Aphid and Mechanical Transmission Properties of Bean Yellow Mosaic Virus Isolates

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Florida Agricultural Experiment Stations Journal Series Paper No. 3536.

The authors thank S. R. Christie for assistance with the electron microscopy. The authors are also grateful for the technical assistance of R. G. Christie and Margaret O. Smyly.

Accepted for publication 5 March 1970.

ABSTRACT

An isolate of bean yellow mosaic virus (BYMV) from Florida (FV) was compared with an isolate of BYMV from Kentucky (KV) and one from Wisconsin (WV). Although both the FV and KV proved aphid-transmissible, no transmission was obtained in parallel trials with the WV, even when a combination of different known aphid vectors and several host-plant species was used. Based on mechanical transmissibility assays and virus particle counts, however, studies showed that the nonaphid-transmissible WV occurred in a relatively higher titer in leaves than did either of the aphid-transmissible FV and KV isolates.

In one experiment, pea leaves infected with the WV resulted in significantly ($P = < .01$) more lesions per inoculated *Chenopodium amaranticolor* leaf than leaves infected with the FV (510 and 129 lesions/leaf, respectively). Also, significantly ($P = < .001$) more particles were found in WV than FV extracts from these same infected pea leaves (10.0 and 2.1 particles/400-mesh grid square, respectively). Despite the significantly greater mechanical transmissibility and particle numbers, however, WV was not aphid-transmitted from any of these same leaves, whereas the FV was aphid-transmitted in every instance. Phytopathology 60: 1170-1174.

Additional key words: virus particles, local lesions.

Although isolates of bean yellow mosaic virus (BYMV) differ in many ways, relatively little attention has been focused on differences in aphid and mechanical transmission properties. Loss of aphid transmissibility was shown for BYMV by Swenson (11) and Swenson et al. (13). Swenson (11) also 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 (*Phaseolus vulgaris*) plants at each dilution. Our study involved a reinvestigation of the aphid and mechanical transmission properties among different isolates of BYMV, and attempted to relate these results to the numerical presence of virus particles in leaf extracts.

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 D. J. Hagedorn at the University of Wisconsin, Madison; and a Kentucky isolate (KV) obtained from S. Diachun at the University of Kentucky, Lexington. Each of these three isolates proved similar to other known isolates of BYMV in (i) host range studies; (ii) general symptomatology; (iii) specific genetic responses of selected immune and susceptible pea (*Pisum sativum* L.) varieties; (iv) physical property studies; (v) optical microscopy of stained epidermal strips from infected leaf tissue; (vi) electron microscope in situ examination of ultrathin sections of infected tissue; and (vii) virus particle measurements from negatively stained leaf dip extracts (3). The isolate of bean common mosaic virus (BCMV) used in this study was originally obtained in 1961 by R. E. Wilkinson at Cornell University, Ithaca, New York. None of the viruses kept in separate greenhouses to reduce the possibility of contamination of one isolate with another showed evidence of contamination in the 2-year study.

Chenopodium amaranticolor Coste & Reyn., a local lesion host of BYMV (5), was used to compare the mechanical transmissibility of the FV, WV, and KV isolates. Although all three isolates of BYMV induced both local and systemic symptoms in *C. amaranticolor*, noticeable differences were observed in the degree of systemic movement of each of the viruses in this plant (Fig. 1-A, B). The FV moved systemically much more rapidly than either the WV or KV, whereas the KV moved systemically much less readily than even the WV. Crude juice expressed from virus-infected pea and bean plants diluted 1:10 in distilled water was used in all assays on *C. amaranticolor*. In the inoculation procedure, the five youngest fully expanded leaves of *C. amaranticolor* were inoculated by rubbing as uniformly as possible in all trials. Fresh inoculum and a new sterile cheesecloth pad dusted with 600-mesh Carborundum were used for each inoculated plant.

A modification of the starch-iodine technique described by Holmes (6) was used for accentuating and counting local lesions. Four days after inoculation, plants were placed for 18 hr 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 2 or more days at approximately 24 C. After chlorophyll removal, 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 (Fig. 1-C, D, E, F). 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.

The aphid species used in this study were (i) the pea aphid, *Acyrthosiphon pisum* (Harris), reared on broadbean, *Vicia faba* L. 'Longpod'; (ii) the melon aphid, *Aphis gossypii* Glover, reared on celeriac, *Apium graveolens rapaceum* DC. 'Giant Prague'; (iii) the cowpea aphid, *Aphis craccivora* Koch, reared on Longpod broadbean; (iv) the spirea aphid, *Aphis spiraecola* Patch, reared on Giant Prague celeriac; (v) the turnip aphid, *Hyadaphis pseudobrassicae* (Davis), reared on mustard, *Brassica juncea* Coss. 'Florida Broadleaf'; and (vi) 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 Louise M. Russell, USDA, Entomology Research Division, at Washington, D.C., and by A. N. Tissot, Department of Entomology, University of Florida, Gainesville.

In trials with aphids allowed single acquisition probes, vigorous nonviruliferous aphids were starved 4 to 6 hr before being transferred individually with a camel's-hair brush to the virus source. Aphids that voluntarily terminated their probes were transferred by brush (one aphid/plant) to a healthy test plant. In no instance was an aphid allowed to make a probe exceeding 1 min; aphids probing at that time were interrupted with the camel's-hair brush and transferred to a healthy test plant.

A more convenient "mass transfer" method was used to study the comparative aphid transmissibility of the three BYMV isolates from pea, bean, and other hosts. This technique consisted of starving nonviruliferous aphids for 4 hr and transferring them collectively to virus sources in groups of 100 or more individuals. After the initial access period of 3 to 12 min, 50 aphids that appeared to be probing were removed individually and transferred singly (one aphid/plant) to healthy test plants. Approximately 9 min were required to transfer 50 aphids individually from the virus source to test seedlings.

In all studies involving aphids, test plants were Red Kidney bean seedlings used within 2 days after emergence. Aphids were allowed to probe on test plants for at least 12 hr 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.

In one experiment, the relative mechanical and aphid transmissibilities of the FV and WV were correlated with virus particle counts. In this study, aphid transmissibility was determined by selecting eight comparable WV- and eight FV-infected Alaska pea leaves (each with four leaflets) as virus sources for aphids. Each leaflet served as a virus source for five individuals of *A. craccivora* permitted 3-min access periods prior to test feedings on Red Kidney bean seedlings. Virus particle numbers were assayed by (i) excising a single disc of tissue from each of these leaflets with a No. 1 (3-mm diam) cork borer; (ii) quartering each disc with a razor blade; and (iii) placing the discs together (four discs/leaf) and negatively staining the exudate in three drops of 1% phosphotungstic acid (pH 6.8) for 2 min. After a thorough stirring, a drop of this material was applied to a 400-mesh Formvar-coated copper specimen grid and left for 30 sec before removing the excess liquid with filter paper. All filamentous particles observed in a fixed number of grid squares in comparable zones on each grid were counted and recorded. The grid squares selected for examination were located in each 400-mesh specimen grid by arbitrarily subdividing each grid into quadrants from the grid center and selecting nine grid squares centrally located in each quadrant for observation; none of the selected grid squares was closer than 0.3 mm nor farther than 0.7 mm from the center of each grid. Mechanical transmissibility 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.—In early trials, the FV and KV, but not the WV, were transmitted by aphids allowed single acquisition probes on infected pea leaves. Individuals of *A. craccivora* transmitted KV to 14 out of 50 inoculated Red Kidney bean seedlings. Transmission of the FV from infected pea leaves to Red Kidney bean seedlings by individuals of different aphid species was as follows: *A. pisum,* three of nine; *A. craccivora,* five of nine; *A. gossypii,* one of nine; *A. spiraecola,* one of nine; *H. pseudobrassicae,* none of nine; and *M. persicae,* four of nine. WV was not transmitted by any of the above aphid species in a parallel trial.

In later studies, the WV remained nonaphid-transmissible, regardless of virus source plant used or aphid species tested (Table 1). 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-min virus-access periods. In an identical trial with the FV, however, transmission of FV and BCMV was 20 and 67 of 150 test plants, respectively.

Of the three BYMV isolates tested for mechanical transmissibility, the nonaphid-transmissible WV proved consistently more infectious than either the aphid-transmissible FV or KV, regardless of whether pea or bean tissue was assayed; the FV proved the least infectious of the three isolates (Table 2). WV-infected pea tissue 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 ($P = < .01$).

The number of particles found in negatively stained

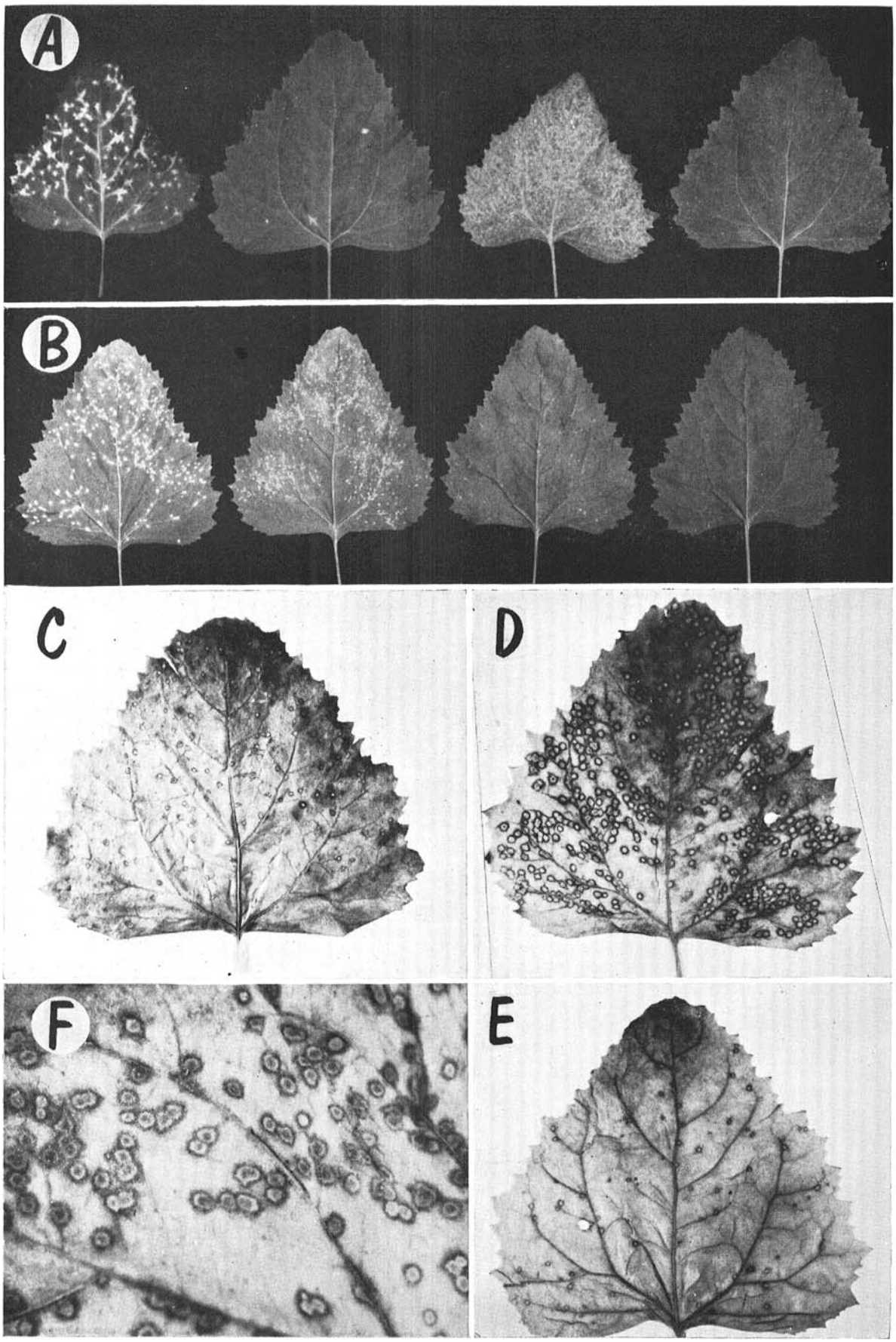
A
B
C
D
F
E

Fig. 1. Local and systemic symptoms on *Chenopodium amaranticolor* induced by each of the three isolates of bean yellow mosaic virus. **A)** Right to left, healthy leaf and systemic symptoms of the Florida, Kentucky, and Wisconsin isolates, respectively; **B)** right to left, healthy leaf and local symptoms on mechanically inoculated leaves induced by the Florida, Kentucky, and Wisconsin isolates, respectively; **C, D, E)** starch-iodine delineated local lesions induced by the Florida, Wisconsin, and Kentucky isolates, respectively; and **F)** magnified view of typical starch-iodine delineated lesions induced by the Wisconsin isolate.

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TABLE 1. Comparative transmissibility of three bean yellow mosaic virus isolates (Florida isolate, Kentucky isolate, and Wisconsin isolate) from different plant species by aphids allowed 3- to 12-min access periods

Aphid species	Virus source plant	Virus isolate FV	KV[b]	WV
Aphis craccivora	pea	12[a]	11	0
		24		0
	bean	1	0	0
		1		0
	Melilotus alba Desr.	7		0
	Crotalaria mucronata	15		0
Acyrthosiphon pisum	pea	9	2	0
	bean	2	0	0
Myzus persicae	pea	6	0	0
	bean	2	0	0

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

[b] The KV proved aphid-transmissible shortly after receipt of this isolate from Kentucky, but lost its ability to be aphid-transmitted in subsequent trials.

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 (Table 3). The results of the aphid and mechanical assays of these same FV- or WV-infected pea leaves corroborated earlier studies: (i) WV-infected tissue proved significantly more mechanically infectious than FV-infected tissue (t value = 5.28; $P = < .01$); and (ii) the WV was never aphid-transmitted, whereas the FV was aphid-transmitted in every instance.

A change similar to that reported by Swenson (11) and Swenson et al. (13) for certain BYMV isolates was noted in the aphid-transmission properties of the KV. After 7 months of routine mechanical transfer of this isolate to pea and bean seedlings at biweekly intervals, a decline and subsequent loss in its aphid transmissibility resulted. After this time, repeated attempts at aphid transmission using at least 300 individuals each of *A. craccivora*, *A. pisum*, and *M. persicae* always failed. This decline in aphid transmissibility of the KV coincided with an apparent increase in the mechanical transmissibility of this isolate (Exp. III-V, Table 2). The changes in the transmission properties, however, were not coincident

TABLE 3. The relation of virus particle numbers to the aphid and mechanical transmissibility of the Florida isolate and Wisconsin isolate

Isolate	Leaf no.[a]	Aphid transmissibility	Mechanical transmissibility	No. 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

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

[b] Number of infected Red Kidney bean test seedlings of four inoculated; each test seedling was inoculated with five individuals of *Aphis craccivora* allowed 3-min access periods on Florida isolate or Wisconsin isolate infected pea leaves.

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

[d] Total number of virus particles on 36 preselected grid squares of 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.

TABLE 2. Comparative mechanical transmissibility of three bean yellow mosaic virus (BYMV) isolates (Florida isolate, Kentucky isolate, Wisconsin isolate) from Alaska pea and Red Kidney bean[a]

Exp. no.	No. leaves inoculated/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 local lesion assays of inoculated and starch-iodine stained leaves of *Chenopodium amaranticolor*.

[b] Average number of lesions per inoculated leaf.

with any appreciable changes in the characteristic symptom expression previously described for the KV in *C. amaranticolor*.

DISCUSSION.—Our study shows that different BYMV isolates can vary significantly in aphid and mechanical transmissibility and in relative numbers of virus particles found in negatively stained leaf-dip extracts.

This study implicates an association of nonaphid transmissibility with a relatively high degree of mechanical transmissibility in support of Swenson's earlier findings (11). Brandes & Bercks (2) 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. The particle sizes of all three BYMV isolates studied herein were similar: the arithmetic mean length of 239, 847, and 517 particles of the FV, KV, and WV isolates were 796, 802, and 781 mμ, respectively (3). Our results therefore suggest an association of nonaphid transmissibility with an occurrence of a relatively high virus titer without an appreciable difference in particle length.

Several workers have suggested that certain stylet-borne virus isolates lost their ability to be aphid-transmitted as a result of spontaneous or induced mutations (1, 4, 11, 12, 13). According to Watson (14) and Badami (1), 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. It is possible, therefore, that the infectious units of the WV and later the KV may have in some way lost their ability to be aphid-transmitted, and that this mutation is coincidental with an increase in virus titer in the host.

The total lack of aphid transmission of the readily mechanically transmissible WV, regardless of host, suggests that the infectious unit transmitted by aphids may differ from the infectious unit transmitted mechanically. In a recent review, Pirone (9) indicated that there is no direct evidence as to whether it is the intact virus or its uncoated nucleic acid that is transmitted by aphids from plants. Working with cucumber mosaic virus, a spherical virus, Pirone & Megahed (10) demonstrated that aphids could acquire and transmit purified preparations of intact virus but not of its uncoated nucleic acid, even though both preparations were of equal mechanical transmissibility. In that same study, however, aphids were unable to transmit readily mechanically transmissible purified preparations of intact turnip mosaic virus, an aphid-transmitted virus with a particle morphology similar to that of BYMV. Our results showed a lack of relation between aphid transmissibility and virus particle counts. Therefore, these results and those of Pirone & Megahed (10) could be interpreted to mean that the stylet-borne flexuous-rod viruses, unlike other stylet-borne viruses such as cucumber mosaic, are transmitted in a form other than intact virus, perhaps as uncoated nucleic acid as suggested by the results of van Hoof (7).

That infectivity based on aphid and mechanical means is not always in agreement is further shown by the results of Normand & Pirone (8). An isolate of cucumber mosaic virus with very low aphid transmissibility but comparable in mechanical transmissibility to two other readily aphid-transmissible isolates was just as aphid transmissible after purification as the other two isolates (8); they concluded that since the properties of the intact virus particle could not account for the lack of transmission, the explanation for this phenomenon must lie in the host-virus relationship.

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