FACTORS AFFECTING THE PRODUCTION OF LOCAL LESIONS BY BEAN COMMON MOSAIC VIRUS (BCMV)

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ABSTRACT

FACTORS AFFECTING THE PRODUCTION OF LOCAL LESIONS BY BEAN COMMON MOSAIC VIRUS (BCMV)

By

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All attempts to consistently obtain local lesions on rub-inoculated leaves of bean as reported by previous workers were unsuccessful. However, local necrotic lesions were consistently obtained on primary leaves of the Monroe bean variety rubbed with the type (V_1), New York 15 (V_{15}), and 123 (V_{123}) strains of bean common mosaic virus (BCMV). Under greenhouse temperature (22-27 C) and light (natural) conditions, necrotic lesions about 1 mm in diameter were initially observed and could be counted 4-5 days after inoculation. Similar lesions were formed on rub-inoculated trifoliolate leaves of Monroe bean. Inoculation of leaves with the rough end of a pestle was superior to other inoculation devices such as cheesecloth, foam rubber pads, and "Q-tips."

The number of local lesions obtained with V_1 and V_{15} on primary leaves of Monroe bean was inversely related to dilution of inoculum, and was relatively uniform among

the four half leaves on a plant. Uniform lesion counts were obtained when inocula were prepared in 0.01 Molar Na_2HPO_4-KH_2PO_4 buffers of pH 6.4-7.8.

Temperature affected the quality, as well as the quantity of BCMV-induced local lesions on Monroe bean. Lesion number and quality (shape and size) were highest at 20 C, good at 24 C, and poor at 28 C and 16 C. Preinoculation shading of plants for 24 hr increased the number of local lesions formed; lesions on such plants were somewhat larger than lesions on unshaded plants.

The optimum age of plants for primary leaf inoculation, as measured by local lesion production, was 9-12 days after planting; 10-days-old plants developed the largest number of local lesions.

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FACTORS AFFECTING THE PRODUCTION OF

LOCAL LESIONS BY BEAN COMMON

MOSAIC VIRUS (BCMV)

By

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TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS ii

LIST OF TABLES v

LIST OF FIGURES vii

Chapter

I. INTRODUCTION 1

Historical Background 1
Symptoms 2
Physico-Chemical Properties 2
Modes of Transmission 4
Seed Transmission 4
Mechanical Transmission 4
Insect Transmission 4
Host Range 4
BCMV Strains 5
Significance and Utility of a BCMV Local Lesion Host 9
Previously Reported Examples of BCMV--Induced Local Lesions 9
Factors Affecting Local Lesion Assay . . . 12
Light 12
Temperature 13
Age of Plant 14
Methods of Virus Preparation and Inoculation 15
Different Rubbing Methods 15
Additives that Increase Infection Efficiency 15
Objectives of This Study 16

II. MATERIALS AND METHODS 17

Plant Material 17
Virus Material 17
Inoculation Procedures 18

Chapter Page

Data Collection 19
Experiments 20
Preliminary Attempts to Reproduce Lesions Reported by Zaumeyer and Goth . . 20
Best Method of Inoculation 20
Inoculum pH 21
Relationship Between Dilution of Inoculum and Lesion Formation 21
Relationship Between Age of Leaf and Lesion Formation 22
Pre- and Post-Inoculation Light Treatment 22
The Effect of Temperature 23

III. RESULTS 25

Preliminary Attempts to Reproduce Lesions Reported by Zaumeyer and Goth 25
Best Method of Inoculation 31
Inoculum pH 33
Relationship Between Dilution of Inoculum and Lesion Formation 34
Relationship Between Age of Leaf and Lesion Formation 35
Pre- and Post-Inoculation Light Treatment 43
Effect of Temperature on Lesion Formation 46
Thermal Inactivation of BCMV 52
Local Lesion Formation by BCMV on Trifoliolate Leaves of Monroe Bean 52
Reaction of Monroe Bean to Other Plant Viruses 56

IV. DISCUSSION 57

V. SUMMARY 66

REFERENCES 68

LIST OF TABLES

Table		Page
1.	Reported strains of BCMV (bean common mosaic virus)	6
2.	Reactions of different bean varieties to strains of BCMV (bean common mosaic virus)	8
3.	The effect of constant air temperature (twelve hour photo period) on symptom response of primary bean leaves inoculated with 3 strains of bean common mosaic virus (BCMV)	27
4.	The effect of constant air temperature (continuous light) on symptom response of primary bean leaves inoculated with 3 strains of bean common mosaic virus (BCMV)	28
5.	Symptom response of primary bean leaves inoculated with 3 strains of bean common mosaic virus (BCMV) under greenhouse conditions (winter season)	29
6.	The effect of inoculation method on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean	32
7.	The effect of inoculation method on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean	32
8.	The effect of inoculum pH on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean	33
9.	The effect of inoculum pH on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean	34
10.	The effect of inoculum dilution on local lesion formation by $BCMV_1$ and $BCMV_{15}$ on primary leaves of Monroe bean	35

Table Page

11. The effect of inoculum dilution on local lesion formation by $BCMV_1$ and $BCMV_{15}$ on primary leaves of Monroe bean 35

12. The effect of inoculum dilution on formation of local lesions by $BCMV_1$ on primary leaves of Monroe bean plants of different ages 36

13. The effect of different light treatments on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean 46

14. The effects of different lengths of pre-inoculation shading on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean 50

15. The effect of constant air temperature on local lesion formation by BCMV on primary leaves of Monroe bean 52

16. Local lesion production by $BCMV_1$ on trifoliolate leaves of Monroe bean inoculated 25 days after planting 53

LIST OF FIGURES

Figure		Page
1A.	Typical symptoms of bean common mosaic virus (BCMV) on trifoliolate leaves of Rainy River	3
1B.	Plant of Rainy River bean showing systemic invasion of BCMV	3
2.	Symptom response of bean leaves inoculated with BCMV at 20 C. (A) Vein necrosis (VN) on Great Northern UI-31 inoculated with $BCMV_{15}$ (B) Ghost Spots (GS) on primary leaves of Great Northern UI-123 inoculated with $BCMV_1$ (arrow)	26
3.	Two types of local lesions on primary leaves of Monroe bean inoculated with BCMV (A) Local necrotic lesion on primary leaf of Monroe bean (B) Ringspot lesion on primary leaf of Monroe bean	30
4.	The effect of dilution of inoculum on local lesion production by two strains of BCMV on primary leaves of Monroe bean	37
5.	Primary leaves of Monroe bean inoculated with different dilutions of $BCMV_1$. Dilution endpoint of BCMV is between 1:800 and 1:1000	38
6.	Seedlings of Monroe bean at various times after planting. From right, 8, 9, 10, 11, 12, and 13 days after planting	40
7.	The effect of inoculum dilution on formation of local lesions by $BCMV_1$ on primary leaves of Monroe bean plants of different ages . .	42

Figure Page

8. The effect of different light treatments on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean
(A) Greenhouse-Greenhouse
(B) Greenhouse-24 hr. dark
(C) 24 hr. dark-24 hr. dark
(D) 24 hr. dark-Greenhouse 44

9. The effect of different light treatments on size of local lesions produced by $BCMV_1$ on primary leaves of Monroe bean 45

10. Ringspot-type lesion on primary leaf of Monroe bean inoculated with $BCMV_1$ at 24 C . 47

11. Severe vein necrosis on primary leaf of Monroe bean inoculated with $BCMV_1$ at 28 C. Local lesions are indistinct and difficult to count 48

12. Hypersensitive-type reaction of primary leaf of Topcrop bean inoculated with $BCMV_1$ at 32 C 49

13. Appearance of BCMV-induced lesions on inoculated primary leaves of Monroe bean at different times of the year
(A) Fall (October) lesions are Ringspot with necrotic center
(B) Winter (January) lesions are local necrotic lesions
(C) Spring (April) lesions are Ringspot with non-necrotic center. All lesions were formed in the greenhouse 51

14. Local lesion production on trifoliolate leaves of Monroe bean inoculated with $BCMV_1$ 54

CHAPTER I

INTRODUCTION

Historical Background

Bean common mosaic virus (BCMV, Bean Virus 1, Marmor phaseoli, Holmes) was first reported in Russia by Iwanowski (22) about 1894. Since that time mosaic has been reported in many countries throughout the world (52) and is almost always present wherever susceptible bean varieties are grown. This seed, insect and mechanically transmissible virus can cause large field losses in susceptible bean varieties.

In 1946 Zaumeyer (47, 51) reported the disease widespread in several western states; the disease was most severe in Colorado, where the yield in some fields was reduced by about one third. Outbreaks of bean mosaic in 1945 and 1947 in Idaho were severe in the variety Red Mexican 3; 100% plant infection was found in at least one field. Field reductions due to mosaic in fields of this same variety were considerable in western Washington. In 1951 Andersen (1) reported bean common mosaic in cranberry beans growing in Montcalm County, Michigan.

Symptoms

The symptoms of BCMV may vary according to the variety of bean infected, time of infection, environmental conditions and strain of the virus involved. Usually mottling and various types of leaf malformation are present (Figure 1); stunting of plant development is usually evident also. Trifoliolate leaves infected with mosaic usually have irregularly shaped, light yellow and green areas (52, 38). Bean plants infected early in the growing season are more likely to be stunted and yield less than plants infected later. Pods on severely infected plants are undersized and contain fewer ovules than those produced on normal plants (52). Usually, high temperatures favor optimum symptom development; symptoms tend to be masked at temperatures above 30 C and below 15 C (38).

Physico--Chemical Properties

The thermal inactivation point of BCMV lies between 56 and 58 C (52). The dilution end point is 1:1,000 and longevity in vitro between 24 to 32 hours at laboratory temperatures. All of these properties were studied, however, without the benefit of a local lesion host. According to Brandes and Quantz (38), BCMV is a typical rod-shaped particle measuring 750 mu in length.

Figure 1A. Typical symptoms of bean common mosaic virus (BCMV) on trifoliolate leaves of Rainy River.

Figure 1B. Plant of Rainy River bean showing systemic invasion by BCMV.

Modes of Transmission

Seed Transmission

Initial establishment of BCMV in a bean field is usually through the planting of virus-infected seed (27). Incidence of seed transmission as high as 30-50% has been reported (51). Fajardo (15) showed that incidence of infected seed harvested is correlated with certain stages of plant growth. Mosaic infection which occurred prior to blossom gave the greatest number of infected seeds.

Mechanical Transmission

BCMV is typical of many mosaic viruses in that it is transmitted through mechanical means, including machinery, man, and animals. The virus is easily transmitted to healthy plants by rub-inoculating leaves with sap expressed from infected leaves.

Insect Transmission

Insect transmission of BCMV has been shown for a number of aphid species (28, 50), including the common *Myzus persicae*.

Host Range

In addition to common bean (*Phaseolus vulgaris* L.), the following plant species have been found experimentally to be susceptible to BCMV:

Species: _Cajanus cajan_ (L) Millsp. (Spring pigeon pea), _Canavalia ensiformis_ (L) D. C. (jack bean), _Cicer aretinum_ L. (chick pea), _Crotalaria spectabilis_ Roth. (crotalaria), _Glycine max_ (L) Merr. (soybean) cv. Black hawk, _Lespedeza striata_ (Thumb.) H. & A. (common lespedeza), _Lupinus albus_ L. (yellow lupine), _Phaseolus acutifolius_ Gray var. _latifolious_ Freeman (tepary bean), P. _calcaratus_ (52). Roxb. (rice bean), P. _lunatus_ L. (lima bean) cv. Henderson, Bush, Thaxter, and Fordhoos 242, P. _mungo_ L. (urd bean), _Sesbania exaltata_ (Raf.) Cory, and _Stizolobium deeringianum_ Bort (velvet bean). In 1961 Quantz (29) reported _Vigna sinensis_ (Torner) Savi (cowpea) to be systemically-susceptible to BCMV. In 1964 Zaumeyer and Goth (49) added these additional hosts: _Cassia tora_, _Lens culinaris_ Medick, and _Trifolium subterraneum_ L. Under natural conditions, BCMV has been isolated from only _Phaseolus vulgaris_ L. (52).

BCMV Strains

A number of strains of BCMV have been reported and are summarized in Table 1. In 1939 Burkholder and Richards (31) found plants showing typical common mosaic virus symptoms in two fields of Michelite Navy (pea) beans near Batavia, New York. Because Michelite was reported resistant to BCMV (14), and because other resistant varieties such as Great Northern UI-15, Red Mexican UI-3, Red Mexican

UI-34 and Norida were also susceptible to the new virus, the authors concluded that this was a new strain of BCMV. The same or very similar strain was found in Idaho in 1946 (9) in a field of Great Northern UI-15 beans: this particular strain of BCMV is commonly called New York 15 (NY 15) or 1943 strain (36). In 1954 (10) another unreported strain of BCMV was discovered in Idaho infecting Great Northern UI-123 beans. Other bean varieties which possessed dominant resistance to the type strain were either resistant or susceptible to the new strain. The varieties Great Northerns UI-16, UI-31, UI-59, UI-81 and UI-123 are resistant to the type and New York 15 strain, but susceptible to the New 1954 strain. This strain has been referred to as 1954 strain, Idaho strain or BCMV 123.

Table 1. Reported strains of BCMV (bean common mosaic virus).

Strain Name	Year When Described	Workers
Type (V_1)	1894	Iwanowski
New York 15 (V_{15})	1943	Richards and Burkholder
123 (V_{123})	1959	Dean and Wilson
Western	1961	Skotland and Burke
Florida	1964	Zaumeyer and Goth
Mexican	1969	Silbernagel

In 1964 Zaumeyer and Goth (49) isolated an additional unreported strain from an experimental pole bean line, seed of which was received from R. A. Conover of Florida. Varieties resistant to the type and N.Y. 15 strains were also resistant to the new strain; it was mainly on the basis of severity of mosaic symptoms that the new strain could be distinguished. Several cowpea varieties were also useful in distinguishing the new strain. Because of the host from which it was first isolated, it is called the Florida strain.

A virus related to BCMV was reported in 1961 (37) from the Pacific Northwest. In addition to common bean, the "new" virus infected lima bean, cowpea var. Ramshorn, chick pea, Tepary bean, and spinach. Topcrop, Sanilac, Idaho Bountiful and Michelite were all immune. The virus was inactivated in 10 minutes between 60-65 C; possessed a dilution end point between 1:10,000 and 1:50,000, and was termed the Western Bean Mosaic Virus (WBMV).

More recently, Silbernagel (36) isolated a virus from the bean line P.I. 197690s', which he named the Mexican strain of BCMV. The bean variety Red Mexican UI-35 is susceptible and the variety Improved Tendergreen is resistant to the Mexican strain, distinguishing it from all of the other reported strains. Silbernagel used cross protection tests to show relationships with the NY-15 and Florida strains.

There are additional reports of other viruses related to BCMV (13, 16). In 1960 (46) Yerkes and Patino reported the severe strain of BCMV from Mexico but Grogan and Kimble (17) later showed that this strain was actually related to southern bean mosaic virus. Snow (39) isolated a virus from gladiolus that was capable of attacking plants possessing the dominant type of resistance derived from Corbett Refugee. Later, however, other workers (49) presented evidence that the gladiolus virus was a strain of yellow mosaic virus. All of the reported strains of BCMV are differentiated on the basis of their reactions on different bean varieties as shown in Table 2.

Table 2. Reactions of different bean varieties to strains of BCMV (bean common mosaic virus).[a]

	BCMV Strain					
Bean Variety	V_1	V_{15}	V_{123}	Florida	Mexican	Western
Bountiful	S	S	S	S	S	S
Great Northern UI-31	R	R	S	R	S	R
Great Northern UI-123	R	R	S	R	S	S
Pinto UI-111	R	S	R	R	R	R
Sanilac	R	S	R	R	R	R
Topcrop	R	R	R	R	R	R

[a]S = susceptible
R = resistant

Significance and Utility of a BCMV Local Lesion Host

Although BCMV has been recognized as a serious bean disease for a long time, its purification has only been partial primarily because no local lesion host is available to estimate virus activity during the purification process.

Quantitative assay using a local lesion host would allow detailed study of the physical and chemical properties of BCMV and its strains, eg. thermal stability, sedimentation constant, isoelectric point, longevity *in vitro*, resistance to certain enzymes and other chemicals. A local lesion host for BCMV would also be useful in cross protection tests to study relationships between the different strains and other viruses. The factors which influence the establishment and multiplication of the virus in a host could also be examined. These are just several of the reasons why a number of workers (35, 40, 48, 49) have attempted to find a local lesion host for BCMV.

Previously Reported Examples of BCMV--Induced Local Lesions

Using high temperatures (32 C) after inoculation, Thomas and Fisher (40) showed that BCMV produced necrosis and occasionally, necrotic lesions on the rubbed leaves of bean varieties such as Idaho Refugee and Topcrop. Only bean varieties which possess the dominant type of mosaic resistance derived from Corbett Refugee give this reaction. The virus later becomes systemic, killing the terminal growth and often killing the plant (18). Although it is a rapid test to detect dominant types of resistance to BCMV, high temperature (32 C) is necessary and the local necrotic lesions which do form are not produced consistently. Quantz in 1957 (29) described faded brown rings and vein necrosis on certain dry bean varieties when rub-inoculated with BCMV. However, these ringed lesions were not formed in reproducible numbers on inoculated leaves, and the lesions required a long period for appearance (3 weeks).

Quantz subsequently reported the production of discrete, brown necrotic lesions by BCMV on detached leaves of Topcrop in closed petri dishes at 32 C. In 1962 Schneider and Worley (35) reported a similar type of lesion which formed on detached leaves of Tender Long 15, as well as Topcrop beans inoculated with BCMV type or New York 15 strain. Detached leaves were placed in a nutrient medium incubated in the dark at 32-35 C. Various factors influenced the number and character of the lesions produced: inoculum concentration, preinoculation or post-inoculation exposure to high temperature and presence or absence of glucose in the medium. The nature and appearance of these lesions on Topcrop suggest that they are a modified hypersensitive reaction of the type initially described by Thomas and Fisher.

Zaumeyer and Goth (48, 49) have reported two types of local lesions. The first type was produced by the type, New York 15 and Florida strains of BCMV on leaves of Stringless Green Refugee, Plentiful, Potomac and Pinto UI-111. The lesions appeared 4 days after inoculation under normal greenhouse conditions, and also in a 27 C growth chamber under continuous light. Because these lesions were not consistently observed, they suggested that certain unknown environmental conditions were necessary for their formation.

The second type of local lesion produced by BCMV and two of its strains was a brown, ring-like lesion 5-7 mm in diameter which appeared 3-4 days after inoculation of leaves of an unnamed pole bean cross (obtained from R. A. Conover of Florida). Later, however, the virus became systemic causing typical mottling of the upper leaves.

Efforts of A. W. Saettler to obtain seed of this unnamed cross were unsuccessful, and, according to R. A. Conover (personal correspondence) "none of these stocks are available now." Dr. Conover, did, however send Dr. Saettler seed of the Dade bean variety which he indicated is almost certainly a sister line of the one used by Zaumeyer and "probably should react as did the stock Bill (Zaumeyer) used."

In 1969 (36) Silbernagel reported a hypersensitive reaction of Mexican BCMV strain on leaves of Topcrop plants maintained at 32 C after inoculation. This hypersensitive reaction of Topcrop is a characteristic for all strains of BCMV.

A. Andersen observed some years ago that Monroe, a Navy (pea) bean variety released by Cornell University, produced well-defined necrotic lesions when inoculated with N.Y. 15 strain (unpublished information). Andersen bulked a large number of single plant selections from plants showing the most ideal lesion development, but did not study the lesions further.

Factors Affecting Local Lesion Assay

Although many factors affect local lesion production qualitatively and quantitatively, light and temperature have been studied most extensively.

Light

Bawden and Roberts (4) reported that shading plants for 24 hours prior to inoculation increased susceptibility to infection with tobacco necrosis, tomato bushy stunt, tobacco mosaic and tomato aucuba viruses on tobacco var. White Burley, *Nicotiana glutinosa*, tomato var. Kondine Red and tobacco and tomato, respectively.

Desjardins (12) working with alfalfa mosaic virus on beans, reported that lesions were larger than normal if the leaves were kept in the dark or shaded immediately after inoculation.

Huguelet and Hooker (21) using potato virus on Gomphrena globosa reported that a period of darkness after inoculation seemed to suppress lesion formation.

Temperature

Bawden (2, 13) stated that the most general effect of increasing temperature is to shorten the interval between infection and the production of lesions. Temperature may affect both the severity and the type of symptom depending on the virus-host combination used.

Kasanis (24) reported that when Nicotiana glutinosa and bean plants were kept at 35 C for about 24 hrs., their subsequent susceptibility to tobacco mosaic, tobacco necrosis and tomato bushy stunt viruses was increased as measured by local lesions.

In 1958 Yarwood (44) reported that lesion counts of TMV were increased 2-3 times when Pinto bean leaves were heated for 30-45 sec. at 50 C, 5-10 hours after inoculation.

Ross (32), working with potato virus Y on Physalis floridana reported that postinoculation temperature exerted a great effect on local lesion formation. Although more local lesions were produced at 27 C than at 18 C, lesions at 18 C were more distinct and more easily counted than those at 27 C. Ross also found that preinoculation temperature had very little effect on the subsequent production of lesions on *P. floridana*.

Age of Plant

Young, well fertilized, rapidly growing plants are usually the best source of inoculum for virus studies (26, 33). Matthews (26) indicates that the susceptibility of leaves to a particular virus may change rapidly as the leaves age.

Bawden (2), working with tobacco necrosis virus on French bean (*P. vulgaris*) showed that 10-day-old plants gave many infection points, but that 13 to 14-day-old plants gave no infection points.

Shein (34) found that susceptibility of beans to TMV, as measured by local lesion numbers, was low at the time of leaf unfolding, maximum when leaves were 20-40% expanded, and low again when leaves were approximately 100% fully expanded.

Ross (32), working with potato virus Y on *Physalis floridana*, found that lesion formation was optimum when plants were inoculated during early flowering.

Crowley (8) found with lettuce necrotic yellow virus on *Nicotiana glutinosa* that the most important factor affecting susceptibility was the age of the plant; young leaves, one-half to three-fourths expanded at the time of inoculation were more susceptible than older leaves.

Methods of Virus Preparation and Inoculation

Different Rubbing Methods

The more common devices used for rub inoculation are: finger, spatula, cheesecloth pad, pestle and brush. The standard rubbing method is to dip the device into virus suspension, and to rub the virus suspension onto the upper surface of young leaves previously dusted with an abrasive. Leaves are quickly rinsed with water after inoculation. Yarwood (43) indicated that all of these methods if properly used will give similar results.

Additives that Increase Infection Efficiency

It has been shown consistently that efficiency of mechanical inoculation is increased when an abrasive material is used (added to the inoculum or sprinkled over the leaves) (43, 44, 45). The most commonly used abrasives are carborundum (400-500 mesh) or diatomaceus earth such as Celite.

In 1952 Yarwood (42) showed that the addition of 1% solution of dipotassium phosphate to the inoculum greatly increased virus infectivity, but the way in which phosphate increases the number of successful infections is not yet understood (45).

Objectives of This Study

The objectives of this investigation were threefold:

1. Attempt to repeat the results of Zaumeyer (49) and others in relation to production of local lesion by BCMV on different bean varieties.

2. To test the utility of Monroe bean as a local lesion host.

3. To determine some of the environmental and physiological factors which influence local lesion formation by BCMV on Monroe.

CHAPTER II

MATERIAL AND METHODS

Plant Material

Seed of various bean varieties was planted in a soil mix composed of equal parts (1:1) muck soil and vermiculite. Plants were contained in 32 oz. wax-lined cardboard cartons, 3 plants per carton (Figure 6). Plants were watered alternately as needed with modified Hoagland's solution, tap water, and iron solution (Sequestrene, Fe as metallic 1.8 ppm.). Unless indicated otherwise, all plants were grown under normal greenhouse conditions of alternating air temperature (22-27 C); natural daylight was supplemented 12 hrs. daily during winter season with fluorescent lamps (60 ft-c).

Virus Material

All of the BCMV strains used in this study were initially established from virus-infected seeds (collected in 1961) or from desiccated plant tissue kept in the freezer since 1961. All of the strains were checked for purity and pathogenicity on the differential bean varieties prior to and during this study. Fresh juice inoculum was prepared from Rainy River (BCMV type), from Pinto UI-111 (N.Y. 15 strain), and from Great Northern 123 (BCMV-123). Young trifoliolate leaves showing good mosaic symptoms (about 20-24 days after plant inoculation) were removed and triturated in a sterilized mortar. The macerated tissue was pressed through one layer of cheesecloth and dilutions prepared in 0.01 M phosphate (Na_2 HPO_4 - KH_2 PO_4) buffer pH 7.4. In some cases, the infected leaves were weighed and buffer added prior to trituration.

Inoculation Procedures

All inoculations were performed within 2.5 hours after inoculum preparation, and most were performed on primary leaves using the half leaf method (25). A summary of this method is as follows: Each plant is a block, each half leaf of opposite primary leaves is a unit, and four treatments in each block of 4 units.

Randomized Blocks: Four Treatments in Blocks of Four Units:

	Plant Number					
Leaf number	1	2	3	4	5	6
1	C D	B D	A C	D A	C A	D B
2	B A	C A	D B	C B	B D	C A

Leaf surfaces were dusted prior to rub inoculation with 500 mesh silicon carbide using a hand-operated atomizer.

The amount of inoculum was carefully measured with a glass pipette. Generally 2 drops (0.1 ml) of inoculum was applied per half leaf. The drops were placed near the petiole base and distributed over the leaf surface with a pestle; strokes were made to the leaf tip and back again two times to insure uniform distribution of inoculum. After inoculation the rubbed leaves were rinsed with a gentle stream of distilled water from a squirt bottle.

Each experiment was repeated at least 3 times, and in each experiment virus purity was determined by inoculating 3 plants each of the differential varieties Rainy River (type), Pinto UI-111 (N.Y.-15), and Great Northern UI-123 (V_{123}). When inoculated plants were maintained in the greenhouse, inoculations were made in the afternoon between 2-4 p.m. in order to have comparable conditions. It has been reported that number of local lesions depends on the time of the day that inoculation is done (2, 26).

Data Collection

In those plants where local lesions were formed, at least two counts were made several days apart. If little or no change in lesion number was noted, no additional counts were made.

Experiments

Preliminary Attempts to Reproduce Lesions Reported by Zaumeyer and Goth

Two pots each of Pinto UI-111, Plentiful, Potomac, Dade, Monroe, Sanilac, Great Northern UI-31, Great Northern U-123, and Stringless Green Refugee beans were inoculated with BCMV type, V15 and V123 strains at 9 days after planting. Inoculated plants were incubated in growth chambers at 3 different air temperatures (20-24-28 C) and 12 hour photoperiod. Inoculum was prepared by grinding young leaves with good mosaic symptoms in a mortar with buffer phosphate pH 7.4 in a ratio of 1:1 (weight of leaf in gm/ml buffer). The inoculation was done by dipping the pestle in the virus preparation and rubbing carborundum-dusted primary leaves. The same experiment was repeated at 24 and 28 C under continuous light and at greenhouse conditions (winter season).

Best Method of Inoculation

Six Monroe plants, 11-days-old, were inoculated with juice inoculum (1:4) of $BCMV_1$ using the same half leaf method previously described. In one randomized block, the inoculation treatments consisted of: (1) half leaves rubbed with a square of polyurethane foam 3 x 3 cm and 0.5 thick, (2) cheesecloth pad, (3) pestle, and (4) finger.

In another randomized block, the treatments consisted of (1) pestle, (2) "Q"-tip, (3) glass spatula, and (4) pestle using "healthy" juice.

Inoculum pH

Phosphate buffers (0.01 m) of different pH values were prepared by altering the ratios of 0.01 M Na_2 HPO_4 and 0.01 m KH_2PO_4 until the desired pH was obtained. Inocula were then prepared by preparing dilutions (1:4) in the different buffers.

Groups of 12 Monroe plants, 12-days-old, were inoculated by the half leaf method (randomized blocks) with BCMV type using the following treatments: A = pH 6.4, B = pH 7.0, C = pH 7.4 and D = pH 7.8.

An additional group of 12 Monroe plants was inoculated by the same method using the following pH: A^1 = pH 5.0, B^1 = pH 6.0, C^1 = pH 7.4, and D^1 = pH 8.5.

Relation Between Dilution of Inoculum and Lesion Formation

A group of 6 Monroe plants (12-days-old) was inoculated with the type strain and another group inoculated with the N.Y.-15 strain, using the previously described pestle method of leaf inoculation. The following dilutions of inoculum were prepared in 0.01 M PO_4 buffer of pH 7.4: 1:1, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256. Six half leaves were inoculated with each dilution.

In another experiment, the dilution end point of $BCMV_1$ and V_{15} were determined. Inoculum dilutions of 1:100, 1:500, 1:1,000, 1:10,000 were prepared in distilled water and applied to primary leaves as described previously.

Relationship Between Age of Leaf and Lesion Formation

Formation.--Primary leaves on each of 6 Monroe plants at 8, 9, 10, 11, 12, 13, 14, 15 and 16 days after planting were inoculated with the type strain of BCMV using the half leaf method. Each plant was inoculated with 4 different inoculum dilutions (1:1, 1:4, 1:8, and 1:16).

Pre- and Post-Inoculation Light Treatment

Because preincubation in darkness, or shading has been shown to markedly affect lesion formation in certain host-virus combinations (12), the following experiments were conducted:

1. In the first experiment the following light-dark regimes were used: (a) Plants growing in normal greenhouse conditions were inoculated and returned to the same greenhouse condition, (b) plants growing in normal greenhouse conditions were inoculated, then incubated 24 hours in dark. (c) Plants preincubated for 24 hours in the dark were inoculated, and then placed in the dark for

an additional 24 hours. (d) Plants preincubated for 24 hours in the dark were inoculated, and then placed at normal greenhouse conditions.

For each treatment 6 Monroe plants (12-days-old) were used, and the concentration of inoculum was 1:4. Darkness during daylight hours was obtained by placing the plants beneath a box (3.5 x 2.0 x 1.5 feet) covered with black plastic.

2. In the second experiment 3 different lengths of preinoculation shading were compared, (a) 48 hours, (b) 24 hours, and (c) 18 hours. After inoculation, all plants were returned to normal greenhouse conditions. Data taken at 7 and 10 days after inoculation included number of local lesions (at 7 days) and lesion diameter (at 10 days-diameter of 10 local lesions per leaf).

The Effect of Temperature

Primary leaves of three Monroe and three Topcrop bean plants, 12-days-old, were inoculated with $BCMV_1$, and then held in different growth chambers at constant air temperatures of 16, 20, 24 and 28 C. All chambers were programmed with 14 hr. of light daily, with a light intensity of 1,150 ft-c at leaf surface.

To compare the local lesions formed by BCMV on Monroe with the hypersensitive lesions formed on Topcrop, 3 plants of each variety were inoculated with the type strain, and subsequently maintained in darkness at 32 C. In some cases detached, young primary leaves (10-days-old) and trifoliate leaves were also inoculated and maintained under the same conditions in petri dishes containing a small amount of water.

CHAPTER III

RESULTS

Preliminary Attempts to Reproduce Lesions Reported by Zaumeyer and Goth

Three different kinds of lesions were obtained in the preliminary experiments. The first kind of lesion (Figure 2B) appeared approximately 7 days after inoculation as barely-visible chlorotic spots of diameter about 2 mm. These lesions gradually became necrotic after 2-3 weeks and are referred to as "Ghost Spots" (GS). GS lesions appeared on primary leaves of Great Northern UI-123, Great Northern UI-31, Pinto UI-111, and Sanilac inoculated with $BCMV_1$ and $BCMV_{15}$ at different light and temperature regimes (Tables 3, 4). This type of lesion, however, was not observed consistently, and was not obtained in the greenhouse (Table 5) (Figure 3).

The second kind of lesion was a necrotic ring spot 1-2 mm in diameter that was clearly visible 5 days after inoculation. These ringspot (RS) lesions (Figure 3B) were observed on primary leaves of Potomac, Rainy River, Plentiful, Stringless Green Refugee, and Monroe bean inoculated with all three strains of BCMV (Tables 3, 4, 5). Although

Figure 2A. Vein necrosis (VN) on Great Northern UI-31 inoculated with $BCMV_{15}$.

Figure 2B. Ghost Spots (GS) on primary leaves of Great Northern UI-123 inoculated with $BCMV_1$ (arrow).

Table 3. The effect of constant air temperature (twelve hour photo period) on symptom response of primary bean leaves inoculated with 3 strains of bean common mosaic virus (BCMV).

	BCMV Strain								
	V_1			V_{15}			V_{123}		
Bean Variety	20°C	24°C	28°C	20°C	24°C	28°C	20°C	24°C	28°C
Dade	-	-	-	-	-	-	-	-	-
Great Northern UI-31	GS^b	GS^b,VN	GS^b,VN	-	GS^b,VN	GS^b,VN	-	-	-
Great Northern UI-123	GS^b	GS^b,VN	GS^b,VN	-	GS^b	GS^b,VN	-	-	-
Monroe	LL	RS	RS,VN	LL	RS	RS,VN	LL	RS,VN	RS,VN
Sanilac	-	GS^b	GS^b	-	-	-	-	-	-
Stringless Green Refugee	RS^b	RS^b,VN	RS^b,VN	-	RS^b,VN	RS^b,VN	-	RS^b,VN	RS^b,VN
Pinto UI-111	GS^b	-	GS^b,VN	-	-	-	-	-	-
Plentiful	-	RS^b	-	RS^b	-	-	-	-	-
Potomac	RS^b	RS^b,VN	-	RS^b	RS^b,VN	RS^b,VN	-	RS^b,VN	RS^b,VN

\- = no symptoms
GS = ghost spot
LL = local lesions
RS = ring spot
VN = vein necrosis
[b] infrequently formed

Table 4. The effect of constant air temperature (continuous light) on symptom response of primary bean leaves inoculated with 3 strains of bean common mosaic virus (BCMV).[a]

	BCMV Strain					
	V_1		V_{15}		V_{123}	
Bean Variety	24°C	28°C	24°C	28°C	24°C	28°C
Dade	VN[b]	VN[b]	-	-	-	-
Great Northern UI-31	GS[b],VN[b]	VN[b],GS[b]	-	VN[b],GS[b]	-	-
Monroe	VN,RS	VSN,RS	VN,RS	VN,RS	VN,RS	VN,RS
Potomac	VN[b],RS	VN[b],RS	VN[b],RS	VN[b]	VN[b]	VN[b]
Sanilac	VN[b]	VN[b]	-	-	-	-
Stringless Green Refugee	VN[b],RS	VN[b],RS	VN[b]	VN[b]	-	-

[a]no symptoms
GS = ghost spot
RS = ring spot
VN = vein necrosis
[b]infrequently formed

Table 5. Symptom response of primary bean leaves inoculated with 3 strains of bean common mosaic virus (BCMV) under greenhouse conditions (Winter season).[a]

Bean Variety	BCMV Strain V_1	V_{15}	V_{123}
Dade	-	-	-
Great Northern UI-31	-	-	-
Great Northern UI-123	-	-	-
Monroe	LL	LL	LL
Rainy River	-	RS[b]	RS[b]
Sanilac	-	VN[b]	VN[b]
Stringless Green Refugee	RS[b]	-	-
Pinto UI-111	-	-	-
Plentiful	-	-	-
Potomac	-	-	-
Topcrop	-	-	-

[a] - no symptoms
LL = local lesions
RS = ring spot
VN = vein necrosis
[b] infrequently formed

Figure 3A. Local necrotic lesion on primary leaf of Monroe bean.

Figure 3B. Ringspot lesion on primary leaf of Monroe bean.

infrequently formed on the first 4 of these varieties, RS lesions were always observed on the Monroe variety when the inoculated plants were held at 24-28 C under 12 hr. light or under continuous light. At 28 C, however, the lesions were always accompanied by strong vein necrosis, making it difficult to count them (Figure 2A).

The third kind of lesion appeared only on leaves of Monroe bean at 16-20 C (12 hr. photoperiod or continuous light) and at greenhouse conditions (winter); these lesions are called local lesions (LL) (Figure 3A). The local lesions were circular and necrotic, well-defined and clearly visible 4-5 days after inoculation. These lesions enlarged in diameter from 0.5 mm at 6 days to nearly 0.8 mm at 10 days after inoculation. Throughout the duration of this study, LL were consistently formed on inoculated primary leaves of Monroe bean maintained either in the greenhouse or in a 20 C growth chamber.

Numerous individual local lesions triturated separately and inoculated to susceptible bean varieties induced typical BCMV symptoms.

Best Method of Inoculation

Of numerous inoculation techniques examined, the pestle appears to give the most consistent and reproducible results (Tables 6, 7). At least a portion of the inoculum applied to each half leaf (0.1 ml) was absorbed when cheesecloth, foam rubber pad, and "Q"-tip devices were used; this absorption could account for the lower lesion counts obtained with these devices. None of the devices tested caused any appreciable leaf damage.

Table 6. The effect of inoculation method on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean.

Inoculation Method	Number Lesions per 6 half leaves	Average Number Lesions per half leaf
Cheesecloth pad	83	13.8 ± 3.0
Finger	38	6.3 ± 2.5
Foam rubber pad	79	13.1 ± 2.3
Pestle	182	30.3 ± 6.6

Table 7. The effect of inoculation method on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean.

Inoculation Method	Number Lesions per 6 half leaves	Average Number Lesions per half leaf
Frosted glass spatula	102	17.0 ± 2.6
Pestle	195	32.5 ± 5.0
Pestle--no inoculum	0	0
"Q"-tip	40	6.6 ± 2.8

Although the data presented in Tables 6 and 7 are from two separate experiments, it is worth noting the consistency of lesion counts (30.3 versus 32.5) obtained with the pestle method of inoculation. Also note that the pestle alone (inoculum replaced with water) caused no damage which could be mistaken for local lesions (Table 7).

Inoculum pH

Within the general pH range of 6.4 to 7.8, local lesion counts were quite uniform and comparable (Tables 8, 9). Inocula prepared in buffer of pH 5.0, 6.0, and 8.5 produced fewer local lesions per half leaf than inocula of the above pH levels. Throughout the remainder of this study, all inocula were prepared in pH 7.4 buffer.

Table 8. The effect of inoculum pH on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean.

Inoculum pH	Number Lesions per 12 half leaves	Average Number Lesions per half leaf
6.4	380	31.6 ± 4.7
7.0	373	31.0 ± 5.1
7.4	371	31.0 ± 4.2
7.8	395	32.0 ± 4.9

Table 9. The effect of inoculum pH on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean.

Inoculum pH	Number Lesions per 12 half leaves	Average Number Lesions per half leaf
5.0	238	19.5 ± 5.8
6.0	304	25.3 ± 5.1
7.4	352	29.3 ± 4.6
8.5	257	21.4 ± 4.5

Relationship Between Dilution of Inoculum and Lesion Formation

Local lesion production on inoculated leaves of Monroe is inversely related to dilution of inoculum; i.e., the lower the inoculum dilution, the higher the lesion counts (Tables 10, 11, 12, Figures 4, 5). Note that with both the V_1 and V_{15} strains of BCMV, a straight line relationship exists between local lesion number and inoculum dilution at dilutions between 1:4 and 1:32. The dilution end point of BCMV, as assayed on Monroe by the V_1 and V_{15} strains, lies between 1:1000 and 1:10,000 (Table 11). Unless indicated otherwise, inocula were diluted 1:4 in pH 7.4 buffer throughout the remainder of this study.

Table 10. The effect of inoculum dilution on local lesion formation by $BCMV_1$ and $BCMV_{15}$ on primary leaves of Monroe bean.

BCMV Strain	Number Lesions per 6 half leaves at several inoculum dilutions							
	1:1	1:4	1:8	1:16	1:32	1:64	1:128	1:256
V_1	428	205	171	149	121	39	21	18
V_{15}	385	196	159	133	113	43	36	23

Table 11. The effect of inoculum dilution on local lesion formation by $BCMV_1$ and $BCMV_{15}$ on primary leaves of Monroe bean.

BCMV Strain	Number Local Lesions per 6 half leaves at several inoculum dilutions			
	1:100	1:500	1:1000	1:10,000
V_1	28	15	5	0
V_{15}	52	21	13	0

Relationship Between Age of Leaf and Lesion Formation

Age of leaf is an important factor in the production of local lesions by BCMV on Monroe bean (Table 12, Figures 6, 7). With the exception of the data for 10-day-old plants, rather uniform local lesion counts were obtained on plants inoculated 8, 9, 11, 12, and 13 days after planting

Table 12. The effect of dilution on formation of local lesions by $BCMV_1$ on primary leaves of Monroe bean plants of different ages.[a]

Inoculum	8		9		10		11		12		13		14		15		16	
Dilution	No	Ave	No	Ave	No	Ave	No	Ave	No	Ave	No	Ave	No	Ave	No	Ave	No	Ave
1:1	202	33.6	451	75.1	587	97.8	716	119.3	451	75.1	243	40.5	195	32.5	208	34.6	176	29.3
1:4	176	29.3	225	37.5	540	90.0	336	56.3	219	36.5	213	36.5	117	19.5	157	26.1	64	10.6
1:8	181	30.1	172	28.5	355	59.1	212	35.3	190	31.6	177	29.5	93	15.5	96	16.0	67	11.1
1:16	155	25.8	165	27.5	308	51.3	178	29.6	151	25.1	103	17.1	50	8.3	45	7.5	55	9.1

[a]No = number lesions per 6 half leaves
Ave = average number lesions per half leaf

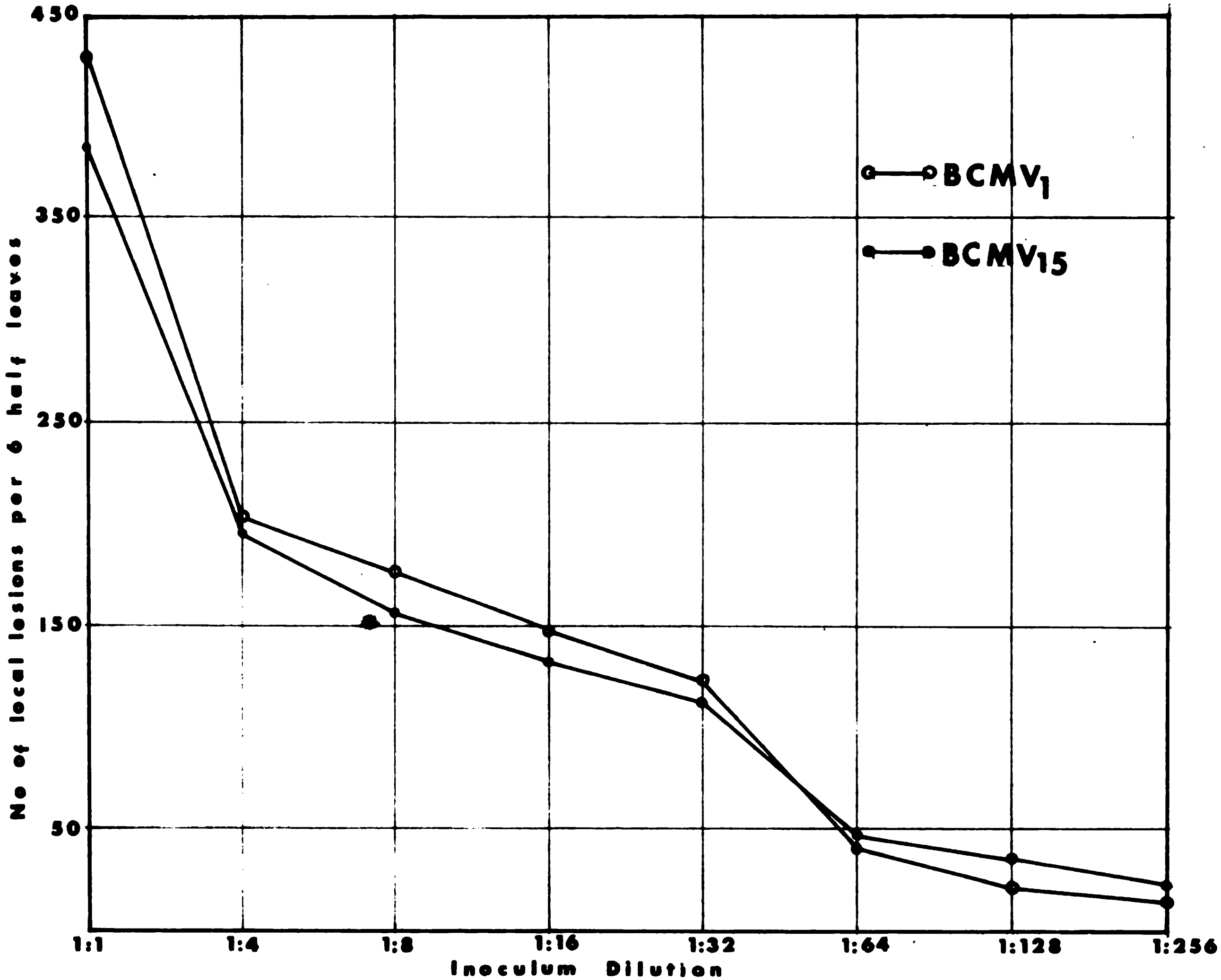
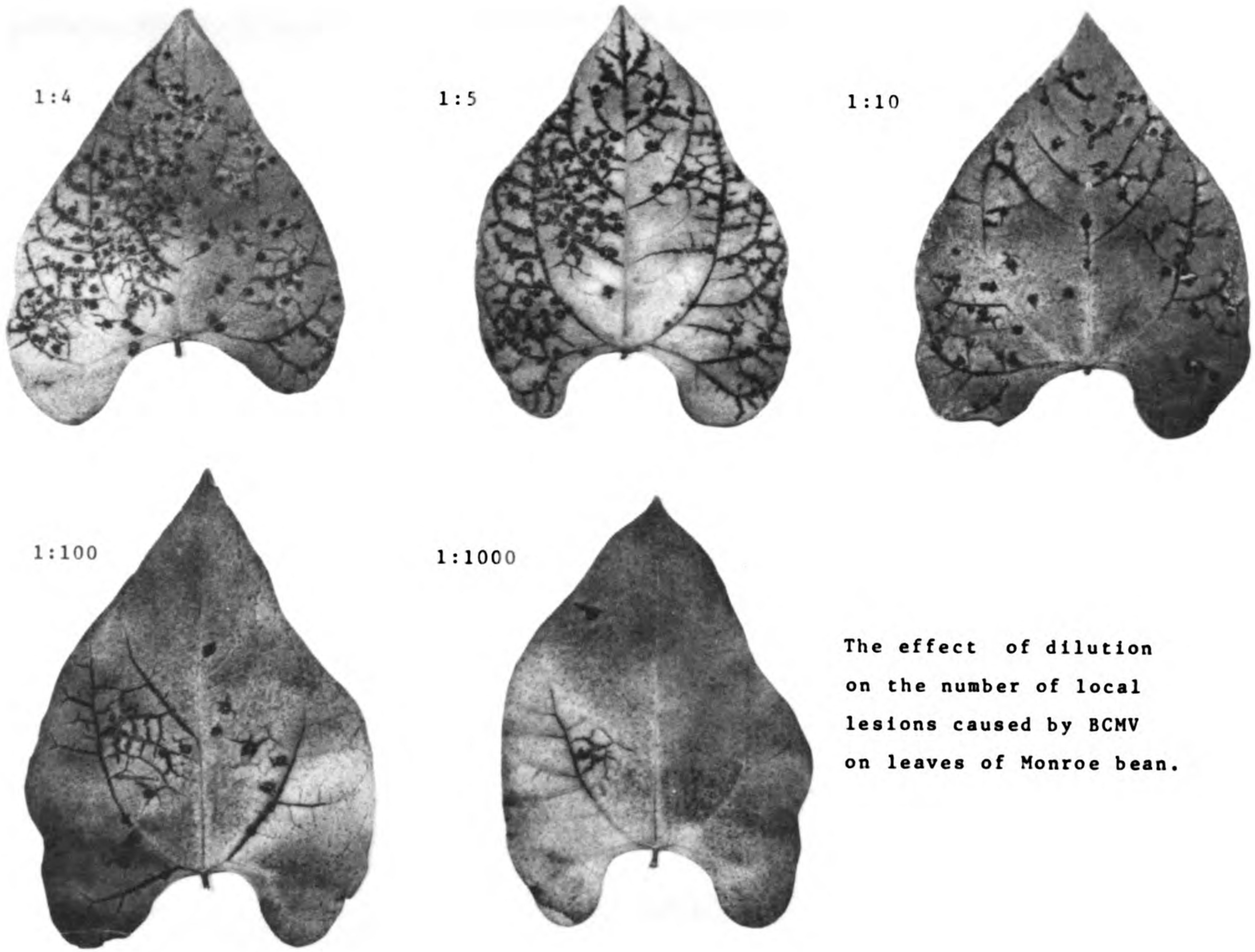


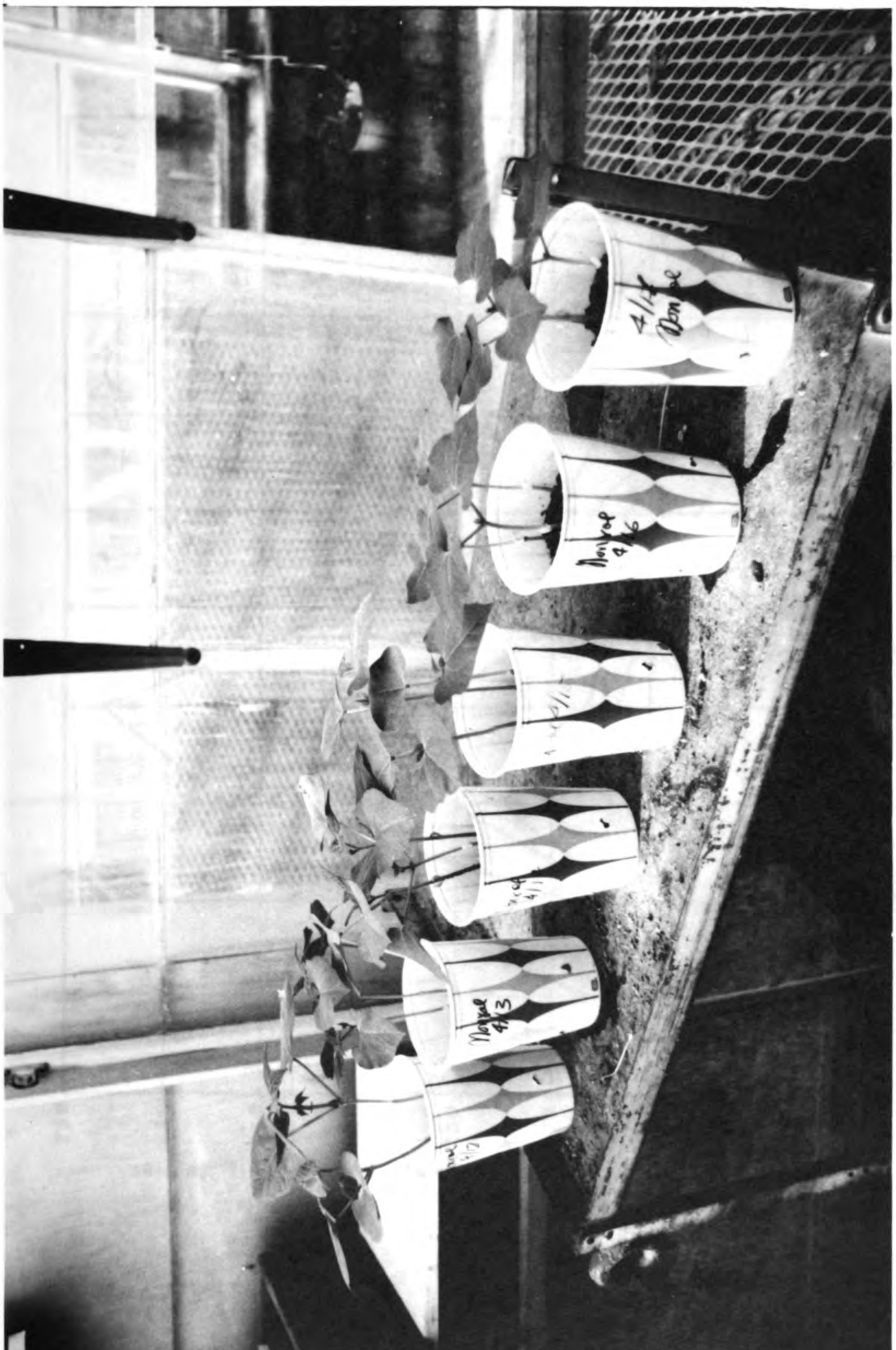
FIGURE 4. The effect of dilution of inoculum on local lesion production by two strains of BCMV on primary leaves of MONROE bean

Figure 5. Primary leaves of Monroe bean inoculated with different dilutions of $BCMV_1$. Dilution end point of BCMV is between 1:800 and 1:1000.



The effect of dilution
on the number of local
lesions caused by BCMV
on leaves of Monroe bean.

Figure 6. Seedlings of Monroe bean at various times after planting. From right, 8, 9, 10, 11, 12 and 13 days after planting.



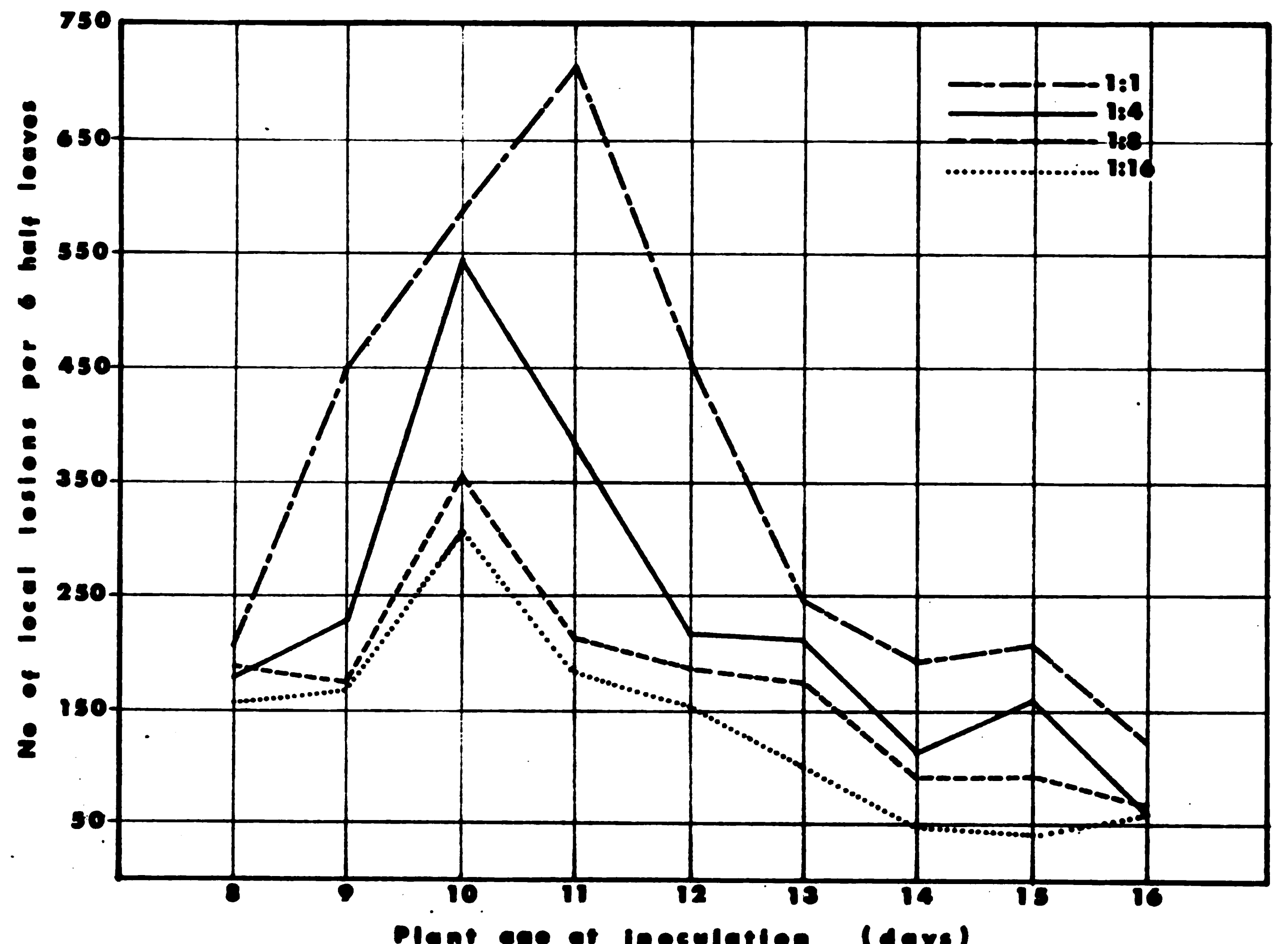


FIGURE 7.

The effect of inoculum dilution on formation of local lesions by $BCMV_1$ on primary leaves of MONROE bean plants of different ages with virus inoculum diluted 1:4. It is quite difficult to count more than 40-60 lesions per half leaf; consequently, the very high counts recorded for 10-day-old leaves may be subject to error. For this reason, and because many of the previous studies employed 12-day-old plants, it was decided to standardize age of bean at inoculation to 12 days after seeding.

Pre- and Post-Inoculation Light Treatment

Interruption of the normal light conditions which exist in the greenhouse (winter) exerts a great influence on the quality, as well as the quantity of local lesions produced on inoculated leaves of Monroe bean (Tables 13, 14, Figures 8, 9). In terms of number of local lesions, post-inoculation shading (24 hr.) of plants had no effect, while preinoculation shading (24 hr.) increased the lesion counts about 40%. A combination of pre- and post-inoculation shading (24 hr. each) reduced lesion counts by about 30%. The optimum period of pre-inoculation shading was found to be 24 hr.; 48 hr. shading drastically reduced lesion formation.

Lesions on leaves shaded prior to inoculation were not only more numerous, but also larger than lesions on leaves under normal conditions (Figures 8, 9). Lesions on leaves receiving the 24 hr. dark-greenhouse treatment were of the ringspot type (RS), averaging 1.56 mm (average of

Figure 8. The effect of different light treatments on local lesion formation by BCMV on primary leaves of Monroe bean. (A) Greenhouse-Greenhouse, (B) Greenhouse-24 hr. dark, (C) 24 hr. dark-24 hr. dark, and (D) 24 hr. dark-Greenhouse.

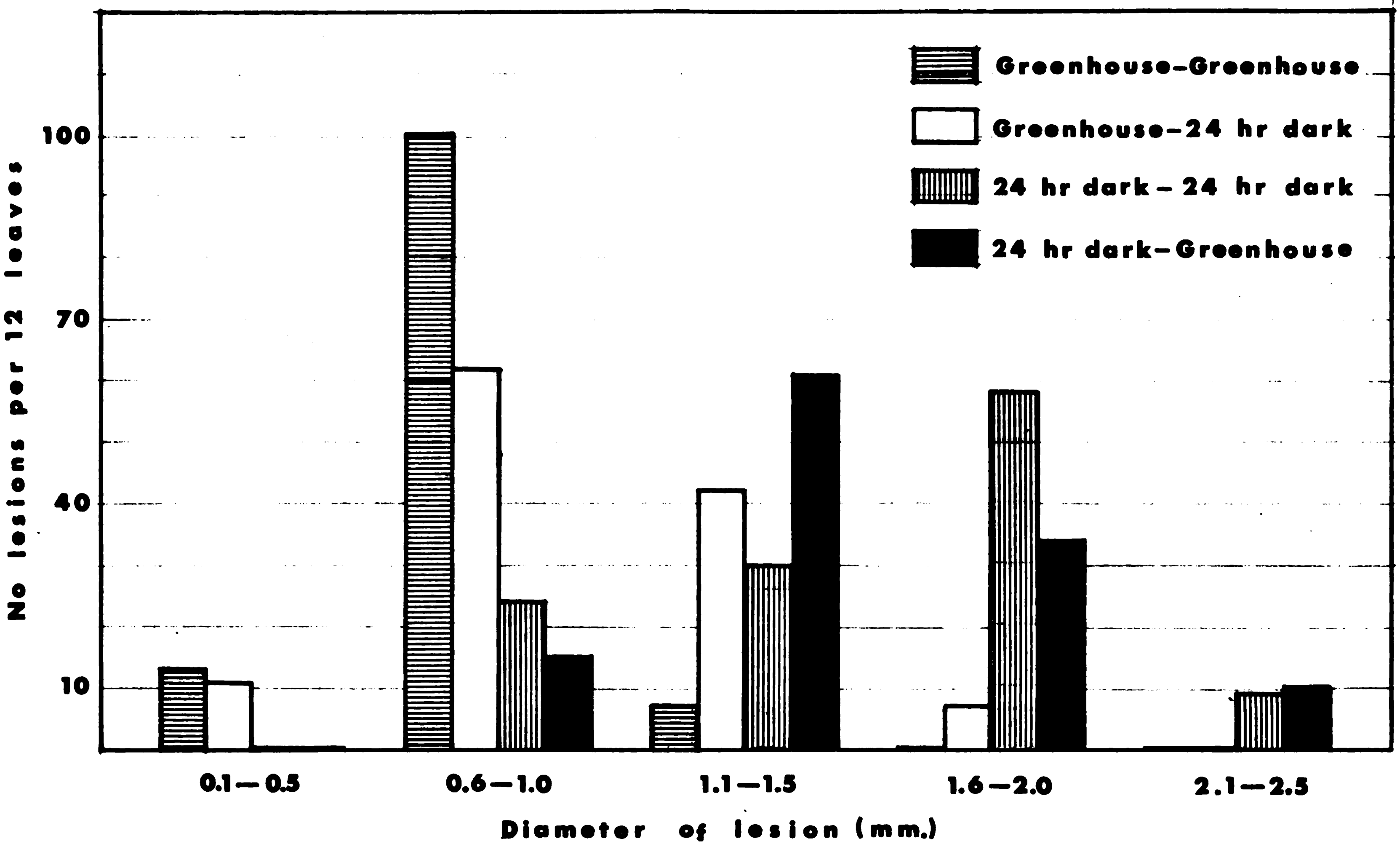


FIGURE 9 The effect of different light treatments on size of local lesions produced by $BCMV_1$ on primary leaves of MONROE bean.

120 lesions) in diameter; lesions from normal conditions (greenhouse-greenhouse) were of the necrotic local lesion type, averaging 0.88 mm in diameter. Lesion types in the remaining two treatments were ringspot (24 hr. dark-24 hr. dark and greenhouse-24 hr. dark). A combination of ringspot and local necrotic lesion was found in some leaves with the treatment greenhouse-24 hr. dark.

Table 13. The effect of different light treatments on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean.[a]

Light Treatments Before and After Inoculation	Number Lesions per 24 half leaves	Average Number Lesions per half leaf
Greenhouse-Greenhouse	702	29.2 ± 4.2
Greenhouse-24 hr. dark	762	31.7 ± 3.2
24 hr. dark-24 hr. dark	510	21.2 ± 5.1
24 hr. dark-greenhouse	891	37.1 ± 3.6

[a]See text for greenhouse conditions--all plants inoculated 2-4 p.m.

Effect of Temperature on Lesion Formation

Air temperature also affects both the quality and quantity of local lesions formed by BCMV on primary leaves of Monroe bean (Table 15, Figures 10, 11, 12). Lesions formed at 16 C and 20 C were completely necrotic, whereas

Figure 10. Ringspot-type lesion on primary leaf of Monroe bean inoculated with $BCMV_1$ at 24°C.

Figure 11. Severe vein necrosis on primary leaf of Monroe bean inoculated with $BCMV_1$ at 28°C. Local lesions are indistinct and difficult to count.

Figure 12. Hypersensitive-type reaction of primary leaf of Topcrop bean inoculated with $BCMV_1$ at 32°C.

those formed at 24 C and 28 C were primarily ringspot-shaped. Lesion diameters at these temperatures were 0.2 mm, 0.5 mm, 1.2 mm, and 2.0 mm, respectively. Lesions were clearly visible 5 days after inoculation at 20 C and 24 C; lesions were visible 7 days after inoculation at 16 C. Lesion numbers were uniform at 20-, decreased slightly at 24 C, and decreased markedly at 16 and 28 C.

Table 14. The effect of different lengths of pre-inoculation shading on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean.

Length of Shading Period	Number Lesions per 24 half leaves	Average Number Lesions per half leaf
48 hr.	230	9.4 ± 3.3
24 hr.	895	37.2 ± 5.1
16 hr.	843	35.2 ± 4.2
0 hr.	689	28.5 ± 3.6

The typical hypersensitive reaction of Topcrop bean was obtained when primary leaves of this and of the Monroe varieties were inoculated with $BCMV_1$ (Figure 13) and incubated at 32 C in the dark; hypersensitivity, evidenced by the formation of necrotic lesions, occurred on both detached and on attached leaves. Topcrop, however, did not form lesions of any type when inoculated with the three strains of BCMV under greenhouse temperatures.

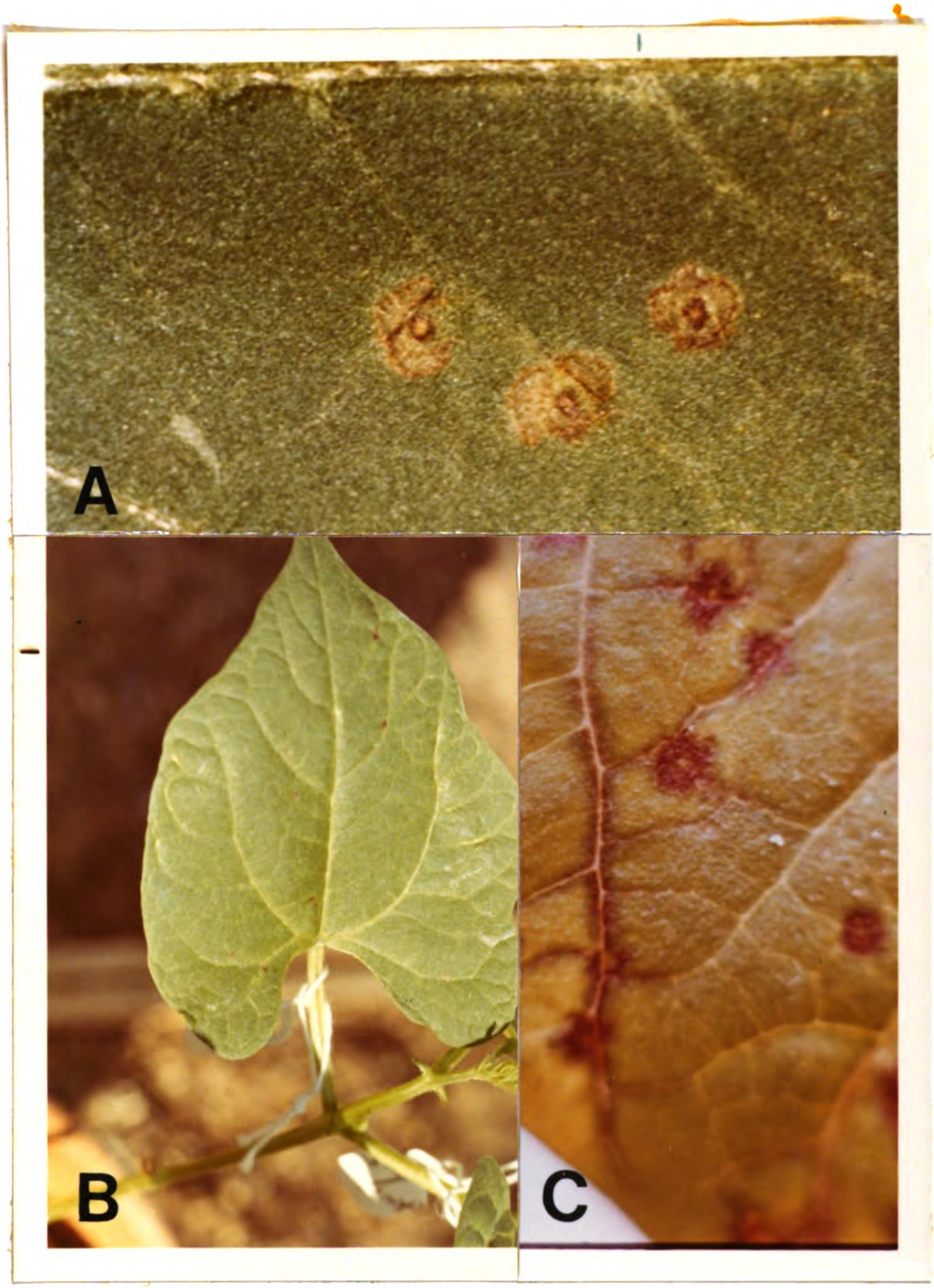


Figure 13. Appearance of BCMV-induced lesions on inoculated primary leaves of Monroe bean at different times of the year. (A) Fall (October) lesions are Ringspot with necrotic center, (B) Winter (January) lesions are local necrotic lesions, and (C) Spring (April) lesions are Ringspot with non-necrotic center. All lesions were formed in the greenhouse.

Table 15. The effect of constant air temperature on local lesion formation by $BCMV_1$ on primary leaves of Monroe bean.

Temperature (0 C)	Number Lesions per 24 half leaves	Average Number Lesions per half leaf	Lesion Appearance	Average Lesion Diameter
16	76	3.1	necrotic spot	0.2 mm
20	569	23.5	necrotic spot	0.5 mm
24	339	14.1	ring spot	1.2 mm
28	180[a]	7.5	ring spot	2.0 mm

[a]Lesions indistinct and difficult to count.

Thermal Inactivation of BCMV

Lesion production by $BCMV_1$ on primary leaves of Monroe bean ceased to occur when the inoculum was heated at 58 C, but not when the inoculum had been heated at 54 and 56 C for 10 minutes.

Local Lesion Formation by BCMV on Trifoliolate Leaves of Monroe Bean

BCMV induces local lesion production on inoculated trifoliolate leaves as well as on inoculated primary leaves (Table 16, Figure 14) of Monroe bean. Although total number of local lesions per trifoliolate leaf increased slightly with age of leaf, there was very little

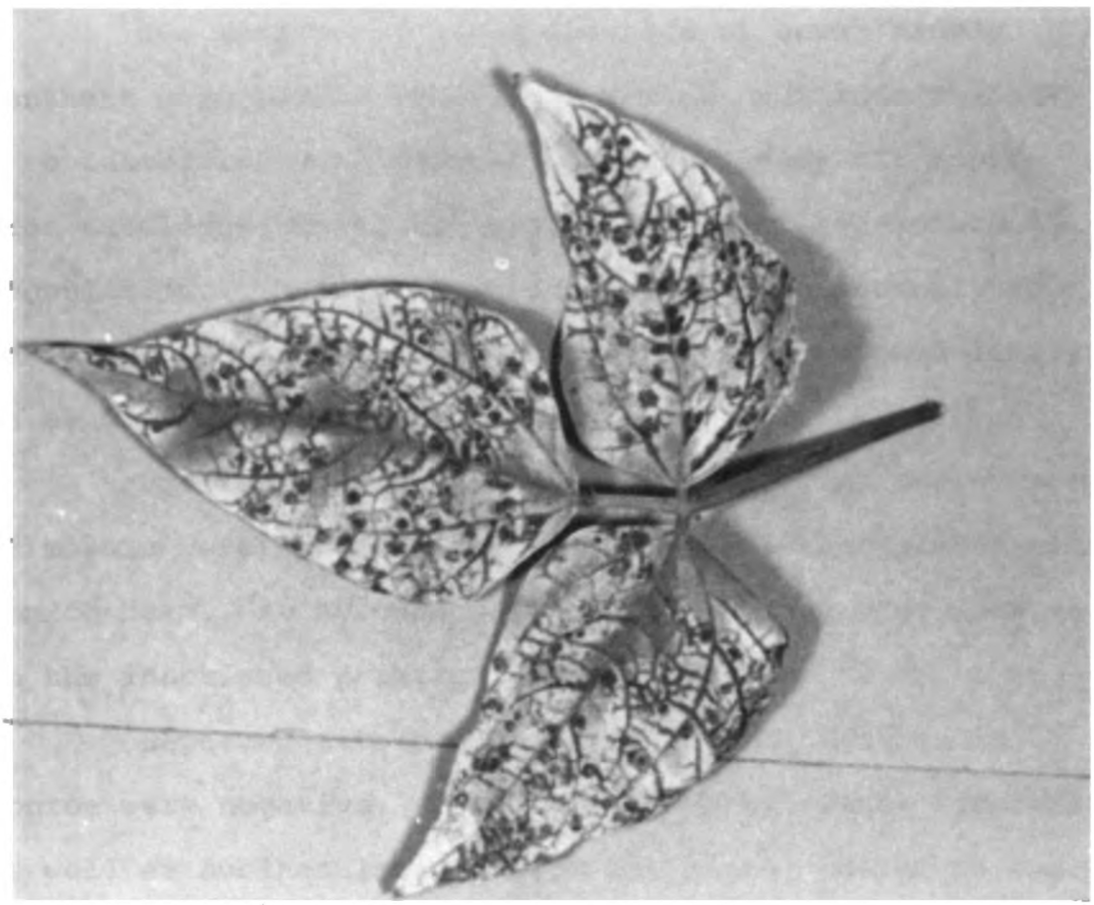
Table 16. Local lesion production by $BCMV_1$ on trifoliolate leaves of Monroe bean inoculated 25 days after planting.

Plant	Trifoliolate Leaf[a] 1 Leaflet Position[b]			2 Leaflet Position			3 Leaflet Position		
No	a	b	c	a	b	c	a	b	c
1	50	58	41	47	60	52	49	41	56
2	26	47	37	41	40	39	13	16	19
3	13	6	12	10	7	13	3	5	2
4	21	27	22	18	18	25	6	9	8
5	13	15	11	23	22	17	6	8	5
6	17	15	17	15	16	16	7	9	8
	140	168	140	154	163	162	84	88	98

[a]1 = bottom (oldest)
2 = middle
3 = top (youngest)

[b]a-c = counterclockwise, looking down

Figure 14. Local lesion production on trifoliolate leaves of Monroe bean inoculated with $BCMV_1$.



variation in lesion counts between the leaflets of the same leaf. There was also very little variation between plants of the same age.

Reaction of Monroe Bean to Other Plant Viruses

Two additional virus diseases of bean, namely southern bean mosaic (SBMV) and yellow bean mosaic (YBMV) were inoculated onto primary leaves of 9-day-old Monroe bean seedlings, using the previously described method of inoculation. These virus diseases are also commonly found in beans, and therefore represent the viruses most likely to be confused with BCMV.

Within two weeks after inoculation, marked symptoms of mosaic were observed on the SBMV-inoculated plants of Monroe bean. In no case were lesions of any type observed on the inoculated primary leaves.

Repeated attempts to obtain YBMV infection in Monroe were negative, as measured both by visual symptoms as well as subinoculations from the Monroe leaves to susceptible bean varieties. Monroe therefore appears resistant to the strain of YBMV tested here.

CHAPTER IV

DISCUSSION

Previous efforts by numerous investigators to find an efficient, useful local lesion host for bean common mosaic virus (BCMV) have been only partially successful. Zaumeyer and Goth (49) reported two types of new local lesions in the mid-sixties; one of the lesions, however, was not consistently produced, and consequently was not studied further. These two authors did report the consistent production of necrotic local lesions by three strains of BCMV on the leaves of an "unnamed bean cross from Florida."

R. A. Conover of Florida who was responsible for making this bean cross was contacted to obtain seed; unfortunately, Conover neither had seed nor knew anyone from whom seed could be obtained. Instead, he sent seed of Dade bean variety which he considered to be a sister line of the cross used by Zaumeyer and Goth, and which he believed might react in the same way as did the cross.

All efforts to obtain lesions on the bean variety Dade with BCMV were fruitless. No combination of temperature and light regime as mentioned by Zaumeyer and Goth was successful in stimulating the production of these lesions on Dade. Since seed of the unnamed bean cross is apparently no longer available, and since its closest genetic relative, Dade, did not form local lesions, attention was then devoted to other reported lesions and lesion hosts.

Although produced only inconsistently, it was thought that a study should be made of the other lesion type reported by Zaumeyer and Goth. Lesions similar to their ghost-like chlorotic spots were formed in the present study (GS lesions) on primary leaves of Great Northerns UI-31 and 123, Pinto UI-111, and Sanilac inoculated with $BCMV_1$ and $BCMV_{15}$; these are the same varieties used by Zaumeyer and Goth. The present study confirmed the inconsistent occurrence of this type of lesion on these varieties. Similarly, lesions (RS lesions) were obtained on primary leaves of Potomac, Plentiful, and Stringless Green Refugee inoculated with all three BCMV strains in the present study. Such lesions are considered similar to the round, white necrotic ringed lesions described by Zaumeyer and Goth (48). No combination of temperature and light regime overcame the inconsistency of formation of these types of lesions.

Another local lesion host for BCMV is thought to be the bean variety Monroe, a Navy (pea) type bean released by Cornell University some years ago. Although apparently resistant to the V_1 and V_{15} strains of BCMV, Monroe developed local lesions when inoculated with the V_{15} strain (Dr. A. L. Andersen, unpublished information). Andersen noted that single plants of Monroe did not react identically, and he therefore made single plant selections for local lesion reaction. In the present study we used the bulked seed of Andersen's single plant selections as well as commercial Monroe seed.

During the course of this study, consistent production of well-defined lesions have been obtained on primary leaves of Monroe grown at temperatures below 28 C and at different light conditions (continuous and 12 hr. photoperiod). Such lesions were easily counted visually, and were different from the "hypersensitive" lesions which develop on Topcrop and other varieties which possess dominant resistance to BCMV derived from Corbett Refugee (40); hypersensitive lesions are formed only at 32 C. It therefore appears that Monroe responds to BCMV inoculation on the primary leaves, as well as the trifoliolate leaves, by the production of local lesions of a type not previously reported.

That the lesions formed on inoculated primary leaves of Monroe are due to BCMV is supported by several lines of evidence. Firstly, juice obtained from healthy plants does not induce lesion formation when rub-inoculated to primary leaves of Monroe. Second, single local lesions removed from inoculated Monroe leaves induce typical BCMV systemic symptoms on susceptible bean varieties. Third, lesion production on inoculated primary leaves occurs if inoculum is heated at 56 C for 10 minutes, but does not occur if inoculum is heated at 58 C for 10 minutes; the thermal inactivation point for BCMV is reported to be between 56 and 58 C (38). Fourth, 5 different isolates of BCMV, 2 of V_1, 2 of V_{15}, and 1 of V_{123}, all induced these lesions when inoculated to Monroe.

Recognizing the potential importance of Monroe as a new local lesion host for bean common mosaic virus, the author has studied several of the more commonly-recognized factors which are known to affect local lesion assays. The most preliminary of these factors is perhaps the method of inoculation to be used in all subsequent work. Although most workers have used pads of cheesecloth or some other absorbent material such as cotton or foam rubber, the rough edge of the pestle seemed to give the most uniform and efficient results in this study. One of the advantages of this method of inoculation is that a half leaf can be inoculated uniformly with a rather small amount of inoculum. This uniformity can be evidenced by the consistent production of 28-35 lesions when 0.1 ml (2 drops) of juice inoculum (1:4 dilution) was applied to half leaves. Another advantage is that the pestle is routinely sterilized with the mortar for the preparation of inoculum, and is therefore immediately ready for use as an inoculator after triturating the tissue for inoculum.

Another equally important factor preliminary to studying the local lesion assay of a plant virus is the inoculum itself. Many plant viruses are particularly susceptible to inactivation because of adverse pH conditions (26). BCMV appears to be quite stable as assayed by local lesion formation when prepared in 0.01 M Na_2HPO_4-KH_2PO_4 solutions buffered at pH 6.4-7.8. Virus inactivation, as measured by fewer local lesions on Monroe leaves, begins to occur at pH 6.0 and 8.5. BCMV therefore appears to be quite stable to a rather wide range of pH.

To be useful as a tool for quantitative measurement of a plant virus, there should exist a direct relationship between number of local lesions and amount of virus in the inoculum. This information is usually obtained by plotting lesion number versus dilution of inoculum, and ascertaining that portion of the graph which represents a rather straight line (25), i.e. where infectivity as measured by number of lesions is directly proportional to dilution. BCMV assayed on Monroe bean leaves produces a characteristic S-shaped curve when number of lesions is plotted against dilution of inoculum (Figure 4); a straight line relationship exists between dilutions of 1:4 and 1:32. At dilutions higher than this, lesion numbers change very rapidly. At dilutions of 1:4 and 1:8, lesion counts per half leaf are in the range of 20-35 where there is little possibility of making a counting error.

With the exception of 10-day-old plants, Monroe responds with a uniform number of local lesions when inoculated with BCMV at 8-12 days after planting. In at least three separate experiments, lesion production was greatest at 10 days after seeding, decreasing at 8, 9, 11, and 12 days. Because plants grow at different rates when maintained at different temperature conditions, it was felt that the period 9-12 days would be most useful to other investigators who might be studying the BCMV-induced lesions on Monroe. For example, investigator "A" may grow his plants at 24 C and inoculate at 10 days, while investigator "B" grows his plants at 28 C and also inoculate at 10 days.

Light conditions before and after inoculation are known to exert a strong influence on the number and the type of lesions formed in certain host-virus combinations (12). In the present study preinoculation darkness for 24 hr. increased lesion production by BCMV on leaves of Monroe. Such stimulation of lesion production has also been reported for tobacco necrosis virus (TNV) and for alfalfa mosaic virus (potato calico strain) on bean leaves (11). This similarity of response by three different local lesion-inducing viruses would suggest that preinoculation shading results in a general increase in susceptibility of bean leaves to lesion formation. It would be interesting to examine the reaction of Monroe to TNV and alfalfa mosaic virus. Preinoculation shading of plants in the dark for 48 hr., however, had a deleterious effect on lesion production by BCMV on Monroe; such is also the case with TNV on bean (5). This would suggest that, among other factors, the photosynthetic economy of the host at the time of inoculation is important in the formation of lesions (41); 48 hr. of shading resulted in leaves showing moderate chlorosis, whereas 24 hr. of shading did not.

Lesion size is another character which is affected by the condition of light before and after inoculation. In the present study, larger lesions were consistently obtained when leaves were shaded for a 24 hr. period prior to inoculation with BCMV. Best (6) using *Nicotiana tabacum*-TMV and Helms (20) using bean-TMV and Desjardins (12) working with alfalfa mosaic virus on bean, all report that size of lesion produced on leaves was greatest when the leaves were preincubated in the dark prior to inoculation. The unusual feature of the larger lesion in the BCMV situation is that the lesion quality was altered; that is to say, the lesions assumed the shape of a ringspot, rather than the shape of a totally necrotic lesion. The reasons for this alteration in lesion appearance by different light conditions are unknown.

Air temperature is another environmental factor which is known to influence local lesion production in plant virus assays (24). Lesion numbers of BCMV on Monroe bean were greatest at 20 C, less at 24 and 16 C, and least at 28 C. These results are similar to those of Crill, *et al*. (7) who reported 20-24 C as the optimum temperature range for alfalfa mosaic virus-induced lesions on Bountiful bean. Harrison (19), however, found that TNV lesions on bean plants were most numerous at 10 C, followed by 14, 18, 22, and 26 C. This suggests that the effect of air temperature will vary depending on the virus being assayed. The time of lesion appearance after inoculation was affected by temperature also. Lesions were first observed at 20 and 24 C, then 28 C (indistinct), and finally, after several days, at 16 C. Best (6) reported that a drop in temperature from 20 to 15 C delayed the appearance of lesions in the two following plant-virus combinations: *Nicotiana glutinosa vs* TMV, and *N. glutinosa vs* tomato spotted wilt virus.

Lesion size, as well as number and time of appearance, was affected by the temperature at which inoculated plants were maintained. Lesions at 16 C were quite small, normal size at 20, slightly larger at 24 C and 28 C. Desjardins noted the opposite response of bean leaves to AMV; lesions decreased in size as temperature increased from 25 to 30 to 32 C (12).

Notwithstanding the various effects which light conditions, air temperature, and age of plant have on lesion formation, the present study suggests that Monroe bean is a superior local lesion host for bean common mosaic virus (BCMV). This bean variety responds to rub-inoculation with several strains of BCMV by the production of local lesions, either ringspot or totally necrotic, which have never been described for this virus on bean. The results of this study suggest that the Monroe bean variety will come to be a very useful tool in the study of BCMV, as well as in the study of virus properties such as replication of virus particles and strain specificity.

CHAPTER V

SUMMARY

A study has been conducted on the formation of local lesions by bean common mosaic virus (BCMV) on primary leaves of various bean varieties. Of numerous varieties tested, only Monroe bean consistently reacted to BCMV inoculation by the formation of local lesions. The lesions formed on Monroe are different from and appear superior to all other previously described lesions induced by BCMV on bean.

Of several inoculation devices tested, the rough end of a pestle seemed to give the most uniform and reproducible lesion counts on inoculated primary leaves of Monroe bean. Inocula of BCMV prepared in buffers of pH 6.4-7.8 were of uniform infectivities as assayed by local lesion counts. Number of local lesions per half leaf was inversely related to dilution of inoculum; optimum inoculum dilutions of 1:4-1:16 are suggested for routine local lesion assays of BCMV.

Preinoculation shading for 24 hr., but not for 48 hr. resulted in better lesion development (number and shape) by BCMV on primary leaves of Monroe bean; such lesions were of a ringspot rather than a totally necrotic type. Ringspot-type lesions were also formed on inoculated plants incubated at 24 C and 28 C. Lesion development was comparable on plants inoculated 8, 9, 11, 12, and 13 days after planting; 10-day-old plants were the most sensitive to local lesion formation.

This study has found the following conditions to be optimum for local lesion assay of BCMV on primary leaves of Monroe bean: (1) Plant age-8-13 days-old, (2) Inoculum-1:4 dilution in pH 6.4-7.8 phosphate buffer (0.01 M), (3) Inoculation device-pestle, (4) Growing conditions-20-24 C, 12 hr. photoperiod or natural light; greenhouse or growth chamber, (4) Preinoculation dark period-24 hr., and (5) Rinsing of inoculated leaves.

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