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AN ASSESSMENT OF THE RESISTANCE OF VARIOUS BEAN CULTIVARS TO AN ISOLATE OF BEAN COMMON MOSAIC VIRUS FROM THE TRANSVAAL AND NATAL

Brian Ross Edington

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree of Master of Science.

Johannesburg, 1988.

ABSTRACT

One of the economically important diseases of beans in South Africa is caused by bean common mosaic virus. The virus occurs as a number of isolates world-wide and breeding for resistance is aided by the identification of the prevalent strain.

The isolate prevalent in South Africa was found to belong to virus pathogenicity group V. It was identified by inoculating a set of bean differential hosts recommended by Drijfhout (1978). Samples collected from the Transvaal and parts of Natal did not differ significantly in symptom development in response to the tests.

The South African isolate causes a temperature-dependant systemic necrosis in plants with the I-gene and without the recessive gene resistance. It was still capable of inducing necrosis in "Nep 2", host resistance group 8, 60 hours post inoculation and 84 hours for "Peru 0257", host resistance group 9, when it transferred from a cold (20 °C day) to a warm glasshouse (30°C). An hour at 30°C was sufficient to induce necrosis in "Nep 2".

The results of a speckled sugar bean breeding programme are included and further suggestions are made for breeding for resistance and the elimination of bean common mosaic virus as a problem in South Africa.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

B.R. Edington

30th day of March, 1988.

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DEFINITIONS

The following definitions are used in the thesis unless further qualified in the text.

Sensitive	An infected plant producing clearly visible symptoms, either local or systemic
Tolerant	An infected plant producing only mild or no clearly visible symptoms, either local or systemic
Susceptible	A plant in which the virus causes a systemic infection
Resistant	A plant in which the virus from does not cause a systemic infection
Cultivar	Preferred term used for subdivision of host genotype, written within inverted commas. A list of cultivars mentioned in the thesis is given in Appendix 2.

CONTENTS

		Page
ABSTRACT	.....	ii
DECLARATION	.....	iii
ACKNOWLEDGEMENTS	.....	iv
DEFINITIONS	.....	iv
LIST OF TABLES	.....	vii
LIST OF FIGURES	.....	viii
1. INTRODUCTION	.....	1
2. MATERIALS AND METHODS	.....	12
2.1 Collection of virus samples	.....	12
2.2 Identification of the isolate present	.....	15
2.3 The nature of the I-gene resistance	.....	18
2.4 The inheritance of the reaction of a number of cultivars to inoculation with BCMV	.....	18
2.5 Breeding for BCMV resistance in speckled sugar beans	.....	19
3. RESULTS	.....	21
3.1 Contamination tests	.....	21
3.2 Symptom development	.....	23
3.3 Differential indicator host studies	.....	31
3.4 Isolate identity	.....	34
3.5 The nature of I-gene resistance	.....	41

3.6 Test crosses 43
3.7 Breeding for BCMV resistance in speckled sugar beans 44

4. DISCUSSION 45

5. SUMMARY 55

APPENDIX 1 :
Dry bean production and consumption in South Africa from 1.1.85 to 31.12.85. 56

APPENDIX 2 :
A list of cultivars referred to in this thesis .
..... 57

APPENDIX 3 :
Results of the "Bonus" backcross check row trials planted at Bethlehem and Delmas 1986/87 59

REFERENCES 60

LIST OF TABLES

		Page
1.	History of BCMV-infected plants used for isolate identification	13
2.	The genotypes of the differential bean hosts used for isolate identification	15
3.	Results of the BCMV samples tested in differential host plants	33
4.	Results of tests for presence of mixture of isolates of BCMV	36
5.	Reactions of the BCMV host resistance groups to infection by known isolates	37
6.	Latent period at 20 °C after which the ability to induce "black root" is lost on transfer to 30 °C. Peru 0257	39
7.	Latent period at 20 °C after which the ability to induce "black root" is lost on transfer to 30 °C. Nep 2	40
8.	Thermo-period at high temperature that will induce "black root" in "Nep 2"	42
9.	Results of isolate tests in South Africa	46

LIST OF FIGURES

		Page
1.	Field symptoms of BCMV	6
2.	Symptoms of "black root" at the infection site	6
3.	Sampling locations in the Transvaal and Natal	14
4.	Micrograph of flexuous virus particles found in a leaf dip preparation from Sanilac plants with typical BCMV symptoms	22
5.	Sanilac: 10 - 14 days post inoculation	25
6.	Diacol Calima: Four weeks post inoculation	26
7.	Diacol Calima: Three weeks post inoculation	26
8.	Sanilac: Necrosis in an older trifoliate leaf	27
9.	Pinto 114: Superficial discolourations associated with the veins	27
10.	Sanilac. Stem necrosis	28
11.	Sanilac. Section through a petiole viewed with Interference contrast	28
12.	Sanilac. Severe leaf symptoms	29

13. Senilac. Severe leaf symptoms 29

14. Peru 0257. Local symptoms six days post inoculation 30

15. Four rows of PC 97 (Nep 2 x (Seafarer x Coffee Bean)), indicated by "P", and one row of Nep 2, indicated by "N" 49

16. A suggested crossing and testing programme for selecting a "speckled sugar-type" line with the $bc1^2$ and I-genes 52

1. INTRODUCTION

Beans are grown world-wide in temperate to tropical regions. In 1984 the production in developed and developing countries was 2,113,000 and 13,356,000 tonnes of dry beans (all types), respectively, while the figures for green beans were 1,534,000 and 1,356,000 tonnes, respectively (Food and Agricultural Organization Yearbook 1985). In 1985 a yield of 58,298 tonnes of common dry beans (Phaseolus vulgaris), 9,901 tonnes of large white kidney beans (Phaseolus coccineus) and 496 tonnes of tepary beans (Phaseolus acutifolius) was produced in South Africa (Dry Bean Board Report, 1986).

Of all the dry bean types, more than 50% of the production in South Africa comes from the Eastern Transvaal Highveld, especially in the Delmas area, and 70% of the total crop in 1985 was produced in the Transvaal. The Orange Free State produced 15.5 % of the National crop in the same period, mainly in the eastern regions. The Cape Province produced 1.9 % of the total in 1985, which varies little from year to year, whilst the production in Natal is increasing, from 0.3 % in 1982 to 4.5 % of the country's total production in 1985. Beans are a convenient summer crop to follow wheat under irrigation in the foothills of the Drakensberg (A.J. Liebenberg, pers. comm.).

Yields over the last five years have been reduced by persistant droughts, in 1983 the total production was only 26,879 tonnes and in 1984, 47,332 tonnes. In spite of a reduction in the area planted to beans of 13 % to 53,500 hectares, due to a lack of moisture during planting time, greater rainfall later in the season led

to an increase in production of 45 % in 1985 compared to 1984. Production in South Africa usually oscillates about 63,000 tonnes, which is approximately the annual consumption. Consumption during 1985 was 60,435 tonnes. The greater production of 76,521 tonnes in 1986 led to lower average prices and hence to a greater consumption of 72,000 tonnes. There is some trading; in 1986 of the 10,581 tonnes exported, 5,126 tonnes were destined for overseas markets, and 739 tonnes were imported.

The large white kidney beans were worst affected by the drought as they are more sensitive to high temperatures and low relative humidity (Lloyd and Liebenberg, 1986), most probably as they are cross-pollinated. Production dropped from 46.8 % (35,761 tonnes) of the total in 1981 (a favourable year) to 14 % (3,757 tonnes) in 1983 and 13.8 % (6,508 tonnes) in 1984, picking up slightly in 1985 to 14.4 % (9,901 tonnes). The small white canning beans (20.2 % of the total in 1985) are mostly used in the canning industry. The other types are usually sold loose in the packaging trade while most of the large white kidney beans go to the mines to be used in rations for the labour force. Speckled sugar beans have been grown on an increasing scale from 45.4 % (16,146 tonnes) of the total in 1980 to 48 % (22,770 tonnes) in 1984 and 45.8 % (31,716 tonnes) in 1985. The actual production area under speckled sugar beans will be greater as they yield about one tonne per hectare and the small white canning beans 200-300 kilogrammes more. In Natal there has been an increase in the plantings of "Broadacres", a green speckled or pinto bean. The areas planted under the various types depend largely on consumer preference, which, with availability, determines the prices of the different types. In 1985 the average price for small white

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canning beans was R765.11 per tonne and speckled sugar beans were R896.10 per tonne. In 1986 the average prices for large white kidney, small white canning, red speckled sugar and brown haricot beans were R685, R655, R927, R613 per tonne respectively. The production figures for 1985 are given in Appendix I and are broken down on a regional and bean type basis.

Bean production in South Africa is hindered by a number of economically important diseases such as bacterial blights, rust, root rots, white mold, anthracnose and a number of viral diseases. One of the most economically important viral diseases in South Africa is bean common mosaic virus (BCMV) as it is readily transmitted by aphids and is also seedborne (Anon, 1987). BCMV was reported in Kenya as early as 1936 (Kulkarni, 1973) and Doidge et al (1953) stated that mosaic was common where beans were grown in South Africa. Klesser (1961) found a number of viruses affecting beans and identified BCMV on the basis of symptom expression and seed infection. Cowper (1983) positively identified BCMV on the basis of serology and made an attempt at strain identification from one locality.

Hampton (1975) found a yield loss of 52.8 % and 70 % from plants with moderate and severe symptoms, respectively, compared to healthy plants in a naturally infected field of "Red Mexican 34". There was a reduction of 21.8 % in the number of seeds per pod for plants with severe symptoms and 9.2 % in seed size for plants with moderate symptoms. The majority of the yield loss was due to pod abscission. There were some plants with no pod set at all. An actual increase in seed size of 11 % was noted for plants with severe symptoms, indicating there was no shortage of photosynthate for the remaining seed (Hampton, 1975). Lockhart

and Fischer (1974) gave grower's estimates of yield loss at about 50 % in Morocco and Omar et al reported a yield loss of 24 % in Egypt (Lopez, 1983).

A mosaic disease of beans was first reported in Russia in 1894 by Iwanowski (Schwartz and Galvez, 1980), and Stewart and Reddick (1919) showed it to be a viral disease. BCMV is a member of the potyvirus group, closely related to bean yellow mosaic virus, but has a very limited natural host range (Bos, 1971). Natural infections have been found in runner beans (P. coccineus) and Rhynchosia minima, a leguminous weed of bean fields in South America (Ainsworth, 1940; Meiners et al, 1978). All coloured dry bean cultivars presently grown in South Africa are susceptible with the exception of "Nuweveld", a brown haricot type. All of the small white canning and major green bean cultivars grown in South Africa are resistant (Internal Report G.C.R.I., 1987).

The virus is aphid transmitted in a non-persistent manner (Bos, 1971). It can be pollen transmitted (Medina and Grogan, 1961; Schwartz and Galvez, 1980), but between-plant transmission without a vector is very poor (Reddick and Stewart, 1919; Fajardo, 1930). The virus is seed transmitted in a number of Phaseolus species, as well as Macroptilium lathyroides, Rhynchosia minima and Vigna radiata (Schwartz and Galvez, 1980; Meiners et al, 1978; Kaiser and Mossahebi, 1974). Drijfhout and Bos (1977) found up to 80 % and Medina and Grogan (1961) up to 86 %, seed transmission in common bean cultivars. The percentage of seed transmission depends on the time of infection, there being no transmission if infection occurs at or after flowering (Fajardo, 1930; Bos, 1971). Germination and seed viability is unaffected by infection and the virus

is found within the embryo (Fajardo, 1930; Bos, 1971). Fajardo (1930) states that since the disease is seed transmitted, it is probable that it occurs wherever beans are grown, and that the spread from one locality to another may be chiefly through commercial transport of bean seed.

The virus can cause one of three of field reactions depending on isolate type and host genotype. Expression of symptoms is strongly dependant on environmental conditions, especially temperature. The reactions of sensitive and susceptible cultivars may vary from stunting and mild leaf epinasty, to more distinct leaf symptoms of mottling to "green islands" and certain forms of rugosity, severe epinasty, lamina size reduction and elongation of the apex. The plant may initially exhibit clear symptoms but young leaves developing later display poor or no symptoms, especially under warmer conditions (> 28 °C). The second type of reaction is tolerance, where no clearly visible symptoms may be expressed by non-sensitive cultivars. The third type of reaction is "black root", a systemic hypersensitivity characterised by the death of the whole plant (Figures 1 and 2).

Resistance to BCMV was found by Corbett in a single plant amongst a stand of "Refugee" green beans (Pierce 1935). After extensive use of "Corbett Refugee" as a resistance source, systemic necrosis was noted by Jenkins (1939) and he reported it a separate virus disease on the basis of symptomology (Jenkins, 1940). Grogan and Walker (1948) showed, however, that it was caused by BCMV in plants with the "Corbett Refugee" resistance by approach grafting. In addition, Zaumeyer and Thomas (1948), working with a variant isolate of the type strain (shiny or greasy pod strain), also found necro-

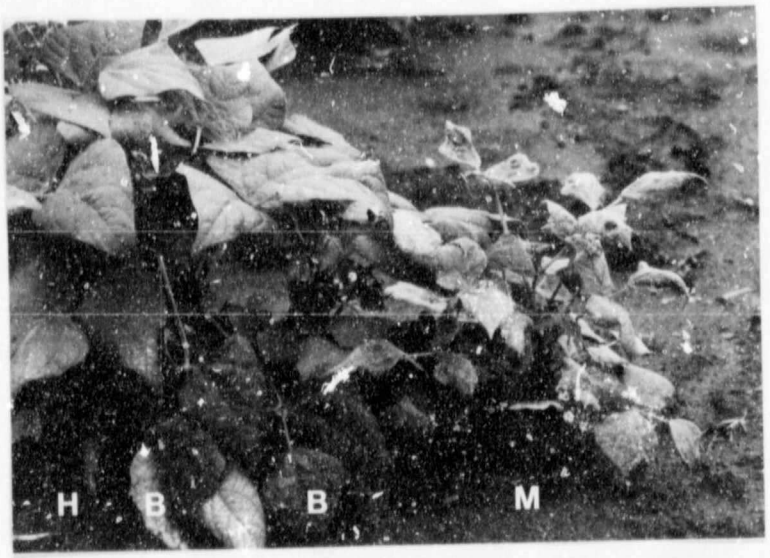


Figure 1. Field symptoms of BCMV
Plants with healthy (H), "black root" (B), "black root" (B) and mottle (M) symptoms, respectively. Note the older leaves of the "black root" plants appear healthy and also the rugosity, epinasty and stunting of the plant displaying mottle symptoms.



Figure 2. Symptoms of "black root" at the infection site. Spreading vein necrosis from the infection point.

sis in beans with the "Corbett Refugee" resistance on inoculation with BCMV. The local symptoms of "black root" are often unclear or cryptic but may be expressed as a veinal necrosis (Figure 2). Once the virus is systemic, causing necrosis in the vascular tissues of the roots as well as the stems, the growth tip dies followed by the death of the whole plant. There is no field transmission, vertical or horizontal, of the virus from plants containing the "Corbett Refugee" resistance gene although Hubbeling isolated BCMV from the pods of such plants (Drijfhout, 1978). In addition, Jenkins (1940) reported 10 % seed transmission from 25 seeds, but the plants were kept in the open and the symptoms only developed seven weeks after sowing. There are no further reports of seed transmission of "black root" in the literature.

Pierce (1935) reported two types of resistance, a dominant form from "Corbett Refugee" and another from "Robust", both of which are inherited independantly and may be influenced by the cytoplasm. Parker (1936) suggested that the "Corbett Refugee" resistance was controlled by a single dominant gene but the resistance of "Robust" is derived from it's cytoplasmic constitution. Both Pierce and Parker found differences in reciprocal crosses. Wade and Andrus (1941) stated that the resistance derived from "Corbett Refugee" was unaffected by any cytoplasmic influence. Ali (1950) also found no differences in reciprocal crosses and suggested that two genes conditioned the plant's reaction to BCMV. One gene was given the symbols A & a, with the recessive allele (a) conferring resistance, and the second was given the symbols I & i, with the dominant allele (I) conferring resistance. In the presence of the A-allele, the I-allele will condition necrosis if the plant is inoculated and kept at high temperatures

or if approach-grafted to an infected plant. Anderson and Down (1954) found resistance to a strain of BCMV capable of overcoming the a-gene of Ali in "Great Northern U.I. 31" and that this was also governed by a single recessive gene. Rudorf (1958) found that resistance in "Bo 19" in a cross with "Saxa" was governed by the epistatic action of two recessive genes but also found independent segregation of a single recessive gene and the I-gene in a cross between "Topcrop" and "Bo 19".

Genetic variation within BCMV has also been found. Zaumeyer and Thomas (1948) described an isolate that differed from the type isolate by inducing pod symptoms, hence shiny pod strain, but as with the type isolate it can only infect cultivars without resistance genes. With the extensive use of recessive genes in breeding programmes, strains have occurred which are able to overcome them. Richards and Burkholder (1943) reported a strain (New York 15) able to overcome the resistance of "Robust" occurring in New York State in 1939 and Dean and Hungerford (1946) reported it's occurrence in Idaho in 1943. Hubbeling (1957) indicated the presence of a strain similar to the NY 15 strain in Holland in a list of cultivar reactions to the prevailing BCMV strain, and later isolated such a strain (Drijfhout, 1978). Other isolates that can overcome the recessive resistance genes but more importantly, give a systemic necrosis in plants with the I-gene at low temperatures have also been identified in Europe (Drijfhout and Bos, 1977; Drijfhout, 1978; Walkey and Innes, 1979). A number of strains have been identified in North America since the NY 15 strain (Dean and Wilson, 1959; Skotland and Burke, 1961; Zaumeyer and Goth, 1964; Silbernagel, 1969) but only recently have isolates been found which also give systemic necrosis

in plants with the I-gene at low temperatures (Kelly et al, 1983; Provvidenti et al, 1983; Tu, 1986). Silbernagel et al (1983 & 1986) reported an isolate in beans from Tanzania which also gave a low temperature necrosis, this isolate being very similar in pathogenicity spectrum to that of Kelly et al (1983).

The numerous reports of isolate identification have differed greatly in the cultivars and environmental conditions (if given) employed. For effective world wide communication on the subject, a standard procedure for identification was necessary, hence the work of Alconero and Meiners (1974) and the definitive work of Drijfhout (1978) and Drijfhout et al (1978). As with other pathogens, differentiation of strains can be elucidated by inoculation of a range of cultivars with different resistance genes grouped into separate host resistance groups. Isolates that infect the same host resistance groups give identical or very similar symptoms and are grouped together in the same pathogenicity group.

In addition to the I-gene there are a number of recessive genes which were given symbols by Drijfhout (1978). There is a strain unspecific gene (bc-u) without which the other recessive genes do not express themselves. There are two genes, each consisting of two alleles which confer resistance to specific strains (bc 1 & bc 1^2; bc 2 & bc 2^2). A third gene bc 3 confers resistance to all presently known strains, unfortunately attempts to obtain cultivars containing this gene have been unsuccessfull to date.

Plant breeders in North America have made extensive use of the I-gene as until recently the threat of loss from low-temperature necrosis-inducing strains has not exis-

ted there as such strains were not prevalent. In Europe, where there is a prevalence of such strains, breeders have used some of the recessive genes. In South Africa the I-gene has been used and this has led t· problems as the prevailing strain(s) will cause a temperature dependant necrosis at higher temperatures.

Due to the absence of vertical or horizontal transmission of BCMV, the sole use of I-gene containing cultivars could lead to the elimination of BCMV. In South Africa, however, presently of all the dry bean cultivars only the small white canning beans and "Nuweveld" (about one third of the total production) have resistance to BCMV. There is also a disease-free certification scheme operating in South Africa but there is no zero tolerance demand for BCMV. In addition, not all cultivars are produced under the seed certification scheme and many farmers still retain some of their crop for replanting. If their previous crop was infected with a seed-borne disease such as BCMV then they will carry over the disease into the next season, thus acting as an inoculum source for neighbouring farms as well. This effect was well demonstrated in 1983 when there was a shortage of certified seed. Seed certification schemes, as well as other agronomic control measures, are also unlikely to have much impact on subsistance farmers.

If it is assumed that there will always be a reservoir of BCMV present in South Africa, then there is always the danger of a total crop failure from "black root" in I-gene containing cultivars. A recessive gene, conferring resistance to the prevailing strain, would prevent such a situation. To prevent the introduction of a foreign strain causing substantial loss, the I-gene should be used in combination with the recessive gene.

The choice of which recessive gene(s) to use requires the prevailing strain(s) to be identified and this forms the subject matter of the work reported here.

2. MATERIALS AND METHODS

2.1 Collection of virus samples

The virus samples were collected from known susceptible cultivars, thereby excluding commercial small white canning and brown haricot beans which have been bred for resistance to BCMV. Plants showing typical BCMV symptoms, namely a mottle and/or mild leaf epinasty, as opposed to a mosaic (the chlorotic areas more sharply defined and angular) were used to inoculate bean stock plants (Diacol Calima) grown at Potchefstroom. Alternatively, seed was collected from plants that had already developed symptoms. Several of the virus samples were not seed transmitted, on the basis of visible symptoms in twenty to several hundreds of seedlings, and thus probably not BCMV. The collection of samples was made with more emphasis on a spatial basis than with time (Table 1 and Figure 3).

There were no reports of BCMV infection at the National Cultivar Trial locations in the Orange Free State (Sandvet, Ficksburg and Bethlehem) for the previous three seasons 1983/84 to 1985/86 (A.J. Liebenberg, pers. comm.). The green beans grown in the George area are mostly under contract to a commercial company, who provide imported "Provider" seed (C. Rappard, pers. comm.). Inoculations of "Provider" have failed to produce symptoms and back-inoculation tests from these plants failed to produce symptoms in susceptible cultivars (Unpublished data). All the other major green bean cultivars have the I-gene (Unpublished data). No incidence of BCMV was noted in December 1985 in the Western Cape where foundation seed is grown for the production of certified seed (W.J. Vermeulen, pers. comm.).

Table 1. History of BCMV-infected plants used for isolate identification.

LOCATION	ABBREVIATION	CULTIVAR	SEASON	COMMENTS
Koster	KOS	BONUS	1983/84	From farmer's field sown
Derby	DER	BONUS	1983/84	with seed from previous
Bapsfontein	BAPS	BONUS	1983/84	year's crop.
Potchefstroom	POT	BONUS	1984/85	From National Cultivar
Sundra	SUN	BONUS	1984/85	Trials. Widespread infec-
Nigel	NIG	BONUS	1984/85	tion. Abundant aphids.
Delmas	DEL	BONUS	1984/85	Seed transmission very low, possibly late infection.
Morgenzon	MOR	BONUS	1985/86	From farmer's field.
Nooitgedacht	NGDT	EXPERIMENTAL SMALL WHITE	1982/83	From experimental station. Near Ermelo
Burgersfort	BUR	BONUS	1982/83	Provided by the U. of the Witwatersrand
Bergville	BERG	BROADACRES	1985/86	Provided by Dr Mellis of Natal University
Winterton	WIN	NATAL SPECKLED SUGAR	1983/84	Collected by Andrew Noble
Weenen	WEEN	NATAL YELLOW SUGAR	1983/84	of Natal University.
Greytown	GREY	NATAL YELLOW SUGAR	1983/84	
Franklin	FRA	NATAL YELLOW SUGAR	1983/84	

Figure 3. Sampling locations in the Transvaal and Natal



2.2 Identification of the isolate present.

The source of the inoculum was plants grown from infected seed. As BCMV is not the only seed transmissible virus in beans, inoculations were made in Chenopodium amaranticolor, small seeded fababeans (Vicia faba minor) and soyabean (Glycine max) to test for multiple infections. BCMV does not cause symptoms in these plants whilst contaminant viruses will. (Hampton et al, 1978). Bean plants from one test with symptoms of ringspotting (incomplete ring of white necrotic tissue up to 4mm. dia.) were subjected to leaf-dip electron-microscopy.

Table 2 : The genotypes of the differential bean hosts used for isolate identification.

Host Resistance Group	Cultivars	Genotype
1	Diacol Calima	Bcu Bc1 Bc2 Bc3 i
2	Redlands Greenleaf C, Bataaf	bcu $bc1_2$ Bc2 Bc3 i
3	Redlands Greenleaf B	bcu $bc1_2$ Bc2 Bc3 i
4	Sanilac	bcu Bc1 bc2 Bc3 i
5	Pinto 114	bcu $bc1_2$ $bc2_2$ Bc3 i
6	Monroe	bcu $bc1_2$ $bc2_2$ Bc3 i
8	Widusa, Nep 2	Bcu Bc1 Bc2 Bc3 I
9	Peru 0257	bcu $bc1_2$ Bc2 Bc3 I
10	Amadia	bcu $bc1_2$ Bc2 Bc3 I

After Drijfhout (1978)

The differential hosts used for isolate identification are listed in Table 2 according to their host resistance groups based on their resistance genotypes. Other members of the same resistance groups were inoculated separately to test for conformity of symptom expression.

All the cultivars of the isolate differential host groups were recently provided by Centro International de Agricultura Tropical with the exception of "Bataaf", "Nep 2" and "The Prince". All experiments were conducted under natural daylength with no supplementary light.

The inoculum source was sown three to four days before the differential cultivars, to be used when the first trifoliate leaf was fully expanded. The seed transmission in the field samples varied from 1 - 13 % in the "Bonus" seed collected from the National Cultivar Trials to about 50% in the "Natal Yellow sugar" seed, therefore seed with an expected low rate of seed transmission was sown at a high density and healthy plants were rogued as they became obvious.

Infected whole plants were blended in a Kenwood Liquidizer with cold 0.01 M dipotassium hydrogen phosphate buffer (pH = 7.0) using a volume to weight ratio of 10:1. Plant material was removed by filtering through a double layer of cotton gauze (c. 1mm. mesh). The filtrate was put into a clean plastic container, the lid of which was provided with a hole. A piece of foam rubber was secured over the hole by cotton gauze and an elastic band. Abrasion was provided by the nature of the cotton gauze.

The plants, usually ten of each type, were inoculated

by wiping the leaves with the gauze-covered foam rubber and were then washed immediately with tap water. The inoculation schedule was as follows :
Beans : Primary leaves; 10-14 days after sowing. Soyabeans : Primary and occasionally first trifoliate leaves; 10-14 days after sowing. Fababeans and C. amaranticolor: Three youngest leaves more than half expanded; 21-28 days after sowing at a height of approximately 10cm.

In the event of a sufficient number of infected plants being available for two inoculations, the test plants were reinoculated three to four days later.

At inoculation, or shortly thereafter, "Diacol Calima", "Nep 2" and "Peru 0257" were sown to be used in a back-inoculation test. After c. two weeks, young uninoculated leaves were taken from the differential plants of host resistance groups 2 to 6, titurated in cold buffer and used to inoculate these plants to check for a systemic infection. Inoculated plants were allowed to grow to at least the flowering stage before roguing if there was no symptom development and no positive back-inoculation results.

The possibility that the BCMV being tested was a mixture of isolates was investigated by inoculating three separate sets of differential host plants, as listed in Table 2, from infected plants of the host resistance groups that gave positive results, namely resistance groups 2,4 & 5.

2.3 The nature of the I-gene resistance.

To examine the nature of the I-gene resistance a number of inoculations were carried out in "Nep 2" and "Peru 0257" plants. One set of each cultivar was inoculated in the cold glasshouse (20 °C day / 20 °C night) and plants were transferred at half daily intervals to the hot glasshouse (30 °C day / 20 °C night). A set of "Nep 2" plants was inoculated and kept in the cold glasshouse for an hour before being transferred to the hot glasshouse; at hourly intervals thereafter plants were transferred back to the cold glasshouse. The first test was designed to determine the period after which the virus no longer induces necrosis and the second test to determine the length of the period in which the temperature must be high enough to induce necrosis.

2.4 The inheritance of the reaction of a number of cultivars to inoculation with BCMV.

"Teebus" is a locally-bred cultivar that has given negative results in previous inoculations. Crosses were made with "Diacol Calima", a member of host resistance group 1 and hence has no resistance genes, to examine the genetics of the resistance.

"Bataaf" is a member of host resistance group 2 and will give good symptoms on infection whereas "Imuna" and "Redlands Greenleaf C", also members of the same resistance group, will not.

Crosses were made between the latter two cultivars and "Bataaf" to investigate the mode of inheritance of the differing reaction.

2.5 Breeding of BCMV resistance in speckled sugar beans

Red speckled sugar beans are the most widely grown type in South Africa but, to date, are still susceptible to BCMV. The breeding of a resistant cultivar, and that of other large-seeded coloured types, has failed in the past due to the very close linkage of the I-gene and an unacceptable seed testa colour gene (Grain Crops Research Institute Report, 1985), the same problem was reported by Temple and Morales (1986). Fortunately, the linkage was broken in the University of Idaho lines "U.I. 50" & "U.I. 51" and these have been used in a backcross programme to introduce the I-gene into "Bonus".

The F_1 of the initial cross "U.I. 50" or "U.I. 51" with "Bonus" was backcrossed with "Bonus". The resultant progeny was tested for the presence of the I-gene by inoculating a detached leaflet. The leaflet was placed on moist filter paper in a plastic tray, sealed with clear plastic and covered with hessian sacking in a glasshouse at a temperature regime of 30 °C day/ 20 °C night. The detached leaf test for the presence of the I-gene was conducted after two backcrosses to "Bonus" and thereafter on two generations of selfs. Seed retained from single plants after the second generation of selfing were tested for homozygosity of the I-gene by planting ten seeds of each single selection. The plants were inoculated and any single plant selection with any plants exhibiting symptoms of mottling were

discarded. The single plant selections were then increased for field trials.

As the new "Speckled Sugar-type" will be similar to "Bonus" on the basis of agronomic characteristics, the pink flower colour of "U.I. 50" & "U.I. 51" was retained. This will help prevent the mixing of the two in the field, which could be potentially disastrous.

The increases were planted out in check row trials in the 1986/87 season at Bethlehem, Delmas and Koster. Ten metre rows of two backcross lines were planted on either side of a 10m row of "Bonus" and this was repeated throughout the trial. The trial spacings were 8cm within rows and 90cm between rows.

3. RESULTS

3.1 Contamination tests

Inoculation of fababean, soybean and C. amaranticolor with the BCMV samples resulted in no visible symptoms in the leaves of these plants. Leaf dip electronmicroscopy revealed no viral particles other than flexuous rods (C. 750 nm length), (figure 4) in inoculated, susceptible bean plants and this was in keeping with the plant's BCMV symptoms. The ringspotting found in one test did not affect the appearance or severity of the BCMV symptoms.

These results are at variance with those of Cowper (1983) who found symptoms in all test plants when working with material taken from a field at Potchefstroom. In the experiments reported here, the inoculum was taken from seedlings raised from infected seed in a glasshouse, thereby reducing the chances of contamination.

Hampton et al (1978) found no symptoms in their inoculations with the type strain and Bos (1971) states that no local lesions, as found by Cowper (1983), are found with C. amaranticolor. Klesser (1961) did, however, find symptoms in large-seeded fababeans but these were chlorotic blotches not a mosaic as reported by Cowper (1983).

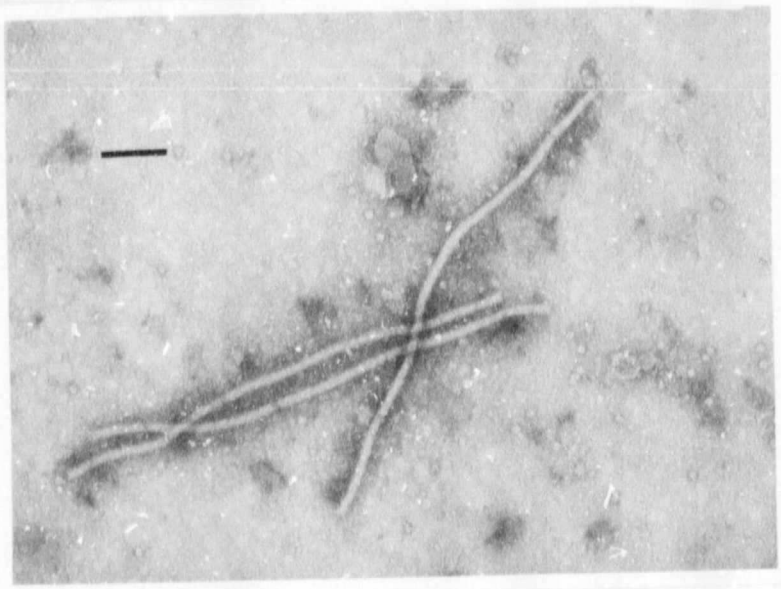


Figure 4. Micrograph of flexuous virus particles found in a leaf dip preparation from Sanilac plants with typical BCMV symptoms. The two shorter particles are 750 nm x 16 nm, dimensions typical of BCMV particles. The particles were negatively stained with 2% Uranyl Acetate. The bar represents 10 nm.

3.2 Symptom development

Inoculated plants were maintained at 20 °C (± 2°C) for a minimum of fourteen days. The Derby isolate was kept at a 22 °C day/ 18 °C night cycle to see if the difference in temperature regime would affect symptom development or severity but, however, it did not. When transferred to a higher temperature (> 25°C), the plants more readily developed leaves with poor or no symptoms. Despite reasonably good temperature control, the absence of adequate humidity hindered better symptom development. This was exacerbated in some cases by root rots and poor soil fertility and necessitated the repeat of the test under better conditions. These factors had an indirect effect on symptom development by adversely affecting the condition of the host plant.

The unifoliates of infected plants grown from seed usually exhibited a very mild mottle and/or slight epinasty. The trifoli tes on the other hand had a distinct mottle, "green island" or distinct dark green rugose formations, and/or leaf lamina reduction with elongated apices (Figures 12 & 13). Symptom severity increased slightly with the first few trifoliates and thereafter often decreased to no visible symptoms in the new leaves depending on the prevailing environmental conditions.

Inoculated leaves of sensitive cultivars without the I-gene (host resistance groups 1 - 6) usually developed spreading necrotic lesions, 1 - 2 mm. in diameter, or veinal lesions after four to seven days (Figures 5 & 6). The necrosis was of a superficial nature and initially found in the epidermis, similar necrosis was described and illustrated by Drijfhout (1978). The

first trifoliate of susceptible cultivars exhibited a slight rugosity and epinasty. On a number of occasions with "Diacol Calima" and "Bataaf" and on one occasion with "Sanilac", the growing point and/or youngest leaves became necrotic and the lamina of the expanding leaf developed with a patchy necrosis (Figure 7). The older trifoliates often developed veinal necrosis or discolouration, in "Sanilac" this was accompanied by necrotic lesions 0.5 - 1.0 mm. in diameter (Figures 8 & 9). Large necrotic lesions of the stem and petiole often developed, and sectioning of a petiole revealed this to be initially associated with the cortex (Figures 10 & 11). Necrotic symptoms were found without fail in infected "Sanilac" plants but the time of appearance and severity was very variable amongst the other test plants.

The trifoliate developing at the time of inoculation exhibited chlorotic veinbanding as it expanded and a general chlorosis at full expansion. Thereafter the plant developed mottling and other symptoms in further leaves as described above (Figures 6, 12 & 13). Necrosis was rarely found in leaves with a mottle.

The necrotic reaction, conditioned by the I-gene in host resistance groups 8 - 10, resulted in pin-point lesions in the hot glasshouse (30 °C , 12 hours day) after three to four days (Figure 14). Necrosis then spread to adjacent veins and unlike that already described, remained confined to the veins. If inoculation occurred directly above a vein the necrosis became systemic after seven days, causing necrosis in the vascular tissue and the death of the growing point and thereafter the rest of the plant. The necrosis was continuous from the point of infection to the necrotic growing point.

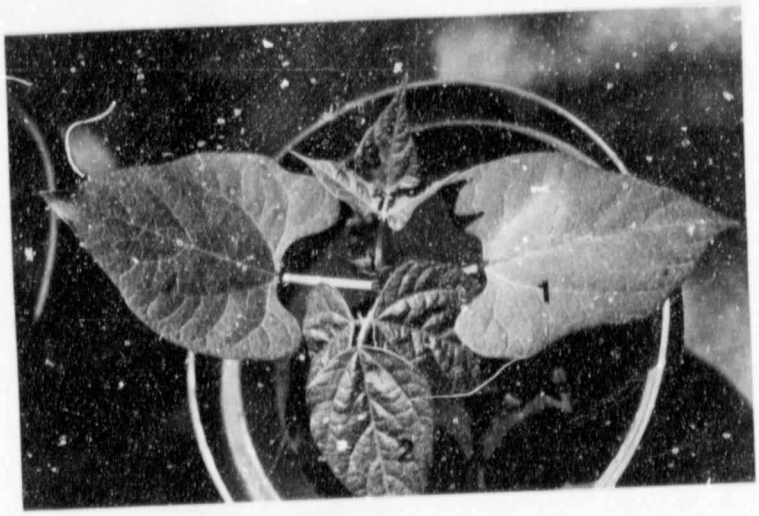


Figure 5. Sanilac. 10 - 14 days post inoculation.

1. Inoculated leaf with necrotic lesions surrounded by a chlorofic area.
2. Older trifoliate with very slight rugosity accompanied by epinasty.
3. Developing trifoliate chlorotic.

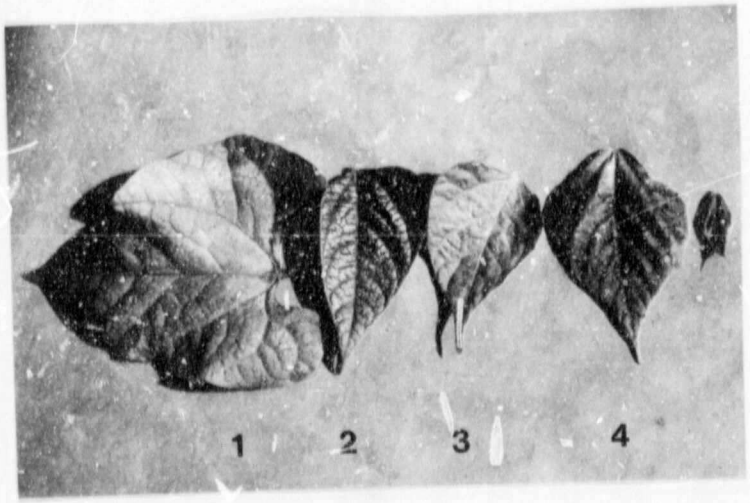


Figure 6. Diacol Calima. Four weeks post inoculation. Key as for Figure 5.
4. Later trifoliates developing with rugose and mottle symptoms.



Figure 7. Diacol Calima. Three weeks post inoculation. Note the necrotic lesions in the second leaf lamina.



Figure 8. Sanilac. Necrosis in an older trifoliate leaf.



Figure 9. Pinto 114. Superficial discolourations associated with the veins. The trifoliate was not inoculated.



Figure 10. Sanilac. Stem necrosis.

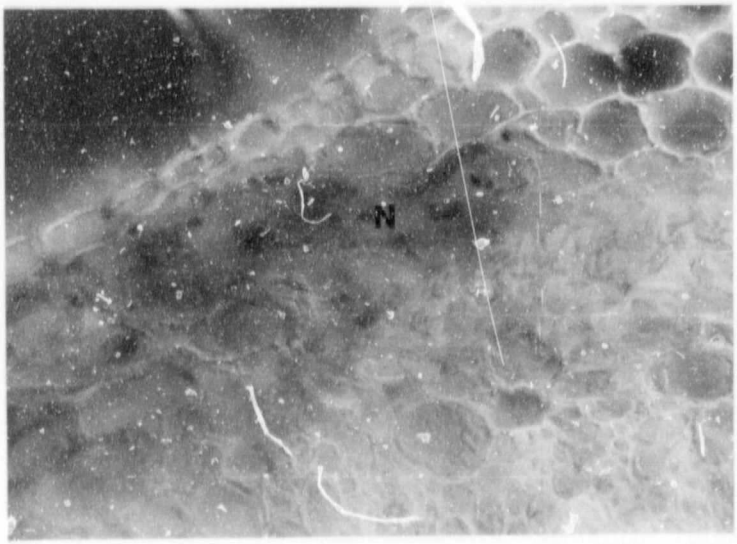


Figure 11. Sanilac. Section through a petiole viewed with interference contrast. Necrosis (N) associated with the cortex.



Figure 12. Sanilac. Severe leaf symptoms. Rugosity (R) adjacent to the mid-rib, and elongation of the leaf apex (A).



Figure 13. Sanilac. Severe leaf symptoms. "Green Island" (GI) and elongated leaf apex (A).

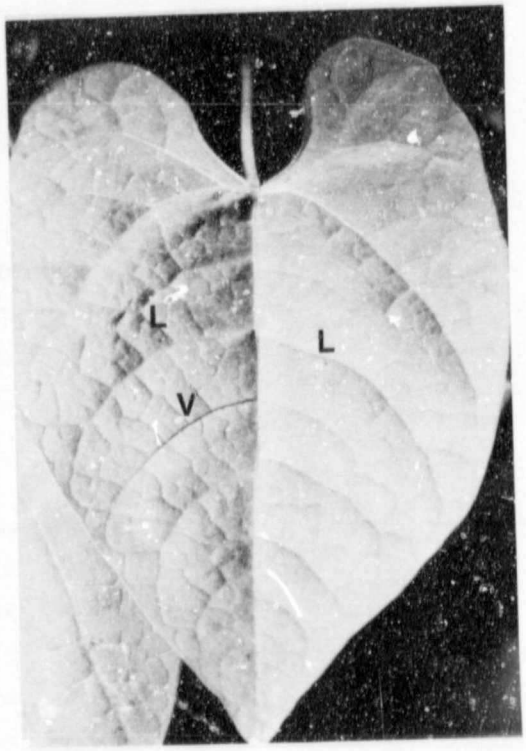


Figure 14. Peru 0257. Local symptoms six days post inoculation. Lesion development (L) is limited except where inoculation occurred above a vein (V).

The spread of necrosis was also dependant on environmental conditions, and also for example, hindered by root rots. If the plant was under stress there was a reduction in the spread of necrosis.

3.3 Differential indicator host studies

Where possible three cultivars of each host resistance group (Table 2) were inoculated as indicated in Materials and Methods and the results are given below.

Group 1

Only two members of this group were inoculated but most of the cultivars in which the virus samples were collected (see Table 1) were probably of this group. "Diacol Calima" exhibited mottle and necrotic symptoms. "The Prince" exhibited less necrosis and more leaf epinasty but the differences were not great.

Group 2

With the exception of slight veinal necrosis of inoculated and first trifoliate leaves for the Nooitgedacht (NGDT) isolate, "Vedlands Greenleaf C" remained symptomless. In the b ck-inoculation tests, 40 % and 20 % infected "Diacol Calima" plants were found for the NGDT and Bapsfontein (BAPS) isolates, respectively. A number of local lesions were also found in "Peru 0257" in the two back-inoculation tests. "Bataaf" was found to be susceptible, giving symptoms similar to those of "Diacol Calima", but "Puregold Wax" and "Imuna" exhibited no symptoms and no positive back-inoculation results were obtained.

Group 3

"Redlands Greenleaf B", "Bigbend" and "Great Northern U.I. 59" remained symptomless and there were no positive back-inoculation results.

Group 4

"Sanilac", "Michelite" and "Red Mexican U.I. 34" exhibited mottle and necrotic symptoms. "Michelite" exhibited less necrosis in the leaves and stem.

Group 5

"Pinto 114" exhibited very mild mottling in only one of the isolates tested but in other tests, the non-I-gene veinal necrosis in older leaves was often observed. Centro Internacional de Agricultura Tropical (CIAT Annual Report, 1982) found that infected Pinto types frequently escaped detection, probably by visual methods. "Pinto 111" (Group 4) gave a clearly discernable mottle whereas "Pinto 114" did not, the latter being the only available member of host resistance group 5.

Group 6

"Monroe" exhibited local necrotic lesions similar to "Sanilac" in some of the tests. "Red Mexican U.I. 35" reacted the same as "Monroe" but "Great Northern U.I. 31" exhibited no symptoms. No positive back-inoculation results were found for any of the three cultivars.

Group7

Host resistance group 7 has the bc 3 gene, but no members of this group are presently available for testing.

TABLE 3: RESULTS OF THE BCMV SAMPLES TESTED IN DIFFERENTIAL HOST PLANTS

	LOCATION OF ISOLATES														
Host Group	KOS[1]	DER	POT	LAPS	SUN	NIG	DEL	MOR	NGOT	BERG	BUR	WIN	WEEN	GREY	FRA
1	M[2]	M	M	M	M	M	M	M	M	M	M	M	M	M	M
REDL C	-[3]	-	-	t[4]	-	-	-	-	t	-	-	-	-	-	-
2															
BATAAF	M	*[5]	*	*	M	M	M	M	*	*	M	M	M	*	M
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	3/10M[6]	7/10M	8/10M	5/10M	7/10M	4/10M	6/10M	5/12M	8/11M	9/12M	8/12M	8/12M	8/10M	7/10M	9/10M
5	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
6	-	4/10N.L[7]	5/10N.L	-	-	3/10N.L	-	3/9N.L	6/10N.L	1/10N.L	5/10N.L	-	-	-	4/10N.L
COLD	-	-	-	-	-	-	-	-	-	-	-	1/12N	-	-	-
8															
HOT	3/10N[8]	3/12N	7/12N	3/10N	3/10N	3/10N	4/10N	3/10N	3/12N	3/10N	3/10N	6/11N	4/10N	6/12N	4/10N
COLD	-	-	-	-	-	3/10N	5/10N	2/10N	-	1/10N	3/10N	3/10N	-	-	-
9															
HOT	2/11N	2/10N	10/10N	3/10N	7/10N	6/10N	7/10N	7/10N	2/12N	8/10N	8/9N	10/14N	8/10N	7/11N	5/10N
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. See table 1 for location abbreviations
2. M = Mottle
3. - = No reaction
4. t = Tolerant or poor symptoms (infection shown by back-inoculation test)
5. * = not tested
6. Number of plants showing symptoms out of number inoculated
7. N.L = Necrotic local lesions
8. N = Systemic necrosis

Group 8

"Widusa", "Nep 2" and "Black Turtle Soup" reacted in the same way in exhibiting a temperature dependent systemic lethal necrosis. Only one "Nep 2" plant exhibited a systemic hypersensitive reaction, but there were a number of local reactions, up to 30 %, in the cold glasshouse (20 ℃).

Group 9

"Peru 0257", "Jubila" and "Topcrop" reacted similarly in symptom development to the previous group but proved to be more sensitive. This was more noticeable in the cold glasshouse (20 ℃ day) where of the fifteen samples tested, six exhibited systemic necrosis. Further differences in sensitivities are reported below.

Group 10

"Amanda" exhibited no systemic necrosis and in only two tests out of seventeen did it exhibit a single local necrotic reaction in one plant. A similar reaction was reported by Kyle and Provvidenti (1987) in their work with the NY 15 isolate.

3.4 Isolate identity

All the samples of BCMV tested exhibited similar results and there was little difference in symptom expression (Table 3). A comparison cannot be made between the different samples when considering infection rate as some were inoculated only once whilst others were inoculated twice.

The more sensitive nature of host resistance group 9 when compared with group 8 is clearly shown in the infection rate of 57 % compared to 37 %, respectively, over all the differential inoculation tests. Host resistance group 4 (Sanilac) had a similar infection rate (63 %) to that of Group 9. Unfortunately, "Bataaf" and "Diacol Calima" cannot be compared as the plants were not grown singly in pots.

Inoculation results for tests of BCMV taken from infected "Redlands Greenleaf C", "Sanilac" and "Pinto 114" plants were very similar and also similar to those of the various samples (Table 4). This suggests a single isolate is present. If another isolate is present concomitantly, it's pathogenicity spectrum is masked by the spectrum of the predominant isolate with no effects on the positive/negative nature of the results obtained. For example if the type strain is present it's sole infection of resistance group 1 will be masked by the South African isolate, symptom development may be affected but on the basis of a negative/positive basis the results will not differ. It is interesting to note that there were no positive results in "Redlands Greenleaf C" for the BCMV originally isolated from "Redlands Greenleaf C" in another set of differential host plants. Perhaps the infection of "Redlands Greenleaf C" is at the borderline of detection.

These results closely resemble those of virus patogenicity group V (Table 5). "Pinto 114" was borderline between tolerant and sensitive, having exhibited a mild mottle in two of the differential tests.

The subdivision of virus pathogenicity group V is, in part, based on a symptomless but detectable infection

TABLE 4 : RESULTS OF TESTS FOR PRESENCE OF MIXTURE OF ISOLATES OF B.C.M.V.

	SOURCE OF B.C.M.V.[1]		
Host Group	Redlands Gr lf C	Sanilac	Pinto 114
1	M	M	M
2	-	-	-
3	-	-	-
4	1/10M	10/10M	5/8M
5	t	t	2/10MM[2]
6	-	6/10N.L.	7/10N.L.
COLD	-	-	-
8 HOT	2/10N	3/10N	6/10N
COLD	-	-	-
9 HOT	3/10N	4/10N	7/10N
COLD	-	-	-
10 HOT/COLD	-	-	-

1. i Redlands Greenleaf C from NGDT.

ii Sanilac and Pinto 114 from BAPS.

Redlands Greenleaf C and Pinto 114 BCMV maintained in "Diacol Calima" stock plants

2. MM = Mild Mottle.

For key see table 3

TABLE 5 REACTIONS OF THE B.C.M.V. HOST RESISTANCE GROUPS TO INFECTION BY KNOWN ISOLATES

Host Resistance Group	Virus Pathogenicity Group										
	I	II	III	IVa	IVb	Va	Vb	VIa	VIb	VII	P[1]
1	+[2]	+	+	+	+	+	+	+	+	+	+
2	-[3]	+	+	+	+	+t[4]	+	+t	+	+	+t
3	-	-	-	+	+	-	-	+	+	+	-
4	-	-	+	-	-	+	+	+	+	-	+
5	-	-	-	-	-	+	+	+	+	-	+t
6	-	-	-	-	-	-	-	-	-	+	-
8	-	-	+n[5]	-	±n[6]	-	-	+n	+n	-	±n
9	-	-	-	-	±n	-	±n	+n	+n	-	±n
10	-	-	-	-	-	-	-	-	+n	-	-

1 P = Reaction of S.A. isolate

2 + = Susceptible with visible symptoms

3 - = No infection

4 +t = Susceptible with poor or no symptoms. (Proven by back-inoculation test)

5 +n = Systemic necrosis

6 ±n = Systemic necrosis dependent on temperature

of host resistance group 2. Drijfhout (1978) listed "Puregold Wax" and "Imuna" as developing a mottle on infection with Hubbeling's Imuna virus isolate (Group Vb). Hubbeling, however, rated "Imuna" as susceptible but "Puregold Wax" as resistant (Drijfhout, 1978). The NY 15 virus isolate (Group Va) is listed as exhibiting a symptomless infection of both cultivars. Both cultivars failed to develop any symptoms on inoculation or positive results in back-inoculation tests with the South African isolate. "Bataaf", also a member of resistance group 2, exhibited good symptom development. This cultivar has been maintained in the germplasm store at Potchefstroom for a long time whereas the other cultivars tested were grown from seed provided from CIAT recently. The cultivar "Monroe" was suggested as a local lesion host for BCMV by Saettler and Trujillo (1972) as it consistently exhibited lesions after inoculation with three isolates, including the NY 15 isolate, under normal glasshouse conditions. The South African isolate consistently caused lesions after inoculation of "Sanilac" but lesion development in "Monroe" was erratic.

Drijfhout (1978), working with the Imuna virus isolate (Group Vb), found systemic necrosis at 30°C but not 26 °C or lower in host resistance group 8. Kelly et al (1983) listed the NY 15 virus isolate (Group Va) as also giving a temperature-dependant necrosis in host resistance group 8 but no reaction with host group 9. Both host resistance groups, 8 and 9, gave a temperature-dependant necrosis when infected with the South African isolate. Hampton (pers. comm.) stated that he has found high temperature necrosis reactions less reliable than those of low temperature mottle symptoms for identifying which pathogenicity group an isolate belongs to.

TABLE 6: Latent period at 20°C after which the ability to induce "black root" is lost on transfer to 30°C. Peru 0257.

	No. plants with systemic necrosis											No. plants local necrosis	Totals	
Hours at 20°C	DPI 10	11	12	13	14	15	16	17	18	19	20	20 DPI	Systemic only	Local + systemic
0	1	0	3	1	1	0	1	0	0	0	0	2/7	8/14 *	9/14
12	1	3	1	0	1	0	0	0	0	0	0	3/7	7/14	10/14
24	0	2	0	0	1	1	1	0	0	0	0	5/11	5/16	10/16
36	0	1	1	0	1	1	0	0	0	0	0	4/10	5/15	9/15
48	0	1	1	1	0	0	0	1	0	0	1	3/9	5/14	8/14
60	0	2	1	1	2	0	0	0	0	2	0	1/6	8/14	9/14
72	0	0	1	0	0	1	1	0	1	1	0	2/10	5/15	7/15
84	0	0	0	0	0	1	2	0	1	0	0	5/10	7/13	6/13
96	0	0	0	0	1	0	0	0	0	0	0	2/9	1/9	3/9
480	0	0	0	0	0	0	0	0	0	0	0	3/15	1/15 *	3/15

Plants were maintained at 20°C day for 0 - 96 hours before transfer to 30°C day. Plants were also kept at 20°C (480 hours) as a control.

All developed local lesions after 3 - 5 days post-inoculation (P.I.).

* Includes one plant with systemic necrosis after 24 days. No further plants exhibited systemic necrosis.

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