MULTIPLICATION AND DISTRIBUTION OF BEAN COMMON MOSAIC VIRUS (BCMV) IN BEAN

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
EPHRAIM J. A. EKPO
1972

S-351

MAY 24 2003

ABSTRACT

MULTIPLICATION AND DISTRIBUTION OF BEAN COMMON MOSAIC VIRUS (BCMV) IN BEAN.

BY

EPHRAIM J.A. EKPO

Various aspects of multiplication and distribution of bean common mosaic virus 1 ($BCMV_1$) in the susceptible bean variety Mich-Cal-Cranberry were studied with the aid of Monroe-local-lesion host. Plant age at time of inoculation affected rates of virus multiplication and movement out of primary leaves. Above-ground parts of young plants (8 day-old) consistently contained higher infectivity than the corresponding parts of older (12 day-old) plants. The reverse was true for below-ground plant parts. The lag or eclipse period between inoculation and subsequent detection of infectious particles in the primary leaf was longer at low temperature (18^{o}) than at high temperature (23^{o} and $28^{o}C$). Greater infectious virus concentrations were associated with high temperatures; however, in some cases the effect of temperature was modified by the virus-tissue combination. At a temperature of 23^{o}, a cyclic pattern of infectivity was demonstrated in the first trifoliolate leaf of inoculated plants; infectivity peaks occurred at 6-7, 9-10 and 12 days after inoculation.

Infectious BCMV particles did not move through sections of the stem previously steamed to kill all living cells (including phloem). This evidence strongly suggests phloem transport of BCMV as opposed to xylem transport. Furthermore, the general inability of infectious particles to move from the inoculated primary leaf across the stem to the opposite uninoculated primary leaf suggests movement in the phloem.

Infectivity was associated with flowers, immature and mature pods and seeds of systemically infected plants. Infectivity was consistently found in dissected cotyledons and embryos of both immature and mature (dry) seeds. Surface decontamination eliminated infectivity in seedcoats but not in cotyledons or embryos. BCMV was transmitted in 46.6% of the seeds from infected plants.

Approved:

Alfred W. Saettler
Major Professor

William B. Drew
Department Chairman

MULTIPLICATION AND DISTRIBUTION OF BEAN COMMON MOSAIC VIRUS (BCMV) IN BEAN

BY

Ephraim J.A. Ekpo

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
(Plant Pathology)

Department of Botany and Plant Pathology

1972

ACKNOWLEDGEMENT

I wish to express my unreserved appreciation to Dr. A.W. Saettler, Research Plant Pathologist (U.S.D.A.) and Assistant Professor, Department of Botany and Plant Pathology, for his guidance, concern and encouragement during the course of the study and at the time of preparing the manuscript.

I gratefully acknowledge Drs. W.J. Hooker and H.H. Murakishi, who served on my guidance committee. Their useful suggestions during the investigation and their readiness to review the manuscript are fully appreciated.

The University of Ibadan/Ford Foundation provided financial assistance to the author during his graduate study programme.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
Chapter	
I. INTRODUCTION	1
Discovery, Distribution and Economic Importance of Bean Common Mosaic Virus (BCMV)	1
Development of Disease Symptoms	3
Means of Transmission	5
Monroe Local-Lesion Host	8
II. MATERIALS AND METHODS	10
Plant Material	10
Virus Maintenance	11
Inoculum Preparation and Routine Inoculations	11
Local Lesion Assays	12
Experiments	14
Effect of Plant Age on Multiplication of $BCMV_1$ in MCC	14
Effect of Air Temperature and Incubation Period on BCMV Distribution	14
Infectivity of BCMV In the First Trifoliolate Leaf	15
Effect of Freezing on BCMV Infectivity in Leaf Tissue	16

Chapter | Page

Movement of $BCMV_1$ from Inoculation Site 16
Direction of movement 16
Time required for Infective Particles to move out of Inoculated leaves 17
Pathway of BCMV Movement in a Susceptible Host 17
Distribution of $BCMV_1$ in Reproductive Organs (Flowers, Pods and Seeds) of a Susceptible Host, MCC 20

III. RESULTS 24

Effect of Plant Age on Multiplication of $BCMV_1$ 24
Effect of temperature 24
Infectivity of BCMV in 1st Trifoliolate Leaf 28
Movement of BCMV from Inoculated Leaves 29
The Pathway of Movement of BCMV . . . 33
Distribution of $BCMV_1$ in Flowers, Pods and Seeds of MCC Plants 37

IV. DISCUSSION 42

V. SUMMARY 54

REFERENCES 56

LIST OF TABLES

Table		Page
1.	Virus infectivity (no. lesions/gm fresh weight) of tissue sections of MCC 10 days after inoculation of 8- and 12-day-old plants	25
2.	Virus infectivity (no. lesions/gm fresh weight) of tissue sections taken from MCC plants after 3, 6, and 9 days post-inoculation incubation at 18°, 23° and 28° C	27
3.	Effect of a 2-hour freezing period on infectivity of BCMV measured as number of lesions/gm f. wt of leaf tissue	31
4.	Infectivity assays of tissue sections taken from plants at various intervals after BCMV inoculation	32
5.	The effect of detaching inoculated leaf on systemic infection of bean by BCMV	34
6.	Absence of BCMV in living tissues above steamed internodes of Mich-Cal-Cranberry plants	35
7.	Infectivity (no. lesions/tissue sample) of various reproductive tissues taken from systemically-infected MCC plants . . .	38
8.	Infectivity (no. lesions/sample) of various parts of seeds taken from systemically-infected MCC plants	39
9.	Seed transmission of BCMV in a sample of field-grown Mich-Cal-Cranberry	41

LIST OF FIGURES

Figure		Page
1.	Typical symptoms of stunting caused by bean common mosaic virus . . .	4
2.	Mich-Cal-Cranberry plants showing method used to steam-kill stem segments	19
3.	Pods and seeds of MCC at various stages of maturation	22
4.	Infectivity levels of BCMV in fresh and frozen tissue samples of the 1st trifoliolate leaf of MCC bean	30

CHAPTER I

INTRODUCTION

Discovery, Distribution and Economic Importance of Bean Common Mosaic Virus (BCMV)

Bean common mosaic virus (BCMV) constitutes a major virus disease of bean (Phaseolus vulgaris L). It attacks a large number of bean varieties, particularly in those countries where no work is done on the development of resistant varieties.

The deleterious effects which BCMV has on bean production vary from reduction in leaf area, flower production and pod set, to reduction in total number and weight of seed per plant.

Iwanowski (25) first reported BCMV occurrence around 1894 on a P. vulgaris variety in Russia. Later Clinton (10) in 1908 observed an "infectious chlorosis" in Connecticut now thought to be common bean mosaic. Several years later, Stewart and Reddick (46) reported that BCMV was responsible for large losses to the bean crop in New York during 1916 and 1917. In 1921, Spragg and Down (45) reported the disease on commercial Navy (pea) bean in Michigan and suggested that the disease must have been present as early as 1908. Barss (3) observed the same disease in Oregon and recorded up to 50%

infection in some fields. In Canada, up to 25% field infection was reported by various workers including Stone and Howitt (47), Archibald (1) and Gussow (18). For the period 1925-1927, BCMV ranked second only to bacterial blight in causing reductions in total yield for the U.S. bean crop (50). Grainger (16) reported the disease in England in 1929 and Chamberlain (8) reported it from New Zealand in 1933.

Geographical distribution of BCMV is extensive, suggesting that the virus is worldwide in distribution. Rands and Brotherton (39) demonstrated bean common mosaic in seedlings grown from seed collected from Argentina, Brazil, Japan, Mexico, England, Ecuador, Chile, Belgium, France, Belgian Congo, Germany, Guatemala, Colombia, Honduras, Java, Czechoslovakia, Peru, Venezuela, Uraguay and the Union of Soviet Socialist Republic. Cass Smith (7) reported the widespread distribution of the disease and its possible menace to the bean industry in Australia. In Nigeria, little work has been done on bean viruses but field experience in local farms, research stations and at Institute of Tropical Agriculture, Ibadan, has revealed the susceptibility of a large number of native bean and cowpea varieties to the bean common mosaic virus, (E.J.A. Ekpo, unpublished information).

The losses caused by BCMV vary considerably depending on cultural practices, environmental factors and availability of disease-free seed. Harter (22) estimated economic loss due to the disease in Idaho at about 5% in 1920 and about

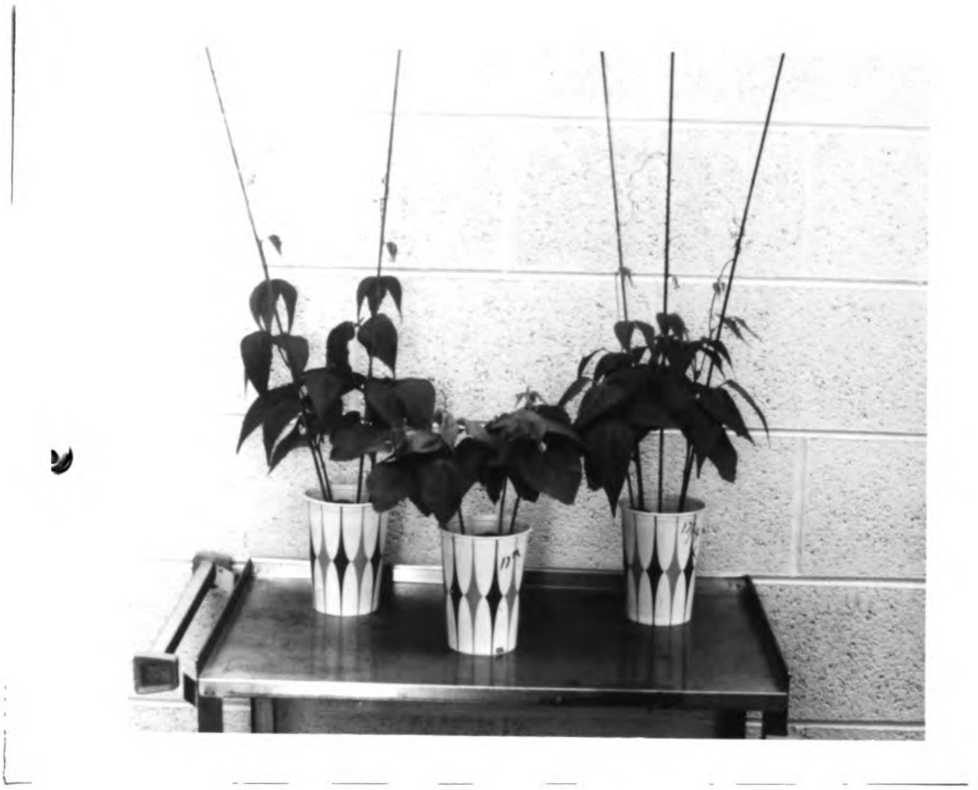

Figure 1. Typical symptoms of stunting caused by bean common mosaic virus.

important factors in the formation of these local lesions (48). Plants inoculated with BCMV 8 and 14 - 16 days after planting produced fewer lesions than those inoculated 9 - 13 days after planting; 10 day-old plants produced the largest number of lesions.

Air temperature also affected both the type and quantity of local lesions formed by BCMV on primary leaves of Monroe bean (48). At 16° and 20° C necrotic-type lesions were formed, whereas at 24° and 28° C lesions were characteristically ringspot-shaped. More lesions were produced at 20° and 24° C. The black root symptom of BCMV infection occurs in some resistant bean varieties in which a systemic necrosis develops; this symptom is believed to be associated with high air temperature (17, 58).

Means of Transmission

The means of transmission of BCMV are generally similar to those of the Southern Bean Mosaic Virus (SBMV) and Yellow Bean Mosaic Virus (YBMV). Like SBMV and YBMV, BCMV is transmitted mechanically by machinery, man and animals. Reddick and Stewart (40) obtained infection by rubbing the upper surface of young leaves with crushed infected leaf tissue. In greenhouse experiments, abrasives are often used to increase the number of infection sites on the leaf surface prior to mechanical rub-inoculation. Zaumeyer and Wade (60) obtained almost 100% infection by the use of carborundum powder dusted

on the leaves prior to inoculation. Under field conditions, however, insects notably aphids, are the primary transmitting agents of these viruses (59).

From the standpoint of seed transmission, BCMV differs markedly from both SBMV and YBMV. There is no record of seed transmission for yellow bean mosaic in the literature. Records of seed transmission for SBMV are of rather rare occurrence (59). In contrast, however, BCMV is known to be commonly seed transmitted. Reddick and Stewart (40) have demonstrated that BCMV is seed transmitted and that plant to plant transmission is rare. Plants rub-inoculated and grown in pots containing control plants resulted in no transfer of the disease to the healthy plants. Similarly, seeds from healthy plants grown in pots with diseased seeds have invariably remained healthy.

Additional evidence of seed transmission of BCMV is indicated by the occurrence of mosaic in plants raised from seeds externally disinfected by immersion in bleaching powder solution, a method suggested by Wilson (52). Fajardo (13) states that spread of BCMV from one locality to another is probably through seed transmission. The disease has not been transmitted through the soil or through root or aerial contact between mosaic-infected and healthy plants. Pierce and Hungerford (34) found that about 33% of the seed from infected plants contained the virus. Nelson (33) in a similar experiment demonstrated up to 50% seed infection in plants grown from infected seed. He stated that the observed variability

in transmission of mosaic in the seed might be due to certain characteristics in the vascular anatomy of the bean pod; this statement assumes that the virus might be present in only certain elements of the vascular bundle and that only seeds having a direct connection with the infected tissue would become infected.

Several workers have demonstrated a possible correlation between percentage of seed infection and certain stages in the growth of the bean plant. Fajardo (13) and Harrison (20) maintain that if infection occurs prior to blossom, the seed is likely to carry infection. Conversely, if the plants become infected after the blossoms are set, no seed infection results. Survival of the virus (BCMV) in the seed for a period of 30 years was demonstrated by Pierce and Hungerford (34).

Detailed studies regarding the mechanics of seed transmission of SBMV are found in the literature. Zaumeyer and Harter (57) demonstrated 5% seed transmission of infectious SBMV in mature seed that had been stored for 7 months. In 1955, Cheo (9) reported the recovery of SBMV from virtually all embryos and seedcoats of immature seeds of P. vulgaris, but from only seedcoats of mature ones. He was led to conclude that infectivity in the immature embryo was inhibited very rapidly upon dehydration of the seed at maturation. McDonald and Hamilton (31) showed that in mature P. vulgaris seed, infectious SBMV is confined to the seedcoat. They,

therefore, propose that the likely mechanism of seed transmission in many hosts of SBMV is from virus on the seedcoat and infection of the embryo during germination.

Although seed transmission of BCMV was reported as early as 1917 (40), no detailed studies have established the actual location of the infectious particles within the seed. This partial neglect may have been due to the absence of a suitable local-lesion host for BCMV.

Monroe Local-Lesion Host

Much information on the biology of SBMV and other mosaic viruses is a result of early discovery of local lesion hosts for these viruses. The lack of a useful local lesion host for BCMV hindered detailed studies on aspects of multiplication and movement of this virus in its hosts. The recent discovery that 'Monroe' bean is an excellent local lesion host for BCMV (49), prompted the author to examine several aspects of virus activity in a susceptible bean variety.

Several areas were of particular interest in this study. Firstly, the effects of air temperature and plant age on BCMV distribution and multiplication were examined.

Secondly, the particular pathway by which the infectious virus particles move throughout the susceptible plant was investigated.

Finally, several aspects relating to seed transmission of BCMV were studied. BCMV distribution in seed developing on infected plants was sequentially assayed during the growing season.

CHAPTER II

MATERIALS AND METHODS

Plant Material

The susceptible test plant used throughout this study was Mich-Cal-Cranberry herein designated 'MCC'. Uniform planting was practised for all comparative studies. Generally 600 ml of vermiculite was placed in a 32 oz wax-lined cardboard carton (Figure 1). Disease-free MCC seeds were spread out on the medium at the rate of 5 per carton and the seeds were covered with an additional 250 ml of vermiculite. Measured amounts (350 ml) of distilled water were added to each carton to insure uniform germination. Seedling number was reduced to 3 per carton after emergence. Watering of plants was done alternately with iron solution (Sequestrene, Fe as metallic 1.8 ppm) and Rapid-Gro (1 teaspoon per 2 liters of water). Unless indicated otherwise, all test plants were grown in growth chambers containing fluorescent and incandescent bulbs programmed to provide 15 hours of daily light.

In the field, MCC was grown in 2 separate plots. The first plot was planted on May 26 and the second on June 20, 1972. In both plots standard fertilizer and planting practices were employed.

Virus Maintenance

The isolate of $BCMV_1$ used in this study was obtained from infected leaf tissues preserved in the freezer since 1961. Infectivity was confirmed on Stringless Green Refugee, a susceptible bean variety. Stock cultures of BCMV were subsequently maintained in MCC by mechanically transferring the virus to young seedlings at 3 - 4 weeks intervals. Contamination of stock culture with another virus was eliminated by continuously maintaining the virus material in insect-free chambers and by preventing accidental contacts with other plants.

Inoculum Preparation and Routine Inoculations

Infected leaves showing typical mosaic symptoms were used as a source of inoculum. Such leaves were weighed and triturated in a sterile mortar with a sufficient amount of phosphate buffer (0.01M, pH 6.9) to give a 1:4 (gm tissue: ml buffer) ratio. This ratio is optimum for local lesion assay of BCMV on primary leaves of Monroe bean (48). The buffer-extract was then filtered through 2 - 3 layers of cheesecloth to remove plant debris. Inoculations were performed immediately after inoculum preparation on primary leaves previously sprayed

with carborundum powder. For all quantitative studies, equal amounts of inoculum (generally 2 drops) were placed on the primary leaves of MCC and rub-inoculated uniformly to the entire surface with the rough end of the pestle. One primary leaf per plant grown in the growth chamber and both primary leaves per plant grown in the field were inoculated. Inoculated surfaces were then rinsed with distilled water to remove excess inoculum.

Local Lesion Assays

'Monroe' bean was used for local lesion assays (49) and all inoculations were performed on 10 day-old plants. Unless otherwise indicated, all seedlings were grown under identical conditions of light (14hr photoperiod) and temperature (23^{o} C). Inoculations were carried out within 1 - 2 hours after inoculum preparation and most were performed on primary leaves using the half-leaf method (28). This method has the advantage of eliminating possible variations in susceptibility from plant to plant, and from leaf to leaf on the same plant. The arrangement essentially consisted of randomized blocks each of four units. Each plant constituted a block and the half leaves of opposite primary leaves formed the units as follows:

	PLANT NUMBER							
	1		2		3		4	
	R	L	R	L	R	L	R	L
Leaf I	A	B	A	C	B	D	C	D
Leaf II	C	D	D	B	C	A	B	A

Surfaces of uniform primary leaves to be inoculated were dusted with carborundum using a hand operated atomizer. A measured amount of inoculum (1 drop or 0.05 ml) was placed on each half leaf and rub-inoculated uniformly with the rough end of the pestle using Trújillo's method of rubbing (48). The inoculated surface was rinsed with a gentle stream of distilled water immediately after inoculation to remove any undiluted sap or toxic materials that might interfere with lesion formation. For purposes of reproducibility, it was advisable to confine inoculations to the same time of the day. Bawden (4) and Matthews (30) state that the number of local lesions formed is related to the time of day at which inoculation is performed.

Each experiment was repeated 2 - 3 times and lesion counts were made 5 - 7 days after inoculation. Lesion counts were made either by visual inspection when the number was low or by the use of an electric counter when lesions were numerous and crowded together.

Experiments

Effect of Plant Age on Multiplication of $BCMV_1$ in MCC

One primary leaf per plant of 8 and 12 day-old MCC plants grown in separate cartons at 24° C were inoculated with $BCMV_1$. Inoculum consisted of a 1:4 buffer dilution of infected leaf tissue extract. Inoculated plants were incubated at 24° C and 15 hour photoperiod.

Ten days after inoculation, plants of each group were individually harvested and immediately dissected into the following plant parts: 1) inoculated primary leaf; 2) uninoculated opposite primary leaf; 3) trifoliolate leaves in order of unfolding; 4) last internode plus terminal bud; and 5) roots. Each plant part was then weighed and assayed for virus infectivity on Monroe bean at a 1:4 dilution. Since certain tissue sections were very small, unfiltered extracts were sometimes assayed for purposes of uniformity. Four half leaves were inoculated with each tissue extract and lesion counts were expressed on a fresh weight basis.

Effects of Air Temperature and Incubation Period on BCMV Distribution

Ten-day-old MCC plants grown at 24° C and 15 hour photoperiod were selected for uniformity. One primary leaf per plant was inoculated using standard procedures. The plants were then distributed randomly among three temperature

regimes -- 18°, 23° and 28° C. All growth chambers were programmed with 15 hours of light daily and infectivity assays of plant parts were performed 3, 6 and 9 days after inoculation.

Infectivity of BCMV in the First Trifoliolate Leaf

Ten-day-old plants grown at 23° C were inoculated (one primary leaf per plant) using standard procedures. Samples of first trifoliolate leaves were harvested 4 days after inoculation and daily then after, until 13 days after inoculation. Three leaf samples were randomly taken each day and fresh weights were quickly determined for each leaf.

Infectivity assays were performed in two ways, namely: (1) by using inoculum prepared from fresh material, and (2) by using inoculum prepared from frozen material. In method (1), assays were performed as soon as leaves were detached from the plants each day. Staggered planting of the Monroe local-lesion assay host was used in this method. In the case of frozen material, leaves were weighed, labelled with group symbols (A, B, C), and then stored in the freezer until all assay samples were obtained. In this case, the Monroe test plants were all planted at the same time.

Effect of Freezing on BCMV Infectivity in Leaf Tissue

To study the possible effect of freezing on virus infectivity, systemically-infected trifoliolate leaves of MCC plants were harvested and divided into two samples along the central vein. Each sample was immediately weighed and labelled. One set of half leaves was placed in a freezer (-5° C) for 2 hours while the other set was placed in petri dish moist chambers for the same period of time. At the end of 2 hours, inoculum was prepared from each half leaf in appropriate volume of buffer. Infectivity assays were then performed on primary leaves of Monroe plant.

Movement of $BCMV_1$ from Inoculation Site

a) Direction of movement

Labelled primary leaves (one per plant) of 10 day-old MCC were inoculated with equal amounts (2 drops) of freshly prepared BCMV inoculum. The inoculated plants were maintained at 23° C and 15 hour photoperiod. At 12 hour intervals, 4 plants were harvested and the different tissue sections were aseptically cut and separately assayed for infectivity on Monroe seedlings.

b) Time required for infective particles to move out of the inoculated leaves

Primary leaves (one per plant) of 10 day-old MCC seedlings were inoculated by standard procedure using equal amounts of inoculum per leaf. Opposite leaves were left uninoculated and their tips were clipped off for purposes of identification. Extra care was exercised to prevent inoculum from running down or splashing on to the petiole of inoculated leaves. Plants with uninoculated leaves served as controls. One and four hours after inoculation and at 4 hour intervals thereafter, inoculated leaves were detached from the intact plants at the petiole-stem junction. Flame sterilized blades (one per leaf) were used for this operation to avoid cross contamination. Comparable leaves were also detached from control plants. All plants were adequately labelled and maintained at 23° C and examined for systemic symptoms during the following 2 - 3 weeks.

Pathway of BCMV Movement in a Susceptible Host

To investigate the apparent pathway of movement of BCMV through MCC, a method used by Schneider *et al* (41) was employed, with several modifications. Essentially, the method consisted of inoculating seedlings some segments of which were previously steamed to kill living tissue (including phloem cells). Plants receiving the steam treatment were supported on poles to prevent falling over. Steaming was performed at

different internodes, namely: between the primary leaf node and the 1st trifoliolate leaf; between the cotyledonary node and the primary leaves; and internode between the 3rd and 4th trifoliolate leaves.

Tissue sections (5 - 10 mm) to be steamed were wrapped with wet cotton over which was wrapped one layer of aluminium foil (Figure 2). Heating was done with a small naked flame from a gas burner which was moved around the aluminium foil to insure complete and uniform steaming of the desired segment. Steaming usually took 3 - 5 minutes. Leaves and/or stems one internode below the steamed internode were then dusted with carborundum powder and rub-inoculated with BCMV extract 30 - 60 minutes after steaming. Plants steamed at comparable areas but left uninoculated and plants not steamed but inoculated at identical segments were included as controls.

The absence of systemic symptoms in tissues above the steamed segments was used to indicate the inability of BCMV to move across the steamed area. In addition to symptom expression, infectivity assays were used to determine the presence or absence of virus above the steamed segments. In such assays, all leaves one internode above the steamed areas on each plant were macerated as one sample in an appropriate amount of buffer (1:4 ratio). Filtered extracts were inoculated to primary leaves of Monroe plants. Leaf extracts from steamed but uninoculated and from plants not steamed but inoculated were similarly prepared and used for infectivity assays.

Figure 2. Mich-Cal-Cranberry plant showing the method used to steam-kill stem segments.

Proper functioning of the xylem vessels was confirmed by the movement of India ink beyond the steamed zone into the leaves and the apical bud. Additional evidence of active xylem transport was the presence of green unwilted tissues above steamed areas 10 - 14 days after steaming.

The presence of living cells (including phloem) in steamed areas that might support BCMV multiplication and movement was assayed using the 2, 3, 5 -- Triphenyl tetrazolium chloride (TTC) method (51). The development of a pink colour by dehydrogenase activity on TTC was used to indicate presence of living cells. No colour reaction was indicative of the absence of living cells. One to two mm sections of steamed and unsteamed portions of the same internode were separately dark-incubated in a 1% solution of TTC prepared in both distilled water and in phosphate buffer (pH 6.9) at room temperature. The tissues were inspected for colour reaction macroscopically and microscopically 2 - 24 hours after incubation.

The Distribution of $BCMV_1$ in Reproductive Organs (Flowers, Pods and Seeds) of a Susceptible Host MCC

MCC seedlings grown in the field were mechanically inoculated by the standard leaf-rubbing technique. Both primary leaves per plant were inoculated. One group of plants was inoculated 13 days after planting and the other group was

inoculated at 38 days after planting. Plants were examined for typical BCMV symptoms three weeks after inoculation and those with no typical symptoms were removed from the test plots.

The distribution pattern of infectious BCMV in flowers, pods and seeds at different stages of development was sequentially determined by quantitatively assaying tissue extracts prepared in buffer on Monroe-local-lesion host. Figure 3 shows the different groups of pods and seeds (except the dry mature group) investigated for virus activity. Pepresentative samples of each group were randomly harvested from both plots and kept in separate bags. When seeds were assayed for infectivity, they were carefully removed from the pods to prevent scratching and possible contamination with pod tissue. Generally, seeds from 3 - 4 pods of each age group were combined and two samples of four seeds, each randomly picked, were obtained for infectivity assays. The seeds in the first set were dissected into seedcoats, cotyledons and embryos. These parts were surface-decontaminated by washing in running water (31). Each seed part was separately wrapped in one layer of cheesecloth and exposed to running distilled water for 10 - 15 minutes. The second set of seeds was similarly dissected but parts were not surface-decontaminated. Flame sterilised blades were used for all dissections.

Figure 3. Pods and seeds of MCC at various stages of maturation.

The following inocula were triturated in minimum volumes of buffer: (a) four unopened flowers; (b) four opened flowers; (c) three pods; (d) four seeds; (e) four seedcoats washed in water; (f) four seedcoats, untreated; (g) four pairs of cotyledons, washed in water; (h) four pairs of cotyledons, untreated; (i) four embryos, washed in water; (j) four embryos, untreated. In several cases the triturate was pressed through two layers of cheesecloth to remove much of the tissue debris. The inocula were applied to Monroe plants (pre-shaded for 24 hours) using the half-leaf technique. Infected leaf extract (k) and phosphate buffer solution (l) were included as controls.

To determine percent seed transmission of BCMV, seeds taken from infected MCC plants were grown in vermiculite at the rate of one pod (3 - 6 seeds) per carton. The seedlings were allowed to grow for 3 - 4 weeks and then examined for mosaic symptoms.

CHAPTER III

RESULTS

Effect of Plant Age on Multiplication of BCMV$_1$

BCMV was detected in all above- and below-ground portions of bean plants 10 days after inoculation of one primary leaf (Table 1). Virus titer was generally higher in above ground tissues of younger plants than in the corresponding tissues of older plants inoculated at the same time (Table 1). However, slightly more infectivity was detected in roots of older plants than in those of younger plants. In only one of six plants was virus infectivity detected in the uninoculated primary leaf opposite the inoculated primary leaf. On a particular plant, virus titer was highest in the terminal bud (leaf) and leaves of increasing age progressively contained less infective virus.

Effect of Temperature

Temperature had a pronounced effect on plant growth, symptom development, and virus titer (Table 2).

Table 1: Virus infectivity (no. lesions/gm fresh weight) of tissue sections of MCC 10 days after inoculation of 8- and 12-day old plants[a]

Tissue section	Plant age at inoculation	
	8 days	12 days
Inoculated primary leaf	171.9 ± 18.7	146.4 ± 27.4
Uninoculated opposite primary leaf	9.6 ± 2.3	0.0
1st trifoliolate leaf	100.2 ± 12.6	44.3 ± 8.6
2nd trifoliolate leaf	247.6 ± 44.0	84.6 ± 13.3
3rd trifoliolate leaf	350.1 ± 33.4	76.3 ± 7.9
Last internode plus terminal bud	375.5 ± 56.2	136.9 ± 21.0
Roots	2.4 ± 1.4	4.9 ± 2.1

[a]Average of 3 separate plants each assayed on 4 half leaves.

3 days after inoculation

At 3 days after inoculation, first trifoliolate leaves were still in the bud stage at 18° while those at 23° and 28° were partially unfolded. Infectivity assays were negative when extracts from inoculated plants grown at 18° were assayed on Monroe plants (Table 2). On the other hand infectivity assays were positive with most extracts obtained from plants maintained at 23° and 28° C. Highest virus concentrations at both temperatures were found in the terminal bud. Downward movement of the virus to roots was relatively rapid and was evident within 3 days after inoculation at 23° and 28°. All tissues examined contained greater virus activity at 28° than at 23° C.

6 days after inoculation

Six days after inoculation, 1st trifoliolate leaves were completely opened at 28° (1.5 - 2.0 x 3.5 - 4.0 cm); partially opened at 23° (0.7 - 1.5 x 2.5 - 3.0 cm); and still folded but separated from the terminal bud at 18° C. By this time the 2nd trifoliolate leaf was still in bud stage at 18° but separated and unfolded at 23° and 28°. Disease symptoms varied from a general chlorosis of inoculated leaves at all temperatures, to mosaic and puckering of 1st trifoliolate leaves at 28° and only puckering of leaves at 23° C. Systemic symptoms were not evident at 18° C. However, tissues of plants at 18° (Table 2) contained infective virus. A direct correlation between infectivity in inoculated primary leaves and temperature of incubation was evident. This relation between infectivity and temperature did not hold in

Table 2: Virus infectivity (no. lesions/gm fresh weight) of tissue sections taken from MCC plants after 3, 6 and 9 days post-inoculation incubation at 18°, 23° and 28° C[a].

Tissue section	Post-inoculation Period: 3 days 18°	3 days 23°	3 days 28°	6 days 18°	6 days 23°	6 days 28°	9 days 18°	9 days 23°	9 days 28°
Inoculated primary leaf	0	81.1 ±11.9	120.8 ±22.1	16.1 ±4.5	149.5 ±32.5	177.4 ±40.3	3.4 ±3.3	394.2 ±52.6	553.4 ±68
Uninoculated opposite primary leaf	0	0	0	0	0	0	0	26.7 ±9.3	70.0 ±15.8
Internode between cotyledon and primary leaf	0	100.4 ±27.8	152.4 ±17.3	30.8 ±4.8	180.4 ±30.8	110.9 ±23.7	293.4 ±42	700.0 ±37.3	95.7 ±32
Hopocotyl	0	8.6 ±1.6	46.1 ±9.2	50.5 ±13.0	109.7 ±23.3	124.6 ±19.7	157.0 ±31.7	260.0 ±23.1	740.0 ±57.3
Roots	0	10.5 ±3.2	39.8 ±8.9	56.4 ±13.8	75.9 ±13.8	65.8 ±12.5	152.7 ±29.3	356.7 ±17.5	146.7 ±18.6
1st trifoliolate leaf	-	153.0 ±45.8	194.4 ±15.7	236.9 ±36.4	296.4 ±47.1	179.9 ±16.3	360.1 ±46.6	107.6 ±26.5	420.0 ±51.3
2nd trifoliolate leaf	0	319.4 ±46.7	528.6 ±30.9	-	764.5 ±66.9	568.5 ±50.8	1,215.5 ±114.9	340.0 ±32.0	390.0 ±51.4
3rd trifoliolate leaf	-	-	-	551.1 ±59.0	2,192.3 ±203.2	1,625.0 ±178.3	-	1,098.9 ±77.8	669.2 ±65.7
Last internode plus terminal bud	-	-	-	-	-	-	1,299.5 ±131.4	1,268.5 ±98.2	1,174.5 ±94.6

[a]Average of 3 plants each assayed on 4 half leaves.

other comparable tissues where concentration peaks more characteristically occurred at 23° than at 28°. Least infectivity was consistently obtained at 18° in all comparable tissues. Infectivity was not found in opposite uninoculated primary leaves at any temperature, suggesting absence of infectious particles in amounts sensitive to our assay technique. Greater infectivity was associated with younger leaves than with older ones at all temperatures. On gram fresh weight basis, infectivity was highest in the terminal buds (Table 2).

9 days after inoculation

At 9 days after inoculation plants grown at 18° possessed two opened trifoliolate leaves while those at 23° and 28° possessed three opened trifoliolate leaves. Typical mosaic symptoms were now observed on plants maintained at 23° and 28° but not at 18° C. The infectivity pattern was similar to that at 6 days after inoculation; however at 9 days, infectivity was found in opposite uninoculated primary leaves of plants grown at 23° and 28° C (Table 2). In inoculated primary leaves greater infectivity was associated with higher temperature. In the other comparable tissues there was no simple relationship between infectivity and temperature. Infectivity in the terminal buds was approximately equal at all three temperatures. (Table 2).

Infectivity of BCMV in 1st trifoliolate leaf at times after inoculation

Infectivity assays of the 1st trifoliolate leaf on

inoculated plants during a 13 day-period suggested a cyclic or fluctuating pattern of virus infectivity (Figure 4). This cyclic pattern was consistently observed, both when frozen and fresh tissue samples were assayed. More infectivity on a fresh weight basis was detected with the latter sample. This was due in part to the deleterious effect which freezing has on BCMV infectivity in the tissue (Table 3). There were slight variations in the time of appearance of infectivity peaks depending on whether fresh or frozen tissue was assayed. Generally, 3 peaks were obtained, these occuring 6-7, 9-10 and 12 days after inoculation (Figure 4).

Movement of BCMV from inoculated leaves

No virus infectivity was detected in any plant parts, including inoculated leaves, during the first 24 hours after inoculation (Table 4). At 36 hours after inoculation, however, virus activity became evident in the inoculated leaves only. By 48 hours, the virus had moved out of the inoculated leaves and BCMV infectivity was detected in all parts of the plant above the inoculated primary leaf. During this 48 hour period, no virus infectivity was detected in tissues below the inoculated leaves or in the opposite uninoculated primary leaves. At 60 hours, (that is 12 hours after detection in apical tissues), the virus had reached the roots. Sixty hours (23^{o}) was therefore sufficient time for the virus to be distributed throughout the entire plant from a single

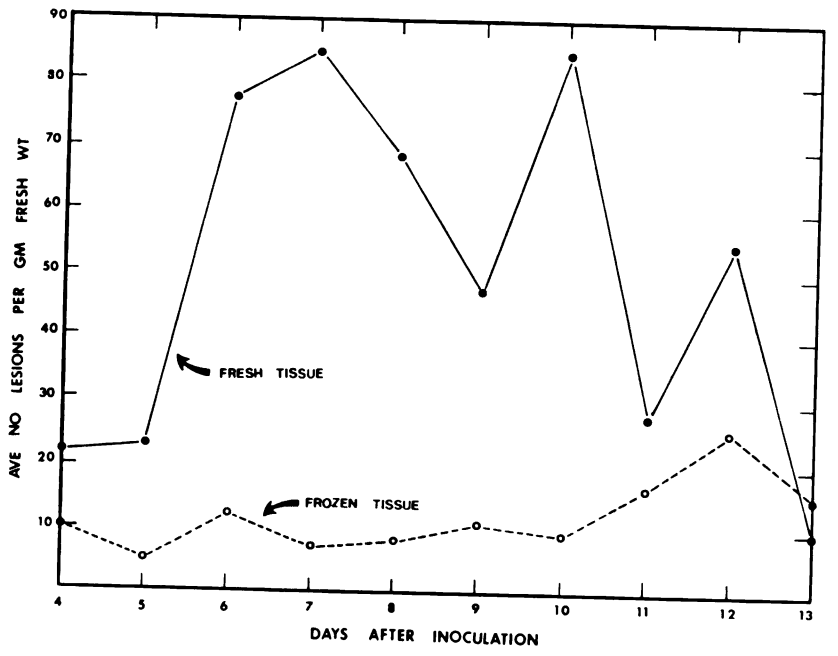

Figure 4. Infectivity levels of BCMV in fresh and frozen tissue samples of the 1st trifoliolate leaf of MCC bean.

Table 3: Effect of a 2 hour freezing period on infectivity of BCMV measured as number of lesions/gm f wt. of leaf tissue[a]

Half leaf No.	Frozen Tissue	Fresh Tissue[b]
1	46.1 ± 12.5	114.8 ± 23.1
2	102.9 ± 10.0	152.6 ± 17.3
3	66.7 ± 20.7	99.4 ± 13.9
4	66.6 ± 23.9	137.3 ± 35.7
5	103.4 ± 19.7	124.2 ± 26.3
6	115.9 ± 21.0	130.7 ± 28.2

[a] mean of 8 half leaves

[b] equal amount of intact tissue held in petri dish at room temperature

Table 4: Infectivity assays of tissue sections taken from plants at various intervals after BCMV inoculation[a]

Tissue Section	Hours after inoculation					
	12	24	36	48	60	72
inoculated primary leaf	-	-	+	+	+	+
petiole of inoculated primary leaf	-	-	-	+	+	+
internode between primary leaves and 1st trifoliolate leaf	-	-	-	+	+	+
1st trifoliolate leaf	-	-	-	+	+	+
apical bud	-	-	-	+	+	+
internode between primary leaves and cotyledonary node	-	-	-	-	+	+
hypocotyl	-	-	-	-	+	+
roots	-	-	-	-	+	+

[a] (+) = local lesion formed on Monroe

(-) = no local lesion formed on Monroe

source in the inoculated primary leaf. By determining the distance moved (between the petiole-lamina junction and the base of the 1st trifoliolate leaf via the stem) and the time between detection of infectivity in inoculated primary leaf and detection in the trifoliolate leaf, the rate of movement from the inoculated primary leaf to the first trifoliolate leaf would be estimated at 6.4×10^{-1} cm/hr at 23^{o} C and 15 hour photoperiod. Visible mosaic symptoms were not observed on the 1st trifoliolate leaves even at 72 hours after inoculation of the primary leaves. The plants were healthy looking. From leaf-detachment studies (Table 5) and observations on systemic symptom development, it was obvious that BCMV required a minimum of 12 - 16 hours to move out of the inoculated leaf through the petiole into the stem for systemic infection.

The pathway of movement of BCMV

Infectivity assays of tissue samples located above and below steamed areas of the stem showed that BCMV did not move through such steam-killed tissue sections (Figure 2, Table 6). No infectivity was obtained in tissues above the steamed areas of inoculated plants. Similarly, no lesions were obtained when tissues above steamed areas from uninoculated plants were assayed. This control was included to demonstrate the total absence of BCMV in test plants prior to controlled inoculation. Infectivity was consistently obtained when tissues from inoculated but unsteamed plants were assayed.

Table 5: The effect of detaching the inoculated leaf on systemic infection of bean by BCMV

Detachment time[a]	No. of plants showing typical mosaic symptoms			
	Trial 1	Trial 2	Trial 3	control[b]
1	0/4	0/4	0/4	0/4
4	0/4	0/4	0/4	0/4
8	0/4	0/4	0/4	0/4
12	1/4	0/4	1/4	0/4
16	2/4	2/4	3/4	0/4
20	2/4	3/4	3/4	0/4
24	3/4	3/4	4/4	0/4
28	4/4	4/4	4/4	0/4
32	4/4	4/4	4/4	0/4
36	4/4	4/4	4/4	0/4
40	4/4	4/4	4/4	0/4

[a] hours after primary leaf was inoculated

[b] uninoculated plants

Table 6: Absence of BCMV in living tissues above steamed internodes of Mich-Cal-Cranberry plants[a,b]

Experiment No.	Plants steamed and inoculated	Plants steamed but uninoculated (control)	Plants not steamed but inoculated (control)
1	0/12	0/6	6/6
2	0/8	0/8	8/8
3	0/6	0/3	3/3

[a] Each ratio represents the total number of MCC plants in which BCMV was detected above steamed areas (numerator) to the total number of plants assayed (denominator).

[b] Infectivity assays were conducted with extracts prepared from leaves above the steamed internode.

This control was used to justify the inoculation technique as well as to confirm the susceptibility of the test plants to BCMV at the different sites of inoculation.

Symptom expression was an additional criterion used for virus presence in tissues above and below steamed areas. Leaves above steamed segments (when leaves below steamed segments were inoculated), and leaves below steamed segments (when leaves above steamed areas were inoculated), never developed typical mosaic symptoms during a 14 day period after inoculation. This observation was true for both inoculated and uninoculated steamed plants. On the other hand, definite mosaic symptoms were seen on leaves of plants inoculated, but unsteamed.

Proper functioning of xylem vessels was shown by the active conduction of India ink beyond steamed areas to the apical growth. Presence of green unwilted tissue up to 14 days after steaming also indicated that such tissue was still being supplied by the xylem. Absence of living tissue in steamed areas was confirmed by the lack of dehydrogenase activity. One to 2 mm thick sections immediately above and below the steamed regions gave a positive red colour reaction with TTC in 1-2 hours at room temperature in the dark. No such colour reaction was obtained with steamed sections examined macroscopically and microscopically even up to 48 hours.

Distribution of $BCMV_1$ in Flowers, Pods and Seeds of MCC plants

Virus infectivity was found in the blossom developing on infected plants, at both the bud stage and at the time when the blossoms were opened (Table 7). Infectivity was also found in pods at various stages of development. Immature green pods (0.2 - 0.5 x 2.5 - 5.0 cm, Figure 3) gave lower infectivity than maturing green pods (1.5 - 1.7 x 10.0 - 12.0 cm). Infectivity was also recovered in the seeds contained in both mature and immature pods and in this case too, older seeds contained higher infectivity than younger (immature) seeds.

The distribution of $BCMV_1$ infectivity in the various seed parts (seedcoats, cotyledon, embryo) is shown in Table 8. The results indicate that little infectivity is associated with seedcoats. Even this little infectivity was completely eliminated when the seedcoats were subjected to surface decontamination in running water. In contrast, very high infectivity was found in cotyledons. Surface decontamination had no apparent effect on the cotyledon infectivity. The data suggest a gradual increase in cotyledon infectivity as the seeds began to mature (dry).

Virus infectivity was also found in the embryos. Surface decontamination was effective in reducing (but not eliminating) this infectivity. In several cases embryo infectivity was reduced by more than one-third by surface

Table 7: Infectivity (no. lesions/tissue sample) of various reproductive tissues taken from systemically infected MCC plants[a,b,c]

Sample No.	Tissue Sample							
	Flowers		Pod length			Seeds from		Infected leaf
	opened	unopened	A 0.2 - 0.3 x 2.5 - 3 cm	B 0.4 - 0.5 x 4.5 - 5 cm	C 1.5 - 1.7 x 10 - 12 cm	B	C	
1	8	12	2	2	11	2	4	12
2	2	3	0	2	8	0	4	14
3	6	7	3	3	8	2	7	20
4	1	2	1	1	11	1	2	17
5	12	11	2	4	10	1	4	10
6	7	1	1	0	13	2	5	16

[a] mean of 4 half leaves/treatment

[b] each assay sample consisted of 4 blossoms, 3 pods or 4 seeds

[c] refer to figure 3 for visual appearance of tissue samples

Table 8: Infectivity (no. lesions/sample) of various parts of seeds taken from systemically-infected MCC plants[a,b]

Pod Appearance	Seed Part						Infected leaf (Control)
	Seedcoat		Cotyledon		Embryo		
	untreated	decontaminated[c]	untreated	decontaminated[c]	untreated	decontaminated[c]	
Green (Immature)	3.3	-	12.0	-	9.5	-	21.3
	2.0	-	11.3	-	5.0	-	17.0
	0	0	12.7	17.5	2.5	2.1	23.9
	0	0	11.8	7.0	4.8	0	15.3
	0	0	5.0	4.0	6.9	3.3	22.0
	0	0	9.0	6.0	2.8	1.5	24.8
Striated (Relatively mature)	4.4	0	18.3	19.1	17.7	10.9	23.9
	0	0	11.3	12.0	6.0	5.8	15.3
	0.1	0	21.2	26.3	12.3	13.0	22.0
	0	0	14.3	29.0	7.8	8.9	24.8
Dry (mature)	0	0	18.2	13.7	13.5	7.3	23.9
	0	0	9.8	9.0	7.8	4.0	15.3
	0	0	21.8	22.3	19.1	7.0	22.0
	0	0	11.5	9.8	11.5	7.8	24.8

[a] mean of 8 half leaves/treatment

[b] all samples but the first two (3 seeds each) consisted of bulked parts from 4 seeds

[c] seed parts decontaminated by immersion in running water for 10 - 15 minutes

decontamination. Generally, more infectivity was recovered in mature, than in immature embryos.

The data on seed transmission (Table 9) indicate that not all seeds in the same pod contain the virus. Occasionally, less than one-third of the seeds transmitted the virus but in a few cases more than half the seeds from the same pod established infection. An incidence of 46.6% seed infection and transmission was estimated for a sample of 58 seeds contained in 20 pods randomly harvested from field infected plants.

Table 9: Seed transmission of BCMV in a sample of field-grown Mich-Cal-Cranberry beans

Pod No.	No. of seeds planted	No. of seedlings with mosaic	No. of seedlings without mosaic
1	4	3	1
2	2	2	0
3	3	1	2
4	3	1	2
5	4	3	1
6	3	0	3
7	2	2	0
8	2	0	2
9	2	0	2
10	1	1	0
11	2	2	0
12	3	0	3
13	2	2	0
14	3	3	0
15	5	2	3
16	2	0	2
17	5	2	3
18	2	0	2
19	5	3	2
20	3	0	3
Totals	58	27 (46.6%)	31 (53.4%)

CHAPTER IV

DISCUSSION

This study examines various aspects of BCMV multiplication, distribution and movement in the susceptible MCC bean variety and adds to our knowledge regarding plant virus-host relationships. [For example, the results show that plant age and temperature strongly influence both the rate at which BCMV moves from the inoculated leaf and the rate of multiplication in various plant parts.] Relative to plant age, above-ground tissues of plants inoculated when 8 days old generally contained higher infectivity than comparable tissues of plants inoculated when 12 days old. In contrast to the above-ground tissues, the below-ground tissues of the older plants contained higher infectivity than corresponding tissues of the younger plants. Bawden (4) in his study of tobacco necrosis virus demonstrated that inoculated leaves of 10 day-old french beans (P. vulgaris) gave numerous local lesions while leaves of 13-14 day-old plants were not susceptible; he did not examine roots in his study.

The reasons for this differential effect of plant age on virus multiplication in above- and below-ground tissues

are not clear. If inhibitory substances are involved, then it is possible that such substances are lacking (or are present at very low concentrations) in the roots of older plants (12 days) while the opposite may be true in the above-ground parts. Another possible explanation for the differential rate of multiplication may be related to the developmental stages of the plant at inoculation time; this might involve translocation patterns from the inoculated leaf to the growing points.

The effect of temperature on virus infectivity seemed to depend on the particular virus-tissue complex examined. Temperature per se seemed to have little influence on the rate and direction of movement of BCMV once infectivity was detected in the inoculated leaf (Table 2). The lag or eclipse period between the time of inoculation and time of detection in the primary leaf was, however, inversely related to temperature tested and could account for the delayed movement of BCMV from inoculated leaves to distant parts at the low temperature. Virus titer of leaves above the inoculated primary leaf showed an inverse relationship between leaf age and virus concentration once systemic infection had developed. The titer was high in young leaves and progressively lower in old leaves. This relationship was true for all 3 temperatures. The infectivity pattern in the inoculated primary leaves was particularly interesting. At each sampling date, a sharp gradient of infectivity existed in these leaves,

the highest concentration being found at the highest temperature. This result is concordant with that obtained by Bancroft and Pound (2) when they determined the concentration of tobacco mosaic virus (TMV) in leaf extracts of susceptible Connecticut Havana No. 38 tobacco plants 4 days after inoculation.

In a somewhat related study, Schneider et al (42) found that temperature influenced not only the extent of multiplication but also the site of multiplication of SBMV in detached leaves of a local lesion host. Exposure to high temperature (32°) after inoculation facilitated SBMV invasion of veinal rather than interveinal cells. They suggested that the effect of high temperature after inoculation was more on a later stage of disease development than on the primary infection, since a 24- and 48-hour delay in high temperature treatment did not eliminate an increase in vein necrosis. With BCMV, it appears that the effect of high temperature is on both the primary and the secondary infection. Infectivity was detected in inoculated leaves 3 days after inoculation at 23 and 28° but not at 18° and higher virus infectivity was obtained in most tissues at 23 and 28° than in tissues at 18°. It is possible that the physiological changes associated with high temperature (23 and 28°) in the presence of BCMV are more advantageous to the multiplication process than are changes at the low temperature (18°). It must be stated,

however, that the differential effect of high and low temperature may partly be on the virus itself.

The effect of temperature on multiplication (infectivity) of viruses, in general, is not a uniform predictable phenomenon. A temperature that favours a high concentration of one virus may be inhibitory to another strain of the same virus. Pound and Walker (37) reported a higher concentration of the cabbage strains of turnip virus I in cabbage plants grown at 28° than in plants grown at 16° C. However, with a horseradish strain of turnip virus I, a reverse gradient was demonstrated in horseradish (35). In Nicotiana species all three strains of turnip virus 1 developed systemically more rapidly and in greater concentration at 16° and 20° than at 24 and 28° C. These differences in temperature response suggested that the effect of temperature is a function of the specific host-virus combination.

Pound and Helms (36) have offered suggestions to explain why variations in virus concentrations occur at different temperatures. They argue that: a) the proportion of infective to non-infective virus particles (or non-lesion-forming virus) may vary under different environmental conditions. At low temperature, host plants may produce a greater proportion of non-infective to infective particles than at higher temperature; b) the proportion of infective virus formed may be approximately the same under all temperature

conditions, but a third substance, an inhibitor that decreases the infectivity of the sap, may be formed at low temperatures.

Relative to (a), the general lack of correlation of results often obtained for concentration of virus when measured by the optical-density and local-lesion techniques seems to favour this arguement. For example, Pound and Helms (36) failed to establish such correlations for concentration of potato virus X using both methods. In the present study, optical density technique was not used and a test for the presence of an inhibitor was not carried out to support the suggestion that an inhibitor molecule may be operative at low temperature. In any case, an inhibitor hypothesis can only be meaningful if such inhibitor principle can be extracted, crystalised and described. Until this is done, we cannot positively and conclusively ascribe the observed decrease in infectivity of $BCMV_1$ at low temperature to a singular effect of an inhibitor. From the present study it can be adduced that temperature appears to influence $BCMV_1$ activity in two stages namely: (a) synthesis and multiplication at site of introduction and (b) movement from the site of introduction (as a function of lag period) to other distant parts like roots and apical buds.

The cyclic pattern of virus activity observed in systemically infected leaf tissue shows a similarity of BCMV to several other mosaic viruses. Wood et al (53) have

recorded a cyclic pattern of infectivity for the W strain of cucumber mosaic virus in leaves of Havana 425 tobacco. Menke and Walker (32) observed that the concentration of cucumber mosaic virus in the second leaf of resistant cucumber plants reached a high level 6 - 8 days after inoculation and and that it afterwards declined steadily to a very low level. In an attempt to explain their observation they postulated that it could be that the production of virus ceases for some reason, that a mechanism inhibitory to virus synthesis builds up or that virus breakdown occurs faster than the virus synthesis in the aging leaves. The inhibitory mechanism appears satisfactory for a bell-shaped infectivity curve but not for the cyclic pattern observed in BCMV. It is possible that variations in the rate of breakdown and synthesis of infective particles may account for the observed daily fluctuations in BCMV infectivity. This cycle of infectivity may not be the general rule for all plant viruses; Pound and Helms (36) failed to demonstrate the cyclic pattern with potato virus X. The appearance of the infectivity peaks may also vary in the same host/virus combination depending on the assay technique employed. In the present study $BCMV_1$ infectivity generally declined in frozen material thus giving rise to lower infectivity peaks. The infectivity curves suggest that the highest virus concentrations in the BCMV-MCC system are 6-7, 9-10 and 12 days after inoculation.

The study on the direction of movement of BCMV from the inoculated leaf indicates that BCMV first moves to the shoot and then to the roots. This pattern of movement is similar to the translocation pattern of C^{14} metabolites in the phloem of the bean plant where Biddulph and Cory (5) report that upward export preceded downward export by several to many hours. The pattern also resembles that reported for tobacco mosaic in tomato plant (23, 24). Of interest is the general inability of BCMV to move from the inoculated primary leaf across the stem to the opposite uninoculated primary leaf. Biddulph et al (5) state that when metabolites flow through the phloem of bean plant (Red kidney), little moves tangentially between bundles except, of course, where anastomosing occurs. Radial loss to the xylem was very low (4%) when export was from a mature primary leaf. Livne and Daly (29) while studying the translocation pattern of labelled C^{14} photosynthate in healthy and rust-infected primary leaf of bean plant found that there was no translocation of labelled material across the stem to the opposite unexposed primary leaf. Our observed pattern of BCMV movement resembles this pattern of translocation and strongly suggests a similar transport pathway.

Other viruses are known to move with difficulty into certain leaves. Schneider (41) demonstrated that while SBMV readily moves to the uninoculated primary leaf of

Black Valentine bean in as short a time as 4 days, tobacco ringspot virus (TRSV) rarely moves into this leaf. He suggested that TRSV for some unknown reason becomes dependent on a slow cell-to-cell invasion in the petiole of the inoculated primary leaf. Karle and Shalla (26) also found that peach yellow bud mosaic virus (a strain of tomato ringspot virus) in inoculated primary leaves of cowpea behaved like TRSV in bean. This differential ability of viruses to move into the opposite primary leaf may be related to their pathway of movement. While SBMV is known to move in both xylem and phloem, TRSV, peach yellow bud mosaic virus and BCMV are likely to move in just the phloem and thus rarely are transported to the opposite primary leaf. The present study strongly suggests that phloem transport is involved in long distance movement and distribution of BCMV. The inability of BCMV to move across steamed sections indicates that a) infectious particles can not move through a section of the stem devoid of living cells, i.e. movement is interrupted by dead tissue; b) movement in the xylem is very unlikely and relatively unimportant; and c) movement of BCMV is mainly in the phloem.

Phloem transport has been described for other plant viruses. Caldwell (6) showed that TMV did not pass through pieces of stem that had been killed with steam. Esau and Cronshaw (12) later demonstrated the presence of TMV particles

in phloem elements of tobacco plant. Movement in the phloem (54) as well as in the xylem (43) has been reported in SBMV.

Associated with phloem transport is the rapid movement and general distribution of BCMV in all parts of the plant. Viruses which move slowly through the plant from infection foci are less likely to survive and spread effectively than those which move rapidly. Speed of movement is important as measured relative to the life-time of the plant. In the case of bean, a rapid movement of BCMV throughout the vegetative tissue into the seed and its survival therein are of ecological advantage in spread and survival of the pathogen. The results of infectivity assays clearly show that absence of symptoms should not be interpreted as absence of infection; high virus titers were detected in many leaves prior to the appearance of visible symptoms.

Seed transmission of BCMV has been reported by numerous workers. However, little definitive work on the biology of such transmission has been done until the present study. This study indicates that the virus (BCMV) is associated with all reproductive organs - flowers, pods and seeds -- of the susceptible host, MCC. The data on infectivity of seed parts show that infectious particles are associated with both cotyledons and embryo of immature and mature seeds. Low levels of infectivity associated with seedcoats were readily eliminated by decontamination. Seed maturation and the associated dehydration apparently had no inhibitory effect

on BCMV infectivity in both cotyledon and embryo. Higher infectivities were consistently obtained in mature than in immature seeds and seed parts. These results differ markedly from those reported for SBMV in bean. Cheo (9) found that SBMV in the embryo was rapidly inhibited when dehydration of the seed occurred; infectious particles in the seedcoat were unaffected during maturation. Gay (15) in a similar study found that cowpea chlorotic mottle virus (CCMV) was rapidly inactivated during drying and suggested that CCMV is not transmitted in mature, dry seeds. In this case the virus was associated specifically with the seedcoat of both immature and mature seeds. No infectivity was associated with embryo or the cotyledons. Pea streak virus (PSV) is another virus which can be transmitted by immature, but not by mature seeds (Ford 1966).

Several theories have been advanced by different workers to explain variation or absence of seed transmission with certain viruses and the probable cause of virus inactivation during seed maturation (9, 11, 14, 15). Two of the most commonly cited are: anatomical isolation of the embryo from the rest of the seed; and inactivation by adsorption onto the seed protein.

It does not appear that anatomical isolation is sufficient to explain why only 46.6% of the seeds from systemically infected MCC plants transmitted BCMV. Obviously, all seeds in a developing pod are anatomically identical, and

yet, some of the seeds contain BCMV and some apparently do not (Table 9). These results are substantiated by numerous studies (13, 20, 34).

If on the other hand, inactivation and/or inhibition is involved, then seed-transmitted viruses such as BCMV probably possess some characteristic which resists the inactivation process (associated with adsorption and drying) or which protects them from the effects of inhibitors or both. Assuming this premise to be true for BCMV, we propose that the high incidence of seed transmission in BCMV is due to one or more of the following: (1) the ability of infectious particles to invade and multiply in the micro- and mega-spore mother cells; (2) the ability of infectious particles to withstand inactivation and inhibition in mature seeds; and (3) the ability to invade and multiply in the cotyledon and embryo. Perhaps the simplest explanation regarding the apparent absence of infectious particles in certain seeds within the same pod is that only seeds supplied by BCMV-invaded vascular connections become infected. Once in the seed the virus is able to survive adverse environmental conditions and then establish primary infections during the growing season. Successful seed transmission is insured not only by infection of the embryo at the time of germination by contact with infected cotyledons, but also by the presence of BCMV in the embryo itself. Such mechanism could explain

why seed transmission is more common in BCMV than in SBMV, where studies (31) indicate that seed transmission is a function of chance-infection of the embryo at germination with virus from the seedcoat.

CHAPTER V

SUMMARY

Monroe-local-lesion host was utilized in a study on the multiplication and distribution of bean common mosaic virus 1 ($BCMV_1$) in a susceptible bean variety, Mich-Cal-Cranberry (MCC). Primary leaves of all test plants were rub-inoculated with virus inoculum, usually at 10 days after planting. BCMV inocula (dilution 1:4) consisted of infected tissues triturated in 0.05M phosphate buffer (pH 6.9).

Plant age and temperature had a marked effect on multiplication and distribution of the virus. Above-ground parts of younger plants (MCC) consistently contained higher infectivity than corresponding parts of older plants. The converse was true in below-ground parts where higher virus infectivity was associated with the older plants. Temperature affected the lag period between inoculation and subsequent detection of BCMV in the primary leaf; the lag period was inversely related to temperature. Infectivity, on the other hand, was directly related to temperature; greater virus concentrations were associated with high (23, 28°) temperatures.

At constant temperature (23°) and photoperiod (15 hr), a cyclic pattern of infectivity was demonstrated in the first trifoliolate leaf. Infectivity peaks occurred at 6-7, 9-10 and 12 days after inoculation.

BCMV did not move across steam-killed stem sections devoid of living cells (including phloem). This evidence strongly suggests that phloem transport, as opposed to xylem transport, is involved in movement of BCMV. With one exception, infectious particles failed to move from inoculated primary leaf across the stem to the opposite uninoculated primary leaf.

Infectivity was obtained with flowers, immature and mature pods and seeds taken from systemically infected plants. Infectivity was consistently associated with dissected cotyledons and embryos. A seed transmission rate of 46.6% was recorded in a sample of 58 seeds harvested from the systemically infected plants.

REFERENCES

1. Archibald, E.S. (1921). Report of the Acting Dominion Botanist. Canada Expt. Farms Rpt. 1920: 58-64.

2. Bancroft, J.B. and Pound, G.S. (1954). Effect of air temperature on multiplication of tobacco mosaic virus in tobacco. Phytopathology 44: 481-482.

3. Barss, H.P. (1921). Bean blight and bean mosaic. Oreg. Agr. Expt. Sta. Crop Pest & Hort. Bien Rpt. (1915-20). 3: 192-194, illus.

4. Bawden, F.C. (1964). "Plant viruses and virus diseases." 4th edition. The Ronald Press Company, New York, 361 pp.

5. Biddulph, O. and Cory, R. (1965). Translocation of C^{14} metabolites in the phloem of the bean plant. Plant Physiology 40: 119-129.

6. Caldwell, J. (1931). The physiology of virus disease in plants. II. Further studies on the movement of mosaic in the tomato plant. Ann. Appl. Biol. 18: 279-298.

7. Cass Smith, W.P. (1945). The bean mosaic menace. West Austral. Dept. Agr. Jour. 22: 20-25, illus.

8. Chamberlain, E.E. (1939). Bean mosaic (Phaseolus Virus I of Smith, 1937). New Zeal. Jour. Sci. & Technol., Sec. A, 20: 381-388.

9. Cheo, P.C. (1955). Effect of maturation on inhibition of southern bean mosaic virus in bean. Phytopathology 45: 17-21.

10. Clinton, G.P. (1909). Notes on fungus diseases etc. for 1908. Conn. (State) Agric. Sta. Bien. Rept. 1907-1908: 849-871.

11. Duggar, B.M. (1930). The problem of seed transmission of typical mosaic of tobacco. Phytopathology 20: 133.

12. Esau, K. and Cronshaw, J. (1967). Relation of tobacco mosaic virus with host cells. J. Cell Biol. 33: 665-678.

13. Fajardo, T.G. (1930). Studies on the mosaic of bean (Phaseolus vulgaris L.) Phytopathology 20: 469-494, illus.

14. Ford, R.E. (1966). Recovery of pea streak virus from pea seed parts and its transmission by immature seeds. Phytopathology 56: 858-859.

15. Gay, J.D. (1969). Effect of seed maturation on the infectivity of cowpea chlorotic mottle virus. Phytopathology 59: 802-805.

16. Grainger, J. (1930). The appearance of bean mosaic in England . Leeds Phil., & Lit. Soc. Proc. 2: 1-32, illus.

17. Grogan, R.G. and Walker, J.C. (1948). The relation of common mosaic to black root of bean. Jour. Agr. Res. 77: 315-331, illus.

18. Gussow, H.T. (1922). Bean mosaic. Canada Expt. Farms, Div. Bot. Interim Rpt. 1921-22: 26-27.

19. Harrison, A.L. (1935). Mosaic of the Refugee bean. N.Y. State Agr. Expt. Sta. Bul. 656, 19 pp., illus.

20. Harrison, A.L. (1935). Transmission of bean mosaic. N.Y. State Agr. Expt. Sta. Techn. Bul. 236, 19pp., illus.

21. Harrison, A.L. & Burkholder, W.H. (1936). Canning bean diseases in New York in 1936. U.S. Bur. Plant Indus. Plant Dis. Reptr. 20: 290-291.

22. Harter, L.L. (1927). Bean diseases in the West. U.S. Bur. Plant Indus., Plant Dis. Rptr. 11: 148-149.

23. Holmes, F.O. (1929). Local lesion in tobacco mosaic. Bot. Gaz. LXXXVII, 39-55.

24. Holmes, F.O. (1929). Inoculation methods in tomato studies. Bot. Gaz. LXXXVII, 56-63.

25. Iwanowski, D. (1894). Ueber die Mosaikkrankheit der Tabakspflanze. Imp. Akad. Nauk Izv. (Acad. Imp. Sci St. Petersbourg Bul.) (n.s.) 35(3): 67-70.

26. Karle, H.P. and Shalla, J.A. (1966). Inability of peach bud mosaic virus and C^{14} to move into opposite non-inoculated primary leaves of cowpea. Photopathology 56: 562-563.

27. Kendrick, J.B. and Gardner, N.W. (1924). Soybean mosaic seed transmission and effect on yield. Jour. Agr. Res. 27: 91-98.

28. Kleczkowski, A. (1967). In Methods in Virology. K. Maramorosch and H. Koprowski, (Ed.). Academic Press, New York. Vol. IV. pp. 615-630.

29. Livne, A. and Daly, J.M. (1966). Translocation in healthy and rust affected beans. Phytopathology 56: 170-175.

30. Matthews, R.E.F. (1970). Plant Virology. XV + 778 pp. Academic Press, New York & London.

31. McDonald, J.G. and Hamilton, R.I. (1972). Distribution of southern bean mosaic virus in the seed of Phaseolus vulgaris. Phytopathology 62: 387-389.

32. Menke, G.H. and Walker, J.C. (1963). Metabolism of resistant and susceptible cucumber varieties infected with cucumber mosaic virus. Phytopathology 53: 1349-1355.

33. Nelson, R. (1922). Transference of the bean mosaic virus by Macrosiphum solanifolii. Science 56: 342-344.

34. Pierce, W.H. and Hungerford, C.W. (1929). Symptomatology, transmission, infection and control of bean mosaic in Idaho. Idaho Agr. Expt. Sta. Bul. 37 pp., illus.

35. Pound, G.S. (1949). The effect of air temperature on virus concentration and leaf morphology of mosaic infected horseradish. Jour. Agr. Res. (U.S.) 78: 161-170.

36. Pound, G.S. and Helms, K. (1955). Effect of temperature on multiplication of potato virus X in Nicotiana sp. Phytopathology 45: 493-499.

37. Pound, G.S. and Walker, J.C. (1945). Effect of air temperature on the concentration of certain viruses in cabbage. Jour. Agr. Res. (U.S.) 71: 471-85.

38. Pound, G.S. and Weathers, L.G. (1953). The effect of air and soil temperatures on the multiplication of turnip virus I in certain Nicotiana species. Phytopathology 43: 550-554.

39. Rands, R.D. and Brotherton, W. Jr. (1925). Bean varietal tests for disease resistance. Jour. Agr. Res. 31: 101-154, illus.

40. Reddick, D. and Stewart, V.B. (1917 & 1918). Transmission of the virus of bean mosaic in seed and observations on thermal death point of seed and virus. Phytopathology 54: 701-705.

41. Schneider, I.R. (1965). Difference in the translocability of tobacco ringspot and southern mosaic virus in bean. Phytopathology 54: 701-705.

42. Schneider, I.R. and Worley, J.F. (1958). Effect of high temperature on site and extent of multiplication of southern bean mosaic virus. Phytopathology 48: 244-248.

43. Schneider, I.R. and Worley, J.F. (1959_a). Upward and downward transport of infectious particles of southern bean mosaic virus through steamed portions of bean stems. Virology 8: 230-242.

44. Schneider, I.R. and Worley, J.F. (1959_b). Rapid entry of infectious particles of southern bean mosaic virus into living cells following transport of the particles in the water stream. Virology 8: 243-249.

45. Spragg, F.A. and Down, E.E. (1921). The Robust bean. Mich. Agr. Expt. Sta. Spec. Bul. 108, 9 pp. illus.

46. Stewart, V.B. and Reddick, J.E. (1917). Bean mosaic (Abstract). Phytopathology 7: 61.

47. Stone, R.E. and Howitt, J.E. (1920). Plant diseases and fungi comparatively new in Ontario. (Abs.) Phytopathology 10: 317-318.

48. Trujillo, G.E. (1971). Factors affecting the production of local lesions by bean common mosaic virus (BCMV). M.S. Thesis, Michigan State Univ. 72 pp.

49. Trujillo, G.E. and Saettler, A.W. (1971). Monroe bean as a new local lesion host for bean common mosaic virus. Phytopathology 61: 1026 (Abstr.).

50. United States Dept. of Agric. (1954). Agricultural Statistics 1954. 607 pp. Washington, D.C.

51. Waugh, T.D. (1948). Staining of stem tissue of plants by TTC. Science, 107:275.

52. Wilson, J.D. (1915). Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. 2: 420-427.

53. Wood, K.R. and Barbara, D.J. (1971). Virus multiplication and peroxidase activity in leaves of cucumber (Cucumis sativus L.) cultivars systemically infected with the W strain of cucumber mosaic virus. Physiol. Pl. Path. 1: 73-81.

54. Worley, J.F. (1965). Translocation of southern mosaic virus in phloem fibers. Phytopathology 55: 1299-1302.

55. Zaumeyer, W.J. (1930. Bean diseases in western United States in 1929. U.S. Bur. Plant Indus., Plant Dis. Rptr. 14: 38-43.

56. Zaumeyer, W.J. (1946). Bean diseases in some of the intermountain States in 1946. U.S. Bur. Plant. Indus., Soils and Agr. Engin. Plant Dis. Rptr. 30: 97-105.

57. Zaumeyer, W.J. and Harter, L.L. (1943). The new virus diseases of beans. Jour. Agr. Res. 67: 305-327, illus.

58. Zaumeyer, W.J. and Thomas, H.R. (1947). Bean diseases in some of the mountain states in 1947. U.S. Bur. Plant Indus., Soils and Agr. Engin., Plant Dis. Teptr. 31: 432-442.

59. Zaumeyer, W.J. and Thomas, H.R. (1957). A monographic study of bean diseases and methods for their control. Tech. Bul. No. 868 (U.S.D.A.) 255 pp., illus.

60. Zaumeyer, W.J. and Wade, B.L. (1935). The relationship of certain legume mosaics to bean. Jour. Agri. Res. 51: 715-749, illus.