STUDIES ON MIXED INFECTION IN BARLEY BY BROMEGRASS MOSAIC VIRUS AND BARLEY STRIPE MOSAIC VIRUS

by Thomas Jack Morris

Abstract

Mixed infections of barley with bromegrass mosaic virus (BNV) and barley stripe mosaic virus (BSMV) were studied in an attempt to detect interaction between the viruses. No effect of the replication of one virus on the concentration of the other was observed, nor was there evidence of phenotypic mixing or genomic masking. These results suggest that the viral replication and assembly systems do not interact. It is hypothesized that the lack of interaction is due to separate sites of synthesis and assembly of the respective viruses in the same host cell.

Master of Science Degree,

Department of Plant Pathology.
Title:
STUDIES ON MIXED INFECTION
IN BARLEY BY BMV AND BSMV
by
T.J. Morris
STUDIES ON MIXED INFECTION IN BARLEY BY BROOMGRASS MOSAIC VIRUS AND BARLEY STRIPE MOSAIC VIRUS

by

Thomas Jack Norris

A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfillment of the requirements for the degree of Master of Science.

Department of Plant Pathology, Macdonald College of McGill University, Montreal, Quebec

June 1970
ABSTRACT

Mixed infections of barley with bromegrass mosaic virus (BMV) and barley stripe mosaic virus (BSMV) were studied in an attempt to detect interaction between the viruses. No effect of the replication of one virus on the concentration of the other was observed, nor was there evidence of phenotypic mixing or genomic masking. These results suggest that the viral replication and assembly systems do not interact. It is hypothesized that the lack of interaction is due to separate sites of synthesis and assembly of the respective viruses in the same host cell.
ACKNOWLEDGEMENTS

The author wishes to express sincere thanks to Dr. R.I. Hamilton for suggesting the problem, and for helpful guidance and advice given during the study.

The author gratefully acknowledges the financial support of the National Research Council of Canada.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>METHODS AND MATERIALS</td>
<td>13</td>
</tr>
<tr>
<td>I. Viruses and Antisera</td>
<td>13</td>
</tr>
<tr>
<td>II. Growth Conditions and Inoculations</td>
<td>13</td>
</tr>
<tr>
<td>III. Purifications</td>
<td>15</td>
</tr>
<tr>
<td>IV. Infectivity Assays</td>
<td>18</td>
</tr>
<tr>
<td>V. Serological Tests</td>
<td>19</td>
</tr>
<tr>
<td>VI. Separation and Analytical Techniques</td>
<td>22</td>
</tr>
<tr>
<td>VII. Reconstitution Studies</td>
<td>28</td>
</tr>
<tr>
<td>VIII. Fractionation of BMV-RNA and Infectivity Tests of the Fractionated RNA</td>
<td>33</td>
</tr>
<tr>
<td>RESULTS AND CONCLUSIONS</td>
<td>35</td>
</tr>
<tr>
<td>I. Studies on the Effect of the Double</td>
<td>35</td>
</tr>
<tr>
<td>Infection on the Plant</td>
<td>35</td>
</tr>
<tr>
<td>II. Studies on the Effect of the Double</td>
<td>37</td>
</tr>
<tr>
<td>Infection on Virus Concentration</td>
<td>37</td>
</tr>
<tr>
<td>III. Studies on Virus-Virus Interactions in Doubly Infected Plants</td>
<td>41</td>
</tr>
<tr>
<td>IV. Studies on the Reconstitution of Barley</td>
<td>61</td>
</tr>
<tr>
<td>Stripe Mosaic Virus Coat Protein with Bromegrass Mosaic Virus Ribonucleic Acid</td>
<td>61</td>
</tr>
<tr>
<td>V. Studies on the Infectivity of Fractionated Bromegrass Mosaic Virus Ribonucleic Acid</td>
<td>69</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>74</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>82</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>83</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Comparison of the properties of BMV and BSMV</td>
<td>23</td>
</tr>
<tr>
<td>2. Fresh weights of barley tissue 14 and 28 days after inoculation</td>
<td>36</td>
</tr>
<tr>
<td>3. The ratio of the amount of BSMV obtained from a real mixture to that obtained from an artificial mixture</td>
<td>41</td>
</tr>
<tr>
<td>4. Results of infectivity assays of artificial mixtures of BSMV and BMV inoculated to barley</td>
<td>44</td>
</tr>
<tr>
<td>5. Results of infectivity assays on barley of the BSMV population after separation from artificial and real mixtures containing BMV</td>
<td>46</td>
</tr>
<tr>
<td>6. Results of infectivity assays on barley of the BMV population after separation from artificial and real mixtures containing BSMV</td>
<td>49</td>
</tr>
<tr>
<td>7. Ultraviolet absorption characteristics of BSMV, reconstituted virus, BSMV protein and BMV-RNA</td>
<td>68</td>
</tr>
<tr>
<td>8. Results of infectivity assays of fractionated BMV-RNA tested on Chenopodium amaranticolor or Chenopodium hybridium</td>
<td>73</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure Page

1. (a) Diagram illustrating the possible types of particles that could result from BMV-BSMV structural interaction. (b), (c) and (d) Diagrams illustrating the separation procedures used in this study................................................................. 40

2. Sucrose density gradient centrifugation profiles:
   (a) Artificial mixture at optimal proportions (black line), and a reference baseline (dashed line) for a gradient which did not contain virus.
   (b) Real mixture with no preliminary separation.
   (c) BSMV population after preliminary separation by MgCl₂ treatment.
   (d) BMV population after preliminary separation by MgCl₂ treatment................................. 50

3. Sucrose density gradient centrifugation profiles. Preliminary separation with MgCl₂.
   (a) BSMV population on a pH 6.5 gradient.
   (b) BSMV population on a pH 8.0 gradient............ 51

   (a) BMV population from a real mixture.
   (b) BSMV population from a real mixture.......... 52

5. Sucrose density gradient zone electrophoresis profiles. Preliminary separation by sucrose density gradient centrifugation on the SW 27 rotor.
   (a) BSMV population of a real mixture.
   (b) BSMV population of an artificial mixture.
   (c) BMV population of a real mixture.
   (d) BMV population of an artificial mixture...................... 53

6. Sucrose density gradient zone electrophoresis profiles.
   (a) Resuspension of the pellet from the MgCl₂ precipitation of a real mixture.
   (b) Resuspension of the pellet from the MgCl₂ precipitation of an artificial mixture.
List of Figures (continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td></td>
</tr>
<tr>
<td>(c) BSMV population of a real mixture after preliminary separation on a 10-40% sucrose gradient in the SW 27 rotor. (d) BSMV population of an artificial mixture after preliminary separation on a 10-40% sucrose gradient in the SW 27 rotor.</td>
<td>54</td>
</tr>
<tr>
<td>7.</td>
<td>55</td>
</tr>
<tr>
<td>Ultraviolet tracing of the elution profiles of BNV-BSMV mixtures from a Sepharose 2 B column.</td>
<td>55</td>
</tr>
<tr>
<td>8.</td>
<td>56</td>
</tr>
<tr>
<td>Sucrose density gradient centrifugation profiles. Preliminary separation on a Sepharose column. (a) Fraction 1 of artificial mixture. (b) Fraction 2 of artificial mixture. (c) Fraction 1 of real mixture. (d) Fraction 2 of real mixture. (e) Fraction 1 of artificial mixture. (f) Fraction 2 of artificial mixture.</td>
<td>56</td>
</tr>
<tr>
<td>9.</td>
<td>57</td>
</tr>
<tr>
<td>Sucrose density gradient zone electrophoresis profiles. Preliminary separation on a Sepharose column. (a) Real mixture, fraction 1. (b) Real mixture, fraction 2. (c) Artificial mixture, fraction 1. (d) Artificial mixture, fraction 2.</td>
<td>57</td>
</tr>
<tr>
<td>10.</td>
<td>62</td>
</tr>
<tr>
<td>Diagram of immunoelectrophoresis patterns.</td>
<td>62</td>
</tr>
<tr>
<td>11.</td>
<td>65</td>
</tr>
<tr>
<td>Sucrose density gradient centrifugation profiles. (a) Reconstituted nucleoprotein from a BNV-RNA-BSMV protein reaction mixture (black line) and a BSMV standard for comparison (dashed line). (b) Polymerized protein from a BSMV-protein reaction mixture without added RNA.</td>
<td>65</td>
</tr>
<tr>
<td>12.</td>
<td>66</td>
</tr>
<tr>
<td>Electron micrographs of reconstitution reaction mixtures stained with neutral 1% phosphotungstic acid. Magnification is 100,000 X. (1) Reconstituted nucleoprotein. (2) Polymerized protein.</td>
<td>66</td>
</tr>
</tbody>
</table>
List of Figures (continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.</td>
<td></td>
</tr>
<tr>
<td>Ultraviolet absorption profiles.</td>
<td>67</td>
</tr>
<tr>
<td>(a) Reconstituted nucleoprotein from a reaction mixture in 0.01M potassium phosphate pH 6.5.</td>
<td></td>
</tr>
<tr>
<td>(b) Polymerized BSNV protein from a reaction mixture in 0.01M potassium phosphate pH 6.5.</td>
<td></td>
</tr>
<tr>
<td>(c) Pure BSNV protein preparation in 0.05M Tris-HCl pH 8.9.</td>
<td></td>
</tr>
<tr>
<td>(d) Pure BSNV preparation in 0.01M potassium phosphate pH 6.5.</td>
<td></td>
</tr>
<tr>
<td>(e) Pure BSNV-RNA preparation in 0.01M Tris, 0.01M KCl, 10^{-4}M MgCl₂ pH 7.4.</td>
<td>72</td>
</tr>
<tr>
<td>14.</td>
<td></td>
</tr>
<tr>
<td>Sucrose density gradient centrifugation profiles of BSNV-RNA and fractionated BSNV-RNA</td>
<td></td>
</tr>
</tbody>
</table>
INTRODUCTION

Studies on mixed infections of two or more plant viruses in one host have been recorded with increasing occurrence in recent literature. This increased interest, although tied to the fact that mixed infections are common in nature, has resulted from the lack of information that exists on plant virus -- virus interactions. The discovery of multicomponent virus systems in plants (54), and the phenomena of hybridization, phenotypic mixing and genomic masking in animal and bacterial viruses, which shall be discussed in the literature review, have shown that viruses do display both functional and structural interactions with other viruses infecting the same cell.

The bromegrass mosaic -- barley stripe mosaic virus system offers a number of advantages for the study of mixed infections. The viruses both occur in relatively high concentrations, therefore offering greater opportunity for interaction. They are both easily isolated from plant tissue and thus readily studied by the techniques currently available. They also represent two diverse structural forms of viruses.

The object of this research was to see if phenotypic mixing or genomic masking could be demonstrated as a result
of mixed infection of the two viruses in barley, a common host. Results will also be presented on the effect of the mixed infection on the host plant and on the relative concentration of the viruses in the host as a result of the mixed infection.
LITERATURE REVIEW

It is well documented that both related and unrelated plant viruses can infect the same host and host cell (2, 36, 45, 51, 65). The results of plant virus interaction have received much attention (11, 53), but studies on the actual nature of the interaction are just beginning to take on significance (54).

Cases of structural and functional interaction such as phenotypic mixing, genomic masking and recombination have been reported for animal and bacterial viruses; however, similar examples among the plant viruses have not been well documented. Recent advances in reconstitution of plant viruses from isolated protein and nucleic acid components "in vitro" (23, 43, 64) have made it necessary to see if hybrid viruses can be demonstrated as a result of virus interaction in mixed infections of two different viruses.

The effects of virus-virus interaction that result from a mixed infection have been reviewed (11, 53) and shall be discussed briefly. Measurable changes in symptomatology of an infected plant or changes in virus concentration or infectivity, as a result of a mixed infection, are the more obvious effects of virus interaction.
Synergism refers to the effect of an interaction where new or more severe symptoms are produced, and the effect on the host is greater than the additive effect that each virus could exert independently. It is often associated with an abnormal increase in the concentration or infectivity of one of the interacting viruses. Many examples of synergism have been reported (2, 41, 45, 59, 70, 74, 77); all cases are the result of interaction between unrelated viruses. The classical potato virus X-potato virus Y interaction resulted in a great increase in symptom severity which was correlated with an induced increase in virus X concentration (27, 31, 80). Similarly, the increase in concentration of dodder latent mosaic virus in the presence of tobacco mosaic or tobacco etch virus was correlated with increased symptom severity (11).

On the other hand, viruses can interact in an antagonistic fashion when in mixed infection. Antagonism is usually associated with interaction between related viruses. This is illustrated by the competitive nature of the interaction between the U₁ and U₂ strains of tobacco mosaic virus in mixed infection, where there is a tendency for the U₁ strain to predominate (28). Similarly, infection of tobacco by two related flexuous rod viruses, tobacco severe etch and potato virus Y, showed a decrease in the concentration of the Y virus (10). In mixed infections of two related viruses, maize dwarf mosaic and sugar cane mosaic, the total amount of viral nucleoprotein synthesized was the same as in single infections. Tu and Ford (86) concluded that this reflected a
sharing of viral synthesis mechanisms and metabolic pools. Presumably, the plant had a capacity to synthesize only so much virus in the single infection. This capacity was unaltered by the mixed infection, but was shared by both of the infecting viruses which were each reduced in concentration to keep the total amount of virus synthesized at the same level as in single infections. However, competitive antagonism between viruses does not necessarily imply relatedness. Replication of tobacco mosaic virus can be retarded by cucumber mosaic virus in double infection (37). Similarly, the replication of bean yellow mosaic virus adversely affects the concentration of tobacco ringspot virus (77).

Cases have also been reported of mixed infections in which no apparent interaction is observed. The mixed infection of bean pod mottle virus and bean yellow mosaic virus caused a reduction in host growth which was the additive result of what each virus could exert alone (74, 77). This showed how two unrelated viruses could act independently in causing a reduction of host growth.

Other results of mixed infections have been reported. Lily symptomless virus was induced to invade epidermal tissue it normally did not infect, in the presence of cucumber mosaic virus (2). Similarly, Brazilian tobacco streak virus became systemic in the presence of anthocyanosis virus of cotton (30). Some interesting morphological changes have been reported in the cucumber mosaic-tobacco mosaic virus inter-
action where cucumber mosaic aggregated into abnormal crystalline masses (45) and TMV was observed as mature particles in the cell nucleus (46) as a result of the mixed infection.

Interacting viruses can affect one another physically when in close proximity in the same cell. Genetic recombination is one such physical interaction that can occur between genetically related viruses infecting the same cell. An exchange of genetic information results in progeny which differ from parental types in some characteristics. This interaction has been recorded for most types of viruses. Influenza viruses have been shown to demonstrate interstrain and intergroup recombination (32, 44). Fraenkel-Conrat (34) pointed out that this was facilitated by the fact that influenza virions contain a number of RNA molecules so that recombination reflected only an encapsulation of different RNA species, rather than a physical cross-over exchange of genetic information between the interacting RNA molecules. Recombination between P22 and P221 phages (94) and poliovirus strains (44) probably represent cases of an actual physical exchange of genetic information by crossing over of the interacting viral genomes. That genetic exchange must occur between the parental viral genomes of these viruses is supported by the fact that these viruses contain only one species of nucleic acid per virion.

Evidence for recombination between plant viruses has been the subject of much criticism (53). Lack of sufficient
evidence may reflect the inability to obtain a high degree of multiple infection of plant cells with related viruses. The evidence for recombination between potato virus Y and potato virus C (93) is doubtful because the results obtained strongly suggest genomic masking of the potato virus C nucleic acid with potato virus Y protein. This would account for the aphid transmission of potato virus C and perpetuation of the mixed culture by the aphid vector would explain the symptomology reported to have been caused by a recombinant strain. Likewise, Thomson (84) showed that mixed infections of a number of pairs of potato virus X strains resulted in new symptoms on tobacco, but he concluded that the evidence was not adequate enough to distinguish genetic recombination from mutation. However, the results of Best (12, 13, 14) with strains of tomato spotted wilt virus were most interesting. He showed that mixed infection of tomato by strains A and E resulted in three new hybrid strains, on the basis of seven marker characteristics, which bred true after single lesion isolation. He concluded that recombination rather than mutation was the more acceptable answer because the incidence of new strains in mixed infection was much more frequent than the observed mutation rate of either strain in single infection. The production of new strains in the multicomponent virus systems of alfalfa mosaic virus (63, 90) and tobacco rattle virus (62) by the separation of the components and the mixing of functional components of different strains resembles the recombination or strain production mechanisms reported for
influenza viruses. It is most probable that recombination occurs between plant virus strains; however the significance of the interaction remains to be studied.

Phenotypic mixing is another physical interaction reported to occur between viruses infecting the same cell. It is defined as an interaction between genetically related viruses which results in the formation of progeny virus carrying the coat proteins of both parents but containing the genome of only one parent (91). Bacteriophage T₂ and T₄ were first shown to interact in this manner (66). Poliovirus types 1 and 2 were later shown to yield particles from mixed infection of which 80 to 90% were neutralized by antisera of both viruses (60). This indicated that most of the particles contained mixed coats and it was concluded that virus assembly occurred from a pool that contained a mixture of the two types of protein subunits. It has also been shown that other related viruses, such as the members of the avian tumor virus group (91), related group A arboviruses (24), strains of φX₁₇₄ phage (48) and the human and simian adenoviruses (3) can produce particles with mixed coat proteins in mixed infection. It is concluded that only closely related (physically, biologically and serologically) viruses will phenotypically mix because this interaction would require that production, storage and assembly of the proteins would have to occur at the same time and in the same area of the cell for mixed association to occur. The protein subunits would also have to be very similar in size, shape and chemical
nature in order to be able to polymerize together with equivalent or quasiequivalent bonding to form stable particles (25). True phenotypic mixing has not been reported for plant viruses except for the "in vitro" formation of particles with mixed coat proteins of bromegrass mosaic virus, cowpea chlorotic mottle virus and broad bean mottle virus (92). It would appear that these viruses are closely related.

Genomic masking is an extreme form of phenotypic mixing. It is defined as an interaction between two viruses infecting the same cell which results in the formation of hybrid progeny virions carrying the coat protein of one parent and the genome of the other (76). The great majority of reported cases of phenotypic mixing on close examination are most probably examples of genomic masking. This interaction does not require that the two viruses be as closely related as for phenotypic mixing, and serological relatedness is not necessary. The examples reported in the literature illustrate that the participants are usually structurally similar. Some examples of genomic masking have been reported between the RNA phages f2 and f24 (87), the DNA phages P22 and P221 (94), poliovirus RNA in coxsackie B1 protein (29) and the "in vitro" formation of masked particles using QB and MS2 phage (61). The only reported cases of genomic masking between two very structurally different viruses was the encapsulation of the simian virus 40 genome in adenovirus protein (33) and the encapsulation of φX174 DNA in phage fd coat protein (58).
Genomic masking in plant viruses has been demonstrated "in vitro" by a number of reconstitution studies. The protein coats of the small spherical plant viruses, brome grass mosaic, cowpea chlorotic mottle and broad bean mottle, were reconstituted around each other's RNA as well as the RNA of tobacco mosaic virus (TMV) and formed infectious particles (43). Similarly, the coat protein of the rod virus, TMV, has been successfully reconstituted with the RNA of bromegrass mosaic virus (7) and potato virus X (23); but it would not coat the RNA of turnip yellow mosaic virus (64) or phage MS₂ RNA (83) to form stable infectious particles.

Plant virus genomic masking has been reported to occur as a result of "in vivo" interactions due to mixed infection. Two cases have been reported and both are between related virus strains. Sarkar (76) showed that the protein of the common strain of TMV would coat the RNA of a temperature sensitive strain during a mixed infection of tobacco at 35°C, which prevented the production of functional coat protein by the temperature sensitive strain. Similarly, Rochow (72, 73) offered evidence for genomic masking between two serologically unrelated strains of barley yellow dwarf virus as a result of mixed infection by the two in barley.

Genomic masking appears to be limited in its occurrence to related groups of viruses except for the simian virus 40-adenovirus interaction. This may be partly due to incompatibility between a viral coat protein and some foreign nucleic acids (23, 61, 64, 83), although the reconstitution
work with plant viruses suggests this is not significant for small RNA viruses. The lack of significant physical interaction of different viruses in the same cell is better explained by the failure of the coat protein and viral nucleic acid from contacting one another because of divergent life cycles that allow synthesis of the different protein and nucleic acid components in different parts of the same cell. The idea of a topographical separation of synthesis sites for different viruses is supported by recent work which suggests that rod viruses such as TMV synthesize nucleic acid in the mitochondria (71) whereas the small spherical viruses use the chloroplast as the site of synthesis (78, 88). In bacterial viruses, QB and MS2 phage were shown to produce masked particles "in vitro" but not "in vivo" on mixed infection (61). It was concluded that some other factor besides physical incompatibility of the viral components was involved in the lack of interaction. The possibility of separate synthesis sites was presented. It might be expected that related viruses would carry out similar life cycles in the same parts of the cell, whereas different viruses might be physically separated into different areas of the cell and so not have a chance to interact.

The significance of these structural interactions to the viral populations is still speculative. Recombination offers the advantage of producing new hybrid strains at a faster rate than normal mutation. Phenotypic mixing and genomic masking have been shown to alter the host range of
certain animal and bacterial viruses (3, 29, 48, 66, 81, 91), however this does not appear to be the case with most plant viruses (23, 43, 50), although some recent evidence with reconstituted hybrid viruses suggests the coat protein can influence the host range of the nucleic acid (7). Phenotypic mixing and genomic masking have been long suspected to play a role in the insect transmission of a virus not normally transmitted by the vector. This occurred when the non-transmissible virus was in mixed infection with a virus that was normally transmitted (52, 79). Recently, Rochow (73) showed that such a mechanism did in fact occur with mixed infections of barley yellow dwarf virus strains. He showed that when the nucleic acid of the non-vectored strain was coated by the protein of the vectored strain "in vivo" to form genomically masked particles, these particles were transmitted by the vector leafhopper. Thus, genomic masking permitted transmission of the non-transmissible strain. The study of virus-virus interaction is becoming increasingly more frequent and should help to create a clearer picture of the nature of viruses.
METHODS AND MATERIALS

I. Viruses and Antisera

The viruses used in this study were the type strain (American Type Culture Collection No. 66) of bromegrass mosaic virus (BWM), the type strain (American Type Culture Collection No. 69) of barley stripe mosaic virus (BSMV) and the VI strain of BSMV derived from a single local lesion on Chenopodium amaranticolor L. after isolation from Hordeum vulgare L. var. Vantage. The viruses and antisera to them were kindly supplied by Dr. R.I. Hamilton of the Plant Pathology Department, McGill University.

II. Growth Conditions and Inoculations

Plants used for virus stock and assays were grown in pasteurized soil in clay pots, in a greenhouse maintained at about 21°C in the winter months and between 24°C and 32°C during the summer months. Supplemental light was supplied by incandescent and fluorescent lamps to give a photoperiod of at least 14 hours. Plants used for virus source were grown in a growth chamber set at a temperature of 24±2°C and a 14 hour photoperiod.
The viruses were maintained in vegetative stock cultures of barley (*Hordeum vulgare* L., var. Blackhulless or Unitan) and regenerated from purified material or from permanent stocks maintained in barley leaves dried over anhydrous calcium chloride at 3°C. Periodically the vegetative stocks were checked for contamination by the immunodiffusion serological assay using antisera to both viruses.

Inocula were made by grinding the leaf tissue with a mortar and pestle in 0.01 M potassium phosphate buffer at pH 7.0. Celite® was added at a concentration of 1 - 2% after grinding as an abrasive. Inoculations were performed by rubbing barley leaves in the one to two leaf stage with sterilized gauze dipped in the inoculum.

In early experiments inoculations were made to barley grown in 10 inch pots with about 50 to 60 plants per pot. In later experiments plants were grown in 6 inch pots with about 20 plants per pot. The following treatments were performed:

1) Control: the plants were rubbed with sap from healthy barley.
2) **BMV** inoculum: the plants were inoculated with sap from **BMV** infected barley diluted 1:1 with sap from healthy barley.
3) **BSMV** inoculum: the plants were inoculated with sap from **BSMV** infected barley diluted 1:1 with sap from healthy barley.
4) **Mixed inocula**: the plants were inoculated with a 1:1 mixture of sap from **BSMV** infected barley and sap from **BMV** infected barley.

*Diatomaceous silica manufactured by Johns-Manville Co.*
5) Challenge inoculation: the plants were inoculated with sap from BSMV infected barley and then challenge inoculated 5 days later with sap from BMV infected barley.

III. Purifications:

The virus purification procedures used throughout this study are presented in stepwise fashion. Centrifugations were performed on a Beckman L2 - 65B Ultracentrifuge for highspeed pelleting and on a Sorvall Superspeed RC-2-B centrifuge with an SS 34 rotor for lowspeed clarification and pelleting.

a) BSMV Purification (R.I. Hamilton, personal communication).

1) Blackhulless barley was inoculated and grown for 10-14 days.

2) The leaves were collected and cut into 2 inch strips.

3) The leaves were crushed in a meat-grinder with a 1/2 weight of 0.02M K2HPO4.

4) The crushed material was strained through two layers of cheese-cloth to remove large debris and fibers.

5) The green extract was heated at 40°C in a water bath for 70 minutes to denature and precipitate host proteins.

6) The heated extract was centrifuged at 6500 rpm for 20 minutes in the SS 34 rotor to pellet the denatured proteins.

7) The clarified supernatant was centrifuged at 30,000 for 2.5 hours in the Beckman TYPE 30 rotor to pellet the virus.
8) The supernatant was discarded and 2 ml. of 0.02 M tris (hydroxymethyl) aminomethane (Tris) - 0.0064 M citrate buffer pH 6.5 was layered on the gelatinous virus pellets which were then allowed to soak overnight at 3°C.

9) The virus pellets were resuspended with stirring and Igepon T-73* was added to a concentration of 0.1% to prevent virus aggregation (20).

10) The virus suspension was then clarified by a lowspeed centrifugation as in step 6.

11) Steps 7, 8, and 9 were repeated.

12) 2 ml. of virus suspension were layered over 5 ml. of 45% sucrose dissolved in 0.02 M Tris-citrate pH 6.5 in a 1 x 3 1/2 inch cellulose nitrate test tube which was filled with mineral oil.

13) Centrifugation was performed in a Beckman SW 27 rotor for 6 hours at 27,000 rpm.

14) After centrifugation the mineral oil and sucrose were discarded and 1 ml. of 0.02M Tris-citrate buffer pH 6.5 plus 0.1M glycine was placed over the virus pellet.

15) The pellets were stored at 3°C overnight, resuspended and clarified as described in step 6. The virus suspension was stored at 3°C with a drop of chloroform to prevent microbial growth.

b) BMV Purification: This procedure was modified after the method described by Bancroft (9).

1) Blackhulless barley was inoculated and grown for 12-14 days.

2) The leaves were collected and cut into 2 inch strips.

3) The leaves were crushed in a meatgrinder with a 1/2 weight of 0.2M sodium acetate buffer at pH 5.0.

4) The crushed material was strained through two

*Igepon T-73: a detergent, Sodium N-methyl N-oleoyl taurate.
layers of cheese-cloth to remove large debris and fibers.

5) The green extract was stored at 3°C overnight to precipitate cellular proteins.

6) The extract was centrifuged at 5000 rpm for 20 minutes in the SS 34 rotor to pellet the de-natured proteins.

7) The clarified supernatant was centrifuged at 30,000 rpm for 3 hours in the Beckman TYPE 30 rotor to pellet the virus.

8) The supernatant was discarded and 2 ml. of 0.1 M sodium acetate buffer pH 5.0, 0.001M CaCl₂ and 0.001M MgCl₂ were layered over each of the virus pellets which were then allowed to soak overnight at 3°C.

9) The virus pellets were resuspended with stirring and the resulting virus suspension was clarified by a lowspeed centrifugation as described in step 6.

10) Steps 7, 8 and 9 were repeated twice. Alternatively, 5 ml. of virus suspension was layered over 3 ml. of 45% sucrose in acetate buffer in a polycarbonate centrifuge tube. The virus was pelleted through the sucrose on centrifugation at 50,000 rpm for 4 hours. The sucrose and virus supernatant were discarded and the virus pellets were resuspended as described in steps 8 and 9.

11) The virus suspension was stored at 3°C with a drop of chloroform to prevent microbial growth.

c) Purification of viruses from mixed infections:
The same purification procedure outlined for BSMV was used on the doubly infected plants with a few modifications. The original tissue extraction was made in 0.02M potassium phosphate buffer at pH 6.5 rather than 0.02M K₂HPO₄ to avoid subjecting BMV to high pH. In most experiments the purification was carried up to Step 10 and then half of the partially
purified virus was set aside for further fractionation as described in section VI of Materials and Methods. The rest of the virus suspension was further purified for ultraviolet absorbance studies and storage.

d) Ultraviolet Spectrophotometry: The purified virus suspensions were diluted in 0.02M Tris-citrate, 0.1 M glycine buffer pH 6.5 and analyzed on a Unicam SP800A Ultraviolet Spectrophotometer with a 1 centimeter quartz cell.

Virus concentrations were determined by measuring the absorbance at 260 nm and relating it to the published values of 2.8 (39) and 4.8 (15) for 0.1% solutions of BSMV and BMV respectively. The ratio of the absorbance at 260 nm divided by the absorbance at 280 nm (260/280 ratio) was calculated and compared to the published values of 1.0-1.2 (39) and 1.67-1.70 (15) for solutions of BSMV and BMV respectively. These values were used to estimate the purity of the preparation and also gave an indication of the proportion of the two viruses in the mixtures when compared to an experimental set of ratios obtained from data of a set of artificially prepared mixtures of the viruses at various proportions.

IV. Infectivity Assays:

Infectivity assays of virus preparations and fractionated samples were made on Blackhulless barley grown in 2 inch peat pots with 8-10 plants per pot. The plants were
inoculated in the one leaf stage with small sterile gauze pads dipped in inoculum containing 1% Celite or in the same manner using cotton applicators (Q-tips). The plants were washed after inoculation and spaced to avoid physical contact. Those plants showing symptoms in 10 to 15 days were collected. The sap of one leaf was extracted into a small test tube by squashing a rolled leaf in a pair of modified pliers between layers of heavy waxed paper. This drop of extracted sap was assayed for both BMV and BSNV by the immunodiffusion technique (see section Va) against BNV and BSNV antisera separately.

V. Serological Tests:

Serological tests were performed routinely throughout this study as an independent method for assaying plant extracts and virus suspensions for the presence of both viruses.

a) Immunodiffusion. The Ouchterlony (67) method of immunodiffusion was used. The plates were prepared by pouring 2.5 ml. of 1% Agar Noble into each quadrant of a quadrant-type Petri dish which had been previously coated with a film of Formvar. Solvent for the agar was phosphate-buffered saline, containing 0.01 M potassium phosphate buffer and 0.14 M NaCl pH 7.1. Sodium azide was added to a concentration of 0.02% as a preservative and 0.5% LSA* was used as a detergent

*LSA: Leonil SA—Sodium dibutylnapthalene sulfonate.
to disperse the BSMV and facilitate the formation of a precipitin line (40). After the agar had hardened, wells were cut with a No. 2 cork borer. The wells were positioned on the circumference of a circle such that the center of each well was 1.0 cm from the center of a central well and the distance between the centers of the six peripheral wells was 1.0 cm. The central well was filled with antiserum and each peripheral well was filled with plant sap or virus preparation. The plates were incubated at 15°C and examined after one and two days for the formation of an antigen-antibody precipitin line.

b) The Microprecipitin Method: The microprecipitin method of van Slogteren (89) was used to estimate the BSMV concentration in the virus preparations prepared from doubly infected tissue. BSMV antiserum was placed in small droplets on the bottom of a flat-bottomed plastic Petri dish. Equally small droplets of two-fold dilutions of antigen in phosphate-buffered saline was added to the antiserum droplets and mixed. The bottom of the dish and the droplets were then covered with a layer of mineral oil to prevent evaporation. The plates were incubated at 36°C for 1 hour, examined by a dark field microscope for the presence of a precipitate, incubated for an additional 12 hours at 3°C and examined again. The precipitatin end point of the sample mixture was compared to a standard dilution series of a mixture of BSMV (2 mg/ml) and BMV (30 mg/ml) and used to estimate the BSMV concentration in the sample mixture.
c) Immunoelectrophoresis: The immunoelectrophoretic technique was carried out in a thin supporting medium of agar gel on microscope slides. The LKB 6800 A immunoelectrophoresis apparatus was used with a Gelman Model 38200 power supply and electrophoresis chamber. Six glass slides were placed in a plastic frame and sealed with a solution of sticker agar (0.1% agar, 0.05% glycerine in distilled water). Then 10 ml. of warm 1% Agar Noble in 0.02M potassium phosphate, 0.1M potassium chloride buffer pH 7.0 (39, 40) was poured into each section of the slide frame. A long trough and two small wells 1.5 mm in diameter on each side of the trough were cut with a gel punch. The virus preparation (0.02 mg/ml at 10-20 mg/ml) was placed in the wells and the frames were then placed in the electrophoresis apparatus. Electrical contact between the gel layer and electrolyte buffer was achieved by means of rayon wicks soaked in buffer. The electrolyte buffer was the same one used as a solvent for the agar. Electrophoresis was performed for 8 hours at 23 milliamperes with a voltage of 120-160 volts. After completion of electrophoresis, the gel was removed from the trough with a small knife and antiserum was then added. The slides were incubated in a humid chamber at 20°C for 12 hours and then examined for the formation of antigen: antibody precipitin lines.
VI. Separation and Analytical Techniques

Differences in the properties of both viruses which could be exploited for their separation from a mixture are shown in Table 1. The following procedures were used alone and in combination in order to separate BMV from BSMV in the mixed virus preparations.

a) Magnesium Chloride Precipitation of BSMV: (6)
Two ml of 0.2M MgCl₂ was added to 2 ml of BMV-BSMV virus suspension. The mixture was allowed to stand at room temperature for 30 to 60 minutes. It was then centrifuged in the SS 34 rotor at 10,000 rpm for 10 minutes. The supernatant, which contained most of the BMV, was dialyzed against 0.02M Tris-citrate buffer pH 6.5 for 12 hours at 30°C to remove the magnesium chloride. The low speed pellet, which contained the BSMV, was resuspended in 2 milliliters of 0.02 M Tris-citrate buffer pH 6.5 and likewise dialyzed. Prolonged incubation of BSMV in magnesium chloride resulted in irreversible aggregation of the virus, so this was avoided.

b) Sucrose Density-Gradient Centrifugation (18, 22):
Preliminary separation of BMV-BSMV mixtures by density gradient centrifugation was done by layering 2.0 ml of a virus suspension on preformed sucrose density-gradient columns. These columns were prepared by layering 5, 8, 8, 8, and 5 ml of 0.02 M Tris-citrate pH 6.5, 0.02% Igepon T-73 containing 400, 300, 200, 100 and 50 mg of sucrose per ml, respectively, in 1 x 3-1/2 inch cellulose nitrate tubes. The columns were incubated at 30°C for at least 12 hours to allow a sucrose
Table 1. Comparison of the properties of BMV and BSMV

<table>
<thead>
<tr>
<th>Property</th>
<th>BMV*</th>
<th>BSMV**</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Structure</td>
<td>Sphere 30 μm</td>
<td>Rod 20 x 130-175 μm</td>
</tr>
<tr>
<td>b) Sedimentation</td>
<td>86 S</td>
<td>189 S</td>
</tr>
<tr>
<td>Coefficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) Particle weight</td>
<td>4.6 x 10^6 daltons</td>
<td>26 x 10^6 daltons</td>
</tr>
<tr>
<td>d) U.V. absorbance</td>
<td>260/280: 1.70</td>
<td>260/280: 1.06</td>
</tr>
<tr>
<td></td>
<td>Ext. coeff. 4.8</td>
<td>Ext. coeff. 2.8</td>
</tr>
<tr>
<td>e) pH stability</td>
<td>Unstable at pH 7.0</td>
<td>Stable at pH 7.0</td>
</tr>
<tr>
<td>f) Isoelectric point</td>
<td>pH 7.9</td>
<td>pH 5.0</td>
</tr>
<tr>
<td>g) Nucleic acid</td>
<td>21% RNA, three species</td>
<td>5% RNA, one species</td>
</tr>
<tr>
<td></td>
<td>1.0, 0.7, and 0.3 x 10^6 daltons</td>
<td>1.0 x 10^6 daltons</td>
</tr>
</tbody>
</table>

*References for BMV: (15, 16, 26, 82).

**References for BSMV: (5, 21, 39, 42, 57).
concentration gradient to form. The columns with 2-3 mg of virus suspension layered on, were centrifuged at 27,000 rpm in the Beckman SW27 rotor for 2 hours. The two virus bands visible after centrifugation were collected into separate fractions by puncturing the bottom of the tube and allowing the gradient to drip out. The collected zone samples were dialyzed against 0.02 M Tris-citrate buffer pH 6.5 and then concentrated by highspeed centrifugation in the type 30 rotor at 30,000 rpm for 3 hours. The supernatant was discarded and the virus pellets were resuspended in 0.02 M Tris-citrate, 0.1 M glycine pH 6.5. This procedure yielded two fractions, one rich in BMV and the other rich in BSV, with traces of contamination of the other virus in each.

Further separation and analysis of the degree of separation was accomplished by another cycle of density-gradient centrifugation. Gradient columns were prepared in the same manner by layering 3 ml each of 400, 300, 200 and 100 mg of sucrose per ml in a 9/16 x 3-3/4 inch cellulose nitrate tube. Sucrose was dissolved in the same buffer as used before. The columns with 0.2 ml of virus suspension layered on top were centrifuged at 40,000 rpm for 90 minutes in the Beckman SW 40 rotor. After centrifugation the density-gradient columns were analyzed on the ISCO Model D Density-gradient Fractionator. This apparatus consisted of a pump which pushed the sucrose gradient at a constant rate past an ultraviolet source. The ultraviolet absorbance was recorded on a chart which allowed visualization of the distribution of ultraviolet
absorbing material down the gradient thus giving a graphical picture of the sedimentation profile of the virus suspension. Using this apparatus, evidence of physical separation of the viruses and fractions for infectivity tests were obtained. The 0.0 - 0.25 scale of U.V. absorbance was used and proved optimal for quantities of BSMV at 0.2 mg and BMV at 0.06 mg.

c) Sucrose Density-gradient Zone Electrophoresis (8, 19, 69). This procedure was to analyse previously separated virus fractions for evidence of electrophoretic heterogeneity. It was also valuable as a second separation step to remove traces of BMV from the BSMV particle population by virtue of the different electrophoretic mobilities of the viruses at pH 7.0. The procedure was performed using the buffer system of 0.0025M potassium phosphate, 0.0016M KCl pH 7.0-7.2 described by Ball (8) in the Isco Model 210 Density Gradient Zone Electrophoresis Apparatus.

The apparatus consisted of a Teflon cylinder which contained a sucrose density gradient and virus sample. The gradient and sample were floated on a dense layer of sucrose. A motor driven syringe pump raised and lowered the gradient past an ultraviolet source which was connected to an external recorder. The position of the virus sample in relation to its origin before electrophoresis was monitored several times during electrophoresis. The absolute location of the origin could be determined from a witness mark electrically recorded on the chart at the same point in the gradient during each scanning operation. The tube containing
the gradient column was surrounded by a jacket in which water at 15°C was circulated as a coolant. Buffer chambers at the bottom of the column and at 2/3 of its height contained platinum wire electrodes in buffer and were separated from the central column by dialysis membranes, making it unnecessary to maintain hydrostatic equilibrium. The electrodes were attached to a constant current power supply.

An 8.1 ml, 5 - 20% preformed sucrose gradient in the phosphate buffer was added to the central column over the supporting buffer solution of 25% sucrose. Usually 0.25 ml of virus at a concentration of 0.2 to 0.4 mg was added to the top of the gradient followed by 16 ml of buffer. Electrophoresis was performed for 30 minute intervals at 3 to 4 milliamperes. After electrophoresis the column was fractionated manually into 6 fractions of 1.6 mls each. The fractions were used for infectivity assays.

d) Sepharose Column Chromatography. This procedure was used as a preliminary separation technique to separate BMV from BSMV in the semi-purified virus preparations. The technique of molecular sieve chromatography or gel filtration involves passing a sample through a glass column containing a suspension of spherical agarose beads in a liquid phase. Molecules larger than the largest pores in the Sepharose beads are excluded from the gel particle and pass down the column in the liquid phase. Smaller molecules penetrate the gel particles to a varying degree depending on their size and shape and are therefore retained. Molecules
are eluted from the Sepharose in the order of decreasing molecular size. Details on theory and applications have been reviewed (1, 4).

Sepharose 2B was selected with an exclusion limit for proteins with a molecular weight of $40 \times 10^6$. This gel excluded BSMV but retained the smaller BNV particles thus enabling mild separation of the two viruses on the basis of size and shape. Sepharose 2B was diluted in 0.02M Tris-citrate buffer pH 6.5 with 0.02% sodium azide added as a preservative. The slurry was poured into a K 25/45 glass column with dimensions of 2.5 cm x 45 cm. The excess buffer was allowed to drain out as the Sepharose packed and the column was then fitted with a flow adaptor that allowed accurate control of the flow rate and semi-automatic sample application. The elution buffer was 0.02 M Tris-citrate pH 6.5 with 0.02% sodium azide added. The experiments were carried out at room temperature. Usually 2 to 3 ml of virus sample was applied to the column at a concentration of 10-15 mg per ml. The flow rate was adjusted to 9 ml per hour by adjusting the level of the inlet eluant reservoir. Eluted fractions were continuously passed through a flow cell attached to an ISCO Model UA-2 Ultraviolet Analyzer and Recorder. This enabled a continuous recording of the absorbance of the effluent and an accurate representation of the position of the eluted virus samples. The effluent was automatically collected into 3 ml fractions with an attached ISCO Model 270 Fraction Collector. The collected fractions were tested for infectivity
on Blackhulless barley, then 8 to 10 fractions representing one zone on the elution profile were pooled and concentrated by highspeed centrifugation in the Type 30 rotor at 30,000 rpm for 3 hours. The resulting pellets were resuspended in 0.02M Tris-citrate, 0.1M glycine pH 6.5 buffer and stored at 3°C. Each sample passed through the Sepharose consisted of three fractions: a BSMV fraction, an intermediate fraction and a BMV fraction (see Fig. 7).

VII. Reconstitution Studies

The following procedures were employed in the isolation of BMV-RNA and BSMV protein and in the reassociation of BSMV protein with BMV-RNA.

a) Isolation of BMV-RNA. The virus was purified from infected Blackhulless barley as indicated in Section III of Materials and Methods. Bentonite, prepared by the method of Frankel-Conrat (35), was used as a means of preventing ribonuclease disruption of the RNA. The RNA was extracted by the following procedure (43).

1) BMV (10 mg/ml) was suspended with an equal volume of bentonite (10 mg/ml) in a glass centrifuge tube.

2) The suspension was allowed to stand for 10 minutes at 37°C to allow the bentonite to adsorb the ribonuclease.

3) One volume of the virus-bentonite suspension was mixed with one volume of phenol saturated in 0.1M sodium acetate, 0.001M CaCl₂, 0.001M MgCl₂ buffer at pH 5.0 in a glass centrifuge tube.
4) The mixture was shaken vigorously on a mechanical shaker for 15 minutes at 30°C.

5) It was then centrifuged at 12,000 G for 5 minutes in the SS34 rotor.

6) After centrifugation the top aqueous phase was removed and the lower meniscus and phenol phase were discarded.

7) To the aqueous phase was added a 1/2 volume of buffer saturated phenol. The mixture was shaken as in step 4 for 5 minutes.

8) Steps 5, 6, 7, and 5 were repeated in that order.

9) The aqueous phase was then treated six times with an equal volume of ether to remove traces of phenol.

10) Nitrogen gas was then bubbled through the aqueous solution to remove traces of ether.

11) The aqueous solution was then centrifuged at 40,000 rpm for 2 hours in the Beckman Type 65 rotor to remove traces of bentonite and undegraded virus.

12) The supernatant of the highspeed centrifugation was saved and to it was added 2 volumes of cold 95% ethanol to which was added a few drops of molar acetate buffer at pH 5.5.

13) The suspension was incubated in the cold at 4°C for 15 minutes.

14) The suspension was then centrifuged at 5000 rpm for 15 minutes in the SS34 rotor to pellet the precipitated nucleic acid.

15) The supernatant of the lowspeed centrifugation was discarded and the light pellet was carefully resuspended in TKM buffer (0.01 M Tris, 0.01 M KCl, 10^-4 M MgCl2 adjusted to pH 7.4 with HCl).

16) The resuspended pellet was then dialyzed against TKM buffer for 24 hours at 4°C.

17) Steps 12, 13, 14 and 15 were repeated.

18) The nucleic acid suspension was stored frozen at -12°C.
Throughout the nucleic acid isolation procedure a number of precautionary measures were taken to avoid contaminating the RNA with ribonuclease. All glassware was heated at 180°C for 5 hours, plastic tubes were heated to 40°C in the presence of 0.1% versene, 0.1% sodium dodecylsulfate for 20 minutes and all dialysis tubing was boiled in the versene solution for 1 hour. The concentration of the nucleic acid and an estimate of its purity were obtained from measurement on an ultraviolet spectrophotometer. The published values of the 260/280 ratio of 2.0, maximum-minimum ratio of 2.5 and extinction coefficient of 25 were used as standards (16, 43).

b) Isolation of BSMV Protein. Two procedures were used in the isolation of BSMV protein. The virus was purified as described in Section III of Materials and Methods and then the protein was isolated by one of the two procedures outlined below: The first procedure is essentially that of Gumpf and Hamilton (39).

1) The virus suspension was dialyzed against 1M CaCl₂ dissolved in 0.02M Tris-citrate buffer pH 6.5 for 12 hours.

2) The suspension was then centrifuged in the SS 34 rotor at 10,000 rpm for 30 minutes. The pellet, which consisted of precipitated virus and nucleic acid, was discarded.

3) The supernatant was centrifuged in the Type 65 rotor at 40,000 rpm for 5 hours and the pellet was discarded.

4) The supernatant from step 3 was dialyzed exhaustively against 400 volumes of 0.02M Tris-citrate buffer pH 6.5 for 24 hours at 3°C to remove the Ca²⁺ and Cl ions.
5) The same sample was then dialyzed exhaustively for several days against a number of changes of 0.01M Tris - HCl buffer pH 8.9 at 3°C.

6) The protein suspension was centrifuged at 12,000 rpm for 30 minutes in the SS 34 rotor and then stored at 3°C.

The second procedure used was that described by Atabekov et al. (6). It was used because it offered a more thorough purification and a better chance for ribonuclease elimination.

1) The final pellet of the last highspeed centrifugation in the BSMV purification procedure was re-suspended in 0.05 M Tris - HCl buffer pH 6.5.

2) To the virus suspension was added 0.1M magnesium chloride to a final concentration of 0.05M.

3) The suspension was allowed to stand at room temperature for 10 minutes to precipitate the BSMV and then it was centrifuged at 10,000 rpm in the SS 34 rotor for 10 minutes.

4) The pellet was resuspended in 0.05 M Tris - HCl, 0.02M EDTA (Ethylenediaminetetraacetic acid) buffer pH 7.0.

5) Steps 2 and 3 were repeated and the pellet was resuspended in 0.05M Tris - HCl pH 6.5.

6) 2 ml of 3 M calcium chloride was added to 4 ml of virus suspension.

7) The mixture was allowed to stand at room temperature for 5 minutes and then it was centrifuged at 10,000 rpm for 10 minutes in the SS 34 rotor.

8) The supernatant was dialyzed exhaustively against 0.05M Tris-HCl pH 5.0 for 2 days.

9) The resulting precipitate was centrifuged at 10,000 rpm for 10 minutes in the SS 34 rotor.

10) The pellet was resuspended in 0.05M Tris-HCl, 0.02 M EDTA buffer pH 8.9.

11) Steps 8 and 9 were repeated and the pellet was resuspended in 0.05 M Tris-HCl pH 8.9.
12) The resuspended pellet was then centrifuged at 40,000 rpm for 1 hour in the Type 65 rotor. The pellet was discarded.

13) The supernatant protein solution was stored at 3°C.

The purity and concentration of the protein solutions were determined by ultraviolet absorption studies. The ultraviolet absorption spectra and data obtained were compared to the published values of a maximum of 280 μm and a minimum at 250 μm with a maximum/minimum ratio of 2.1-2.43 and a 260:280 ratio of 0.47-0.57 (6, 39).

c) Reconstitution Experiments. In these experiments, protein, nucleic acid and buffer were mixed in critical proportions to a volume of 1.0 to 1.5 ml. The reagent mixture was then dialyzed against an appropriate buffer for 16-20 hours. After dialysis, 0.5 ml of suspension was layered on an SW40 gradient prepared as described in Section VI of Materials and Methods. The gradients were centrifuged at 25,000 rpm for 60-70 minutes and then examined on the ISCO Model D Density Gradient Fractionator. Infectivity assays on barley var. Blackhulless and Chenopodium amaranticolor L. were performed on fractions obtained from the gradients and on the reagent mixtures after dialysis. In some cases the reagent mixture was incubated with 0.05 ml of ribonuclease at 1 mg/ml for one hour before centrifugation.

In a typical experiment, 0.4 ml of BSMV coat protein (1.7 mg/ml) was mixed with 0.25 ml of BMV-RNA (0.14 mg/ml) in TKM buffer and 0.5 ml of 0.05 M Tris-HCl, 2M MgCl₂ pH 8.9
was added to it. The protein control consisted of the same reagents except that TKM buffer without RNA was added. The reagent mixture was dialyzed at 26°C against 0.1M potassium phosphate buffer pH 6.5 for 22 hours and then centrifuged on SW40 gradients with sucrose dissolved in 0.01M potassium phosphate buffer pH 6.5 containing 0.02% Igepon T-73. A number of variations of the above procedure were employed without significant differences in the results.

VIII. Fractionation of BMV-RNA and Infectivity Tests of the Fractionated RNA

Brome grass mosaic virus ribonucleic acid was extracted as described in section VI of Materials and Methods. It was then separated into three RNA component species (17) by a series of three sucrose density gradient centrifugations. The separated components were tested alone and in mixtures on leaves of Chenopodium amaranticolor L. and Chenopodium hybrids L. grown at 22°C with an 18 hour photoperiod. The SW40 gradients were prepared by layering 3 ml each of 200, 150, 100 and 50 mg/ml of ribonuclease-free sucrose solutions dissolved in 0.01M TKM buffer 0.01% polyvinyl sulfate pH 7.4 in 9/16x3 3/4 inch cellulose nitrate centrifuge tubes. SW65 gradients were also prepared in 1/2x2 inch cellulose nitrate tubes using 1 ml of each sucrose solution. The centrifugation series consisted of one 7 hour centrifugation in the SW 40 rotor at 40,000 rpm and two 5 hour centrifugations in the SW65 rotor at 40,000 rpm. The nucleic acid zones
illustrated in Fig. 14 were carefully fractionated on the ISCO Model D Density Gradient Fractionator and the RNA was concentrated from each zone by precipitation with 95% ethanol as described in Section VII of Materials and Methods on BMV-RNA purification. Fractionated zones from the third density gradient centrifugation were collected and assayed directly on leaves of plants. Assay plants were shaded for 72 hours before inoculation and then lightly dusted with carborundum. A small drop of inoculum was applied to a glass spatula which was then rubbed on one leaf (75). The leaves were washed with tap water after inoculation.
RESULTS AND CONCLUSIONS

I. Studies on the effect of the Double Infection on the Plant

When two or more viruses infect one host the disease reaction they initiate may be termed "synergistic" if some new type of symptom is observed or there is a reduction of tissue yield, greater than the additive effects that the two viruses would have produced alone (53). The term "tissue" is used throughout this paper to refer to the stem and leaf portions of the barley plant and it is only on this portion of the plant that fresh weight measurements were made. In the course of this investigation a comparison of the fresh weights of singly infected plants and doubly infected plants was made. Virus-infected plants generally show a reduction in fresh weight when compared to healthy plants of the same age. In Table 2 the fresh weights of healthy, single and doubly infected plants are compared on the basis of fresh weight per pot. A pot of barley plants was selected as an equivalent for comparison because on a large scale it could be assumed that each pot would contain an equal number of plants. The results presented are the average from 6 experiments.

The results in Table 2 show that doubly infected plants are lower in fresh weight than singly infected plants.
Table 2. Fresh weights of barley tissue 14 and 28 days after inoculation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>14 days</th>
<th></th>
<th>28 days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight of tissue</td>
<td>Weight difference</td>
<td>Weight of tissue</td>
<td>Weight difference</td>
</tr>
<tr>
<td></td>
<td>in grams (10&quot; pot)</td>
<td>(% of control)</td>
<td>in grams (6&quot; pot)</td>
<td>(% of control)</td>
</tr>
<tr>
<td>Healthy control</td>
<td>56</td>
<td>--</td>
<td>8.3</td>
<td>--</td>
</tr>
<tr>
<td>BHV-inoculated</td>
<td>41</td>
<td>27</td>
<td>6.2</td>
<td>25</td>
</tr>
<tr>
<td>BSMV-inoculated</td>
<td>32</td>
<td>43</td>
<td>5.5</td>
<td>34</td>
</tr>
<tr>
<td>Doubly inoculated</td>
<td>28</td>
<td>50</td>
<td>3.3</td>
<td>60</td>
</tr>
</tbody>
</table>
However, the difference between singly and doubly infected plants is not synergistic as was reported by Lal and Sill (59), but less than additive at 14 days. When the infection was allowed to progress for 4 weeks the difference in fresh weight between the single and doubly infected plants approached a true additive effect. It would appear that BMV and BSMV in mixed infection on barley do not affect it synergistically, but act independently to cause a reduction in fresh weight which is the additive effect of what each virus could exert independently of the other.

II. Studies on the Effect of the Double Infection on Virus Concentration

It has been shown that in a number of mixed virus infections the concentration of the viruses increases or decreases significantly compared to their concentration in a single infection. In order to determine if the concentration of one of the viruses changed in the doubly infected plants, the yield of each virus in a mixed infection was compared with that from a single infection of the same number of plants. It was assumed that on a large scale the number of plants in many pots would be constant and so the results are expressed as the average yield of each virus in milligrams from one pot of plants.

a) BMV Concentration: Purified preparations of virus from doubly infected plants and purified preparations of artificially mixed virus from an equivalent number of
BMV and BSMV singly infected plants were compared using the ultraviolet spectrophotometer. In all cases the amount of ultraviolet-absorbing nucleoprotein was about the same. When the BSMV was removed from both the real and artificial mixtures with magnesium chloride the total amounts of BMV remaining in suspension were the same. In a series of 5 experiments the yield of BMV was compared by this manner. From 10 pots of doubly infected plants (268 gm), 310 milligrams of BMV was recovered, compared to 320 milligrams of BMV recovered from 10 pots of plants (454 gm) infected with BMV only. These results suggest that a doubly infected plant produced as much BMV as a singly infected plant. In addition, equivalent amounts of BMV were recovered from single and double infections when estimates of the amounts of virus were made by ultraviolet analysis of the BMV fraction after separation from the BSMV by Sepharose (Fig. 7) and electrophoresis (Fig. 5c and d).

It is concluded on the basis of virus yield per plant, that the amount of BMV obtained from a doubly infected plant was the same as the amount of BMV obtained from a singly infected plant.

d) BSMV Concentration. In order to detect BSMV by sucrose density gradient centrifugation (Fig. 2a) it was necessary to remove most of the BMV which was in such high concentration that it sedimented into the BSMV zone and obscured its identification on the ultraviolet absorption tracing of the gradient column (Fig. 2b). Using the
separation techniques described in Section VI of Materials and Methods and diagramed in Fig. 1(c, d, and e), BSMV was separated from BMV. The amount of BSMV obtained from a real mixture was compared to the amount of BSMV obtained from the same number of plants infected with BSMV only.

To prepare an artificial mixture, an equal number of BMV singly infected plants was added to the BSMV singly infected plants and this mixture was then purified. The ratios of the amount of BSMV in the real mixture to that in the artificial mixture for several experiments are reported in Table 3. The results show that the amount of BSMV produced in a mixed infection is not significantly different from the amount of BSMV produced in an equal number of singly infected plants.

When serial dilutions of real and artificial mixtures prepared from an equivalent number of BSMV-infected plants were compared by the microprecipitation method the antigen dilution end points were always similar, indicating that the quantities of serologically active nucleoprotein were the same. In addition, equivalent amounts of BSMV were recovered from singly and doubly infected plants when estimates of the amounts of virus were made by ultraviolet analysis of the BSMV fraction after separation from BMV by Sepharose (Fig. 7) and electrophoresis (Fig. 6). The results indicate that the quantity of BSMV from a singly infected plant is the same as that in a doubly infected plant.
Figure 1

Possible structural interactions between BMV and BSMV and techniques used in their separation.

(a) Diagram illustrating the possible types of particles that could result from BMV-BSMV structural interaction. The spherical particles represent the BMV population and the rod shaped particles represent the BSMV population. BMV protein coat is drawn as a white outer shell and the BMV-RNA is represented by the enclosed white band. BSMV protein coat is drawn as a black outer shell and BSMV-RNA is represented as an enclosed black band.

(b) Separation procedure using MgCl₂ treatment followed by sucrose density-gradient zone electrophoresis and sucrose density-gradient centrifugation in the SW40 rotor.

(c) Separation procedure using sucrose density-gradient centrifugation on the SW27 rotor followed by sucrose density gradient zone electrophoresis and sucrose density-gradient centrifugation in the SW40 rotor.

(d) Separation procedure using Sepharose column chromatography followed by sucrose density-gradient zone electrophoresis and sucrose density-gradient centrifugation in the SW40 rotor.
(A) GENOMIC MASKING
PHENOTYPIC MIXING

(B) Electrophoresis

S.W.40

MgCl₂

Infectivity

Infectivity
Table 3. The ratio of the amount of BSMV obtained from a real mixture to that obtained from an artificial mixture.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>MgCl₂</th>
<th>Technique of Separation</th>
<th>Density</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.79</td>
<td>Centrifugation</td>
<td>0.85</td>
<td>Density</td>
</tr>
<tr>
<td>2</td>
<td>0.70</td>
<td></td>
<td>1.10</td>
<td>Gradient</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td>Gradient</td>
</tr>
<tr>
<td>4</td>
<td>0.87</td>
<td></td>
<td>0.87</td>
<td>Density</td>
</tr>
<tr>
<td>5</td>
<td>0.60</td>
<td></td>
<td></td>
<td>Electrophoresis</td>
</tr>
</tbody>
</table>

In conclusion it would appear that a plant doubly infected with both BMV and BSMV is capable of supporting the synthesis of equivalent amounts of both viruses that are normally produced in a single infection. A barley plant is able to support both BMV and BSMV replication without any significant effect of one replication system on the other.

III. Studies on Virus-Virus Interactions in Doubly Infected Plants

The main object of this research was to examine for the possibility of "in vivo" physical mixing between BMV and BSMV as a result of mixed infection. The following experiments were designed to isolate any physical intermediates displaying phenotypic mixing or genomic masking. The types of expected particles are illustrated in Fig. 1(a).
a) Genomic Masking. Young barley plants were inoculated with BMV, BSMV and a mixture of the two viruses. After 2 to 3 weeks of growth the plants were harvested and the virus was isolated from the plant tissue as described in Section III of Materials and Methods. Artificial or "in vitro" mixes were prepared by mixing equal numbers of infected plants before grinding the tissue or by mixing the virus suspensions derived from equal numbers of plants after the first highspeed centrifugation pelleting. These artificial mixtures were used as a control for estimating the degree of cross contamination after the virus populations were separated.

The real mixed virus preparations and the artificially mixed virus preparations were then subjected to a number of separation procedures in order to separate the BMV virus population from the BSMV virus population. The methods of separation employed are outlined in Fig. 1(b, c and d). The purpose of separating the virus populations was to see if any genomically masked particles could be detected. If BMV-RNA was coated in BSMV protein to form a genomically masked particle, it would exist as a rod shaped particle inseparable from the BSMV population. Therefore, after all of the spherical BMV particles were removed from the mixture, the genomically masked BMV-RNA would be expressed as BMV infectivity originating in the rod virus population of only the real mixture. The artificial mixture would act as a check on the level of BMV contamination. Similarly, if
BSMV-RNA was coated by BMV coat protein it would express itself as BSMV infectivity originating from the separated spherical virus population of the real mixtures.

Before the results could be interpreted it was necessary to determine the lowest concentration at which BMV infectivity could be detected in a predominantly BSNV inoculum. This was determined by mixing small amounts of BMV with BSNV at 1 mg/ml and then inoculating Blackhulless barley. BMV could be detected at a level between 0.01 to 0.001% of the BSMV inoculum as indicated in Table 4. The results show that when BMV is present in the BSNV rod population at a concentration lower than 0.01% it cannot be readily detected by infectivity assays. Therefore, if genomically masked BMV-RNA particles were to be detected by this system they would have to be present at a level greater than 0.001% of the normal BSNV population of particles. Table 4 also shows that BSNV does not appreciably interfere with BMV infectivity in the mixed inoculum even at relatively high proportions of BSNV.

After the BMV and BSNV viral populations were separated, infectivity tests of the separated populations of particles were performed on Blackhulless barley. When symptoms developed, each plant was tested for the presence of BMV and BSNV by the immunodiffusion serological test using both BMV and BSNV antisera as described in Section V of Materials and Methods. A comparison of the infectivity results for the BSNV population of the artificial and real
Table 4. Results of infectivity assays of artificial mixtures of BSMV and BMV inoculated to barley.

<table>
<thead>
<tr>
<th>Inoculum BSJV mg/ml</th>
<th>BMV mg/ml</th>
<th>Tested</th>
<th>Infected with BMV</th>
<th>Infected with BSMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 + 0.1</td>
<td>11</td>
<td>10(91)</td>
<td></td>
<td>10(91)</td>
</tr>
<tr>
<td>1.0 + 0.01</td>
<td>23</td>
<td>14(61)</td>
<td></td>
<td>22(96)</td>
</tr>
<tr>
<td>1.0 + 0.001</td>
<td>24</td>
<td>10(42)</td>
<td></td>
<td>22(92)</td>
</tr>
<tr>
<td>1.0 + 0.0001</td>
<td>23</td>
<td>4(17)</td>
<td></td>
<td>22(96)</td>
</tr>
<tr>
<td>0 + 0.1</td>
<td>12</td>
<td>11(92)</td>
<td></td>
<td>--</td>
</tr>
<tr>
<td>0 + 0.01</td>
<td>12</td>
<td>10(83)</td>
<td></td>
<td>--</td>
</tr>
<tr>
<td>0 + 0.001</td>
<td>12</td>
<td>7(58)</td>
<td></td>
<td>--</td>
</tr>
</tbody>
</table>

a Numbers in parenthesis represent percent of plants infected.
mixtures is presented in Table 5. The BMV infectivity in the artificial mixtures represents the degree of contamination of BMV spheres in the BSMV rod population after separation by the various procedures indicated in the table. Table 6 is a comparison of the infectivity results for the BMV population of the artificial and real mixtures. Similarly any BSMV infectivity in the artificial mixtures represents the degree of contamination of BSMV rods in the BMV population.

Examination of Table 5 shows that after the BMV and BSMV populations have been separated by MgCl₂ precipitation and sucrose density gradient centrifugation at pH 6.5 or by two cycles of sucrose density gradient centrifugation at pH 6.5, more BMV infectivity is present in the BSMV population of the real mixes than in the artificial mixes. It could be argued that because this BMV infectivity is above the level of contamination indicated by the artificial mixture, it represents genomically masked BMV. The degree of separation indicated in Figs. 2(c) and 4(b) would support the idea as well. However, when the same samples concentrated by MgCl₂ were run on sucrose density gradients at pH 8.0, all of the contaminant BMV in the artificial mixtures was removed along with all of the BMV infectivity in the real mixtures. This is explained by the fact that BMV is unstable at a pH above 7.0 and tends to swell and become inactivated (49). The swelling and inactivation is manifested by a decrease in density and decrease in sedimentation rate as
Table 5. Results of infectivity assays on barley of the BSMV population after separation from artificial and real mixtures containing BMV.

<table>
<thead>
<tr>
<th>Technique of separation</th>
<th>ARTIFICIAL mixture</th>
<th>REAL mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of plants tested</td>
<td>Infected with BMV</td>
</tr>
<tr>
<td>a) MgCl₂-SW 40 pH 6.5</td>
<td>63</td>
<td>7(11) a</td>
</tr>
<tr>
<td>b) MgCl₂-SW 40 pH 8.0</td>
<td>32</td>
<td>0(0)</td>
</tr>
<tr>
<td>c) SW 27-SW 40 pH 6.5</td>
<td>57</td>
<td>1(2)</td>
</tr>
<tr>
<td>d) MgCl₂-Electrophoresis</td>
<td>6</td>
<td>0(0)</td>
</tr>
<tr>
<td>e) SW 27-Electrophoresis</td>
<td>7</td>
<td>0(0)</td>
</tr>
<tr>
<td>f) Sepharose</td>
<td>225</td>
<td>21(9)</td>
</tr>
<tr>
<td>g) Sepharose-SW 40 pH 6.5</td>
<td>96</td>
<td>1(1)</td>
</tr>
<tr>
<td>h) Sepharose-Electrophoresis</td>
<td>149</td>
<td>3(2)</td>
</tr>
<tr>
<td>i) Sepharose-BMV anti-serum</td>
<td>64</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

a Numbers in parenthesis represent percent of plants infected.
indicated in Fig. 3. These results suggest that all of the BMV infectivity was present as normal spherical particles which contaminated the BSMV population and not as genomically masked rods which would be expected to withstand the high pH as do normal BSNV rods.

The results in Table 5 also indicate that no BMV infectivity could be detected in the BSMV population of either mixture when sucrose density gradient zone electrophoresis was used as a final step in the separation procedure. Figs. 5 and 6 illustrate the separation obtained by this method after preliminary separation by MgCl₂ and density gradient centrifugation on SW27 gradients. The infectivity results strongly indicate that no genomically masked particles were detectable in the BSMV population.

Sepharose column chromatography offered the best method for mild bulk separation of the mixed population of virus particles. Fig. 7 shows an ultraviolet absorption tracing of fractions of nucleoprotein eluted from the column in 120 ml of effluent. The first peak is the rod population which is well separated from the larger second peak containing the sphere population. Fig. 8 shows sucrose density gradient profiles of the first two fractions marked 1 and 2 in Fig. 7. Fig. 8(a) illustrates that the BSMV population from the sepharose column contained only a small amount of BMV. Table 5 shows the infectivity level of this degree of contamination, which is not appreciably different in the real and artificial mixes. Further separations following
Sepharose indicated in Table 5 shows that the real mixtures contain little or no BMV infectivity above the level of contamination indicated in the artificial mixtures. This is further evidence that genomically masked BMV-RNA does not exist in the BSMV rod population in significant quantity to be detected by infectivity assays.

On the basis of the above evidence one would have to conclude that no sufficient evidence exists for the possibility that genomically masked BMV-RNA occurred in detectable amounts. The possibilities that such particles existed below a level of 0.001% of the particle population, or that they were too unstable to survive the purification and separation procedures, cannot be ruled out.

Examination of Table 6 clearly indicates that in both the real and artificial mixtures all of the BSMV infectivity was readily removed from the BMV population by the separation procedures. Figs. 2(d), 4(a) and 5(c and d), indicate that this is correlated with complete removal of all of the rod virus population. It must therefore be concluded that no BSMV-RNA was genomically masked in BMV coat protein in any of the real mixtures tested.

The results indicate that genomically masked particles could not be demonstrated as a result of the BMV and BSMV mixed infection in barley. It is conceivable that such an interaction could have occurred but at such a low level as to be non-detectable by the methods employed.
Table 6. Results of infectivity assays on barley of the BMV population after separation from artificial and real mixtures containing BSMV

<table>
<thead>
<tr>
<th>Technique of separation</th>
<th>ARTIFICIAL mixture</th>
<th>REAL mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of plants tested</td>
<td>Infected with BHV</td>
</tr>
<tr>
<td>a) SW 27</td>
<td>17</td>
<td>17(100)</td>
</tr>
<tr>
<td>b) SW 27-SW 40 pH 6.5</td>
<td>36</td>
<td>32 (89)</td>
</tr>
<tr>
<td>c) MgCl₂-SW 40 pH 6.5</td>
<td>33</td>
<td>28 (85)</td>
</tr>
<tr>
<td>d) Electrophoresis</td>
<td>62</td>
<td>53 (86)</td>
</tr>
<tr>
<td>e) Sepharose-BSMV</td>
<td>18</td>
<td>14 (78)</td>
</tr>
</tbody>
</table>

a Numbers in parenthesis represent percent of plants infected.
Sucrose density gradient centrifugation profiles in a 10-40% sucrose gradient in 0.02% Tris-citrate pH 6.5 containing 0.02% Igepon T-73 after centrifugation in an SW 40 rotor at 40,000 rpm for 70 minutes. Direction of sedimentation is to the left.

a) Artificial mixture at optimal proportions (black line), and a reference baseline (dashed line) for a gradient which did not contain virus.

b) Real mixture with no preliminary separation.

c) BSHV population after preliminary separation by MgCl₂ treatment.

d) BNV population after preliminary separation by MgCl₂ treatment.
Figure 3.

Sucrose density gradient centrifugation profiles in a 10-40% sucrose gradient in 0.02M Tris-citrate containing 0.02% Igepon T-73 after centrifugation in an SW 40 rotor at 40,000 rpm for 90 minutes. Direction of sedimentation is to the left. Preliminary separation with MgCl₂ treatment.

a) BSMV population on a pH 6.5 gradient.

b) BSMV population on a pH 8.0 gradient.
Sucrose density gradient centrifugation profiles in a 10-40% sucrose gradient in 0.02M Tris-citrate pH 6.5 containing 0.02% Igepon T-73 after centrifugation in an SW 40 rotor at 40,000 rpm for 90 minutes. Direction of sedimentation is to the left. Preliminary separation was on a 10-40% sucrose gradient in the SW 27 rotor.

a) BNV population from a real mixture.

b) BSMV population from a real mixture.
Sucrose density gradient zone electrophoresis profiles in a 5-20% sucrose gradient in 0.0025 M potassium phosphate, 0.0016 M KCl pH 7.0 at 3 mA, 280V, for 90 minutes. Anode is at the left. Preliminary separation was on a 10-40% sucrose gradient in the SW 27 rotor.

a) BSMV population of a real mixture.
b) BSMV population of an artificial mixture.
c) BMV population of a real mixture.
d) BMV population of an artificial mixture.

Note: The sharp break in the absorbance profile is the electrically recorded witness mark.
Figure 6.

Sucrose density gradient zone electrophoresis profiles in a 5-20% sucrose gradient in 0.0025 M potassium phosphate, 0.0016 M KCl pH 7.0 at 4 mA for 90 minutes. Anode is at the right.

a) Resuspension of the pellet from the MgCl₂ precipitation of a real mixture.

b) Resuspension of the pellet from the MgCl₂ precipitation of an artificial mixture.

c) BSNAV population of a real mixture after preliminary separation on a 10-40% sucrose gradient in the SW 27 rotor.

d) BSNAV population of an artificial mixture after preliminary separation on a 10-40% sucrose gradient in the SW 27 rotor.
Ultraviolet tracing of the elution profiles of artificial and real mixtures of BNV and BSNV from a Sepharose-2B column in 0.02 M Tris-citrate pH 6.5 containing 0.02% Na azide. Elution was at a flow rate of 9 ml/hr. Numbers between arrows refer to pooled fractions which were concentrated by differential centrifugation for further analysis.
Sucrose density gradient centrifugation profiles in a 10-40% sucrose gradient in 0.02 M Tris-citrate pH 6.5 containing 0.02% Igepon T-73 after centrifugation in an SW 40 rotor at 40,000 rpm for 90 minutes. Direction of sedimentation is to the left. Preliminary separation was on the Sepharose column. The fraction numbers refer to the pooled fractions from the Sepharose column.

a) Fraction 1 of artificial mixture (prepared at the time tissue was homogenized).

b) Fraction 2 of artificial mixture (as in a).

c) Fraction 1 of real mixture.

d) Fraction 2 of real mixture.

e) Fraction 1 of artificial mixture (prepared from purified preparations at the time of Sepharose separation).

f) Fraction 2 of artificial mixture (as in e).
Figure 9.

Sucrose density gradient zone electrophoresis profiles in a 5-20% sucrose gradient in 0.0025 M potassium phosphate, 0.0016 M KCl pH 7.0 at 3 mA for 90 minutes. Anode is at the left. Preliminary separation was on the Sepharose column. The fraction numbers refer to the pooled fractions from the Sepharose column.

a) Real mixture, fraction 1.
b) Real mixture, fraction 2.
c) Artificial mixture, fraction 1.
d) Artificial mixture, fraction 2.
b) Phenotypic Mixing: Phenotypic mixing is defined as an interaction between two viruses where the product contains one of the parent nucleic acids and a protein coat that is a mixture or mosaic of protein contributed by both of the parent viruses (91). If such particles exist as a result of a BMV-BSMV interaction in a doubly inoculated barley plant (Fig. 1a), they would be intermediate in electrophoretic mobility and they should also be precipitated by both antisera to the respective viruses.

Immunoelectrophoresis was performed in a thin layer of agar gel to separate the BMV and BSMV populations from a mixed preparation and to test the separated populations against antisera to the viruses. Fig. 10 illustrates the type of antigen antibody precipitin lines obtained after electrophoretic separation followed by immunodiffusion with the virus antisera. These results failed to reveal any signs of a significantly large population of intermediates with respect to electrophoretic mobility or antigenicity in the real mixtures as compared to the artificial mixtures. Fig. 10a, bottom well, shows a slight tail on the BMV precipitin line; this will be discussed in the next section. Immunoelectrophoresis was considered unsatisfactory because of the large quantities of virus required for this technique and because BSMV produced such a diffusely tailed pattern that it tended to prevent expression of any intermediates. The results, although negative, were not considered very significant.
Sucrose density gradient zone electrophoresis offered a more sensitive method for detecting small populations of electrophoretically intermediate particles. Examination of Figs. 5, 6 and 9 shows that no electrophoretically intermediate populations of particles could be demonstrated as a characteristic of only the real mixes. Particles intermediate in electrophoretic mobility were detected in both populations of real and artificial mixes. This will be discussed in the next section.

In conclusion it would appear that phenotypic mixing did not occur to any detectable extent between BMV and BSMV in the mixed infections.

c) "In vitro" Association between BMV and BSMV: Some of the results presented in previous sections suggested that BMV and BSMV may form a complex on incubation together "in vitro". If one considers that the isoelectric points of BMV and BSMV are pH 7.9 and pH 5.0 respectively, it is evident that at pH 6.5, BMV would be positively charged and BSMV would be negatively charged. A similar phenomena was reported by Kassanis (55) between TMV, a rod virus similar to BSMV, and BMV in solutions of low ionic strength.

Pure preparations of BSMV were homogenous when examined on the electrophoresis apparatus, however both real and artificial mixtures tended to show a slower migrating shoulder in the BSMV zone (Figs. 6 and 9). Similarly, immunoelectrophoresis showed that purified BMV was homogenous but when it was mixed with BSMV (Fig 10d) it tended to show a slight tail not seen
Figure 10.

Immunoelectrophoresis patterns in 1% agar dissolved in 0.02 M potassium phosphate, 0.1 M KCl pH 7.0. Electrophoresis was for 8 hours at 23 mA, 120 V. Anode is to the left.

a) BMV in both wells against BMV antiserum in the center trough.

b) BSMV at 10 mg/ml (top well) and 20 mg/ml (bottom well) against BSMV antiserum in the center trough.

c) BSMV from a real mixture (top well) and purified BSMV (bottom well) against BSMV antiserum in the center trough.

d) Purified BMV (top well) and BMV from a real mixture (bottom well) against BMV antiserum in the center trough.
in the single preparation. It should be noted that BMV normally appeared heterogenous at a pH above 7.0 in zone electrophoresis because of swelling of the virion and exposure of the nucleic acid resulting in a decrease of the positive charge on the virus particle.

Additional indirect evidence for an "in vitro" association was obtained from density gradient profiles of sepharose fractions illustrated in Fig. 8. Fig. 8(a and c) represents artificial and real mixtures in which the viruses were mixed at the time the plant tissue was homogenized while 8(e) represents an artificial mixture prepared after purification and mixed just before the sample was applied to the sepharose column. Note that in those preparations in which association occurred very early in the purification (Fig. 8(a and c)), aggregation is much more conspicuous in comparison to preparations in which the association was brief (Fig. 8(e)).

It is hypothesized, on the basis of the above evidence, that there is a tendency for the positively charged BMV particles to associate with the negatively charged BSMV particles when a mixture of the two viruses is incubated in the pH range of 6.0-7.0. This "in vitro" association accounted for some of the BSMV aggregation observed as well as the presence of electrophoretically intermediate populations in the mixed preparations. It might also have accounted for much of the BMV contamination in the BSMV population after sucrose density gradient centrifugation. The absence of such
apparent contamination after electrophoresis might have re­sulted from the separation of the complex by virtue of the applied electric charge.

No conclusive proof for the association is offered in the form of electron micrographs or refined serological studies. It is hypothesized only as a likely explanation for some of the results observed.

IV. Studies on the Reconstitution of Barley Stripe Mosaic Virus Coat Protein with Bromegrass Mosaic Virus Ribonucleic Acid

These studies were attempted to see if BSMV coat protein could coat BMV-RNA "in vitro". If such hybrid particles (Fig. 1a), could be formed "in vitro", then it could be assumed that physical compatibility of the protein and the RNA would not prevent genomic masking "in vivo".

Nucleoprotein-like particles were obtained under a number of variations of the reconstitution procedure. In 11 experiments performed, the BSMV and BMV-RNA checks were infectious, while no indication of any infectivity was observed for the reconstituted particles on barley or Chenopodium amaranticolor L.

The results of the experiment outlined in Section VIII of Materials and Methods are presented. Fig. 9(a) shows the density gradient sedimentation profile of reconstituted protein and nucleic acid with a control BSMV sedimentation profile superimposed on the graph. It indicates that reconstituted
particles sedimented as a variable population of rods which were smaller than the monomer of BSMV. The polymerized protein sedimentation profile is given in Fig. 9(b). It shows that the protein without nucleic acid was much smaller than the reconstituted particles or that it was unstable in the gradient. Fig. 12 shows electron micrographs of the reconstituted particles and the protein. There were many rods of various sizes in both preparations; however, in the absence of RNA the length of the polymerized protein was indeterminate. Furthermore, these rods broke up to form light weight protein in a density gradient containing Igepon T-73. The reconstituted nucleoprotein, on the other hand, showed a more diffuse but distinctly faster sedimenting band in the density gradient.

It is significant that no portion of the reconstituted rods sedimented as far as BSMV indicating that no stable rods enclosing 1.0 x 10^6 daltons of RNA were produced. This would appear to be due to the fact that stable completely coated rods were not formed. As indicated in Fig. 12(a), the RNA was only incompletely coated and the reconstituted particles contained gaps and kinks. This result indicates that BSMV protein could coat the BMV-RNA only partially to form imperfect and unstable rods with exposed portions of nucleic acid. The exposed RNA would be susceptible to ribonuclease, thus accounting for the lack of infectivity in the reconstituted product.

It is concluded that the imperfect rods formed were nucleoprotein particles. Ultraviolet absorption measurements
(260/280 ratios and maximum/minimum ratios) presented in Fig. 13 and Table 7 indicate that this was the case.

The results suggest two possible explanations:
1) The reconstitution procedure was not refined enough to permit complete encapsulation which might occur under more refined conditions. It should be noted that BSNV coat protein has not been shown to coat even its own nucleic acid "in vitro" (7).
2) The BSNV coat protein may not be compatible with BMV-RNA for absolute encapsulation of the total BMV-RNA genome and thus reconstitution does not produce a stable infectious particle. This possibility is unlikely in the light of the recent evidence for the reconstitution of BMV-RNA with TMV coat protein (7).
Figure 11.

Sucrose density gradient centrifugation profiles in a 10-40% sucrose gradient in 0.01M potassium phosphate pH 6.5 containing 0.02% Igepon T-73 after centrifugation at 40,000 rpm for 70 minutes. Direction of sedimentation is to the left.

a) Reconstituted nucleoprotein from a BMW-RNA-BSMV protein reaction mixture (black line) and a BSMV standard for comparison (dashed line).

b) Polymerized protein from a BSMV-protein reaction mixture without added RNA.
Figure 12.

Electron micrographs of reconstitution reaction mixtures stained with neutral 1% phosphotungstic acid. Magnification is 100,000 X.

a) Reconstituted nucleoprotein.

b) Polymerized protein.
Ultraviolet absorption profiles from a Unicam SP 800 A Ultraviolet Spectrophotometer.

a) Reconstituted nucleoprotein from a density gradient in 0.01M potassium phosphate pH 6.5.

b) Polymerized BSMV protein from a density gradient in 0.01M potassium phosphate pH 6.5.

c) Pure BSMV protein preparation in 0.05M Tris-HCl pH 8.9.

d) Pure BSMV preparation in 0.01M potassium phosphate pH 6.5.

e) Pure BMV-RNA preparation in 0.01M Tris, 0.01M KCl, 10⁻⁴M MgCl₂ pH 7.4.
Table 7. Ultraviolet absorption characteristics of BSMV, reconstituted virus, BSMV protein and BMV-RNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>260/280 Ratio</th>
<th>Maximum Absorbance (nm)</th>
<th>Minimum Absorbance (nm)</th>
<th>Max./Min. Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted virus</td>
<td>1.02</td>
<td>270</td>
<td>250</td>
<td>1.02</td>
</tr>
<tr>
<td>BSMV</td>
<td>1.06</td>
<td>260</td>
<td>250</td>
<td>1.01</td>
</tr>
<tr>
<td>Polymerized protein</td>
<td>0.80</td>
<td>280</td>
<td>250</td>
<td>1.30</td>
</tr>
<tr>
<td>Pure BSMV protein</td>
<td>0.57</td>
<td>280</td>
<td>250</td>
<td>2.15</td>
</tr>
<tr>
<td>Pure BMV-RNA</td>
<td>2.10</td>
<td>256</td>
<td>230</td>
<td>2.40</td>
</tr>
</tbody>
</table>
V. Studies on the Infectivity of Fractionated Bromegrass Mosaic Virus Ribonucleic Acid

It has been reported that BMV-RNA exists as a multicomponent entity (16) and several papers have been written to explain the significance of the multicomponent nature of the nucleic acid (17, 56). The multicomponent nature of the nucleic acid is illustrated after extraction from the virus with phenol and sucrose density gradient centrifugation in Fig. 14(a). The three components, labeled S for small, M for medium and L for large are indicated in the centrifugation profile. They approximated the reported sedimentation coefficients of 14, 22 and 27 S (17) using TMV-RNA as a standard. The reported S, M and L components have molecular weights of 0.2, 0.7 and 1.0 x 10^6 daltons respectively. Infectivity tests on C. hybridum and C. amaranticolor were performed on the portion of the nucleic acid component indicated by the stippled areas on the sucrose density gradient profiles of Fig. 14. The infectivity results of the isolated components are reported in Table 8. After two cycles (indicated by run 2 in the table) of density gradient centrifugation, incomplete separation was obtained as evidenced by the shoulders present on the sides of the main component peak. Infectivity results showed the greatest amount of infectivity resided in the middle component peak and the enhancement produced by mixing components as reported by Bockstahler and Kaesberg (17) was not very apparent. However, after three cycles of sucrose density gradient centrifugation infectivity studies showed that no
infectivity could be demonstrated in S, M and L alone, but that on mixture of these components infectivity was restored. The mixture of M and L produced fewer numbers of local lesions when the S component was added to them. The components were mixed in equal proportions so that the absolute amount of each component was the same in the mixtures.

It is concluded from these results that in order for BMV-RNA to be infectious and cause local lesions, both the middle and larger component RNA species are required and not just the large RNA molecule as has been suspected. If this is the case then both the middle and large RNA molecules carry different and essential pieces of genetic information for the virus and it would seem that BMV would require at least $1.7 \times 10^6$ daltons of RNA to be functional. It is known that each BMV particle carries only $1.0 \times 10^6$ daltons of nucleic acid (16, 56). It would appear that BMV infection could not be initiated by only one virus particle; rather two would be required, one carrying the L component and the other carrying the M and S components. It can also be seen from Table 8 that the addition of the S component in equal proportion to the M and L components appears to induce an inhibition of virus infection. The explanation for this may be the same phenomenon as was recently reported by Huang and Baltimore (47). They suggested that defective virus particles or small nucleic acids which arise as a result of infection in most animal viruses inhibit the replication of their parent viruses when they reach a high enough concentration to cause
interference. The high concentration of S component in the inoculum may represent an artificially high proportion of small nucleic acid molecules that could be inhibitory.
Figure 14.

Sucrose density gradient centrifugation profiles in a 5-20% sucrose gradient in 0.01 M Tris, 0.01 M KCl, 10^{-4} M MgCl2, pH 7.4 containing 0.01% polyvinyl sulfate after centrifugation at 40,000 rpm. Direction of sedimentation is to the left.

a) Unfractionated BMV-RNA centrifuged for 7 hours in the SW 40 rotor. The letters designate the positions of the small, middle and large RNA components.

b, c, d) Profiles of the RNA zones fractionated in (a) and centrifuged for 5 hours in the SW 65 rotor. Arrows indicate the estimated positions of the absent components and the stippled areas represent the portion which was collected and recentrifuged on a second set of gradients for infectivity assays.

b) Small (S) component fraction. (c) Middle (M) component fraction. (d) Large (L) component fraction.
Table 8. Results of infectivity assays of fractionated BMV-RNA tested on *Chenopodium amaranticolor* or *Chenopodium hybridum*.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Number of cycles of centrifugation</th>
<th>Assay host</th>
<th>Number of local lesions from fractionated zones indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td><em>amaranticolor</em></td>
<td>-- 60 5 14 4</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td><em>amaranticolor</em></td>
<td>0 42 6 2 24</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td><em>amaranticolor</em></td>
<td>0 0 0 22 7</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td><em>hybridum</em></td>
<td>0 39 11 29 46</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td><em>hybridum</em></td>
<td>0 0 0 49 2</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td><em>hybridum</em></td>
<td>0 351 151 414 523</td>
</tr>
</tbody>
</table>
DISCUSSION

The original aim of this work was to examine viral populations for possible interactions that might have occurred between two viruses as a result of mixed infection. The results presented suggest that BMV and BSMV do not interact in any significant manner when in mixed infection in barley.

The effects of possible viral interaction on the plant were studied by measuring the fresh weights of infected plant tissue. Healthy plants normally weighed more than virus infected plants. The result of virus infection caused the plant to gain weight less rapidly than it would have if it were not infected. The mixed infection caused a greater reduction in the growth of the barley plants than either single infection alone. This reduction in growth rate was shown to be the additive result that each virus infection could exert alone. Lal and Sill (59) reported that a mixed infection of BMV and BSMV caused a synergistic decrease in the growth rate of wheat plants. However, examination of their results revealed that this was not the case; in reality their results suggested that the reduction was additive. The
fact that these two viruses exerted their potential to reduce host growth additively, suggests that they acted on different parts of the plant or plant cell. It is concluded that these two viruses did not interact with respect to ability of inhibiting the growth rate of the plant.

In single infection viruses have a certain production potential and they produce a relatively constant amount of virus under a given set of conditions. In mixed infection this production potential may be altered in a manner which results in a decrease or increase in virus production depending on the combination of interacting viruses (10, 31, 37). In mixed infections of BMV and BSMV there did not appear to be any significant change in the concentration of either virus in comparison to singly infected plants. The comparisons of virus yield were done on a per plant basis rather than on the basis of an equal weight of tissue because singly infected plants weighed more than an equal number of doubly infected plants. It was important to compare the virus yield on the basis of an equal number of plants because at 10 days of infection both singly and doubly infected plants were in the same stage of maturity and both would have had equal numbers of replication sites. However, because the same amount of each virus was produced in single and mixed infections, it is argued that the sites of replication of both viruses must have been different or there would have been some competition for sites and utilization of common metabolic pools. The idea of separate replication sites is
supported by some evidence in the literature. Replication of the rod viruses has been associated with mitochondria (71), while replication of the small spherical viruses probably occurs in association with the cell chloroplasts (78, 88).

It is concluded that the reason for the lack of interaction between the BMV and BSMV replication systems in mixed infection is due to a physical isolation of the replication sites for the two viruses in the cell. It is because of these different replication sites that the plant was able to support, independently, the replication of both viruses without effect of one on the other.

The search for structural interactions between the two viruses also failed to reveal any significant indication that phenotypically mixed or genomically masked particles were produced. To explain the lack of interaction at this level three aspects of the problem must be considered: 1) the level of mixed cell infection; 2) structural incompatibility of the viral components; and 3) isolation of the sites of assembly of the viral components.

If two viruses are going to interact structurally to produce phenotypically mixed or genomically masked particles they must be in close proximity during the assembly process, that is, they must infect the same cell. It is unlikely that a lack of mixed infection in the same cell was a factor in preventing the interaction between BMV and BSMV. Little quantitative work has been done to assess the degree of multiple cell infection with different plant viruses (2, 36, 45, 46, 51) and no studies were made along these lines in
this study. However, Paliwal (68) has shown that BMV invades almost every host cell of the barley plant and because there is no reduction in the amount of BMV synthesized in the mixed infection it is likely it would invade most of the cells also infected with BSMV. If this assumption is correct, then it is unlikely that the lack of interaction between BMV and BSMV was due to isolation of the viruses in different cells.

The possibility that viral components may not be compatible with other foreign viral components has been discussed in the literature review. That BMV coat protein could associate with BSMV coat protein around one of the viral nucleic acids to form a stable phenotypically mixed virus particle is theoretically unlikely. Tremaine and Goldsack (85) suggest that small isometric viruses like BMV have protein subunits which are approximately 18Å in diameter and spherical, while the BSMV protein subunit is known to resemble a prolate ellipsoid 75Å by 25Å (6). It would be illogical to expect such structurally different, and certainly, chemically different protein molecules to polymerize together with equivalent or quasi-equivalent bonding (25) to form a stable particle. It is concluded that phenotypic mixing did not occur between these two viruses because of marked structural differences in the protein subunits of the two viruses.

The inability to demonstrate the production of genomically masked particles could not be explained by
structural incompatibility. Genomic masking would only be limited by incompatibility between the coat protein and a foreign nucleic acid and would not be subjected to the strict limitations imposed by protein-protein interactions involved in phenotypic mixing. Most of the evidence in the literature indicates that the small, isometric, virus coat protein can coat any foreign nucleic acid of the right dimensions to produce a stable infectious hybrid virion (43). Therefore, genomic masking of BS\textsubscript{MV}-RNA in B\textsubscript{MV} coat protein is theoretically possible. There is some evidence in the literature to suggest that rod virus coat protein can only successfully coat nucleic acids which are similar in their chemical nature and size to the parent nucleic acid (23, 64, 83). However, Atabekov et al. (6) have shown that BS\textsubscript{MV}-RNA could successfully be reconstituted with TMV coat protein to form stable, infectious rod-shaped particles. Reconstitution studies with BS\textsubscript{MV} coat protein and BS\textsubscript{MV}-RNA failed to produce stable infectious particles. It is suggested that this was not due to incompatibility, but more probably, unsatisfactory reconstitution procedures. The failure to produce stable infectious particles on reconstitution of BS\textsubscript{MV} protein with BS\textsubscript{MV} nucleic acid, or any other nucleic acid, supports this idea (6). It is concluded that incompatibility problems were not responsible for the lack of genomic masking.

The most likely explanation for the lack of genomic masking is the lack of physical interaction of the different viral components due to the physical separation of the
subcellular, protein-nucleic acid, assembly sites for Bl'IV and BShV. There is no direct evidence in the literature about these assembly sites, but some indirect evidence supports the idea. Ling et al. (61) alluded to the idea as a reason why genomically masked particles were not produced in doubly infected *Escherichia coli* cells with MS₂ and QB phage. They could produce the genomically masked particles "in vitro" but could not detect them "in vivo" as a result of the mixed infection and therefore concluded that isolation of subcellular sites of synthesis and assembly might be responsible for the lack of interaction. It is concluded that the same mechanism is responsible for the lack of structural interaction between Bl'IV and BShV.

Some evidence was presented that Bl'IV may be a multicomponent virus. Studies on the infectivity of fractionated bromegrass mosaic virus RNA suggest that none of the three nucleic acid molecules isolated from the virus particles were infectious alone. However, when the 1.0 x 10⁶ dalton and the 0.7 x 10⁶ dalton species were mixed, infectivity was restored. Now because each Bl'IV particle is known to contain only 1.0 x 10⁶ daltons of RNA (17), it follows that two particles of Bl'IV would be required to initiate infection. This is an interesting possibility because it implies that this virus carries its complement of genetic information in two separate but identical packages, unlike the previously reported cases of multicomponent viruses (54, 62, 63).

The idea that BShV might also be a multicomponent virus is much more difficult to prove. It is known that
3 modal lengths of BSMV rods exist (42), but their similarity in size does not permit easy separation for infectivity studies. It has been suggested by Gibbs (38) that BSMV may actually be present as two or more functional complements of RNA totaling $2.0 \times 10^6$ daltons like THV. If both of these viruses prove to be multicomponent systems, then the chance for detection of structural interactions like genomic masking would be reduced. This is because infectivity assays would require that two genomically masked particles, each carrying the correct complement of RNA, infect the same cell to initiate infection. Therefore, the multicomponent nature of the viruses would tend to decrease the sensitivity of the assay system and would make genomic masking harder to detect by the methods used in this study.

In conclusion, it is hypothesized that the reason for the lack of BHV-BSMV interaction is because these viruses are very different and unrelated. This unrelatedness is probably expressed in the mixed infection by divergent requirements from the host cell and by different life cycles which require different parts of the cell for nucleic acid replication and virus assembly. This topographical separation reduces the chance of encounter that would be required for functional or structural interaction. Moreover, structural and chemical differences between the viral components, which is another indication of their unrelatedness, probably prevent cross association of the protein components. It is for these reasons that BHV and BSMV failed to interact in mixed infection.
It was concluded that the lack of structural inter-
action was due to isolation of the different subcellular
sites of virus assembly.

It is hypothesized that the lack of interaction,
on all levels, expressed by ENV and BSMV is a reflection of
the degree of unrelatedness of the two.
SUMMARY

Studies on mixed infections of BMV and BSHV in barley failed to reveal any interaction between the two viruses. The decrease in growth rate of the doubly infected plant was the additive result of the decrease that each virus could exert alone. It was concluded that the detrimental effects of the viruses on the plant were exerted independently. Similarly, a doubly infected plant was shown to produce as much BMV and BSHV as the corresponding, singly infected plant. These results suggested that the replication systems of the two viruses acted independently. Attempts to isolate phenotypically mixed and genomically masked particles as an indication of structural interactions were unsuccessful. It was therefore concluded that the respective viral assembly systems did not interact. It is hypothesized that the lack of interaction expressed by BMV and BSHV in doubly infected plants is a reflection of the degree of unrelatedness of the two viruses.
LITERATURE CITED


protein from bacteriophage fd. III: Specificity 
of protein-DNA association "in vivo". J. Mol. 
Biol. 21: 305-312.

59. Lal, S.B. and W.H. Sill, Jr. 1959. Combination re-
actions of three small grain viruses on wheat. 
Phytopathology 49: 214-220.

of HeLa cells with polio viruses types 1 and 2. 
Virology 24: 207-209.

Independent assembly of QB and \( \text{NS}_2 \) phages in 
doubly infected \textit{Escherichia coli}. Virology 40: 
920-929.

and dependence in three related strains of tobacco 

63. Majorana, G. and H.L. Paul. 1969. The production of 
new types of symptoms by mixtures of different 
components of two strains of alfalfa mosaic virus. 
Virology 38: 145-151.

64. Matthews, R.E.F. and J.D. Hardie. 1966. Reconstitu-
tion of RNA from spherical viruses with tobacco 

two different plant viruses can multiply simultaneous-

identical phenotype but different genotype. 
Science 113: 34-35.

immunological analysis; in Progress in Allergy. 

68. Paliwal, Y.C. 1970. Electro microscopy of brome-
grass mosaic virus infected leaves. J. Ultra-
structural Res. 30: 491-502.

Chapter 14, pp. 391-425. Ed. K. Karamorosch and 
H. Kowprowski. Academic Press. N.Y.


STUDIES ON MIXED INFECTION IN BARLEY BY BROMEGRASS MOSAIC VIRUS AND BARLEY STRIPE MOSAIC VIRUS

by Thomas Jack Norris

Abstract

Mixed infections of barley with bromegrass mosaic virus (BMV) and barley stripe mosaic virus (BSMV) were studied in an attempt to detect interaction between the viruses. No effect of the replication of one virus on the concentration of the other was observed, nor was there evidence of phenotypic mixing or genomic masking. These results suggest that the viral replication and assembly systems do not interact. It is hypothesized that the lack of interaction is due to separate sites of synthesis and assembly of the respective viruses in the same host cell.

Master of Science Degree,
Department of Plant Pathology.