COMPARATIVE APHID AND MECHANICAL TRANSMISSIBILITY OF BEAN YELLOW MOSAIC VIRUS ISOLATES

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INTRODUCTION

The flexuous rod viruses affecting legumes assignable to the "potato virus Y" (PVY) group of Brandes and Bercks (14) are numerous, and considerable confusion exists regarding their identities and relationships to one another. Indeed, many of these viruses have distinctive properties that have profound influences on their potentials as plant pathogens, and the control of these legume viruses in cultivated crops may depend on one's ability to identify them. Compounding the confusion is the fact that certain properties of different isolates of a given virus may differ significantly from one another. This study was designed primarily to assess isolate variability, using different bean yellow mosaic virus (BYMV) isolates and emphasizing relative aphid and mechanical transmissibilities.

REVIEW OF LITERATURE

Bos (10) has suggested that all legume viruses of the PVY group are related in one way or another to BYMV. Indeed, in an attempt to clarify the confusion that exists in the literature regarding the identification of legume viurses, Bos et al. (12) suggested definite procedures to be used in their international identification. Pierce (51) was the first to accurately describe and distinguish BYMV from bean common mosaic virus (BCMV), both of which induce mosaic symptoms in bean. His differentiation was based on symptomatology, varietal susceptibility, host range and seed transmissibility; physical property studies, however, failed to show significant differences between these viruses. Although Grogan and Walker (27) suggested that a relation existed between BYMV and BCMV based on cross protection studies, Bos (10) in a later study of 3 legume viruses, including BYMV, considered evidence of cross protection not to be particularly meaningful in establishing mutual relationships. Evidence from serological tests has also given conflicting results as to the relatedness of BYMV to other similar, but distinct, viruses such as BCMV. Kahn et al. (38), for example, indicated that BYMV and BCMV were unrelated serologically, contrary to the results of Bercks (8) and

Beemster and Van der Want (7). Further contradiction surrounding the identity of BYMV is apparent when the information on the distinction between the BYMV/Pea mosaic virus (PMV) complex is reviewed. Goodchild (25) distinguished BYMV from PMV isolates and from each other on the basis of a single difference in host range. Hull (34) also distinguished BYMV from PMV and considered them distinct primarily on the basis of differences in host range, although he admitted that the symptoms of these viruses on mutually susceptible hosts were almost identical. Taylor and Smith (67) also distinguished BYMV from PMV isolates mainly on the basis of symptomatology, but concluded that their PMV isolates should be regarded as strains of BYMV. Schroeder and Provvidenti (57) likewise concluded that PMV isolates were only strains of BYMV.

It appears, therefore, that no 2 isolates of BYMV are identical; each may differ from another in properties such as host range, symptomatology, physical properties, virus-induced inclusion bodies and aphid transmissibility (10, 17, 18, 25, 28, 47, 62, 65). Taylor and Smith (67), for example, have shown that isolates of BYMV can vary in particle length and that this property is therefore of questionable value in establishing strain relationships. Loss of

aphid transmissibility of some BYMV isolates was shown by Swenson (62) and Swenson et al. (65). Other stylet-borne viruses have been reported as having lost their ability to be aphid transmitted (3, 31, 36, 63). Swenson (62) showed that a nonaphid-transmissible isolate of BYMV, when compared in a dilution series on bean with an aphid-transmissible isolate, consistently infected about 10 to 20% more bean plants at each dilution. According to Steere (61), who based his information on tobacco mosaic virus, this method of assay is subject to a 20 - 50% error in the estimation of virus titer.

The role of the host in affecting the properties of BYMV has received relatively little study. Adlerz (1) showed broadbean to be a consistently better source of BYMV for aphid transmission than pea, bean or clover. Similarly, Corbett (18) found differences among lupine species as sources of BYMV in aphid-transmission studies. Swenson et al. (65) showed that, among broadbean plants infected with the same BYMV isolate, plants with severe symptoms were better sources of virus for aphids than plants with mild symptoms. He also correlated this symptom expression with greater numbers of local lesions induced on Chenopodium amaranticolor Coste and Reyn. in mechanical assays of the BYMV infected plants. Normal particle length determinations

are also affected by host related factors. Taylor and Smith (67) found BYMV particle length to vary according to the host plant; from legumes their isolate was 742 - 756 mμ in length whereas from C. amaranticolor particles were 794 - 800 mμ.

MATERIALS AND METHODS

Three isolates of BYMV were used in this study: a Florida isolate (FV) collected from naturally infected plants of Crotalaria mucronata Desv., a Wisconsin isolate (WV) obtained from Dr. D. J. Hagedorn at the University of Wisconsin, Madison, and a Kentucky isolate (KV) obtained from Dr. S. Diachun at the University of Kentucky, Lexington. The isolate of bean common mosaic virus (BCMV) was obtained from Dr. F. W. Zettler at the University of Florida, Gainesville.

All 3 isolates of BYMV used in the comparative study were maintained concurrently in pea (Pisum sativum L. 'Alaska') and bean (Phaseolus vulgaris L. 'Red Kidney'). The viruses were kept in separate greenhouses to reduce the possibility of contamination of one isolate with another. Each isolate was routinely transferred from infected pea plants to recently emerged healthy pea and bean seedlings every 2 to 3 weeks. No evidence of contamination was observed in the 2 year study involving these isolates.

Mechanical Inoculations

Host Range Studies

All host range studies were conducted during the spring months in a greenhouse with a temperature range of 15 to 30° C. Mechanical inoculations were made by dusting test plant species with 600-mesh Carborundum and abrading the leaves with a sterile cheesecloth pad dipped into tap-water diluted, Carborundum-dusted juice from infected pea or bean plants. A minimum of ten individuals of each test plant species was inoculated. Noninoculated test plants were placed in the same greenhouse in all trials as controls.

Symptom expression in most susceptible species became apparent between 5 and 10 days after inoculation. After 3 weeks, all inoculated test plant species, irrespective of symptom expression, were checked for virus infection by mechanical inoculation onto seedlings of 'Red Kidney' bean and cowpea, Vigna sinensis (L.) Endl. 'Early Ramshorn Black-eye'. Test plant species that failed to become infected with BYMV but were reported by others as being susceptible were retained for up to 6 weeks and rechecked for susceptibility by back inoculating to bean test plants.

Physical Property Studies

The determination of the thermal inactivation point, longevity in vitro and dilution end point of the FV and WV was carried out by using crude juice from systemically infected 'Red Kidney' bean seedlings according to the procedures suggested by Ross (56). The thermal inactivation point was determined by pipetting 2-ml samples of crude juice into thin walled 5-ml serological tubes. These tubes were then immersed in a constant-temperature water bath for 10 minutes either at 50° or 60° C. The heated juice was cooled immediately in cold running water and subsequently mechanically inoculated onto replicates of 10 healthy 'Red Kidney' bean seedlings. In determining the longevity in vitro of each isolate, crude juice was maintained at 24° C in stoppered flasks and inoculated at 12-hour intervals up to 96 hours onto groups of 10 healthy 'Red Kidney' bean seedlings. This procedure was repeated 3 times. The dilution end point determination was carried out by diluting the crude sap in distilled water at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} using replicates of 50 'Red Kidney' bean seedlings for the assay of each dilution.

Local Lesion Assays

Chenopodium amaranticolor Coste and Reyn., a local lesion host of BYMV (32), was used to compare the mechanical infectivity of the FV, WV and KV isolates. Crude juice expressed from virus infected pea and bean plants diluted 1/10 in distilled water was used in all but 2 of the earlier local lesion assays on C. amaranticolor. Undiluted juice from pea or bean infected with either isolate in infectivity trials resulted in much lower and more erratic lesion numbers than dilutions of this juice. In the inoculation procedure, the 5 youngest fully expanded leaves of C. amaranticolor were inoculated with as uniform a rubbing procedure as could be maintained in all trials. Fresh inoculum and a new sterile cheesecloth pad dusted with 600-mesh Carborundum was used for each inoculated plant; this was done in order to minimize possible buildup of virus inhibitors on the cheesecloth pads (19, 45, 68).

The WV and KV both induced clearly discernible necrotic lesions on inoculated leaves of C. amaranticolor, whereas the FV induced indistinct chlorotic lesions of varying intensity. Frequently the WV induced so many lesions on inoculated leaves of C. amaranticolor as to cause collapse and rapid dehydration of the inoculated leaves before conditions for

the counting of lesions were optimum. In order to circumvent these drawbacks, a starch-iodine technique was used (6, 33) for counting local lesions.

Preparatory to starch-iodine accentuation of the expected local lesions, plants were placed 4 days after inoculation for 18 hours in a completely dark chamber maintained at 21° C. To allow for complete chlorophyll removal, the inoculated leaves were excised and immersed in 95% ethanol for at least 2 days at approximately 24° C. Subsequent to dechlorophyllization, the leaves were placed in a 2% aqueous iodine/potassium iodide (IKI) solution, thus staining the starch delineated local lesions and greatly facilitating lesion assays. The lesions after staining in IKI typically were darkly delineated circles frequently surrounding another smaller circle or dot. Such lesions resulted only after inoculation with BYMV-infected pea or bean tissue but never after inoculation with similarly treated noninfected pea or bean tissue.

Aphid Transmissions

The aphid species used in this study were reared on virus free plants in cages kept in a growth chamber maintained at around 24° C. Listed with the host plants on which they were reared, they were as follows: 1) the pea

aphid, Acyrthosiphon pisum (Harris), reared on broadbean, Vicia faba L. 'Longpod'; 2) the melon aphid, Aphis gossypii Glover, reared on celeriac, Apium graveolens rapaceum DC 'Giant Prague'; 3) the cowpea aphid, A. craccivora Koch, reared on 'Longpod' broadbean; 4) the spirea aphid, A. spiraecola Patch reared on 'Giant Prague' celeriac; 5) the turnip aphid, Hyadaphis pseudobrassicae (Davis) reared on mustard, Brassica juncea Coss. 'Florida Broadleaf'; and 6) the green peach aphid, Myzus persicae (Sulzer) reared on tobacco, Nicotiana tabacum L. 'Samsun NN'. The identities of all the above aphid species were confirmed by Miss Louise M. Russell, U.S.D.A., Entomology Research Division at Washington, D.C., and by Dr. A. N. Tissot, Department of Entomology, University of Florida at Gainesville.

In trials with aphids allowed single acquisition probes, vigorous nonviruliferous aphids were starved 4 to 6 hours before being transferred individually with a camel-hair brush to the virus source. Each aphid was observed through a binocular microscope as it made a timed probe on the virus source. An aphid was considered to have commenced probing when its labium was lowered into contact with the leaf surface and its antennae were arched backwards over the body. Aphids that voluntarily terminated their probes were transferred by brush (one aphid per plant) to a healthy test

plant. In no instance was an aphid allowed to make a probe exceeding 1 minute in duration; aphids probing at that time were interupted with the camel-hair brush and transferred to a healthy test plant. Aphid probes were limited to 60 seconds in duration in order to maximize transmission probabilities, during which aphid stylets have penetrated epidermal and possibly mesophyll tissue as well (48, 66, 78). 'Red Kidney' bean plants used as virus sources for aphids were mechanically inoculated with virus within 2 days after emergence from seedling pots,and the second almost fully expanded trifoliolate leaves to develop after inoculation were used as virus sources. When 'Alaska' pea was used, the virus source was the second fully expanded leaf to form above the leaves mechanically inoculated with BYMV. In both pea and bean, virus source leaves were generally available for aphids about 15 to 20 days after mechanical inoculation.

A more convenient "mass transfer" method was used to study the comparative aphid transmissibility of the 3 BYMV isolates from pea, bean and other hosts. This technique consisted of starving nonviruliferous aphids for 4 hours and transferring them collectively to virus sources in groups of 100 or more individuals. After the initial access period of 3 to 12 minutes, 50 aphids that appeared to be probing were

removed individually and transferred singly (1 aphid per plant) to healthy test plants. Approximately 9 minutes were required to transfer 50 aphids individually from the virus source to test seedlings. The virus source tissue for this technique consisted of the entire pea or bean shoot showing BYMV induced symptoms that formed subsequent to mechanical inoculation. Comparably infected tissue was used when C. amaranticolor, C. mucronata and white sweet clover (Melilotus alba L.) were tested as virus sources for aphids.

In all studies involving aphids, test plants were 'Red Kidney' bean seedlings used within 2 days after emergence from seedling pots. Aphids were allowed test feedings of at least 12 hours following virus access periods after which they were killed by spraying with malathion. Noninoculated 'Red Kidney' bean seedlings were used as controls in all studies and never numbered less than half the number of test seedlings used.

Light Microscopy

Stained epidermal strips taken from the leaves of healthy and virus infected plants were examined using an optical microscope for the presence of virus-induced inclusions. These epidermal strips were prepared for microscopy by a method described by Christie (16) which included staining the epidermal strips in calcomine orange and

"luxol" brilliant green, rinsing them briefly in 95% ethyl alcohol and mounting them in Euparal on a glass slide. The material thus treated was examined at a maximum magnification of 940X.

Electron Microscopy

All material was examined using a Philips model EM 200 electron microscope. Speciment grids were coated with Formvar and strengthened by arc-vaporized carbon (50).

Size determinations were obtained by comparing projected electron micrographs of selected material and leaf extracts with projected micrographs of a 54,864 line/inch diffraction grating.

Sectioned Material

Preparatory to *in situ* investigations, sections 2 x 3 mm were cut out of FV, WV and noninoculated leaves of 'Red Kidney' bean, 'Alaska' pea, crimson clover (*Trifolium incarnatum* L.), white sweet clover and *C. amaranticolor*. The techniques used in preparing tissue for ultrathin sectioning were modified from those used by previous workers (7, 35, 46, 72). The tissue pieces were fixed for 10 minutes at 25° C. under tap-water induced vacuum in a solution containing 6.5% glutaraldehyde buffered in 0.1 M phosphate at pH 6.8. The pieces were then rinsed in a 0.1 M phosphate buffer

solution at pH 6.8 and postfixed for 3 hours at 4° C in a 1.0% OsO_4 solution buffered in 0.1 M phosphate at pH 6.8. The tissue was subsequently stained overnight in a 0.5% uranyl acetate solution at 4° C. The tissue pieces were then progressively dehydrated in a series of increasing concentrations of ethanol. After 30 minutes in absolute ethanol, the specimens were placed into propylene oxide, which was changed 3 times over a period of 2 hours. The final embedding of the tissue pieces was in a 65:33:2 mixture of Maraglas plastic, Cardolite and benzyldimethylamine, respectively. This material was then allowed to harden at least 48 hours in an oven at 60° C.

Sections of the embedded tissue were made with a diamond knife mounted on a Sorvall model MT-1 ultramicrotome. They were transferred to specimen grids and stained for 1 hour in 1% uranyl acetate. They were then stained for 15 minutes in lead citrate, after which the specimens were rinsed in distilled water and examined with the electron microscope.

Leaf Extracts

All specimen grids divulging particle morphology were obtained from negatively stained leaf dip preparations. Mounts were prepared by cutting up small pieces of infected or healthy leaf tissue in a few drops of 1% phosphotungstic

acid (PTA) neutralized to pH 6.8 with KOH and containing 0.025% bovine serum albumen. A drop of the resulting liquid was then placed with a pipette on a Formvar coated specimen grid. After 30 seconds, excess fluid was absorbed with filter paper after which the grid was examined with the electron microscope.

The same technique was used in studying virus-induced striated inclusions found in leaf extracts, except that the PTA stain was substituted with a 1.0% ammonium molybdate stain adjusted to pH 6.95 and containing 0.025% bovine serum albumen. Although PTA has proved effective in enhancing virus particle contrast for electron microscopy, ammonium molybdate was selected when studying virus-induced inclusions on the basis of a study by Purcifull (55), who showed the latter stain to be more effective in preserving the integrity of striated inclusions.

An attempt was made to determine whether virus particle counts from leaf extracts corresponded with assays based on mechanical means or with assays based on aphid transmissibility. In this study, aphid transmissibility was determined by selecting 8 comparable WV and 8 FV infected 'Alaska' pea leaves (each with 4 leaflets) as virus sources for aphids. Each leaflet served as a virus source for 5 individuals of

A. craccivora permitted 3-minute access periods prior to test feeding on 'Red Kidney' bean seedlings. Virus particle numbers were assayed by 1) excising a single disc of tissue from each of these same above leaflets with w #1 (3 mm diameter) cork borer, 2) quartering each disc with a razor blade and 3) placing the discs together (4 discs per leaf) in 3 drops of 1% PTA for 2 minutes and stirring them thoroughly with a pipette. After stirring, a drop of this material was applied to a 400-mesh Formvar coated copper specimen grid and left for 30 seconds before removing the excess liquid with filter paper. All particles encountered in a fixed number of grid squares in comparable pre-selected zones on each grid were counted and recorded.

The grid squares selected for examination were located in each 400-mesh specimen grid as follows: each grid was arbitrarily subdivided into 4 equal quadrants from the grid center, and 9 grid squares centrally located in each quadrant were selected for observation; none of the selected grid squares were closer than 0.30 mm nor farther than 0.70 mm from the center of each grid. Mechanical infectivity was determined by taking the same leaflets from which discs had been removed, triturating them in 2-ml of distilled water, mechanically applying the resulting juice to 10 selected

leaves of C. amaranticolor and accentuating and recording the resulting local lesions as described previously.

RESULTS

Isolate Identification

The verification of FV, KV, and WV as isolates of the same virus, BYMV, was effected by using the following criteria in comparative studies: 1) host range and symptomatology, 2) the susceptibility of known BYMV resistant and susceptible pea varieties, 3) physical property determinations, 4) virus-vector relationships, 5) optical microscopy of stained epidermal tissues and 6) electron microscopy of negatively stained leaf extracts and ultrathin sections.

Host Range, Symptomatology and Varietal Susceptibility

A list of the plants inoculated with the FV, KV, and WV together with the resulting reactions induced by each isolate are given in Table 1. With the exception of the 2 Chenopodium species tested, all susceptible plants were in the family Leguminosae. The host range and symptom expression of the 3 isolates are in close agreement with the reports of others for BYMV (18, 25, 28, 32). Moreover, the resistance of 'Little Marvel' and 'Midway' pea to infection by all 3 isolates and the uniform susceptibility of the

other tested pea varieties are in complete agreement with the reports of Corbett (18) and Ford (23) for the differential susceptibility of these pea varieties to BYMV.

Symptoms induced by all 3 isolates were similar, except that the FV usually induced less severe symptoms in inoculated plants than either the KV or WV (Figs. 1 and 2). Although all 3 isolates induced both local and systemic symptoms in C. amaranticolor, noticeable differences were observed in systemic movement of each of the viruses in this plant. Even though local symptoms induced by the KV and WV were much more intense than those induced by the FV, neither virus moved systemically as readily as the FV (Fig. 2 A). Systemic symptoms typical of FV infection were a pronounced and persistent general mottle accompanied by a leaf distortion and, over a period of time, a stunting of infected plants. The WV induced prominent necrotic systemic lesions that frequently caused a pronounced leaf distortion, and occasionally death of the growing tip; as new leaves developed, however, lesion number decreased progressively until no lesions occurred in succeeding leaves. Back inoculations from such symptomless leaves, using healthy C. amaranticolor as test plants, never resulted in infection, thereby indicating an absence of virus from symptomless tissue.

Table 1. Susceptibility of plants mechanically inoculated with the Florida (FV), Kentucky (KV) and Wisconsin (WV) isolates of bean yellow mosaic virus.

Test plants	Test plant susceptibility to virus[a]		
	FV	KV	WV
Chenopodium amaranticolor Coste & Reyn.	LS	LS	LS
C. quinoa Willd.	L	L	L
Crotalaria mucronata Desv.	S	S	LS
C. spectabilis Roth.	-	S	LS
Pumpkin, Cucurbita pepo L. 'Small Sugar'	-		-
Cucumber, Cucumis sativus L. 'A. & C.'	-		-
Globe amaranth, Gomphrena globosa L.	-		-
Blue lupine, Lupinus angustifolius L.	SN	SN	SN
Yellow lupine, L. luteus L.	S	S	S
White sweet clover, Melilotus alba Desr.	S		S
N. clevelandii Gray x N. glutinosa L. 'Christie'[b]	-	-	-
Wild tobacco, N. rustica L.	-	-	-
Tobacco, N. tabacum L. 'Samsun NN'	-		-
Bean, Phaseolus vulgaris L. 'Great Northern U.I. 31'	-		L

Table 1. Continued.

Test plants	FV	KV	WV
Bean, *Phaseolus vulgaris* L. 'Great Northern U.I. 59'	S		S
'McCaslan 42'	S		SN
'Michelite 62'	S		S
'Perry Marrow'	S		S
'Dark Red Kidney'	S	S	S
'Light Red Kidney'	S	S	S
Pea, *Pisum sativum* L. 'Alaska'	S	S	S
'Midfreezer'	S	S	S
'Ranger'	S		S
'Thomas Laxton'	S		S
'Little Marvel'	-	-	-
'Midway'	-	-	-
Crimson clover, *Trifolium incarnatum* L.	S	S	S
White clover, *T. repens* L.	-		-
Garden nasturtium *Tropaeolum majus* L.	-		-
Broadbean, *Vicia faba* L. 'Longpod'	S	S	S
Cowpea, *Vigna sinensis* (L.) Endl.			
'Early Ramshorn Blackeye'	-	-	-
'Black Local'	-	-	-

Table 1. Continued.

Test plants	FV	KV	WV
Cowpea, *Vigna sinensis* (L.) Endl.			
'Alabama Giant'	-	-	-

[a] S = systemic infection; L = local symptoms; N = lethal necrosis; - = no infection. All results were checked by back inoculations to 'Red Kidney' bean and 'Early Ramshorn Blackeye' cowpea.

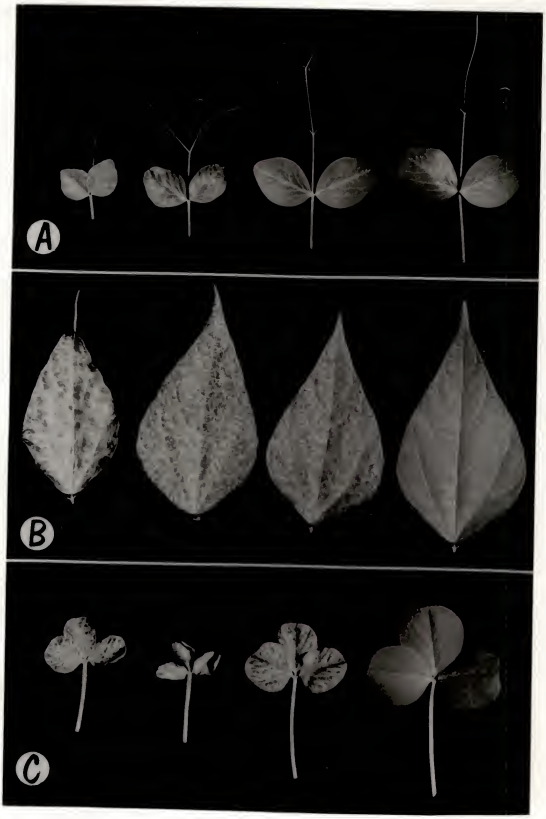
[b] Hybrid developed by S. R. Christie, Plant Pathology Department, University of Florìda, Gainesville.

Figure 1. Leaves of pea, bean and crimson clover systemically infected by each of the three virus isolates.

A. Right to left, healthy leaf and Florida isolate, Kentucky isolate and Wisconsin isolate infected leaves of pea.

B. Right to left, healthy leaf and Florida isolate, Kentucky isolate and Wisconsin isolate infected leaves of bean.

C. Right to left, healthy leaf and Florida isolate, Kentucky isolate and Wisconsin isolate infected leaves of crimson clover.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B
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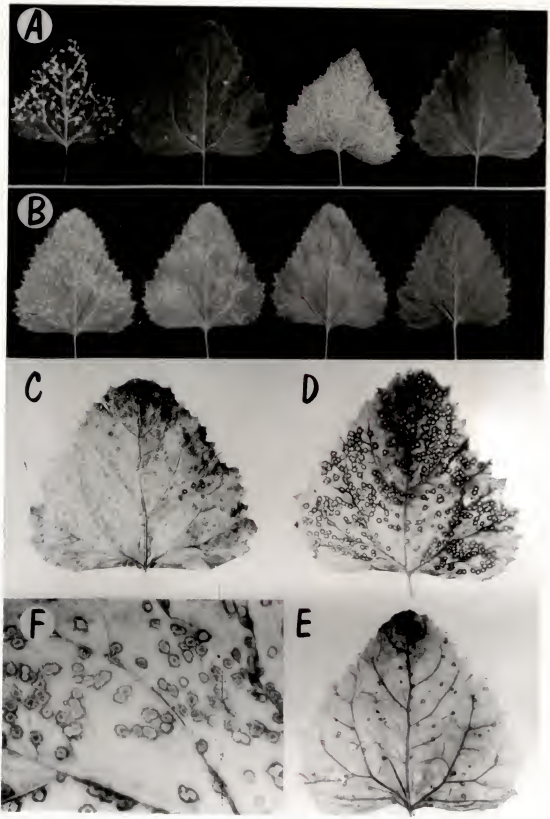
Figure 2. Local and systemic symptoms and starch delineated local lesions on Chenopodium amaranticolor induced by each of the 3 isolates of bean yellow mosaic virus.

A. Right to left, healthy leaf and systemic symptoms of Florida isolate, Kentucky isolate and Wisconsin isolate, respectively in C. amaranticolor.

B. Right to left, healthy leaf and local symptoms on mechanically inoculated leaves of C. amaranticolor induced by Florida isolate, Kentucky isolate and Wisconsin isolate, respectively.

C, D, and E. Local lesions induced by Florida isolate, Wisconsin isolate and Kentucky isolate, respectively on C. amaranticolor accentuated by starch/iodine treatment.

F. Close-up of 'typical' starch/iodine accentuated lesions induced by one of the bean yellow mosaic virus isolates on C. amaranticolor.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Systemic symptoms induced by the KV in C. amaranticolor were similar to those described for the WV except that KV moved systemically even less readily than WV.

Attempts were made to recover virus from all inoculated plants listed in Table 1 by back inoculation to seedlings of 'Red Kidney' bean and 'Early Ramshorn Blackeye' cowpea. With the exception of C. amaranticolor and C. quinoa, virus was recovered in bean seedlings from all plants expressing symptoms. Repeated attempts to recover each of the 3 isolates of BYMV from C. amaranticolor and C. quinoa when inoculating seedlings of 'Red Kidney' bean and 'Alaska' pea resulted in failure; a single exception was noted when 1/10 tap-water diluted juice from KV infected plants of C. amaranticolor infected an 'Alaska' pea seedling. Similar difficulties in recovering virus from C. amaranticolor have been noted by others (19, 45, 68). Milne et al. (45) were able to recover watermelon mosaic virus 2 from local lesions of C. amaranticolor only after layering centrifuged juice into an agarose column previously equilibrated with phosphate buffer and assaying the resulting eluant in 1-ml fractions for virus infectivity. In this study, recovery of virus from C. amaranticolor and C. quinoa was consistently effected only when plants of C. amaranticolor were used in

back inoculations. That C. amaranticolor was virus infected was further substantiated by the detection of virus particles typical of BYMV in negatively stained leaf extracts from FV, KV and WV infected C. amaranticolor leaves.

Although cowpeas have been reported as being susceptible to BYMV (41), none of the 3 isolates of BYMV used in this study was found to infect the 3 cowpea varieties tested in Table 1. Moreover, even though 'Red Kidney' bean seedlings used in recovery tests always became infected when inoculated with juice from plants with BYMV symptoms (with the exception of the 2 Chenopodium species as noted above), in no instance did 'Early Ramshorn Blackeye' cowpea seedlings used in the same tests ever become infected.

Physical Property Studies

The FV and WV had similar physical properties, which are in agreement with those known for BYMV (18, 60). Both isolates were infectious after 10 minutes at 50° C but not at 60° C. The FV was infectious after 24 hours at around 24° C (7 out of 50 bean seedlings infected), but not after 36 hours; the WV infected 9 of 50 bean seedlings after 48 hours, but infectivity was lost after 60 hours. Both viruses were infectious at a 10^{-2} dilution of crude juice (1 of 50 seedlings infected with FV and 9 of 50 seedlings

infected with WV); neither virus was infectious at a 10^{-3} dilution.

Aphid Transmissibility

The FV and KV, but not the WV, were transmitted by aphids allowed single acquisition probes on infected pea leaves. In preliminary trials, individuals of A. craccivora transmitted KV to 14 out of 50 inoculated 'Red Kidney' bean seedlings. In similar trials, transmission of the FV by individuals of different aphid species was as follows: A. pisum, 3 of 9; A. craccivora, 5 of 9; A. gossypii, 1 of 9; A. spiraecola, 1 of 9; H. psuedobrassicae, 0 of 9; and M. persicae, 4 of 9. Results in tests with the WV were as follows: A. pisum, 0 of 9; A. craccivora, 0 of 9; A. gossypii, 0 of 9; A. spiraecola, 0 of 9; H. psuedobrassicae, 0 of 9; and M. persicae, 0 of 9.

Transmission of the KV and FV resulted from single acquisition probes as brief as 15 seconds. This indicates that these isolates are aphid transmissible in a stylet-borne manner as is typical of BYMV (40). The lack of aphid transmission of the WV corroborates earlier reports of the nonaphid transmissibility of certain BYMV isolates (62, 65).

Light Microscopy

Stained epidermal strips taken from plants of several

different species infected with FV or WV revealed the presence of prominent amorphous cytoplasmic inclusion bodies such as those reported by Bos (10, 11) for BYMV (Fig. 3). In pea, cytoplasmic inclusions were always readily apparent despite the relatively inconspicuous systemic foliar symptoms typical of FV and WV infected peas. In bean, however, no inclusions were ever detected, even though epidermal strips were removed from leaves with pronounced and typical BYMV symptoms. Amorphous cytoplasmic inclusions were detected in bean only when epidermal strips were removed from leaves of plants singly infected with BCMV or from plants doubly infected with either BCMV and FV or BCMV and WV.

Neither nuclear nor nucleolar crystals reportedly characteristic of infection with certain BYMV isolates were apparent in stained epidermal strips taken from pea, bean, crimson clover, yellow lupine and broadbean infected with the FV or WV (44, 47).

Electron Microscopy

Sectioned Material: Ultrathin sections of FV or WV infected pea, bean, broadbean, white sweet clover and crimson clover leaf tissue revealed the presence of "cylindrical inclusions" (21) in the cytoplasm of epidermal and mesophyll tissues. These inclusions resembled those reported by

Figure 3. Stained amorphous cytoplasmic inclusions induced by the Florida isolate and Wisconsin isolate in the epidermal cells of several plant species.

A and B. Stained epidermal cells from healthy pea and bean, respectively. Note discrete nuclei. Scale line = 0.02 mm.

C and F. Amorphous cytoplasmic (i) inclusions induced by Florida isolate in epidermal cells of pea and yellow lupine, respectively.

D and G. Amorphous cytoplasmic (i) inclusions induced by Wisconsin isolate in epidermal cells of pea and broadbean, respectively.

E. Note the absence of cytoplasmic inclusions in Wisconsin isolate infected bean cell.

H. Cytoplasmic inclusions (i) induced in epidermal cell of bean by bean common mosaic virus infection.

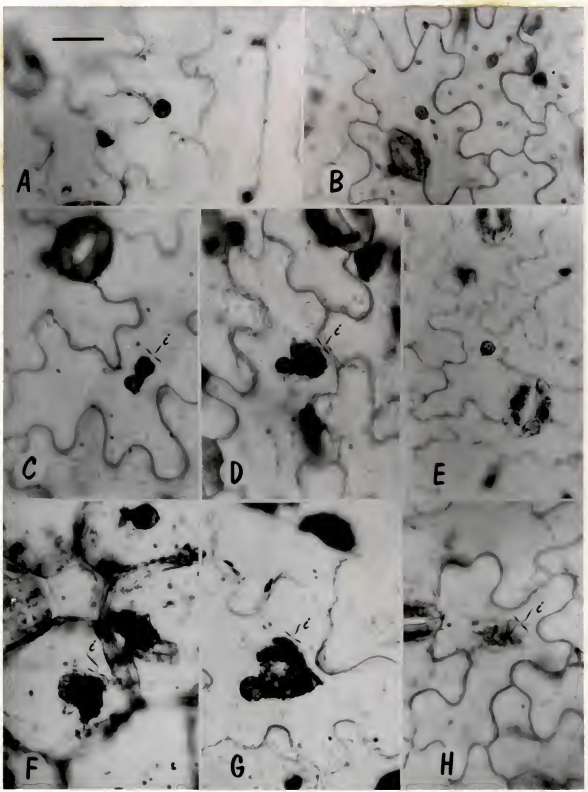
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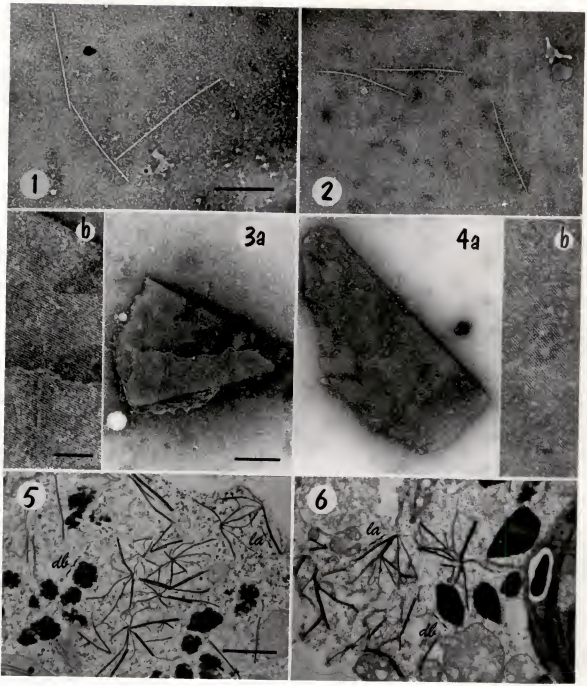
Figure 4. Electron micrographs of negatively stained leaf extracts from plants infected with Florida isolate and Wisconsin isolate, and thin sections of Florida isolate and Wisconsin isolate infected pea leaves.

1 and 2. Negatively (phosphotungstate) stained filamentous particles from leaf extracts of Florida isolate and Wisconsin isolate infected pea, respectively. Scale line = 0.5μ.

3a and 4a. Flattened striated structures from negatively (molybdate) stained leaf extracts of Florida isolate and Wisconsin isolate infected pea, respectively. Scale line = 0.25μ. Insets 3b and 4b of the striated structures showing the regular 5 mμ periodicity for both Florida isolate and Wisconsin isolate. Scale line = 0.1μ.

5 and 6. Ultrathin sections of fixed stained cell areas from Florida isolate and Wisconsin isolate infected pea leaves. Note dense bodies (db) and laminated aggregates (la) presumed substructures of the inclusions in Fig. 3. Scale line = 1μ.


1
2
b
3a
4a
b
5
db
la
6
la
db

others (20, 39, 73, 74) for BYMV. These inclusions, presumably substructures of the amorphous cytoplasmic inclusions apparent in epidermal strips examined with the light microscope (21), consisted of "laminated aggregates," "pinwheels" and "bundles" as defined by Edwardson et al. (21); neither "tubes" nor "circular inclusions" were ever observed in FV or WV infected tissue, although such structures were abundant in BCMV infected bean tissue.

In addition to cylindrical inclusions, electron dense bodies (db) were seen in FV and WV infected tissues (Fig. 4). These were of indeterminate morphology and were similar to the "virus crystals" reported by Weintraub and Ragetli (73, 74) and the "dense bodies" by Kamei et al. (39) for BYMV. Such structures were never seen in BCMV infected tissues.

Inclusions were never observed in sections from non-inoculated control plants.

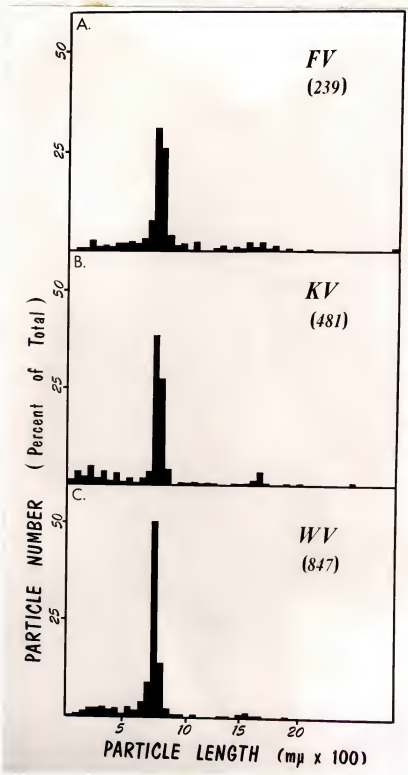
It was noted earlier that amorphous cytoplasmic inclusions were apparently absent in FV or WV infected bean epidermis as resolved with the light microscope. Examination of such tissue with the electron microscope, however, showed that amorphous virus-induced inclusions do indeed occur in bean epidermis but cannot be distinguished from other cell constituents with the light microscope because of

their relatively small size in bean.

Leaf Extracts: Electron microscopic examination of negatively stained leaf dip preparations from 'Red Kidney' bean, 'Alaska' pea, 'Longpod' broadbean, crimson clover, and C. amaranticolor infected with the FV, KV or WV revealed the presence of filamentous rods (Fig. 4) such as those reported by others for BYMV (5, 14, 15, 67). The main maxima of particle lengths in the case of each isolate in 'Alaska' pea were between 670 and 890 mμ. The percentage of each isolate falling into this category, excluding apparent diamers and trimers, was 70% of 239 particles for FV, 72% of 847 particles for KV, and 78% of 517 particles for WV (Fig. 5). The arithmetic mean length of FV, KV and WV particles lying between 670 and 890 mμ was 796, 802 and 781 mμ, respectively. These lengths are in general agreement with other reports for BYMV (5, 14, 15, 67).

Negatively stained molybdate leaf dip preparations from 'Alaska' pea leaves systemically infected with FV or WV revealed, in addition to cell organelle remnants and virus particles, the presence of flattened striated structures (Fig. 4). These were generally somewhat triangular in outline and had a striation periodicity of approximately 5 mμ for both isolates. These structures are similar to

Figure 5. Histograms showing the distribution of particle lengths of each of the three isolates of bean yellow mosaic virus in relation to particle length and number of particles (expressed in percent).


A.
FV
(239)
B.
KV
(481)
C.
WV
(847)
50
25
PARTICLE NUMBER (Percent of Total)
5
10
15
20
PARTICLE LENGTH (mμ x 100)

the "plates" described by Edwardson et al. (21) for tobacco etch virus; and, based on the studies of Edwardson et al. (21) and Purcifull and Edwardson (54), they are assumed to be equivalent to the cylindrical inclusions found in ultra-thin sections of FV or WV infected 'Alaska' pea leaves.

Neither particles nor striated inclusions were observed in extracts from noninoculated control plants.

Pea Compared With Bean as a Virus Source

Even though, as noted previously, mosaic symptoms of each of the 3 BYMV isolates were somewhat less pronounced in pea than bean, pea proved to be a much better virus source than bean based on aphid and mechanical transmissibility.

Mechanical Transmissibility

In preliminary trials, pea proved a better virus source than bean when assayed onto leaves of 'Red Kidney' bean seedlings. Crude pea and bean juice resulted in 47/50 and 19/50 infected test plants, respectively; similarly, pea and bean juice diluted to 1/10 with distilled water resulted in 29/50 and 7/50 infected plants, respectively.

Likewise, comparison of lesion numbers (Table 2) resulting from inoculating leaves of C. amaranticolor with 1 in 10 dilutions of pea and bean juice infected with the

Table 2. Comparative mechanical infectivity of 3 bean yellow mosaic virus isolates (Florida isolate, Kentucky isolate, Wisconsin isolate) from 'Alaska' pea and 'Red Kidney' bean.[a]

Experiment no.	No. of leaves inoculated per treatment	BYMV isolate FV pea	FV bean	KV pea	KV bean	WV pea	WV bean
I	25	27[b]	6			845	46
II	25	74	9			741	21
III	100	49		65		309	
IV	50	71	6	147	11	689	48
V	50	51	11	463	86	706	83

[a] Infectivity based on lecal lesion assays of inoculated and starch-iodine stained leaves of C. amaranticolor.

[b] Average number of lesions per inoculated leaf.

FV, KV or WV showed pea to be a significantly greater virus source than bean ($P = < .01$, $< .01$, and $< .01$) for the FV, KV and WV, respectively.

Aphid Transmissibility

Aphid transmission of the FV and KV isolates from pea and bean seemingly reflected titer derived from results of mechanical infectivity trials; the WV, as tested earlier, proved nonaphid transmissible.

Individuals of A. craccivora and M. persicae were able to acquire and transmit FV more readily from pea or lupine than from bean (Table 3), thereby indicating that bean is a relatively deficient source of BYMV. Individuals of H. pseudobrassicae proved inefficient vectors of the FV regardless of virus source (Table 3), even though their acquisition probing behavior as perceived through a binocular microscope was inducive of virus transmission. Pea also proved a better virus source than bean in similar trials with individuals of A. craccivora tested as vectors of the KV (14/50 and 2/50 test plants infected from pea and bean, respectively). That pea is a better virus source of BYMV isolates for aphids is still further indicated by tests involving aphids allowed 3- to 12-minute access periods on FV, KV or WV infected pea or bean plants (Table 4). Again,

Table 3. Transmission of Florida isolate from pea, bean and lupine by aphids allowed single acquisition probes.

Virus source	Aphid species		
	A. craccivora	M. persicae	H. pseudobrassicae
'Alaska' pea	37/100[a]	25/100	1/50
'Red Kidney' bean	8/100	7/100	0/50
Yellow lupine	22/100	11/100	0/50

[a] Ratio is number of infected 'Red Kidney' bean test plants infected of inoculated (1 aphid per test plant).

Table 4. Comparative transmissibility of 3 bean yellow mosaic virus isolates (Florida isolate, Kentucky isolate, Wisconsin isolate) from 'Alaska' pea and 'Red Kidney' bean by aphids allowed 3- to 12-minute access periods.

Aphid species[b]	Virus isolate					
	FV		KV		WV	
	pea	bean	pea	bean	pea	bean
A. craccivora	12[a]	1	11	0	0	0
A. pisum	9	2	2	0	0	0
M. persicae	6	2	0	0	0	0

[a] Number of infected plants of 100 'Red Kidney' bean test plants inoculated using 1 aphid per test plant.

[b] The comparative transmission trials of the 3 bean yellow mosaic virus isolates with A. craccivora, A. pisum and M. persicae were spaced at 3 month intervals. These aphid-transmissibility trials were carried out in conjunction with the mechanical infectivity experiments III, IV and V, respectively, in Table 2.

in no instance did FV or KV transmission from bean exceed that from pea, regardless of aphid species tested; indeed, no transmission was ever obtained from KV infected bean plants in this trial.

Several workers have reported that the curved epidermal hairs typical of bean leaves ensnare aphids, thereby preventing them from establishing colonies on this plant (22, 37, 42). In order to eliminate aberrant aphid behavior on bean foliage as a major causal factor of the observed low transmission of BYMV from beans, trials with BCMV were initiated. The results of these studies (Table 5) show that BCMV is efficiently transmitted from either plants singly infected with BCMV or from plants doubly infected with BCMV and FV; moreover, the results show that FV, relative to BCMV, is not as readily transmitted from beans, presumably because of the occurrence of low titers of that virus in bean. The results also show that H. pseudobrassicae, relative to A. craccivora and M. persicae, is an ineffective vector of BCMV; these results parallel earlier studies with these aphid species as vectors of BYMV (Table 3).

Variability of Different BYMV Isolates as Virus Sources

Marked differences were found in the relative aphid and mechanical infectivities of the 3 BYMV isolates. It was also

Table 5. Transmission of virus by aphids from 'Red Kidney' bean plants singly infected with bean common mosaic virus or doubly infected with bean common mosaic virus and the Florida isolate.

	Virus transmitted by aphids			
	From plants singly infected with BCMV	From plants doubly infected with BCMV and FV[a]		
Aphid species	BCMV	BCMV	FV	BCMV/FV
A. craccivora	74/100[b]	55/150	8/150	12/150
M. persicae	47/100	56/150	3/150	7/150
H. pseudobrassicae	1/100			

[a] Test plants infected with bean common mosaic virus were identified on the basis of a typical abaxial cupping of systemically infected leaves (29, 51, 60); plants infected with the Florida isolate were verified on the basis of infection of pea and/or C. amaranticolor indicator plants (32,60).

[b] Ratio is number of infected 'Red Kidney' bean test plants of total inoculated; each test plant was inoculated by a single aphid allowed a single timed virus acquisition probe.

noted that virus particle counts of negatively stained leaf dip extracts reflected the results of mechanical transmission assays but had no apparent relation to aphid transmissibility.

Aphid and Mechanical Transmissibility

Whereas, noted earlier, both the FV and KV were transmitted by aphids, the WV repeatedly was not, regardless of virus source plant used or aphid species tested (Tables 4 and 6). Moreover, the WV was never transmitted by aphids that probed plants doubly infected with WV and BCMV; transmission of WV and BCMV from doubly infected bean plants was, respectively, 0 and 24 of 100 test plants inoculated by single individuals of A. craccivora allowed 3- to 12-minute virus access periods. This nonaphid transmissibility resulted despite the occurrence of numerous and prominent amorphous inclusions, earlier correlated with aphid transmissibility (75, 76), in WV infected pea, broadbean or lupine epidermis (Fig 2). Additionally, virus particles were readily detected in negatively stained extracts of pea, bean, broadbean or lupine epidermal strips examined with the electron microscope.

Of the 3 BYMV isolates tested for mechanical infectivity, however, the nonaphid-transmissible WV proved consistently more infectious than either the aphid-transmissible

Table 6. Comparative aphid transmissibility of Florida isolate and Wisconsin isolate from different plant species by individuals of A. craccivora allowed 3- to 12-minute virus access periods.

Virus source	BYMV isolate	
	FV	WV
'Alaska' pea	24[a]	0
'Red Kidney' bean	1	0
White sweet clover	7	0
C. amaranticolor	0	0
C. mucronata	15	0

[a] Number of 'Red Kidney' bean test plants infected of 100 inoculated by single aphids.

FV or KV, regardless of whether pea or bean tissue was assayed; the FV, conversely, proved the least infectious of the 3 isolates (Table 2). WV infected pea leaves proved significantly more infectious than either FV ($P = < .01$) or KV ($P = < .01$) infected pea tissues. Similarly, WV infected bean leaves proved significantly more infectious than FV infected bean tissue ($P = < .01$).

A progressive increase in mechanical infectivity from pea and bean leaves was observed in 3 succeeding trials with the KV (Table 2). This increase in mechanical infectivity coincided with a corresponding decline and eventual loss of aphid transmissibility of this isolate (Table 4), thereby suggesting a rapid change in the transmission properties of this isolate of BYMV similar to findings reported by Swenson (62), and Swenson et al. (65). Aphid transmission of the KV in initial trials, for example, was readily effected using individuals of A. craccivora allowed access to KV infected pea leaves (14 of 50 and 11 of 100 'Red Kidney' bean test plants infected of inoculated, respectively). After 7 months, following routine mechanical transfer to pea and bean seedlings at biweekly intervals, however, aphid transmissibility was lost. Repeated attempts at aphid transmissions, using at least 300 individuals each of A. craccivora, A. pisum

and M. persicae allowed access to KV infected pea and bean plants, always failed. The apparent change in aphid and mechanical transmissibility of the KV appeared not to be associated with any appreciable change in symptom expression of KV infected plants; indeed, the distinctive symptoms described previously for the KV in C. amaranticolor remained unchanged throughout this study.

Virus Particle Numbers Related to Aphid and Mechanical Transmissibility

In view of the observed incongruity between aphid and mechanical infectivity with respect to the FV and WV isolates of BYMV, an attempt was made to determine whether relative virus particle counts more closely reflected aphid or mechanical transmissibility. The results of this study show that particle counts closely reflect mechanical transmission assays but have no apparent relation to aphid transmissibility (Table 7). The number of particles found in negatively stained leaf dip extracts from WV infected tissue significantly exceeded that of the FV (t value = 7.57, $P = < .001$); indeed, in no instance did the number of FV particles equal or exceed that of the WV. The results of the aphid and mechanical assays of these same FV or WV infected pea leaves corroborated earlier studies: 1) WV infected tissue proved significantly more infectious than FV infected tissue

Table 7. The relation of virus particle numbers to the aphid and mechanical transmissibility of the Florida isolate and Wisconsin isolate.

BYMV isolate	Leaf number[a]	Aphid transmissibility	Mechanical transmissibility	Number of virus particles
FV	1	2[b]	77[c]	88[d]
	2	3	178	60
	3	4	78	114
	4	3	215	37
	5	1	28	75
	6	3	122	77
	7	4	138	59
	8	2	197	108
	totals	22	1,033	618
WV	1	0	496	258
	2	0	759	279
	3	0	716	484
	4	0	351	356
	5	0	100	547
	6	0	642	359
	7	0	394	341
	8	0	621	261
	totals	0	4,079	2,885

Table 7. (continued)

[a] 'Alaska' pea leaves (each leaf with 4 leaflets) systemically infected with either the Florida isolate or Wisconsin isolate.

[b] Number of infected 'Red Kidney' bean test seedlings of 4 inoculated; each test seedling was inoculated with 5 individuals of A. craccivora allowed 3-minute access periods on Florida isolate or Wisconsin isolate infected pea leaves.

[c] Average number of lesions per leaf on 10 leaves of C. amaranticolor inoculated mechanically with Florida isolate or Wisconsin isolate infected pea leaves.

[d] Total number of virus particles on 36 selected grid squares or a 400-mesh electron microscope specimen grid; virus particles were extracted from discs of Florida isolate or Wisconsin isolate infected pea leaves and negatively stained in phosphotungstic acid.

(t value = 5.28, P= <.01), and 2) the WV was never aphid transmitted, whereas the FV was aphid transmitted in every instance.

DISCUSSION

The results of this study have shown that variations among BYMV isolates can occur with regard to symptomatology, physical properties, aphid transmissibility, specific host range and prominence of amorphous virus-induced cytoplasmic inclusions seen with the light microscope. Similar variabilities among BYMV isolates with regard to these properties have also been shown by other workers (10, 18, 25, 47, 53, 65). In addition to these variables, it was found that different BYMV isolates can also vary significantly in mechanical infectivity and in relative numbers of virus particles found in negatively stained leaf dip extracts.

Stable criteria found in common in this study among the BYMV isolates were the unvarying genetic responses of certain pea varieties, general host range and the fact that pea was a consistently better source of virus than bean in aphid- and mechanical-transmission trials. In addition, electron microscopy showed normal particle lengths and ultrastructure of virus-induced cylindrical inclusions in sectioned material and in molybdate stained leaf extracts to be stable criteria among the BYMV isolates investigated in this study. Sectioned cylindrical inclusions found in FV, KV and WV

infected tissues were remarkably similar to those published by others for BYMV isolates (20, 39, 73, 74). These inclusions consisted of pinwheels and laminated aggregates. Neither tubes nor circular inclusions such as have been found in association with other flexuous rod viruses such as BCMV (unpublished data), watermelon mosaic virus 2 (21), papaya distortion ringspot virus (77) and an unidentified virus of cowpeas (76) were ever found in BYMV infected tissues. In view of such differences, it is possible that inclusion morphology may serve as a useful criterion in the future for distinguishing BYMV from other similar, but distinct, filamentous legume viruses.

In aphid-transmissibility trials, the percentage transmission of BYMV isolates from pea was many times the percentage transmission obtained from bean. This effect of source plant on the degree of virus transmissibility has also been shown by others for BYMV and other viruses (1, 4, 18, 58, 59). In this study, the presence and/or prominence of virus-induced inclusions present in epidermal cells of pea leaves, but not in bean, appeared to be coincident with high virus titers as measured by aphid and mechanical transmissibilities. This disparity between pea and bean existed despite the fact that much more striking symptoms were apparent with all 3 isolates in bean than pea, thereby

indicating that symptom expression is not necessarily an indication of virus titer and thus differing from the results of Swenson et al. (65) for broadbeans.

Pea mosaic virus (PMV) is similar to BYMV except that it does not infect the more common varieties of bean; this criterion is claimed by Schroeder and Provvidenti (57) to be the sole basis on which this virus is distinguishable from the BYMV isolates; and, indeed, many workers such as McWhorter (43), Schroeder and Provvidenti (57), Taylor and Smith (67) and Van Regenmortel (69) conclude that PMV in fact is a strain of BYMV. Isolates of BYMV which do not infect one or more hosts that are susceptible to other BYMV isolates have been reported by several workers (18, 25, 26). In this study, for example, the reaction of each BYMV isolate on C. spectabilis differed markedly, ranging from insusceptibility to FV, local lesion response and sometimes systemic infection with WV, to being highly systemically susceptible to KV. That the insusceptibility of most bean varieties to PMV may be merely a variable in the behavior of BYMV is supported by the results of this study which indicate that bean is a relatively poor host of BYMV and therefore BYMV may not be particularly adapted to bean.

A potential source of confusion as to the identity of

different flexuous rod viruses of legumes can result from serological studies which demonstrate varying degrees of relationship between such dissimilar viruses as BYMV and potato virus Y, watermelon mosaic virus, and beet mosaic virus (8, 9, 14, 51, 70). For example, a reported serological relatedness led to the blackeye cowpea mosaic virus described by Anderson (2) to be referred to as being a strain of BYMV. In this study, however, none of the BYMV isolates infected any of the tested cowpea varieties. It is therefore feasible that cowpeas are in fact insusceptible to BYMV and that a distinct virus (possibly in many ways different from BYMV) is responsible for the disease of cowpeas studied by others and assumed to be BYMV based on this reported serological relationship (30, 41). Indeed, Gibbs (24), obtaining much of his information from serological work on tobacco mosaic virus, states that only a very small amount of the virus coat protein is involved in serological reactions. According to his information, a maximum of 4% of the total nucleotide sequence of different viruses is being indirectly compared in serological tests. This then may account for the fact that serological relationships may not always correlate with relationships based on other properties, which Gibbs presumes to reflect information from other parts of the viral nucleic acid. This study with BYMV

emphasizes the need to take into consideration as many criteria as possible in characterizing viruses. It is apparent that no 1 criterion, such as serological relatedness or inability of an isolate to infect a particular plant species, should form an arbitrary basis for classification of viruses in this group where variability in behavior appears to be a rule and not an exception.

This study implicates an association of nonaphid transmissibility with a relatively high degree of mechanical infectivity. Brandes and Bercks (14) indicated that rod shaped viruses with a normal particle length of 730-790 mμ typically are aphid transmitted in a stylet-borne manner and have relatively low dilution end points; rod shaped viruses with normal lengths less than 730 mμ, conversely, typically are not aphid transmitted and have relatively high dilution end points. The results of this study suggest an association of nonaphid transmissibility with a coincidental occurrence or a relatively high virus titer without any change in particle length. It is possible that nonaphid transmissibility is associated with a breakdown in some regulatory host/virus interaction limiting virus titer in infected plants.

Several workers (13, 64) have reported certain stylet-borne virus isolates as losing their ability to be aphid transmitted,

presumably as a result of spontaneous or induced mutations (3, 31, 62, 63, 65). According to Watson (71) and Badami (3) working with nonaphid-transmissible isolates of potato virus Y and cucumber mosaic virus, respectively, lack of aphid transmission may be due to a chemical or configurational change in the virus particles. Pirone and Megahed (52) demonstrated that aphids could acquire and transmit purified preparations of intact cucumber mosaic virus but not cucumber mosaic virus-RNA. They tentatively suggested that the intact virus is the transmissible form rather than the non-coated nucleic acid. An isolate of cucumber mosaic virus with very low aphid transmissibility but compapable in mechanical infectivity to 2 other readily aphid-transmissible isolates was shown by Normand and Pirone (49) to be just as aphid-transmissible after purification as the other 2 isolates. They concluded that since the properties of the virus particle could not account for the lack of transmission, then the explanation for this phenomenon must lie in the host/virus relationship. If in this study with BYMV, conclusions on virus titer were to be based solely on the FV isolate it could be inferred that aphid transmissibility was correlated with mechanical infectivity from pea and bean tissue, respectively, similar to findings by Simons for cucumber mosaic virus (59) and Swenson et al. for BYMV (65).

This could lead to the interpretation that the infectious units involved in aphid and mechanical transmissibility were in some way comparable. However, the nonaphid transmissibility of the WV, coupled with the higher virus particle counts and infectivities than the aphid-transmissible FV, does not lead to this conclusion. The total lack of aphid transmissibility of the WV isolate from pea, bean and other hosts suggests the possibility that the infectious unit as transmitted by aphids may differ qualitatively from the infectious unit involved in mechanical transmission and is subject to loss by continued mechanical transfer. Alternatively, the intact particles of the WV may have in some way lost their ability to be aphid-transmitted and the mutation that led to this loss of aphid transmissibility is coincidental with the increase in virus titers in the host.

SUMMARY

An isolate of bean yellow mosaic virus (BYMV) from Florida (FV) was compared with an isolate of BYMV from Wisconsin (WV). The identities of both viruses as isolates of BYMV was substantiated as follows: 1) host range studies; 2) specific genetic responses of certain immune and susceptible pea (Pisum sativum) varieties; 3) physical property studies; 4) optical microscope examination of stained epidermal strips from infected leaf tissue; 5) electron microscope in situ examination of infected tissue; 6) virus particle measurements from negatively stained leaf dip extracts and 7) establishment of virus-vector relationships.

Juice from pea leaves infected with either the FV or WV was significantly ($P = < .01$) more infectious than juice from bean leaves. This was based on average numbers of local lesions induced on mechanically inoculated leaves of Chenopodium amaranticolor (57 and 729 lesions per leaf from pea and 8 and 55 lesions per leaf from bean, for the FV and WV, respectively).

Virus-induced cytoplasmic inclusions were abundant and prominent in epidermal cells of FV- or WV- infected pea, but

were nor found in bean.

Aphid transmission of the FV further indicated a higher virus titer in pea than bean. Aphis craccivora and Myzus persicae readily transmitted FV from pea (37% and 25% transmission, respectively) but not readily from bean (8% and 7% transmission, respectively). That aberrant aphid behavior on bean was not a factor in the low transmission of the FV from bean was confirmed by trials involving bean plants doubly infected with the FV and bean common mosaic virus (BCMV). Transmission of BCMV and FV by A. craccivora (allowed access to doubly infected bean leaves) was 45% and 13%, respectively; similarly, transmission by M. persicae (allowed access to the same leaves) was 42% and 7%, respectively.

Although the FV was proved aphid transmissible under our conditions, no transmission was ever obtained in parallel trials with the WV, even when a combination of different known aphid vectors and several plant species infected with the WV were tested for virus transmission. Moreover, in bean plants doubly infected with the WV and BCMV, only BCMV was transmitted.

Further studies, including a third BYMV isolate from Kentucky (KV), indicated the apparently nonaphid-transmissible

WV occurred in much higher titer in pea or bean than either of the aphid-transmissible FV and KV isolates. Leaves of C. amaranticolor inoculated with 1/10 diluted juice from bean plants infected with the FV, KV and WV averaged only 9, 49 and 65 lesions per leaf, but those inoculated with 1/10 diluted juice from pea plants averaged 61, 305 and 698 lesions per leaf, respectively.

Virus particle counts of leaf dip extracts from FV or WV infected pea leaves reflected the results of mechanical transmission assays but had no apparent relation to aphid transmissibility. Pea leaves infected with the WV resulted in significantly ($P = < .01$) more lesions per inoculated C. amaranticolor leaf than leaves infected with the FV (510 and 129 lesions per leaf, respectively). Similarly, significantly ($P = < .001$) more particles were found in leaf dip extracts from these same WV and FV infected pea leaves (361 and 77 particles per 400-mesh grid, respectively). Despite significantly greater mechanical infectivity and particle numbers, WV was never aphid transmitted, whereas the FV was aphid transmitted in every instance.

LITERATURE CITED

1. Adlerz, W. C. 1958. Factors affecting transmission of bean yellow mosaic virus. J. Econ. Entomol. 52:260-262.

2. Anderson, C. W. 1955. Vigna and crotalaria viruses in Florida. II. Notations concerning cowpea mosaic virus (Marmor vignae). Plant Dis. Reptr. 39:349-353.

3. Badami, R. S. 1958. Changes in the transmissibility by aphids of a strain of cucumber mosaic virus. Ann. Appl. Biol. 46:554-562.

4. Bagnall, R. H., and R. H. E. Bradley. 1958. Resistance to virus Y in the potato. Phytopathology 48:121-125.

5. Bancroft, J. B., and P. Kaesberg. 1959. Partial purification and association of filamentous particles with the yellow mosaic disease of bean. Phytopathology 49:713-715.

6. Bawden, F. C. 1964. Plant viruses and virus diseases. 4th ed., Ronald Press, New York. 361 p.

7. Beemster, A. B. R., and J. P. H. Van der Want. 1951. Serological investigations on the phaseolus viruses 1 and 2. Leeuwenhock Ned. Tijschr. 17:285-296.

8. Bercks, R. 1960a. Serlolgische Untersuchungen zur Differenzierung von Isolaten des Phaseolus-Virus 2 und ihrer Verwandrschaft mit Phaseolus-Virus 1. Phytopathol. Z. 39:120-128.

9. Bercks, R. 1960b. Serological relationships between beet mosaic virus, potato virus Y and bean yellow mosaic virus. Virology 12:311-313.

10. Bos, L. 1968. The problem of variation within the potato virus Y group, with special reference to bean yellow mosaic. 3rd meeting IWGLV, London (Abstr.).

11. Bos, L. 1969. Inclusion bodies of bean yellow mosaic virus, some less known closely related viruses and

beet mosaic virus. Neth. J. Plant Pathol. 75:137-143.

12. Bos, L., D. J. Hagedorn, and L. Quantz. 1960. Suggested procedures for international identification of legume viruses. T. Pl. ziekten 66:328-343.

13. Bradley, R. H. E. 1964. Aphid transmission of stylet-borne viruses, p. 148-174. In M. K. Corbett and H. D. Sisler (ed.), Plant Virology. University of Florida Press, Gainesville.

14. Brandes, J., and R. Bercks. 1965. Gross morphology and serology as a basis for classification of elongated plant viruses. Adv. Virus Res. 11:1-24.

15. Brandes, J., and C. Wetter. 1959. Classification of elongated plant viruses on the basis of particle morphology. Virology 8:99-115.

16. Christie, R. G. 1967. Rapid staining procedures for differentiating plant virus inclusions in epidermal strips. Virology 31:268-271.

17. Corbett, M. K. 1957. Local lesions and cross-protection studies with bean yellow mosaic virus. Phytopathology 47:573-574.

18. Corbett, M. K. 1958. A virus disease of lupines caused by bean yellow mosaic virus. Phytopathology 48:86-91.

19. Demski, D. W. 1968. Local lesion reactions of Chenopodium species to watermelon mosaic virus 2. Phytopathology 58:1196-1197.

20. Edwardson, J. R. 1966. Electron microscopy of cytoplasmic inclusions in cells infected with rod-shaped viruses. Amer. J. Bot. 53:359-364.

21. Edwardson, J. R., D. E. Purcifull, and R. G. Christie. 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34:250-263.

22. Fluiter, H. J. de, and G. W. Ankersmit. 1948. Gegevens

betreffende de aantasting van bonen (Phaseolus vulgaris L.) door de zwarte bonenluis (Aphis (Doralis) fabae Scop.). Tijdschr. PlZiekt. 54:1-13.

23. Ford, R. E. 1963. Susceptibility of perfection-type peas to bean yellow mosaic virus. Plant Dis. Reptr. 47:384-388.

24. Gibbs, A. 1969. Plant virus classification. Adv. Virus Res. 14:263-328.

25. Goodchild, D. J. 1956. Relationships of legume viruses in Australia. I. Strains of bean yellow mosaic virus and pea mosaic virus. Australian J. Biol. Sci. 9: 213-230.

26. Grogan, R. G., and J. C. Walker. 1948. A pod-distorting strain of the yellow mosaic virus of bean. J. Agr. Res. (U. S.) 77:301-314.

27. Grogan, R. G., and J. C. Walker. 1948. Interrelation of bean virus 1 and bean virus 2 as shown by cross-protection tests. Phytopathology 38:489-493.

28. Hagedorn, D. J., and J. C. Walker. 1950. The relation of bean virus 2 to pea mosaic in Wisconsin. Phytopathology 40:684-698.

29. Harrison, A. L. 1935. The physiology of bean mosaic. N. Y. State (Geneva) Agr. Exp. Sta. Tech. Bull 235.

30. Harrison, A. N., and R. T. Gudauskas. 1968. Identification of viruses isolated from cowpeas in Alabama. Plant Dis. Reptr. 52:34-36 (Abstr.).

31. Hollings, M. 1955. Investigation of chrysanthemum viruses. I. Aspermy flower distortion. Ann. Appl. Biol. 43:86-102.

32. Hollings, M. 1956. Chenopodium amaranticolor as a test plant for plant viruses. Plant Pathol. 5:57-60.

33. Holmes, F. O. 1931. Local lesions of mosaic in Nicotiana tabacum L. Boyce Thompson Inst. Contrib. 3: 163-172.

34. Hull, R. 1965. Virus diseases of sweet peas in England. Plant Pathol. 14:150-153.

35. Huxley, H. E., and G. Zubzy. 1961. Preferential staining of nucleic acid-containing structures for electron microscopy. J. Biophys. Biochem. Cytol. 11:273-296.

36. Jensen, D. D. 1959. Insects, both hosts and vectors of plant viruses. Pan-Pacific Entomol. 35:65-82.

37. Johnson, B. 1953. The innurious effects of the crooked epidermal hairs of French beans (Phaseolus vulgaris L.) on Aphis craccivora Koch. Bull Entomol. Res. 44: 779-788.

38. Kahn, R. P., H. A. Scott, F. F. Smith, and J. J. Higgins. 1963. Sunn hemp mosaic incited by the bean yellow mosaic virus. Plant Dis. Reptr. 47:364-368.

39. Kamei, T., Y. Honda, and C. Matsui. 1969. Intracellular appearance of turnip mosaic and bean yellow mosaic virus particles. Phytopathology 59:139-144.

40. Kennedy, J. S., M. F. Day, and V. F. Easop. 1962. A conspectus of aphids as vectors of plant viruses. Commonwealth Institute of Entomology, London, 114 p.

41. Kuhn, C. W. 1964. Separation of cowpea virus mixtures. Phytopathology 54:739-740.

42. McKinney, K. B. 1938. Physical characteristics on the foliage of beans and tomatoes that tend to control some small insect pests. J. Econ. Entomol. 31:630-631.

43. McWhorter, F. P. 1941. Isometric crystals produced by Pisum virus 2 and Phaseolus virus 2. Phytopathology 31:760-761.

44. McWhorter, F. P. 1965. Plant virus inclusions. Ann. Rev. Phytopathol. 3:287-312.

45. Milne, K. S., R. G. Grogan, and K. A. Kimble. 1969. Identification of viruses infecting cucurbits in California. Phytopathology 59:819-827.

46. Mollenhauer, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Tech. 39:111-114.

47. Mueller, W. C., and R. Koenig. 1965. Nuclear inclusions produced by bean yellow mosaic virus as indicators of cross-protection. Phytopathology 55:242-243.

48. Nault, L. R., and R. H. E. Bradley. 1969. Acquisition of maize dwarf mosaic virus by the greenbug, Schizaphis graminum. Ann. Entomol. Soc. Amer. 62:403-406.

49. Normand, R. A., and T. P. Pirone. 1968. Differential transmission of strains of cucumber mosaic virus by aphids. Virology 36:538-544.

50. Pease, D. C. 1964. Histological techniques for electron microscopy. 2nd. ed., Academic Press, New York. 381 p.

51. Pierce, W. H. 1943. Viruses of the bean. Phytopathology 24:87-115.

52. Pirone, T. P., and E. S. Megahed. 1966. Aphid transmissibility of some purified viruses and viral RNA's. Virology 30:631-637.

53. Pratt, M. J. 1969. Clover yellow vein virus in North America. Plant Dis. Reptr. 53:210-212.

54. Purcifull, D. E., and J. R. Edwardson. 1967. Watermelon mosaic virus: tubular inclusions in pumpkin leaves and aggregates in leaf extracts. Virology 32: 393-401.

55. Purcifull, D. E. 1968. Disruption of watermelon mosaic virus-induced inclusions by phosphotungstate. Virology 36:690-693.

56. Ross, A. F. 1964. Identification of plant viruses, p. 68-92. In M. K. Corbett and H. D. Sisler (ed,), Plant virology. University of Florida Press, Gainesville.

57. Schroeder, W. T., and R. Provvidenti. 1966. Further evidence that common pea mosaic (PV2) is a strain of bean yellow mosaic virus (BV2). Plant Dis. Reptr. 50: 337-340.

58. Simons, J. N. 1957. Three strains of cucumber mosaic

virus affecting bell pepper in the Everglades area of south Florida. Phytopathology 47:145-150.

59. Simons, J. N. 1958. Titers of three nonpersistent aphid-borne viruses affecting pepper in south Florida. Phytopathology 48:265-268.

60. Smith, K. M. 1957. A textbook of plant virus diseases. 2nd ed., F. and A. Churchill Ltd., London. 652 p.

61. Steere, R. L. 1955. Concepts and problems concerning the assay of plant viruses. Phytopathology 45:196-208.

62. Swenson, K. G. 1957. Transmission of bean yellow mosaic virus by aphids. J. Econ. Entomol. 50:727-731.

63. Swenson, K. G., and R. L. Nelson. 1959. Relation of aphids to the spread of cucumber mosaic virus in gladiolus. J. Econ. Entomol. 52:421-425.

64. Swenson, K. G. 1963. Effects in insect and virus host plants on transmission of viruses by insects. Ann. N. Y. Acad. Sci. 105:730-740.

65. Swenson,K. G., S. S. Sohi, and R. E. Welton. 1963. Loss of transmissibility by aphids of bean yellow mosaic virus. Ann. Entomol. Soc. Amer. 57:378-382.

66. Sylvester, E. S., and J. Richardson. 1964. Transmission of cabbage mosaic virus by green peach aphids - stylet transmission studies. Virology 22:520-538.

67. Taylor, R. H., and P. R. Smith. 1968. The relationship between bean yellow mosaic virus and pea mosaic virus. Australian J. Biol. Sci. 21:429-437.

68. Thompson, A. D., and Barbara A. Peddie. 1965. Studies on a virus inhibitor from Chenopodium leaves. New Zealand J. Agr. Res. 8:825-831.

69. Van Regenmortel, M. H. V. 1966. Plant virus serology. Adv. Virus Res. 12:207-271.

70. Van Regenmortel, M. H. V., J. Brandes, and R. Bercks.

1962. Investigations on the properties of watermelon mosaic virus. Phytopathol. Z. 45:205-216.

71. Watson, M. A. 1958. The specificity of transmission of some nonpersistant viruses. 10th Int. Congr. Entomol. (Montreal, 1956), Proc., 3:215-219.

72. Wehrmeyer, W. 1957. Darstellung und Strukturordnung eines Tabak-Mosaikvirus-Einschlusskorpers in der Zelle. Naturwissenschaften 19:519-520.

73. Weintraub, M., and H. W. J. Ragetli. 1966. Fine structure of inclusions and organelles in Vicia faba infected with bean yellow mosaic virus. Virology 28: 290-302.

74. Weintraub, M., and H. W. J. Ragetli. 1968. Intracellular characterization of bean yellow mosaic virus-induced inclusions by differential enzume digestion. J. Cell. Biol. 38:316-328.

75. Zettler, F. W. 1969. The heterogeneity of bean leaves as sources of bean common mosaic virus for aphids. Phytopathology (in press).

76. Zettler, F. W., R. G. Christie, and J. R. Edwardson. 1967. Aphid transmission of virus from leaf sectors correlated with intracellular inclusions. Virology 33:549-552.

77. Zettler, F. W., J. R. Edwardson, and D. E. Purcifull. 1968. Ultramicroscopic differences in inclusions of papaya mosaic virus and papaya ringspot virus correlated with differential aphid transmission. Phytopathology 58:332-335.

78. Zettler, F. W., and R. E. Wilkinson. 1966. Effect of probing behavior and starvation of Myzus persicae on transmission of bean common mosaic virus. Phytopathology 56:1079-1082.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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