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IDENTIFICATION OF NECROSIS-INDUCING STRAINS OF
BEAN COMMON MOSAIC VIRUS AND BREEDING FOR RESISTANCE

By

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ABSTRACT

IDENTIFICATION OF NECROSIS-INDUCING STRAINS OF BEAN COMMON MOSAIC VIRUS AND BREEDING FOR RESISTANCE

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Four necrosis-inducing isolates of bean common mosaic virus (BCMV) were characterized to obtain information as to the identity of strain (pathotype) of the virus.

SM (Sanilac-Mosaic) and SF (Sanilac-Foundation) isolates induced more severe symptoms on differential bean cultivars than SN (Sanilac-Necrotic) and SB (Sanilac-Breeder) isolates. When compared with known BCMV strains NL-3 and NY-15, the isolates showed different pathogenicity patterns. The new isolates induced reactions on a wider host range than the NY-15 strain. Although reactions were narrower in host range than the NL-3 strain, the isolates tend to resemble this strain more. The reaction induced by SM was more virulent than that of the other three isolates.

Data indicate the need to incorporate the recessive resistant genes $bc-2^2$ and $bc-3$ along with dominant I gene in order to provide adequate levels of resistance to the necrosis-inducing strains of BCMV.

To My Lovely Daughter, Wendy.

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I. INTRODUCTION

Bean Common Mosaic Virus (bean virus 1) is a disease of major importance worldwide in dry edible beans (Phaseolus vulgaris L.) The virus is seed-borne and readily transmitted by aphids. Infected plants may exhibit either "typical mosaic" symptoms, usually consisting of various leaf-malformations, or severe vascular necrosis, which is generally known as "Top Necrosis" or "Black Root." The latter frequently results in plant death, and in severely infected fields, yield may be reduced 50-80% (25,35).

Sixteen different strains of the pathogen have been classified to date. The sixteen strains are divided into seven pathogenicity groups (and subgroups), depending on the reactions induced in differential bean varieties. Four of the virus groups (plus one subgroup) induce only systemic mosaic in susceptible varieties while the remaining three groups (plus one subgroup) in addition to inducing systemic mosaic, induce black root or top necrosis and are known as necrotic virus strains. In Michigan, only the Type and New York-15 strains have previously been isolated and identified (15).

Since 1980 Michigan dry bean farmers have experienced an increase in the damage caused by common mosaic virus disease. A two year contract market with Mexico for black and pinto bean types, which began in 1980, apparently gave rise to a dramatic increase in the planting of BCMV susceptible varieties such as vine Black Turtle Soup and Pinto UI-111.

This was accompanied by an increase in the incidence of the black root reaction in production fields of I gene-carrying cultivars such as Seafarer and Fleetwood navy and T-39 black beans. This occurrence suggested the possibility that a necrotic strain of BCMV now exists in Michigan since the Type and New York-15 strains do not induce this reaction under field conditions.

Resistance to BCMV depends on the interaction between the virus strain and specific genes of the bean plant. Two types of resistance, known as "recessive" and "dominant" resistance, have been identified and used in the breeding of resistant varieties. The recessive or strain specific (vertical) resistance is governed by six recessive genes. Five of these genes, designated as $bc-1$, $bc-1^2$, $bc-2$, $bc-2^2$, and $bc-3$ are strain specific genes; and one recessive gene, $bc-u$, is a strain non-specific gene (5). Resistant varieties may carry either a single gene or combinations of genes, but a variety can not carry $bc-1/bc-1^2$ or $bc-2/bc-2^2$ as these genes are allelic. Also, the strain specific genes need the presence of $bc-u$ to be effective.

In the U.S.A. the dominant or "I gene" resistance has been the most commonly used form of resistance in breeding programs. Varieties carrying the I gene show a resistant reaction when infected with nine of the sixteen known BCMV strains. The remaining seven necrotic strains induce the black root (top necrosis) reaction in these cultivars. This reaction results in the death of tissue and thus prevents spread of the virus to neighboring healthy plants. For this reason, bean breeders in Europe, where necrotic strains are frequently detected, have not relied on the I gene as much as American breeders. Consequently, they have utilized the specific resistant genes to a greater degree (7).

The following study was undertaken in four general areas to investigate the possible occurrence of a necrosis-inducing strain of BCMV in Michigan, and breeding for resistance to this type of strain:

- a) Identification of several of the more severe necrosis-inducing isolates currently affecting Michigan dry bean production.
- b) Screen varieties, segregating populations and MSU elite breeding lines for presence of I dominant gene and recessive resistant (bc) genes.
- c) Study the genetics of resistance of dry bean cultivars to the BCMV strain(s) identified.
- d) Initiate breeding efforts to incorporate the recessive genes bc-2² and bc-3 into dominant I gene breeding materials.

II. LITERATURE REVIEW

1. The Virus

Bean common mosaic virus was first reported in Russia by Iwanowski (13) about 1899. In the United States the pathogen was first recognized as a virus disease by Stewart and Reddick (29) in 1917, who assigned to the disease the epithet of bean mosaic virus. In 1934, Pierce (19) described the biological properties of the virus as compared with bean yellow mosaic virus adding the epithet of "common." The virus is now known to occur in many countries, is evidently worldwide in distribution and probably coexistent with the host. Other names used as synonyms to designate the virus have been: common bean mosaic, bean mosaic virus, bean virus 1 and Phaseolus virus 1 (5).

In nature, infection seems confined to Phaseolus vulgaris. Since the virus is seed-borne, it is established in the field through the planting of diseased seed. Several species of aphids have been found to transmit the virus (8,34) from diseased to healthy plants. Among these are the common species Aphis gossypii, Aphis rumicis and Myzus persicae. Artificially, the virus is readily transmitted to healthy plants by sap inoculation (within Phaseolus vulgaris).

Seed transmission of the virus was first reported by Reddick and Stewart (24) in 1919, who found that 50% or more of the seeds from infected parents produced mosaic plants. In 1933, Nelson and Down (17) found that the virus was also transmitted to offspring from healthy bean

plants through the pollen or ovule from infected plants. Seed transmission is irregular, depending on stage of growth at the time of infection, cultivar and virus strain. If infection occurs after flowering, the virus does not usually reach the seed (17). Flower buds, which become infected just before or after fertilization, never produce seed infected with BCMV (8).

BCMV readily develops new strains by mutation, which infect previously resistant cultivars (7).

Most infectivity is usually lost around 60°C in expressed sap after heating for 10 minutes, but, depending on virus source, virus strain and environmental conditions, thermal inactivation may occur between 50 and 65°C. The dilution end-point is usually between 10^{-3} and 10^{-4} and ageing in vitro at room temperature 1-4 days. In crude sap the particles can now easily be seen with the electron microscope. They are flexuous filaments about 75nm long and 15 nm wide. In particle morphology, mode of transmission and other biological and biophysical properties, BCMV resembles members of the potato virus Y group of viruses (potyvirus group) and is serologically related to several of them, especially to bean yellow mosaic virus (BYMV). The latter virus has a wider host range and is not seed transmitted in Phaseolus beans (5).

2. Host Range

The following plant species have been found susceptible to BCMV by artificial inoculation:

Species: Cajanus cajan L. (Spring pigeon pea), Canavalia ensiformis (L) D.C. (Jack bean), Cicer arietinum L. (Chick pea), Glycine max (L) Merr (Soybean) cv. Black Hawk, Lespedeza striata (thumb) H. and H. (common

lespedeza), Phaseolus acutifolius Gray var. latifolius Freeman (Tepary bean), P. calcaratus Roxb (rice bean), P. lunatus L. (lima bean) cvs. Henderson Bush, Thaxter and Fordhook 242, P. mungo L. (urd bean), Sesbania exaltata (raf.) C. and Stizolobium deeringeanum (velvet bean) (36), Phaseolus artropurpureus, P. radiatus (= P. aureus), some Vigna spp., Crotalaria spectabilis (crotalaria) and Lupinus albus (23), Cassia tora, Lens culinaris Medick and Trifolium subterraneum L. (33), Chenopodium quinoa, Gomphrena globosa and Nicotiana clevelandii (2), and Tetragonia expansa (6).

3. Host Reaction

The reaction of host plants inoculated with BCMV may vary according to the bean genotype, time of infection, environmental conditions and strain of virus involved.

The virus causes mottling and various types of leaf malformations to susceptible bean genotypes not possessing the dominant type of resistance derived from cv. Corbet Refugee. Stunting of plant development is usually evident and plants infected early are more likely to be stunted, yielding less than plants infected later (21).

According to Grogan and Walker (9), systemic necrosis only appears in cultivars having the dominant type of resistance. They found that such plants were generally resistant to the Type and NY-15 strains but might show systemic necrosis if grafted on plants of cv. Stringless Green Refugee, which were inoculated with one of these strains, developing mosaic. Common mosaic was not found in plants with dominant resistance after inoculation with BCMV. Conversely, no systemic necrosis appeared in genotypes showing mosaic or possessing the resistance of the cvs. Robust or Michelite.

The black root (or top necrosis) reaction may affect all of the vascular bundles of the plant which will usually die if infected while young. If infected later, parts of the plant may die and many of the pods, even on the apparently healthy stems, show black discolorations in the pod wall due to vascular necrosis. Even low percentages of such pods are unacceptable in mechanically harvested beans for food market processing, because they have to be manually removed and this leads to considerable economic loss (5).

After inoculation, local lesions may be found prior to development of systemic necrosis. They initially appear as dark-brown pin-point restricted lesions, then enlarging to star-like vein necrosis by brown discoloration of the adjacent veinlets. The number of lesions is influenced by temperature. Drijfhout (5) tested five strains at five temperatures ($^{\circ}\text{C}$) on a group of I dominant gene cultivars and found differences in their ability to induce top necrosis. Cultivars that were not affected at 20-26 $^{\circ}\text{C}$ became affected by the same strains at 30 $^{\circ}\text{C}$. Zaumeyer and Goth (33) found a strain (Florida) which did not induce necrotic lesions at high temperatures while Hubbeling (10) reported other strains in the Netherlands (NL-3, NL-5) which induced a severe systemic necrosis at lower (15-20 $^{\circ}\text{C}$) temperatures in cultivars with gene I.

Local lesions are not restricted only to plants with the dominant resistance from Corbet Refugee. Another type of local lesion is sometimes induced in I gene recessive (I^+I^+) plants. Saettler and Trujillo (26) reported the development of very small local lesions in cv. Monroe. They were described as circular dark-red spots of about 4mm diameter on the upper side of the leaves, arising about 4-5 days after inoculation. The lesions enlarged during the next 4-5 days to 0.8-1mm. Number and

clearness of lesions were high at 20°C, good at 24°C and poor at 16 and 28°C. At the higher temperatures, more ring-shaped spots and superficial vein necrosis developed.

Thus, there are two types of local lesions. One type arises as pin-point lesions, mostly enlarging to star-like vein necrosis, sometimes followed by systemic necrosis but never by mosaic and only occurring in genotypes with the dominant resistance from Corbet Refugee. The second type may start as pin-point lesions but mostly as bigger spots, sometimes ring-shaped, first white or chlorotic, later brownish extending in a superficial necrosis about the veins. Sometimes this local reaction, only arising in genotypes not carrying the dominant resistance from Corbet Refugee, is followed by mosaic in younger leaves but never by systemic necrosis (5).

4. BCMV Strains

Most BCMV strains reported in the literature are from the United States and from the Netherlands (Table 1). A new strain of the virus was reported in 1943 by Richards and Burkholder (25). They mentioned that as early as 1939, plants of cv. Michelite, a navy bean resistant to the Type strain, were observed with symptoms of BCMV in two fields near Batavia, New York. The resistance of cv. Robust was also broken down by this strain termed the New York-15 strain.

Dean and Wilson (4) reported an Idaho strain in 1954 infecting cvs. Great Northern-123 and Great Northern-31, which was later recorded as Idaho or B strain. They stated that cultivars carrying dominant resistance to Type strain were either resistant or susceptible to the new strain.

In 1961, Skotland and Burke (28) described a virus in the western United States that attacked cv. Great Northern-123, which cvs. Michelite, Sanilac, Pinto UI-111, RM-34 and Great Northern-31 proved resistant. Later in 1969, Silbernagel (27) considered this virus as a new strain of BCMV and designated it as the Western strain (WBCMV).

Zaumeyer and Goth (33) reported strain Florida in 1964. This strain produced symptoms on susceptible cultivars that were more severe than those caused by the Type, NY-15, or Idaho strains. Cv. Stringless Green Refugee was susceptible to Florida strain but cvs. Pinto UI-111, Michelite, Sanilac, RM-34, Great Northern-123 and Great Northern-31 were resistant. Plants of cv. Topcrop did not show local necrosis when inoculated with Florida strain at 32°C, as they did after inoculation with Type or NY-15 strains.

In 1964, the Mexican strain was isolated by Silbernagel (27) from a Mexican bean line (P.I. 197690S) being increased in a greenhouse at Prosser, Washington. This strain differed from those previously reported in being seed-transmitted through cv. Red Mexican 35 and by its inability to infect cv. Improved Tendergreen. This strain also induced necrosis in plants of cv. Topcrop when subjected to 32°C for 3 days.

Table 1. BCMV Strains Reported In The Literature

Strain Name	Year When Described	Reference
Type	1943	Richards and Burkholder (25)
New York-15	1943	Richards and Burkholder (25)
Idaho or B	1954	Dean and Wilson (4)
Western	1961	Skotland and Burke (28)
Florida	1964	Zaumeyer and Goth (33)
Mexican	1969	Silbernagel (27)
Imuna (NL-2)	1963	Hubbeling (10)
Michelite (NL-3)	1963	Hubbeling (10)
Great Northern (NL-4)	1963	Hubbeling (10)
Jolanda (NL-5)	1972	Hubbeling (11)
Colana (NL-6)	1972	Hubbeling (11)
NL-7	1977	Drijfhout and Bos (6)
NL-8	1977	Drijfhout and Bos (6)

In the Netherlands, strains have been isolated that induce systemic necrosis at moderate temperatures (18°- 20°C) with a wider host range. They comprise the group called the necrotic-inducing strains. In 1963, Hubbeling (10) described the Imuna (NL-2), Michelite (NL-3) and Great Northern (NL-4) strains from cvs. Imuna, Michelite and Great Northern-123, respectively. He compared these strains on cvs. Dubbele Witte, Imuna, Michelite, Great Northern-123 and Widusa. All three strains attacked cv. Dubbele Witte. Also, Imuna and Michelite strains each produced symptoms both in Imuna and Michelite. Both isolates did not affect Great Northern-123, which was susceptible only to the Great Northern strain. Michelite strain differed from Imuna in giving local and systemic necrosis at 20°C in Widusa and other cultivars with dominant resistance. Thus, this strain produced systemic necrosis at the moderate temperature.

In addition, Hubbeling (11) isolated two strains in 1972 from pods of Jolanda and Colana cultivars that were known as having dominant resistance. Jolanda (NL-5) strain, like Michelite (NL-3) strain, produced local and systemic necrosis at 20°C in cultivars with dominant resistance. It differed from Michelite strain in inducing rapid systemic necrosis at 20°C in cv. Jubila (a new added differential), in which Michelite strain induced local vein necrosis only. Colana (NL-6) strain differed from Great Northern strain in its inability to infect Great Northern-31 and its ability to induce systemic necrosis in Jubila, and from Jolanda and Michelite strains in not attacking cvs. Michelite and Sanilac.

Finally, two strains, coded NL-7 and NL-8, were isolated by Drijfhout and Bos (6) in 1977. Strain NL-7 did not attack I gene differentials Jubila, Topcrop, Improved Tendergreen 40031, Widusa, Black Turtle Soup, and Amanda. It produced typical mosaic symptoms in the differentials

Dubbele Witte and Stringless Green Refugee, both susceptible to all strains. Strain NL-8, in addition to infecting cvs. Dubbele Witte and Stringless Green Refugee, also infected cvs. Sanilac, Michelite and RM-34. It induced local necrosis at 20°C in all differentials with dominant I, but systemic necrosis in Widusa and Black Turtle Soup only. Actually, this strain was recently isolated in Sanilac in western New York (22).

4.1 Differentiation of Strains of BCMV

The foregoing BCMV strains and other reported isolates were classified on the basis of reaction induction on a group of differential cultivars developed by Drijfhout, et al, (7). Seven pathogenicity groups (and subgroups) were established in which groups of strains vary in symptoms produced on differential cultivars. Also, one of the primary goals of that study was to develop a standard set of bean cultivars for strain differentiation. Thus, differential cultivars used by various authors were classified into nine host groups. Tables 2 and 3 summarizes these results.

Table 2. Bean Host Groups Used for Differentiation
of BCMV Strains*

Host Group	Cultivar	Origin
Cultivars with recessive allele (I^+I^+) of the necrosis gene		
1	Dubbele Witte	Netherlands
	Sutter Pink	U.S.A.
	Stringless Green Refugee	U.S.A.
2	Redlands Greenleaf-C	Australia
	Puregold Wax	U.S.A.
	Imuna	Germany
3	Redlands Greenleaf-B	Australia
	Great Northern UI-123	U.S.A.
4	Sanilac	U.S.A.
	Red Mexican UI-34	U.S.A.
	Michelite 62	U.S.A.
5	Pinto 114	U.S.A.
6	Monroe	U.S.A.
	Great Northern UI-31	U.S.A.
	Red Mexican UI-35	U.S.A.
Cultivars with dominant alleles (II) of the necrosis gene		
7	Widusa	Netherlands
	Black Turtle Soup	Mexico
8	Jubila	Germany
	Topcrop	U.S.A.
	Improved Tendergreen	U.S.A.
9	Amanda	Netherlands

*From Drijfhout, et al, (7)

I^+I^+ = New symbolism replacing ii, adopted by Drijfhout, et al, (7)

Table 3. Differentiation and Grouping of the Strains of BCMV*

Resistance group of the host	Differential Cultivar	Pathogenicity group of the virus														
		I	II		III	IVa Florida US 5	IVb Western US 4	Idaho or B US 3	Colana NL 6	Va NY US 2	Vb Imuna NL 2	VIa Michelite NL 3	VIb Jolanda NL 5	VII Mexico US 6	Great North. NL 4	
			Westlandia NL 1	Type US 1												Puerto Rico PR 1
Cultivars with recessive alleles (I ⁺) of the necrosis gene																
1	Dubbele Witte Str. Gr. Ref.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Redl. Gr. C Puregold Max Imuna	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
3	Redl. Gr. B Gr. North. 123	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+
4	Sanilac Michelite 62 Red Mex. 34	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
5	Pinto 114	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
6	Monroe Gr. North. 31 Red Mex. 35	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Cultivars with dominant alleles (II) of the necrosis gene																
7	Widusa Bl. Turtle S.	-	-	-	+	+	-	±n	±n	±n	-	±n	±n	-	-	-
8	a Jubila	-	-	-	-	-	-	±n	±n	±n	±n	±n	±n	-	-	-
b	Topcrop Imp. Tendergr.	-	-	-	-	-	-	±n	±n	±n	±n	±n	±n	-	-	-
9	Amanda	-	-	-	-	-	-	-	-	-	-	-	±n	-	-	-

+ = susceptible, sensitive, systemic mosaic; +t = susceptible, tolerant, systemic symptoms questionable or very weak, virus recovered from uninoculated leaves by back-inoculation onto Dubbele Witte; - = resistant, no systemic symptoms, virus not recovered from uninoculated leaves by back-inoculation; +n = susceptible, sensitive, usually all plants with systemic necrosis, not clearly dependent on temperature; ±n = susceptible or resistant, dependent on temperature, from none to all but mostly only a few plants with systemic necrosis, the number varying in repeated tests and increasing with temperature. Greenhouse mean temperature 22-26°C, day and night fluctuation at most 20-24°C in winter and 20-30°C in summer.

*From Drijfhout et al (7)

5. Genetics of Resistance to BCMV

The genetic basis of resistance to BCMV was initially studied in the United States. Pierce (20) studied the inheritance of mosaic resistance of three cultivars, Corbet Refugee, Great Northern UI No. 1 and Robust, which had previously been identified as resistant to BCMV. Actually, Corbet Refugee and Robust had been mentioned as having different types of resistance. He, in fact, showed that there were two types of resistance: one inherited dominantly as in Corbet Refugee, and the other recessively as in cvs. Robust and Great Northern UI-1.

Ali (1) studied the genetic basis of resistance to bean virus 1 (BCMV) and was the first to propose a genetic explanation for the segregations found in the F_2 of his crosses after inoculation with the "Zaumeyer" strain of BCMV. The F_2 of Stringless Green Refugee $\times$ US5 Refugee and Stringless Green Refugee $\times$ Idaho Refugee segregated in a 3:1 ratio of resistance to susceptible suggesting the segregation of a single dominant gene for resistance (Table 4).

Table 4. F_2 Distribution From Crosses Between Susceptible $\times$ Resistant Varieties Following Rub-Inoculation*

Matings	F_2 Plants			χ^2 (R:S)	P. Range
	Healthy	Diseased	Total		
Stringless Green Refugee $\times$ No. 5 Refugee	630	219	849	0.266(3:1)	0.70-0.50
Stringless Green Refugee $\times$ Idaho Refugee	409	150	559	0.859(3:1)	0.50-0.30
Stringless Green Refugee $\times$ Robust	195	547	742	0.650(1:3)	0.50-0:30

*From Ali (1)

Crosses between cvs. Stringless Green Refugee and Robust gave an F_2 ratio of 1:3 (R:S). Thus, the gene conferring resistance in Robust was inherited recessively. The F_2 of crosses between cvs. US5 Refugee or Idaho Refugee and Robust segregated in a ratio of 13:3 (R:S). The last two crosses between two resistant types gave evidence of two pairs of independently inherited genes. The ratio 13:3 was broken down into 3 plants with mosaic, 9 with top necrosis and 4 healthy, after the graft inoculation approach was used (Table 5).

Table 5. F_2 Distributions From Crosses Between Two Resistant Types*

Parents		F_2 Plants Tested	F_2 Segregation		$\chi^2(13:3)$	P. Range
			Healthy	Diseased		
Rub-inoculation technique						
Robust $\times$ U.S. No. 5 Refugee	-1	84	62	22	13.047	0.01
	-2	80	65	15	0.000	0.99
	-3	82	66	16	0.000	0.99
	-4	142	115	27	0.000	0.99
	Pooled	411	328	83	0.320	0.70-0.50
Total		799	636	163	1.232	0.30-0.20
Idaho Refugee $\times$ Robust	-1	143	117	26	0.000	0.99
	-2	170	134	36	0.799	0.50-0.30
	-3	209	166	43	0.398	0.70-0.50
	Pooled	667	537	130	0.300	0.70-0.50
Total		1189	954	235	0.862	0.50-0.30
Parents		F_2 Plants Tested	F_2 Segregation		$\chi^2(9:4:3)$	P. Range
			Top- necrotic	Healthy Mottle		
Approach-graft inoculation technique						
Robust $\times$ U.S. No. 5 Refugee		103	57	26	20	0.070 0.98-0.95
Idaho Refugee $\times$ Robust		150	83	40	27	0.326 0.90-0.80

*From Ali (1)

Ali interpreted the results as follows: A dominant gene A is required for virus infection, rendering the tissues susceptible. Another dominant gene I, when present with gene A, inhibits symptom expression following rub-inoculation and conditions systemic necrosis after approach-graft inoculation when there is a continuous supply of virus inoculum. With aa, the plant becomes resistant to both development of typical mosaic and systemic necrosis. On this basis, the genotypes of the four cultivars are: Stringless Green Refugee AAii (susceptible); US5 Refugee and Idaho Refugee AAIi (resistant, systemic necrosis if grafted); Robust aaii (resistant, no systemic necrosis if grafted). The necrotic reaction after graft-inoculation is governed by gene I in the presence of A, most likely through a hypersensitivity mechanism. Plants with the genotype aaI, aaii or Aii do not react in this way. The first two genotypes remain healthy, while the third develops mosaic symptoms.

More recently, Drijfhout (5) carried out an extensive investigation on the inheritance of resistance to BCMV, since none of the earlier researchers studied the inheritance of resistance to different strains of the virus. In fact, from the literature, he noted that only two recessive and one dominant resistance genes have been reported from work done in different areas of the world. He performed diallel crosses among the group of differential varieties selected in the previous section, giving preference to those mentioned in the literature (Table 2). The F_1 generation of the diallel crosses among the I^+I^+ materials was tested with strains of all pathogenicity groups and the F_2 with strains of all groups and subgroups (Tables 6 & 7).

After an extensive study of the segregation ratios, he theorized six recessive resistance genes, five of which are strain-specific: bc-1 (Imuna

Table 6. Testing F₁ of crosses in both directions between I⁺I⁺ differentials with strains of all pathogenicity groups.*

Cross		Pathogenicity group and strain														
P ₁	P ₂	I		II		III		IV		V		VI		VII		
		NL 1		NL 7		NL 8		NL 6		NL 2		NL 5		NL 4		
		P ₁	P ₂	F ₁	P ₁	P ₂	F ₁	P ₁	P ₂	F ₁	P ₁	P ₂	F ₁	P ₁	P ₂	F ₁
1 DW	x Imuna	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
2 DW	x RG-B	S	R	S	S	R	S	S	S	S	S	R	S	S	S	S
3 DW	x Michelite	S	R	S	S	R	S	S	R	S	S	S	S	S	S	R
4 DW	x Pinto 114	S	R	S	S	R	S	S	R	S	S	S	S	S	S	R
5 DW	x GN 31	S	Rd	S	S	Rd	S	S	Rd	S	S	Rd	S	S	R	S
6 DW	x IVT 214	S	R	S	S	R	S	S	R	S	S	R	S	S	R	S
7 Imuna	x RG-b	R	R	S	S	R	S	S	S	S	S	R	S	S	S	S
8 Imuna	x Michelite	R	R	S	S	R	S	S	R	S	S	S	S	S	S	R
9 Imuna	x GN 31	R	Rd	S	S	Rd	S	S	Rd	S	S	Rd	S	S	R	S
10 Imuna	x IVT 7214	R	R	(S)	S	R	S	S	R	S	S	R	S	S	R	S
11 RG-B	x Michelite	R	R	S	S	R	S	S	R	S	S	R	S	S	S	R
12 RG-B	x GN 31	R	Rd	S	S	Rd	S	S	Rd	S	R	Rd	S	S	R	S
13 RG-B	x IVT 7214	R	R	(S)	R	R	S	S	R	S	R	R	S	S	R	S
14 Michelite	x GN 31	R	Rd	S	S	Rd	S	S	Rd	S	S	Rd	S	S	R	S
15 Michelite	x IVT 7214	R	R	S	S	R	S	S	R	S	S	R	S	S	R	R
16 Pinto 114	x GN 31	R	Rd	S	S	Rd	S	R	Rd	S	S	Rd	S	S	Rd	S
17 GN 31	x IVT 7214	Rd	R	S	S	Rd	S	Rd	R	S	Rd	R	S	R	R	S

S = Susceptible, systemic mosaic, or systemic infection detectable by back-inoculation.

(S) = Susceptible, systemic infection detected by back-inoculation in some experiments only.

R = Resistant, no symptoms, no systemic infection detectable.

Rd = Resistant, local discoloration, no systemic infection detectable.

*From Drijfhout (1978) (5)

Table 7. Scheme of tests of F₂ progenies of the crosses between I⁺I⁺ differentials.*

F ₂ progenies tested	Pathogenicity group and strain											Numbers of strains used per F ₂ progeny
	I NL 1	II NL 7	III NL 8	IVa US 5	IVb NL 6	Va US 2	Vb NL 2	VIa NL 3	VIb NL 5	VII NL 4		
1 DW	x	x	x				x		x	x	6	
2 DW	x	x	x	x	x	x	x		x	x	7 + 2	
3 DW	x	x	x				x	x	x	x	7 + 1	
4 DW	x	x	x				x		x	x	6	
5 DW	x	x	x		x		x	x	x	x	7 + 1	
6 DW	x	x			x				x	x	5	
7 Imuna	x	x	x		x		x		x	x	7	
8 Imuna	x	x	x	x	x		x		x	x	6 + 1	
9 Imuna	x	x	x		x	x	x			-	5 + 1	
10 Imuna	x		x		x					x	4	
11 RG-B	x	x	x		x	x	x				5 + 1	
12 RG-B	x	x	x	x	x		x		x	x	7 + 1	
13 RG-B	x	x	x		x		x			x	6	
14 Michelite	x	x	x		x		x			x	7 + 1	
15 Michelite	x	x			x		x		x	x	6	
16 Pinto		x	x		x		x				4	
17 GN 31			x		x		x		x		4	
Total no. of F ₂ progenies tested	15	15	15	3	15	3	14	3	12	13	99 + 9	
1. x = F ₂ progeny of cross mentioned in row tested with strain noted in column.												

*From Drijfhout (1978) (5)

gene), bc-1² (RG-B gene), bc-2 (Michelite gene), bc-2² (GN-31 gene), and bc-3 (IVT 7214 gene). The remaining bc-u gene was strain-nonspecific. Based on this interpretation, the genotype of the differentials was determined (Table 8). In addition, the II differentials of Table 2 were crossed with Imuna, Michelite and GN-31, which carry genes bc-1, bc-2, and bc-1² plus bc-2², respectively. The F₂ generations of these crosses were tested with appropriate strains and the genotypes of these differentials was also determined (Table 9). Furthermore, Drijfhout worked out the pathogenicity genes, on a gene-for-gene host/virus parasite relationship basis, and suggested that twelve (if some are allelic) or sixteen strain genotypes are possible to be formed, seven of these types are presently known. Of the remaining nine, five are likely to be detected with the known set of differential varieties.

Table 8. Genotypes for Resistance (Host) and Pathogenicity (Virus) With a Gene-For-Gene Relationship Between the Strain-Specific Genes for Resistance and the Supposed Pathogenicity Genes.* One Allele for Each Pair of Resistance Genes is Mentioned.

Differential Cultivar	Resistance Genes	Strain group, virus strain and pathogenicity gene code									
		I	II	III	IV	V	VI	VII	VIII		
		NI 1	NL 7	NL 8	NL 6 or US 5	NL 2 or US 2	NL 5 or NL 3	NL 4			
		PO	P1	P2	P1.1 ²	P1.2	P1.1 ² .2	P1.1 ²	P1.1 ²	.2 ²	
Dubbele Witte	bc-u ⁺ bc-1 ⁺ bc-2 ⁺ bc-3 ⁺ I ⁺	+	+	+	+	+	+	+	+		
Imuna	bc-u bc-1 bc-2 ⁺ bc-3 ⁺ I ⁺	-	+	-	+	+	+	+	+		
RG-B	bc-u bc-1 ² bc-2 ⁺ bc-3 ⁺ I ⁺	-	-	-	+	-	+	+	+		
Michelite	bc-u bc-1 ⁺ bc-2 bc-3 ⁺ I ⁺	-	-	+	-	+	+	-	-		
Pinto 114	bc-u bc-1 bc-2 bc-3 ⁺ I ⁺	-	-	-	-	+	+	-	-		
GN 31	bc-u bc-1 ² bc-2 ² bc-3 ⁺ I ⁺	-	-	-	-	-	-	+	+		
IVT 7214	bc-u bc-1 ⁺ bc-2 bc-3 I ⁺	-	-	-	-	-	-	-	-		

*From Drijfhout (1978) (5)

+ = Positive relationship resulting in systemic infection.

Table 9. Observed and theoretical differentials, carrying II and combinations of the strain-specific genes, and their observed or expected positive reactions with the necrosis-inducing strains found, resulting in systemic necrosis.* Temperature 30°C.

Resistance group	Theoretical Combinations of strain-specific genes ¹	Observed differentials	Pathogenicity group, virus strain and supposed pathogenicity genes			
			III NL 8 P2	IVb NL 6 P1.1 ²	Vb NL 2 P1.1	VIa, VIb NL 3, NL 5 P1.1 ² .2
8		Widusa	+	+	+	+
9	bc-1	Jubila,	-	+	+	+
10	bc-1 ²	Topcrop				
		Amanda	-	+	-	+
(11) ²	bc-2		+	-	+	+
	bc-2 ²		-	-	-	-
	bc-3		-	-	-	-
	bc-1 bc-2		-	-	+	+
	bc-1 bc-2 ²		-	-	-	-
11	bc-1 bc-3		-	-	-	-
	bc-1 ² bc-2		-	-	-	+
	bc-1 ² bc-2 ²	IVT 7233	-	-	-	-
	bc-1 ² bc-3		-	-	-	-
	bc-2 bc-3		-	-	-	-
	bc-2 ² bc-3		-	-	-	-
	bc-1 bc-2 bc-3		-	-	-	-
	bc-1 bc-2 ² bc-3		-	-	-	-
	bc-1 ² bc-2 bc-3		-	-	-	-
	bc-1 ² bc-2 ² bc-3		-	-	-	-
1. As long as bc-1 and bc-1 ² , and bc-2 and bc-2 ² are allelic.						
2. Obtained from a genotype mixture of the original IVT 7233.						

*From Drijfhout (1978) (5)

6. Gene Combinations Suggested in the Literature for Resistance to BCMV

In 1974, Burke and Silbernagel (3) had indicated the need to incorporate the dominant (hypersensitive) type of resistance, conferred by the I gene, into all bean types. This recommendation was based on the fact that at that time BCMV strains prevalent in the U.S. did not induce the hypersensitive reaction (black root or top necrosis) at normal temperature (20°-25°C). However, Drijfhout (5) suggested the incorporation into II genotypes of strain-specific genes not overcome by the necrosis-inducing or temperature-independent strains. Specifically, he recommended the bc-2² gene, which is present in GN-31 and other cultivars of host group 6 (Tables 2 & 8).

Morales (16) suggested the incorporation of genes bc-1² and bc-2² into I gene materials. He considered this a good strategy since Table 3 indicates that cultivars carrying bc-1² and bc-2² (no I gene) are only susceptible to NL-4, but this strain is unable to attack II dominant carriers.

However, to provide the plants with a more lasting resistance since simultaneous mutation of more than one pathogenicity gene would break down more than one corresponding resistance gene, Drijfhout recommended the incorporation of one or two genes for resistance not yet overcome by the pathogens such as gene bc-3. Based on these considerations, he recommended bc-u bc-1² bc-2² bc-3 I as the best combination.

III. MATERIAL AND METHODS

1. Inoculum Preparation and Inoculation

A preliminary study was conducted to determine the optimum inoculum concentration to be used in this research study. Two inoculum concentrations, three tissue ages, and three bean varieties as sources of infected tissues were compared. The results indicated slight differences between the two concentrations used, namely, 1:5 and 1:10 (gm infected tissue : ml buffer). Also, infected tissue contained highest infectivity during the first three weeks after inoculation. Thus, the 1:5 concentration was selected for inoculum both when screening materials and when identifying isolates of the virus. Inoculum was prepared by triturating severely infected leaf tissue in 0.1 M Na_2HPO_4 - KH_2PO_4 buffer, pH 7.2, at the ratio of 1gm infected tissue per 5ml buffer. All materials used in the inoculations (mortar, pestle, buffer) were sterilized by autoclaving for 30 minutes.

Inoculations were performed by dipping the rough surface of the pestle in the inoculum and then rubbing the upper surface of expanded primary leaves previously dusted lightly with 320 mesh carborundum. Leaves were rinsed immediately after inoculation with distilled water. When making inoculations with different isolates, hands were washed between each inoculation. High greenhouse temperatures were maintained (25-30°C), with 16 hours per day supplemental lighting.

2. Strains of BCMV

The NL-3, New York-15 and Type strains of BCMV used in this study were kindly provided by Dr. M. J. Silbernagel, USDA/ARS, Prosser, Washington. NL-3 is a necrosis-inducing strain independent of temperature and induces systemic necrosis (top necrosis) in varieties carrying the I dominant gene, at both normal (20-25°C) and high (30°C) temperatures. This strain has not been reported in the U.S.A. NY-15 and Type strains do occur in the U.S.A., but both strains are unable to induce systemic necrosis in I dominant genotypes at normal temperature (25°C). At high temperature (30°C), they may induce systemic necrosis in I dominant genotypes. Also, the three strains have different patterns of reactions in susceptible I recessive genotypes (I^+I^+ differentials, Table 2); NL-3 strain is the most virulent.

These three strains comprised the control strains used in the identification studies. In addition, the NL-3 strain was used to screen varieties, breeding lines, and segregating populations for the presence of I dominant and recessive resistant (bc) genes.

3. Maintenance of Strains

Throughout the course of this study, the strains were maintained separately in inoculated plants of cultivars Sutter Pink, Dubbele Witte, or Sanilac. Also, infected seeds of these cultivars were harvested and stored for future use.

4. Collection of Isolates

The isolates studied in this research were obtained by two different methods. Two isolates, Sanilac Mosaic (SM) and Sanilac Necrotic (SN), were isolated from one infected plant of the Sanilac cultivar found in

the field at the Saginaw Valley Bean and Beet Research Farm, Saginaw, Michigan. In the summer of 1982, this variety was severely infected with virus at this experiment station. The canopy of leaves on the infected plant exhibited two different types of symptoms. Upper trifoliate leaves were mosaic infected, and the lower part of the canopy had relatively large, irregular necrotic lesions (Figure 1). Therefore, the sample taken from the upper leaves were designated as Sanilac Mosaic and the sample from the lower leaves as Sanilac Necrotic. These samples were then brought to the laboratory and refrigerated overnight. The following day inocula were prepared separately and were rub-inoculated onto the leaves of seedlings of Sutter Pink and Sanilac cultivars.

The second method of obtaining isolates was by sampling Foundation and Breeder seed lots. A sample of the Sanilac Foundation seed used for planting during the summer of 1982 at the Bean and Beet Farm was obtained. The seed was planted in a flat in the greenhouse and mosaic infected seedlings were observed. Infected tissue inoculum was prepared from these seedlings and rub-inoculated onto Sutter Pink and Sanilac cultivars. This isolate was designated Sanilac Foundation (SF). By the same procedure, another isolate was obtained from Breeder seed and designated Sanilac Breeder (SB).

5. Differential Host Cultivars

Differential cultivars employed in this study are those recommended by E. Drijfhout, et al, (7) to differentiate BCMV strains (Table 2). The cultivars are organized into nine groups depending on the allele of the I gene, and/or the possible recessive resistance genes in their genotypes. Host cultivars of groups 1 through 6 are I recessive carriers (I^+I^+) and,

if susceptible to any BCMV strain, they exhibit mosaic symptoms. Groups 7 through 9 possess the I dominant allele (II) exhibiting a hypersensitive reaction (called black root or top necrosis) when attacked by necrosis-inducing BCMV strains.

Based on the recommendations of E. Drijfhout, et al, (7) preference was given to the first cultivar of each group. They were inoculated with the test isolate, and symptom development was observed during the following two weeks.

Host group 1 cultivars are susceptible to all BCMV strains, and are used primarily to maintain the isolates and to reproduce the symptoms present in infected leaf samples. Another use given to them is to detect infection in symptomless plants by back-inoculation.

6. Methodology of Isolates Identification

A two step approach was employed as follows:

6.1 Confirmation of Isolates as BCMV:

To establish that BCMV was the pathogen causing the observed symptoms, isolates SM, SN and SF were rub-inoculated onto plants of the differential cultivars Sutter Pink and Sanilac (host groups 1 and 4). Inoculated plants of each differential cultivar were examined for development of mosaic symptoms one and two weeks after inoculation. Mosaic infected plants were retained and grown to maturity (Figure 2). The harvested seed was then planted and seedlings were closely examined for development of mosaic symptoms (seed transmission), a distinctive characteristic of BCMV.



Figure 1: Original Necrotic Lesions on Sanilac Cultivar;
Source of SN Isolate



Figure 2: Sanilac Plant Infected by SM Isolate

6.2 Identification of Isolates to Strain:

These tests were carried out by inoculating series of differential cultivars (Table 2). The reaction of each isolate on these cultivars was compared with those caused by NL-3, NY-15 and Type reference strains. This enabled their identification and differentiation.

A preliminary identification test was conducted by inoculating seedlings of cv. Black Magic, an I dominant gene carrier probably belonging to host group 7 (cvs. Black Turtle Soup and Widusa). These tests were carried out primarily to determine whether SM and SN were, in fact, necrosis-inducing isolates. The ability of an isolate to induce necrosis was confirmed when inoculated seedlings developed local lesions or top necrosis within the first week after inoculation (Figure 3). Inocula for this test was obtained from the infected plants of the previous step.

Based on the results of the preliminary test, more detailed identification tests were performed. These tests involved the use of the II differentials of Table 2 (host groups 7-9). Young infected tissues of isolates SM and SN were produced by transferring old infected tissue to seedlings of cv. Sutter Pink. Two growth chambers with constant air temperatures of 24° and 30°C were used. Cultivars used in these studies were: C-20, Black Turtle Soup, Widusa, Jubila, Topcrop, and Amanda. A test consisted of 48 seedlings (12 pots) rub-inoculated with the test isolate and 48 seedlings (12 pots) inoculated with NL-3 strain. Plants were assessed for development of local lesions or top necrosis 5-7 days after inoculation. SN was tested at both temperatures, whereas, SM and SN were tested only at the high temperature.

Additional identification tests were conducted in the greenhouse and included the complete set of differential host cultivars. At this stage

isolate SB was included. Four of the experiments were carried out at a mean day temperature of 26.6°C with 20°C overnight. The last two experiments were replicated over this temperature range (normal) and over a higher mean day temperature of 30°C with night temperature of 24.4°C. Four plants (1 pot) of each differential host cultivar were rub-inoculated. The first reading of the differentials for symptoms was made 5-7 days after inoculation. A second reading was made 10-12 days after inoculation to evaluate the remaining cultivars for development of systemic mosaic. In most cases, the first differential cultivar of each group was used for inoculation. However, in the last experiment, two differentials each of host groups 2, 3, 6, 7 and 8b and the three differentials of host group 4 were rub-inoculated per each isolate. In these experiments, strains NL-3 and NY-15 were used as checks in most cases.

7. Infectivity Tests

Differential cultivars in host groups 2, 3 and 6 not showing distinct mosaic symptoms after inoculation were assessed for presence of infective virus by back-inoculation to susceptible cultivars of host group 1.

The inoculations were performed only in the last two experiments. From each of the four inoculated plants, the third or fourth trifoliolate leaf was excised. Then, sap from these leaves were rub-inoculated onto seedlings of cvs. Dubbele Witte and Sutter Pink. Hands were washed between each back-inoculation. Assessment of mosaic symptoms on inoculated plants were done in the first and second week after inoculation.

8. Crossing Techniques

Crosses were performed in the bud stage without emasculation by the following pollination technique: the standard of an unopened flower bud was lifted up with a pair of fine forceps. The left and right wings were pressed downward, forcing the stigma out of the keel. The stigma was then rubbed with the desired pollen using a pollen-covered stigma from an open flower of the male plant.

9. Screening for Sources of Genetic Resistance to NL-3 Strain of BCMV

The NL-3 strain was used to accomplish this objective because it is a necrosis-inducing strain independent of temperature. When rub-inoculated on test plants, NL-3 was found to induce three reaction types: 1) Materials carrying the I gene recessive allele (I^+I^+) are susceptible and react with typical systemic mosaic; 2) Materials carrying only the dominant resistant (I gene) are hypersensitive and develop local lesions and top necrosis (black root); 3) Finally, materials carrying the recessive genes bc-2² and/or bc-3 show no symptoms (recessive resistant reaction), or, in the presence of gene I, they may develop scattered pin-point or restricted necrotic lesions on the inoculated primary leaf, which is considered a resistant reaction.

Four plants of each variety and breeding line were screened by rub-inoculation with this strain (NL-3). At the same time, four plants of a susceptible check were rub-inoculated. Five to seven days after inoculation, plants were observed for development of local lesions and top necrosis. Two weeks after inoculation, the remaining plants were examined either for systemic mosaic or no reaction at all. Varieties and breeding lines screened are shown in Tables 25 and 26.

In F_2 populations, all test plants were rub-inoculated together with the parents and one susceptible check. F_2 populations represent crosses made in spring and summer 1982. Thus, populations screened were divided into two groups. Spring crosses (made by Dr. J. Kelly) involved ten MSU elite breeding lines and the introduced variety Orfeo (Chile). The breeding lines possess the I dominant gene and Orfeo is a black-cultivar donor for both I dominant and bc-2² recessive resistant genes. The incorporation of this gene into I dominant gene materials confers resistance to all known strains of the virus (5). The F_1 generation was grown in the field during summer, 1982 and the F_2 screened in fall, 1982 in the greenhouse (Figure 4). The second group of F_2 populations (summer) represent crosses between MSU varieties and breeding lines with five CIAT (Centro Internacional de Agricultura Tropical) lines. The MSU materials carry the I dominant gene, whereas, CIAT lines are donors for both the dominant I and the recessive resistance genes bc-2² and bc-3. The bc-3 alone confers resistance to to all known BCMV strains and to other strains which theoretically may arise in the future (5). The F_1 generation was grown in fall, 1982 in the greenhouse and the F_2 screened in early spring, 1983, also in the greenhouse. The resistant plants were grown to maturity and back-crossed to MSU breeding lines and varieties. These results are shown in Tables 27, 28 and 29.



Figure 3: Top Necrosis Induced on Cv. Black Magic by SM Isolate



Figure 4: F₂ Plants Segregating for I Dominant (Top Necrosis) and bc-2² (Non-Reaction) Genes After Inoculating With NL-3 Strain of BCMV

IV. RESULTS AND DISCUSSION

1. Inoculum

1.1 Dilution

Although throughout this study the 1:5 inoculum concentration (gm infected tissue : ml buffer) was satisfactorily used, the 1:10 concentration proved to be effective, as well. The amount of virus particles in both inoculum concentrations was not counted but the effect on test plants was observed. When symptoms on these plants were compared, they were essentially the same. Trujillo (31) tested the effect of several inoculum dilutions on the number of local lesions on primary leaves of cv. Monroe and showed that the number of local lesions was inversely related to dilution of inoculum. At dilutions between 1:4 and 1:32, he found a straight line relationship between local lesion number and inoculum dilution. The 1:4 inoculum dilution was used in most of his work.

1.2 Age of Tissue

Tissue age is a second factor to be considered. We observed a reduction of infectivity in infected tissue taken at blossom or beyond (28 days after inoculation). To obtain best results in screening and in identification tests; it is recommended that infected tissue be taken prior to the fourth week after inoculation.

2. Confirmation of Isolates as BCMV

The results of planting seed harvested from mosaic-infected cultivars Sutter Pink and Sanilac plants are shown in Table 10. SM isolate was recovered in 40 and 25.4% of the infected seed planted of Sutter Pink and Sanilac, respectively; whereas, SN isolate was recovered in 54.7 and 100%. Also, seed infection of 34.8% was obtained from cv. Sutter Pink seed infected with SF isolate. These percentages of seed transmission indicate the seed-borne nature of the three isolates, thus confirming them as BCMV (Figure 5). These results agreed with the incidence of seed infection reported in the literature (8,24).

Table 10. Percent Transmission of BCMV in Seed Harvested From Mosaic-Infected Plants of Sanilac and Sutter Pink Cultivars

BCMV Isolate	Cultivars							
	Sutter Pink				Sanilac			
	No. Healthy	No. Infected	Total # Seed Planted	% Infected	No. Healthy	No. Infected	Total # Seed Planted	% Infected
SM	12	8	20	40.0	38	13	51	25.0
SN	19	23	42	54.7	0	13	13	100.0
SF	28	15	43	34.8	--	--	--	--

3. Identification of BCMV Isolates to Strain

3.1 Preliminary Test

A preliminary test conducted on cv. Black Magic (Table 11) at the high temperature range revealed that isolates SM and SN induced top



Figure 5: Seed Transmission of SM Isolate Through Seedlings of Sanilac Cultivar

necrosis (black root) on cultivars carrying the I dominant allele for hypersensitivity (Figure 3). In 1948, Grogan and Walker (9) reported that bean cultivars resistant to Type and NY-15 strains at normal temperature (20°- 28°C), developed the hypersensitive reaction, known as "black root," at higher temperatures (about 30°C). The induction of top necrosis at normal temperature by SM and SN was the first evidence suggesting that these isolates differ from the NY-15 and Type strains.

Table 11. Reaction of Black Magic Cultivar to SM and SN Isolates of BCMV

BCMV Isolate	No. Plants Inoculated	No. Plants Showing	
		Top Necrosis	No Reaction
SM	12	3	9
SN	12	11	1

3.2 Growth Chamber Tests

The first identification experiment, conducted at 30°C (Table 12), indicated that SN isolate induced top necrosis in cultivars of host group 7 (Widusa, Black Turtle Soup and N76006), similar to NL-3 strain. In addition, NL-3 strain induced top necrosis in plants of differential cultivars Jubila and Topcrop (host group 8a,b).

Table 12. Reaction of Differential Cultivars to
SN and NL-3 Isolates of BCMV at 30°C

Host Group	Differential Cultivar	BCMV Isolate							
		Sanilac Necrotic				NL-3			
		No. Plants Showing				No. Plants Showing			
		TN	VN	LL	NR	TN	VN	LL	NR
7	N76006* Widusa Black Turtle Soup	7 5 8	2			7 7 7			
8	a Jubila b Topcrop		7	6		1 6	5		
9	Amanda			7				6	

TN = Top necrosis VN = Vein necrosis mostly on inoculated leaf

LL = Local lesions or pin-point restricted necrotic
lesions on inoculated leaf

NR = No reaction or symptoms observed

* = MSU breeding line carrying the I gene

A second experiment, involving the same isolates was conducted in a 24°C growth chamber. The results (Table 13) indicate that SN isolate also induced top necrosis in plants of host group 7 at this temperature. Again, NL-3 strain caused top necrosis to plants in host group 7 and 8b, but not in host group 8a (cv. Jubila).

Table 13. Reaction of Differential Bean Cultivars
to SN and NL-3 Isolates of BCMV at 24°C

Host Group	Differential Cultivar	BCMV Isolate							
		Sanilac Necrotic (SN)				NL-3			
		No. Plants Showing				No. Plants Showing			
		TN	VN	LL	NR	TN	VN	LL	NR
7	C-20*	3	1			4			
	Widusa	6	2			8			
	Black Turtle Soup	4	2			8			
8	a Jubila		8				7		
	b Topcrop		8			8			
9	Amanda			5	1		6		

TN = Top necrosis VN = Vein necrosis mostly on inoculated leaf

LL = Local lesions or pin-point restricted necrotic lesions
on inoculated leaf

NR = No reaction or symptoms observed

* = Recently released variety from MSU carrying the I gene

In the final experiment isolates SM, SF and NL-3 were compared at 30°C. The results (Table 14) indicate that SF induces top necrosis in plants of host group 7, whereas, SM induced this reaction in plants of host groups 7 and 8b (cv. Topcrop). The differential cultivar Amanda (host group 9) is infected very slightly by SM (scattered pin-point lesions on inoculated primary leaves). NL-3 showed similar results to those of the previous experiment, namely, top necrosis induced in plants of host groups 7 and 8b. Thus, SM isolate resembles somewhat NL-3 strain but the pin-point restricted lesions (LL) caused by NL-3 on

cv. Amanda are larger and more numerous. NL-3 also causes a strong stunting of cv. Jubila with the first trifoliolate leaf exhibiting vein necrosis.

Table 14. Reaction of Differential Bean Cultivars to SM, SF and NL-3 Isolates of BCMV at 30°C

Host Group	Differential Cultivar	BCMV Isolate											
		Sanilac Mosaic				NL-3				Sanilac Foundation*			
		No. Plants Showing				No. Plants Showing				No. Plants Showing			
		TN	VN	LL	NR	TN	VN	LL	NR	TN	VN	LL	NR
7	C-20 Widusa Black Turtle Soup	6	1			8				1		2	5
		7		1		8				4			2
		8		1		8				2		4	
8	a Jubila b Topcrop	6	8			8	8				3	2	3
			1								3		
9	Amanda			3	5				8		3	3	2

TN = Top necrosis

VN = Vein necrosis mostly on inoculated leaf

LL = Local lesions or pin-point restricted necrotic lesions on inoculated leaf

NR = No reaction or symptoms observed

* = Inoculum source of infected tissue was older than the other two isolates

3.3 Greenhouse Experiments

The first of these experiments was carried out at the normal mean (26.6°C) ambient temperature. SN and SM isolates were tested with NL-3 strain as a check. This was, in fact, the first identification test performed. The results (Table 15) showed that both isolates infected plants of host groups 1 and 4 (cvs. Sutter Pink and Sanilac). SN induced vein necrosis on plants of host groups 7 and 8(a,b) while causing local lesions on plants of cv. Amanda (host group 9). SM induced no symptoms whatsoever on plants of cultivars carrying the I dominant gene. This inability of SM was probably due to a low concentration of virus particles in the source of infected tissue (old infected plants). NL-3 induced typical mosaic on plants of host groups 1, 4 and 5 (cvs. Sutter Pink, Sanilac and Pinto 114). It induced top necrosis to host groups 7 and 8(a,b); while host group 9 (cv. Amanda) was affected with local lesions.

Table 15. Reaction of Differential Bean Cultivars to SN, SM and NL-3 BCMV Isolates at the Normal Mean (26.6°C) Greenhouse Temperature.

Host Group		Differential Cultivar	BCMV Isolate																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
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1		Sutter Pink	4								1																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														

M = System mosaic TN = Top necrosis VN = Vein necrosis mostly on inoculated leaf

LL = Local lesions or pin-point restricted NR = No reaction or symptoms observed

necrotic lesions on inoculated leaf - = Different inoculated cultivar

A second experiment compared SN isolate and NY-15 strain at the normal mean (26.6°C) ambient temperature (Table 16). In addition to host groups 1 and 4, SN induced mosaic in plants of cv. Pinto 114 (host group 5) and top necrosis in plants of cvs. Widusa and Black Turtle Soup (host group 7). Host groups 8(a,b) and 9 were again affected with vein necrosis (cvs. Jubila and Topcrop) and restricted local lesions (cv. Amanda). Since NY-15 strain does not induce necrotic lesions at normal greenhouse temperature, the reaction induced by this strain was recorded as susceptible (systemic mosaic) or resistant (no symptoms at all). This strain (NY-15) only infected host groups 1, 4 and 5; the others groups were resistant. Obviously, the induction of necrotic lesions by SN separated this isolate from NY-15 strain.

Table 16. Reaction of Differential Bean Cultivars to SN and NY-15 Isolates of BCMV at the Normal Mean (26.6°C) Greenhouse Temperature.

Host Group		Differential Cultivar	BCMV Isolate					Reaction	
			Sanilac Necrotic						NY-15
			No. Plants Showing						
			M	TN	VN	LL	NR		
1		Sutter Pink	4					S	
2		Imuna					4	R	
3		Great Northern-123					3	R	
4		Sanilac	4					S	
5		Pinto 114	4					S	
6		Great Northern-31					4	R	
7		Widusa		3				*	
		Black Turtle Soup		4				*	
8	a	Jubila			4			R	
	b	Topcrop			4			R	
9		Amanda				4		R	

M = Systemic mosaic TN = Top necrosis

VN = Vein necrosis mostly on inoculated leaf

LL = Local lesions or pin-point restricted necrotic lesions on inoculated leaf

NR = No reaction or symptoms observed

* = Not tested

The results of the first experiment conducted with SF isolate (Table 17) at the normal mean (26.6°C) ambient temperature indicate that it induces vein necrosis on inoculated leaves of cvs. Widusa (host group 7), Jubila and Topcrop (host group 8a,b) as well as local lesions on inoculated leaves of cv. Amanda (host group 9). Also, SF produced systemic mosaic on plants of host groups 1, 4 and 5 (cvs. Sutter Pink, Sanilac and Pinto 114).

Table 17. Reaction of Differential Bean Cultivars to BCMV Isolate SF at the Normal Mean (26.6°C) Greenhouse Temperature.

Host Group	Differential Cultivar	BCMV Isolate Sanilac Foundation (SF)				
		No. Plants Showing				
		M	TN	VN	LL	NR
1	Sutter Pink	3				
2	Redlands Greenleaf-C					3
3	Redlands Greenleaf-B					4
4	Sanilac	1				
5	Pinto 114	3				1
6	Great Northern-31					3
7	Widusa			1		2
8 a	Jubila			3		
b	Topcrop			2		2
9	Amanda				2	

M = Systemic mosaic

TN = Top necrosis

VN = Vein necrosis mostly on inoculated leaf

LL = Local or pin-point restricted lesions on inoculated leaf

NR = No reaction or symptoms observed

Another experiment compared SN and SM isolates with NL-3, NY-15 and Type strains at the normal mean (26.6°C) ambient temperature. These results are shown in Table 18. SM only induced vein necrosis on inoculated leaf of cvs. Jubila and Topcrop (host group 8a,b), but no symptoms resulted on plants of cvs. Amanda (host group 9) and Black Turtle Soup (host group 7). Again, SN induced top necrosis on plants of host group 7 (cv. Black Turtle Soup), vein necrosis on plants of host group 8(a,b), and restricted local lesions on plants of host group 9. Host groups 1, 4 and 5 (cvs. Sutter Pink, Sanilac and Pinto 114) were affected with systemic mosaic by both isolates. In general, all symptoms were mild, probably influenced by the cool weather during fall 1982. The NL-3 strain induced top necrosis in cvs. Black Turtle Soup and Topcrop, but did not induce this reaction in cv. Jubila. Vein necrosis was produced on inoculated leaves of cv. Amanda. NL-3 also produced typical mosaic symptoms on plants in host groups 1, 4 and 5. Symptoms caused by NY-15 were essentially the same as in the previous experiment; namely, typical mosaic on plants of host groups 1, 4 and 5. The Type strain only infected (typical mosaic) plants of host group 1, as was expected.

Table 18. Reaction of Differential Cultivars to Five BCMV Isolates at the Normal Mean (26.6°C) Greenhouse Temperature

Host Group	Differential Cultivar	BCMV Isolate																			
		Sanilac Necrotic				Sanilac Mosaic				NL-3				NY-15				Type			
		No. Plants Showing				No. Plants Showing				No. Plants Showing				No. Plants Showing				No. Plants Showing			
		M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR
1	Sutter Pink	3				1	1					5					3	1			
2	Redlands Greenleaf-C					4					4					4					4
3	Redlands Greenleaf-B					4					4					4					4
4	Sanilac	3					2					1					2				4
5	Pinto 114	3					1					2					3				4
6	Monroe					3					4										4
7	Black Turtle Soup		2	2			4	4					4				3				4
8 a	Jubila		4	4			3														4
b	Topcrop		1	1		3	2						3			2					4
9	Amanda				4		4		1							3					1

M = Systemic mosaic
 TN = Top necrosis
 VN = Vein necrosis mostly on inoculated leaf
 LL = Local or pin-point restricted necrotic lesions on inoculated leaf
 NR = No reaction or symptoms observed

A study was then conducted at two greenhouse mean air temperatures (26.6°C and 30°C). Four isolates, namely, SM, SN, SF and SB, were compared with NL-3 and NY-15 strains. Results at the high temperature (Table 19) indicate that SM induced top necrosis on plants of host groups 7 (cv. Widusa) and 8b (cv. Topcrop). Extremely small restricted local lesions were induced on a few plants of cv. Amanda (host group 9) and vein necrosis on inoculated leaf of cv. Jubila (host group 8a). SN induced top necrosis on plants of host group 7, vein necrosis in host group 8a and restricted local lesions on plants of host groups 8b and 9. SF also induced top necrosis on plants of host groups 7 and 8b. Host groups 8a and 9 were affected with vein necrosis and restricted local lesions on inoculated leaves, respectively. SB isolate (Sanilac Breeder) induced top necrosis on plants of host group 7, but only vein necrosis on inoculated leaves of plants in host groups 8(a,b) and 9. Typical mosaic symptoms were produced by the four Sanilac isolates on plants of host groups 1, 4 and 5. NY-15 strain gave results that differed somewhat from those in the normal temperature tests. This time NY-15 induced top necrosis on plants of host group 7, as well as severe stunting and defoliation of primary leaves on cv. Jubila (host group 8a). This strain induced restricted local lesions on inoculated primary leaves of cvs. Topcrop (host group 8b) and Amanda (host group 9). The NL-3 strain induced top necrosis on plants of host groups 7 and 8b, and severe stunting and rapid defoliation of inoculated primary leaves (including vein necrosis on trifoliolate leaves) on cv. Jubila (host group 8a). Strain NL-3 appeared to induce more severe general stunting than the other isolates. Finally, this strain produced typical symptoms of mosaic in host groups 1, 4, and 5.

Differential cultivars in host groups 2 and 3 not reacting to the isolates (SN, SM, SF, SB and NL-3) were tested for infectivity by back-inoculation onto the susceptible cultivar Dubbele Witte (Table 24).

Table 19. Reaction of Differential Bean Cultivars to Six BCMV Isolates at the High Mean (30°C) Greenhouse Temperature

Host Group		BCMV Isolate																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
		Sanilac Necrotic				Sanilac Mosaic								Sanilac Foundation								Sanilac Breeder				NL-3				NY-15																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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		M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
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M = Systemic mosaic

TN = Top necrosis

VN = Vein necrosis on inoculated leaf

LL = Local or pin-point restricted necrotic lesions on inoculated leaf

NR = No reaction or symptoms observed

* = Virus recovered by back-inoculation onto cv. Dubbele Witte (Table 24)

The results of the experiment conducted at normal mean (26.6°C) temperature are generally similar to those obtained at the high mean temperature (Table 20), with two differences. Firstly, isolate SF did not induce top necrosis in plants of cv. Topcrop (host group 8b). Secondly, strain NY-15 did not induce top necrosis on plants of host group 7 or necrotic lesions to host groups 8(a,b) and 9. Cv. Jubila, which was recorded as exhibiting vein necrosis with NY-15, possessed only slight vein necrosis on one primary leaf. This may have resulted from the high temperature registered during some sunny days of winter 1982-83, when a temperature of 33°C was registered. Host groups 1, 4 and 5 were recorded as susceptible to the isolates and strains of this test. However, some days later, plants in these host groups suddenly died, which was unexpected under any condition.

Table 20. Reaction of Differential Bean Cultivars to Six BCMV Isolates at the Normal Mean (26.6°C) Greenhouse Temperature

Host Group	Differential Cultivar	BCMV Isolate																			
		Sanilac Necrotic				Sanilac Mosaic				Sanilac Foundation				Sanilac Breeder				NL-3			
		No. Plants Showing				No. Plants Showing				No. Plants Showing				No. Plants Showing				No. Plants Showing			
		M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR
1	Dubbele Witte	3					4					2					3	4			
2	Redlands Greenleaf-C					4					4					4		4			4
3	Redlands Greenleaf-B					4					4					4		4			4
4	Sanilac	4					4					4					4	4			
5	Pinto 114	4					4					4					4	4			
6	Monroe										4										
7	Widusa						3	1					4				4	4			
8	a Jubila		4				4							4					1		
	b Topcrop		4											4							
9	Amanda					4										4				3	

M = Systemic mosaic

TN = Top necrosis

VN = Vein necrosis mostly on inoculated leaf

LL = Local or pin-point restricted necrotic lesions on inoculated leaf

NR = No reaction or symptoms observed.

The last experiment was performed with SN, SB, NL-3 and NY-15, including several cultivars per host group and two ranges of greenhouse temperatures (15.5°-37.8°C and 23.3°-37.8°C). It should be noted that both temperature ranges were high; by contrast, what was initially planned as having a normal and a high greenhouse temperature. The frequent sunny days of March, 1983 raised the temperature of the greenhouse that had been designated as normal temperature.

Results are shown in Tables 21 and 22. In both replications, SM induced top necrosis on plants of cultivars in host groups 7 and 8b; vein necrosis was induced in plants of cv. Jubila (host group 8a). Once again, SM did not induce any type of restricted necrotic lesion on plants of cv. Amanda (host group 9). SB induced top necrosis on plants of cultivars in host group 7 and vein necrosis and restricted local lesions on inoculated leaves of cultivars in host groups 8 (a,b) and 9 (Figure 6). The NL-3 strain induced top necrosis on plants of cultivars in host groups 7 and 8(a,b); whereas, inoculated leaves of cv. Amanda plants developed vein necrosis and restricted local lesions.

NY-15 induced top necrosis in plants of host group 7 in both replications. Host group 8(a,b) exhibited leaf vein necrosis, although one plant of cv. Topcrop (host group 8b) died of top necrosis in one replication (Table 21). NY-15 also caused restricted local lesions on inoculated leaves of cv. Amanda in the same replication. The two isolates, along with the strains (checks), produced systemic mosaic in plants of cultivars in host groups 1, 4 and 5 (Sutter Pink, Sanilac and Pinto 114).

In both replications, plants in host groups 2, 3 and 6 not reacting to these isolates were assessed for virus infection by back-inoculation on susceptible cultivars Dubbele Witte and Sutter Pink. These results are shown in Table 24.

Table 21. Reaction of Differential Bean Cultivars to Four BCMV Isolates at High (23.3° - 37.8°C) Greenhouse Temperature Range (1st Rep.)

Host Group	Differential Cultivar	BCMV Isolate															
		Sanilac Mosaic				Sanilac Breeder				NL-3				NY-15			
		No. Plants Showing				No. Plants Showing				No. Plants Showing				No. Plants Showing			
		M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR	
1	Sutter Pink	4					4					4					/
2	Imuna					1*											4*
	Redlands Greenleaf-C					4*											4*
3	Redlands Greenleaf-B					4*											4*
	Great Northern-123					2*											2*
4	Sanilac	4					4					4					
	Michelite 62	4					4					4					
	Red Mexican-34	4					4					4					
5	Pinto 114	4					4					3					
6	Monroe					4*											4*
	Great Northern-31					4*											3*
7	Widusa		4					4					4				
	Black Turtle Soup		4					4					4				
8	a Jubila			3											4		
	b Topcrop		4													3	
	Improved Tendergreen		4											1			
9	Amanda					4										4	

M = Systemic mosaic TN = Top necrosis VN = Vein necrosis mostly on inoculated leaf

LL = Local or pin-point restricted lesions on inoculated leaf NR = No reaction or symptoms observed

/ = Not inoculated * = Tested for infectivity by back-inoculation onto cv. Dubbele Witte (Table 24)

Table 22. Reaction of Differential Bean Cultivars to Four BCMV Isolates at High (15.5° - 37.8°C) Greenhouse Temperature Range (2nd Rep.)

Host Group	Differential Cultivar	BCMV Isolate															
		Sanilac Mosaic				Sanilac Breeder				NL-3				NY-15			
		No. Plants Showing				No. Plants Showing				No. Plants Showing				No. Plants Showing			
		M	TN	VN	LL	NR	M	TN	VN	LL	NR	M	TN	VN	LL	NR	
1	Sutter Pink	4					4					4					
2	Imuna					4*					4*					3*	
	Redlands Greenleaf-C					4*					4*					4*	
3	Redlands Greenleaf-B					4*					4*					3*	
	Great Northern-123					2*					3*					3*	
4	Sanilac	4					4					4					
	Michelite 62	3					4					3					
	Red Mexican-34	4					4					4					
5	Pinto 114	3					4					4					
6	Monroe					4*					4*					4*	
	Great Northern-31					4*					4*					4*	
7	Widusa		4					4					4				
	Black Turtle Soup		4					4					4				
8	a Jubila			4					3					4			
	b Topcrop		4						3					3			
	Improved Tendergreen		3						4					3			
9	Amanda					4											4

M = System mosaic TN = Top necrosis VN = Vein necrosis mostly on inoculated leaf

LL = Local or pin-point restricted necrotic lesions on inoculated leaf NR = No reaction or symptoms observed

* = Tested for infectivity by back-inoculation onto cv. Sutter Pink (Table 24)

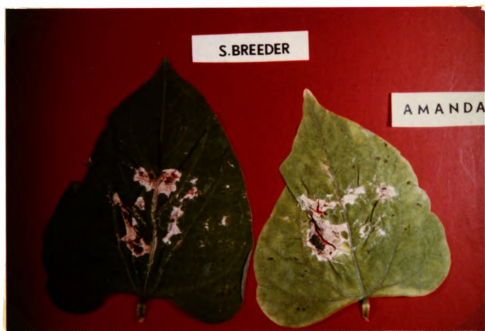


Figure 6: Restricted Local Lesions (Pin-Point) and
Vein Necrosis on Cv. Amanda Induced by
SB Isolate

Isolates SN and SB produced similar reactions on the differential cultivars, but did not induce top necrosis on cv. Topcrop (host group 8b), whereas, SM and SF did. This response of cv. Topcrop thus differentiated SM and SF from SN and SB isolates. However, there were two differences between SM and SF isolates. Firstly, SM induced top necrosis on cv. Topcrop at both mean temperatures (independence of temperature), whereas, SF only induced this reaction at the high mean temperature. Secondly, isolate SF produced restricted local lesions (pin-point) on inoculated leaves of cv. Amanda, whereas, SM did not. However, SM proved to be the most virulent isolate, being more virulent than NY-15 strain (Table 23).

Throughout this study, strain NL-3 remained independent of temperature in inducing the black root symptom. The results of other work reported in the literature (7,32) have shown that it is the second most virulent BCMV strain (to NL-5 strain) so far identified. Also, in this study, an increase in virulence was noticed when NL-3 interacted with high temperature. In such a condition, it induced the killing reaction (top necrosis) on cv. Jubila (host group 8a). This effect was not produced by any of the isolates studied herein (Table 23). Moreover, the reactions of plants in host groups 2 and 3 were characterized as tolerant on the basis of infectivity tests.

The Sanilac isolates tend to resemble NL-3 more than the NY-15 strain. The chief difference from NY-15 is the ability of Sanilac isolates to induce top necrosis in plants in host groups 7 and 8b (SM and SF) at normal temperature.

A comparison of this data with that of Drijfhout, et al, (7) indicates that isolates of this study are probably related to Strain Groups V and VI (Table 3). These strain groups are the only ones capable of infecting cv.

Pinto 114 (host group 5). However, in I gene cultivars (host groups 7-9), Strain Group V does not induce top necrosis to cultivars in host group 7 (Widusa and Black Turtle Soup). NL-3 strain belongs to Strain Group VI and the differences with this strain are clearly indicated by the foregoing discussion. Thus, these isolates represent two pathogenicity patterns (SN-SB and SM-SF) that do not closely fit those in Table 3, based on reaction in host groups 3 and 8b.

The question arises how did these two potential new pathotypes of BCMV originate here in Michigan. The answer to such a question may be connected with the increase of MSU Sanilac Breeder seed class in Idaho. M. J. Silbermagel, in personal communication with Dr. A. W. Saettler, has indicated that a pathotype similar to NL-8 strain was found among BCMV isolates that he and a colleague collected in Idaho in 1977 (unpublished data). Also, he noted that among the Idaho isolates and other isolates from Tanzania, pathotypes that infect host groups 1, 4 and 5 (mosaic symptoms) were found, and that these induce top necrosis in cvs. Black Turtle Soup (host group 7) and Topcrop (host group 8b). This is, in fact, the pathogenic range of isolates SM-SF. On the other hand, a recent report (22) on the isolation of NL-8 strain in New York from Sanilac seed class grown in Michigan, suggests the possibility that this strain also exists in Michigan. Actually, the main difference between SN-SB isolates and NL-8 strain is the inability in the latter of infecting cv. Pinto 114 (host group 5). It seems unlikely that so many different strains can be introduced in an area (Michigan) in a relatively short period of time. Moreover, since NY-15 strain infects cv. Pinto 114, it may be that SN and SB isolates really represent a mixture of NL-8 and NY-15 strains. Such a mixture will induce mosaic in cv. Pinto 114 and top necrosis in cv.

Black Turtle Soup. Although this possibility was not investigated in this study, it may be confirmed by subculturing virus from SN and SB infected Pinto 114 plants onto the differential cultivars. This would clarify whether there was a mixture of NY-15 and NL-8 strains, or whether the isolates (SN-SB) represent a unique pure culture. The possible presence of a mixture may also account for the variability in reaction between four necrotic-inducing isolates (SM, SF, SN and SB).

Table 23. Reactions of Differential Bean Cultivars to Six Isolates of Bean Common Mosaic Virus Over Two Mean Temperatures

Host Group	Differential Cultivar	Mean-Air Temperature (°C)	BCMV Isolates					
			Sanilac Mosaic	Sanilac Necrotic	Sanilac Foundation	Sanilac Breeder	NY-15	NL-3
1	Dubbele Witte	26.6 30	S S	S S	S S	S S	S S	S S
2	Redlands Greenleaf-C	26.6 30	R* T	R* T	R* T	R* T*	R* R	R* T
3	Redlands Greenleaf-B	26.6 30	R* T	R* T	R* T	R* T*	R* R	R* T
4	Sanilac	26.6 30	S S	S S	S S	S S	S S	S S
5	Pinto 114	26.6 30	S S	S S	S S	S S	S S	S S
6	Monroe	26.6 30	R R	R R	R R	R R	R R	R R
7	Widusa	26.6 30	N N	N N	N N	N N	R N	N N
8 a	Jubila	26.6 30	I I	I I	I I	I I	R I	I N
b	Topcrop	26.6 30	N N	I I	I N	I I	R I	N N
9	Amanda	26.6 30	R R	I* I*	I* I*	I* I*	R I*	I* I*

S = Susceptible, moderate to severe mosaic symptoms

T = Tolerant, mild, atypical, delayed or absent

T* = Tolerant or resistant, virus recovered once out of three infectivity tests

R = Resistant, no systemic symptoms of mosaic

R* = Resistant or tolerant, no attempts to recover virus

N = Necrotic tip kill of most/all plants

I = No systemic top necrosis, vein necrosis on inoculated leaves

I* = No systemic top necrosis, restricted local lesions and/or restricted vein necrosis on inoculated leaves

3.4 Infectivity Tests

The results of the three infectivity tests carried out are summarized in Table 24. Plants in host groups 2, 3 and 6 exhibiting no symptoms of infection were assessed for presence of virus by back-inoculation onto leaves of a susceptible check plant (host group 1). In the first experiment, cultivars Redlands Greenleaf-C and Redlands Greenleaf-B (host groups 2 and 3), previously inoculated with isolates SM, SN, SF and SB and with NL-3 strain (Table 18), were back-inoculated onto leaves of the Dubbele Witte cultivar. Presence of mosaic symptoms in these tester plants revealed that the tested cultivars were, in fact, infected but symptomless. Host group 6 comprises those cultivars carrying the recessive resistant gene $bc-2^2$, one of the genes on which the present breeding effort is based. This gene has only been overcome by NL-4 strain (7,32). Thus, to confirm the assumption that these isolates do not attack this gene ($bc-2^2$), host group 6 cultivars were added to host groups 2 and 3 in the last two tests. Cultivar Dubbele Witte was the susceptible check in the second test and isolates tested were SM and SB along with the NY-15 and NL-3 strains (Table 21). The third infectivity test included the same isolates (SM and SB) as well as the same strains (NY-15 and NL-3) (Table 22); but the susceptible check was cv. Sutter Pink.

Similar results were obtained in both tests with SM infecting plants of cultivars in host groups 2 (Imuna and Redlands Greenleaf-C) and 3 (Redlands Greenleaf-B and Great Northern-123); but not in host group 6 (Monroe and Great Northern-31). The reaction obtained from cultivars inoculated with NL-3 was essentially the same as those for SM, but with a few differences. These differences occurred in the third infectivity test where NL-3 infected cvs. Imuna and Great Northern-123 and SM did not.

SB did not infect any of these cultivars. This lack of reaction in the last two tests was unexpected, since in the first test this isolate infected plants in host groups 2 and 3 (Table 18). Moreover, cv. Pinto 114, which possesses bc-u, bc-1 and bc-2 recessive resistant genes (Table 8), was infected by the four isolates; thus, it was expected that each isolate, at least, would infect host group 2 (bc-u, bc-1 gene cultivars). More biological indexing of plants in host groups 2 and 3, inoculated with isolates SN, SF and SB, will be needed before a definite conclusion on the infectivity of these strains can be reached. Finally, the reaction of NY-15 strain was similar to SB isolate.

Table 24. Reaction of Cultivars Dubbele Witte (D.W.) and Sutter Pink (S.P.) Inoculated with Sap from Symptomless Plants in Host Groups 2, 3 and 6 at the High Mean (30°C) Greenhouse Temperature.

Host Group	Differential Cultivar	Test 1 (D.W.)						Test 2 (D.W.)						Test 3 (S.P.)					
		Isolates						Isolates						Isolates					
		SM	SN	SF	SB	NL-3		SM	SB	NL-3	NY-15			SM	SB	NL-3	NY-15		
2	Redlands Greenleaf-C Imuna	+	+	+	+	+		+	-	+	-			+	-	+	-		
		nt	nt	nt	nt	nt		+	-	nt	nt			-	-	+	-		
3	Redlands Greenleaf-B Great Northern-123	+	+	+	+	+		+	-	+	-			+	-	+	-		
		nt	nt	nt	nt	nt		+	-	nt	-			-	-	+	-		
6	Monroe Great Northern 31	nt	nt	nt	nt	nt		-	-	-	-			-	-	-	-		
		nt	nt	nt	nt	nt		-	-	-	-			-	-	-	-		

SM = BCMV isolate Sanilac Mosaic

SN = BCMV isolate Sanilac Necrotic

SF = BCMV isolate Sanilac Foundation

SB = BCMV isolate Sanilac Breeder

NL-3 = BCMV strain

NY-15 = BCMV strain

D.W. = Dubbele Witte

S.P. = Sutter Pink

+ = Systemic mosaic

- = No reaction

nt = Not tested

4. Screening for Sources of Genetic Resistance to NL-3 Strain of BCMV

4.1 Varieties and MSU Elite Breeding Lines

Screening with NL-3 strain provided a rapid technique to detect not only I dominant gene materials, but also materials segregating for recessive resistant genes not overcome by this strain, such as bc-2² and bc-3. Using the NY-15 strain, Thomas and Fisher (30) screened single plant selections (snap beans) for the presence of I gene but the inoculated seedlings had to be incubated at 90°F. This screening technique, though effective, thus requires special conditions (90°F) and may have space limitations. The advantage of using a temperature-independent necrosis-inducing strain to avoid absence of systemic necrosis was also pointed out by E. Drijfhout (5) and Walkey and Innes (32).

The reactions of the varieties and breeding lines screened against strain NL-3 were scored as: hypersensitive or dominant resistance (+h), recessive resistance or no reaction (-) and susceptible or mosaic symptoms (+). The results in Table 25 indicate that most varieties tested fall into either the dominant or the recessive resistance reaction. Susceptible varieties included: Tall Bunyan, Kentwood, D-79054, Agate, Pindak, Michigan Improved Cranberry, Taylor, Charlevoix, MSU 0688, Manitou and Ruddy. Those that showed the recessive resistant reaction were: D79147, Rufus, Garnet, Olathe, Ouray, Red Kloud, U. of Calif. 2602 and Orfeo. Thus, these cultivars may carry the strain-specific resistance genes bc-2² and/or bc-3. Cv. Orfeo, however, yielded several plants that developed top necrosis, suggesting, heterogeneity at the bc-2² locus in this variety. Plants exhibiting the top necrosis reaction (+h) resulted from the presence of the unprotected I dominant gene; thus, cv. Orfeo needed to be purified. This was accomplished by harvesting the seed from the resistant plants (no reaction or pin-point restricted lesions on inoculated primary leaf).

Table 25. Bean Varieties Screened With the NL-3 Strain of BCMV for the Presence of I Dominant Gene for Resistance.

Variety	Seed Type	Reaction
C-20	Navy	+h
Ex-Rico	Navy	+h
Tall Bunyan	Navy	+
Fleetwood	Navy	+h
Kentwood	Navy	+
Artic	Navy	+h
Opal	Navy	+h
Swan Valley	Navy	+h
D-79054	Navy	+
D-77196	Navy	+h
WGT 8001	Navy	+h
Sanilac	Navy	+
LA-182	Navy	+h
WGT 7901	Navy	+h
D79147	Small Red	-
Rufus	Small Red	-
Garnet	Small Red	-
Agate	Pinto	+
Olathe	Pinto	-
Ouray	Pinto	-
Pindak	Pinto	+
Mich. Improv. Cran.	Cranberry	+
Taylor	Cranberry	+
028	Cranberry	+h
Mo. Res. Vine Cran.	Cranberry	+h
Black Magic	Black	+h
Black Beauty	Black	+h
Midnight	Black	+h
Domino	Black	+h
Ebony	Black	+h
D78214	Black	+h
Orfeo	Black	(+h) and (-)
Negro Argel	Black	+h
Montcalm	Dark Red Kidney	+h
Charlevoix	Dark Red Kidney	+
Royal Red	Dark Red Kidney	+h
Red Kloud	Light Red Kidney	-
MSU 068	Light Red Kidney	+
Manitou	Light Red Kidney	+
Ruddy	Light Red Kidney	+
Linden	Light Red Kidney	+h
2602	Light Red Kidney	-

+h = Hypersensitive reaction (top necrosis)

+ = Susceptible (typical mosaic) - = Resistant, no reaction

Breeding lines tested possessed either the dominant type of resistance (top necrosis) conferred by the I dominant gene or the recessive allele of this gene that confers susceptibility (typical mosaic) to NL-3 (Table 26). The hypersensitive reaction induced by this gene was referred to as "black root" by Grogan and Walker (9). Then, Ali (1) showed that this gene, from cv. Corbet Refugee, was responsible for this reaction in the absence of recessive resistant genes. Susceptible lines were N81038 and N81095. Lines N79023, N81017, N81023, N81037 and N81064 occasionally showed both mosaic infection and top necrosis. This indicates that these lines are heterogeneous for the I gene locus.

Table 26. MSU Elite Breeding Lines Screened With the NL-3 Strain of BCMV for the Presence of I Dominant Gene for Resistance.

MSU Breeding Line	Seed Type	Reaction
N74001	Navy	+h
N76006	Navy	+h
N76008	Navy	+h
N79021	Navy	+h
N79023	Navy	+h (+)
N79028	Navy	+h
N79034	Navy	+h
N80014	Navy	+h
N80038	Navy	+h
N80038	Navy	+h
N80043	Navy	+h
N80054	Navy	+h
N80058	Navy	+h
N80059	Navy	+h
N80061	Navy	+h
N80068	Navy	+h
N81002	Navy	+h
N81004	Navy	+h
N81016	Navy	+h
N81017	Navy	+h (+)
N81023	Navy	+h (+)
N81026	Navy	+h
N81037	Navy	+h (+)
N81038	Navy	+
N81052	Navy	+h
N81058	Navy	+h
N81062	Navy	+h
N81064	Navy	+h (+)
N81077	Navy	+h
N81086	Navy	+h
N81095	Navy	+
AL-16*	Pompadour	+h
AL-17*	Pompadour	+h
AL-24*	Pompadour	+h

+h = Hypersensitive reaction (top necrosis)

+h (+) = Presence of both hypersensitive reaction and mosaic infected Plants

* = Dominican Republic breeding lines

4.2 F₂ Populations

The F₂ populations screened comprised two groups of materials. One group represents crosses made to introduce strain-specific resistance gene bc-2² from cv. Orfeo. The results of the ten F₂ populations screened are summarized in Table 27. The original concept was to incorporate the recessive resistant gene into I dominant gene materials. Therefore, if cv. Orfeo and all breeding lines were homozygous dominant for the I gene, then this gene had been fixed and bc-2² would have been the only one segregating. Thus, a segregation ratio of 3:1 (TN:R) was expected. However, the population number 81N188 was the only one that really fulfilled this assumption (Figure 4). A chi-square (χ^2) test performed on the segregation of top necrosis to resistant plants (84:42) showed that this cross fits the theoretical 3:1 ratio. Populations 81N186 and 81N189 had fewer resistant plants (122:2 and 121:1, respectively) to fit this ratio. All plants of populations 81N185, 81N187, 81N191 and 81N193 were killed by the hypersensitive reaction (top necrosis). This suggests that, in fact, these populations represent crosses between breeding lines carrying I dominant gene and the few Orfeo plants not carrying the recessive resistant gene bc-2² (unprotected I dominant gene).

Innes and Walkey (8) tested F₂'s of several crosses involving I gene parents with the NVRS strain of BCMV (Pathogen Group VIb, Table 3), and all plants were resistant or did not react, which is equivalent to a necrosis-inducing strain, such as NL-3. Results from populations 81N184, 81N190 and 81N194 are less clear and harder to explain. These populations yielded both mosaic infected plants and a few plants that were not infected (resistant). This only could have happened if the selected plants of Orfeo were heterogeneous for bc-2² and the female plants (MSU breeding lines) had been heterogeneous

for the I gene locus. However, it is more likely that these few plants somehow escaped from mosaic infection or top necrosis. Two events may have occurred to allow this: (a) selections of Orfeo used carried only the I dominant gene; and (b) the plants of MSU breeding lines chosen to make the crosses happened to be homozygous recessive for the I gene (I^+I^+) (i.e., individuals selected from a heterogeneous parent population).

Finally, it is clear that if these plants that did not react were eliminated from the calculations, the resulting ratio of top necrosis : mosaic infected plants would fit the theoretical 3:1 (R:S) ratio (Table 27). The resistance here refers to segregation of the dominant I gene.

These results suggest the need of checking the parental lines for homogeneity prior to crossing. This was demonstrated with the second group of F_2 plants screened where no mosaic infected plants segregated.

Table 27. Reaction of Ten F₂ Populations to NL-3 Strain of BCMV (Spring Crosses)

Population Number	Number of Plants Showing				No. of Plants Tested	Ratio	Probability Between
	M	TN	LL	NR		TN:R	
81N184	42	114		7	163	(3:1*)	.500-.750
81N185		125			125		
81N186		122	2		124		
81N187		122			122		
81N188		84	38	4	126	3:1	.050-.100
81N189		121	1		122		
81N190	58	205		20	283	(3:1*)	.250-.500
81N191		121			121		
81N193		116			116		
81N194	17	52		5	74	(3:1*)	.950-.975

M = Typical mosaic symptoms (=S)

TN = Top necrosis (dominant resistance)

LL = Local lesions on inoculated leaf
(recessive resistance reaction or R)

NR = No reaction or symptoms observed
(recessive resistance reaction or R)

(*) = Hypothetical ratio of top necrosis to mosaic infected plants if non-reacting plants are ignored.

The resistant plants of populations 81N184 and 81N188 were back-crossed to their recurrent parents, C-20 and N81017, respectively; and also crossed to other MSU elite breeding lines and varieties. Similarly the resistant plants of populations 81N190 and 81N194 were crossed back to MSU materials. Back-crosses and crosses to MSU materials were as follows:

Back-Crosses

C-20 * 2 / Orfeo
N81017 * 2 / Orfeo

Crosses to MSU Varieties and Breeding Lines

Domino	//	C-20	/	Orfeo
C-20	//	N81017	/	Orfeo
Midnight	//	N81017	/	Orfeo
N79021	//	N81017	/	Orfeo
N79012	//	N81017	/	Orfeo
Domino	//	N81017	/	Orfeo
Black Magic	//	N81017	/	Orfeo
Black Magic	//	N81038	/	Orfeo
Domino	//	N81038	/	Orfeo
C-20	//	N81095	/	Orfeo
Domino	//	N81095	/	Orfeo

The second group of screened F_2 populations represents crosses made with CIAT early generation materials, probably F_3 . Thus, the populations comprised a heterogeneous mixture of genotypes relative to the presence of recessive genes $bc-2^2$ and $bc-3$. As a result of a necrosis test performed with NL-3 on the MSU and CIAT lines prior to crossing, both sources of parents proved to be homogeneous for the I gene dominant allele. Therefore, the results (Table 28) show no evidence of mosaic infected plants when inoculated with NL-3; by contrast, the segregation observed (hyper-sensitive to resistant reaction) is that characteristic for recessive resistant genes.

Table 28. Reaction of Seventeen F₂ Populations to NL-3 Strain of BCMV (Summer Crosses)

Population Number	No. of Plants Showing				No. Plants Tested	Reaction TN:R
	M	TN	LL	NR		
82M001		24	2	10	36	24:12
82M002		41	4	18	63	41:22
82M003		40	6	25	71	40:31
82M004		37	4	7	48	37:11
82M005		20	1	8	29	20:9
82M006		35		17	52	35:17
82M007		54		21	75	54:21
82M008		64	2	21	87	64:23
82M009		35		24	59	35:24
82M010		34		7	41	34:7
82M011		58	14	27	99	58:41
82M012		49		9	58	49:9
82M013		6		6	12	6:6
82M014		57	4	12	73	57:16
82M015		52		15	67	52:15
82M016		32		12	44	32:12
82M017		88	2	9	99	88:11

M = Typical mosaic symptoms
TN = Top necrosis

LL = Local lesions on inoculated leaf
NR = No Reaction (=R)

F₂ plants from twelve populations segregated in a 3:1 ratio for hypersensitive (TN) and resistant reactions. Possibly the gene segregating here is the bc-3, since few restricted local lesions (pin-point) were observed on inoculated plants. E. Drijfhout (5) obtained 3:1 ratios when the strain used (NL-3 or NL-5) was effective against one of two recessive genes present in the segregating population. In our case, NL-3 strain attacked neither bc-2² nor bc-3; thus, larger numbers of recessive resistant plants were expected. Since the CIAT lines were F₃ generation, it can be assumed they were not homozygous for the two recessive genes; perhaps some of them just carried either bc-2² or bc-3. Selfing in population 82M017 (large number of top necrosis plants) prevented a correct determination of segregation ratios. The segregation of bc-2² and bc-3 is clearly indicated by the remaining population ratio of top necrosis to resistant plants. Four populations (82M003, 82M006, 82M009 and 82M011) appeared to fit a 9:7 complementary gene ratio (Table 29). In addition, population number 82M013 segregated with equal numbers of top necrosis and resistant plants (no reaction). It is likely that had the population been larger (20 plants), the ratio would more closely have fit 9:7. Drijfhout (5) also obtained a 9:7 ratio in segregating populations screened with NL-5 for the recessive genes bc-1², bc-2, bc-2², and bc-3. He pointed out that strain NL-5 overcame the recessive genes bc-1² and bc-2, accounting for the observed ratio. In the present case, NL-3 strain attacked those I dominant gene plants not protected by bc-2² and/or bc-3.

Table 29. Observed Number and Theoretical Ratios of Top Necrosis to Resistant Plants in F₂ Populations Segregating for the Recessive Resistant Genes bc-2² and bc-3.

Cross Number	Pedigree	Observed Results			Ratio TN:R	Probability Between
		TN	R	Total		
82M001	BAT338/ /IVT7233/IVT7214/3/N79023	24	12	36	3:1	.100-.250
82M002	BAT338/ /IVT7233/IVT7214/3/N81064	41	22	63	3:1	.050-.100
82M003	BAT338/ /IVT7233/IVT7214/3/Midnight	40	31	71	9:7	.950-.975
82M004	A23/ /IVT7233/IVT7214/3/Black Magic	37	11	48	3:1	.500-.750
82M005	BAT44/ /IVT7233/IVT7214/3/Domino	20	9	29	3:1	.250-.500
82M006	BAT44/ /IVT7233/IVT7214/3/Sawn Valley	35	17	52	9:7	.100-.250
82M007	BAT44/ /IVT7233/IVT7214/3/N79021	54	21	75	3:1	.500-.750
82M008	BAT44/ /IVT7233/IVT7214/3/N79023	64	23	87	3:1	.500-.750
82M009	BAT44/ /IVT7233/IVT7214/3/Swan Valley	35	24	59	9:7	.100-.250
82M010	BAT44/ /IVT7233/IVT7214/3/Black Magic	34	7	41	3:1	.100-.250
82M011	C-20/3/BAT338/ /IVT7233/IVT7214	58	41	99	9:7	.500-.750
82M012	C-20/3/BAT44/ /IVT7233/IVT7214	49	9	58	3:1	.050-.100
82M013	N79021/3/BAT338/ /IVT7233/IVT7214	6	6	12	---	---
82M014	N79021/3/BAT44/ /IVT7233/IVT7214	57	16	73	3:1	.500-.750
82M015	N79023/3/BAT44/ /IVT7233/IVT7214	52	15	67	3:1	.500-.750
82M016	Swan Valley/3/BAT44/ /IVT7233/IVT7214	32	12	44	3:1	.500-.750
82M017	Domino/3/BAT338/ /IVT7233/IVT7214	88	11	99	---	---

TN = Hypersensitive reaction (top necrosis) of I gene materials.

R = Recessive resistant reaction.

V. CONCLUSION

Two general patterns of symptoms were induced by BCMV isolates identified in this research work: (a) SN and SB isolates induce systemic msoaic on plants in host groups 1, 4 and 5 (Table 23) and top necrosis in plants of host group 7; and (b) SM and SF isolates affected the same host groups plus top necrosis was induced on plants in host group 8b (Topcrop and Improved Tendergreen). SM was recovered in three infectivity tests carried out on symptomless plants in host groups 2 and 3, whereas, the other isolates, though recovered once, will require more biological indexing.

The severe induction of top necrosis in cvs. Widusa, Black Turtle Soup and Topcrop is comparable only with that induced by the European strains NL-3, NL-5 and NL-8 as reported by Drijfhout, et al, (7). Thus, these represent new necrosis-inducing strains and are of greater host range as compared with Type, NY-15 and Western strains (previously identified U.S.A. BCMV strains); in addition, they induced the hypersensitive reaction (black root or top necrosis) at normal mean greenhouse temperature (26.6 C). Recent reports on the occurrence of new BCMV strains with the necrosis-inducing ability property of European strains [NL-8 in New York (22); and an unknown necrotic strain in Puerto Rico, Dr. G. Freytag, MITA, personal communication] seem to indicate that new BCMV strains are finding entrance into this part of the world.

The ability of these new BCMV strains to induce top necrosis constitutes a major new threat not only for Michigan dry beans but also for bean production (dry and snap beans) in the U.S.A., since American breeders have favored the use of the unprotected I gene as a source of BCMV resistance. The recessive type of resistance (strain specific) along with the I dominant gene need to be considered for designing future breeding strategy. Although the combination of I gene plus the recessive resistant gene bc-2² provided a good source of resistance to BCMV, the presence of pin-point restricted necrotic lesions in materials possessing both genes indicates that the bc-3 gene may be a more reliable source of resistance (no reaction at all). Both genes are now present in MSU breeding lines. Also, there is a possibility of combining the three genes (I, bc-2² and bc-3) into bean genotypes possessing good performance in other traits. It should be noted that among screened varieties the presence of recessive resistant genes was detected among pinto (Olathe and Ouray) and small red types (D79147, Rufus and Garnet).

Finally, since SB isolate was obtained from breeder seed stocks grown and maintained in Idaho, it suggests that this could be the original source of these new strains with the subsequent introduction into the Foundation seed stocks in Michigan.

Thus, based on these results, the new breeding approach underlined in this research to stop the spread of these strains in Michigan and elsewhere is summarized in the following statements:

- a) Deployment of new dry bean cultivars possessing
I dominant gene and recessive resistant genes
that have previously been tested against

actual occurring strains, such as in the present case of bc-2² and bc-3.

- b) Cooperation between breeders and plant pathologists of different breeding programs to exchange resistant materials that may provide both with more reliable resistant genes and more virulent strains with which to screen. This will allow the detection of new sources of resistance ahead of the virus mutation. Moreover, screening techniques, like the one used in this study, should be developed to make sure that selected parent stocks carry the required resistance (long-term objective).
- c) Because of the seed transmission nature of BCMV, there is a continued danger of introducing new strains, particularly , when seed is imported from areas where BCMV is known to be prevalent. Therefore, seed stocks increased in certain Western states should be monitored for seed-borne BCMV, since the activity of aphid vector is greater in those states.
- d) Eliminate current BCMV susceptible (I^+I^+) varieties from the production area. Actually, cultivars of different seed types screened in this study showed either the dominant type of resistance conferred by the I dominant gene or the recessive type (bc) genes. These cultivars should be substituted for the susceptible ones immediately.

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VI. LIST OF REFERENCES

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