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MECHANICAL TRANSMISSION OF AN APPLE MOSAIC VIRUS

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Virus diseases of fruit trees are rarely transmitted by mechanical means. A virus from apple mosaic has been transmitted mechanically to tobacco, *Nicotiana glutinosa,* tomato, cucumber, globe amaranth, sunflower, broad bean, French bean, cowpea, guar, and pokeweed, but not to apple. Evidence that the virus which was transmitted mechanically was actually apple mosaic rests on the transmission of the infection from tobacco to apple by means of dodder, with symptoms on apple similar to those of the original natural infection.

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MECHANICAL TRANSMISSION OF AN APPLE MOSAIC VIRUS[1]

C. E. YARWOOD[2]

INTRODUCTION

JUICE OR mechanical transmission of virus diseases of woody plants is usually unsuccessful (Cochran and Reeves, 1953)[3] although several reports of such transmission do exist (Grieve, 1931; Christoff, 1935; Moore, Boyle, and Keitt, 1948; Willison, 1951; Varney and Moore, 1952; Fulton, 1952; McWhorter, 1953; Milbrath, 1953; Yarwood, 1953; Brierley, 1954; Yarwood and Thomas, 1954). In some of these reports a virus was transmitted from the woody plant to a herbaceous plant, but transmission back to the woody plant was unsuccessful. The report by Christoff (1935) that apple mosaic was transmitted by juice is regarded with skepticism here and elsewhere (Bawden, 1950). The present report may be the first case where a virus disease of a fruit tree has been unequivocally and readily transmitted by mechanical means.

If a virus is defined as an infective particle with at least one dimension less than 200 mu (Bawden, 1950) then perhaps no virus disease of fruit trees has been established. However, if a mosaic infection reveals no conventional microörganism in microscopic examination and is transmissible by the methods successful with known virus diseases, it is usually thought to be caused by a virus, even though no virus-like particles are detected by aid of the electron microscope. The mosaic disease of apples, described here, is such an infection, and in the absence of specific proof is tentatively assumed to be a virus disease.

SOURCE OF VIRUS

The apple mosaic virus (AMV) studied was from a single infected tree, also showing rough bark, in Tulare County, California. Scions from this tree were grafted to potted apple trees in Berkeley by H. E. Thomas. The inoculated apple trees became systemically infected and showed mild to severe symptoms of mosaic, chlorosis, vein clearing, and ring spotting, but did not

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[3] See "Literature Cited" for citations referred to in the text by author and date.

show rough bark. Symptoms were most pronounced in the flush of growth after dormancy, whereas growth formed late in the season was sometimes free of symptoms. Inoculum for the present study was taken from one of these potted trees. The identity of this with other apple mosaics has not been established, and mechanical inoculations with a similar but different apple mosaic from Butte County were unsuccessful. In appearance Tulare apple mosaic resembles that illustrated by Bradford and Joly (1933) but is quite different from that illustrated by Christoff (1935). At least three strains of apple mosaic are reported in England (Posnette and Cropley, 1952), and probably a similar situation prevails in America.

METHOD OF INOCULATION

A standard method of plant virus inoculation (Rawlins and Takahashi, 1952) is to rub juice from infected plants over the carborundum-dusted upper leaf surface of young leaves of test plants. The methods described here will be variants of this basic method. Per cent concentration of inoculum refers to the per cent by weight of tissue in the final inoculum.

For juice inoculation a few square centimeters of tissue from diseased leaves were ground with a few drops of water in a mortar, and diluted as desired with water or with water solutions of K_2HPO_4 or Na_2SO_3 (Yarwood, 1952*a*). This tissue suspension was stroked over the upper surfaces of test leaves with a stiff brush. For quick tissue inoculations, four 11 mm. diameter disks were cut from infected leaves by means of a cork-borer, stacked one on top of the other, held by means of cover-glass forceps with the cut edges protruding, trimmed to a straight edge with a scalpel, and rubbed over the carborundum-dusted and water-sprayed surface of the suscept (Yarwood, 1953).

For inoculations by means of dodder (Bennett, 1944*b*) *Cuscuta subinclusa* D. and H., and *C. campestris* Yunck growing on systemically infected tobacco were trained on the healthy host. Dodder free of AMV was maintained on healthy tobacco or on broad bean (*Vicia faba* L).

RESULTS

Apple-to-apple Inoculation Unsuccessful. All attempts to transmit AMV from apple to apple or from tobacco to apple by mechanical means were unsuccessful. These included ordinary rubbing with infective juice, using phosphate (Yarwood, 1952*a*), carborundum (Rawlins and Tompkins, 1936), sulfite (Bald and Samuel, 1934), quick tissue-to-tissue inoculation (Yarwood, 1953), quick drying (Yarwood, 1952*b*), and several unconventional treatments. In all, 132 apple seedlings were inoculated by various mechanical methods without success.

Electron Microscopy. No virus-like or microörganism-like particles associated with the infection in apple, tobacco, or bean were consistently detected with the electron microscope or with the conventional microscope.

Graft Transmission. Inoculation by conventional bark grafting is a thoroughly proved procedure in transmitting viruses of woody plants, including apple mosaic (Thomas, 1937). This method is considered outside the scope of

the present study, but was used in one trial for comparing symptoms on apple resulting from dodder transmission. Of four apple seedlings inoculated by inserting the bark of mosaic apples into the cambial region, three showed mosaic within 38 days. The symptoms on these three trees were slightly more severe than those on seedlings inoculated by dodder, but this difference in symptoms due to method of inoculation is not now regarded as significant.

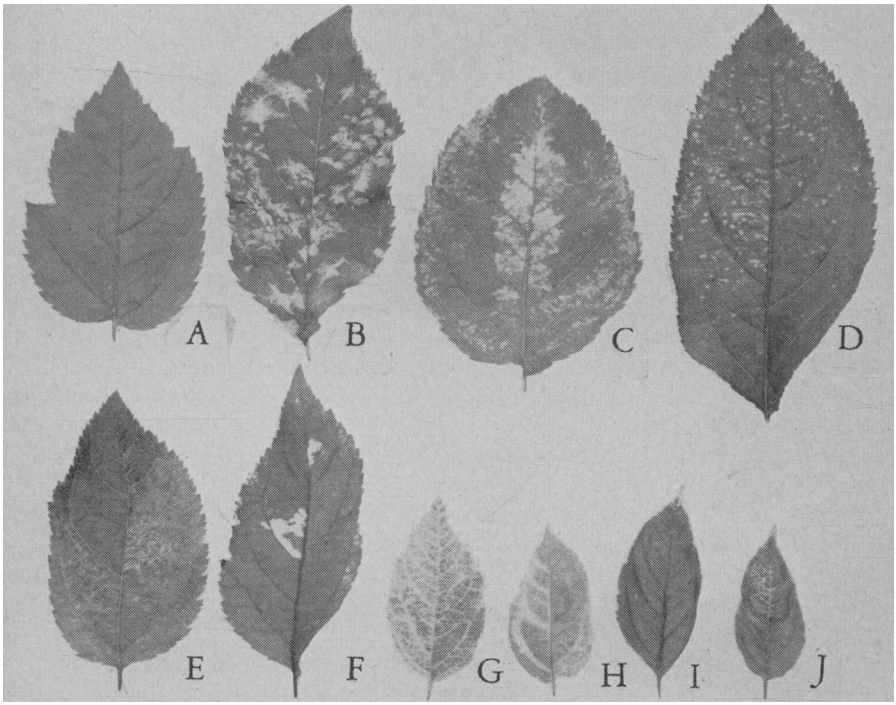

Fig. 1. Apple mosaic virus in apple: *A*, healthy leaf of apple seedling; *B*, mosaic from graft inoculation from apple to Golden Delicious; *C*, mosaic from graft inoculation from apple to Newtown; *D, E, F*, mosaic from transmission by *Cuscuta subinclusa*. Each leaf is from a different apple seedling which was trained with dodder growing on tobacco systematically infected with apple mosaic virus; *G, H, I, J*, mosaic from graft transmission to apple seedlings. Each leaf is from a different apple seedling which was inoculated with apple mosaic by means of a bark graft.

Identity of the Virus in Tobacco as Apple Mosaic Virus. Since tobacco presumed to be infected with apple mosaic virus was the source of virus for most of the studies, evidence that the infected tobacco actually contained apple mosaic virus will be presented first. *Cuscuta subinclusa* growing on tobacco which had developed symptoms after mechanical inoculation with apple mosaic was trained on healthy apple seedlings. Of eight seedlings thus colonized January 20 to February 2, 1954, all produced symptoms of apple mosaic (fig. 1). The shortest period from inoculation (training of dodder) to first appearance of symptoms was 11 days; the longest period was 25 days; and the average period was 18 days. Quick tissue-to-tissue inoculation of

bean with leaves from these apple seedlings yielded lesions typical of those resulting from inoculations from the original apple tree. Bark graft inoculations from these apple seedlings to healthy apple seedlings yielded typical apple mosaic on five out of six trees. Five apple seedlings trained with AMV-free dodder growing on healthy tobacco did not develop symptoms. Ten uninoculated apple seedlings did not become infected. No accidental infection with apple mosaic virus on apple or any other host was observed during this study.

Inoculations made during the period February 2 to February 22 on apple seedlings by means of *Cuscuta subinclusa* were not uniformly successful. Of 17 seedlings inoculated, only six showed clear symptoms of mosaic. The poor success of these inoculations is believed to be due to the greater age and slower growth rate of these inoculated apple seedlings than of those inoculated earlier.

The *Cuscuta subinclusa* used in the January 20 to February 22 inoculations, by training on broad bean, was later shown to contain a latent virus, believed to be tomato spotted wilt virus and different from that described by Bennett (1944*a*). Another strain of *C. subinclusa,* collected August 5, 1954, at Los Altos, California, and as yet showing no evidence of carrying spotted wilt virus, was therefore tested as a vector of apple mosaic. Of 10 apple seedlings trained with this dodder growing on AMV-infected tobacco, seven showed symptoms of apple mosaic within 24 days. Bountiful beans inoculated with tissue from two of these infected apples showed local lesions typical of apple mosaic virus.

Two out of five apple seedlings inoculated by means of *Cuscuta campestris* growing on tobacco infected with AMV became systemically infected. The *C. campestris* was later shown to be carrying the same latent virus or one similar to that carried by *C. subinclusa,* but no dodder latent virus has been shown to produce symptoms on apple.

Bean as an Assay Host. When young primary unifoliate leaves of Bountiful or Pencil Pod bean (*Phaseolus vulgaris*) were suitably inoculated with a source of AMV, necrotic local lesions usually formed in one day. The formation of these lesions (fig. 2) was the criterion usually used for the presence of and concentration of AMV in an active form in apple, tobacco, and other hosts. No lesions resulted from inoculation of beans with juice or tissue of healthy apple or tobacco.

Inoculations from bean to bean were usually unsuccessful, but if AMV-infected rusted bean was used as the test source the virus could be readily recovered. Bountiful bean leaves which were inoculated with rust (*Uromyces phaseoli* (Pers.) Wint.) on one side of the midrib were inoculated over their entire upper surfaces with AMV from apple. The lesions produced on the rusted half of the leaf were larger and less necrotic than those produced on the nonrusted half (fig. 2) (Yarwood, 1951). When these lesions on rusted bean were used as inoculum on healthy Bountiful beans, 71 lesions typical of AMV resulted in one test, while in comparable inoculations with local lesions from nonrusted bean as inoculum, only one lesion resulted. Inoculations of AMV from rusted bean to tobacco resulted in the same symptoms as inoculations direct from apple to tobacco.

The size of the AMV lesions on Pencil Pod bean appeared to vary with the source of virus in some tests. In two trials, inoculum from the youngest, healthiest-appearing apple leaves from shoots showing symptoms on the

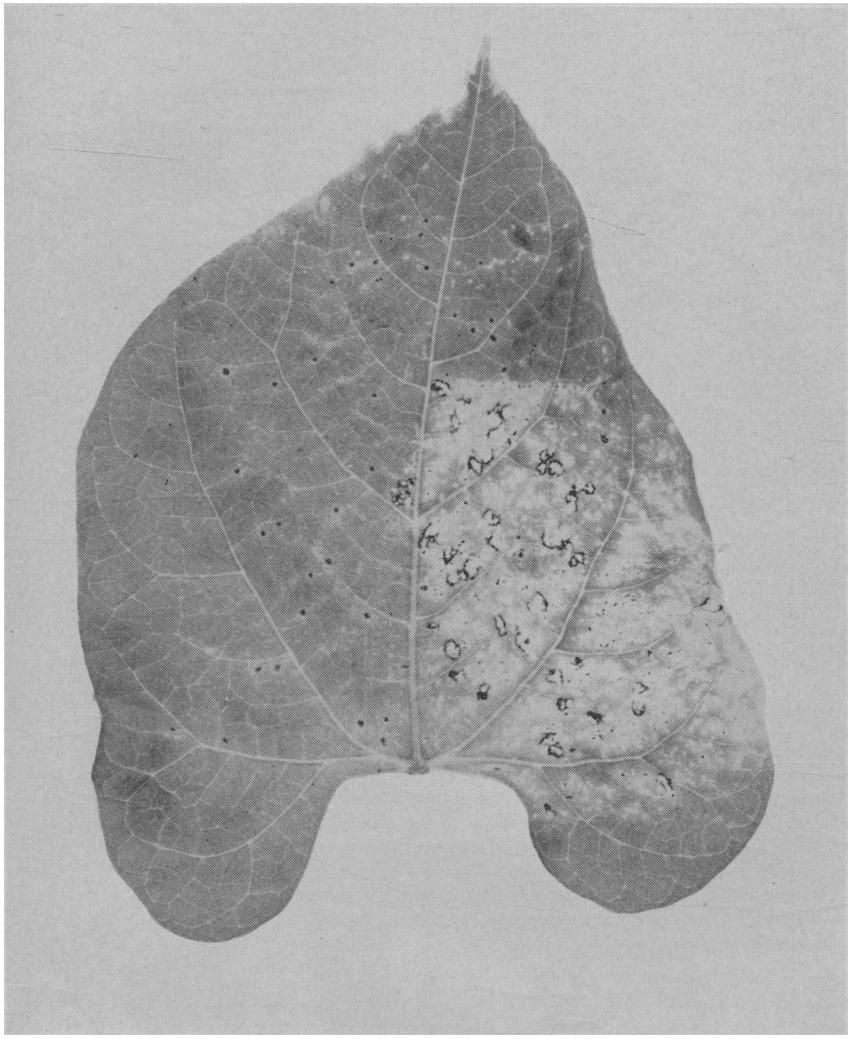

Fig. 2. Relation of rust to apple mosaic virus on Bountiful bean. Right side of leaf was heavily inoculated with rust on June 7; entire leaf was inoculated with apple mosaic on June 8. (Photographed June 12, 1953.)

basal leaves yielded larger lesions on Pencil Pod than did inoculum from tobacco. In one trial the lesions formed by inoculum from vigorously growing, systemically infected tobacco were larger than those formed by inoculum from slow-growing tobacco (fig. 3*A*).

Effectiveness of Sources of Inoculum. While apple was necessarily the original source of inoculum, inoculum from infected tobacco and cucumber yielded more lesions than inoculum direct from apple in most later trials. In all trials the number of lesions per bean leaf from comparable mechanical inoculations of juice from infected apple, dodder, cucumber, and tobacco

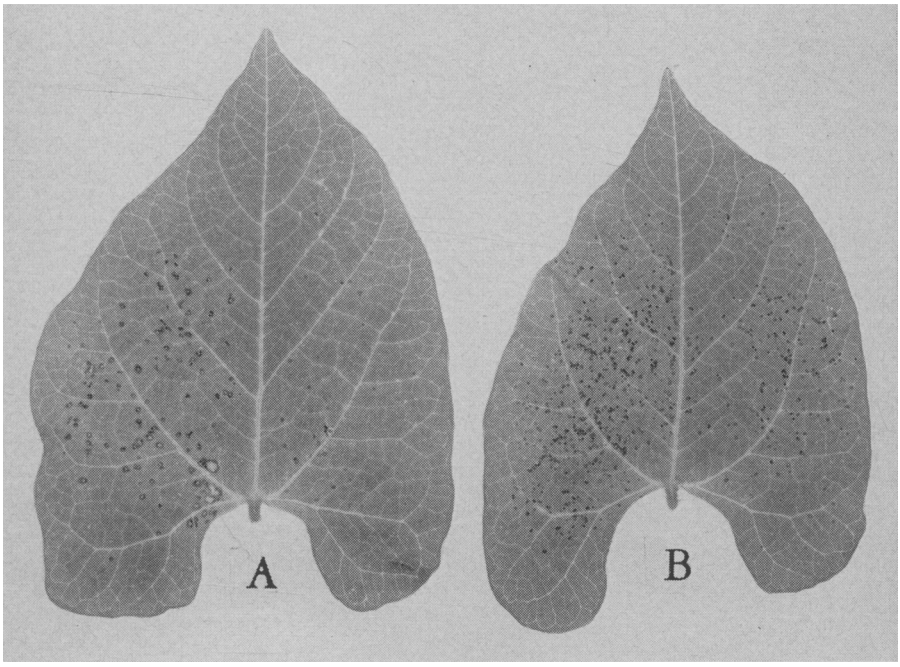

Fig. 3. Apple mosaic virus on Pencil Pod bean. February 3, 1954, result of February 1, 1954, inoculation by quick tissue method: *A*, left, inoculum from recovered leaf of tobacco plant inoculated August 23, 1953, and showing vigorous growth on February 1; *A*, right, inoculum from recovered leaf of tobacco plant inoculated August 23, 1953, but showing poor chlorotic growth on February 1. Lesions resulting from inoculum from the vigorous tobacco are larger and more numerous than those from the chlorotic tobacco. *B*, left, inoculum from shock leaf of tobacco inoculated January 26, 1954; *B*, right, inoculum from inoculated leaf of tobacco inoculated January 26. These lesions are of the same age, but are much smaller than those on leaf *A*.

were 7, 11, 41, and 166, respectively. Young, succulent symptom-free apple leaves of mosaic plants were a richer source of virus than were older leaves expressing symptoms. In one representative test using comparable quick tissue inoculations, the youngest apple leaves which showed only indistinct symptoms yielded 57 lesions, whereas leaves only slightly older, but which showed clear mosaic symptoms, yielded only four lesions. In another test using juice in phosphate, inoculum from the youngest leaves with no symptoms yielded 27 lesions, whereas leaves only slightly older but with good symptoms yielded no lesions.

Inoculation with juice of AMV-free *Cuscuta subinclusa* and *C. campestris* frequently yielded local lesions on Pinto, Bountiful, and Pencil Pod beans,

which were much larger than those of AMV. These lesions, believed to be caused by the tomato spotted wilt virus, can be readily distinguished from those of AMV.

Quick Inoculation. Rubbing carborundum-dusted, K_2HPO_4-sprayed leaves with cut edges of leaf pieces of a donor host has been a useful technique in securing infection with apple mosaic (Yarwood, 1953). This technique was adopted because of frequent failures in early trials to secure transmission by ordinary juice inoculation methods. During the early trials apple leaf juice or tobacco leaf juice was believed to inhibit virus activity, and the ground virus tissue was usually diluted to about 1 to 100 with water or phosphate solution to secure a compromise between the dilution of the virus and the dilution of inhibitors. The *in vitro* inhibition of virus activity by leaf juice was overestimated, however, and in several subsequent trials the number of lesions from the juice of infected apple or tobacco leaves has increased progressively with the concentration of the juice. In a total of 17 trials with inoculum from apple or tobacco where the juice versus quick tissue method was believed to be the only contrasting variable, but where the concentration of the juice has varied, 5,453 lesions resulted from juice inoculation, and 13,662 lesions resulted from quick tissue inoculation of the same number of bean leaves.

Differences between trials also included speed of drying of inoculated leaves, washing of inoculated leaves, species and age of donor host source, species and age of assay host, time from preparation of juice inoculum to use, time of day, kind of weather, and chemical supplements to inoculum. The role of these factors in the success of quick tissue inoculation versus juice inoculation has not been adequately studied, but their role as separate variables has been shown to be important in several cases.

The Dilution Effect. Inoculations with juice from mosaic apple leaves were quantitatively so erratic that no satisfactory data on the effect of dilution on the infectivity of apple juice have been secured. With juice from tobacco infected with AMV, the number of lesions on bean from 0.4, 2, 10, and 50 per cent juice in water were 47, 59, 199, and 807, respectively, in a typical trial.

The Quick-drying Effect. Quick drying after inoculation increased infection with tobacco mosaic virus, spotted wilt virus, and tobacco necrosis virus (Yarwood, 1952*b*). With apple mosaic virus, the effect of quick drying was difficult to measure in ordinary juice inoculations because such inoculations, especially with inoculum from apple, were so often unsuccessful. In one composite trial with inoculum from tobacco (table 1) the per cent increase in infection from quick drying was 1,200 with leaf juice in water, 150 with quick tissue inoculation with water, 270 with leaf juice in 0.1 per cent Na_2SO_3, and 280 with quick tissue-to-tissue inoculation plus Na_2SO_3. No significant increase in infection resulted from quick drying when K_2HPO_4 was used in the inoculum.

The quick-drying effect was much greater with dilute than with concentrated inoculum. In one trial with 0.3 per cent inoculum in water the number of AMV lesions on two bean leaves was 18 for ordinary inoculation and 98 for quick-dried inoculation, whereas with 30 per cent inoculum the number

of lesions was 490 for the ordinary inoculation, and 510 for the quick-dried inoculation. In another trial with 0.2 per cent inoculum, 80 lesions developed on the leaves which dried slowly, whereas 681 lesions developed on the same number of quick-dried leaves.

The Phosphate Effect. The addition of K_2HPO_4 to the inoculum resulted in greater increases in infection with several viruses (Yarwood, 1952*a*) than

Table 1

EFFECT OF QUICK DRYING AND CHEMICALS ON MECHANICAL INOCULATION WITH APPLE MOSAIC VIRUS*

Inoculation method	Post-inoculation treatment and number of lesions per leaf	
	None	Quick dried§
Standard juice method†		
Tissue suspended in water	1	13
Tissue suspended in 0.5 per cent K_2HPO_4	72	51
Tissue suspended in 0.2 per cent Na_2SO_3	28	104
Quick tissue method‡		
Leaves pretreated with water	61	152
Leaves pretreated with 0.5 per cent K_2HPO_4	379	479
Leaves pretreated with 0.2 per cent Na_2SO_3	41	155

* Inoculum from tobacco, inoculations on bean; carborundum used throughout.
† 1 per cent suspension of ground tobacco leaves brushed over bean leaves.
‡ Disks of tobacco leaves rubbed directly over bean leaves.
§ Leaves held in blast from compressed air outlet, and all visible free water removed within 5 seconds of inoculation.

any other modifications of the standard inoculation procedure tested. With AMV the increase in infection due to phosphate (table 1) was much greater in juice inoculations than with quick tissue inoculations, and much greater when the leaves were allowed to dry naturally after inoculation than when quickly dried by an air blast. In all 10 trials of the phosphate effect in which infection occurred, only 270 lesions developed on leaves inoculated without phosphate, and 1,832 lesions on the same number of twin leaves inoculated with phosphate. In four of the 10 trials no infection occurred on the leaves inoculated without phosphate.

The Sulfite Effect. Bald and Samuel (1934), well supported by experimental evidence, were perhaps the first to show that the addition of Na_2SO_3 to the inoculum increased the longevity of the relatively unstable spotted wilt virus they were studying. That this may not be the entire explanation is indicated by the great increase in infection resulting from the addition of sulfite to inoculum of the relatively stable tobacco mosaic virus (Yarwood, unpublished). Regardless of the interpretation, sulfite greatly increased the infectivity of AMV in juice inoculations but not in quick tissue inoculations (table 1).

In juice inoculations, K_2HPO_4 and Na_2SO_3 together added to the inoculum brought about a greater increase in infectivity than either added alone. In

seven trials the number of lesions on comparable bean leaves was 157 for inoculum suspended in water, 1,572 for inoculum suspended in 0.5 per cent K_2HPO_4, 725 for inoculum suspended in 0.5 per cent Na_2SO_3, and 2,329 for inoculum suspended in 0.25 per cent K_2HPO_4 + 0.25 per cent Na_2SO_3. In all but one of the trials the infection from inoculum with phosphate plus sulfite was greater than for phosphate alone. Several concentrations and ratios of phosphate and sulfite were tested. At the optimum concentration of phosphate (about 1 per cent) or of sulfite (about 0.5 per cent) the addition of the other chemical increased infection. This indicated that the two chemicals acted independently in part, although their separate effects on infection were not additive in combination.

The Shade Effect. Shade-induced susceptibility to plant viruses has been studied by Samuel and Bald (1933). In the present study, inoculations were usually made in the early morning. A few inoculations made around midday and late afternoon were less successful, but a study of time of day of inoculation was not made. In one case, in bright weather the number of lesions resulting from inoculations on bean plants exposed to the normal greenhouse environment was six, while the number on comparable plants which had been held in darkness for 23 hours prior to inoculation was 128.

Longevity in-Vitro. Juice inoculations from apple to bean were usually unsuccessful. The maximum period from preparation of apple leaf juice that would still permit successful inoculation was 8 minutes when neither phosphate nor sulfite was added to the inoculum. With inoculum from tobacco, juice inoculations were more regularly successful than from apple. In one representative trial the numbers of lesions resulting from inoculation of two bean leaves with a 30 per cent virus suspension from tobacco were 139 at 0.5 minutes; 37 at 6 minutes; 7 at 12 minutes; 13 at 18 minutes; and 0 at 25 minutes. The half-life of the virus (time for half the virus to be inactivated) in this trial was thus about 6 minutes. In all nine successful trials the half-life of the virus ranged from 0.5 to 10 minutes and averaged 4.8 minutes.

Host Range and Symptoms. Hosts and symptoms of apple mosaic virus infections observed in this study were as follows:

Host	Symptoms
Apple, *Malus sylvestris* Mill.	Systemic mosaic, vein clearing, occasional ring spot, chlorosis.
Tobacco, *Nicotiana tabacum* L.	Local etched rings, systemic necrotic shock, recovery with abundant virus.
Nicotiana glutinosa L.	Chlorotic local lesions, systemic mosaic.
Tomato, *Lycopersicon esculentum* Mill.	Large chlorotic to necrotic local lesions, systemic die-back.
Strawberry, *Fragaric vesca* var. *alpina*	Systemic mosaic, stunting.
Pokeweed, *Phytobacca americana* L.	Large chlorotic local lesions.
Sunflower, *Helianthus annuus* L.	Necrotic local lesions on some resistant plants, systemic mosaic and ring spot patterns, recovery with virus.
Globe amaranth, *Gomphrena globosa* L.	Chlorotic to necrotic local lesions, systemic vein banding, recovery with virus.
Cucumber, *Cucumis sativus* L.	Chlorotic local lesions on cotyledons, systemic mosaic.

Broad bean, *Vicia faba* L.	Large necrotic local lesions.
Bean, *Phaseolus vulgaris* L.	Small necrotic local lesions on some varieties.
Cowpea, *Vigna sinensis* (Torner) Savi.	Small necrotic local lesions.
Guar, *Cyamopsis tetragonoloba* (L.) Taub.	Small necrotic local lesions.
Dodder, *Cuscuta subinclusa* D. and H.	None recognized.
Dodder, *Cuscuta campestris* Yunck	None recognized.

Apple mosaic virus has been transmitted mechanically from apple to tobacco, cucumber, tomato, bean, and cowpea; from tobacco to tobacco, cucumber, bean, cowpea, broad bean, sunflower, *Nicotiana glutinosa, Gomphrena globosa,* and guar; and has been transmitted mechanically from apple, strawberry, tobacco, cucumber, sunflower, *Nicotiana glutinosa,* and rusted bean to bean. Mechanical inoculations from apple to apple and barley yielded no symptoms. Inoculations of bean with apple leaves which had been previously inoculated mechanically with apple mosaic virus were unsuccessful. The virus has been transmitted by *Cuscuta subinclusa* from tobacco to apple, strawberry, tobacco, and sunflower. Dodder inoculations to peach, apricot, and onion were unsuccessful. The following bean varieties yielded local lesions when inoculated by the quick tissue-to-tissue method (Yarwood, 1953) with AMV-infected tobacco leaves: Bountiful, Brittle Wax, Dwarf Horticultural, Large White Marrowfat, Pencil Pod, Red Kidney, and Top Notch. The following bean varieties yielded no symptoms: Black Valentine, Dwarf Small White, Golden Cluster, Great Northern, Kentucky Wonder Wax, Old Homestead, Refugee, and Striped Scotia.

Ordinary Pinto bean leaves were usually immune to AMV, but rusted Pinto leaves were highly susceptible. In one test, inoculation on four otherwise healthy halves of primary leaves of Pinto bean produced no lesions, whereas 66 lesions appeared on the rusted halves of the same leaves. It seems likely that some of the other bean varieties listed as resistant to AMV would be susceptible if inoculations were made on rusted tissue.

Infections sometimes became systemic in apple, tomato, cucumber, tobacco, sunflower, *Cuscuta subinclusa, C. campestris,* and *Gomphrena globosa,* but remained local in bean, cowpea, guar, and Phytolacca. *Cuscuta subinclusa* and *C. campestris* acquired the virus from systemically infected tobacco but showed no symptoms.

Inoculations on tobacco yielded etched rings on the inoculated leaves. These increased in size for several days and were zonate in appearance (fig. 4). A severe shock effect manifested as a systemic necrosis, usually appeared about three days after the primary lesions. The shock effect usually involved about three leaves, and new growth after the shock effect showed no symptoms.

Inoculation to cucumber seedlings by quick tissue or juice method yielded chlorotic local lesions, followed by systemic infection (fig. 5) and sometimes death. The severity of infection on cucumber plants decreased markedly as the age of the cucumber plants at inoculation was increased (see also Boyle, Moore, and Keitt, 1954). The symptoms of AMV on cucumber appear to differ mainly in a quantitative way only from symptoms on cucumber

produced by alfalfa mosaic virus, tobacco ring spot virus, a ring spot virus from apricot, and a virus from peach yellow bud mosaic (fig. 5).

Inoculation of AMV by the quick tissue method to tomato, cucumber, tobacco, and Gomphrena usually resulted in systemic infection of every plant inoculated, but inoculations to sunflower usually did not result in systemic

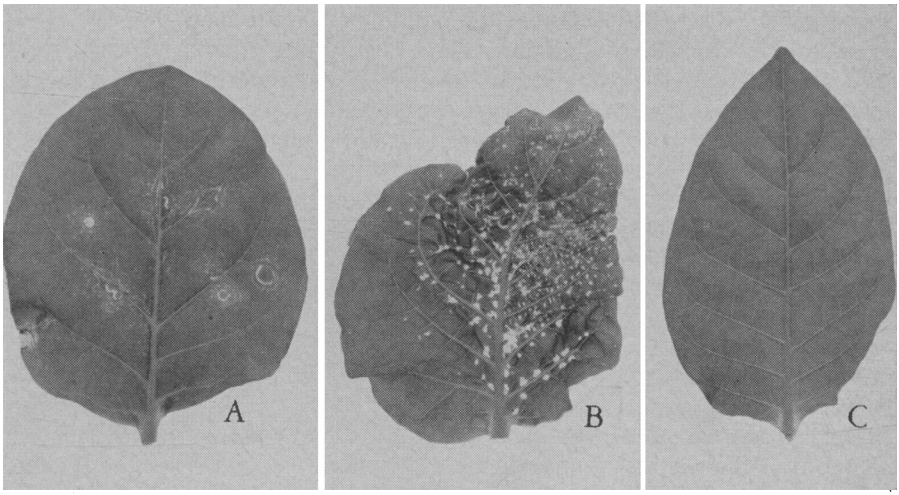

Fig. 4. Symptoms of apple mosaic virus in Turkish tobacco: *A*, local lesions on inoculated leaf; *B*, shock symptoms on fourth leaf above inoculated leaf; *C*, recovery without symptoms on eighth leaf above inoculated leaf. (Photographed 15 days after inoculation.)

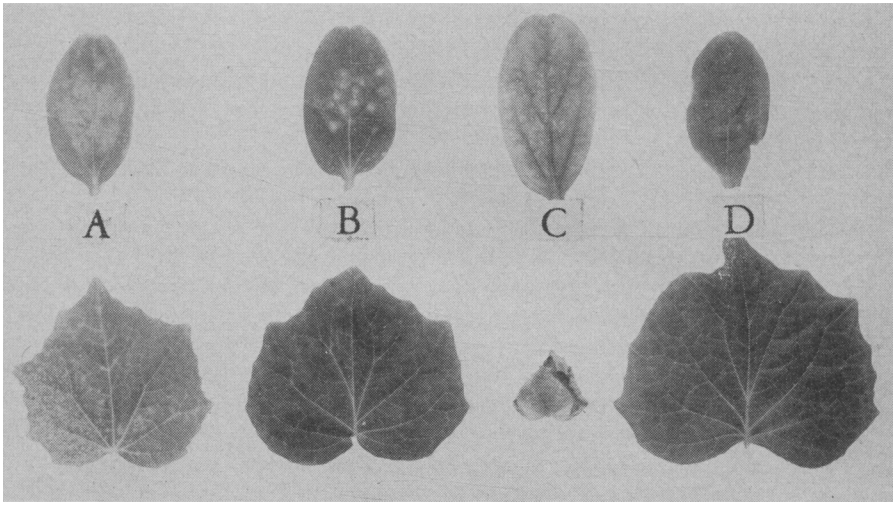

Fig. 5. Comparative behavior on cucumber of three tree viruses. In the upper row are the inoculated cotyledons, and in the lower row are the first true leaves of the same plants: *A*, inoculated with apple mosaic virus; *B*, inoculated with a virus from peach yellow bud mosaic; *C*, inoculated with a virus from ring spot on apricot; *D*, not inoculated. (All were photographed June 24, at 10 days after inoculation.)

infection. Of a total of 187 sunflowers inoculated in eight trials during 10 months, only 25 became systemically infected. In later trials systemic infection was observed to result only on plants which did not show local necrotic lesions on the inoculated leaves. These local necrotic lesions were never numerous (a maximum of 10 was observed on the two inoculated leaves of one plant) and many plants showed neither necrotic lesions nor systemic infection. It is therefore believed that the sunflower population consisted of resistant and susceptible individuals. Of the susceptible individuals some were partially resistant. One type of resistance was sometimes manifested by the formation of necrotic lesions. This is similar to the situation with southern bean mosaic in bean, where some varieties (for example, Pinto) react with necrotic local lesions without systemic infection, and some (for example, Bountiful) react with systemic infection without necrotic local lesions.

In-arch grafting of systemically infected sunflowers with sunflowers on which mechanical inoculation had failed to produce systemic infection failed to transmit AMV in the three pairs of plants tested. This further supports the idea that most sunflower plants are resistant to systemic infection.

Graft transmission of AMV from tobacco to tobacco yielded symptoms similar to those resulting from mechanical inoculations, except that in graft inoculation there were no local symptoms.

Of 21 apples inoculated by inserting stem or leaf tissue of AMV-infected tobacco into the cambial region of the apple seedlings, one became infected.

Comparison of AMV with Tobacco Ring Spot Virus. The zonate primary lesions, shock symptoms, and recovery of tobacco inoculated with AMV as observed in this study suggested that AMV might be related to tobacco ring spot virus (TRSV) as observed by Price (1932, 1935). AMV and TRSV were therefore compared directly. While the similarities of AMV and TRSV in bean and tobacco are impressive, a statement of differences may be more appropriate here:

1) The local lesions formed by AMV on the primary leaves of Bountiful and Pencil Rod beans were much smaller than those formed by TRSV on these same varieties.

2) AMV never produced systemic infection on bean, whereas TRSV usually did.

3) The new growth of infected tobacco following the shock symptoms was usually symptom free with AMV, but only partially so with TRSV.

4) Inoculum from the new growth of recovered AMV-infected Turkish tobacco yielded more lesions on bean than did inoculum from leaves bearing shock symptoms or inoculated leaves. In 10 trials on different days with different sources of inoculum, and with a total of 11,607 lesions counted, the average number of lesions from inoculum from shock leaves and from leaves of recovered growth respectively was 1.5 and 1.8 times the number of lesions from inoculated leaves of the same plants. This may be an important difference, because Price (1935) found that with TRSV the amount of virus in the recovered tissue was distinctly less than in the primary local lesions. This difference between AMV and TRSV might indicate that the basis of acquired immunity may be basically different for these two viruses. However, Fulton

(1949) found that with Havana 38 tobacco the concentration of TRSV was as great in recovered (tolerant) as in necrotic (shock) leaves.

5) AMV was less stable in a water suspension than was TRSV. In comparable tests the half-life (time for half of the virus to be inactivated as measured by assay on bean) of AMV was about 5 minutes, while that of TRSV was about 16 hours.

6) In electron micrographs, spherical particles were found consistently associated with TRSV infections of tobacco, but not with AMV infections.

7) Cross-protection trials by the methods of Price (1932) indicated that AMV and TRSV were not closely related. When inoculated with TRSV, tobacco systemically infected with AMV yielded typical local lesions of TRSV, and later, systemic infection, and conversely. Both viruses were recovered from the above types of mixed infections.

Parallel host range comparisons of AMV with viruses from plum rough bark, peach yellow bud mosaic, Himalaya blackberry mosaic, and apricot ring spot, all of which were infections on cucumber, indicated that AMV is distinct from these viruses.

Comparison of AMV with Alfalfa Mosaic Virus. The similarity in host range of apple mosaic virus and alfalfa mosaic virus (Price, 1940) might suggest that these two viruses are related. They were directly compared in this study. Both attacked bean, tobacco, tomato, and cucumber with differential symptoms on each host. When tobacco plants systemically infected with AMV were inoculated with alfalfa mosaic virus, typical symptoms of alfalfa mosaic resulted in several cases. When tobacco plants systemically infected with alfalfa mosaic virus were inoculated with AMV, typical symptoms of AMV resulted in most instances. The finding of several cases where symptom expression of AMV in tobacco plants previously infected with alfalfa mosaic virus was slower than in previously healthy tobacco is believed to be because tobacco plants weakened by any factor are slow to show symptoms of AMV. It is concluded that AMV and alfalfa mosaic virus are distinct from each other.

Comparison of AMV with Tobacco Streak Virus. The writer's present opinion that the apple mosaic discussed here is caused by a strain of tobacco streak virus is supported by the following evidence:

1) The similarity in shock, recovery symptoms, and acquired immunity of AMV to those illustrated and described by Johnson (1937) for tobacco streak virus (fig. 4).

2) The similarity in the lability of AMV (half-life of 5 minutes) to that of tobacco streak virus (half-life of 7 minutes) as reported by Fulton (1948).

3) The similarly greater concentration of virus in recovered than in inoculated tobacco leaves of tobacco plants affected with tobacco streak (Fulton, 1949) and with apple mosaic virus (this study).

4) The apparent similarity of host range of tobacco streak virus (Fulton, 1948) to host range of apple mosaic virus. Both viruses attack tobacco, cucurbits, bean, and guar with similar symptoms. Tobacco streak virus has been transmitted to three species in the Rosaceae. Unfortunately, apple mosaic virus and tobacco streak virus have not been directly compared.

SUMMARY

A virus-like infection from a mosaic apple was transmitted mechanically to tobacco, *Nicotiana glutinosa,* tomato, cucumber, globe amaranth, sunflower, broad bean, French bean, cowpea, guar, and pokeweed with characteristic symptoms. It was acquired by dodder (*Cuscuta subinclusa* and *C. campestris*) without symptoms, and was transmitted by means of dodder from tobacco to apple, strawberry, and tobacco. Mechanical inoculations from apple to apple were unsuccessful. Inoculations on Pencil Pod and Bountiful bean resulted in characteristic local lesions, and these beans were used as assay hosts to measure the concentration of infective virus. Infection on bean was favored by quick tissue inoculation, by quick drying of inoculated leaves, by the addition of K_2HPO_4 and Na_2SO_3 to the inoculum, by rust infection, and by shade treatment of the beans before inoculation. The half-life of the virus in water suspension was about 5 minutes. Inoculations on tobacco resulted in large etched rings on the inoculated leaves, followed by a systemic necrosis of the new growth and then by new growth without symptoms. The new symptom-free growth of tobacco was immune to further inoculation and contained more virus than the inoculated tobacco leaves, or more than any other source of the virus tested. Cross-protection tests on tobacco have shown the virus to be different from tobacco ring spot virus and alfalfa mosaic virus. On the basis of published accounts of tobacco streak virus, but in the absence of direct comparison, this apple mosaic virus is believed to be the tobacco streak virus.

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LITERATURE CITED

BALD, J. G., and GEOFFREY SAMUEL
1934. Some factors affecting the inactivation rate of the virus of spotted wilt. Ann. Appl. Biol. **21**: 179–80.

BAWDEN, F. C.
1950. Plant viruses and plant virus diseases. 335 p. Chronica Botanica, Waltham, Mass.

BENNETT, C. W.
1944*a*. Latent virus of dodder and its effect on sugar beet and other plants. Phytopathology **34**: 77–91.
1944*b*. Studies of dodder transmission of plant viruses. Phytopathology **34**: 905–32.

BOYLE, J. S., J. DUAIN MOORE, and G. W. KEITT
1954. Cucumber as a plant host in stone fruit virus research. Phytopathology **44**: 303–12.

BRADFORD, F. C., and LLOYD JOLY
1933. Infectious variegation in the apple. Jour. Agr. Res. **46**: 901–08.

BRIERLEY, PHILIP
1954. Symptoms in the florists' hydrangea caused by tomato ringspot virus and an unidentified sap-transmissible virus. Phytopathology **44**: 696–99.

CHRISTOFF, A.
1935. Mosaikfleckigkeit, Chlorose und Stippenfleckigkeit bei Apfeln, Birnen und Quitten. Phytopath. Ztschr. **8**: 285–96.

COCHRAN, L. C., and E. L. REEVES
1953. Virus diseases of stone fruits. U. S. Dept. Agr. Yearbook of Agr.: 714–21.

FULTON, ROBERT W.
1948. Hosts of the tobacco streak virus. Phytopathology **38**: 421–28.
1949. Virus concentration in plants acquiring tolerance to tobacco streak. Phytopathology **39**: 231–43.
1952. Mechanical transmission and properties of rose mosaic virus. Phytopathology **42**: 413–16.

GRIEVE, B. J.
1931. "Rose wilt" and "dieback." A virus disease of roses occurring in Australia. Austral. Jour. Exp. Biol. and Med. Sci. **8**: 107–21.

JOHNSON, JAMES
1937. An acquired partial immunity to the tobacco streak disease. Wisconsin Acad. Sci. Arts, Letters Trans. **30**: 27–34.

MCWHORTER, FRANK P.
1953. The utility of flower tissues for making inocula for difficult virus inoculations. Phytopathology **43**: 479. (Abstract)

MILBRATH, J. A.
1953. Transmission of components of the stonefruit latent virus complex to cowpea and cucumber from cherry flower petals. Phytopathology **43**: 479–80. (Abstract)

MOORE, J. DUAIN, J. S. BOYLE, and GEO. KEITT
1948. Mechanical transmission of a virus disease to cucumber from sour cherry. Science **108**: 623–24.

POSNETTE, A. F., and R. CROPLEY
1952. A preliminary report on strains of the apple mosaic virus. East Malling [Kent.] Res. Sta. Ann. Rpt. 1951: 128–30.

PRICE, W. C.
1932. Acquired immunity to ring-spot in Nicotiana. Boyce Thompson Inst. Contrib. **4**: 359–403.
1935. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopathology **26**: 503–29.
1940. Comparative host ranges of six plant viruses. Amer. Jour. Bot. **27**: 530–41.

RAWLINS, T. E., and W. N. TAKAHASHI
1952. Technics of plant histochemistry and virology. 125 p. National Press, Millbrae, Calif.
RAWLINS, T. E., and C. M. TOMPKINS
1936. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology. **26**: 578–87.
SAMUEL, GEOFFREY, and J. C. BALD
1933. On the use of primary lesions in quantitative work with two plant viruses. Ann. Appl. Biol. **20**: 70–99.
THOMAS, H. EARL
1937. Apple mosaic. Hilgardia **10**(14) : 581–88.
VARNEY, E. H., and J. D. MOORE
1952. Strains of tomato ringspot virus from American elm. (Abstract) Phytopathology **42**: 476–77.
WILLISON, R. S.
1951. The effects of some stone-fruit viruses on cucumber. U. S. Dept. Agr. Plant Dis. Rpt. **35**: 254–55.
YARWOOD, C. E.
1951. Associations of rust and virus infections. Science **114**: 127–28.
1952*a*. The phosphate effect in virus inoculations. Phytopathology **42**: 137–43.
1952*b*. Deleterious action of water in plant virus inoculations. Nature **169**: 502.
1953. Quick virus inoculation by rubbing with fresh leaf disks. U. S. Dept. Agr. Plant Dis. Rpt. **37**: 501–02.
YARWOOD, C. E., and H. E. THOMAS
1954. Mechanical transmission of 3 fruit tree viruses. Phytopathology **44**: 511 (Abstract)

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