Monoclonals		Reactions of polyclonal antisera to standard BCMV strains and isolate 30 at two sap dilutions (1/1 and 1/10)															
				NL1		NL4		NL6		NY15		NL3		NL5		NL8		30	
		197	I2	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10	1/1	1/10
Wild legume isolates																			
	BCMV strain-type																		
465	NL1	+++	-	+	-	+	-	++++	-	+	++	-	-	-	-	-	-	-	-
145	NL6	++++	-	+	+	++++	+++	++	-	+++	-	+	+	+++	-	-	-	+++	-
956	NY15	++++	-	+	+	+	++	-	-	++++	-	+	+	-	-	-	-	+++	-
963	Novel	++++	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
499	NL3	++++	++++	+	-	-	-	+	-	+++	-	+	+	+++	-	+++	+	++++	++++
28	Novel	+++	++++	+	+	++	-	++++	-	++++	+	+	+	+++	++	++++	++	++++	++++
30	Novel	++++	++++	+	-	+	-	-	-	+++	-	+	+	+++	-	++++	+	++++	++++
38	Novel	++++	++++	+	-	++	-	++++	-	++++	++	+	+	++	-	++++	+	++++	++++
820	Novel	++	+++	-	-	-	-	-	-	++	-	+	+	-	-	++++	++	++++	++++
830	Novel	++++	++++	+	+	+	++	-	-	++	++	+	+	-	-	+++	++	++++	++++
Viruses other than BCMV																			
CSMV		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BLCMV-N		+	-	-	-	+	-	-	-	+++	-	-	-	-	-	-	-	-	-

*ELISA absorbence values read at 405 nm after 90 minutes; +, <0.3; ++, 0.3–0.7; +++, 0.7–1.1; ++++, >1.1; -, negative (ie value less than twice the mean healthy control)

NY15 strain in that it was detected by the antiserum raised against NL1, NL4 and NY15 strains, but not by the antiserum raised against NL6. However, isolate 956 differed from the standard NY15 strain in that it was also detected by the antiserum raised against NL3 and isolate 30.

Isolate 963 (a novel 'B' serotype) was weakly detected at the 1/1 dilution by only the antiserum raised against NL1; this suggests that it is quite distinct from the standard NY15 strain. Isolate 499 (a NL3 strain-type) was detected in a similar way to standard NL3 strain, except it was not detected by the antiserum raised against NL4 and was weakly detected by the antiserum raised against NL6.

The 'A' serotypes from wild legumes (28, 30, 38, 499, 820 and 830) were all detected strongly by both monoclonal antibodies and the antisera raised against NL5, NL8 and isolate 30 at both sap dilutions, with the exception of the antiserum raised against NL5 which detected isolates 30, 38, 499, 820 and 830 only at the 1/1 dilution. Isolates 30, 820 and 830 were not detected by the antiserum raised against NL6, a result which was typical of the standard NL8 strain. However, all five 'A' serotypes were detected by the antiserum raised against NY15 which was not typical of the standard NL8 strain. Also, isolate 820 was not detected by the antisera raised against NL1, NL5 and NL4.

CSMV was not detected by any of the antisera but was detected by monoclonal antibody 197, suggesting it was a potyvirus but not related to BCMV. This and other evidence (Section 4) suggests that this isolate is not a strain of BCMV and is possibly a previously undescribed potyvirus. The necrotic ringspot strain of blackeye cowpea mosaic virus (BLCMV-NR) was weakly detected by the antiserum raised against NL4 and strongly detected by the antiserum raised against NY15 (Table 36). BLCMV is known to have a close serological relationship with non-necrotic strains of BCMV, particularly NL1 and NY15 and a more distant relationship with necrosis-inducing strains such as NL3 (Lana *et al.*, 1988). This result was therefore in agreement with these findings. It is also interesting to note that none of the antisera prepared against the 'A' serotype strains of BCMV detected the standard NY15 strain. Thus, although the NY15 antiserum was not capable of distinguishing between BCMV and BLCMV-NR, the polyclonal antisera raised against the 'A' serotype strains did distinguish the 'A' serotype of BCMV from BLCMV-NR.

The antiserum raised against BLCMV-NR detected isolates 820, 830, 956 and 963 from wild legumes, but not isolates 28, 30, 38, 145, 465, 499 and 531 (Table 36). As isolate 956 is a NY15 strain-type, it is not surprising that it should react with BLCMV-NR antiserum, but the detection of 830 (a novel strain-type) suggested that this isolate was distinct from isolates 28, 30 and 38 (also a novel strain-type) and other 'A' serotype isolates.

The results of the present study showed that the polyclonal antiserum raised against NL8 was highly specific for the 'A' serotype strains of BCMV, whether standard strains or isolates from wild legumes, and was as sensitive and as specific as the monoclonal antibody I2. Similarly, the antisera raised against NL5 and isolate 30 were specific for the 'A' serotype standard BCMV strains and detected all the 'A' serotype wild legume isolates. However, the antiserum raised against NL5 failed to detect isolates 820 and 830, but did detect isolate 145 (a NL6 strain-type). The antiserum raised against isolate 30 detected isolates 145 and 956 (a NY15 strain-type) which the monoclonal antibodies differentiated as 'B' serotypes.

The variation in the reactions of the polyclonal antisera to the isolates from wild legumes compared with standard BCMV strains, reflected the variation in the phenotypic pathogenicity of these isolates compared to those of the standard BCMV strains belonging to the same pathogenicity groups (Section 3). This is

consistent with the variation in phenotype observed in the reactions of these isolates in the differential hosts and is probably consistent with what should be expected in a natural virus population infecting different wild legume hosts. The reactions of the monoclonal antibodies did not appear to reflect this pathogenic variability and although useful in distinguishing the BCMV serotypes, they may be of less value than polyclonal antisera in evaluating the variability of isolates of BCMV from wild legumes.

Western blotting

Comparison of the reactions of the standard BCMV strains, isolates from wild legumes and viruses other than BCMV using the monoclonal antibodies 197 and I2 in Western blots (see Table 37) and ELISA tests (Tables 35 and 36) showed that Western blotting was as sensitive as ELISA in differentiating the 'A' and 'B' serotypes of BCMV. However, the blots also showed the migration pattern of the viral coat proteins, based on their molecular weight and so provided additional information for the characterization of the BCMV strains. Some antisera detected several virus coat protein bands, consisting of a major band, which was more darkly staining, plus one or more minor bands which were not visible in extracts from healthy leaves (Plates 28 and 29). However, some minor bands were visible in the track containing the healthy control in addition to the tracks containing virus isolates; this was due to the detection of healthy plant proteins by contaminating antibodies in the antiserum.

Table 37 Reactions of standard BCMV strains, isolates from wild species of legumes and viruses other than BCMV in Western blots with monoclonal antibodies and polyclonal antisera

Antigen		Monoclonals		Polyclonal antisera to standard BCMV strains and isolate 30							
		197	I2	NL1	NL4	NL6	NY15	NL3	NL5	NL8	30
Standard BCMV strains											
NL1		+	-	+	+	+	+	+	+	-	-
NL4		+	-	+	+	+	+	+	+	-	-
NL6		+	-	+	+	+	+	+	+	-	-
NY15		+	-	+	+	+	+	+	+	-	-
NL3		+	+	+	+	+	+	+	+	+	+
NL5		+	+	+	+	+	+	+	+	+	+
NL8		+	+	+	+	+	+	+	+	+	+
Wild legume isolates											
	BCMV strain-type										
465	NL1	+	-	+	-	+	+	+	+	-	-
145	NL6	+	-	+	+	+	+	+	+	-	-
956	NY15	+	-	+	+	+	+	+	+	-	-
963	Novel	+	-	+	+	+	+	+	+	-	-
499	NL3	+	+	+	+	+	+	+	+	+	+
28	Novel	+	+	-	-	+	+	+	-	+	+
30	Novel	+	+	-	-	+	+	+	+	+	+
38	Novel	+	+	-	-	+	+	+	-	+	+
820	Novel	+	+	+	+	+	-	+	+	+	+
830	Novel	+	+	+	+	+	+	+	+	+	+
Viruses other than BCMV											
CAbMV		+	-	nt	nt	nt	nt	nt	nt	nt	nt
CSMV		+	-	-	-	-	-	-	-	-	-
BLCMV-NR		+	-	+	-	-	+	-	-	-	-

Notes: + viral protein detected
- viral protein not detected
nt not tested

The reactions of the standard BCMV strains in Western blots with polyclonal antisera raised against NL1, NL4, NL8 and isolate 30 (Table 37) were also similar to the ELISA results (Table 41), in that in the Western blots the antisera raised against NL1 and NL4 detected all standard strains of BCMV and the antisera raised against NL8 and isolate 30 detected only necrotic 'A' serotypes. In contrast, the antiserum raised against NL6, which in ELISA detected only the standard NL6 strain, also detected all of the standard BCMV strains in Western blots. However, of the other isolates tested, it detected only isolate 145 (a NL6 strain-type). This could be explained by the serological reaction in the Western blots being an indirect one and therefore less specific than the direct method of DAS-ELISA. Further evidence for this comes from the fact that the antisera raised against NY15 and NL3 detected all standard BCMV strains, whereas in ELISA the antiserum raised against NY15 failed to detect NL8, and the antiserum raised against NL3 failed to detect NY15.

The number of coat protein bands detected by different antisera varied. Only one protein band was detected in the standard NL1 strain by all the polyclonal and monoclonal antibodies, whereas one, two or sometimes three bands were detected in the standard NL5 strain according to the antibodies used. BCMV produces a single polypeptide, which is proteolytically cleaved into at least eight functional proteins, including the coat protein (Section 1, p. 9) and there may be a number of protein bands produced following degradation of the viral protein during electrophoresis. The epitope specificity of individual antisera or antibodies would determine which of these bands were visualized. Monoclonal antibody I2 consistently detected two protein bands in samples of 'A' serotype isolates. This result was as expected, as monoclonal antibodies are produced to only one epitope. However, the antisera raised against the standard NL8 strain and against isolate 30 also consistently detected two bands in 'A' serotype isolates, suggesting that these antisera contained antibodies to the same epitope as monoclonal I2.

CSMV, BLCMV-NR and CAbMV were all detected in Western blots by monoclonal 197, as in ELISA, but CSMV was not detected by any other antisera, and BLCMV-NR was detected only by the antisera raised against NL1 and NY15 antisera.

Virobacterial agglutination

Virobacterial agglutination (VBA) tests were carried out to determine if the test would detect BCMV in crude sap extracts and to determine if it would differentiate between BCMV strains and other viruses. Homologous and heterologous tests of antisera/antigen were carried out with five strains of BCMV (NL1, NL3, NL4, NL6, NY15), peanut mottle (PnMoV), cassia severe mosaic (CSMV) and bean yellow mosaic (BYMV) viruses. Antisera to the French (Fr) and Italian necrotic (It.N.) strains of BYMV were used and an unidentified strain of BYMV was used as the antigen. In addition, potato virus Y (PVY), turnip mosaic (TuMV) and zucchini yellow mosaic (ZYMV) virus antigens were also tested against the above-mentioned antisera, and healthy sap of the appropriate host species was tested against each antiserum as a control.

The results showed that antisera prepared against all five BCMV strains used in the tests readily detected their homologous and heterologous BCMV antigens (see Plate 30 and Table 38). Antisera to BCMV strain NL1, NL3, NL4 and NL6 did not react with the antigens to any of the other potyviruses used in the tests. In contrast, antiserum raised against BCMV-NY15 also reacted with BYMV, PnMoV and CSMV, three other viruses that infect legume species, but did not react with the more distantly related potyviruses, PVY, TuMV and ZYMV. CSMV antigen was detected by all the antisera tested, but the antiserum raised against CSMV

Table 38 The detection of bean common mosaic virus strains and other viruses by virobacterial agglutination*

Antigen†	Antiserum								
	BCMV NL1	BCMV NL3	BCMV NL4	BCMV NL6	BCMV NY15	BYMV FR.	BYMV It.N	PnMoV	CSMV
BCMV-NL1	+++	+++	+++	+++	+++	-	-	-	-
BCMV-NL3	+++	+++	++	+++	+++	-	-	-	-
BCMV-NL4	++	++	+++	++	+++	-	-	-	-
BCMV-NL6	++	++	++	+++	+++	-	-	-	-
BCMV-NY15	+++	+++	+++	+++	+++	+++	++	++	+++
BYMV	-	-	-	-	+	+	+++	-	-
PnMoV	-	-	-	-	++	-	-	+++	+++
CSMV	+++	+++	+++	+++	+++	+++	++	+++	+++
PVY	-	-	-	-	-	-	-	-	-
TuMV	-	-	-	-	-	-	-	-	-
ZYMV	-	-	-	-	-	-	-	-	-
Healthy sap	-	-	-	-	-	-	-	-	-

Notes: * Agglutination reaction after 3 min incubation, - no agglutination, +slight agglutination, ++moderate agglutination, +++strong agglutination

† Antigen used as crude sap prepared by grinding c. 2 g leaf in 0.5 ml PB5 buffer

only detected its homologous antigen and NY15. Antiserum raised against PnMoV detected only its homologous antigen, CSMV and the NY15 strain. Similarly, antisera raised against BYMV (Fr) and BYMV (It.N) detected their homologous antigens, CSMV and the NY15 strain.

These results showed that the simple and economical VBA test may be used as a rapid procedure to detect BCMV in diseased bean leaves, but it did not differentiate between different BCMV strains. This result is similar to earlier studies with the test, which showed that it was highly effective in specifically detecting unrelated viruses, but did not differentiate different BYMV strains (Walkey *et al.* 1992). The usefulness of the VBA test for the rapid detection of BCMV in diseased bean material, was further demonstrated in the present survey, when the test was used successfully in the field in Uganda.

Our results also showed that care must be taken in the selection of the BCMV antiserum used for the general detection of the virus in VBA tests. Antiserum to a strain which reacts only with other BCMV strains should be used. The broad spectrum reaction of the antiserum to the NY15 strain in our tests, showed that it would be unsuitable for detecting BCMV alone, although it might be useful for detecting the other closely related potyviruses that infect legume species. The heterologous, cross-reactions observed between the NY15 strain, CSMV, PnMoV and BYMV potyviruses indicated the close relationship of these legume-infecting potyviruses.

Electrophoresis

The mean molecular weights of the major coat protein bands of the standard necrotic BCMV strains NL3, NL5 and NL8 were lower (33–34 kDa) than those of the major coat proteins of the non-necrotic strains NL1, NL4 and NY15 (36 kDa) (Table 39). The NL6 strain had three protein bands of identical molecular weight to the three bands of the NY15 strain, but the major protein of the NL6 strain had a molecular weight of 31 kDa, whereas the major protein band of NY15 was 36 kDa.

Table 39 Molecular weight of viral coat proteins of standard BCMV strains, isolates from wild species of legumes and viruses other than BCMV

Isolate		Mean molecular weight (kDa) of major and minor coat protein bands	
Standard BCMV strains			
NL1		Major	36.0 ± 1.03
NL4		Major	36.0 ± 0.87
		minor	34.0 ± 0.57
NL6		minor	36.0 ± 0.58
		minor	34.0 ± 0.76
		Major	31.0 ± 1.52
NY15		Major	36.0 ± 1.08
		minor	34.0 ± 0.85
		minor	31.0 ± 0.5
NL3		Major	34.0 ± 0.9
		minor	36.0 ± 1.2
NL5		Major	34.0 ± 0.57
NL8		Major	33.0 ± 1.48
Isolates from wild species of legumes			
	BCMV strain-type		
465	NL1	Major	35.0 ± 0.35
145	NL6	Major	31.5 ± 1.08
499	NL3	Major	33.0 ± 0.00
28	Novel	Major	35.0 ± 0.76
30	Novel	Major	35.0 ± 0.35
38	Novel	Major	35.0 ± 0.35
Viruses other than BCMV			
CSMV*		Major	34.5 ± 1.3
BLCMV-NR		Major	36.0 ± 0.0

*CSMV, cassia severe mosaic virus; BLCMV-NR, blackeye cowpea mosaic virus (necrotic strain)

The isolates from wild legume and viruses other than BCMV all had only one visible coat protein band. Isolate 145 (a NL6 strain-type) had a band of low molecular weight (31.5 kDa) which was similar to the major protein band of NL6. The proteins of isolates 28, 30 and 38 (novel isolates) had identical molecular weights (35 kDa) which were larger than that of the standard NL8 strain. The protein of isolate 499 (a NL3 strain-type) had a band of 33 kDa which was more comparable with the standard NL3 and the other standard necrotic BCMV strains, than with the standard non-necrotic strains.

The proteins of CSMV and BLCMV-NR both had molecular weights which were typical of potyviruses, the molecular weight of BLCMV-NR was higher (36 kDa) than that of CSMV and comparable to the standard non-necrotic BCMV isolates.

Electron microscopy

Virus particle length

A comparison was made of the particle lengths of standard BCMV strains and BCMV strain-types from wild legume species, together with non-BCMV potyviruses isolated from beans and other legumes.

The particle length measurements were used to produce frequency distributions for each isolate according to how many particles were within the range of specific intervals of length. The distribution of particle lengths for each isolate

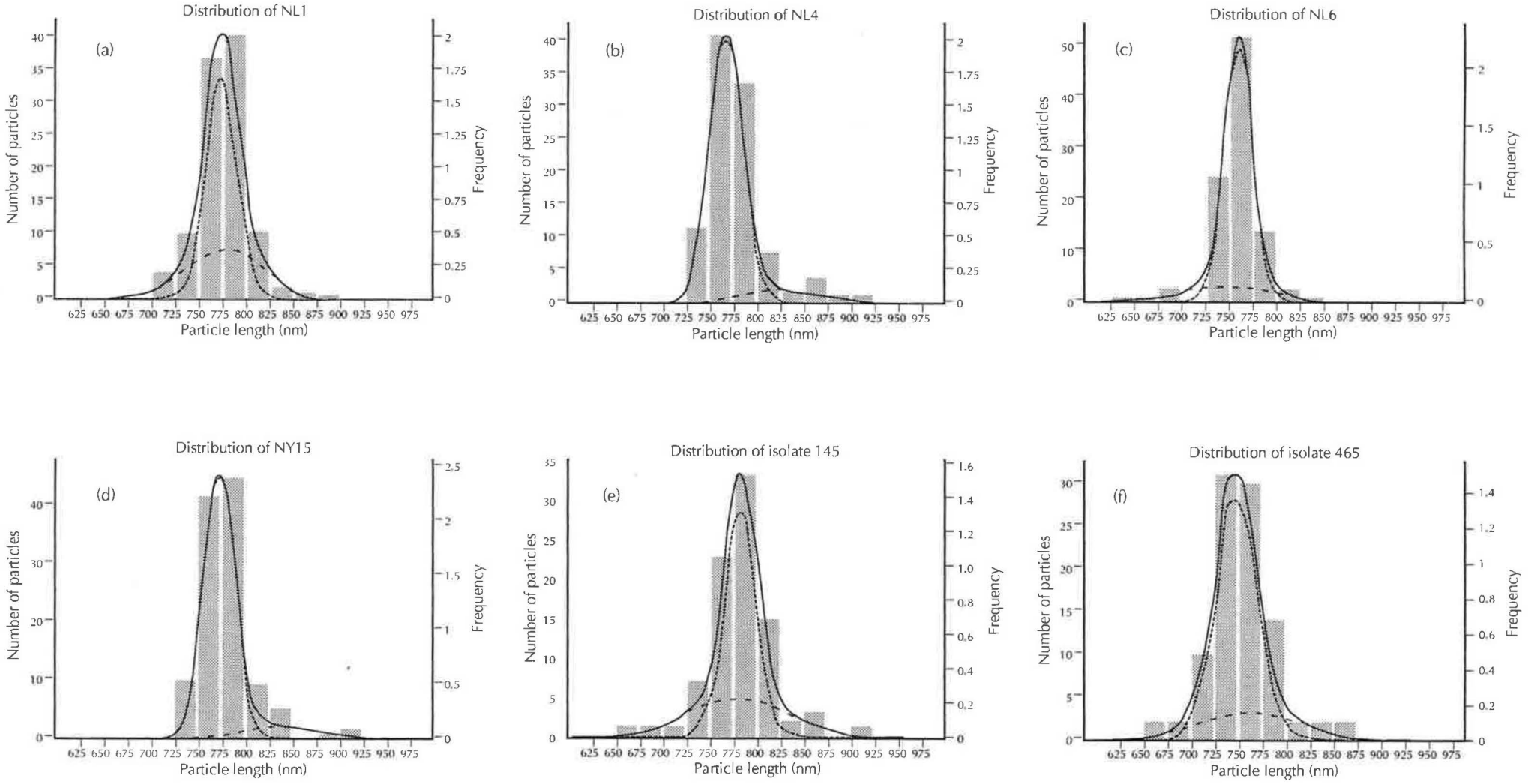


Figure 12 Frequency Distribution of virus particle lengths of (a) NL1; (b) NL4; (c) NL6; (d) NY15; (e) isolate 145; (f) isolate 465: ————, total distribution; – – – – – –, whole particle distribution, — — — — —, broken particle distribution.

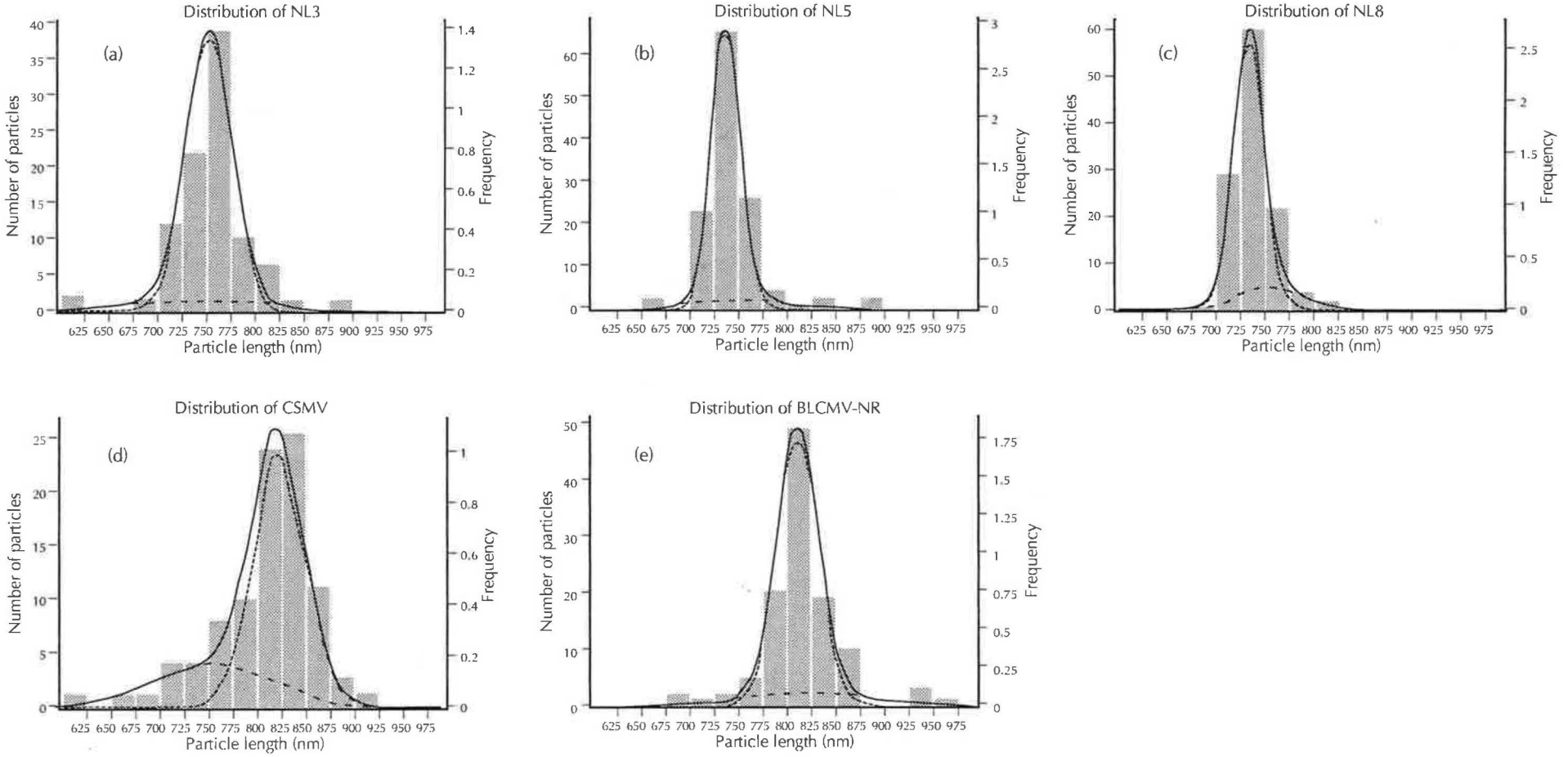


Figure 13 Frequency Distribution of virus particle lengths of (a) NL3; (b) NL5; (c) NL8; (d) CSMV; (e) BLCMV-NR; ————, total distribution; – – – – – –, whole particle distribution, — — — — —, broken particle distribution.

Table 40 Mean virus particle lengths and standard deviations of standard BCMV strains, isolates from wild species of legumes and viruses other than BCMV

Isolate identity		Number of particles measured	Virus particles in the first distribution ('whole')				Virus particles in the second distribution ('broken')			
			Mean length (nm)	Standard Deviation	Proportion of whole distribution	95% Confidence Interval	Mean length	Standard Deviation (nm)	Proportion of whole distribution	95% Confidence Interval
Standard BCMV strains										
NL1		105	774.8	16.31	0.637	(742.8, 806.8)	780.8	41.17	0.363	(700.1, 861.5)
NL4		98	771.9	18.06	0.873	(736.5, 807.3)	832.3	42.04	0.127	(749.9, 914.7)
NL6		100	759.7	15.34	0.811	(729.6, 789.8)	748.1	55.46	0.189	(639.4, 856.8)
NY15		113	773.8	16.88	0.888	(740.7, 806.9)	837.8	43.18	0.112	(753.2, 922.4)
NL3		94	755.2	24.55	0.888	(707.1, 803.3)	743.4	93.61	0.112	(559.9, 926.9)
NL5		126	737.8	15.23	0.872	(707.9, 767.7)	773.4	66.76	0.128	(642.6, 904.2)
NL8		116	734.3	15.85	0.854	(703.2, 765.4)	754.4	33.22	0.146	(689.3, 819.5)
Wild legume isolates										
	BCMV strain-type									
465	NL1	95	750.2	21.95	0.773	(707.2, 793.2)	764.9	54.05	0.227	(659.0, 870.8)
145	NL6	88	782.3	17.28	0.654	(748.4, 816.2)	781.7	54.14	0.346	(675.5, 887.8)
Viruses other than BCMV										
CSMV*		94	830.3	27.01	0.719	(777.4, 883.2)	764.3	61.20	0.281	(644.3, 884.3)
BLCMV-NR		115	813.2	21.92	0.826	(770.2, 856.2)	819.5	81.89	0.174	(659.0, 980.0)

*CSMV, cassia severe mosaic virus; BLCMV-NR, blackeye cowpea mosaic virus (necrotic strain)

was compared with various simple distribution models, but none of these provided a satisfactory description of particle lengths. However, a complex distribution model combining two normal distribution functions with unequal variances was found to model the distribution of virus particle lengths for all isolates. The first distribution, comprising the majority of particle lengths, had the smaller standard deviation of variance, and was interpreted at the true distribution of particle length. This distribution was subsequently used for all particle length comparisons. The second distribution was interpreted as the distribution of broken particles and/or particles with broken pieces joined to them and this always had a much larger standard deviation (Figures 12 and 13).

Only 43 particles of isolate 30 were photographed and measured because of the low concentration of virus in the host. The mean particle length was 758.9, with a standard deviation of 66.7. Because of the small number of particles measured, however, this result may not be a valid indication of the length of this isolate, so it was not included in the analysis. The mean particle lengths of CSMV (830.3 nm) and BLCMV-NR (813.2 nm) were longer than the BCMV strains and other isolates, and although they were still within the range for potyviruses, the lengths indicated they were not closely related to the standard strains of BCMV and the BCMV isolates from the wild legumes (Table 40). The standard necrotic NL5 (737.8 nm) and NL8 (734.3 nm) strains had mean particle lengths which were shorter than the means of the standard non-necrotic NL1 (774.8 nm), NL4 (771.9 nm) and NY15 (773.8 nm) strains. However, the mean particle lengths of the standard NL3 (755.2 nm) and NL6 strains (759.7 nm) and isolate 465 from *Cassia hirsuta* (750.2 nm) were intermediate between the necrotic and non-necrotic strains, although the NL3 strain and isolate 465 had much larger standard deviations than the other isolates. In contrast, the mean particle length of isolate 145 (782.3 nm, a NL6 strain-type) was slightly longer than that of the standard NL6 strain and closer to the other standard non-necrotic strains of BCMV (Table 39).

Cytology studies

Cytoplasmic inclusion bodies consisting of pinwheels and scrolls were seen in tissues infected with the standard strains NL6 and NL8 and with isolates 30, 145 and CSMV (see Plate 13). The inclusion bodies observed in all the samples were of the pinwheel-associated scroll type typical of the Group I potyviruses described by Edwardson and Christie (1978) (see Section 1, p. 9). No such inclusion bodies were seen in the healthy leaf material.

DISCUSSION

The results showed that the standard BCMV strains could be distinguished into two serological groups, 'A' and 'B', on the basis of their reactions with monoclonal antibodies and polyclonal antisera in ELISA and Western blots, which was in agreement with Wang *et al.* (1984; 1985). Also, the 'A' and 'B' serotypes of the standard BCMV strains were distinguishable according to the molecular weight of their protein and their mean particle length. Our results were generally in agreement with Vetten *et al.* (1992) who found that the major protein band of the 'A' serotype strains of BCMV had a lower molecular weight than that of the 'B' serotypes and that the particle lengths of 'A' serotype isolates had shorter particles (810–818 nm) than isolates of the 'B' serotype (847–886 nm). They concluded that this, as well as the serological and peptide sequence data, provided sufficient evidence for strains of the 'A' serotype of BCMV to be re-classified as strains of a distinct virus (bean necrosis mosaic virus, see p. 14). However, in our study the 'B' serotype strains NL6 and NY15 had mean particle lengths closer to those of the 'A' serotype.

The relationships of the NL6 strain to the other BCMV strains were also not clearly distinguished in the present study. The molecular weight of the major coat protein band of the NL6 strain was closer to that of an 'A' than a 'B' serotype and the NL6 strain had a mean particle length that was not typical of a 'B' serotype but intermediate between an 'A' and 'B' serotype. Serologically, however, the NL6 strain was differentiated as a 'B' serotype, and the homologous NL6 antiserum was highly specific for the NL6 strain. Also, this strain had an intermediate pathogenicity, being normally non-necrotic but inducing necrosis in some *I* gene cultivars usually only at temperatures above 30 °C (p. 12).

These results showed that the classification of BCMV according to necrotic 'A' and non-necrotic 'B' serotypes is not as clearly defined as has been previously described. Also, the discovery of 'A' and 'B' serotypes of BCMV in various wild legume species has provided more diverse isolates to compare with the standard BCMV strains. Although some of these isolates were found to be typical of 'A' or 'B' serotypes, others showed deviation in their serological relationships, molecular weights of their coat protein and virus particle length, which place them in an intermediate position between the two serotypes.

The proposed re-classification of BCMV also included grouping strains of BLCMV with the 'B' serotype of BCMV on the basis of peptide sequence data (McKern *et al.*, 1992b), with the 'B' serotype strains of BCMV having special adaptation to *Phaseolus* beans. However, comparison of the necrotic ringspot strain of BLCMV (BLCMV-NR) with standard BCMV strains and isolates from wild legumes in our study has indicated that BLCMV-NR was related only to NL4 and NY15 in ELISA and NL1 and NY15 in Western blots. The molecular weight of the BLCMV-NR protein was similar to a 'B' serotype of BCMV, but its mean particle length showed it to be quite different to the standard BCMV strains and BCMV isolates from wild legumes.

Plates

Plate 1

Traditional farming system in Rwanda. Crops include *Phaseolus* beans, maize and banana often grown as interplants

Plate 2

Typical bean/banana interplant in Uganda

Plate 3

Bean rust (*Uromyces appendiculatus*) symptoms

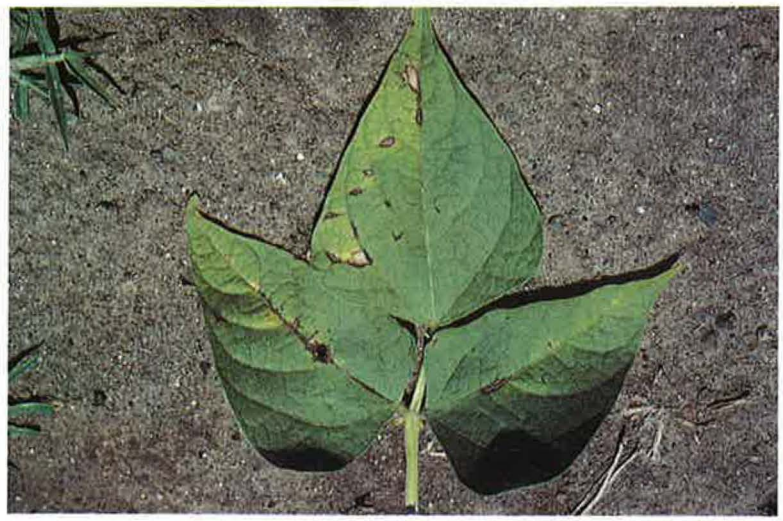


Plate 4

Anthracnose (*Colletotrichum lindemuthianum*) symptoms

Plate 5

Ascochyta (*Phoma exigua* var. *diversispora*) symptoms

Plate 6

Necrotic lesions caused by angular leaf spot (*Phaeoisariopsis griseola*)

Plate 7

Symptoms caused by common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*)

Plate 8

Haloblight (*Pseudomonas syringae* pv. *phaseolicola*) symptoms

Plate 9

Mild mosaic symptoms and growth reduction caused by BCMV in *Phaseolus vulgaris* in Swaziland

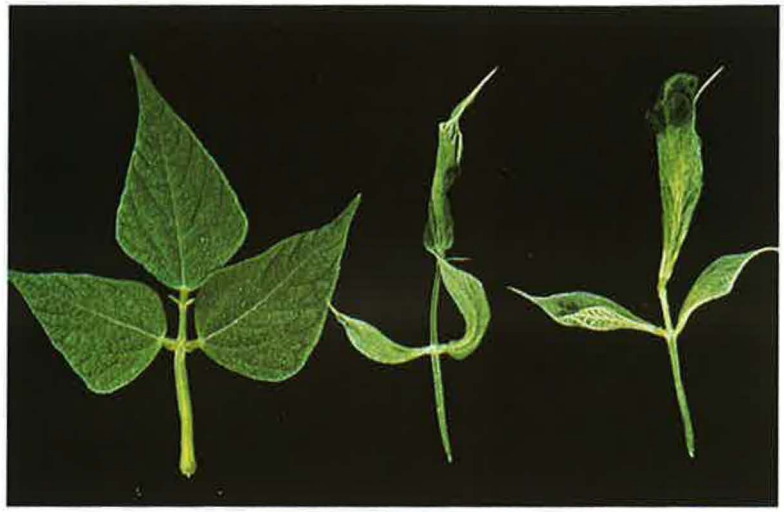


Plate 10

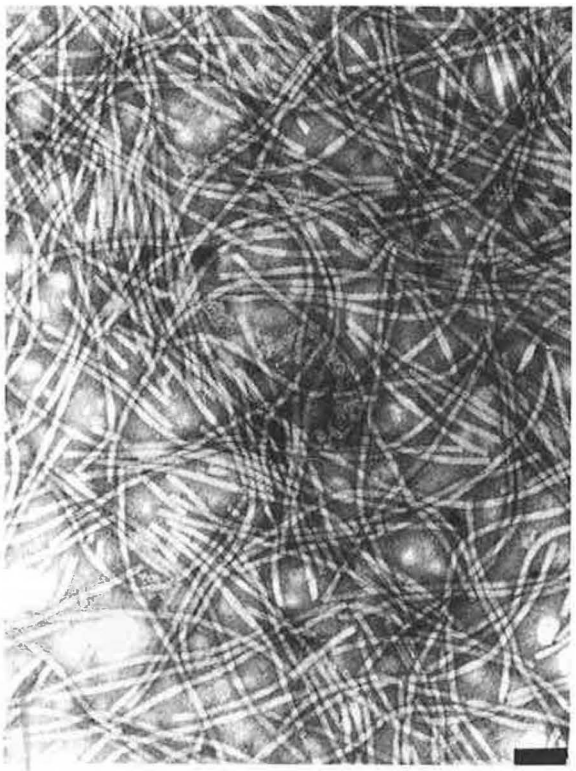
Severe mosaic and leaf distortion symptoms caused by BCMV. Healthy leaf on left

Plate 11

Systemic veinal necrosis (blackroot symptoms) in *P. vulgaris* caused by the hypersensitive resistance reaction of an *I* gene cultivar infected with BCMV in Zimbabwe. NL3 strain-types were isolated from plants showing these symptoms

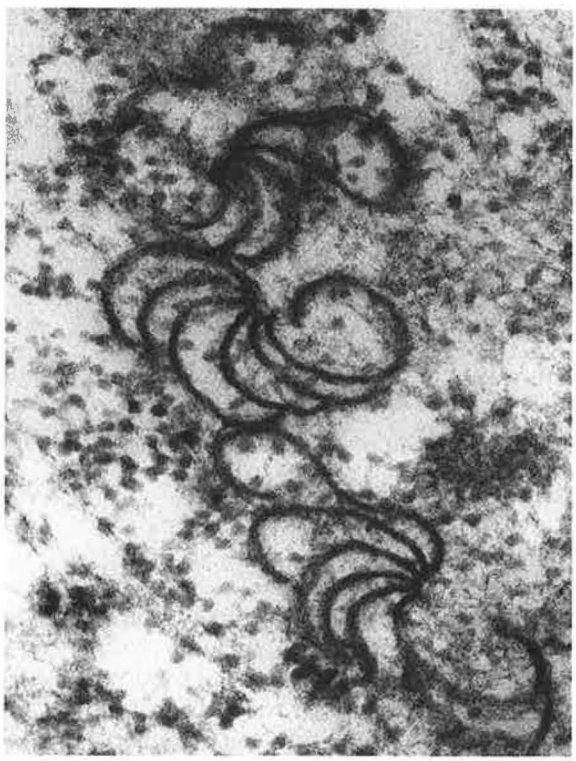
Plate 12

Close-up of systemic necrosis shown in Plate 11

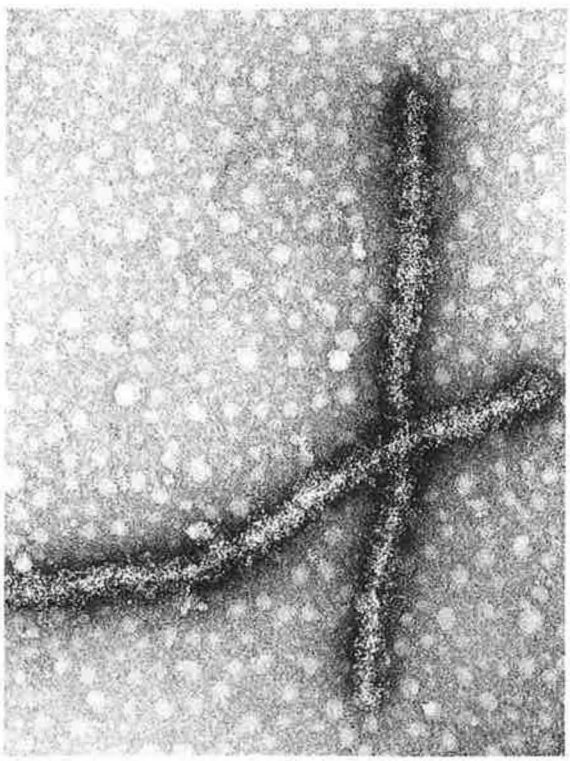


(a)

(b)



(c)



(d)

Plate 13

Electron micrographs of **a**, a purified preparation of particles of the standard NL3 strain, **b**, cylindrical cytoplasmic inclusion bodies in an ultra-thin section of bean infected with the standard NL8 strain; **c**, cylindrical inclusion bodies of isolate 145 (a NL6 strain-type) from a *Rhynchosia* sp., **d**, particles of the standard NL3 strain decorated with NL3 antibodies

Plate 14

Typical bean mixtures on sale in a Tanzanian market

Plate 15

Infected *Crotalaria incana* plant growing as a weed near a bean plot at Nakabango, Uganda. Isolate 28 (a novel 'A' serotype) was isolated from this plant

Plate 16

Close-up of leaf symptoms of infected *C. incana*

Plate 17

Systemic necrosis induced in a non-*I* gene differential cultivar (Sanilac) by isolate 30, a novel 'A' serotype isolated from *Crotalaria incana*

Plate 18

Local chlorotic lesions caused by isolate 30 from *Crotalaria incana* in inoculated leaves of *Chenopodium quinoa* seedlings

Plate 19

Cassia sophera leaves with chlorotic blotch symptoms from which isolate 820 (a novel 'A' serotype of BCMV) was isolated in Rwanda

Plate 20

Tephrosia vogelii (sample 806) from Rwanda showing very mild leaf mottle symptoms in which an 'A' serotype of BCMV was detected by ELISA

Plate 21

An unidentified, wild legume species found in Burundi, in which an 'A' serotype of BCMV was detected by ELISA

Plate 22

Pigeon pea (*Cajanus cajun*) growing in Kenya with mild chlorotic blotch symptoms in which an 'A' serotype of BCMV was detected by ELISA

(a)

(b)

(c)

Plate 23

Various wild legume species inoculated in the glasshouse with standard strains of BCMV (a) *Crotalaria incana* inoculated with the NL3 strain showing inter-veinal chlorosis, (b) *Cassia hirusta* inoculated with NL3 showing veinal chlorosis, and (c) *Indigofera hirsuta* inoculated with the NL4 strain showing systemic chlorotic spots

(a)

(b)

Plate 24

(a) *Cassia sophera* infected with peanut mottle virus (isolate 225) in Ethiopia
(b) Close-up of infected leaves

Plate 25

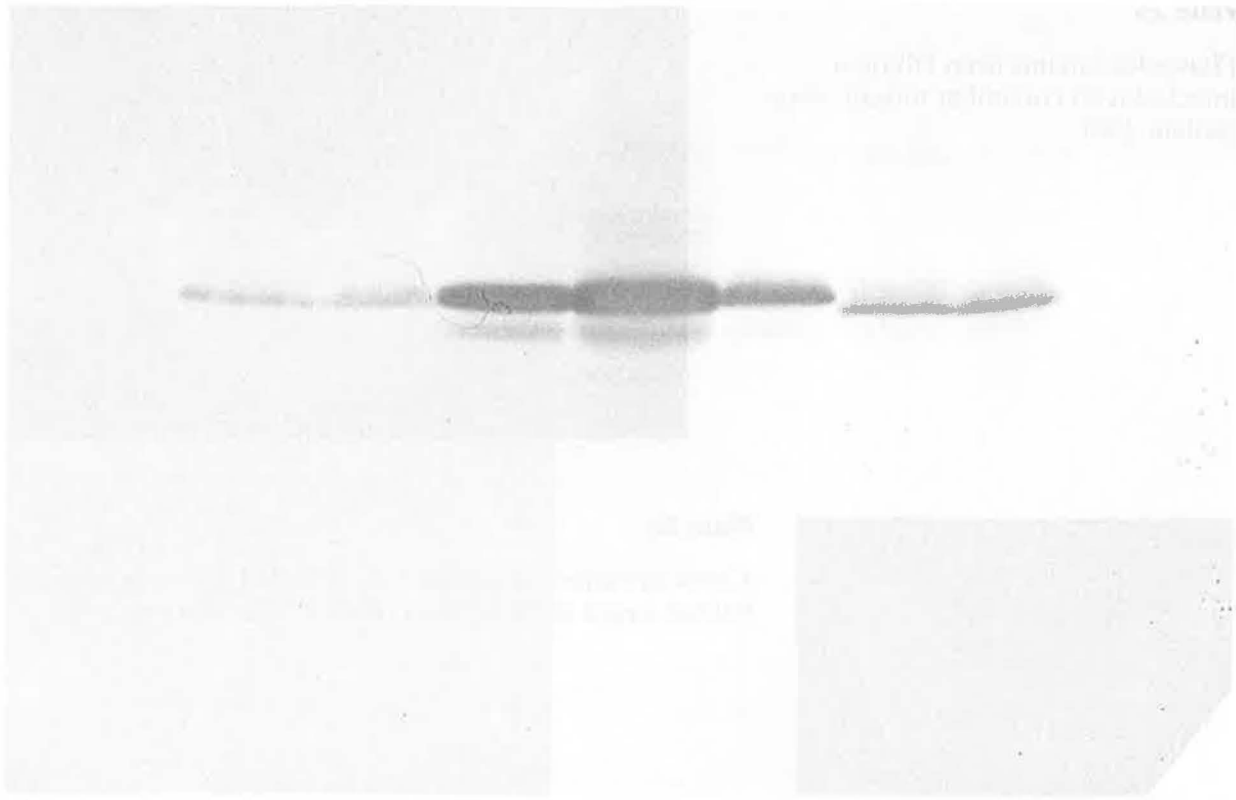
Phaseolus lunatus from Ethiopia infected with cucumber mosaic virus (isolate 250)

Plate 26

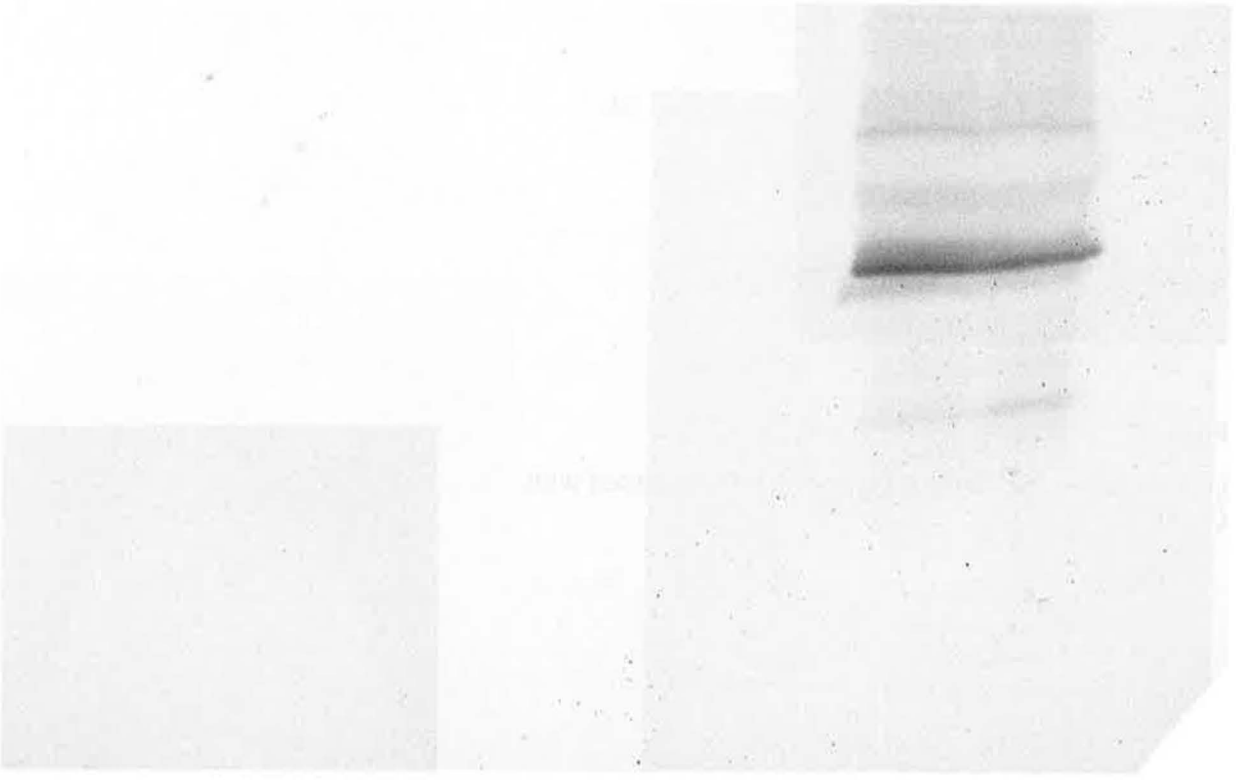
Cassia occidentalis systemically infected with a potyvirus named cassia severe mosaic virus (CSMV) (isolate 316)

Plate 27

Leaf of *Nicotiana clevelandii* systemically infected with CSMV



(a)



(b)

Plate 28

Western blots of crude leaf samples infected with wild legume isolates and probed with monoclonal antibodies. (a) monoclonal 197; (b) monoclonal I2. Track 1, isolate 820 (a novel 'A' serotype); 2, isolate 830 (a novel 'A' serotype); 3, isolate 956 (a NY15 strain-type); 4, isolate 961 (a novel 'B' serotype); 6, CAbMV; 7, BLCMV-NR; 8, healthy bean leaf. N.B. monoclonal I2 only reacts with the 'A' serotype isolates

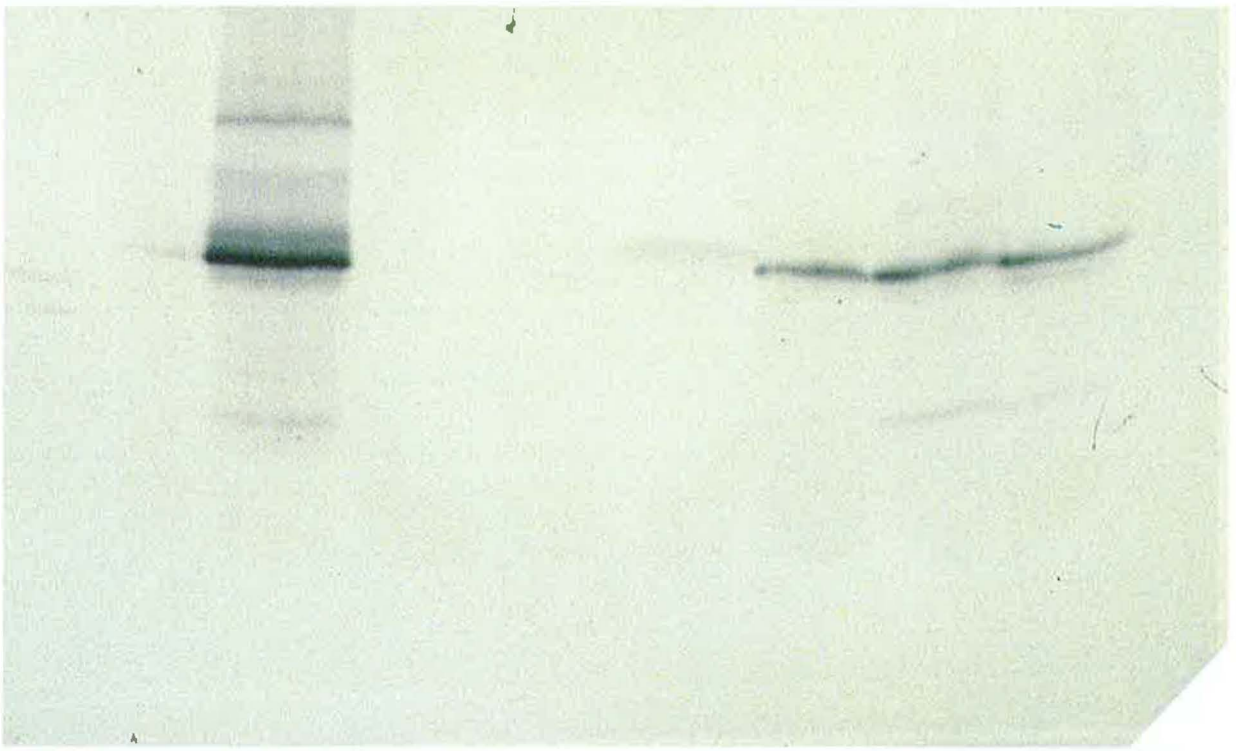


Plate 29

Western blots of crude leaf samples inoculated with wild legume isolates and probed with monoclonal antibodies (a) monoclonal 197; (b) monoclonal I2

Track 1, isolate 28 (a novel 'A' serotype); 2, isolate 30 (a novel 'A' serotype); 3, 38 (a novel 'A' serotype), 4, isolate 145 (a NL6 strain-type) 5, CSMV; 6, isolate 465 (a NL1 strain-type); 7, isolate 499 (a NL3 strain-type 'A' serotype) 8, healthy bean leaf N.B. Monoclonal I2 only reacts with the 'A' serotype isolates

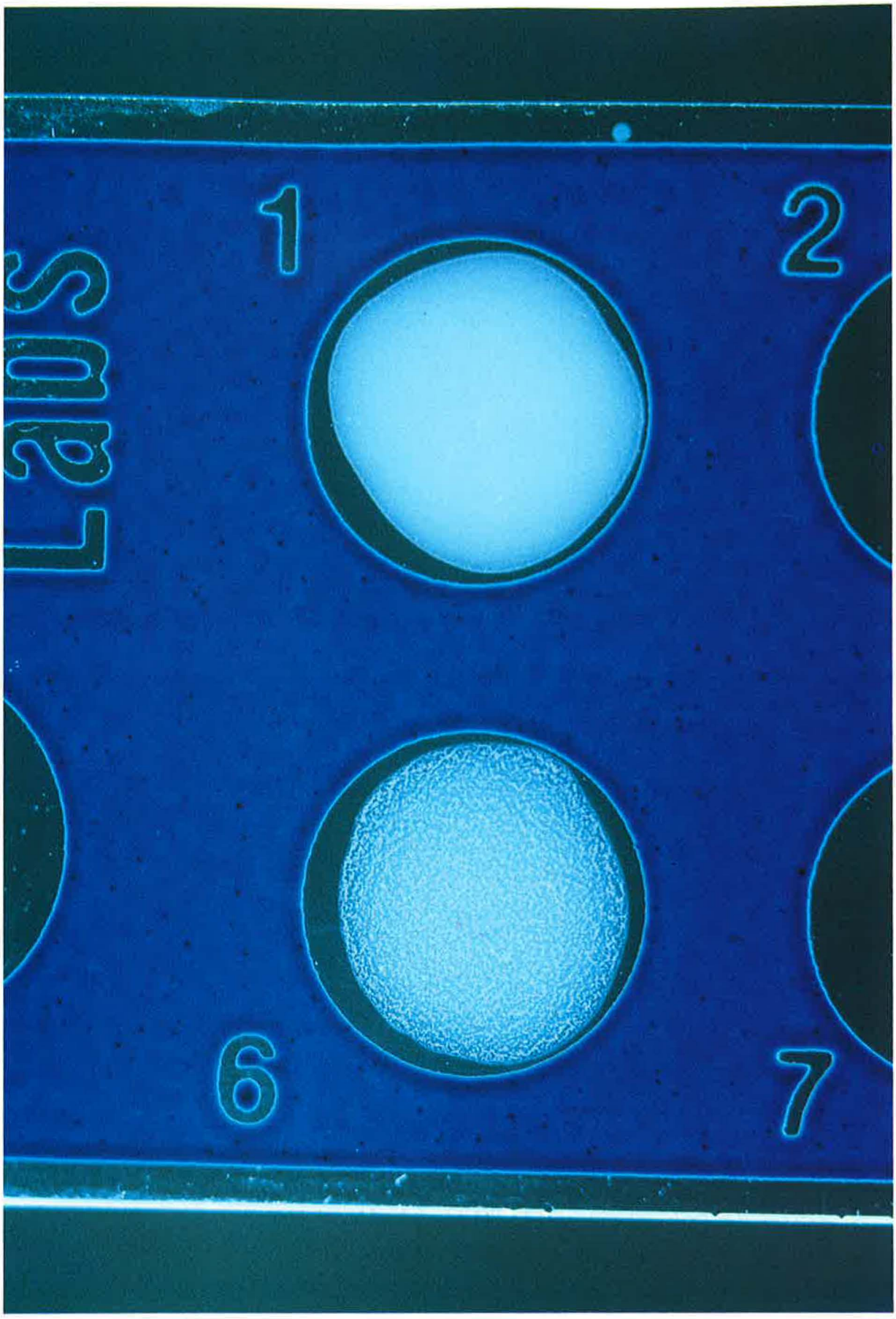


Plate 30

Virobacterial agglutination (VBA) test

(a) Control: healthy bean sap mixed with *Staphylococcus aureus*/BCMV antiserum conjugate, in which no bacterial clumping has occurred (container 1);

(b) Sap from BCMV infected bean plant mixed with *S. aureus*/BCMV antiserum conjugate (clumping of bacteria has occurred showing a positive reaction) (container 6)

Section 6

General Discussion and Conclusions

THE DISTRIBUTION, VARIATION AND ORIGINS OF BCMV STRAINS IN AFRICA

Strain identification and variation determined by phenotype response in differential hosts

In general, the reactions of the standard strains of BCMV in the bean differential hosts were consistent with the expected host reactions described by Drijfhout (1978) (see Tables 3 and 4). The small differences that were observed between our results and those of Drijfhout could probably be attributed to minor climatic variations (particularly temperature) under which the tests were made.

The variation in the phenotypic reactions induced by many of the isolates found in the survey suggested that each isolate should be designated a distinct strain of BCMV. Such a scheme of strain classification would, however, be very complex and as an alternative the isolates have been grouped into 'strain-types' according to the standard strain to which they were most closely related. Many of the BCMV isolates found infecting *P. vulgaris* beans in Africa induced phenotype responses in the differential host cultivars that allowed them to be clearly identified as being related to a specific, previously described BCMV pathogenicity group (see Tables 6–18 and Tables 21–25). Some of these isolates induced reactions that were identical to those of the standard strain, while others showed small but distinct differences in pathogenicity phenotype from the standard strain. For example, this variation might involve the failure of an isolate to infect one individual cultivar of a host group, even though it infects other cultivars of the same differential host group. Isolates whose phenotype deviated from that of the standard strain by such small variation were considered in this study to belong to the same strain-type as the standard strain. A significant number of isolates, however, showed phenotypic expression that differed considerably from that of the standard strains or any other previously well characterized BCMV isolate. The phenotype of these isolates was considered distinct from the standard strain if they failed to infect all cultivars of a particular differential host group. Such isolates were considered to be novel and if two or more such isolates had identical novel phenotypic responses in the differential host cultivars, they were considered to belong to the same novel strain (see Tables 41–45). The significance and origins of these novel isolates and strains are discussed in Section 6, p. 94.

In ELISA and other serological tests the isolates of identifiable BCMV strain-types and the novel isolates were readily identified as 'A' or 'B' serotypes (see Section 5).

The results of the present study clearly demonstrate the widespread occurrence and predominance of necrotic, 'A' serotype strains of BCMV in central, eastern and southern Africa in both beans and wild species of legumes. Strain-

Table 41 The reactions of differential host cultivars to various BCMV isolates compared with the standard NL3 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 strain and necrosis-inducing novel isolates							
Group	Cultivar	NL3	100 S	874 B	191 Z	3515 Za	3507 Za	161 M	162 M
1	The Prince	+	+	+	+	+	+	+	+
	SGR	+	nt	nt	nt	+	+	nt	nt
	Double White	+	nt	+	nt	+	+	nt	nt
	Sutter Pink	+	nt	+	nt	nt	nt	nt	nt
	CRM	+	+	nt	+	+	+	+	+
2	PGW	+	-	-	-	-	-	-	+
	RGC	+	+t	-	-	-	-	+	+
3	RGB	+	-	-	-	-	-	+	+
4	Michelite	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	-	+	+
6	Monroe	-	-	nt	-	-	-	-	-
	RM 35	-	-	-	-	-	-	-	-
	GN 31	-	nt	-	nt	-	-	nt	nt
8	BTS	N	N	N	N	N	n	N	N
	Widusa	N	N	N	N	N	n	n	N
9a	Jubila	N	n	n	n	n	n	n	n
9b	ITG	N	N	N	N	N	N	n	n
	TC	N	n	N	N	N	N	-	n
10	Amanda	-	n	-	-	-	-	-	-
Pathogenicity genes		*P0* *P1* *P1*^{2} *P2* *Px*	*P0* *P1* *P2* *Px* *Px*^{2}	*P0* *P1* *P2* *Px* *Px*^{2}?	*P0* *P1* *P2* *Px* *Px*^{2}?	*P0* *P2* *Px* *Px*^{2}?	*P0* *P1* *P2* *Px* *Px*^{2}	*P0* *P1* *P1*^{2} *P2* *Px* *Px*^{2}	*P0* *P1* *P1*^{2} *P2* *Px* *Px*^{2}

Notes: + host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA
N systemic necrosis at 26 °C and 32 °C
n systemic necrosis only at 32 °C
Origin of isolates: B, Burundi; M, Malawi; S, Swaziland; Z, Zimbabwe; Za, Zaire

types similar to the NL3 strain were prevalent in much of the area, although the NL8 strain-type was also widespread in Burundi, Rwanda and Tanzania. Isolates belonging to the temperature-dependent NL6 strain-type, although not as prevalent as NL3, were also common in most countries surveyed. It is interesting to note that this strain-type was frequently isolated as seed-transmitted virus from local farmers' land races of beans.

The widespread occurrence of the necrosis-inducing NL3 and NL8 strain-types and other novel 'A' serotype isolates throughout the region, emphasizes the importance of protecting new bean cultivar introductions against these potentially devastating necrotic strains. Many improved cultivars that are being introduced into national bean improvement programmes in Africa carry only the dominant *I* resistance gene which will not protect them against infection from these 'A' serotype isolates. In such areas it is essential to protect dominant *I* germplasm with the recessive resistance genes *bc*3 or *bc*2^2 to obtain complete resistance against all BCMV strain-types. The exception appears to be Ethiopia, where our survey confirmed the absence of NL3 and NL8 strain-types. It should

Table 42 *Reactions of differential hosts to the standard NL3 and NL8 strains and necrosis-inducing novel isolates**

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 and NL8 strains and necrosis-inducing novel isolates																		
Group	Cultivar	NL3	NL8	820 R	550 Za	437 T	444 T	450 T	521 R	528 R	532 R	836 R	587 B	277 E	286 E	319 E	50 L	830 R	28 U	30 U	38 U
1	The Prince	+	+	+	+	-	+	-	+	+	-	+t	+	+	+	+	+	+	-	-	-
	SGR	+	+	nt	+	+	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
	Double White	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	nt	+	+	+	+
	Sutter Pink	+	+	+	nt	nt	nt	+	+	+	+	-	nt	+	+	+	nt	+	+	+	+
	CRM	+	+	nt	nt	+	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	+	nt	nt	+	+
2	PGW	+	-	-	-	+	+	-	+t	+t	-	-	+	+	-	-	-	-	-	-	-
	RGC	+	-	+t	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-
3	RGB	+	-	+t	+	-	-	-	-	-	-	+t	+	-	+t	+t	-	-	-	-	-
4	Michelite	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
5	Pinto 114	+	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-
6	Monroe	-	-	nt	-	-	-	-	nt	nt	-	nt	nt	nt	nt	nt	-	nt	-	-	-
	RM 35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GN 31	-	nt	-	nt	nt	nt	nt	-	-	nt	-	nt	-	-	-	nt	-	-	-	-
8	BTS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	n	N	n	n	n
	Widusa	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	n	N	n	n	n
9a	Jubila	N	-	n	-	-	-	-	-	n	-	-	-	-	-	-	-	-	-	-	-
9b	ITG	N	-	N	-	-	-	-	-	-	-	-	-	-	-	n	-	n	-	-	-
	TC	N	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-
10	Amanda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
		P1		*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*		*P1*	*P1*							
		P1[2]		*P1*[2]	*P1*[2]							*P1*[2]	*P1*[2]		*P1*[2]	*P1*[2]					
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*					*P2*	*P2*	*P2*	*P2*
		Px	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*	*Px*		*Px*			
										Px[2](?)						*Px*[2]	*Px*[2]	*Px*[2]?	*Px*[2]	*Px*[2]	*Px*[2]

Notes: + Host susceptible to systemic infection
nt Not tested
- Host resistant to systemic infection
N Systemic necrosis at 26 °C and 32 °C
n Systemic necrosis only at 32 °C
+t Symptomless host but virus detected by ELISA
Origin of isolates: B, Burundi; E, Ethiopia; L, Lesotho; R, Rwanda; T, Tanzania; U, Uganda; Za, Zaire

* Isolates 820, 830 and 836 were isolated from *Cassia sophera*; isolates 28 and 30 from *Crotalaria incana*; isolate 38 from *Glycine max*; all other isolates were obtained from *P.vulgaris*

Table 43 The reactions of differential host cultivars to various BCMV isolates compared with the standard NL6 strain

Differential hosts		Infectibility of differential hosts inoculated with the standard NL6 strain and temperature-dependent novel isolates											
Group	Cultivar	NL6	127 Z	403 T	404 T	887 B	149 M	178 M	407 T	1008 Zm	179 M	151 M	853 B
1	The Prince	+	+	+	+	+	+	+	+	+	+	+	+
	SGR	+	nt	nt	+	nt	nt	nt	nt	nt	nt	nt	nt
	Double White	+	nt	+	+	+	nt	nt	+	+	nt	nt	+
	Sutter Pink	+	nt	+	nt	+	nt	nt	+	+	nt	nt	+
	CRM	+	+	nt	+	nt	+	+	nt	nt	+	+	nt
2	PGW	+	-	-	-	-	-	-	+	+	-	-	-
	RGC	+	-	-	-	-	-	-	+	+	+t	+	+
3	RGB	+	-	-	-	-	-	-	-	-	-	-	-
	GN 59	+	-	nt	-	nt	-	-	nt	nt	-	nt	nt
4	Michelite	-	-	-	-	-	-	-	-	-	-	-	-
	Sanilac	-	-	-	-	-	-	-	-	-	-	-	-
5	Pinto 114	-	-	-	-	-	-	-	-	-	-	-	+
6	Monroe	-	-	-	-	nt	-	-	-	nt	-	-	nt
	RM 35	-	-	-	-	-	-	-	-	-	-	-	-
	GN 31	nt	nt	nt	-	-	nt	nt	nt	-	nt	nt	-
8	BTS	n	N	n	n	n	n	n	N	n	N	N	n
	Widusa	n	N	n	n	n	n	n	N	n	N	n	n
9a	Jubila	n	n	-	n	-	n	n	-	-	-	n	n
9b	ITG	n	n	n	n	n	n	n	n	n	n	N	n
	TC	n	n	n	n	n	n	n	-	n	n	N	n
10	Amanda	-	-	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0* *P1* *P1*[2] *Px*[2]	*P0* *Px*[2] *Px*	*P0* *Px*[2]	*P0* *Px*[2]	*P0* *Px*[2]	*P0* *Px*[2]	*P0* *Px*[2]	*P0* *P1* *Px*[2] *Px*	*P0* *P1* *Px*[2]	*P0* *P1* *Px*[2] *Px*	*P0* *P1* *Px*[2]	*P0* *P1* *P2* *Px*[2]

Notes: + host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
N systemic necrosis at 26 ° and 32 °C
n systemic necrosis at 32 °C but not at 26 °C
+t symtomless host but virus detected by ELISA
Origin of isolates: B, Burundi; M, Malawi; R, Rwanda; T, Tanzania; Z, Zimbabwe; Zm, Zambia

be noted, however, that three novel isolates were found there that did induce necrosis in host group 8 cultivars although they reacted serologically in ELISA as 'B' serotypes (see Table 9). In Ethiopia, germplasm carrying the *I* gene alone may be sufficient to confer resistance against strains of BCMV in most areas, but plant breeders need to be aware of the existence of these variant strains for they may influence the durability of any new resistant bean germplasm that is introduced.

The prevalence of NY15 strain-types and other non-necrosis inducing 'B' serotypes in both beans and wild species of legumes in a relatively wide geographical area around Kisii and Kakamega in western Kenya, is of interest. Some of these isolates had pathogenicity phenotypes typical of the NY15 strain, while others were novel non-necrotic 'B' serotypes. Previously, the NY15 strain had only been found in North America and its occurrence only in this region of Africa raises the question of the original source of the NY15 strain. Could it have originated in this area of western Kenya? Perhaps its occurrence in the Kisii area and in New York state may be associated with the strong influence of American

Table 44 Reactions of differential hosts to the standard NL3 and NY15 strains and non-necrosis inducing novel isolates

Differential hosts		Infectibility of differential hosts inoculated with the standard NL3 and NY15 strains and non-necrosis-inducing novel isolates										
Group	Cultivar	NL3	NY15	961 K	963 K*	964 K	967 K	970 K	959 K	953 K	973 K	847 B
1	The Prince	+	+	+	+	+	+	+	+	+	+	+
	Double White	+	+	+	+	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	+	+	+	+	-	+
2	PGW	+	+t	+	-	+	-	-	-	-	-	-
	RGC	+	+	+	+t	-	+t	-	-	-	-	-
3	RGB	+	-	+	+t	+	+t	+t	+	-	-	-
4	Michelite	+	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+	+	+	-
6	Monroe	-	-	-	-	nt	nt	nt	-	nt	nt	nt
	RM 35	-	-	-	-	-	-	-	-	-	-	-
	GN 31	-	nt	-	-	-	-	-	-	-	-	-
8	BTS	N	-	-	-	-	-	-	-	-	-	-
	Widusa	N	-	-	-	-	-	-	-	-	-	-
9a	Jubila	N	-	-	-	-	-	-	-	-	-	-
9b	ITG	N	-	-	-	-	-	-	-	-	-	-
	TC	N	-	-	-	-	-	-	-	-	-	-
10	Amanda	-	-	-	-	-	-	-	-	-	-	-
Pathogenicity genes		*P0* *P1* *P1*[2] *P2* *Px*	*P0* *P1* *P2*	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P1*[2] *P2*	*P0* *P1* *P2*	*P0* *P1* *P2*	*P0* *P2*

Notes: + host susceptible to systemic infection
nt not tested
- host resistant to systemic infection
+t symptomless host but systemic infection detected by ELISA, tolerant reaction
N systemic necrosis at 26 °C and 32 °C
Origin of isolates: K, Kenya; B, Burundi
* isolated from *Crotalaria comanestiana*, all other isolates obtained from *P. vulgaris*

Churches in the development of religion in this area of Kenya, in contrast to the influence of European Churches elsewhere in Kenya. The regular movement of people and goods between western Kenya and the United States over a relatively long period of time could have involved movement of bean seed and virus isolates.

All the virus isolates characterized during the course of this study have been stored in liquid nitrogen and freeze-dried at Wellesbourne so that they are available to other workers. Many isolates have already been returned to their country of origin for use during selection for resistance in national breeding programmes. Strain-types that were isolated locally are certainly more appropriate for screening local germplasm than standard BCMV strains because of their variation in pathogenicity compared with the standard strain's that was demonstrated in the present study.

The occurrence of BCMV isolates in wild species of legumes and other non-*Phaseolus vulgaris* hosts

A result of major significance in the current survey has been the isolation of BCMV from wild species of legumes and certain non-*Phaseolus* legume crop

Table 45 Observed and theoretical genotypes for resistance (host) and pathogenicity (virus) for the strain-specific resistance genes to BCMV

Theoretical differential host genotypes				Observed differential cultivars	Theoretical virus pathogenicity genotypes and observed strains																Theoretical number of infecting genotypes	Observed number of infecting genotypes
					V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16		
					P0	*P0* *P1*	*P0*	*P0*	*P0*	*P0* *P1*	*P0* *P1*	*P0* *P1*	*P0*	*P0*	*P0*	*P0* *P1*	*P0* *P1*	*P0* *P1*	*P0*	*P0* *P1*		
							P1^2			*P1*^{2}			*P1*^{2}	*P1*^{2}		*P1*^{2}	*P1*^{2}		*P1*^{2}	*P1*^{2}		
								P2			*P2*		*P2*		*P2*	*P2*		*P2*	*P2*	*P2*		
									P2^2			*P2*^{2}		*P2*^{2}	*P2*^{2}		*P2*^{2}	*P2*^{2}	*P2*^{2}	*P2*^{2}		
					NL1	NL7		NL8		NL6 US5	NL2 US2 NY15					NL3 NL5	NL4					
H1				Double White	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16	7
H2	*bc*-1			RGC	-	+	-	-	-	+	+	+	-	-	-	+	+	+	-	+	8	5
H3	*bc*-1^2			RGB	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	8	3
H4		*bc*-2		Michelite	-	-	-	+	-	-	+	-	+	-	+	+	-	+	+	+	8	3
H5		*bc*-2^2			-	-	-	-	+	-	-	+	-	+	+	-	+	+	+	+	8	1
H6			*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H7	*bc*-1	*bc*-2		Pinto 114	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	4	2
H8	*bc*-1	*bc*-2^2			-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+	4	1
H9	*bc*-1		*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H10	*bc*-1^2	*bc*-2		'X'	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	4	1
H11	*bc*-1^2	*bc*-2^2		Monroe	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+	4	1
H12	*bc*-1^2		*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H13		*bc*-2	*bc*-3	IVT7214	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H14		*bc*-2^2	*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H15	*bc*-1	*bc*-2	*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H16	*bc*-1	*bc*-2^2	*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H17	*bc*-1^2	*bc*-2	*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
H18	*bc*-1^2	*bc*-2^2	*bc*-3		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0

Note. It is assumed that *bc*-1 and *bc*-1^2, and *bc*-2 and *bc*-2^2 are allelic. Table modified from Drijfhout (1978).

plants. The survey has shown that virus-like symptoms were often common in wild legume species growing as weeds in bean-growing areas. Such species were more prevalent in the wetter, humid areas of Uganda, Rwanda, Malawi, Burundi and the wetter areas of Kenya and Ethiopia, than in the drier areas of southern Africa, Tanzania and the eastern parts of Kenya and Ethiopia.

A number of naturally occurring BCMV isolates were obtained from these wild species of legumes. These included novel isolates from *Crotalaria incana* and *Glycine max* in Uganda and *Cassia sophera* in Rwanda, and the NL3 strain-types from *Macroptilium atropurpureum* in Uganda and *Vigna unguiculata* in Rwanda.

A non-necrotic, 'B' serotype isolate conforming to the NY15 strain-type was obtained from *Vigna vexillata* in western Kenya, and a novel 'B' serotype isolate from *Crotalaria comanestiana* in the same area. In addition, further 'B' serotypes identified as NL1 and NL6 strain-types were isolated from *Cassia hirsuta* in Uganda and a *Rhynchosia* species in Malawi, respectively.

The widespread occurrence and predominance of necrotic, 'A' serotypes of BCMV in Africa, infecting both beans and wild species of legumes, raises the question of the geographical centre of origin of the necrotic strains. This question is of particular significance considering the recent proposal that the necrotic strains should be considered to belong to a virus distinct from BCMV (McKern *et al.*, 1992a and Vetten *et al.*, 1992). These strains are not thought to occur naturally in South America, the centre of origin of *P. vulgaris*. When they have been recorded there, or in North America, then occurrence can usually be traced to infected imported seed (F. Morales, personal communication).

The necrotic strains of BCMV were first described by workers in the Netherlands, although Grogan and Walker (1948) described temperature-sensitive necrosis in a cultivar carrying the *I* gene infected with the common mosaic and greasy pod strains of BCMV (see Section 1, p. 11). Strains NL3 and NL5 were isolated from cultivars carrying the *I* gene by Hubbeling (1963, 1972) in bean varietal trials conducted by a commercial company that at the time was not conducting bean seed multiplication in Africa. However, as cultivars carrying the *I* gene do not transmit virus in their seed (Morales, 1989), they were probably not the source of the virus. It is more likely that the virus was aphid-transmitted from different cultivars included in the trial from other seed companies. These other companies were known at the time to be multiplying seed in Africa and it is likely that these trials included seed which had come from Africa. This could have been the source of seed-transmitted virus which was then in turn aphid-transmitted to the cultivars carrying the *I* gene from which the NL3 and NL5 strains were isolated (L. Bos, personal communication).

The NL8 strain first described by Drijfhout and Bos (1977), was isolated from the progeny of a breeding experiment in a private breeder's field in Holland. This strain may also have been introduced from Africa via the same route as the NL3 and NL5 strains but there is no further evidence to confirm or dispute this possibility.

The laboratory and glasshouse studies have shown that a range of wild species of legumes that commonly occur in Africa, notably of the genera *Cassia, Crotalaria, Macroptilium, Vigna* and *Rhynchosia*, are susceptible to a number of the standard strains of BCMV. Some are particularly susceptible to the necrotic 'A' serotype strains, NL3 and NL8, and the temperature-dependent strain NL6, which were prevalent in the areas where these genera occur. These tests also showed that the necrotic strains NL3 and NL8 induced latent infection in many wild legume species that was only detectable in ELISA tests (see Table 22). Such latent infection may indicate host adaptation to these virus strains resulting from

long-term exposure, an indication that these strains may have originated in this region. In addition, our tests showed that several of the BCMV isolates from wild legumes were aphid- and seed-transmitted to various wild species of legumes and *P. vulgaris* and that the standard BCMV strains were seed-transmitted in wild species of legumes (see Section 3). It is therefore possible that in bean-growing areas in Africa, BCMV could be transmitted from cultivated or wild species of legumes to beans or from beans to the other legume species. Infected wild species of legumes could provide a source of inoculum to infect bean crops which are grown twice a year in some areas. They may also act as alternative hosts in which BCMV may adapt or evolve, resulting in the occurrence of novel virus isolates. Such changes in virus pathogenicity could have important implications for plant breeders developing new resistant cultivars in Africa.

Although it is possible that the necrotic 'A' serotypes might have originated in *P. vulgaris* beans introduced into Africa and then subsequently transmitted by aphids to the wild species of legumes, the bulk of the evidence provided by the present survey would suggest otherwise. The survey has confirmed that the necrotic strains are the prevalent strain-types occurring in most of eastern and central Africa and that except for the temperature-dependent NL6 strain, the occurrence of non-necrotic 'B' serotype isolates of BCMV is uncommon in these areas. In contrast, in the new world the 'B' serotypes are prevalent and the occurrence of 'A' serotype isolates is rare and can often be traced to imported seed. Similarly, the original 'A' serotypes (NL3, NL5 and NL8) isolated in Holland, could well have originated from Africa.

The widespread occurrence of necrotic 'A' serotypes in wild legume weed species in Africa, and the observed tolerance of some of these wild species of legumes to BCMV infections is a further indication that eastern and central Africa may be close to the centre of origin of the 'A' serotype isolates.

Also, the large number of novel 'A' serotype isolates that have been isolated in this survey from both beans and wild species of legumes is probably a further indication that BCMV strain-types originated in and are possibly evolving in the survey area.

More studies on the ecology of BCMV isolate in wild legume species in Africa are needed to resolve this question and an investigation of the occurrence of such isolates in legumes in non-bean growing locations could be informative. The great abundance of wild legume species in Uganda would suggest that this region would be a suitable area for further ecological studies.

The significance and possible origins of the novel isolates found in the survey

A result of major interest in the current survey has been the isolation of a number of isolates with previously unrecorded pathogenicity phenotypes. In some instances, two or more isolates had the same novel pathogenicity phenotypes suggesting that they belong to the same strain. The majority of the isolates with novel phenotypes induced a temperature-independent necrotic reaction (referred to as N in Tables 41–44) in cultivars carrying the *I* gene and reacted as 'A' serotypes in ELISA and other serological tests. The pathogenicity phenotypes of these isolates are summarized in Tables 41 and 42, and compared with those of the standard NL3 and NL8 BCMV strains.

A number of novel isolates were also found that induced a temperature-dependent necrotic reaction (referred to as n in Tables 41 and 44) in the differential host cultivars carrying the *I* gene and the pathogenicity phenotypes of these isolates are compared with those of the standard NL6 strain in Table 43.

Fewer non-necrotic 'B' serotype novel isolates were obtained and all but one of these was found in western Kenya. The reactions of differential hosts to these isolates are compared with those of the standard NY15 and NL3 strains in Table 44.

The gene-for-gene hypothesis developed by Drijfhout (1978) to explain the relationship between BCMV and host genotypes of *Phaseolus vulgaris* (see Section 1), goes some way towards explaining the genetics of pathogenicity of some of the novel isolates found in this study, but does not explain the full range of pathogenic variation observed in the novel 'A' serotype isolates.

Drijfhout presented the total theoretical number (64) of virus genotypes that were possible on the basis of a gene-for-gene relationship for the available combinations of the *P*1, *P*1^2, *P*2 and *P*2^2 genes (see Table 45). It was assumed that all pathogenicity genes were at different loci. It should also be noted that a pathogenicity gene *P*3 has so far not been found. At the time Drijfhout (1978) prepared his manuscript, only 7 of the possible 64 theoretical genotypes had been identified and only three (NL3, NL5 and NL8) temperature-independent strains were known. The pathogenicity phenotypes of isolates 286 (V3) and 319 (V3) from Ethiopia and 836 (V9) from Rwanda (see Table 42) indicated that these novel isolates corresponded to the theoretical BCMV strains represented by V3 and V9 respectively.

The large number of variant, novel 'A' serotype strains found in this survey capable of inducing a temperature-independent necrosis in differential host cultivars carrying the *I* gene, indicated that additional pathogenicity genes must be carried by these necrosis-inducing isolates. It is proposed that two genes may be responsible for the necrotic reactions. The theoretical gene inducing the temperature-independent necrosis (N) is designated *Px* and the theoretical gene inducing the temperature-dependent necrosis (n) is designated *Px*^{2}.

The *Px* and *Px*^{2} pathogenicity genes are included in Table 46 to explain the previously described temperature-independent and temperature-dependent necrosis-inducing BCMV strains NL8, NL6/US5, NL2/US2 and NL3/NL5. If *Px* and *Px*^{2} are not allelic, the 64 theoretical combinations of the *P*1, *P*1^2, *P*2 and *P*2^2 pathogenicity genes proposed by Drijfhout is expanded to result in 256 possible virus genotypes.

If this hypothesis is correct, the novel BCMV isolates found in our survey could be carrying the combinations of *P*1, *P*1^2, *P*2, *Px* or *Px*^{2} pathogenicity genes listed in Tables 41–44. It should be noted that the reactions of group 2 and 3 host cultivars and those of cv Pinto 114 were sometimes inconsistent. The susceptiblity of cv Pinto 114 to isolates 874, 191 and 3515 (see Table 41) indicated that these isolates carried *P*1 and *P*1^2 and should therefore also have caused systemic infection of group 2 and 3 cultivars. Their failure to infect these cultivars may be due to the relatively high level of resistance these cultivars have when inoculated with some BCMV isolates. Systemic symptoms in these cultivars are usually mild and frequently it is necessary to confirm the systemic infection by ELISA.

It will be noted from these tables, that some of the novel isolates induce either a temperature-independent (N) necrotic reaction (similar to the standard NL3 and NL8 strains) or a temperature-dependent (n) necrotic reaction (similar to the standard NL6 strain) in all the host cultivars of groups 8 and 9 that they infect. This indicates that the isolates are carrying either *Px* or *Px*^{2}. In contrast, other novel isolates appear to induce a temperature-independent (N) necrotic reaction in host group 8 cultivars and a temperature-dependent (n) necrotic reaction in host group 9 cultivars (see isolates 127 and 179 in Table 43). It is possible that these isolates carry both *Px* and *Px*^{2}.

Table 46 Table of observed and theoretical genotypes for resistance (host) and pathogenicity (virus), in which strain-specific resistance genes are in combination with the dominant *I* gene. The observed or expected positive reactions result in systemic necrosis at 30 °C. The proposed *Px* and *Px*^{2} genes have been added for the genotypes observed prior to the present survey.

Theoretical differential host genotypes				Observed differential cultivars	Theoretical virus pathogenicity genotypes and observed strains																Theoretical number of infecting genotypes	Observed number of infecting genotypes
					V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16		
					P0	*P0* *P1*	*P0* *P1*^{2}	*P0* *P2* *Px* NL8	*P0* *P2*^{2}	*P0* *P1* *P1*^{2} *Px*^{2} NL6 US5	*P0* *P1* *P2* *Px*^{2} NL2 US2 NY15	*P0* *P1* *P2*^{2}	*P0* *P1*^{2} *P2*	*P0* *P1*^{2} *P2*^{2}	*P0* *P2* *P2*^{2}	*P0* *P1* *P1*^{2} *P2* *Px* NL3 NL5	*P0* *P1* *P1*^{2} *P2*^{2}	*P0* *P1* *P2* *P2*^{2}	*P0* *P1*^{2} *P2* *P2*^{2}	*P0* *P1* *P1*^{2} *P2* *P2*^{2}		
8	*I*			Widusa	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-	4	4
9	*I*	*bc*-1		Jubila	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	3	3
10	*I*	*bc*-1^2		Amanda	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	2	2
	I		*bc*-2		-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	3	3
11	*I*		*bc*-2^2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I			*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1	*bc*-2		-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	2	2
	I	*bc*-1	*bc*-2^2		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1		*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1^2	*bc*-2		-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	1	1
	I	*bc*-1^2	*bc*-2^2	IVT 7233	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1^2		*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I		*bc*-2	*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I		*bc*-2^2	*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1	*bc*-2	*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1	*bc*-2^2	*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1^2	*bc*-2	*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0
	I	*bc*-1^2	*bc*-2^2	*bc*-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0

Note: It is assumed that *bc*-1 and *bc*-1^2, and *bc*-2 and *bc*-2^2 are allelic. Table modified from Drijfhout (1978).

The results of this survey do not provide conclusive evidence for the occurrence of pathogenicity genes *Px* and *Px*^{2} for the control of temperature-sensitive and temperature-insensitive necrosis. An alternative explanation could be that there is a single *Px* pathogenicity gene which interacts with different host determinants and this is the basis for whether the necrotic response is temperature-sensitive or not. Further genetic studies using these novel isolates and crosses between differential host cultivars are required to determine the genetic basis of the necrotic reaction. Such studies would also allow the pathogenicity phenotype of the novel isolates to be confirmed and the relationship between strains to be determined. It should also be noted that the response of cv Jubila (group 9a) is frequently inconsistent when inoculated with isolates that induce a temperature-independent (N) necrosis in all other group 8 and 9 cultivars (see Tables 16 and 18). This cultivar often responds by developing necrotic symptoms following inoculation with these isolates only at 32 °C and not at 26 °C. It is possible therefore, that there may be an additional gene or genes in this host cultivar that modify the development of the necrotic symptoms, or that local fluctuations in temperature may influence gene expression.

The considerable genetic diversity in the local landraces and bean cultivars found in these regions, together with the abundance of wild legume species in Africa, may have resulted in a co-evolution of the BCMV strain-types and novel isolates and their hosts. Potyviruses in general, have a known ability to produce new variant strains by the rapid recombination of their RNA leading to heterogeneous RNA populations within the infected plants (Lecoq and Purcifull, 1992; Goldbach, 1992). The prevalence of suitable legume host species and the aphid vectors of the virus in central Africa, would have provided an ideal ecological situation for the evolution of these novel strains of BCMV.

The recent evidence that suggested the necrotic 'A' and non-necrotic 'B' serotypes of BCMV are two distinct viruses has resulted in a proposal to re-classify BCMV into BCMV and BNMV (bean necrosis mosaic virus), with BNMV comprising the necrotic 'A' serotypes (McKern *et al.*, 1992b; Vetten *et al.*, 1992). The basis of these proposals, however, are the comparison of the peptide profiles of only a few isolates (NL3, NL5 and NL8) which are the standard 'A' serotype strains of BCMV with those of non-necrotic 'B' serotype strains. The results of this study support the contention that the two serotypes are distinct and that they may have two separate origins. The prevalence of the necrotic 'A' serotype isolates in central and eastern Africa is indicative that they may have evolved in this region, whereas the 'B' serotype of BCMV would probably have had their centre of origin, along with *Phaseolus vulgaris* beans, in central or south America. However, the status and relationships of the NL6 strain and the other temperature-dependent necrosis-inducing novel strains is not so clear. The reactions of the differential hosts to these strains, some of their serological reactions and their physical properties were intermediate between the 'A' and 'B' serotype; this may be indicative that the NL6 strain and some of the novel strains may be intermediate in their evolutionary position between the 'A' and 'B' serotype.

It is essential that the investigation of peptide profiles is extended to include the novel BCMV isolates from beans and wild legumes in Africa for comparison with the strains already characterized by McKern *et al.* (1992b). Information from such studies may provide a clearer understanding of the relationship of the 'A' and 'B' serotype and could provide further evidence about the evolutionary origins of the strains of BCMV. These studies should be carried out before an official re-classification of BCMV into BCMV and BNMV occurs.

The selection of the most suitable virus isolate or isolates for screening beans for resistance to BCMV in Africa by plant breeders is important. At present, most

breeders in national and regional breeding programmes are using isolates of the NL3 strain-type to select the resistance to protect germplasm carrying the *I* gene against the locally occurring necrosis-inducing isolates. This selection regime is probably suitable for most purposes, although it is probably sensible to use a locally prevalent NL3 strain-type isolate rather than the standard NL3 strain. As the NL6 strain-type and other temperature-dependent novel isolates were found to be widespread in Africa, it is also important to consider these strains in relation to regional breeding programmes.

THE DIFFERENTIATION OF BCMV STRAINS BY SEROLOGY AND PHYSICAL CHARACTERISTICS

The results of this study showed that the standard BCMV strains, BCMV strain-types and novel isolates from *P. vulgaris* and wild species of legumes in Africa could be differentiated into two distinct serotypes, 'A' and 'B', using monoclonal antibodies in ELISA and Western blots. This was in agreement with the earlier studies reported by Wang *et al.* (1984). However, our results also showed that polyclonal antisera could similarly differentiate the 'A' and 'B' serotype, both in ELISA and Western blots. Several antisera had broad-spectrum reactivity with BCMV, while antiserum raised against the standard NL8 strain was highly specific for the 'A' serotype only (see Section 5). The 'A' and 'B' serotypes of BCMV were also differentiated by their particle length and the molecular weight of their coat protein, which is in agreement with the findings of Vetten *et al.* (1992), who in common with our study showed that the NL6 strain appeared to be intermediate between the 'A' and 'B' serotype.

The necrotic ringspot strain of blackeye cowpea mosaic virus (BLCMV-NR) was shown in our study to have a close serological relationship with the 'B' serotype NY15 strain and NY15 strain-types from Africa. BLCMV-NR had a more distant serological relationship with the 'A' serotype strains in ELISA and Western blots and this is in agreement with the findings of Lana *et al.* (1988) and Khan *et al.* (1990). However, the 'A' serotype isolates from *Cassia sophera* in Rwanda reacted strongly with BLCMV-NR antisera. Also in our study BLCMV-NR had considerably longer virus particles than the 'A' and 'B' serotypes of BCMV. Therefore, before BLCMV can be included in the same group as the 'B' serotypes of BCMV, as proposed by Mckern *et al.* (1992b), it is important that more isolates of BLCMV are studied and their relationship to the standard BCMV strains and strain-types isolated in Africa is clearly defined.

VIRUSES OTHER THAN BCMV IDENTIFIED IN THE SURVEY

Although BCMV was the virus most commonly isolated from *P. vulgaris* in Africa, peanut mottle virus (PnMoV), alfalfa mosaic virus (AlfMV) and cucumber mosaic virus (CMV) were also found infecting *P. vulgaris*. PnMoV was also isolated from wild legume species collected in bean-growing areas which may be a significant factor in developing new bean cultivars with resistance to PnMoV. The incidence of AlfMV was limited to Morocco, which reflects the possible spread of the virus from widely grown alfalfa forage crops to bean crops. None of the other African countries surveyed grew alfalfa to the same extent and so it would be interesting to determine if AlfMV occurs more frequently in beans in countries where alfalfa is widely grown (see Section 4).

The host range, serological and other physical properties of the potyvirus isolated from *Cassia occidentalis* in Ethiopia indicate that it is possibly a previously undescribed virus. It has been tentatively named *Cassia* severe mosaic virus and is similar to several potyvirus isolates from *Cassia occidentalis*

in Yemen (Walkey, 1992). As Ethiopia and Yemen are geographically close, and *Cassia occidentalis* occurs commonly in both countries, it is possible that this virus may have spread between one or other of the two countries. Its characterization needs to be completed to confirm that it is a new virus.

Several isolations of other potyviruses were made from *P. vulgaris* and various wild legume species during the survey which were not related to BCMV or any of the other known potyviruses identified in the study (see Section 4). Further studies are now necessary to characterize these isolates fully and to determine if they are strains of a known virus or are new viruses.

Appendix 1

Methodology

COLLECTION OF VIRUS SAMPLES

The following field surveys were carried out in Africa in collaboration with CIAT and national bean programme personnel, to collect virus-infected bean and wild legume samples for virus isolation and identification at Wellesbourne. In addition, numerous samples were received directly from collaborators in Africa.

January 1990:	Uganda, Lesotho, Swaziland, Zimbabwe and Malawi.
September 1990:	Ethiopia.
November 1990:	Morocco.
May 1991:	Tanzania, Uganda, Rwanda and Zaire.
November 1991:	Rwanda, Burundi and Kenya.

Plant material was normally collected as leaves, showing distinct virus symptoms. If samples other than bean (*Phaseolus vulgaris*) were collected, additional herbarium material was preserved in a flower press and photographs taken to aid identification. For each specimen collected, records were taken of location, altitude, host species, cultivar (if known) and disease severity.

Before any collections of plant material were made, an experiment was carried out to investigate the most suitable collecting method for maintaining short-term virus viability, which would allow the samples to be collected in Africa and returned to Wellesbourne for identification. The results of this experiment are detailed below.

The infectivity of BCMV during short-term storage

Plants of the bean cv The Prince were inoculated with the standard NL4 strain of BCMV. One infected trifoliate leaf was removed 21 days after inoculation and stored under different conditions. The infected plants were then kept in the glasshouse so that fresh infected leaves could be sampled at intervals as controls. The experimental treatments were as follows:

- Leaves stored in sealed polythene bags between sheets of damp filter paper at 4 °C;
- Leaves stored as above but at 20 °C;
- Leaves air-dried between sheets of dry filter paper at 4 °C;
- Leaves air-dried between sheets of dry filter paper at 20 °C;
- Leaves placed in screw-topped plastic scintillation vials containing 10 g of calcium chloride at 20 °C.

There were twenty-four replicate samples of each treatment of which eight were tested at each of three sampling dates. At each sampling date (7, 14 and 49 days after storage), samples were ground in 1% phosphate inoculating solution (K_2HPO_4) containing 0.1% Na_2SO_3 (1 ml of buffer/g of plant material), and inoculated onto two bean plants of cv The Prince. Test plants were recorded for symptoms for up to 4 weeks after inoculation.

Table A1 The infectivity of the NL4 strain of BCMV under different short-term storage conditions

Storage treatment	No. of plants inoculated	No. of test plants infected following storage		
		7 days	14 days	49 days
Damp, 4 °C	8	8*	3	2
Damp, 20 °C	8	8	2	0
Dry, 4 °C	8	2	3	0
Dry, 20 °C	8	0	1	0
Calcium chloride	8	8	7	7
Control	8	8	8	8

* all infected test plants developed systemic mosaic symptoms

The results indicated that air-drying infected leaf samples at 4 °C or 20 °C was unsatisfactory for maintaining infectivity (Table A1), which was greatly reduced after 7 or 14 days and completely lost after 49 days storage. Storing samples in damp conditions maintained the infectivity of the virus although infectivity was greatly reduced after 14 days at 4 °C and 20 °C and completely lost after 49 days storage at 20 °C. The virus was still infective in some damp samples stored at 4 °C for 49 days. Most samples stored over calcium chloride at room temperature were still infective after 49 days.

As a result of this experiment, infected material collected in Africa was stored both as fresh leaves refrigerated at about 4 °C and by drying over calcium chloride.

Storage of survey samples

Young leaves with virus symptoms were removed from the plant and placed between two sheets of filter paper (Whatman no.1), the paper was lightly moistened with water and then placed in a polythene bag. The samples were stored in cool bags in the field and later refrigerated at 4–5 °C, if possible, until they reached the laboratory. Experience showed that refrigerated fresh samples would normally yield viable virus for 2 weeks after collection. Identical leaves were also taken from the plant and immediately dried in plastic scintillation vials (Hughes and Hughes Ltd., U.K.) containing calcium chloride. Approximately 10 g of calcium chloride was placed in each vial covered by a small amount of non-absorbent cotton wool to prevent its contact with the plant material. The vial was sealed with a screw top. The amount of plant material added to each vial was limited to about 5 g; if too much plant material was added the calcium chloride became deliquescent and the sample did not dry satisfactorily.

Upon arrival at Wellesbourne each fresh sample was divided into three portions. One portion was homogenized in 1% K_2HPO_4 solution containing 0.1% Na_2SO_3 (in the ratio 1 g leaf to 1 ml solution), immediately placed in a plastic vial and stored in liquid nitrogen. A second portion was examined by electron microscopy to observe any virus particles and the third was used for sap transmission to appropriate test plants (see p. 102).

In addition to fresh and dried leaf samples, BCMV isolates were also obtained by collecting seed. The virus is seed-transmitted with varying frequency depending on the bean cultivar and virus strain. Seed was normally bought in local markets where the varieties for sale were usually local landraces. BCMV isolates from such landraces were more likely to be typical of local strains, than those isolated from recently imported seed. Seed was stored at 8 °C and 50% relative humidity until it was germinated to isolate the virus.

Storage of virus isolates

Following identification by differential hosts and serological studies (see Appendix 1) the virus isolates were stored in liquid nitrogen and freeze-dried. Infected leaf samples to be stored in liquid nitrogen were ground with a pestle and mortar in 1% K_2HPO_4 phosphate solution containing 0.1% Na_2SO_3. The sap was poured into clearly labelled screw-top cryogenic tubes (Sterilin, U.K.) which were immediately plunged into liquid nitrogen flasks (H 123/27, Jencons, U.K.). Samples were recovered from liquid nitrogen by allowing them to thaw for about 10 minutes at room temperature, before directly inoculating the sap onto test plants.

For freeze-drying, infected leaf samples (about 2 g) were placed in 0.5 ml glass ampoules (Samco, U.K.) and dried using an Edwards 5 PS centrifugal freeze-drier. The specimens were stored in the ampoules under vacuum and were revived by cutting open the vial with a glass cutter and grinding the contents in 1% K_2HPO_4 solution containing 0.1% Na_2SO_3 (1 ml buffer to 1 g dried material), before inoculating onto test plants.

ISOLATION AND HOST RANGE IDENTIFICATION OF VIRUS ISOLATES

Test plants and virus inoculation

A range of host species was used to isolate virus from the collected samples and the choice of these species was often determined by the species of plant from which the infected sample was collected. Two bean seedlings of cv The Prince or Double White (cultivars susceptible to all strains of BCMV) which had partly expanded primary leaves, but no visible trifoliate leaf, were used to isolate virus from all bean samples, together with one seedling each of *Chenopodium quinoa* and *Nicotiana clevelandii*. The following hosts were also used to isolate virus from non-*Phaseolus vulgaris* hosts: cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), various *Cassia* species and *Nicotiana benthamiana*.

Seeds of test plants were germinated in plastic boxes on damp cellulose wadding by incubating for four days at 25 °C. The germinated seed was sown in individual 3-inch plastic pots in M2 compost (Fisons, U.K.) and kept in an insect-proof glasshouse at 26 °C. From October to March supplementary lighting was provided to give a daylength of 16 hours. Seeds of the genera *Chenopodium* and *Nicotiana* were sown directly in sifted peat compost and germinated seedlings pricked out into individual pots.

Infected samples (either fresh or dried) were ground in ice-cold 1% phosphate solution (K_2HPO_4) containing 0.1% Na_2SO_3 (1 ml of buffer per 1 g of material). Approximately 2 ml of this inoculum was stored in liquid nitrogen and the remainder was inoculated to carborundum (300 mesh) – dusted leaves of the test plants, by gently rubbing the leaf surface with a muslin pad moistened with inoculum. After inoculation, the leaf surface was immediately watered using a watering can to improve test plant infection (Yarwood, 1973).

The test plants were observed regularly for symptoms for a period of 4 weeks after inoculation and tested for the presence of virus by electron microscopy and ELISA (see Appendix 1). Isolates were maintained by regular sub-inoculation to host seedlings and if a virus isolate was lost or the host plant died prematurely, another test plant was inoculated with the original inoculum stored in liquid nitrogen.

The symptoms produced on test plants by unknown isolates were recorded and compared with those produced by standard BCMV strains and related

viruses. To assist identification, the results were also compared with previous literature descriptions, particularly the AAB/CMI Descriptions of Plant Viruses.

Identification of BCMV isolates by host reactions

The only method currently available to identify strains of BCMV is to study host symptom reactions in a range of selected differential *P. vulgaris* cultivars (Drijfhout, 1978). The system of differential bean cultivars was devised by Drijfhout after the genetic basis of host resistance in beans had been determined (see Section 1, p. 17) The known strains of BCMV have been divided into ten pathogenicity groups (I to VII, some with subdivisions), according to the pathogenicity genes present (Table 1). Correspondingly, host genotypes are divided into eleven resistance groups (1 to 11) according to their resistance genotype (i.e. the combination of resistance genes carried by a particular host).

A BCMV strain may be characterized by the set of reactions it causes in a selected range of differential cultivars, this is described as its pathogenicity phenotype. The pathogenicity phenotype is the reaction that a virus strain induces in a standard series of differential cultivars, determined by whether or not the pathogen systemically infects each cultivar (Drijfhout, 1978) (see Table 2). Correspondingly, each differential cultivar in the series has a resistance phenotype, which is the reaction of the cultivar to a standard range of virus strains, determined by whether or not individual strains can systemically infect the cultivar.

When an unknown strain of BCMV is inoculated onto plants of this differential series, its pathogenicity phenotype can be compared with that produced by standard strains (see Table 2). The unknown strain may then be identified as being identical or closely related to a standard strain.

Seeds of the differential host cultivars were kindly supplied by E. Drijfhout and multiplied annually at Wellesbourne in insect-proof polythene tunnels. Ten plants of each cultivar were planted, which yielded a total of 2 to 6 kg of seed depending on the cultivar.

Up to 22 cultivars (Table A2) were used for screening the unknown BCMV isolates, with a minimum of two cultivars per resistance group being used in any one test.

Four seedlings of each cultivar were inoculated with each unknown isolate as soon as their primary leaves unfolded (as described in Appendix 1). These plants were kept in a insect-proof glasshouse at 26 °C. In addition, two further plants of

Table A2 Differential host resistance group cultivars used to identify BCMV strains

Host resistance group	Bean cultivar
1	The Prince, Common Red Mexican (CRM), Double White (DW), Stringless Green Refugee (SGR), Sutter Pink (SP)
2	Pure Gold Wax (PGW), Redlands Greenleaf C (RGC)
3	Redlands Greenleaf B (RGB), Great Northern 59 (GN 59), Great Northern 123 (GN 123)
4	Michelite, Sanilac
5	Pinto 114 (P114)
6	Monroe, Red Mexican 35 (RM35), Great Northern 31 (GN31)
8	Black Turtle Soup (BTS), Widusa
9a	Jubila
9b	Top Crop (TC), Improved Tendergreen (ITG)
10	Amanda

cultivars in resistance group 8, 9a, 9b and 10 (cultivars which possess the dominant *I* gene) were inoculated with each isolate and grown in a controlled environment cabinet at 32 °C to identify those strains which cause systemic necrosis only at this higher temperature.

Prior to inoculation of the complete differential host series, virus isolates were propagated in cvs The Prince or Double White. Only when these isolates had been positively identified as BCMV (by host symptoms, ELISA and ISEM studies), were they inoculated onto the bean differential host series.

The standard BCMV strains NL1, NL3, NL4, NL5, NL6, NL8 and NY15 were all tested on the differential host series to ensure that they induced reactions according to those described by Drijfhout (1978). Cultures of these strains were then maintained in bean cvs The Prince or Double White and in liquid nitrogen. The appropriate standard strain was used for comparison of reactions when unknown isolates were being identified. In addition, isolates of several other viruses closely related to BCMV and known to infect legumes were also maintained for comparison of symptoms and other characteristics (Table A3). These included blackeye cowpea mosaic (BLCMV-NR strain), cowpea aphid-borne mosaic (CAbMV) and peanut mottle (PnMoV) viruses. The groups of bean cultivars used to differentiate BCMV pathogenicity groups and strains are shown in Table A2.

Table A3 List of standard viruses and virus strains used

Virus strain	Source	Propagation host	Country of origin
BCMV NL1	E. Drijfhout	*Phaesolus vulgaris*	Netherlands
BCMV NL3	E. Drijfhout	*P. vulgaris*	Netherlands
BCMV NL4	E. Drijfhout	*P. vulgaris*	Netherlands
BCMV NL5	H.J. Vetten	*P. vulgaris*	Germany
BCMV NL6	E. Drijfhout	*P. vulgaris*	Netherlands
BCMV NL8	E. Drijfhout	*P. vulgaris*	Netherlands
BCMV NVRS	D.G.A. Walkey	*P. vulgaris*	U.K.
BCMV NY15	H.J. Vetten	*P. vulgaris*	Germany
BLCMV NR	H.J. Vetten	*Nicotiana clevelandii*	Germany
CAbMV	H.J. Vetten	*N. clevelandii*	Germany
PnMoV	H.J. Vetten	*N. clevelandii*	Germany
AlfMV	D.G.A. Walkey	*N. clevelandii*	Yemen

Table A4 Antibodies used in ELISA

Name	Type	Source
BCMV NL1	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL3	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL4	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL5	Pab	H.J. Vetten, Germany
BCMV NL6	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL8	Pab	N.J. Spence, HRI Wellesbourne
BCMV NY15	Pab	H.J. Vetten, Germany
BCMV NVRS	Pab	D.G.A. Walkey, HRI Wellesbourne
BLCMV-NR	Pab	H.J. Vetten, Germany
CAbMV	Pab	H.J. Vetten, Germany
PnMoV	Pab	H.J. Vetten, Germany
bc-1-3	Mab	H.J. Vetten, Germany
bc-1-1A4	Mab	H.J. Vetten, Germany
I2	Mab	G.I. Mink, Washington U.S.A.
197	Mab	G.I. Mink, Washington U.S.A.

Pab = polyclonal antibody, Mab = monoclonal antibody

Table A5 System used for scoring symptoms of differential host cultivars

Symptom score	Description of symptom
0	No visible symptoms on any part of the plant
1	Local chlorotic lesions only
1^{n}	Local necrotic lesions only
2	Mild systemic mosaic of leaves, no deformity of leaves or stunting of the plant
2^{n}	Systemic necrotic lesions affecting small areas of the leaves, no stunting of the plant
3	Severe systemic mosaic of leaves, and leaf deformity, with or without stunting of the plant
3^{n}	Systemic necrosis affecting large areas of the leaves, with or without stunting of the plant
4	Severe systemic mosaic of leaves, severe leaf deformity and plant severely stunted
4^{n}	Systemic necrosis affecting most of the leaf area, plant severely stunted with necrosis of the stem ('black root' symptoms) and/or growing tip
5^{n}	Leaves totally necrotic, growing tip necrotic and necrosis of the stem and sometimes the roots, plant dying

^{n}=necrosis

All plants were scored for symptoms at intervals up to 4 weeks, using the system shown in Table A5. The time taken for symptoms to develop varied according to environmental conditions at different times of the year. Differential cultivars that developed questionable symptoms were back-inoculated to plants of the cv The Prince or Double White to check for virus infection, or the suspect plants were tested for infection by ELISA.

VIRUS PURIFICATION

Although numerous methods have been described for the purification of BCMV many have given unsatisfactory results (Day, 1984). The method used in this study was based on a modification of an unpublished procedure developed by Dr. X. Ding at Wellesbourne. It was satisfactory for all standard BCMV strains and isolates purified for antiserum production or electrophoresis. The same method was also used for the purification of *Cassia* severe mosaic virus (CSMV, see Section 4).

Leaf material was homogenized with phosphate solution (0.5 M K_2HPO_4, pH 7.5) containing 0.02 M Na_2SO_3) and solvent (chloroform) in a glass blender, in the ratio of 1 g leaf: 2 ml of 0.5 M phosphate solution: 1 ml chloroform. The homogenate was centrifuged in an MSE GF-8 at 500 *g* for 10 min at 4 °C and then filtered through one layer of paper tissue (Kimwipe). The filtrate was again centrifuged at 5000 *g* for 10 min at 4 °C and the supernatant filtered through one layer of paper tissue. The volume was measured and polyethylene glycol (mol. wt. 6000) and sodium chloride were added to concentrations of 6% and 4% respectively. The supernatant was stirred for 90 min at 4 °C and centrifuged at 12 000 *g* for 10 min at 4 °C. The resultant pellets were each resuspended in 2 ml of 0.5 M phosphate buffer containing 0.01 M EDTA (ethylenediaminetetra-acetic acid), pH 7.5 and a further 2 ml of the same buffer which was used to rinse the tubes was added. The preparation was stirred overnight at 4 °C.

The preparation was divided between two 36 ml centrifuge tubes which were then filled with 0.5 M phosphate buffer (pH 7.5) containing 0.01 M EDTA. It was centrifuged at 9000 *g* for 10 mins at 4 °C before the supernatant was recovered and further centrifuged at 65 000 *g* for 120 mins at 4 °C. The resultant pellets were each resuspended in 2 ml of 0.25 M phosphate buffer (pH 7.5) containing

0.01 M EDTA and a further 2 ml of the same buffer used to rinse the tubes was added. The preparation was stirred overnight at 4 °C.

The following day the preparation was centrifuged at 6000 *g* for 10 min at 4 °C, the supernatant removed and diluted to a total volume of 9 ml with 0.25 M phosphate buffer. The preparation was then mixed with 4 g caesium chloride and centrifuged at 110 000 *g* for 18 h at 4 °C. The virus bands were drawn off with a syringe and diluted with 0.25 M phosphate buffer to fill two 36 ml centrifuge tubes, before being centrifuged at 65 000 *g* for 120 min at 4 °C. The resultant pellets were each resuspended in 0.5 ml distilled water.

The yield of virus in each purified preparation varied according to the quantity and virus content of infected plant material. The final concentration of virus in the preparation was determined by ultraviolet spectorphotometry using the extinction coefficient for potyviruses of 2.4–2.9. The absorption coefficient (A_{260}/A_{280}) was also determined for each purified preparation for comparison with the range of A_{260}/A_{280} values already determined for BCMV (i.e. 1.12–1.27) (Morales and Bos, 1988). The absorption coefficient is the ratio of the absorbence of a virus preparation in light of wavelengths 260 nm and 280 nm. This gives an estimate of the proportion of nucleic acid in the nucleoprotein preparation which can be of value in characterizing virus particles, and also gives an estimate of the purity of the preparation. The absorption coefficient increased with the proportion of RNA in the nucleoprotein; this is also true of the relationship between the extinction coefficient and RNA content (Gibbs and Harrison, 1976).

The purified preparations were used for antiserum production and in electrophoresis.

PRODUCTION OF POLYCLONAL ANTISERUM

Female, New Zealand white rabbits were used to produce antisera and prior to the first injection they were bled from the marginal ear vein to obtain a small quantity of normal serum. The purified virus antigen for antiserum production was thoroughly mixed with an equal volume of Freund's incomplete adjuvant (usually 0.5 ml virus to 0.5 ml adjuvant) and injected into the thigh muscle of the rabbit. A course of six to seven injections was given, in alternate thigh muscles, at weekly intervals. Ten days after the final injection the rabbit was bled from the marginal ear vein and blood was taken at weekly intervals for the next 4 to 5 weeks (approximately 30 ml of blood were taken each time). Each blood sample was allowed to coagulate at room temperature overnight before the serum was drawn off and centrifuged at 5000 *g* for 10 min to separate it from any remaining red blood cells. The serum was preserved in an equal volume of glycerol and stored in a glass container at 4 °C.

PURIFICATION AND CONJUGATION OF ANTISERUM

Antiserum purification

To use polyclonal antiserum in ELISA it must first be purified to concentrate the antibodies (primarily the immunoglobulin IgG) and to remove other proteins such as plasma proteins which are not immunogenic.

One ml of crude antiserum was mixed with 9 ml of distilled water and 10 ml of saturated ammonium sulphate solution and left at room temperature for 1 hour. The mixture was then centrifuged at 9000 *g* for 10 min at 4 °C and the pellet resuspended in 2 ml of half-strength PBS (sodium phosphate-buffered saline, pH 7.3, see Table A15). Three 10 cm lengths of visking tubing were cut

and boiled for 3 minutes in 0.001 M EDTA. The serum mixture was dialysed in the tubing in 1 l of half-strength PBS for two separate 3 h periods and once overnight in 1.5 l of half-strength PBS, before being fractioned on a separating column.

To prepare a separating column, 10 g of cellulose (Whatman DE 52) was mixed with 100 ml of PBS (×10 concentration). After the cellulose had settled, the concentrated PBS was poured off and the procedure was repeated twice more with fresh PBS (×10). This procedure was repeated three times using half-strength PBS. The mixture was pipetted into a 10 ml fast-flow pipette which had been broken off at the 5 ml level and which had a small glass wool plug at the narrow end to allow the cellulose to accumulate up to the 4 ml mark. The column was equilibrated to pH 7.3–7.4 by running half-strength PBS through it and when the correct pH was achieved the dialysed antiserum mixture was pipetted into the column, 1 ml at a time, followed by half-strength PBS. Ten 1 ml fractions were collected in separate test tubes and the absorbance at 280 nm (A_{280}) of each fraction determined with a spectrophotometer. The fractions were mixed according to their individual absorbances to produce one sample with an absorbance of 1.36, as this is equivalent to 1 mg IgG /ml (Johnstone and Thorpe, 1987). Aliquots of purified immunoglobulin were freeze-dried and stored at 4 °C.

Conjugation of purified antisera

For direct ELISA it is necessary to conjugate the specific purified IGg to an enzyme, usually alkaline phosphatase. The following procedure was used to conjugate the IGg of all polyclonal antisera used.

One ml of purified immunoglobulin from the purification of crude antiserum described in Appendix 1. Alkaline phosphatase (Type VII-S, Sigma) (0.25 ml) was centrifuged at 5000 *g* for 10 min at 4 °C and the pellet was resuspended in 1 ml of the purified immunoglobulin and dialysed three times in half-strength PBS as described in Appendix 1. The contents of the dialysis tubing were mixed with 25% glutaraldehyde to a concentration of 0.006% and left at room temperature in a fume cupboard for 4 h, before being dialysed against half-strength PBS a further three times. The resulting conjugate was stored in silicone-coated glass Durham tubes (Samco Ltd) at 4 °C.

IDENTIFICATION OF BCMV ISOLATES BY SEROLOGY

Introduction to the antisera used in serological tests

On arrival at Wellesbourne, most of the original fresh and dried survey samples were tested initially by indirect-plate trapped antigen ELISA (PTA-ELISA) using monoclonal antibodies (see Table A4). In addition, fresh samples was ground and loaded onto ELISA plates during field surveys in Africa and these plates were then developed on return to the laboratory. These initial PTA-ELISA tests indicated whether or not the sample was infected with BCMV, and to which serotype the isolate belonged. If a virus was subsequently isolated, further ELISA tests and electron microscopy were carried out to confirm the initial serology result. In the present survey, virus was not isolated from many samples, so the initial ELISA result was the only diagnostic information obtained for these particular samples (see Table A14). For these samples, the serological data were confirmed by a second ELISA test on the portion of leaf sample that had been initially stored in liquid nitrogen.

A number of monoclonal antibodies and polyclonal antisera (described in Table A4) were used in ELISA and electron microscopy tests to aid the identification of the BCMV strains and other viruses. Monoclonal antibodies bc-1-3 (BC3) and 197 were broad-spectrum antibodies which reacted in PTA-ELISA with all standard BCMV strains and with some other potyviruses including BLCMV, CAbMV and PnMoV. A sample which produced a positive reaction with either of these monoclonal antibodies was considered a possible strain of BCMV or another potyvirus. In contrast, monoclonal antibodies bc-1-1A4 (BC1) and I2 were highly specific and reacted in ELISA only with BCMV strains NL3, NL5 and NL8, the temperature-independent necrotic strains. The monoclonal antibodies BC3 and BC1 were used during the early surveys of 1990 and 1991 but were later replaced by 197 and I2. Each survey sample was tested by PTA-ELISA in two duplicate wells, with one of each of these two types of monoclonal antibodies (i.e. BC1 and BC3 or 197 and I2). If the sample reacted with both monoclonal types, it was designated an 'A' serotype, necrotic strain of BCMV; if the sample reacted only with BC3 or 197, and not BC1 and I2, it was designated a 'B' serotype, non-necrotic strain of BCMV, or an unknown potyvirus. However, if the sample, very exceptionally reacted only with either BC1 or I2, and not with BC3 or 197, it was designated a possible 'A' serotype of BCMV. Negative or inconclusive tests were repeated and if the result was still not conclusive it is indicated as such in the Tables (see Table A14).

Samples were also tested in direct double-antibody sandwich ELISA (DAS-ELISA) with a number of polyclonal antisera raised to specific BCMV strains. Most of these polyclonal antisera reacted specifically with BCMV, although not always with specific strains (see Section 6). The antiserum to the NL3 strain had a broad-spectrum reaction with all BCMV strains and the antiserum to NL8 reacted only with 'A' serotypes of BCMV. The antiserum to the NY15 strain reacted with all strains of BCMV except strain NL8 and also with CAbMV and BLCMV. If a sample reacted in ELISA with NY15 and none of the other polyclonal antisera to BCMV it could have been infected with CAbMV or BLCMV. BLCMV infection was confirmed using a polyclonal antiserum to BLCMV. In some samples, from which no virus was isolated, it was difficult to determine by ELISA if they were infected with BCMV, BLCMV or a mixture of the two viruses. In these cases, the samples were designated BCMV?, BLCMV? or BCMV/BLCMV?. A polyclonal antiserum to alfalfa mosaic virus (AlfMV) was used in ELISA to investigate samples from Morocco. In some samples both AlfMV and BCMV were detected and these were designated AlfMV/BCMV (Table 33).

Procedures for enzyme-linked immunosorbent assay (ELISA)

ELISA tests were carried out routinely with a range of different polyclonal and monoclonal antibodies (Table A4) using modifications of the method of Clark and Adams (1977). Initially, the purified antisera and conjugate of each of the polyclonal antisera prepared to standard BCMV strains were tested in DAS-ELISA and the monoclonal antibodies were tested in PTA-ELISA (Section 2). This allowed a determination of the working dilutions which gave an absorption of at least 1.0 at 405 nm and was at least twice the value of the mean healthy control. These dilutions varied for each antiserum according to the titre, but were normally 1.0 µg/ml for the coating antisera and 1/1000 for the conjugate. However, the absorbence values of the antisera to standard strains NL1 and NY15 were lower than 1.0 even when the concentrations were increased to 5 µg/ml and 1/500, respectively. Increasing the concentration further, did not significantly increase the absorbence value. In all ELISA tests a reaction was considered to be positive if the absorption value of the sample was at least twice that of the mean healthy control sample, which varied for each antiserum.

For direct double-antibody sandwich ELISA (DAS-ELISA), microtitre plates (Dynatech, U.K.) were coated with purified IgG (immunoglobulin G, see Appendix 1, p. 106) diluted to 1 µg/ml in 0.05 M sodium carbonate buffer pH 9.6, using 100 µl per well (see Table A15). The plates were incubated with coating antiserum at 37 °C for three hours, then washed 3 times for 3 minutes each with phosphate-buffered saline containing 0.5% Tween-20 (PBS-T), pH 7.3 (see Table A15). Samples were ground in PBS-T containing 2% polyvinylpyrrolidone (mol. wt. 44 000) (see Table A15) and loaded into duplicate wells, using 100 µl per well and incubated overnight at 4 °C. Infected and healthy control samples were always included on each plate. After a repeat of the washing procedure, the plates were coated with purified IgG conjugated to the alkaline phosphatase (Appendix 1), diluted to 1/1000 in PBS-T containing 0.05% bovine serum albumin (BSA) as a blocking agent. Plates were incubated at 37 °C for 4 h, then the washing procedure was repeated. The colour reaction was developed by adding 100 µl *p*-nitrophenyl phosphate in 10% diethanolamine buffer (see Table A15), pH 9.8, to each well and the reactions read with a Titertek Multiskan MCC/340 plate reader at an absorbence of 405 nm.

For plate-trapped antigen ELISA (PTA-ELISA) with polyclonal antisera, samples were ground in 0.05 M sodium carbonate buffer and loaded onto the plates as described above. After overnight incubation at 4 °C followed by washing, the plates were coated with IgG diluted as above, but using PBS-T instead of carbonate buffer. After 2 h at room temperature the plates were washed again and then coated with anti-rabbit antiserum conjugated to alkaline phosphatase (supplied by Sigma Ltd) diluted in PBS-T containing 0.05% BSA according to the manufacturer's instructions. After a further 2 h at room temperature the plates were washed and developed with substrate as described for the direct ELISA method.

Monoclonal antibodies were used in PTA-ELISA at a dilution of 1/1000 or 1/2000 in PBS-T, and goat anti-mouse conjugate (supplied by Sigma Ltd) was used diluted in PBS-T containing 0.05% BSA according to the manufacturer's instructions.

Western blotting

The method was that of Torrance (1992). After elecrophoresis, gels were equilibrated for 1 h in transfer buffer (see Table A16). Then the proteins were transferred electrophoretically from the gel to a 45 µm nitrocellulose membrane (supplied by Schleicher and Schuell Ltd), using a Trans-Blot™ apparatus (Bio-Rad Ltd) for 60 min at 100 V (see Appendix 1). After blotting, the membrane was air-dried overnight between two pieces of filter paper.

The membrane was blocked with 0.05 M Tris-buffered saline solution, pH 7.4, containing 0.1% Tween 20 (TBS-T) (see Table A16) and 5% skimmed milk for 60 min at room temperature on a shaker. The membrane was then washed three times for 5 min each in TBS-T on a shaker before incubation with a monoclonal antibody or purified polyclonal antiserum, diluted in TBS-T containing 0.5% bovine serum albumin (BSA), for 2 h at room temperature on a shaker. The membrane was washed as before, then incubated with an anti-mouse or anti-rabbit antibody conjugated to alkaline phosphatase (Sigma) diluted in TBS-T containing 0.5% BSA for 2 h on a shaker. After a final washing, the substrate was made up by taking 10 ml of substrate buffer and adding 330 µl of nitro blue tetrazolium (NBT) stock solution and 33 µl of bromo-chloro-indolyl phosphate (BCIP) stock solution (see Table A16). The substrate was gently pipetted over the membrane and after the blue colour had developed sufficiently, the reaction was stopped by rinsing the membrane in water and air-drying it between two pieces of filter paper.

Virobacterial agglutination test (VBA)

A simple, rapid virobacterial agglutination (VBA) test was developed to identify BCMV in crude sap extracts. The test visualizes the agglutination of *Staphylococcus aureus* bacteria particles, which have been pre-conjugated with specific virus antibodies, when mixed with infected crude leaf sap, containing the specific antigen to the antibody-conjugated bacteria. Agglutination occurs rapidly (within 0.5–3 min) (Walkey *et al.*, 1992).

The BCMV antiserum was prepared for conjugation with the bacterial particles by diluting in phosphate-buffered saline (PBS) pH 7.2, containing 2 mg/ml sodium azide in the ratio 1 vol of antiserum (50:50 antiserum/glycerol mixture) to 24 vols of PBS buffer. A suspension of formalin-treated *S. aureus* was then mixed with the diluted antiserum in the ratio of 1 vol bacteria suspension to 5 vols of antiserum. This conjugate may be coloured to improve the visualization of the test, by adding several drops of alcohol-saturated basic fuchsin stain.

Approximately 2 µl of this conjugate was mixed with 2 µl of antigen (infected crude plant sap) on a blue multitest slide (Flow Laboratories Ltd). A negative control test, using healthy crude sap mixed with the conjugate was prepared at the same time. A positive reaction was indicated by agglutination of the particles within 0.5–3 min (see Plate 30) and was observed with a hand-lens with the slide held over a black background lit by diffuse light.

GEL-ELECTROPHORESIS

Polyacrylamide gel electrophoresis (PAGE) was carried out using the sodium dodecyl sulphate (SDS) discontinuous gel system of Laemmli (1970) with a Mini-Protean II™ dual slab cell apparatus (Bio-Rad Ltd.). Gels were made with 12.5% acrylamide-bisacrylamide in the resolving gel and 4.5% acrylamide-bisacrylamide in the stacking gel (see Table A17).

Partially or fully purified virus samples were diluted 1:4 in SDS reducing sample buffer (Laemmli, 1970) and crude virus preparations were made by grinding 1 g of fresh leaf tissue in liquid nitrogen and adding 1 ml of sample buffer (see Table A17). All samples were boiled in Eppendorf tubes with the sample buffer for 3 min and then centrifuged in a bench centrifuge for 5 min. Seven molecular weight markers were used: Bovine albumin (66 000 Da), egg albumin (45 000 Da), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 000 Da), bovine erythrocyte carbonic anhydrase (29 000 Da), bovine pancreas trypsinogen (24 000 Da), soybean trypsin inhibitor (20 100 Da) and bovine milk lactalbumin (14 200 Da) (MW-SDS-70L kit, Sigma Ltd). Electrophoresis of proteins took place at 200 V for about 45 min at room temperature in electrode buffer (see Table A17). Gels were stained overnight on a shaker in 0.2% Coomassie blue in 40% methanol and 10% acetic acid, then destained with 40% (v/v) methanol and 10% (v/v) acetic acid for approximately 3 h on a shaker with several changes of destaining solution. Finally, the gels were rehydrated in 7% acetic acid. Alternatively, gels were equilibrated in transfer buffer and then blotted onto a nitrocellulose membrane before staining.

For each gel the relative mobility (R_f) of the molecular weight markers was used to construct a calibration curve from which the molecular weight of the virus coat proteins was determined. For all isolates the mean molecular weight of the major protein subunit was determined, to the nearest 0.5 kDa, from at least five gels of the same purified preparation (see Table 38). Some standard BCMV strains also had additional bands of minor protein subunits, which were interpreted as being degradation products of the viral protein since they were not present in purified extracts from healthy leaves.

ELECTRON MICROSCOPY

'Quick-dip' examination

If a sample was thought to be virus-infected it was first examined for virus particles in the electron microscope (EM) using the 'quick-dip' method (Brandes, 1957). This method was used with both crude sap from infected leaf sample and with purified virus preparations.

Crude sap was prepared by grinding a small portion of leaf (approximately 2–3 mm^2) in one drop of wetting agent (0.05% bacitracin) and two drops of 2% methylamine tungstate stain (MT) on a glass slide using a disposable plastic rod. The sample was then loaded directly onto the carbon-coated surface of a copper grid and blotted dry with filter paper. Grids were stored on filter paper in petri dishes until viewed. Purified preparations of virus were prepared for electron microscopy by mixing 0.005 ml of purified preparation with 0.02 ml of MT stain and 0.005 ml of wetting agent on a glass slide and then loading the sample directly onto the carbon-coated grid as described above.

Immunosorbent electron microscopy (ISEM)

ISEM was used only when virus particles had been seen in a sample by 'quick-dip' electron microscopy and was based on the method of Derrick (1973). Dilutions of 1/50 and 1/1000 of the crude antiserum were prepared in 50 mM potassium phosphate buffer (K_2HPO_4) pH 7.5. Copper EM grids coated with cellulose nitrate were floated, with the coated side down, on a drop of 1/1000 dilution of antiserum ('attracting' antiserum) and incubated in a moist chamber for 1 h at room temperature. Samples were prepared by grinding a small piece (approx. 2–3 mm^2) of leaf material in two drops of the phosphate buffer, or by mixing 0.005 ml of purified virus with two drops of phosphate buffer. The grids were blotted with filter paper (Whatman no. 1) then washed with a few ml of the phosphate buffer and blotted again. The grids were floated on a drop of the leaf sap sample and incubated in the moist chamber for 1 h. Grids were blotted and washed again as described above and floated on a drop of 1/50 antiserum ('decorating antiserum') for 1 h. The grids were blotted and washed with buffer and finally washed with double-distilled water, and blotted again. They were stained by adding a few drops of saturated uranyl acetate (approximately 5% solution) to each grid. The grids were immediately blotted and stored in a petri dish or grid holder until observed in the EM.

For all ISEM tests a control reaction was made using known virus particles decorated with their homologeous antibodies. If the same antisera also decorated the unknown virus particles to a similar intensity (see Plate 13d), the reaction was interpreted as an indicating a close relationship between the unidentified virus and the antiserum. If the unidentified particles were only faintly decorated or if the decoration was patchy, the relationship was considered more distant. If the antiserum failed to decorate the virus particles it was considered unrelated to the virus under test.

A JEOL JEM-100CX II transmission electron microscope was used for examining the grids and taking photographs.

Particle measurement

The number of virus particles seen in 'quick-dip' preparations was too few to allow accurate measurement. However, since complete purification of the isolates may have modified their morphology, particles were concentrated by the following procedure.

Infected leaf material was homogenized in 0.5 M phosphate buffer, containing 0.002 M Na_2SO_3 pH 7.5, in a ratio of 1 g leaf to 2 ml of buffer and then filtered through one layer of muslin. The supernatant was centrifuged at 9000 *g* for 10 min at 4 °C and then filtered through one layer of paper tissue (Kimwipe) before being centrifuged again at 65 000 *g* for 120 min at 4 °C. The resultant pellets were not resuspended, but a small amount of pellet was mixed with a drop of wetting agent (0.05% bacitracin) and two drops of MT stain (2% methylamine tungstate) on a glass slide using a disposable plastic rod. The sample was then loaded directly onto the cellulose nitrate coated surface of a copper grid and blotted dry with filter paper. Grids were stored on filter paper in petri dishes.

The virus particles were photographed at a magnification of ×28 000 and a diffraction replica grating (462.963 nm) was also photographed to calibrate the microscope and calculate the exact magnification. The photographic film was developed and the negatives were projected onto the graphics tablet of a Hewlett Packard HP 85 computer using a photographic enlarger. The particles were measured with the graphics tablet and the actual lengths calculated using the diffraction grating replica. Approximately 100 particles of each virus isolate were measured.

Cytological studies

Small samples, approximately 4 mm in diameter, were cut from infected and healthy leaves avoiding the large veins. The samples were fixed in glutaraldehyde (2% in 0.1 M sodium cacodylate buffer pH 7.2) for 60 min, then rinsed three times for 10 min in the same buffer before fixing in osmium tetroxide (1% in buffer) for 120 min followed by the same rinsing procedure. Samples were then dehydrated in 50% and 75% ethanol diluted in distilled water for 30 min at each concentration and dehydrated twice for 30 min in 100% ethanol.

After dehydration, the samples were infiltrated with a 1:1 mixture of 100% ethanol and medium-grade LR White acrylic resin (London Resin Co. Ltd, Basingstoke, Hants, UK) for 24 h at 4 °C, and then infiltrated with LR White resin only on three occasions over the next 72 h. Finally, samples were polymerized in resin at 58 °C for 20 h, initially in a nitrogen-enriched atmosphere without oxygen for approximately 1 h.

After polymerization, the resin blocks were trimmed with a pyramitome (model 11800, LKB-Produkter AG, Sweden) using glass knives prepared with a knifemaker (LKB model 7801B). Ultra-thin sections (approximately 100 nm thick) were cut with an ultramicrotome (Reichert-Jung Ultracut, C. Reichert AG, Austria) using glass or diamond knives. The sections were trapped on the surface of uncoated copper grids and stained with uranyl acetate followed by lead citrate in an automatic Ultrostainer (LKB model 2168).

APHID TRANSMISSION

BCMV is transmitted by a number of aphid species in a non-persistent manner (Section 1, p. 10). *Myzus persicae* was used as the vector in aphid transmission experiments. It was maintained in pure culture on *Brassica perviridis* cv Tendergreen Mustard and removed from the host plants by placing them under the heat from an anglepoise lamp for a few minutes to stop them feeding, after which they were gently shaken off into plastic boxes. They were starved for up to 2 h and then allowed to feed on the virus-infected leaves. After an access period of about 10 min the aphids were removed from the infected leaf and placed on healthy test plants using a paintbrush. They were allowed an inoculation access

feed on the test plants of 24 hours before being killed by fumigation with nicotine.

Myzus persicae was used for aphid transmission of virus isolates to and from wild legume species using the procedures described above.

SEED TRANSMISSION OF BCMV AND OTHER VIRUS ISOLATES

Wild species of legumes were inoculated with the standard strains of BCMV as described in Section 3. After symptom development, plants were repotted into 18 cm diameter pots. Flowering was induced under Wellesbourne conditions by growing the plants under a short day of 8 h. The plants were either allowed to self-pollinate or were hand-pollinated with a paint-brush. The resultant seed was harvested and subsequently chitted as described in Section 4. Four weeks after sowing, virus symptoms were recorded and leaf samples from every plant were tested for virus by indirect ELISA with monoclonal antibody 197 (see p. 108).

Seed transmission of the wild legume virus isolates in *P. vulgaris* was tested by inoculating them to 4 plants of the bean cvs Double White and Sutter Pink. At flowering, about 3 weeks after sowing, the plants were repotted into 7 in pots. All plants were self-pollinated and the resultant seed was collected and subsequently tested for seed-transmitted virus by visually recording virus symptoms in germinated seedlings and by testing them by ELISA.

Appendix 2

Survey collection and differential cultivar data

Table A6 contains a summary of the collection data relating to all the bean (*P. vulgaris*) samples from which BCMV isolates were obtained. This includes the date of collection; country, location and map grid reference (latitude and longitude), host species and description of leaf sample symptoms. Table A7 describes the serological reactions with various mono- and polyclonal antisera. Table A8 and A9 present details of the symptom reactions induced by each isolate on the bean host differential cultivars at 26 °C and 32 °C for host groups 1 to 6 and 8 to 10, respectively, together with the BCMV strain-type that these reactions identified. Tables A10–A13 presents the collection data for each wild legume species from which BCMV strain-types were isolated, together with information on the host species in which the viruses were isolated; their serological reactions with various mono- and polyclonal antisera; electron microscopy results; the reactions the isolates induced at 26 °C and 32 °C in the differential bean cultivars and the BCMV strain-type identified. Table A14 lists the collection details of all other samples collected in the survey, from which no virus was isolated; a virus other than BCMV was isolated; or in which the presence of BCMV or another potyvirus was identified by ELISA but no virus isolated.

The following abbreviations have been used in Table A6–A14.

Symptoms

BYMV-like	Symptom similar to bean yellow mosaic virus
Chl	Chlorosis
CVB	Chlorotic vein banding (MCVB, mild; SCVB, severe)
GVB	Green vein banding (MGVB, mild; SGVB, severe)
HBSusp	Haloblight suspected
LD	Leaf distortion (MLD, mild; SLD, severe)
Mos	Mosaic (MMos, mild; SMos, severe)
Mot	Mottle (MMot, mild; SMot, severe)
Nec	Necrosis (LNec, local; SNec, severe)
CM	Chlorotic mottle (SCM, severe)
St	Stunting (MSt, mild; SSt, severe)

General

*	Virus isolated and identity confirmed (see Table A14)
?	Questionable result
+	Positive result
+t	Symptomless host but systemic infection detected by ELISA
–	Negative result
197	Monoclonal antibody 197
750r	Rod-shaped particles approximately 750 nm long seen
A	'A' serotype isolate

Ag. St.	Agricultural Station
AlfMV	Alfalfa mosaic virus
B	'B' serotype isolate
Bact.	Bacteria
BC1	Monoclonal antibody BC1
BC3	Monoclonal antibody BC3
BCMV	Bean common mosaic virus (A, 'A' serotype; B, 'B' serotype)
BLCMV	Blackeye cowpea mosaic virus
CBI	Crop Breeding Institute
CBS	Crop Breeding Station
Cent.	Centre
Commune	District
C.q.	*Chenopodium quinoa*
CSMV	*Cassia* severe mosaic virus
Dec	Homologous decoration by antiserum following ISEM (FDec, faint; MDec, moderate)
EM	Electron microscopy
Fld.St.	Field Station
Fm	Farm
I2	Monoclonal antibody I2
Inst.Agr.	Institute of Agriculture
ISABU	Institut des Sciences Agronomiques du Burundi
ISEM	Immunosorbent electron microscopy
Jct	Junction
N.b.	*Nicotiana benthamiana*
N.c.	*Nicotiana clevelandii*
NI	No virus isolated
Nr	Near
n	Local necrotic symptoms
ns	nothing seen in EM
NT	Not tested
PnMoV	Peanut mottle virus
Poty	Potyvirus
P.v.	*Phaseolus vulgaris*
RC	Research Centre
Res.St.	Research Station (also RS)
r	Rod-shaped virus particle
S1	Site 1 etc.
sp.	Species
Univ.	University
V.m.	*Vigna mungo*
V.r.	*Vigna radiata*
V.u.	*Vigna unguiculata*

Tables A15, A16 and A17 contain details for the preparation of the various chemicals required for the ELISA, electrophoresis and western blot procedures, respectively.

Table A6 Summary of collection information for each *Phaseolus vulgaris* sample from which BCMV was isolated

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom
4	21/01/90	Uganda	Bukalasa Fld. St	00 43 N 32 30 E	*P. vulgaris*	Mos
5	21/01/90	Uganda	Bukalasa Fld. St	00 43 N 32 30 E	*P. vulgaris*	Mot, St, LD
50	25/01/90	Lesotho	Maseru Res. St.	29 30 S 28 00 E	*P. vulgaris*	Mot
57	25/01/90	Lesotho	Maseru Res. St.	29 30 S 28 00 E	*P. vulgaris*	Mot
65	26/01/90	Lesotho	Roma	29 27 S 27 42 E	*P. vulgaris*	Mos
88	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	St, LD
100	29/01/90	Swaziland	Nr Mbekelwen	Unknown	*P. vulgaris*	Mos
114	31/01/90	Zimbabwe	CBI, Harare	18 23 S 31 51 E	*P. vulgaris*	Mos
115	31/01/90	Zimbabwe	CBI, Harare	18 23 S 31 51 E	*P. vulgaris*	Mos, LD
119	05/01/90	Zimbabwe	Gwebi College	17 41 S 30 52 E	*P. vulgaris*	Mos
121	31/01/90	Zimbabwe	Gwebi College	17 41 S 30 52 E	*P. vulgaris*	Mos
122	31/01/90	Zimbabwe	Zvimba	17 39 S 30 07 E	*P. vulgaris*	MMos, LD
127	31/01/90	Zimbabwe	Zvimba	17 39 S 30 07 E	*P. vulgaris*	SMos, LD
128	31/01/90	Zimbabwe	CBS Kadoma	17 40 S 29 25 E	*P. vulgaris*	Mos
133	02/02/90	Zimbabwe	Tavistock Farm	18 05 S 29 55 E	*P. vulgaris*	MMos, LD
134	02/02/90	Zimbabwe	Tavistock Farm	19 05 S 29 55 E	*P. vulgaris*	MMos
136	02/02/90	Zimbabwe	Tavistock Farm	18 05 S 29 55 E	*P. vulgaris*	MMos
139	05/02/90	Malawi	Champhira	12 20 S 33 37 E	*P. vulgaris*	LD
140	05/02/90	Malawi	Champhira	12 20 S 33 37 E	*P. vulgaris*	SMos
149	06/02/90	Malawi	Linthipe	14 10 S 34 10 E	*P. vulgaris*	Mos
151	06/02/90	Malawi	Linthipe	14 10 S 34 10 E	*P. vulgaris*	Mos
161	06/02/90	Malawi	Dedza	14 22 S 34 20 E	*P. vulgaris*	MMot
162	06/02/90	Malawi	Dedza	14 22 S 34 20 E	*P. vulgaris*	MMot
164	06/02/90	Malawi	Dedza	14 22 S 34 20 E	*P. vulgaris*	SMot
166	07/02/90	Malawi	Makapwa Res. St.	16 10 S 35 50 E	*P. vulgaris*	Mos
167	07/02/90	Malawi	Makapwa Res. St.	16 10 S 35 25 E	*P. vulgaris*	Mos
168	07/02/90	Malawi	Makapwa Res. St.	16 10 S 35 25 E	*P. vulgaris*	Mot
169	07/02/90	Malawi	Makapwa Res. St.	16 10 S 35 25 E	*P. vulgaris*	Mot
178	07/02/90	Malawi	Thyolo	16 05 S 35 12 E	*P. vulgaris*	MMot
179	07/02/90	Malawi	Thyolo	16 05 S 35 12 E	*P. vulgaris*	Mot
190	01/12/89	Zimbabwe	Bikita Dist.	20 15 S 31 50 E	*P. vulgaris* seed	Mos, LD
191	12/12/89	Zimbabwe	Buhera Dist.	19 39 S 32 00 E	*P. vulgaris* seed	Mot

192	12/12/89	Zimbabwe	Charter Dist.	18 50 S 31 00 E	*P. vulgaris* seed	Mos, LD
193	22/01/90	Uganda	Jinja market	00 23 N 32 33 E	*P. vulgaris* seed	GVB, LD
194	22/01/90	Uganda	Jinja market	00 23 N 32 33 E	*P. vulgaris* seed	SMos
195	26/01/90	Swaziland	Manzini market	26 15 S 31 30 E	*P. vulgaris* seed	MMot, LD
197	05/02/90	Malawi	Dedza	14 22 S 34 20 E	*P. vulgaris* seed	MMos
226	25/08/90	Ethiopia	Jima, Site 2	07 55 N 37 05 E	*P. vulgaris*	MMos
277	28/08/90	Ethiopia	Nr Zway	07 56 N 38 43 E	*P. vulgaris*	SMos
286	28/08/90	Ethiopia	40 km Nr Awasa	07 03 N 38 28 E	*P. vulgaris*	MMos
289	29/08/90	Ethiopia	Awasa, Site 4	07 03 N 38 28 E	*P. vulgaris*	SMos
319	30/08/90	Ethiopia	14 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	SMos, St
400	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	Mos, St
402	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	Mot, LD
403	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	MMos, GVB
404	02/05/91	Tanzania	Sokione Univ.	06 50 S 37 45 E	*P. vulgaris*	MMos, GVB
405	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	LD, CVB
406	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	Mot, SLD
407	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	Mos, LD
412	04/05/91	Tanzania	Mbimba Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	MMos, SLD
437	06/05/91	Tanzania	Moshi-Arusha	03 15 S 37 20 E	*P. vulgaris*	SLD
442	07/05/91	Tanzania	Arusha, Suye	03 22 S 36 41 E	*P. vulgaris*	Mos, LD
444	07/05/91	Tanzania	Arusha, Site 1	03 22 S 36 41 E	*P. vulgaris*	Mos, GVB
450	07/05/91	Tanzania	Arusha, Site 2	03 22 S 36 41 E	*P. vulgaris*	SLD
451	07/05/91	Tanzania	Arusha, Site 2	03 22 S 36 41 E	*P. vulgaris*	SLD
454	07/05/91	Tanzania	Arusha, Site 4	03 22 S 36 41 E	*P. vulgaris*	MMos, SLD
456	07/05/91	Tanzania	Arusha, Site 4	03 22 S 36 41 E	*P. vulgaris*	Mot, LD
458	13/05/91	Uganda	Kawanda Res. St.	00 26 N 32 32 E	*P. vulgaris*	Mos
459	13/05/91	Uganda	Kawanda Res. St.	00 26 N 32 32 E	*P. vulgaris*	CVB, LD
460	13/05/91	Uganda	Kawanda Res. St.	00 26 N 32 32 E	*P. vulgaris*	GVB, LD
464	13/05/91	Uganda	Bukalasa Fld. St	00 43 N 32 30 E	*P. vulgaris*	SMos
471	13/05/91	Uganda	Kalule	00 38 N 32 32 E	*P. vulgaris*	MMos, LD
481	14/05/91	Uganda	Kategule	00 12 S 30 18 E	*P. vulgaris*	Mos, LD
482	14/05/91	Uganda	Kategule	00 12 S 30 18 E	*P. vulgaris*	GVB, LD
497	15/05/91	Uganda	Rubaare Ag. St.	01 01 S 30 12 E	*P. vulgaris*	MMos, LD
504	16/05/91	Uganda	Kyanga	00 13 S 31 03 E	*P. vulgaris*	GVB, LD, Mot
505	16/05/91	Uganda	Kyanga	00 30 N 30 30 E	*P. vulgaris*	Mos
510	19/05/91	Rwanda	Karama Res. St.	02 32 S 29 46 E	*P. vulgaris*	SMos, LD
511	19/05/91	Rwanda	Karama Res. St.	02 32 S 29 46 E	*P. vulgaris*	GVB

Table A6 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom
512	19/05/91	Rwanda	Karama, Farm 1	02 32 S 29 46 E	*P. vulgaris*	GVB
513	19/05/91	Rwanda	Karama, Farm 1	02 32 S 29 46 E	*P. vulgaris*	MMos, LD
519	19/05/91	Rwanda	Kanzenze	02 05 S 30 06 E	*P. vulgaris*	Mot, LD
520	19/05/91	Rwanda	Kanzenze	02 05 S 30 06 E	*P. vulgaris*	Mot, LD
521	19/05/91	Rwanda	Kanzenze	02 05 S 30 06 E	*P. vulgaris*	Mot, GVB
523	19/05/91	Rwanda	Tambwe	02 12 S 29 47 E	*P. vulgaris*	Mos, LD
524	19/05/91	Rwanda	Tambwe	02 12 S 29 47 E	*P. vulgaris*	Mos, LD
526	20/05/91	Rwanda	Runinya, Farm 1	02 39 S 29 37 E	*P. vulgaris*	Mot
528	20/05/91	Rwanda	Runinya, Farm 3	02 39 S 29 37 E	*P. vulgaris*	Mos, LD
529	20/05/91	Rwanda	Runinya, Farm 3	02 39 S 29 37 E	*P. vulgaris*	Mos, GVB
530	20/05/91	Rwanda	Runinya, Farm 3	02 39 S 29 37 E	*P. vulgaris*	Mos, GVB, LD
532	20/05/91	Rwanda	Bukarama	02 42 S 29 00 E	*P. vulgaris*	MMos, GVB
535	20/05/91	Rwanda	Ngoma	02 04 S 29 56 E	*P. vulgaris*	Mos, St, LD
537	20/05/91	Rwanda	Ngoma	02 04 S 29 56 E	*P. vulgaris*	GVB, LD
550	21/05/91	Zaire	Mulungu, Farm 5	02 20 S 28 47 E	*P. vulgaris*	MMos, GVB
564	22/05/91	Rwanda	Gikongoro	02 29 S 29 34 E	*P. vulgaris*	Mos, GVB, LD
565	22/05/91	Rwanda	Gikongoro	02 29 S 29 34 E	*P. vulgaris*	Mos, GVB, LD
567	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos, GVB, LD
568	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos, GVB, LD
569	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos, GVB, LD
575	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos, GVB, LD
578	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mot
583	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mot
584	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mot
586	19/06/91	Burundi	Kisozi	03 34 S 29 41 E	*P. vulgaris*	Mot
587	19/06/91	Burundi	Kisozi	03 34 S 29 41 E	*P. vulgaris*	Mot
802	20/11/91	Rwanda	Gikongoro	02 29 S 29 34 E	*P. vulgaris*	Mos, LD
835	21/11/91	Rwanda	Shyorongi	01 52 S 29 29 E	*P. vulgaris*	LD
838	22/11/91	Burundi	Kayanza	02 55 S 29 37 E	*P. vulgaris*	SMos
842	22/11/91	Burundi	Banga	03 08 S 29 38 E	*P. vulgaris*	SMos
847	22/11/91	Burundi	Ruzize	03 16 S 29 14 E	*P. vulgaris*	Mot

852	22/11/91	Burundi	Ruzize	03 16 S 29 14 E	*P. vulgaris*	SMos
853	22/11/91	Burundi	Nr Ruzize	03 16 S 29 14 E	*P. vulgaris*	Mos
854	22/11/91	Burundi	Nr Ruzize	03 16 S 29 14 E	*P. vulgaris*	Mos
869	24/11/91	Burundi	Nyamiyaga	03 55 S 29 47 E	*P. vulgaris*	Mos
872	24/11/91	Burundi	35 km Bujumbura	03 00 S 29 22 E	*P. vulgaris*	Mos
873	24/11/91	Burundi	35 km Bujumbura	03 00 S 29 22 E	*P. vulgaris*	Mos
874	24/11/91	Burundi	44 km Bujumbura	02 55 S 29 20 E	*P. vulgaris*	Mos
875	24/11/91	Burundi	Kumusho	02 55 S 29 29 E	*P. vulgaris*	Mos
876	24/11/91	Burundi	76 km Bujumbura	02 50 S 29 10 E	*P. vulgaris*	Mos
877	24/11/91	Burundi	76 km Bujumbura	02 55 S 29 22 E	*P. vulgaris*	Mos
878	24/11/91	Burundi	91 km Bujumbura	02 50 S 29 10 E	*P. vulgaris*	Mos
879	25/11/91	Burundi	Nr Isabu RC	03 23 S 29 22 E	*P. vulgaris*	MMos
880	25/11/91	Burundi	Nr Isabu RC	03 23 S 29 22 E	*P. vulgaris*	Mos
881	25/11/91	Burundi	Nr Isabu RC	03 23 S 29 22 E	*P. vulgaris*	Mos
884	25/11/91	Burundi	Nr Bujumbura	03 23 S 29 22 E	*P. vulgaris*	Chl
887	25/11/91	Burundi	Bujumbura	03 23 S 29 22 E	*P. vulgaris*	Mos
896	28/11/91	Kenya	Kinoo	01 02 S 36 52 E	*P. vulgaris*	Mos
897	28/11/91	Kenya	Ndumbeni	00 19 S 37 43 E	*P. vulgaris*	Mos?
946	02/12/91	Kenya	Timau	00 09 N 37 02 E	*P. vulgaris*	Mos
947	02/12/91	Kenya	Timau	00 09 N 37 02 E	*P. vulgaris*	Mos
950	04/12/91	Kenya	Cheplanget	00 09 S 35 08 E	*P. vulgaris*	SMos
951	04/12/91	Kenya	Cheplanget	00 09 S 35 08 E	*P. vulgaris*	SMos
952	04/12/91	Kenya	Sotik	00 41 S 35 07 E	*P. vulgaris*	Mos
953	04/12/91	Kenya	Nyangusu	00 56 S 34 51 E	*P. vulgaris*	Mos
955	04/12/91	Kenya	Nyangusu	00 56 S 34 51 E	*P. vulgaris*	Mos
959	04/12/91	Kenya	Kisii	00 41 S 34 46 E	*P. vulgaris*	SMos
961	04/12/91	Kenya	Nyangina	00 55 S 34 25 E	*P. vulgaris*	SMos
964	04/12/91	Kenya	Kisii Dist.	00 45 S 34 50 E	*P. vulgaris*	SMos
967	05/12/91	Kenya	Nr Oyugis	00 30 S 34 43 E	*P. vulgaris*	Mos
970	05/12/91	Kenya	Kakamega	00 15 N 34 45 E	*P. vulgaris*	SMos
973	06/12/91	Kenya	Kakamega	00 15 N 34 45 E	*P. vulgaris*	SMos
987	01/01/88	Rwanda	Unknown	Unknown	*P. vulgaris* seed	Mos
1003	17/02/92	Zambia	Msandile Ag. St.	13 29 S 32 43 E	*P. vulgaris*	Mos
1004	17/02/92	Zambia	Lutembwe	13 18 S 32 04 E	*P. vulgaris*	Mos
1007	17/02/92	Zambia	Thanila	Unknown	*P. vulgaris*	Mos
1008	17/02/92	Zambia	Mangwe	09 07 S 31 47 E	*P. vulgaris*	Mos
1011	17/02/92	Zambia	Kalichero	13 33 S 32 24 E	*P. vulgaris*	Mos

Table A6 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom
1014	17/02/92	Zambia	Msekera Ag. St.	13 39 S 32 34 E	*P. vulgaris*	Mos
1301	01/01/88	Rwanda	Ikiringiti	Unknown	*P. vulgaris* seed	Mos
1317	01/01/88	Rwanda	Nyiramukara	Unknown	*P. vulgaris* seed	Mos
1322	01/01/88	Rwanda	Nyerakabundi	Unknown	*P. vulgaris* seed	Mos
1329	01/01/88	Rwanda	Nyarutembe	01 36 S 29 37 E	*P. vulgaris* seed	Mos
1334	01/01/88	Rwanda	Nyagakecuru	Unknown	*P. vulgaris* seed	Mos
1356	01/01/88	Rwanda	Urujenone	Unknown	*P. vulgaris* seed	Mos
2046	01/01/88	Burundi	Unknown	Unknown	*P. vulgaris* seed	Mos
2433	01/01/88	Burundi	Unknown	Unknown	*P. vulgaris* seed	Mos
3496	01/01/88	Zaire	Unknown	Unknown	*P. vulgaris* seed	Mos
3507	01/01/88	Zaire	Unknown	Unknown	*P. vulgaris* seed	Mos
3515	01/01/88	Zaire	Unknown	Unknown	*P. vulgaris* seed	Mos

see Appendix 2 p. 114 for symptom abbreviations used above

Table A7 Summary of host symptoms, ELISA and EM results of *Phaseolus vulgaris* isolates

Isolate number	Symptoms produced on *Phaseolus vulgaris*	ELISA results with various antisera	EM 'quick dip' results	ISEM decoration results
4	MMos, LD	BC1+ BC2? BC3+ NY15?		
5	Mos, LD	BC3+ BC1+ NL3+ NL4–		
50	Mos, VB	NL3+ NL4–		
57	SMos, LD	NL3+ NL4–		
65	Mos, St, LD C.q.–	NL3+ NL4–		
88	CVB C.q.–	NL3+ NL4– NVRS–		
100	Mos, LD C.q.–	NL3+ NVRS–		NL3 Dec 750r
114	Mos C.q.–	NL3? NVRS+		NL3 Dec 750r
115	Mos C.q.–	BC1+ NL3+ NL4–		
119	Mos, LD	BC1+ NL3+ NL4–		
121	Mos	NL3+ NL4–		
122	CVB C.q.–	NL3+ NVRS–	750r	
127	Mos C.q.–	NL3+ NVRS+	750r	
128	Mos C.q.–			
133	Mos C.q.–	NL3+ NVRS–		NL3 Dec 750r
134	SMos, St C.q.–	NL3+ NVRS–		NL3 Dec 750r
136	Mos, LD, GVB	NL3+ NL4–	ns	NL3 ns
139	Mos, LD C.q.–	NL3? NVRS–		NL3 FDec 750r
140	Mos, GVB C.q.–	NL3+ NVRS–	ns	NL3 ns
149	SMos C.q.–	NL3?	NL3 ns	
151	Mos C.q.–			
161	Mos	NL3+ NL4–	750r	
162	Mos, LD, GVB	NL3+ NVRS–		NL3 MDec 750r
164	Mos, St, LD	NL3+ NVRS–		NL3 MDec 750r
166	Mos, LD C.q.–	NL3? NVRS–		NL3 FDec 750r
167	Mos C.q.–	NL3+ NVRS–		NL3 MDec 750r
168	Mos, LD	NL3+ NL4– NVRS–		
169	Mos, St, GVB	NL3+ NVRS–		NL3 ns
178	Mos C.q.–	NL3+ NVRS–		NL3 FDec 750r
179	Mos C.q.–	NL3+ NVRS–		NL3 ns
190	Mos, LD	NL3+		

Table A7 (contd.)

Isolate number	Symptoms produced on *Phaseolus vulgaris*	ELISA results with various antisera	EM 'quick dip' results	ISEM decoration results
191	Mot	NL3+		
192	Mos, LD	NL3+		
193	SMos	NL3+ NY15+		
194	SMos			
195	Mos, St,	NL3+ NL4+	750r	
197	SMos			
226	Mos, LD	BC3– BC1– NL3+ NY15+		
277	Mos, LD C.q.–	BC1+ BC3– 197+ I2–		
286	Mos, LD	197+ I2–		
289	Mos, LD	BC3+ BC1+ NL3+		
319	Mos, LD	197+ I2–		
400	Mos, St, LD	NL3+ NVRS–		
402	Mos, St, LD	NL3+ NY15?		
403	SMos, St, LD	NL3+ NY15+		
404	SMos, St, LD	NL3? NY15–		
405	SMos, LD	NL3? NY15– NVRS–		
406	Mos, St, LD	NL3– NY15?		
407	Mos, St, LD	NL3+ NY15+		
412	?	BC3+ BC1– NL3– NY15– NVR–		
437	SMos, St, LD	BC3+ BC1+ NL3+ NY15–		
442	Mos, St, LD	NL3+ NY15–		
444	Mos	BC3+ BC1+ NL3? NY15?		
450	Mos	BC3+ BC1+ NL3+ NVRS–		
451	Mos	BC3+ BC1+ NL3+ NVRS–		
454	Mos	BC3+ BC1+ NL3+ NVRS–		
456	Mos	BC3+ BC1+ NL3? NVRS–		
458	Mos, St, LD	NL3? NVRS+		
459	Mos, St, LD	BC3– BC1– NL3? NY15– NVRS–		
460	Mos, St, LD	BC3– BC1– NL3? NVRS+		
464	SMos	BC3+ BC1– NL3– NVRS+		
471	Mos, St, LD	BC3– BC1– NL3? NVRS–		

481	Mos, St, LD	BC3–	BC1–	NL3–	NVRS–
482	SMos, St, LD	BC3?	BC1–	NL3?	NVRS+
497	Mos, St, LD	BC3+	BC1+	NL3–	NVRS–
504	SMos, St, LD	BC3+	BC1+	NL3?	NVRS+
505	Mos, St, LD	BC3+	BC1+	NL3+	NVRS+
510	SMos, St, LD	BC3+	BC1+	NL3+	NVRS+
511	Mos, St, LD	BC3+	BC1+	NL3–	NVRS+
512	Mos, St, LD	BC3+	BC1+	NL3–	NVRS+
513	Mos, St, LD	BC3+	BC1–	NL3–	NVRS+
519	Mos, St C.q.CM	BC3+	BC1+	NL3+	NVRS–
520	Mos, St, LD	BC3+	BC1+	NL3+	NVRS+
521	Mos, LD	BC3+	BC1+	NL3?	NVRS–
523	Mos, St, Ld C.q.CM	BC3–	BC1+	NL3+	NVRS+
524	Mos	BC3+	BC1+	NL3+	NVRS–
526	Mos	BC3–	BC1–	NL3+	NVRS–
528	Mos, St	BC3+	BC1+	NL3+	NVRS+
529	Mos, St, LD	BC3+	BC1+	NL3?	NVRS–
530	Mos	BC3+	BC1+	NL3+	NVRS–
532	Mos	BC3+	BC1+	NL3+	NVRS–
535	Mos, St, LD	BC3+	BC1+	NL3+	NVRS–
537	Mos, St, LD	BC3+	BC1+	NL3–	NVRS–
550	Mos	BC3+	BC1–	NL3?	NVRS–
564	Mos, St	BC3+	BC1–	NL3+	NVRS–
565	SMos, St, LD	BC3+	BC1+	NL3+	NVRS–
567	Mos, St, LD	BC3+	BC1+	NL3?	NVRS–
568	SMos	BC3+	BC1+	NL3?	NVRS–
569	SMos, St, LD	NL3+	NVRS–		
575	SMos, St, LD	BC3+	BC1+	NL3?	NVRS–
578	SMos, St, LD	BC3+	BC1+		
583	MMos	BC3+	BC1?	NL3+	
584	SMos, St, LD	BC3+	BC1+		
586	Mos, St	BC3+	BC1+	NL3+	
587	SMos, St, LD	BC3+	BC1+	NL3+	
802	Mos, LD, GVB	197+	I2+		
835	Mos, GVB	197+	I2–		
838	Mos, St, LD	197+	I2+		
842	Mos, LD	197+	I2+		

Table A7 (contd.)

Isolate number	Symptoms produced on *Phaseolus vulgaris*	ELISA results with various antisera	EM 'quick dip' results	ISEM decoration results
847	Mos, LD	197+ I2–		
852	Mos, LD	197+ I2–		
853	Mos, LD	197+ I2–		
854	Mos, LD	197+ I2–		
869	Mos, LD	197+ I2+		
872	Mos, LD	197+ I2+		
873	Mos, LD	197+ I2+		
874	Mos, LD	197+ I2+		
875	Mos, LD	197+ I2+		
876	Mos, LD	197+ I2+		
877	Mos, LD	197+ I2+		
878	Mos, LD	197+ I2+		
879	Mos, LD	197+ I2+		
880	Mos, LD	197+ I2+		
881	Mos, LD	197+ I2+		
884	Mos, LD	197+ I2+		
887	Mos, LD	197+ I2–		
896	Mos, LD	197+ I2+		
897	Mos, St	197+ I2+		
946	Mos, LD, GVB	197+ I2+		
947	Mos, LD, GVB	197+ I2+		
950	Mos, LD	197+ I2+		
951	?	197+ I2+		
952	Mos, LD	197+ I2+		
953	Mos, LD	197+ I2–		
955	Mos, LD, LNec	197+ I2–		
959	Lnec, Mos	197+ I2–		
961	SMos, Nec	197+ I2–		
964	Mos	197+ I2+		
967	Mos	197+ I2–		
970	SMos, LD, LNec	197+ I2–		

973	SMos	197+ I2–
987	Mos	
1003	Mos	197– I2–
1004	Mos	197+ I2–
1007	SMos	197+ I2+
1008	SMos	197+ I2–
1011	SMos, LD	197– I2–
1014	Mos	197+ I2+
1301	Mos	
1317	Mos	
1322	Mos	
1329	Mos	
1334	Mos	
1356	Mos	
2046	Mos	
2433	Mos	
3496	Mos	
3507	Mos	
3515	Mos	

See Appendix 2, p. 114 for symptom abbreviations used above

Table A8 Summary of reactions of *Phaseolus vulgaris* isolates in differential host cultivars at 26 °C

Isolate number	Group 1					Group 2		Group 3			Group 4		Group 5	Group 6			BCMV strain-type identified
	Prince	SGR	DW	SP	CRM	PGW	RGC	RGB	GN59	GN123	MICH	SAN	P114	MON	RM35	GN31	
4	4	4	NT	NT	4	2	0	2	0	NT	4	4	3	1	0	NT	NL3
5	4	NT	NT	NT	4	2	2	2	+t	NT	4	4	4	0	0	NT	NL3
50	4	NT	NT	4	4	0	0	1n	0	NT	0	0	0	0	0	NT	Novel
57	4	4	NT	NT	4	3	3	3	NT	NT	4	4	3	0	0	NT	NL3
65	4	NT	NT	NT	4	0	2	3	+t	NT	4	4	4	1n	0	NT	NL3
88	4	NT	NT	NT	4	0	2	2	0	NT	4	4	4	1	0	NT	NL3
100	4	NT	NT	NT	4	0	+t	0	0	NT	4	4	4	0	0	NT	Novel
114	4	NT	NT	NT	4	0	3	4	0	NT	4	4	4	0	0	NT	NL3
115	4	NT	NT	NT	4	2	2	3	2	NT	0	0	0	0	0	NT	US5
119	4	NT	NT	NT	4	3	+t	3	+t	NT	4	4	3	1n	1n	NT	NL3
121	4	NT	NT	NT	4	+t	2	2	+t	NT	4	4	4	1	0	NT	NL3
122	4	NT	NT	NT	4	+t	3	+t	0	NT	4	4	4	1n	0	NT	NL3
127	4	NT	NT	NT	4	0	0	0	0	NT	0	0	0	1n	1n	NT	Novel
128	4	NT	NT	NT	4	3	3	3	+t	NT	4	4	4	0	0	NT	NL3
133	4	NT	NT	NT	4	0	3	0	3	NT	0	0	0	0	0	NT	NL6
134	4	NT	NT	NT	4	+t	+t	+t	0	NT	4	4	4	1n	0	NT	NL3
136	4	NT	NT	NT	4	2	2	2	+t	NT	0	0	0	1n	1n	NT	NL6
139	4	NT	NT	NT	4	+t	3	+t	0	NT	4	4	4	0	0	NT	NL3
140	4	NT	NT	NT	4	3	3	3	3	NT	4	4	4	1n	0	NT	NL3
149	4	NT	NT	NT	4	0	0	0	0	NT	0	0	0	1n	1n	NT	Novel
151	4	NT	NT	NT	3	0	3	0	NT	NT	0	0	0	1n	1n	NT	Novel
161	4	NT	NT	NT	4	0	2	2	0	NT	4	4	4	1	0	NT	Novel
162	3	NT	NT	NT	3	3	3	3	0	NT	4	4	4	1n	0	NT	Novel
164	4	NT	NT	NT	4	1	2	2	+t	NT	4	4	4	0	0	NT	NL3
166	4	NT	NT	NT	4	+t	+t	+t	0	NT	4	4	4	0	0	NT	NL3
167	4	NT	NT	NT	4	+t	0	+t	0	NT	4	4	4	0	0	NT	NL3
168	4	NT	NT	NT	4	3	3	3	3	NT	4	4	4	1	0	NT	NL3
169	4	NT	NT	NT	4	3	3	3	3	NT	4	4	4	1	1	NT	NL3
178	4	NT	NT	NT	4	0	0	0	0	NT	0	0	0	1n	1n	NT	Novel
179	4	NT	NT	NT	4	0	+t	0	0	NT	0	0	0	1n	1n	NT	Novel
190	4	NT	NT	NT	4	0	3	3	0	NT	4	4	4	0	0	NT	NL3

191	4	NT	NT	NT	4	0	0	0	0	NT	4	4	4	0	0	NT	Novel
192	4	NT	NT	NT	4	+t	+t	+t	0	NT	4	4	4	0	0	NT	NL3
193	4	3	NT	NT	4	3	3	+t	+t	NT	1	1	0	1n	1n	NT	NL6
194	4	4	NT	NT	4	+t	0	+t	+t	NT	0	0	0	1n	1n	NT	NL6
195	4	NT	NT	NT	4	2	3	3	3	NT	0	0	0	1n	1n	NT	NL6
197	4	1	NT	NT	4	3	0	3	0	NT	0	0	0	0	1n	NT	NL6
226	4	NT	4	4	NT	0	0	0	NT	NT	0	0	0	NT	0	0	NL1
277	2	NT	4	3	NT	2	2	1	NT	NT	1	1	1	NT	0	0	Novel
286	2	NT	4	3	NT	1	1	+t	NT	NT	1n	1n	0	NT	0	0	Novel
289	4	NT	4	4	NT	3	2	2	NT	NT	0	0	0	NT	0	0	NL6
319	2	NT	4	3	NT	1	1	+t	NT	NT	1n	1n	0	NT	0	0	Novel
400	4	NT	4	4	NT	3	3	3	NT	NT	4	4	3	1	0	NT	NL3
402	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	1n	1	NT	NL3
403	4	NT	4	4	NT	0	0	0	NT	NT	0	0	0	1n	1n	NT	Novel
404	4	4	4	NT	4	0	0	0	0	0	1	1	0	1n	1n	0	Novel
405	4	4	4	NT	4	3	3	3	NT	NT	4	4	1	1	0	NT	NL3
406	4	4	4	NT	3	0	0	3	0	0	4	4	3	1n	1n	0	NL3
407	4	NT	4	4	NT	3	2	0	NT	NT	0	1	0	1	1	NT	Novel
412	0	0	4	NT	0	0	0	0	NT	NT	0	0	0	0	0	NT	NL1
437	0	2	4	NT	4	2	0	0	0	0	4	4	1	1n	0	0	Novel
442	2	0	4	NT	4	0	0	0	0	0	4	4	0	1n	0	0	NL8
444	3	NT	4	NT	NT	3	2	0	NT	NT	4	4	0	0	0	NT	Novel
450	0	NT	4	4	NT	0	0	0	NT	NT	4	4	3	1	0	NT	Novel
451	3	NT	4	NT	NT	2	1	2	NT	NT	4	4	2	1	1	NT	NL3
454	3	NT	4	NT	NT	2	3	3	NT	NT	4	4	1	1	1	NT	NL3
456	2	NT	4	4	NT	0	0	0	NT	NT	4	4	0	1n	1	NT	NL8
458	4	NT	4	4	NT	+t	+t	3	NT	NT	4	4	3	1n	0	NT	NL3
459	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
460	4	4	4	NT	3	+t	+t	+t	NT	NT	4	4	4	1	1	NT	NL3
464	4	NT	4	4	NT	0	0	0	NT	NT	0	0	0	NT	0	0	NL1
471	4	4	4	NT	4	3	3	3	NT	NT	4	4	3	1	1	NT	NL3
481	4	4	4	NT	4	3	+t	+t	NT	NT	4	4	3	1	1	NT	NL3
482	4	4	4	NT	3	3	3	3	NT	NT	4	4	3	1	1	NT	NL3
497	4	4	4	NT	3	0	0	+t	NT	NT	4	4	3	1	1	NT	NL3
504	4	4	4	NT	3	3	3	3	NT	NT	4	4	3	1	1	NT	NL3
505	4	NT	4	3	NT	3	2	3	NT	NT	4	4	4	NT	1	1	NL3
510	4	4	4	NT	4	3	3	3	NT	NT	4	4	3	0	0	NT	NL3

Table A8 (contd.)

Isolate number	Group 1					Group 2		Group 3			Group 4		Group 5	Group 6			BCMV strain-type identified
	Prince	SGR	DW	SP	CRM	PGW	RGC	RGB	GN59	GN123	MICH	SAN	P114	MON	RM35	GN31	
511	4	NT	4	4	NT	+t	+t	+t	NT	NT	4	4	4	1n	0	NT	NL3
512	4	4	4	NT	3	3	+t	+t	NT	NT	4	4	3	1	1	NT	NL3
513	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
519	4	NT	4	NT	NT	3	3	4	NT	NT	4	4	1	1	0	NT	NL3
520	4	NT	4	3	NT	3	3	2	NT	NT	4	4	4	1	0	0	NL3
521	2	NT	4	4	NT	+t	0	0	NT	NT	4	4	0	NT	0	0	Novel
523	0	NT	4	3	NT	0	0	0	NT	NT	4	4	0	NT	0	0	NL8
524	3	NT	4	4	NT	0	0	0	NT	NT	4	4	0	1n	0	NT	NL8
526	4	NT	4	NT	NT	3	2	3	NT	NT	4	4	4	1	1	NT	NL3
528	2	NT	4	4	NT	+t	0	0	NT	NT	4	4	0	NT	0	0	Novel
529	0	NT	4	4	NT	0	0	0	NT	NT	4	4	0	1n	0	NT	NL8
530	3	NT	4	NT	NT	3	3	3	NT	NT	4	4	4	1	1	NT	NL3
532	0	NT	4	4	NT	0	0	0	NT	NT	4	4	2	1	0	NT	Novel
535	4	NT	4	NT	NT	3	3	3	NT	NT	4	4	4	1	1	NT	NL3
537	4	4	4	NT	4	3	3	2	NT	NT	4	4	3	1	1	NT	NL3
550	3	3	4	NT	4	0	0	3	NT	NT	4	4	3	1	0	NT	Novel
564	4	4	4	NT	4	2	4	3	NT	NT	4	4	4	1	0	NT	NL3
565	4	NT	3	NT	NT	2	2	3	NT	NT	4	4	3	1	1	NT	NL3
567	4	4	4	NT	4	2	3	3	NT	NT	4	4	4	1	0	NT	NL3
568	3	4	4	NT	4	0	0	+t	NT	NT	NT	4	2	1	1	NT	NL3
569	4	4	4	NT	NT	0	0	+t	NT	NT	4	4	0	0	0	NT	NL3
575	4	4	4	NT	NT	3	3	3	NT	NT	0	0	0	1n	0	NT	NL6
578	4	NT	4	NT	NT	3	3	3	NT	NT	4	4	3	1	1	NT	NL3
583	2	2	4	NT	4	0	0	0	NT	NT	4	4	0	1n	1n	NT	NL8
584	0	NT	4	4	NT	0	0	0	NT	NT	4	4	0	0	0	NT	NL8
586	1	0	4	NT	4	0	0	0	NT	NT	4	4	0	1n	0	NT	NL8
587	3	NT	3	NT	NT	2	3	3	NT	NT	4	4	3	1	1	NT	Novel
802	4	NT	4	4	NT	3	3	3	NT	NT	0	0	0	NT	0	0	NL6
835	4	NT	4	4	NT	0	0	0	NT	NT	4	4	0	NT	0	0	NL8
838	2	NT	4	2	NT	0	0	0	NT	NT	4	4	0	NT	0	0	NL8

842	0	NT	4	4	NT	0	0	0	NT	NT	4	4	0	NT	0	0	NL8
847	4	NT	4	4	NT	0	0	0	NT	NT	3	3	0	NT	1n	0	Novel
852	4	NT	4	4	NT	2	2	2	NT	NT	1	0	0	NT	1n	0	NL6
853	4	NT	4	4	NT	0	3	0	NT	NT	0	0	4	NT	0	0	Novel
854	3	NT	4	3	NT	0	2	2	NT	NT	1n	0	0	NT	1n	0	NL6
869	4	NT	4	4	NT	3	3	3	NT	NT	4	4	3	NT	0	0	NL3
872	4	NT	4	3	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
873	4	NT	4	4	NT	3	3	3	NT	NT	4	4	2	NT	0	0	NL3
874	4	NT	4	4	NT	0	0	0	NT	NT	4	4	4	NT	0	0	Novel
875	4	NT	4	3	NT	3	3	+t	NT	NT	4	4	4	NT	0	0	NL3
876	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
877	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
878	0	NT	4	2	NT	0	0	0	NT	NT	4	4	0	NT	0	0	NL8
879	4	NT	4	2	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
880	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
881	0	NT	4	2	NT	0	0	0	NT	NT	4	4	0	NT	0	0	NL8
884	2	NT	4	2	NT	0	0	0	NT	NT	4	4	0	NT	0	0	NL8
887	4	NT	4	2	NT	0	0	0	NT	NT	1	0	0	NT	1n	0	Novel
896	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
897	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
946	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
947	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
950	4	NT	4	4	NT	3	3	3	NT	NT	4	4	4	NT	0	0	NL3
951	4	NT	4	3	NT	3	3	3	NT	NT	3	3	3	NT	0	0	NL3
952	4	NT	4	4	NT	3	3	3	NT	NT	4	4	2	NT	0	0	NL3
953	4	NT	4	4	NT	0	0	0	NT	NT	4	4	3	NT	1n	1n	Novel
955	4	NT	4	4	NT	+t	0	0	NT	NT	4	4	4	NT	1n	1n	NY15
959	4	NT	4	4	NT	0	0	3	NT	NT	4	4	4	0	0	0	Novel
961	4	NT	4	4	NT	3	2	2	NT	NT	3	3	3	0	0	0	Novel
964	4	NT	4	4	NT	2	1	2	NT	NT	4	4	3	NT	1n	1n	Novel
967	3	NT	4	2	NT	1	+t	+t	NT	NT	3	3	3	NT	1n	0	Novel
970	4	NT	4	4	NT	1	1	+t	NT	NT	4	4	3	NT	1n	1n	Novel
973	4	NT	4	1	NT	1	1	1	NT	NT	3	3	2	NT	0	1	Novel
987	3	NT	4	4	4	0	2	2	1n	NT	4	4	4	1n	1n	0	NL3
1003	4	NT	4	3	NT	3	3	3	NT	NT	1	1	0	NT	0	0	NL6
1004	4	NT	4	3	NT	3	3	3	NT	NT	3	3	3	NT	0	0	NL3
1007	4	NT	4	3	NT	3	3	3	NT	NT	4	4	3	NT	0	0	NL3

Table A8 (contd.)

Isolate number	Group 1					Group 2		Group 3			Group 4		Group 5	Group 6			BCMV strain-type identified
	Prince	SGR	DW	SP	CRM	PGW	RGC	RGB	GN59	GN123	MICH	SAN	P114	MON	RM35	GN31	
1008	4	NT	4	3	NT	2	2	0	NT	NT	0	0	0	NT	1n	0	Novel
1011	4	NT	4	4	NT	1	+t	+t	NT	NT	4	4	2	NT	0	0	NL3
1014	4	NT	4	4	NT	3	3	3	NT	NT	4	4	3	NT	0	0	NL3
1301	3	NT	NT	NT	4	0	0	0	0	NT	4	4	4	0	0	NT	NL3
1317	4	NT	NT	NT	4	0	0	1	0	NT	4	4	4	1n	0	NT	NL3
1322	4	NT	NT	NT	4	1	0	0	1	0	4	4	4	1	1n	NT	NL3
1329	2	NT	NT	NT	4	1	1	1	0	NT	4	4	4	1n	1n	NT	NL3
1334	4	NT	NT	NT	4	2	2	2	2	NT	4	4	4	1n	1n	NT	NL3
1356	4	NT	NT	NT	4	0	0	0	0	0	4	4	4	1	0	NT	NL3
2046	3	NT	NT	4	4	1	2	2	1	NT	4	4	4	1n	1	NT	NL3
2433	4	NT	NT	NT	4	0	0	0	0	0	0	0	0	1n	0	NT	NL1
3496	4	4	4	NT	4	0	0	3	3	0	4	4	4	1	1	1	NL3
3507	3	3	3	NT	3	0	0	0	0	0	4	4	0	1	1	1	Novel
3515	4	4	4	NT	4	0	0	0	0	0	4	4	4	1	1n	1	Novel

See Appendix 2, p. 114 for abbreviations used above and Table A5 for explanation of symptom scores

Table A9 Summary of reactions of *Phaseolus vulgaris* isolates in differential host cultivars at 26 °C and 32 °C

Isolate number	Reactions at 26 °C						Reactions at 32 °C						BCMV strain-type identified
	Group 8		9a	9b		10	Group 8		9a	9b		10	
	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	
4	5n	4n	1n	4n	1n	0	5n	5n	3n	5n	5n	1n	NL3
5	5n	5n	1n	5n	4n	0	5n	5n	3n	5n	3n	0	NL3
50	0	0	0	0	0	0	5n	5n	0	0	0	0	Novel
57	5n	3n	1n	4n	4n	0	5n	5n	3n	5n	5n	2n	NL3
65	5n	3n	1n	NT	2n	0	5n	5n	3n	5n	3n	0	NL3
88	5n	5n	1n	5n	5n	1n	5n	5n	1n	5n	5n	1n	NL3
100	5n	3n	1n	5n	0	0	5n	5n	3n	4n	4n	2n	Novel
114	5n	5n	3n	5n	5n	0	5n	5n	1n	5n	5n	0	NL3
115	0	0	0	0	0	0	0	0	0	0	0	0	US5
119	5n	5n	2n	5n	5n	0	5n	5n	3n	5n	5n	1n	NL3
121	5n	5n	3n	1n	0	0	5n	5n	1n	4n	2n	1n	NL3
122	5n	5n	2n	5n	5n	1n	5n	5n	3n	5n	4n	1n	NL3
127	5n	2n	0	0	1n	0	5n	4n	2n	4n	5n	1n	Novel
128	5n	5n	2n	5n	0	0	5n	5n	4n	5n	5n	0	NL3
133	5n	4n	0	0	0	0	5n	5n	2n	5n	5n	0	NL6
134	5n	5n	1n	5n	5n	0	5n	5n	4n	5n	5n	2n	NL3
136	0	0	1n	0	0	0	5n	5n	1n	5n	2n	0	NL6
139	5n	5n	3n	5n	5n	0	5n	5n	5n	5n	5n	2n	NL3
140	5n	5n	0	5n	5n	1n	5n	5n	0	5n	5n	2n	NL3
149	0	0	0	0	0	0	5n	5n	4n	5n	5n	1n	Novel
151	5n	0	1n	5n	4n	0	5n	5n	1n	5n	5n	1n	Novel
161	5n	1n	1n	0	0	0	5n	5n	4n	5n	1n	1n	Novel
162	5n	4n	1n	NT	1n	0	5n	5n	3n	5n	5n	1n	Novel
164	5n	5n	1n	5n	4n	0	5n	5n	1n	5n	5n	1n	NL3
166	5n	5n	1n	2n	5n	0	5n	5n	5n	5n	5n	0	NL3
167	5n	5n	2n	0	5n	0	5n	5n	3n	5n	5n	2n	NL3
168	5n	5n	NT	5n	1n	0	5n	5n	3n	5n	4n	0	NL3
169	5n	5n	1n	5n	5n	0	5n	5n	3n	5n	5n	1n	NL3
178	0	0	0	0	0	0	5n	2n	2n	4n	3n	1n	Novel

Table A9 (contd.)

Isolate number	Reactions at 26 °C						Reactions at 32 °C						BCMV strain-type identified
	Group 8		9a	9b		10	Group 8		9a	9b		10	
	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	
179	2n	2n	1n	3n	0	0	5n	5n	0	5n	2n	0	Novel
190	5n	5n	3n	NT	5n	0	5n	5n	3n	5n	5n	0	NL3
191	5n	5n	1n	2n	5n	0	5n	5n	5n	5n	5n	0	Novel
192	5n	5n	1n	2n	5n	0	5n	5n	5n	5n	5n	0	NL3
193	0	0	0	0	0	0	5n	0	1n	5n	5n	0	NL6
194	0	0	0	0	0	0	5n	5n	1n	5n	5n	0	NL6
195	4n	0	0	0	0	0	5n	0	1n	4n	0	0	NL6
197	0	0	0	0	0	0	5n	5n	1n	5n	5n	1n	NL6
226	0	0	0	0	0	0	2n	0	0	0	0	0	NL1
277	5n	2n	1n	1n	1n	1n	5n	5n	1n	1n	1n	1n	Novel
286	5n	4n	1n	1n	1n	0	5n	5n	1n	1n	1n	1n	Novel
289	0	0	1n	4n	0	0	5n	2n	1n	5n	5n	0	NL6
319	5n	4n	1n	1n	1n	1n	5n	5n	1n	3n	1n	0	Novel
400	5n	5n	1n	5n	NT	0	5n	5n	3n	5n	5n	1n	NL3
402	5n	3n	1n	3n	NT	0	5n	5n	2n	3n	5n	0	NL3
403	1n	0	0	0	NT	0	5n	5n	1n	5n	5n	0	Novel
404	0	0	0	0	0	0	5n	5n	3n	5n	5n	0	Novel
405	5n	5n	5n	4n	4n	0	5n	5n	2n	5n	5n	2n	NL3
406	5n	5n	3n	5n	5n	0	5n	5n	3n	5n	4n	0	NL3
407	4n	5n	0	0	NT	0	5n	5n	1n	5n	1n	0	Novel
412	0	0	0	0	0	0	0	0	0	0	0	0	NL1
437	5n	5n	1n	1n	1n	0	5n	5n	3n	3n	1n	1n	Novel
442	5n	5n	3n	1n	1n	1n	5n	5n	3n	1n	1n	0	NL8
444	5n	5n	0	0	0	0	5n	5n	1n	1n	1n	1n	Novel
450	5n	5n	1n	1n	NT	1n	5n	5n	1n	1n	1n	1n	Novel
451	5n	5n	1n	5n	5n	1n	4n	1n	1n	2n	1n	2n	NL3
454	5n	5n	1n	1n	1n	1n	5n	5n	2n	2n	1n	2n	NL3
456	5n	5n	1n	1n	1n	1n	5n	5n	3n	1n	1n	0	NL8
458	5n	5n	1n	4n	2n	1n	5n	5n	2n	5n	3n	1n	NL3

459	4n	3n	1n	5n	4n	0	5n	3n	3n	5n	4n	1n	NL3
460	5n	5n	1n	5n	5n	0	5n	5n	3n	5n	5n	1n	NL3
464	0	0	0	0	0	0	0	0	0	0	0	0	NL1
471	5n	5n	1n	5n	5n	1n	5n	5n	2n	5n	5n	2n	NL3
481	5n	5n	1n	5n	4n	1n	5n	5n	2n	5n	5n	1n	NL3
482	5n	5n	1n	5n	5n	0	5n	5n	3n	5n	5n	2n	NL3
497	5n	5n	1n	5n	5n	0	5n	5n	2n	5n	5n	2n	NL3
504	5n	5n	1n	5n	5n	1n	5n	5n	3n	5n	5n	2n	NL3
505	5n	2n	2n	5n	3n	0	5n	5n	5n	5n	3n	2n	NL3
510	5n	5n	1n	5n	5n	0	5n	5n	2n	4n	4n	1n	NL3
511	5n	5n	1n	NT	5n	0	5n	5n	3n	5n	5n	0	NL3
512	5n	5n	1n	2n	3n	0	5n	5n	2n	5n	5n	2n	NL3
513	5n	4n	3n	5n	5n	0	5n	5n	2n	5n	5n	2n	NL3
519	5n	5n	NT	5n	5n	1n	5n	4n	4n	5n	5n	1n	NL3
520	5n	1n	1n	3n	1n	1n	5n	5n	2n	5n	5n	0	NL3
521	4n	5n	1n	1n	1n	0	5n	5n	1n	1n	1n	1n	Novel
523	5n	5n	1n	1n	1n	0	5n	5n	1n	1n	1n	0	NL8
524	5n	5n	1n	1n	1n	1n	5n	4n	1n	1n	1n	0	NL8
526	5n	5n	2n	5n	5n	1n	5n	5n	3n	5n	5n	1n	NL3
528	4n	4n	1n	1n	1n	0	5n	5n	2n	1n	1n	0	Novel
529	5n	5n	1n	1n	1n	1n	5n	5n	1n	1n	1n	0	NL8
530	5n	5n	1n	1n	1n	0	5n	5n	1n	2n	1n	1n	NL3
532	5n	5n	1n	1n	1n	1n	5n	5n	1n	1n	1n	1n	Novel
535	5n	3n	1n	5n	NT	0	5n	5n	3n	5n	5n	0	NL3
537	5n	5n	1n	5n	5n	0	5n	5n	3n	5n	5n	2n	NL3
550	5n	5n	1n	1n	1n	1n	5n	5n	1n	1n	1n	1n	Novel
564	5n	5n	1n	3n	5n	1n	5n	5n	3n	5n	5n	2n	NL3
565	5n	5n	NT	1n	5n	0	5n	5n	3n	3n	3n	2n	NL3
567	5n	5n	1n	4n	5n	1n	5n	5n	2n	4n	4n	1n	NL3
568	5n	4n	1n	NT	4n	0	0	5n	1n	2n	3n	0	NL3
569	5n	5n	1n	5n	NT	0	5n	5n	1n	5n	NT	0	NL3
575	5n	5n	1n	0	5n	0	5n	5n	2n	5n	5n	0	NL6
578	5n	5n	NT	4n	5n	0	5n	5n	1n	5n	NT	0	NL3
583	5n	5n	1n	1n	0	1n	5n	5n	1n	1n	1n	1n	NL8
584	5n	1n	1n	1n	1n	1n	5n	5n	2n	1n	1n	1n	NL8
586	5n	5n	1n	1n	1n	0	5n	5n	1n	1n	1n	1n	NL8
587	5n	5n	1n	0	1n	1n	5n	5n	1n	1n	1n	0	Novel

Table A9 (contd.)

Isolate number	Reactions at 26 °C						Reactions at 32 °C						BCMV strain-type identified
	Group 8		9a	9b		10	Group 8		9a	9b		10	
	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	
802	0	0	0	0	0	0	5n	3n	1n	3n	3n	0	NL6
835	5n	5n	1n	1n	1n	1n	5n	5n	1n	1n	1n	0	NL8
838	5n	5n	1n	1n	1n	0	5n	5n	2n	1n	1n	0	NL8
842	5n	3n	1n	1n	1n	0	5n	5n	1n	1n	1n	1n	NL8
847	0	0	0	0	0	0	0	0	0	0	0	0	Novel
852	0	0	0	0	0	0	5n	5n	2n	3n	3n	0	NL6
853	0	0	0	0	0	0	5n	5n	1n	5n	5n	0	Novel
854	2n	0	0	2n	0	0	5n	3n	1n	5n	5n	0	NL6
869	5n	5n	1n	4n	3n	0	5n	3n	2n	5n	3n	0	NL3
872	5n	5n	3n	5n	4n	0	5n	5n	3n	4n	5n	0	NL3
873	5n	5n	1n	5n	4n	0	5n	3n	2n	5n	5n	1n	NL3
874	5n	5n	1n	5n	3n	0	5n	5n	3n	5n	3n	0	Novel
875	5n	5n	2n	5n	3n	0	5n	5n	3n	4n	5n	1n	NL3
876	5n	5n	2n	4n	3n	1n	5n	5n	3n	5n	5n	1n	NL3
877	5n	3n	1n	5n	2n	0	5n	5n	3n	5n	5n	1n	NL3
878	5n	5n	1n	1n	1n	1n	5n	5n	3n	1n	1n	1n	NL8
879	5n	2n	1n	5n	4n	1n	5n	5n	1n	5n	5n	0	NL3
880	5n	5n	1n	3n	3n	0	5n	5n	3n	5n	5n	0	NL3
881	5n	5n	1n	1n	1n	1n	5n	3n	0	0	0	0	NL8
884	5n	5n	1n	0	1n	0	5n	5n	2n	0	1n	0	NL8
887	0	0	0	0	0	0	4n	3n	1n	5n	5n	0	Novel
896	5n	5n	1n	3n	2n	0	5n	5n	3n	5n	4n	1n	NL3
897	5n	5n	1n	5n	5n	0	5n	4n	3n	5n	5n	1n	NL3
946	5n	5n	1n	4n	3n	0	5n	5n	3n	4n	3n	1n	NL3
947	5n	5n	3n	5n	5n	0	5n	5n	3n	5n	5n	1n	NL3
950	5n	4n	2n	4n	3n	0	5n	5n	2n	5n	5n	1n	NL3
951	5n	5n	3n	5n	5n	0	5n	5n	3n	5n	5n	0	NL3
952	5n	4n	2n	4n	4n	1n	5n	5n	1n	5n	5n	1n	NL3
953	0	0	0	0	0	0	0	0	0	0	0	0	Novel

955	0	0	0	0	0	0	0	0	0	0	0	0	NY15
959	0	0	0	0	0	0	0	0	0	0	0	0	Novel
961	0	0	0	0	0	0	0	0	0	0	0	0	Novel
964	0	0	0	0	0	0	0	0	0	0	0	0	Novel
967	0	0	0	0	0	0	0	0	0	0	0	0	Novel
970	0	0	0	0	0	0	0	0	0	0	0	0	Novel
973	0	0	0	0	0	0	0	0	0	0	0	0	Novel
987	5n	5n	1n	5n	4n	0	5n	5n	4n	5n	5n	0	NL3
1003	0	0	5n	3n	0	0	5n	5n	5n	5n	5n	1n	NL6
1004	5n	5n	2n	5n	4n	0	5n	5n	4n	5n	5n	1n	NL3
1007	5n	5n	3n	3n	5n	1n	5n	5n	0	5n	5n	1n	NL3
1008	0	0	0	0	0	0	5n	5n	1n	5n	5n	0	Novel
1011	5n	5n	2n	3n	4n	0	5n	5n	3n	5n	5n	0	NL3
1014	5n	5n	3n	5n	1n	1n	5n	5n	5n	5n	5n	1n	NL3
1301	5n	5n	1n	5n	NT	0	5n	5n	4n	3n	NT	1n	NL3
1317	5n	5n	1n	5n	NT	0	5n	5n	1n	5n	NT	0	NL3
1322	5n	5n	1n	4n	3n	1n	4n	5n	2n	0	3n	1n	NL3
1329	5n	5n	1n	3n	NT	1n	5n	5n	5n	5n	NT	0	NL3
1334	5n	5n	1n	5n	NT	1n	5n	5n	1n	5n	NT	1n	NL3
1356	5n	5n	2n	1n	2n	0	5n	5n	3n	4n	5n	0	NL3
2046	5n	5n	2n	5n	5n	0	5n	5n	4n	5n	5n	1n	NL3
2433	0	0	0	0	0	0	0	0	0	0	0	0	NL1
3496	5n	5n	5n	5n	5n	0	5n	5n	5n	5n	5n	0	NL3
3507	0	0	1n	5n	5n	0	5n	5n	5n	5n	5n	0	Novel
3515	5n	5n	1n	5n	5n	0	5n	5n	1n	5n	5n	0	Novel

See Appendix 2, p. 114 for abbreviations used above and Table A5 for explanation of symptom scores

Table A10 Summary of collection information for each wild legume sample from which BCMV was isolated

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom
28	22/01/90	Uganda	Nakabango	00 23 N 32 33 E	*C. incana*	SMos
30	22/01/90	Uganda	Nakabango	00 23 N 32 33 E	*C. incana*	Mos,St
38	22/01/90	Uganda	Nakabango	00 23 N 32 33 E	*Glycine max*	Mos
145	05/02/91	Malawi	Champhira	12 20 S 33 37 E	*Rhynchosia* sp.	Mot
465	13/05/91	Uganda	Bukalasa Res.St	00 43 N 32 30 E	*Cassia hirsuta*	SMos
499	16/05/91	Uganda	Mubuku	00 16 N 30 07 E	*M.atropurpureum*	SMos
531	20/05/91	Rwanda	Runinya, Farm 3	02 39 S 29 37 E	*V.unguiculata*	Mot,LD
820	20/11/91	Rwanda	Mbazi	02 22 S 29 35 E	*Cassia sophera*	SMos
830	21/11/91	Rwanda	Nemba	01 38 S 29 47 E	*Cassia sophera*	MMos
836	21/11/91	Rwanda	Musambira	02 03 S 29 51 E	*Cassia sophera*	Mos
956	04/12/91	Kenya	Nyangusu	00 56 S 34 51 E	*Vigna vexillata*	SMos
963	04/12/91	Kenya	Nyangina	00 55 S 34 25 E	*C.comanestiana*	Mot

See Appendix 2, p. 114 for abbreviations used above
C.=Crotalaria; M.=Macroptilium; V.=Vigna

Table A11 Summary of host symptoms, ELISA and EM results of virus isolates from wild species of legumes

Isolate number	Symptoms produced on *Phaseolus vulgaris*	ELISA results with various antisera	EM 'quick dip' results	ISEM decoration results
28	Nec C.q.Mot	197+ I2+ NL3+ BLCMV–	750r	NL3 Dec,BLCMVns
30	Nec C.q.Mot	197+ I2+ NL3+ BLCMV–	750r	NL3 Dec,BLCMVns
38	Nec C.q.Mot	197+ I2+ NL3+ BLCMV–	750r	NL3 Dec,BLCMVns
145	Mos,LD C.q.– N.c.–	NL3+ NL4– 197+ I2+ BLCMV–		
465	SMos,LD	BC3– BC1– NL3+ 197+ I2–	750r	
499	Mos. C.q.–	BC3+ BC1– NL3+ 197+ I2–	750r	
531	SMos	BC3+ BC1– 197+ I2+ BLCMV–		
820	Mos C.q.– N.c.– N.b.–	197+ I2– BLCMV+		
830	Mos,LD,GVB	197+ I2+ BLCMV–		
836	Mos,LD N.c.– C.q.–	197+ I2+ BLCMV+		
956	Mos,LD,LNec	197+ I2– BLCMV+		
963	Mos,Nec	197+ I2– BLCMV+	750r	NL3 Dec

See Appendix 2, p. 114 for abbreviations used above

Table A12 Summary of reactions of virus isolates from wild species of legumes in differential host cultivars at 26 °C

Isolate number	Group 1					Group 2		Group 3			Group 4		Group 5	Group 6			BCMV strain-type identified
	Prince	SGR	DW	SP	CRM	PGW	RGC	RGB	GN59	GN123	MICH	SAN	P114	MON	RM35	GN31	
28	0	NT	4n	4n	NT	0	0	0	0	NT	4n	4n	0	0	0	0	Novel
30	0	NT	4n	4n	4n	0	0	0	0	NT	4n	4n	0	0	0	0	Novel
38	0	NT	4n	4n	4n	0	0	0	0	NT	4n	4n	0	0	0	0	Novel
145	4	NT	4	4	4	3	0	2	+t	NT	0	0	0	NT	0	0	NL6
465	4	3	4	3	3	0	0	0	NT	NT	0	0	0	1n	1n	NT	NL1
499	4	4	4	4	4	2	2	4	NT	NT	4	NT	4	1n	0	NT	NL3
531	4	NT	4	4	NT	0	+t	+t	NT	NT	4	4	4	1	0	NT	NL3
820	2	NT	4	4	NT	1	+t	+t	NT	NT	4	4	0	NT	1	1	Novel
830	2	NT	4	4	NT	0	0	0	NT	NT	4	4	0	NT	0	0	Novel
836	+t	NT	4	0	NT	0	0	+t	NT	NT	4	4	0	NT	0	0	Novel
956	4	NT	4	4	NT	2	0	0	NT	NT	4	4	4	NT	1n	1n	NY15
963	4	NT	4	4	NT	1	+t	+t	NT	NT	3	3	3	0	0	0	Novel

See Appendix 2, p. 114 for abbreviations used above and Table A5 for explanation of symptom scores

Table A13 Summary of reactions of virus isolates from wild species of legumes in differential host cultivars at 26 °C and 32 °C

Isolate number	Reactions at 26 °C						Reactions at 32 °C						BCMV strain-type identified
	Group 8		9a	9b		10	Group 8		9a	9b		10	
	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	BTS	WIDUSA	JUBILA	ITG	TC	AMANDA	
28	1n	1n	0	0	0	0	5n	5n	0	0	0	0	Novel
30	1n	1n	0	0	0	0	4n	5n	0	0	0	0	Novel
38	1n	1n	0	0	0	0	5n	5n	0	0	0	0	Novel
145	4n	2n	0	0	0	0	5n	5n	2n	5n	5n	0	NL6
465	0	0	0	0	0	0	0	0	0	0	0	0	NL1
499	5n	5n	1n	5n	5n	0	5n	5n	2n	5n	5n	1n	NL3
531	5n	0	1n	NT	5n	0	5n	5n	3n	5n	5n	2n	NL3
820	5n	5n	1n	1n	1n	1n	5n	5n	1n	1n	1n	1n	Novel
830	5n	4n	1n	1n	2n	0	5n	5n	1n	3n	3n	0	Novel
836	5n	4n	1n	1n	1n	0	5n	3n	1n	1n	1n	1n	Novel
956	0	0	0	0	0	0	0	0	0	0	0	0	NY15
963	0	0	0	0	0	0	0	0	0	0	0	0	Novel

See Appendix 2, p. 114 for abbreviations used above and Table A5 for explanation of symptom scores

Table A14 Summary of collection information and results for samples from which either no virus was detected by ELISA, a virus other than BCMV was isolated, or a virus was detected by ELISA but not isolated

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
1	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	MMos,St	BlCMV?
2	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Mos,St,LD	BlCMV?
3	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Mos,St,LD	BCMV?
6	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Chl	NI
7	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Nec	NI
8	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Nec	NI
9	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Mos,LD	NI
10	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	Legume weed	MMot	NI
11	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	Legume weed	Mos?	NI
12	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Mot	NI
13	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*Lotononis* sp.	MMot	NI
14	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*Lotononis* sp.	MMot	NI
15	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*Lotononis* sp.	MMot	NI
16	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*Lotononis* sp.	MMot	NI
17	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	Mot	NI
18	21/01/90	Uganda	Bukalasa Fld.St	00 43 N 32 30 E	*P. vulgaris*	LD	NI
20	21/01/90	Uganda	Makerere Univ.	00 20 N 32 34 E	*Pueraria* sp.	Mot,St	NI
26	21/01/90	Uganda	Makerere Univ.	00 20 N 32 34 E	Legume weed	MMot,Ld	NI
27	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	MMos	NI
29	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	Mot,Mos	NI
31	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	*Arachis hypogea*	Mos	PnMoV*
32	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	MMos	NI
33	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	MMos	NI
34	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	SMos	NI
35	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	Mot	NI
36	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	Mot	NI
37	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	Mot	NI
39	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	*Cassia* sp.	Mos?	NI
40	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	Chl	NI
41	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	Legume weed	Mot	NI
42	22/01/90	Uganda	Nakabango Fld.S	00 23 N 32 33 E	*Cassia* sp.	Mos?	NI

43	22/01/90	Uganda	Jinja	00	23	N	32	33	E	Legume weed	Mos?	NI
45	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Chl	NI
46	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	MMot	NI
47	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Mot	NI
48	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Chl	NI
49	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Mos?	NI
51	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Mot	NI
52	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Chl	NI
53	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Chl	NI
54	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Chl	NI
55	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	Chl	NI
56	25/01/90	Lesotho	Maseru Res.St.	29	30	S	28	00	E	*P. vulgaris*	SMos	NI
58	26/01/90	Lesotho	20 km Maseru	29	25	S	27	40	E	*P. vulgaris*	MMos	NI
59	26/01/90	Lesotho	20 km Maseru	29	25	S	27	40	E	*P. vulgaris*	MMos	NI
60	26/01/90	Lesotho	20 km Maseru	29	25	S	27	40	E	*P. vulgaris*	MMos	NI
61	26/01/90	Lesotho	9 km Roma	29	26	S	27	41	E	*P. vulgaris*	Chl	NI
62	26/01/90	Lesotho	9 km Roma	29	26	S	27	41	E	*P. vulgaris*	MMos	NI
63	26/01/90	Lesotho	9 km Roma	29	26	S	27	41	E	*P. vulgaris*	Mos	NI
64	26/01/90	Lesotho	9 km Roma	29	26	S	27	41	E	*P. vulgaris*	Mos	NI
66	26/01/90	Lesotho	9 km Roma	29	26	S	27	41	E	*P. vulgaris*	Mos	NI
67	26/01/90	Lesotho	Nr Machache	29	25	S	27	45	E	*P. vulgaris*	MMos	NI
68	26/01/90	Lesotho	Nr Machache	29	25	S	27	45	E	*P. vulgaris*	MMos,St	NI
69	26/01/90	Lesotho	Nr Machache	29	25	S	27	45	E	*P. vulgaris*	Chl,St	NI
70	26/01/90	Lesotho	Nr Machache	29	25	S	27	45	E	*P. vulgaris*	Chl	NI
71	26/01/90	Lesotho	Machache Res.St	29	22	S	27	55	E	*P. vulgaris*	Mos	NI
72	26/01/90	Lesotho	Bushmen's Pass	29	26	S	27	57	E	*P. vulgaris*	Mos?	NI
73	26/01/90	Lesotho	Bushmen's Pass	29	26	S	27	57	E	*P. vulgaris*	Chl	NI
74	26/01/90	Lesotho	Bushmen's Pass	29	26	S	27	57	E	*P. vulgaris*	Mos?	NI
75	26/01/90	Lesotho	Bushmen's Pass	29	26	S	27	57	E	*P. vulgaris*	Chl,St	NI
76	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	SMot	NI
77	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	SMot	NI
78	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	Chl	NI
79	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	MMot	NI
80	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	MMot	NI
81	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	Chl,St	NI
82	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	Mot	NI
83	29/01/90	Swaziland	Mahlangatsha	26	47	S	31	12	E	*P. vulgaris*	Mot	NI

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
84	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	MMos	NI
85	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	Chl	NI
86	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	Chl	NI
87	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	Chl	NI
89	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	Chl	NI
90	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	Chl	NI
91	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	MMos	NI
92	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	MMos	NI
93	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	SMos	NI
94	29/01/90	Swaziland	Mahlangatsha	26 47 S 31 12 E	*P. vulgaris*	MMos	NI
95	29/01/90	Swaziland	Mbekelwen	Unknown	*P. vulgaris*	Mos,St	NI
96	29/01/90	Swaziland	Mbekelwen	Unknown	*P. vulgaris*	Mos	NI
97	29/01/90	Swaziland	Mbekelwen	Unknown	*P. vulgaris*	Mos	NI
98	29/01/90	Swaziland	Mbekelwen	Unknown	*P. vulgaris*	Mos	NI
99	29/01/90	Swaziland	Mbekelwen	Unknown	*P. vulgaris*	Mos	NI
101	29/01/90	Swaziland	Nr Mbekelwen	Unknown	*P. vulgaris*	Mos	NI
102	29/01/90	Swaziland	Low Veld	Unknown	*P. vulgaris*	Mos	NI
103	29/01/90	Swaziland	Low Veld	Unknown	*P. vulgaris*	Mos	NI
104	29/01/90	Swaziland	Low Veld	Unknown	*P. vulgaris*	Mos	NI
105	29/01/90	Swaziland	Low Veld	Unknown	*P. vulgaris*	Mos	NI
106	29/01/90	Swaziland	Low Veld	Unknown	*P. vulgaris*	MMos	NI
107	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	MMos	NI
108	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	Nec	NI
109	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	SMos	BCMV?
110	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	SMos	NI
111	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	Nec	NI
112	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	LN	NI
113	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	MMos	NI
116	31/01/90	Zimbabwe	CBI Harare	18 23 S 31 51 E	*P. vulgaris*	Mos	NI
117	31/01/90	Zimbabwe	Gwebi College	17 41 S 30 52 E	*P. vulgaris*	Nec	Poty
118	31/01/90	Zimbabwe	Gwebi College	17 41 S 30 52 E	*P. vulgaris*	Mos	NI
120	31/01/90	Zimbabwe	Gwebi College	17 41 S 30 52 E	*P. vulgaris*	Mos	NI

123	31/01/90	Zimbabwe	Zvimba	17 39 S	30 07 E	*P. vulgaris*	SMos	NI
124	31/01/90	Zimbabwe	Zvimba	17 39 S	30 07 E	*P. vulgaris*	MMos,LN	NI
125	31/01/90	Zimbabwe	Nr Zvimba	17 39 S	30 07 E	*P. vulgaris*	Mos	NI
126	31/01/90	Zimbabwe	Nr Zvimba	17 39 S	30 07 E	*P. vulgaris*	Mos	NI
129	31/01/90	Zimbabwe	CBS Kadoma	17 40 S	29 25 E	*P. vulgaris*	Mos	NI
130	02/02/90	Zimbabwe	Nr Beatrice	18 17 S	30 52 E	*P. vulgaris*	MMos,LD	NI
131	02/02/90	Zimbabwe	Nr Beatrice	18 17 S	30 52 E	*P. vulgaris*	MMos,LD	NI
132	02/02/90	Zimbabwe	Tavistock Farm	18 05 S	29 55 E	*P. vulgaris*	MMos,LD	NI
135	02/02/90	Zimbabwe	Tavistock Farm	18 05 S	29 55 E	*P. vulgaris*	Mos	NI
137	02/02/90	Zimbabwe	Nr Harare	18 23 S	31 51 E	*P. vulgaris*	MMos	NI
138	05/02/90	Malawi	Champhira	12 20 S	33 37 E	*P. vulgaris*	Mos	NI
141	05/02/90	Malawi	Champhira	12 20 S	33 37 E	*P. vulgaris*	Mos	NI
142	05/02/90	Malawi	Champhira	12 20 S	33 37 E	*P. vulgaris*	Mot	BCMV?
143	05/02/90	Malawi	Champhira	12 20 S	33 37 E	*P. vulgaris*	Mot	NI
144	05/02/90	Malawi	Nr Champhira	12 20 S	33 37 E	*Rhynchosia* sp.	SMot	NI
146	05/02/90	Malawi	Nr Champhira	12 20 S	33 37 E	*Rhynchosia* sp.	Mot	NI
147	05/02/90	Malawi	Nr Champhira	12 20 S	33 37 E	*Rhynchosia* sp.	Mot	NI
148	05/02/90	Malawi	5 km Kasunga	13 02 S	33 29 E	*P. vulgaris*	Mos	NI
150	06/02/90	Malawi	Linthipe	14 10 S	34 10 E	*P. vulgaris*	MMos	NI
152	06/02/90	Malawi	Linthipe	14 10 S	34 10 E	*P. vulgaris*	SMos	NI
153	06/02/90	Malawi	20 km Dedza	14 20 S	34 15 E	*P. vulgaris*	MMos	NI
154	06/02/90	Malawi	20 km Dedza	14 20 S	34 14 E	*P. vulgaris*	Chl	NI
155	06/02/90	Malawi	20 km Dedza	14 20 S	34 14 E	*P. vulgaris*	MMos	NI
156	06/02/90	Malawi	20 km Dedza	14 20 S	34 14 E	*P. vulgaris*	LD	NI
157	06/02/90	Malawi	Nr Dedza	14 20 S	34 15 E	Weed species	Mos,HBSusc	NI
158	06/02/90	Malawi	20 km Dedza	14 20 S	34 14 E	Legume weed	Mos	NI
159	06/02/90	Malawi	Nr Dedza	14 22 S	34 20 E	*P. vulgaris*	MMos	NI
160	06/02/90	Malawi	Nr Dedza	14 22 S	34 20 E	*P. vulgaris*	MMos	NI
163	06/02/90	Malawi	Dedza	14 22 S	34 20 E	*P. vulgaris*	MMot	NI
165	06/02/90	Malawi	Dedza	14 22 S	34 20 E	*Desmodium* sp.	MMos	NI
170	07/02/90	Malawi	4 km Makapwa	16 05 S	35 20 E	*P. vulgaris*	MMot	NI
171	07/02/90	Malawi	9 km Makapwa	16 05 S	35 20 E	*P. vulgaris*	MMos	NI
172	07/02/90	Malawi	9 km Makapwa	16 05 S	35 20 E	*P. vulgaris*	MMos	NI
173	07/02/90	Malawi	9 km Makapwa	16 05 S	35 20 E	*P. vulgaris*	MMos	NI
174	07/02/90	Malawi	9 km Makapwa	16 05 S	35 20 E	*P. vulgaris*	MMos	NI
175	07/02/90	Malawi	9 km Makapwa	16 05 S	35 20 E	*P. vulgaris*	MMos	NI
176	07/02/90	Malawi	14 km Makapwa	16 00 S	35 20 E	*P. vulgaris*	SMot	NI

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
177	07/02/90	Malawi	14 km Makapwa	16 00 S 35 20 E	*P. vulgaris*	MMos	NI
180	07/02/90	Malawi	14 km Makapwa	16 00 S 35 20 E	*P. vulgaris*	Mot	NI
181	07/02/90	Kenya	Nairobi Univ.	01 17 S 36 49 E	*P. vulgaris*	Chl	NI
182	07/02/90	Kenya	Nairobi Univ.	01 17 S 36 49 E	Cowpea	Mos	NI
183	07/02/90	Kenya	Nairobi Univ.	01 17 S 36 49 E	Cowpea	Chl	NI
184	07/02/90	Kenya	Nairobi Univ.	01 17 S 36 49 E	*P. vulgaris*	GVB,St,LD	NI
185	07/02/90	Kenya	Nairobi Univ.	01 17 S 36 49 E	Cowpea	GVB	NI
186	07/02/90	Kenya	Nairobi Univ.	01 17 S 36 49 E	Legume species Omto	MMos	NI
201	23/08/90	Ethiopia	Ijaji	09 00 N 37 20 E	*C. occidentalis*	MMot	Poty
202	23/08/90	Ethiopia	Ijaji	09 00 N 37 20 E	*C. occidentalis*	MMot	Poty
203	23/08/90	Ethiopia	Ijaji	09 00 N 37 20 E	*C. occidentalis*	MMot	Poty
204	23/08/90	Ethiopia	Bako	09 08 N 37 03 E	*Crotalaria* sp.	MMos	NI
205	23/08/90	Ethiopia	Nr Nekemte	09 05 N 36 33 E	*C. occidentalis*	MMos	BCMV?
206	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*P. vulgaris*	Mos?	BCMV A?
207	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*P. vulgaris*	MMos	NI
208	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*P. vulgaris*	MMos	NI
209	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*P. vulgaris*	MMos	NI
210	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*P. vulgaris*	MMos	Poty
211	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*P. vulgaris*	SMos	Poty
212	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*P. vulgaris*	MMos	Poty
213	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*Cassia sophera*	SMot	Poty
214	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*Cassia sophera*	SMot	Poty?
215	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*Cassia sophera*	SMot	NI
216	24/08/90	Ethiopia	Nr Metu	08 18 N 35 35 E	*Cassia sophera*	SMot	Poty
217	25/08/90	Ethiopia	Bulubla	07 51 N 36 39 E	*C. occidentalis*	Mot	NI
218	25/08/90	Ethiopia	Bulubla	07 51 N 36 39 E	*Cassia sophera*	Mot	Poty?
219	25/08/90	Ethiopia	Bulubla	07 51 N 36 39 E	*Crotalaria* sp.	MMos,St	Poty?
220	25/08/90	Ethiopia	Bulubla	07 51 N 36 39 E	*Cassia sophera*	Mot	NI
221	25/08/90	Ethiopia	Bulubla	07 51 N 36 39 E	*C. occidentalis*	Mot	Poty
222	25/08/90	Ethiopia	Agaro	07 51 N 36 39 E	*P. vulgaris*	SMos	BCMV/BlCMV?
223	23/08/90	Ethiopia	Agaro	07 51 N 36 39 E	*P. vulgaris*	MMot,St	NI
224	25/08/90	Ethiopia	Agaro	07 51 N 36 39 E	*P. vulgaris*	MMos	Poty

225	25/08/90	Ethiopia	Nr Jima	07 40 N 36 50 E	*Cassia sophera*	SMot	PnMoV*
227	25/08/90	Ethiopia	Nr Jima	07 40 N 36 50 E	*P. vulgaris*	MMos	NI
228	25/08/90	Ethiopia	Nr Jima	07 40 N 36 50 E	*Cassia sophera*	SMot	PnMoV*
229	26/08/90	Ethiopia	Inst.Agr. Jima	07 40 N 36 50 E	*P. vulgaris*	Mos	NI
230	26/08/90	Ethiopia	Inst.Agr. Jima	07 40 N 36 50 E	*P. vulgaris*	MMos	BCMV?
231	26/08/90	Ethiopia	Inst.Agr. Jima	07 40 N 36 50 E	*Cassia sophera*	SMot	Poty?
232	26/08/90	Ethiopia	Seka	07 36 N 36 44 E	*P. vulgaris*	Mos?	BCMV/BlCMV?
233	26/08/90	Ethiopia	Seka	07 36 N 36 44 E	*P. vulgaris*	MMos	NI
234	26/08/90	Ethiopia	Seka	07 36 N 36 44 E	*P. lunatus*	MMos	BCMV/BlCMV?
235	26/08/90	Ethiopia	Ataro nr Seka	07 36 N 36 44 E	*P. coccineus*	MMos	Poty
236	26/08/90	Ethiopia	Ataro nr Seka	07 36 N 36 44 E	*P. vulgaris*	MMos	Poty
237	26/08/90	Ethiopia	Ataro nr Seka	07 36 N 36 44 E	*P. vulgaris*	MMos	NI
238	26/08/90	Ethiopia	Sebaka nr Seka	07 36 N 36 44 E	*P. lunatus*	SMos	NI
239	26/08/90	Ethiopia	Sebaka nr Seka	07 36 N 36 44 E	*P. vulgaris*	Mos	NI
240	26/08/90	Ethiopia	Sebaka nr Seka	07 36 N 36 44 E	*P. vulgaris*	Mos	NI
241	26/08/90	Ethiopia	Sebaka nr Seka	07 36 N 36 44 E	*P. vulgaris*	Mos	NI
242	26/08/90	Ethiopia	Bonga	07 16 N 36 15 E	*Cassia sophera*	SMos	PnMoV
243	26/08/90	Ethiopia	Bonga Shet'	08 11 N 34 51 E	*P. vulgaris*	Mos,St	Poty
244	26/08/90	Ethiopia	Bonga Shet'	08 11 N 34 51 E	*P. vulgaris*	SMos	NI
246	26/08/90	Ethiopia	Bonga Shet'	08 11 N 34 51 E	*P. vulgaris*	SMot	BCMV?
247	26/08/90	Ethiopia	Bonga Shet'	08 11 N 34 51 E	*P. vulgaris*	MMot	NI
248	26/08/90	Ethiopia	Bonga Shet'	08 11 N 34 51 E	*P. vulgaris*	MMot	NI
249	26/08/90	Ethiopia	Diri Goma	07 23 N 36 17 E	*P. lunatus*	Mos	NI
250	26/08/90	Ethiopia	Diri Goma	07 23 N 36 17 E	*P. lunatus*	SMos	CMV
251	26/08/90	Ethiopia	Diri Goma	07 23 N 36 17 E	*P. lunatus*	SMos	NI
252	27/08/90	Ethiopia	Abelti	08 10 N 37 34 E	*P. vulgaris*	Mos?	NI
253	27/08/90	Ethiopia	Abelti	08 10 N 37 34 E	*P. vulgaris*	Mos?	NI
254	27/08/90	Ethiopia	Gibe Valley	08 14 N 37 39 E	Legume weed	Mot	NI
255	28/08/90	Ethiopia	3 km Mojo Jct	08 30 N 39 10 E	*P. vulgaris*	MMos	NI
256	28/08/90	Ethiopia	3 km Mojo Jct	08 30 N 39 10 E	*P. vulgaris*	MMos	NI
257	28/08/90	Ethiopia	3 km Mojo Jct	08 30 N 39 10 E	*P. vulgaris*	MMot	NI
258	28/08/90	Ethiopia	3 km Mojo Jct	08 30 N 39 10 E	*P. vulgaris*	MMos	BCMV/BlCMV?
259	28/08/90	Ethiopia	3 km Mojo Jct	08 30 N 39 10 E	*P. vulgaris*	MMos	BCMV/BlCMV?
260	28/08/90	Ethiopia	5 km Mojo Jct	08 30 N 39 10 E	*P. vulgaris*	MMos	NI
261	28/08/90	Ethiopia	11 km Mojo	08 20 N 39 10 E	*P. vulgaris*	MMos	NI
262	28/08/90	Ethiopia	11 km Mojo	08 20 N 39 10 E	*P. vulgaris*	MMos	NI
263	28/08/90	Ethiopia	20 km Mojo	08 20 N 39 10 E	*C. occidentalis*	SMos	NI

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
264	28/08/90	Ethiopia	20 km Mojo	08 20 N 39 10 E	*C. didymobotrya*	Mot	Poty?
265	28/08/90	Ethiopia	22 km Mojo	08 20 N 39 10 E	*P. vulgaris*	MMos?	NI
266	28/08/90	Ethiopia	Nr Tuka Koramtu	08 10 N 39 00 E	*P. vulgaris*	MMos?	BCMV/BICMV?
267	28/08/90	Ethiopia	58 km Mojo	08 05 N 38 50 E	*P. vulgaris*	MMos	NI
268	28/08/90	Ethiopia	58 km Mojo	08 05 N 38 50 E	*P. vulgaris*	MMos	NI
269	28/08/90	Ethiopia	58 km Mojo	08 05 N 38 50 E	*P. vulgaris*	Mos	NI
270	28/08/90	Ethiopia	82 km Mojo	08 00 N 38 45 E	*P. vulgaris*	SMos	Poty
271	28/08/90	Ethiopia	82 km Mojo	08 00 N 38 45 E	*P. vulgaris*	MMos	BCMV/BICMV?
272	28/08/90	Ethiopia	82 km Mojo	08 00 N 38 45 E	*P. vulgaris*	MMos	NI
273	28/08/90	Ethiopia	82 km Mojo	08 00 N 38 45 E	*P. vulgaris*	SSt	BCMV/BICMV?
275	28/08/90	Ethiopia	82 km Mojo	08 00 N 38 45 E	*P. vulgaris*	SSt	NI
276	28/08/90	Ethiopia	82 km Mojo	08 00 N 38 45 E	*C. occidentalis*	SMos	NI
278	28/08/90	Ethiopia	Nr Zway	07 56 N 38 43 E	*P. vulgaris*	MMos	BCMV?
279	28/08/90	Ethiopia	Nr Zway	07 56 N 38 43 E	*P. vulgaris*	MMos	BCMV/BICMV?
280	28/08/90	Ethiopia	115 km Mojo	07 40 N 38 40 E	*P. vulgaris*	SSt	Poty
281	28/08/90	Ethiopia	115 km Mojo	07 40 N 38 40 E	*P. vulgaris*	SMos,LD	Poty
282	28/08/90	Ethiopia	115 km Mojo	07 40 N 38 40 E	*P. vulgaris*	Mos,SSt	Poty
283	28/08/90	Ethiopia	115 km Mojo	07 40 N 38 40 E	*P. vulgaris*	SMos	Poty
284	28/08/90	Ethiopia	115 km Mojo	07 40 N 38 40 E	*P. vulgaris*	SMos	Poty
285	28/08/90	Ethiopia	146 km Mojo	07 30 N 38 40 E	*P. vulgaris*	SMos,St	Poty
287	28/08/90	Ethiopia	146 km Mojo	07 30 N 38 40 E	*P. vulgaris*	St	NI
288	28/08/90	Ethiopia	146 km Mojo	07 30 N 38 40 E	*P. vulgaris*	Mos	Poty
290	29/08/90	Ethiopia	Awasa	07 03 N 38 28 E	*P. vulgaris*	SMos,St	Poty
291	29/08/90	Ethiopia	Awasa	07 03 N 38 28 E	*P. vulgaris*	SMos	Poty
292	29/08/90	Ethiopia	Awasa	07 03 N 38 28 E	*P. vulgaris*	SMos	Poty
293	29/08/90	Ethiopia	Awasa	07 03 N 38 28 E	*P. vulgaris*	SMos	NI
294	29/08/90	Ethiopia	7 km Shashmene	07 02 N 38 38 E	*P. vulgaris*	St	NI
295	29/08/90	Ethiopia	7 km Shashmene	07 02 N 38 38 E	*P. vulgaris*	SMos,St	Poty
296	29/08/90	Ethiopia	7 km Shashmene	07 02 N 38 38 E	*P. vulgaris*	SMos,St	Poty
297	29/08/90	Ethiopia	14 km Shashmene	07 02 N 38 38 E	*P. vulgaris*	MMos	NI
298	29/08/90	Ethiopia	23 km Shashmene	07 10 N 38 20 E	*P. vulgaris*	MMos	NI
299	29/08/90	Ethiopia	23 km Shashmene	07 10 N 38 20 E	*P. vulgaris*	MMos	NI

300	29/08/90	Ethiopia	23 km Shashmene	07 10 N 38 20 E	*P. vulgaris*	MMos	BCMV/BlCMV?
301	29/08/90	Ethiopia	23 km Shashmene	07 10 N 38 20 E	*P. vulgaris*	MMos	NI
302	29/08/90	Ethiopia	37 km Shashmene	07 10 N 38 15 E	*P. vulgaris*	St,LD	BCMV/BlCMV?
303	29/08/90	Ethiopia	Nr Geshgola	07 05 N 38 30 E	*P. vulgaris*	MMos	Poty?
304	29/08/90	Ethiopia	Nr Geshgola	07 05 N 38 30 E	*P. vulgaris*	MMos	NI
305	29/08/90	Ethiopia	Nr Geshgola	07 05 N 38 30 E	*P. vulgaris*	MMos	NI
306	29/08/90	Ethiopia	Nr Geshgola	07 05 N 38 30 E	*Cassia sophera*	SMos	Poty?
307	29/08/90	Ethiopia	82 km Shashmene	07 05 N 38 30 E	*P. vulgaris*	MMos	NI
308	29/08/90	Ethiopia	82 km Shashmene	07 05 N 38 30 E	*P. vulgaris*	MMos	NI
309	29/08/90	Ethiopia	80 km Shashmene	07 05 N 38 30 E	*P. vulgaris*	MMos	NI
310	29/08/90	Ethiopia	80 km Shashmene	07 05 N 38 30 E	*P. vulgaris*	MMos	BCMV?
311	29/08/90	Ethiopia	80 km Shashmene	07 05 N 38 30 E	*P. vulgaris*	MMos	NI
312	30/08/90	Ethiopia	Lake Shala	07 20 N 38 30 E	*P. vulgaris*	SMos	NI
313	30/08/90	Ethiopia	Lake Shala	07 20 N 38 30 E	*P. vulgaris*	SMos	PnMoV*
314	30/08/90	Ethiopia	Lake Shala	07 20 N 38 30 E	*P. vulgaris*	SMos	PnMoV*
315	30/08/90	Ethiopia	23 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	MMos,St	NI
316	30/08/90	Ethiopia	14.5 km Nazreth	08 33 N 39 16 E	*C. occidentalis*	SMos	CSMV*
317	30/08/90	Ethiopia	14 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	SMos,St	NI
318	30/08/90	Ethiopia	14 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	SMos,St	NI
320	30/08/90	Ethiopia	14 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	SMos,St	Poty
321	30/08/90	Ethiopia	14 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	SMos,St	NI
322	30/08/90	Ethiopia	10 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	SMos	NI
323	30/08/90	Ethiopia	5 km Nazreth	08 33 N 39 16 E	*P. vulgaris*	SMos	BCMV?
324	02/09/90	Ethiopia	Dire Dawa	09 30 N 41 56 E	*P. vulgaris*	MMos	NI
325	02/09/90	Ethiopia	Dire Dawa	09 30 N 41 56 E	*P. vulgaris*	Mos?	NI
326	02/09/90	Ethiopia	Dire Dawa	09 30 N 41 56 E	*P. vulgaris*	Mos?	NI
327	02/09/90	Ethiopia	Harer-Awash S3	09 30 N 41 40 E	*P. vulgaris*	Mos?	Poty/BCMV?
328	02/09/90	Ethiopia	Harer-Awash S3	09 30 N 41 40 E	*P. vulgaris*	Mos?	NI
329	02/09/90	Ethiopia	Harer-Awash S7	09 30 N 41 20 E	*P. vulgaris*	Mos?	Poty/BCMV?
330	02/09/90	Ethiopia	Harer-Awash S7	09 30 N 41 20 E	*P. vulgaris*	Mos?	Poty/BCMV?
331	02/09/90	Ethiopia	Harer-Awash S8	09 25 N 41 00 E	*P. vulgaris*	Mos	Poty/BCMV?
332	02/09/90	Ethiopia	Harer-Awash S8	09 25 N 41 00 E	*P. vulgaris*	Mos	NI
333	02/09/90	Ethiopia	Harer-Awash S8	09 25 N 41 00 E	*P. vulgaris*	Mos	Poty
334	02/09/90	Ethiopia	Harer-Awash S10	09 20 N 40 50 E	*P. vulgaris*	Mos?	BCMV/BLCMV?
335	02/09/90	Ethiopia	Harer-Awash S11	09 10 N 40 40 E	*P. vulgaris*	Mos?	NI
336	03/09/90	Ethiopia	Nazreth Res.St.	08 33 N 39 16 E	*C. laburnifolia*	MMos	Poty*
337	03/09/90	Ethiopia	Nazreth Res.St.	08 33 N 39 16 E	Legume weed	MMos	NI

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
338	03/09/90	Ethiopia	Nazreth Res.St.	08 33 N 39 16 E	*C. laburnifolia*	MMos	Poty*
341	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	Mos	BCMV A
342	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	Mos	BCMV B
343	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	Mos	BCMV A
344	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	Mos	AlfMV*/BCMV
345	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	Mos	BCMV B
346	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	Mos	BCMV?
347	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	Mos	AlfMV*/BCMV A
348	05/11/90	Morocco	Beni Mallal S1	32 10 N 06 45 E	*P. vulgaris*	BYMV-like	AlfMV*/BCMV
349	05/11/90	Morocco	Beni Mallal S2	32 10 N 06 45 E	*P. vulgaris*	Mos,St	BCMV B
350	05/11/90	Morocco	Beni Mallal S2	32 10 N 06 45 E	*P. vulgaris*	Mos,St	BCMV A
351	05/11/90	Morocco	Beni Mallal S2	32 10 N 06 45 E	*P. vulgaris*	Mos,St	BCMV B
352	05/11/90	Morocco	Beni Mallal S2	32 10 N 06 45 E	*P. vulgaris*	Mos,St	BCMV B
353	05/11/90	Morocco	Beni Mallal S3	32 10 N 06 40 E	*P. vulgaris*	BYMV-like	BCMV?
354	05/11/90	Morocco	Beni Mallal S3	32 10 N 06 40 E	*P. vulgaris*	St	Poty
355	05/11/90	Morocco	Beni Mallal S4	32 10 N 06 30 E	*P. vulgaris*	Mos	BCMV?
356	05/11/90	Morocco	Beni Mallal S4	32 10 N 06 30 E	*P. vulgaris*	Mos	BCMV?
357	05/11/90	Morocco	Beni Mallal S4	32 10 N 06 30 E	*P. vulgaris*	SMos	Poty
358	05/11/90	Morocco	Beni Mallal S4	32 10 N 06 30 E	*P. vulgaris*	SMos	BCMV?
359	05/11/90	Morocco	Beni Mallal S4	32 10 N 06 30 E	*P. vulgaris*	SMos	BCMV B
360	05/11/90	Morocco	Beni Mallal S4	32 10 N 06 30 E	*P. vulgaris*	SMos	BCMV A
361	05/11/90	Morocco	Beni Mallal S5	32 10 N 06 30 E	*P. vulgaris*	MMos	NI
362	05/11/90	Morocco	Beni Mallal S5	32 10 N 06 30 E	*P. vulgaris*	Mos,LD	NI
363	05/11/90	Morocco	Beni Mallal S5	32 10 N 06 30 E	*P. vulgaris*	Mos,SST	BCMV B
364	05/11/90	Morocco	Beni Mallal S5	32 10 N 06 30 E	*P. vulgaris*	SMos	BCMV?
365	05/11/90	Morocco	Beni Mallal S6	32 10 N 06 25 E	*P. vulgaris*	SMos,St	NI
366	05/11/90	Morocco	Beni Mallal S7	32 10 N 06 25 E	*P. vulgaris*	SMos	AlfMV*
367	05/11/90	Morocco	Beni Mallal S7	32 10 N 06 25 W	*P. vulgaris*	SMos	NI
368	07/11/90	Morocco	20 km Fez	33 50 N 06 45 W	*P. vulgaris*	Chl	NI
369	07/11/90	Morocco	20 km Fez	33 50 N 06 45 W	*P. vulgaris*	Chl	BCMV A
370	07/11/90	Morocco	20 km Fez	33 50 N 06 45 W	*P. vulgaris*	Chl	NI
371	07/11/90	Morocco	20 km Fez	33 50 N 06 45 W	*P. vulgaris*	Chl	BCMV/BlCMV?

372	05/11/90	Morocco	Beni Mellal S1	32 10 N 06 45 W	*P. vulgaris*	SMos	BCMV?
401	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	MMos,GVB	BCMV?
408	02/05/91	Tanzania	Sokoine Univ.	06 50 S 37 45 E	*P. vulgaris*	Mot	NI
409	04/05/91	Tanzania	Mbimba Res. St.	08 50 S 33 30 E	*P. vulgaris*	CVB,LD	Poty
410	04/05/91	Tanzania	Mbimba Res. St.	08 50 S 33 30 E	*P. vulgaris*	Mos,LD	NI
411	04/05/91	Tanzania	Mbimba Res. St.	08 50 S 33 30 E	*P. vulgaris*	Mot	NI
413	04/05/91	Tanzania	Mbimba Res. St.	08 50 S 33 30 E	*P. vulgaris*	CVB,LD	NI
414	04/05/91	Tanzania	Mbimba Res. St.	08 50 S 33 30 E	*P. vulgaris*	SMot	NI
415	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMos,LD	NI
416	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMos,LD	NI
417	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMos,LD	NI
418	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	Mos,LD	NI
419	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMot	NI
420	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMot,SSt	NI
421	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMot,SSt	NI
422	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMos,SLD	NI
423	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMos	NI
424	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	SMos	NI
425	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	MMos,SLD	NI
426	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	MMos,SLD	NI
427	04/05/91	Tanzania	Nr Mbeya	08 50 S 33 30 E	*P. vulgaris*	LD	NI
428	06/05/91	Tanzania	Nr Moshi	03 15 S 37 30 E	*P. vulgaris*	SMos,LD	Poty*
429	06/05/91	Tanzania	Nr Moshi	03 15 S 37 30 E	*P. vulgaris*	Mos	Poty*
430	06/05/91	Tanzania	Nr Moshi	03 15 S 37 30 E	*P. vulgaris*	GVB,Mot	Poty*
431	06/05/91	Tanzania	Nr Moshi	03 15 S 37 30 E	*P. vulgaris*	Mos,GVB	NI
432	06/05/91	Tanzania	Nr Moshi	03 15 S 37 30 E	*P. vulgaris*	GVB,LD,SSt	Poty?
433	06/05/91	Tanzania	Nr Moshi	03 15 S 37 30 E	*P. vulgaris*	GVB,SSt	BCMV A?
434	06/05/91	Tanzania	Nr Moshi	03 15 S 37 30 E	*P. vulgaris*	SMot	BCMV A?
435	06/05/91	Tanzania	Masasani	03 15 S 37 20 E	*P. vulgaris*	St,LD	Poty*
436	06/05/91	Tanzania	Moshi-Arusha	03 15 S 37 20 E	*P. vulgaris*	MMos,LD	Poty*
438	06/05/91	Tanzania	Moshi-Arusha	03 15 S 37 20 E	*P. vulgaris*	GVB	BCMV?
439	06/05/91	Tanzania	Moshi-Arusha	03 15 S 37 20 E	*P. vulgaris*	GVB,LD	NI
440	06/05/91	Tanzania	30 km Same	04 04 S 37 44 E	*Indigofera* sp.	Mot	NI
441	07/05/91	Tanzania	Arusha Site1	03 22 S 36 41 E	*P. vulgaris*	Mos,GVB	BCMV/BICMV?
443	07/05/91	Tanzania	Arusha Site1	03 22 S 36 41 E	*P. vulgaris*	Mot	BCMV A
445	07/05/91	Tanzania	Arusha Site1	03 22 S 36 41 E	*P. vulgaris*	Mos,LD	NI
446	07/05/91	Tanzania	Arusha Site1	03 22 S 36 41 E	*P. vulgaris*	MMot,St	BCMV A

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
447	07/05/91	Tanzania	Arusha Site2	03 22 S 36 41 E	*P. vulgaris*	Mot,St,SLD	NI
448	07/05/91	Tanzania	Arusha Site2	03 22 S 36 41 E	*P. vulgaris*	Mot,SLD	BCMV?
449	07/05/91	Tanzania	Arusha Site2	03 22 S 36 41 E	*P. vulgaris*	SChl	NI
452	07/05/91	Tanzania	Arusha Site3	03 22 S 36 41 E	*P. vulgaris*	Mot	BCMV?
453	07/05/91	Tanzania	Arusha Site3	03 22 S 36 41 E	*P. vulgaris*	Mot,GVB	BCMV A
455	07/05/91	Tanzania	Arusha Site4	03 22 S 36 41 E	*P. vulgaris*	MMos,SLD	BCMV A
457	07/05/91	Tanzania	Arusha Site4	03 22 S 36 41 E	*P. vulgaris*	Mot,LD	BCMV A
461	13/05/91	Uganda	Kawanda Res.St.	00 26 N 32 32 E	*C. ochroleuca*	Mot,LD,CVB	Poty
462	13/05/91	Uganda	Kawanda Res.St.	00 26 N 32 32 E	*Indigofera* sp.	MMot,LD	NI
463	13/05/91	Uganda	Bukalasa Fld.St.	00 43 N 32 30 E	*Cassia* sp.	SMos	Poty
466	13/05/91	Uganda	Bukalasa Fld.St.	00 43 N 32 30 E	*P. vulgaris*	SMos,LD	BCMV?
467	13/05/91	Uganda	Kawanda Res.St.	00 26 N 32 30 E	*C. incana*	Mot	BCMV A
468	13/05/91	Uganda	Kawanda Res.St.	00 26 N 32 30 E	*Desmodium* sp.	Mos?	BCMV?
469	13/05/91	Uganda	Bukalasa Fld.St.	00 43 N 32 30 E	*Desmodium* sp.	Mot	BCMV A
470	13/05/91	Uganda	Nr Bukalasa	00 43 N 32 30 E	*P. vulgaris*	MMos	NI
472	13/05/91	Uganda	Kalule	00 38 N 32 32 E	*P. vulgaris*	SMos,St,LD	Poty
473	13/05/91	Uganda	Kalule	00 38 N 32 32 E	*Calopogonium* sp.	MMot	NI
474	14/05/91	Uganda	Kikone	00 23 S 31 30 E	*P. vulgaris*	Mot	NI
475	14/05/91	Uganda	Kikone	00 23 S 31 30 E	*P. vulgaris*	Mos,LD	NI
476	14/05/91	Uganda	Kikone	00 23 S 31 30 E	*P. vulgaris*	Mot	NI
477	14/05/91	Uganda	Kikone	00 23 S 31 30 E	*Desmodium* sp.	MMos	NI
478	14/05/91	Uganda	Mbirizi	00 23 S 31 27 E	*P. vulgaris*	CVB,LD	BCMV?
479	14/05/91	Uganda	Mbirizi	00 23 S 31 27 E	*P. vulgaris*	Mos,SLD	BCMV A?
480	14/05/91	Uganda	Mbirizi	00 23 S 31 27 E	*P. vulgaris*	MMos,SLD	BCMV A?
483	14/05/91	Uganda	Kategula	00 12 S 30 18 E	*P. vulgaris*	Mos,SLD	BCMV A
484	14/05/91	Uganda	Itohzo	00 38 S 30 21 E	*P. vulgaris*	Mos,LD	NI
485	14/05/91	Uganda	Itohzo	00 38 S 30 21 E	*Crotalaria* sp.	Mot	NI
486	15/05/91	Uganda	Kabale	01 20 S 30 00 E	*P. vulgaris*	MMos,LD	BCMV?
487	15/05/91	Uganda	Kabale	01 20 S 30 00 E	*P. vulgaris*	MMos, LD	BCMV?
488	15/05/91	Uganda	Mukateme	01 20 S 30 00 E	*P. vulgaris*	Mot,SLD	BCMV A?
489	15/05/91	Uganda	Mukateme	01 20 S 30 00 E	*P. vulgaris*	Mos	BCMV A
490	15/05/91	Uganda	Karukara	01 20 S 30 00 E	*P. vulgaris*	SMos,LD	BCMV?

491	15/05/91	Uganda	Karukara	01 20 S 30 00 E	*P. vulgaris*	SMos,LD	BCMV?
492	15/05/91	Uganda	Karukara	01 20 S 30 00 E	*P. vulgaris*	Mos,LD	BCMV?
493	15/05/91	Uganda	Mourole	01 20 S 30 00 E	*P. vulgaris*	Mot,LD	BCMV?
494	15/05/91	Uganda	Kalangyere RS	01 20 S 30 00 E	*P. vulgaris*	SMos,LD	BCMV A
495	15/05/91	Uganda	Rubaare Ag. St.	01 01 S 30 12 E	*P. vulgaris*	SMos,LD	BCMV?
496	15/05/91	Uganda	Rubaare Ag. St.	01 01 S 30 12 E	*P. vulgaris*	SMos,LD	BCMV?
498	15/05/91	Uganda	Rubaare Ag. St.	01 01 S 30 12 E	*P. vulgaris*	SMos,LD	BCMV A
500	16/05/91	Uganda	Mubuku	00 16 N 30 07 E	*P. vulgaris*	LD	BCMV?
501	16/05/91	Uganda	Mubuku	00 16 N 30 07 E	*P. vulgaris*	Mot,LD	NI
502	16/05/91	Uganda	Mubuku	00 16 N 30 07 E	*Calopogonium* sp.	Mos	NI
503	16/05/91	Uganda	Ruimi	00 20 N 30 17 E	*P. vulgaris*	Mot,LD	BCMV?
506	16/05/91	Uganda	Kyanga	00 30 N 30 30 E	*P. vulgaris*	SMos	BCMV A
507	16/05/91	Uganda	Kicucu	00 30 N 30 35 E	*P. vulgaris*	MMot	BCMV?
508	16/05/91	Uganda	Kicucu	00 30 N 30 35 E	*P. vulgaris*	MMot	HB
509	16/05/91	Uganda	Kicucu	00 30 N 30 35 E	*Dolichos* sp.	CVB,LD	NI
514	19/05/91	Rwanda	Karama Farm 1	02 32 S 29 46 E	*P. vulgaris*	GVB	BCMV A
515	19/05/91	Rwanda	Karama Farm 2	02 32 S 29 46 E	*P. vulgaris*	Mos,LD	BCMV?
516	19/05/91	Rwanda	Karama Farm 3	02 32 S 29 46 E	*P. vulgaris*	Mos,LD	BCMV A
517	19/05/91	Rwanda	Karama Farm 3	02 32 S 29 46 E	*P. vulgaris*	GVB,Chl	BCMV A
518	19/05/91	Rwanda	Kanzenze	02 05 S 30 06 E	*P. vulgaris*	SMot,LD	BCMV?
522	19/05/91	Rwanda	Musambira	02 12 S 29 47 E	*Vigna* sp.	Mos	Isometric
525	20/05/91	Rwanda	Runinya Farm 1	02 39 S 29 37 E	*P. vulgaris*	Mot	BCMV?
527	20/05/91	Rwanda	Runinya Farm 2	02 39 S 29 37 E	*P. vulgaris*	Mos,St	BCMV A
533	20/05/91	Rwanda	Bukarama	02 42 S 29 00 E	*P. vulgaris*	Mot,LD	BCMV?
534	20/05/91	Rwanda	Bukarama	02 42 S 29 00 E	*P. vulgaris*	MMos	BCMV?
536	20/05/91	Rwanda	Ngoma	02 04 S 29 56 E	*P. vulgaris*	Mos,LD	NI
538	20/05/91	Rwanda	Mbazi Commune	02 35 S 29 45 E	*P. vulgaris*	MMos,LD	NI
539	20/05/91	Rwanda	Mbazi Commune	02 35 S 29 45 E	*P. vulgaris*	MMos	BCMV?
540	20/05/91	Rwanda	Mbazi Commune	02 35 S 29 45 E	*P. vulgaris*	Mot,LD	NI
541	20/05/91	Rwanda	Mbazi Commune	02 35 S 29 45 E	*P. vulgaris*	Mot,LD	BCMV?
542	21/05/91	Zaire	Mulungu Farm 1	02 20 S 28 47 E	*P. vulgaris*	Mos,GVB	NI
543	21/05/91	Zaire	Mulungu Farm 1	02 20 S 28 47 E	*P. vulgaris*	CM	NI
544	21/05/91	Zaire	Mulungu Farm 2	02 20 S 28 47 E	*P. vulgaris*	Mos,SLD	NI
545	21/05/91	Zaire	Mulungu Farm 3	02 20 S 28 47 E	*P. vulgaris*	SMos,LD	BCMV?
546	21/05/91	Zaire	Mulungu Farm 3	02 20 S 28 47 E	*P. vulgaris*	MMos,LD	NI
547	21/05/91	Zaire	Mulungu Farm 4	02 20 S 28 47 E	*P. vulgaris*	Mos,SLD	NI
548	21/05/91	Zaire	Mulungu Farm 5	02 20 S 28 47 E	*P. vulgaris*	Mos,SLD	NI

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
549	21/05/91	Zaire	Mulungu Farm 5	02 20 S 28 47 E	*P. vulgaris*	MMos	BCMV?
551	21/05/91	Zaire	Mulungu Farm 6	02 20 S 28 47 E	*P. vulgaris*	MMos,SGVB	NI
552	21/05/91	Zaire	Mulungu Farm 6	02 20 S 28 47 E	*P. vulgaris*	SMos,St	Poty
553	22/05/91	Zaire	Mulengesa Fm 1	02 20 S 28 47 E	*P. vulgaris*	Mos,LD	BCMV?
554	22/05/91	Zaire	Mulengesa Fm 1	02 20 S 28 47 E	*P. vulgaris*	Mos,GVB,LD	BCMV A
555	22/05/91	Zaire	Mulengesa Fm 1	02 20 S 28 47 E	*P. vulgaris*	Mot,LD	NI
556	22/05/91	Zaire	Mulengesa Fm 2	02 20 S 28 47 E	*P. vulgaris*	MMos,LD	NI
557	22/05/91	Zaire	Mulengesa Fm 2	02 20 S 28 47 E	*P. vulgaris*	Mos,LD	NI
558	22/05/91	Zaire	Mulengesa Fm 3	02 20 S 28 47 E	*P. vulgaris*	Mot,St,LD	NI
559	22/05/91	Zaire	Mulengesa Fm 4	02 20 S 28 47 E	*P. vulgaris*	Mos,LD	NI
560	22/05/91	Zaire	Mulengesa Fm 5	02 20 S 28 47 E	*P. vulgaris*	SMos,LD	NI
561	22/05/91	Zaire	Mulengesa Fm 6	02 20 S 28 47 E	*P. vulgaris*	GVB,SLD	NI
562	22/05/91	Zaire	Mulengesa Fm 6	02 20 S 28 47 E	*P. vulgaris*	GVB,SLD	NI
563	22/05/91	Zaire	Mulengesa Fm 6	02 20 S 28 47 E	*P. vulgaris*	GVB,SLD	NI
566	22/05/91	Rwanda	Gikongoro	02 29 S 29 34 E	*P. vulgaris*	Mos,GVB,LD	NI
570	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos,GVB,LD	BCMV A
571	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	MMos	NI
572	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	MMos	BCMV A
573	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos,GVB,LD	BCMV A
574	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos,GVB,St	BCMV A
576	23/05/91	Rwanda	Rubona Res. St.	02 29 S 29 46 E	*P. vulgaris*	Mos,GVB,LD	BCMV A
577	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mot	BCMV?
579	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mot	BCMV?
580	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mos	NI
581	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mot	NI
582	19/06/91	Burundi	Parambo	Unknown	*P. vulgaris*	Mot	BCMV A
585	19/06/91	Burundi	Kisozi	03 34 S 29 41 E	*P. vulgaris*	Mos	NI
588	11/07/91	Zambia	Msekera Ag.St.	13 39 S 32 34 E	Cowpea	?	BICMV?
589	11/07/91	Zambia	Msekera Ag.St.	13 39 S 32 34 E	Cowpea	?	BICMV?
590	11/07/91	Zambia	Msekera Ag.St.	13 39 S 32 34 E	Cowpea	?	BICMV?
591	11/07/91	Zambia	Msekera Ag.St.	13 39 S 32 34 E	Cowpea	?	BCMV?
592	09/09/91	Zimbabwe	Guruwe	16 44 S 30 51 E	*P. vulgaris*	?	NI

593	09/09/91	Zimbabwe	GBS Kadoma	17 40 S 29 25 E	*P. vulgaris*	?	NI
594	09/09/91	Zimbabwe	Chegutu	Unknown	*P. vulgaris*	?	NI
595	09/09/91	Zimbabwe	Harare	18 00 S 30 55 E	*P. vulgaris*	?	BCMV?
596	09/09/91	Zimbabwe	Gwebi	17 41 S 30 52 E	*P. vulgaris*	?	BCMV?
597	09/09/91	Zimbabwe	Gwebi	17 41 S 30 52 E	*P. vulgaris*	?	NI
598	09/09/91	Zimbabwe	Gwebi	17 41 S 30 52 E	*P. vulgaris*	?	NI
599	09/09/91	Zimbabwe	Mazowe	17 10 S 31 00 E	*P. vulgaris*	?	NI
600	09/09/91	Zimbabwe	Mazowe	17 10 S 31 00 E	*P. vulgaris*	?	BCMV?
601	09/09/91	Zimbabwe	Mapotos	Unknown	*P. vulgaris*	?	BCMV?
602	09/09/91	Zimbabwe	Mapotos	Unknown	*P. vulgaris*	?	NI
603	09/09/91	Zimbabwe	Honde Valley	18 31 S 31 10 E	*P. vulgaris*	?	BCMV?
604	09/09/91	Zimbabwe	Honde Valley	18 31 S 31 10 E	*P. vulgaris*	?	BCMV?
605	09/09/91	Zimbabwe	Honde Valley	18 31 S 31 10 E	*P. vulgaris*	?	NI
606	09/09/91	Zimbabwe	Honde Valley	18 31 S 31 10 E	*P. vulgaris*	?	NI
607	09/09/91	Zimbabwe	Honde Valley	18 31 S 31 10 E	*P. vulgaris*	?	NI
608	09/09/91	Zimbabwe	Nyanyadzi	19 44 S 32 42 E	*P. vulgaris*	?	NI
609	09/09/91	Zimbabwe	Nyanyadzi	19 44 S 32 42 E	*P. vulgaris*	?	NI
801	20/11/91	Rwanda	Gikongoro	02 29 S 29 34 E	*P. vulgaris*	Mot	NI
803	20/11/91	Rwanda	Gikongoro	02 29 S 29 34 E	*P. vulgaris*	MMos	NI
804	20/11/91	Rwanda	Ngoma	02 04 S 29 56 E	*P. vulgaris*	Mos	NI
805	20/11/91	Rwanda	Ngoma	02 04 S 29 56 E	*C. lachnophora*	MMot	NI
806	20/11/91	Rwanda	Ngoma	02 04 S 29 56 E	*T. vogelii*	MMot	BCMV A
807	20/11/91	Rwanda	Kibungo	02 10 S 30 32 E	*T. vogelii*	MMot	BCMV A
808	20/11/91	Rwanda	Kibungo	02 10 S 30 32 E	*P. vulgaris*	MMos	BCMV A
809	20/11/91	Rwanda	Kibungo	02 10 S 30 32 E	*P. vulgaris*	MMos	BCMV A
810	20/11/91	Rwanda	Nr Kibungo	02 10 S 30 32 E	*P. vulgaris*	MMos	BCMV A
811	20/11/91	Rwanda	Nr Kibungo	02 10 S 30 32 E	*P. vulgaris*	MMos,LD	BCMV A
812	20/11/91	Rwanda	Nr Kibungo	02 10 S 30 32 E	*P. vulgaris*	SMos	BCMV A
813	20/11/91	Rwanda	Nr Kibungo	02 10 S 30 32 E	*P. vulgaris*	SMos	NI
814	20/11/91	Rwanda	Nr Kibungo	02 10 S 30 32 E	*P. vulgaris*	MCVB	NI
815	20/11/91	Rwanda	Maraba	02 45 S 29 39 E	*P. vulgaris*	MMos	NI
816	20/11/91	Rwanda	Cyumba	02 17 S 29 41 E	*P. vulgaris*	SMos	NI
817	20/11/91	Rwanda	Sovu	02 00 S 30 26 E	*P. vulgaris*	SMos	NI
818	20/11/91	Rwanda	Sovu	02 00 S 30 26 E	*P. vulgaris*	SMos	NI
819	20/11/91	Rwanda	Sovu	02 00 S 30 26 E	*P. vulgaris*	SMos	NI
821	20/11/91	Rwanda	Mbazi	02 22 S 29 35 E	*P. vulgaris*	Mot	Poty
822	21/11/91	Rwanda	Nr Rubona R.S.	02 29 S 29 46 E	*C. agetiflora*	SMos	NI

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
823	21/11/91	Rwanda	Nr Rubona R.S.	02 29 S 29 46 E	*C. agetiflora*	SMos	NI
824	21/11/91	Rwanda	Ndago	02 42 S 29 34 E	*P. vulgaris*	MMos	NI
825	21/11/91	Rwanda	3 km Cyuru	01 40 S 30 12 E	*P. vulgaris*	Mos?	Poty
826	21/11/91	Rwanda	Nr. Cyuru	01 40 S 30 12 E	*P. vulgaris*	MMot	NI
827	21/11/91	Rwanda	Rweru	02 24 S 30 19 E	*P. vulgaris*	Mos	Poty
828	21/11/91	Rwanda	Rweru	02 24 S 30 19 E	*P. vulgaris*	MMos	Poty
829	21/11/91	Rwanda	Kabingo	01 42 S 29 50 E	*P. vulgaris*	St,LD	NI
831	21/11/91	Rwanda	Nyamugari	02 24 S 29 00 E	*P. vulgaris*	MMos	Poty
832	21/11/91	Rwanda	Nyamugari	02 24 S 29 00 E	*P. vulgaris*	MMos	Poty
833	21/11/91	Rwanda	Shyorongi	01 52 S 29 29 E	*P. vulgaris*	CVB	Poty
834	21/11/91	Rwanda	Shyorongi	01 52 S 29 29 E	*P. vulgaris*	LD	NI
837	21/11/91	Rwanda	Musambira	02 03 S 29 51 E	*Cassia sophera*	Mos	Poty
839	22/11/91	Burundi	Kayanza	02 55 S 29 37 E	*P. vulgaris*	SMos	BCMV A
840	22/11/91	Burundi	Kayanza	02 55 S 29 37 E	*P. vulgaris*	Mos	Poty
841	22/11/91	Burundi	Banga	03 08 S 29 38 E	*P. vulgaris*	SMos	Poty
843	22/11/91	Burundi	Mageyo	03 19 S 29 29 E	*P. vulgaris*	Mos?	Poty
844	22/11/91	Burundi	Mageyo	03 19 S 29 29 E	*P. vulgaris*	Mos?	BCMV A
845	22/11/91	Burundi	Mageyo	03 19 S 29 29 E	*P. vulgaris*	Mos	BCMV A
846	22/11/91	Burundi	Mageyo	03 19 S 29 29 E	*P. vulgaris*	Mos	Poty
848	22/11/91	Burundi	Ruzibe	03 21 S 29 17 E	*Desmodium* sp.	Mot	BCMV A
849	22/11/91	Burundi	Ruzibe	03 21 S 29 17 E	*Crotalaria* sp.	Mos?	NI
850	22/11/91	Burundi	Ruzibe	03 21 S 29 17 E	Legume weed	MMot	NI
851	22/11/91	Burundi	Ruzibe	03 21 S 29 17 E	*N. whiteii*	SMos	Poty
855	23/11/91	Burundi	Giheta	03 22 S 29 52 E	*P. vulgaris*	MMot	Poty
856	23/11/91	Burundi	Nr. Giheta	03 22 S 29 52 E	*P. vulgaris*	MMos	NI
857	23/11/91	Burundi	Nr. Gitega	03 20 S 29 55 E	*P. vulgaris*	MMos	NI
858	23/11/91	Burundi	Nr. Gitega	03 20 S 29 55 E	*P. vulgaris*	MMos	Poty?
859	23/11/91	Burundi	Nr. Gitega	03 20 S 29 55 E	*P. vulgaris*	LD	NI
860	23/11/91	Burundi	Nr. Gitega	03 20 S 29 55 E	*P. vulgaris*	MMos	NI
861	23/11/91	Burundi	Gitega	03 20 S 29 55 E	*C. agetiflora*	MMot	NI
862	23/11/91	Burundi	Nr. Gitega	03 20 S 29 55 E	*P. vulgaris*	MMot	Poty?
863	23/11/91	Burundi	Nr. Gitega	03 20 S 29 55 E	*P. vulgaris*	MMot	NI

864	23/11/91	Burundi	Gishora	03 22 S	29 55 E	*P. vulgaris*	MMos	NI
865	23/11/91	Burundi	Nr. Gitega	03 20 S	29 55 E	*P. vulgaris*	LD	NI
866	23/11/91	Burundi	Giheta	03 22 S	29 52 E	*P. vulgaris*	MMos	NI
867	24/11/91	Burundi	3 km Bujumbura	03 23 S	29 22 E	*P. vulgaris*	Mos	BCMV A
868	24/11/91	Burundi	3 km Bujumbura	03 23 S	29 22 E	*E. psorateoides*	Mot	BCMV A
870	24/11/91	Burundi	Nyamiyaga	03 55 S	29 47 E	*P. vulgaris*	Mos	Poty
871	24/11/91	Burundi	Nyamiyaga	03 55 S	29 47 E	Legume weed	SMos	BCMV A
882	25/11/91	Burundi	Bujumbura	03 23 S	29 22 E	*P. vulgaris*	Mos	Poty
883	25/11/91	Burundi	Bujumbura	03 23 S	29 22 E	*P. vulgaris*	Mos	BCMV A
885	25/11/91	Burundi	Bujumbura	03 23 S	29 22 E	*P. vulgaris*	MMos	Poty
886	25/11/91	Burundi	Bujumbura	03 23 S	29 22 E	*Desmodium* sp.	Chl	BCMV A
888	25/11/91	Burundi	Bujumbura	03 23 S	29 22 E	*P. vulgaris*	Mos	Poty
889	25/11/91	Burundi	Bujumbura	03 23 S	29 22 E	*P. vulgaris*	Mot	NI
890	25/11/91	Burundi	Bujumbura	03 23 S	29 22 E	*P. vulgaris*	Mos	Poty
891	28/11/91	Kenya	Thabu	00 49 S	37 18 E	*P. vulgaris*	Mos	NI
892	28/11/91	Kenya	Gitan	00 49 S	37 18 E	*P. vulgaris*	Mos	NI
893	28/11/91	Kenya	Kinoo	01 02 S	36 52 E	*Cassia* sp.	MMos	NI
894	28/11/91	Kenya	Kinoo	01 02 S	36 52 E	*P. vulgaris*	Mos	NI
895	28/11/91	Kenya	Kinoo	01 02 S	36 52 E	*P. vulgaris*	Mos	BCMV A
898	28/11/91	Kenya	Thika Res. St.	01 03 S	37 05 E	*P. vulgaris*	LD	NI
899	28/11/91	Kenya	Thika Res. St.	01 03 S	37 05 E	*P. vulgaris*	Mot	NI
900	28/11/91	Kenya	Thika Res. St.	01 03 S	37 05 E	*P. vulgaris*	St,LD	NI
901	28/11/91	Kenya	Thika Res. St.	01 03 S	37 05 E	*P. vulgaris*	St,LD	NI
902	28/11/91	Kenya	Thika Res. St.	01 03 S	37 05 E	*P. vulgaris*	St,LD	NI
903	29/11/91	Kenya	Katumani Res. St.	01 35 S	37 15 E	*P. acutifolius*	Mos	NI
904	29/11/91	Kenya	3 km Katumani	01 35 S	37 15 E	*Cajanus cajan*	Chl	NI
905	29/11/91	Kenya	3 km Katumani	01 35 S	37 15 E	*Cajanus cajan*	Chl	BCMV A
906	29/11/91	Kenya	3 km Katumani	01 35 S	37 15 E	*P. vulgaris*	SMos	NI
907	29/11/91	Kenya	3 km Katumani	01 35 S	37 15 E	*P. vulgaris*	SMos	NI
908	29/11/91	Kenya	3 km Katumani	01 35 S	37 15 E	*P. vulgaris*	SMos	NI
909	29/11/91	Kenya	Prof Mbithi's	01 35 S	37 15 E	*Cajanus cajan*	Chl	BCMV A
910	29/11/91	Kenya	Kavumbo	01 35 S	37 45 E	*P. vulgaris*	SMos	NI
911	30/11/91	Kenya	Mutomo Dist.	01 45 S	38 04 E	*Lacuna* sp.	Mot	NI
912	30/11/91	Kenya	4 km Kitui	01 30 S	38 30 E	*P. vulgaris*	LD	NI
913	30/11/91	Kenya	4 km Kitui	01 30 S	38 30 E	*P. vulgaris*	LD	NI
914	30/11/91	Kenya	4 km Kitui	01 30 S	38 30 E	*P. vulgaris*	LD	NI
915	30/11/91	Kenya	4 km Kitui	01 30 S	38 30 E	*P. vulgaris*	LD	NI

Table A14 (contd.)

Isolate number	Date of collection	Country of collection	Place of collection	Grid reference	Host species	Symptom	Virus detected by ELISA
916	01/12/91	Kenya	East of Meru	00 00 37 50 E	*P. vulgaris*	Mos,LD	NI
917	01/12/91	Kenya	East of Meru	00 00 37 50 E	*P. vulgaris*	Mos,LD	Poty
918	01/12/91	Kenya	East of Meru	00 00 37 50 E	*Cajanus cajan*	Chl	BCMV A
919	01/12/91	Kenya	East of Meru	00 00 37 50 E	*Cajanus cajan*	Chl	BCMV A
920	02/12/91	Kenya	Embu	00 35 S 37 40 E	*P. vulgaris*	LD	NI
921	02/12/91	Kenya	Embu	00 35 S 37 40 E	*P. vulgaris*	LD	NI
922	02/12/91	Kenya	Embu	00 35 S 37 40 E	*P. vulgaris*	LD	NI
923	02/12/91	Kenya	Embu	00 35 S 37 40 E	*P. vulgaris*	Chl	NI
924	02/12/91	Kenya	10 km Embu	00 30 S 37 45 E	*P. vulgaris*	Mot	NI
925	02/12/91	Kenya	10 km Embu	00 30 S 37 45 E	*P. vulgaris*	Mot	NI
926	02/12/91	Kenya	10 km Embu	00 30 S 37 45 E	*P. vulgaris*	MMot	NI
927	02/12/91	Kenya	10 km Embu	00 30 S 37 45 E	*P. vulgaris*	Mot,LD	NI
928	02/12/91	Kenya	20 km Embu	00 25 S 37 45 E	*P. vulgaris*	LD	BCMV A
929	02/12/91	Kenya	20 km Embu	00 25 S 37 45 E	*P. vulgaris*	LD	NI
930	02/12/91	Kenya	28 km Embu	00 25 S 37 45 E	*P. vulgaris*	LD	Poty
931	02/12/91	Kenya	49 km Embu	00 20 S 37 45 E	*P. vulgaris*	LD	NI
932	02/12/91	Kenya	49 km Embu	00 20 S 37 45 E	*P. vulgaris*	LD	BCMV A
933	02/12/91	Kenya	49 km Embu	00 20 S 37 45 E	*Indigofera* sp.	Mot	NI
934	02/12/91	Kenya	49 km Embu	00 20 S 37 45 E	*Indigofera* sp.	Mot	NI
935	02/12/91	Kenya	Tharaka	00 19 S 38 04 E	*P. vulgaris*	LD	BCMV A
936	02/12/91	Kenya	Tharaka	00 19 S 38 04 E	*P. vulgaris*	Mos	NI
937	02/12/91	Kenya	Tharaka	00 19 S 38 04 E	*P. vulgaris*	Chl	Poty
938	02/12/91	Kenya	85 km Embu	00 10 S 38 00 E	*P. vulgaris*	LD	NI
939	02/12/91	Kenya	85 km Embu	00 10 S 38 00 E	*P. vulgaris*	LD	NI
940	02/12/91	Kenya	85 km Embu	00 10 S 38 00 E	*P. vulgaris*	Mos	NI
941	02/12/91	Kenya	Ntima	00 02 S 37 38 E	*P. vulgaris*	MMos	NI
942	02/12/91	Kenya	5 km Meru	00 02 S 34 38 E	*Cassia* sp.	SMot	NI
943	02/12/91	Kenya	Ntima	00 02 S 37 38 E	*Indigofera* sp.	SMot	NI
944	02/12/91	Kenya	Ntima	00 02 S 37 38 E	*P. vulgaris*	Mot,LD	NI
945	02/12/91	Kenya	14 km Meru	00 09 S 37 02 E	*Cassia* sp.	Mot	PnMoV*
948	03/12/91	Kenya	5 km Nyeri	00 25 S 36 57 E	*P. vulgaris*	LD	NI
949	03/12/91	Kenya	5 km Nyeri	00 25 S 36 57 E	*P. vulgaris*	Mot	NI

954	04/12/91	Kenya	2 km Nyangusu	00 56 S	34 51 E	*P. vulgaris*	Mos	Poty
957	04/12/91	Kenya	Kisii	00 41 S	34 46 E	Cowpea	SMos	Poty
958	04/12/91	Kenya	Kisii	00 41 S	34 46 E	Cowpea	SMos	Poty
960	04/12/91	Kenya	Kisii Cent.	00 45 S	34 50 E	*P. vulgaris*	SMos	Poty
962	04/12/91	Kenya	Nyangina	00 55 S	34 25 E	*P. vulgaris*	SMos	Poty
965	05/12/91	Kenya	Kitutu	00 38 S	34 50 E	*P. vulgaris*	SMos	Poty
966	05/12/91	Kenya	Kitutu	00 38 S	34 50 E	*P. vulgaris*	SMos	Poty
968	05/12/91	Kenya	Kendu Bay	00 21 S	34 38 E	Cowpea	Mos	Poty
969	05/12/91	Kenya	Kakamega	00 15 N	34 45 E	*P. vulgaris*	SMos	Poty
971	06/12/91	Kenya	Kakamega	00 15 N	34 45 E	*P. vulgaris*	SMos	Poty
972	06/12/91	Kenya	Kakamega	00 15 N	34 45 E	*P. vulgaris*	SMos	Poty
974	06/12/91	Kenya	Malikisi	00 46 N	34 28 E	*P. vulgaris*	SMos	Poty
975	06/12/91	Kenya	Maliki	00 46 N	34 28 E	*P. vulgaris*	SMos	Poty
1001	17/02/92	Zambia	Msandile Ag. St.	13 29 S	32 43 E	*P. vulgaris*	?	Poty
1002	17/02/92	Zambia	Chalumbe	Unknown		*P. vulgaris*	?	Poty
1005	17/02/92	Zambia	Jerusalemu	Unknown		*P. vulgaris*	?	Poty
1006	17/02/92	Zambia	Jerusalemu	Unknown		*P. vulgaris*	?	NI
1009	17/02/92	Zambia	Selemani vill.	08 28 S	29 52 E	*P. vulgaris*	?	NI
1010	17/02/92	Zambia	Kalichero	Unknown		*P. vulgaris*	?	NI
1012	17/02/92	Zambia	Mugabe village	Unknown		*P. vulgaris*	?	NI
1013	17/02/92	Zambia	Mteleza village	Unknown		*P. vulgaris*	?	NI
1015	09/04/92	Swaziland	Mangcongco	26 34 S	30 53 E	*P. vulgaris*	Mos?	BCMV A
1016	09/04/92	Swaziland	Mangcongco	26 34 S	30 53 E	*P. vulgaris*	Mos?	NI
1017	09/04/92	Swaziland	Luyengo	Unknown		*P. vulgaris*	Mos?	NI
1018	09/04/92	Swaziland	Big Bend	26 49 S	31 56 E	*P. vulgaris*	Mos?	NI
1029	15/04/92	Kenya	Nairobi	01 17 S	36 49 E	*N. wightii*	Mos	NI
1030	15/04/92	Kenya	Nairobi	01 17 S	36 49 E	*N. wightii*	Mos	NI
1031	30/04/92	Tanzania	Arusha	03 22 S	36 41 E	*L. purpureus*	Mos?	NI
1032	22/05/92	Tanzania	Arusha	03 22 S	36 41 E	*P. vulgaris*	Mos?	Poty
1033	18/06/92	Tanzania	Nkundi	07 51 S	31 26 E	*N. wightii*	Mos?	NI

See Appendix 2, p. 114 for abbreviations used above. C. = *Cassia* (*occidentalis, didymobotrya*) or *Crotalaria* (*agetiflora, incana, laburnifolia, lachnophora, ochroleuca*); E. = *Eriosema*; L. = *Lablab*; P. = *Phaseolus*; T. = *Tephrosia*

Table A15 ELISA solutions

Sodium phosphate-buffered saline (PBS)	
NaCl	8.0 g
Na_2HPO_4. 12 H_2O	2.9g
KH_2PO_4	0.2 g
KCl	0.2 g
NaN_3	0.2 g
Distilled water	1 l
Sodium phosphate-buffered saline 'Tween (PBS-T)	
PBS	1 l
'Tween 20	5.0 ml
Grinding buffer for DAS-ELISA samples	
PBS-T	100 ml
Polyvinylpyrollidone (PVP)	2.0 g
Coating buffer (also grinding buffer for PTA-ELISA samples)	
Na_2CO_3	0.16 g
$NaHCO_3$	0.32 g
Distilled water	100 ml
Diethanolanime substrate buffer	
$[CH_2(OH).CH_2]_2NH$ (diethanolamine)	9.7 ml
Distilled water	80 ml
Adjusted to pH 9.8 with 1 M HCl	
Distilled water	Made up to 100 ml

Table A16 Western blotting solutions

Transfer buffer	
Tris	3.03 g
Glycine	14.4 g
Methanol	200 ml (20% w/v)
Distilled water	Made up to 1 litre
Tris-buffered saline 'Tween (TBS-T)	
Tris	6.0 g
NaCl	8.0 g
Adjusted to pH 7.4 with 1 M HCl	
Distilled water	Made up to 1 l
'Tween 20	1.0 ml
Substrate buffer	
Tris	12.1 g
NaCl	5.84 g
$MgCl_2$	1.0 g
Adjusted to pH 9.5 with 1 M HCl	
Distilled water	Made up to 1 l
NBT stock solution	
p-nitro blue tetrazolium chloride	1 tablet (Sigma)
Distilled water	1.0 ml
BCIP stock solution	
5-bromo-4-chloro-3-indolyl phosphate	1 tablet (Sigma)
100% Dimethyl formamide	0.5 ml

Table A17 Electrophoresis solutions

Resolving gel (12.5%)	
Protogel™ (30% acrylamide, 0.8% bisacrylamide)	9.37 ml
3 M Tris-HCl pH 8.8	2.8 ml
10% $C_{12}H_{25}O_4SNa$ (SDS, sodium dodecylsulphate)	225 l
Distilled water	8.96 ml
After degassing for 10 minutes:	
1.5% $(NH_4)_2S_2O_8$ (APS, ammonium persulphate)	1.13 ml
N,N,N'N'-Tetramethylethylenediamine (TEMED)	11 l
Stacking gel (4.5%)	
Protogel™ (30% acrylamide, 0.8% bisacrylamide)	1.25 ml
0.5 M Tris-HCl pH 6.8	2.5 ml
10% SDS	100 l
Distilled water	5.65 ml
After degassing for 10 minutes:	
1.5% APS	0.5 ml
TEMED	7.5 l
SDS sample buffer	
10% SDS	3.2 ml
C_2H_6OS (2-Mercapto-Ethanol)	0.8 ml
50% Glycerol	0.4 ml
0.1% Bromophenol blue	0.4 ml
Distilled water	4.4 ml
Electrode buffer pH 8.3	
$C_4H_{11}NO_3$ (Tris hydroxymethylaminomethane)	1.51 g
Glycine	7.2 g
SDS	0.5 g
Distilled water	Made up to 500 ml

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