TOP NECROSIS

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W. E. C.

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INTRODUCTION

Guar, Cyamopsis psoraloides DC., is a native of India where it is extensively grown as a food and forage crop. It has been grown experimentally in the United States since 1903 (U.S.D.A., 1937). It has recently become of importance as a source of mucilaginous material found in the seed. It normally yields between 500 and 1000 pounds of seed per acre in California and Arizona, where most of the commercial crop (about 2,000 acres) is grown (McKee, 1944).

In July, 1944, an undescribed disease was observed by K. Starr Chester, producing necrosis in an experimental planting of guar in Stillwater, Oklahoma. His preliminary infection experiments indicated that the disease was due to a virus. A search of the literature failed to disclose a disease of this nature affecting this crop. Following preliminary studies the disease was reported as a new virus of guar (Chester and Cooper, 1944), and was later given the name of "top necrosis" (Weiss, 1945).

As reported by Chester and Cooper (1944) the disease was causing an estimated 75 per cent loss. Symptoms consisted of vein-clearing, wilting, early stipple necrosis, and abscission of young leaves, necrosis of growing point and stem, chlorosis and abscission of older leaves, and severe stunting, followed by death. The virus is mechanically transmissible to bean, cowpea, soybean, mungbean, petunia, and tobacco, N. tabacum L. It does not infect N. glutinosa, L. It is not destroyed by field temperatures of 42° - 45° C. for several days. The infection is masked in hot weather with spectacular return of symptoms in the fall. Symptoms in petunia and tobacco differ from those of tobacco ring spot.

There was less than 0.1% infection when the disease was first observed on July 7; however, by September 27, approximately 40% of the plants were showing severe symptoms and a total of 90% were showing definite necrotic symptoms. (Fig. 1). Those plants that were severely infected had completely ceased bearing, and the immature pods present when necrosis began either shrivelled or were decayed by secondary pathogens. There is a close relationship between the date of infection and the yields, since a large part of the seeds of healthy plants are set after fall rains begin (U.S.D.A., 1917). The yield of the plot under consideration was so low that no harvest was made.

This study was undertaken with the object of identifying the virus causing the disease, by determining its host range and symptoms on those plants infected, and ascertaining its infectivity, transmissibility, thermal inactivation point, dilution end-point, and other biological characteristics.

SYMPTOMS AND PROGRESS OF NATURAL INFECTION OF GUAR

When first observed July 7, 1944, less than 0.1% of the plants showed definite infection. The symptoms, as observed on the leaves, were yellowing, stunting to about one-third normal size, occasional vein-clearing, or a faint oak-leaf pattern; still more rarely a light chlorotic mottling. By very careful observations one can distinguish a stipple of very small chlorotic depressions on the leaves. These chlorotic depressions begin to die from the center of each outwardly, thus producing necrotic stipple, which is usually more prominent on basal halves of leaves. Unequal infection over the leaf may result in skewness or rugosity of the leaflet. Many of the more mature leaves not developing necrosis became bronzed in general appearance, with a marked intercostal chlorosis.

Counts on August 18 showed 2.2% of the plants with definite symptoms. However a much larger percentage was thought to be infected in the masked condition. This assumption was apparently borne out by the rapidity and severity with which the disease developed during somewhat cooler weather. (See Fig. 1.) It was at about this time that a new symptom was observed: dark brown, almost black lesions appeared on the petioles and stems of the plants. This symptom became more prominent as the season advanced, i.e., about September 1, when many plants developed a very severe necrosis of the stem and growing tip, accompanied by severe leaf abscission, which left the bare stems with a few chlorotic leaves attached to the lower half of the plant.

According to counts made September 13, about 22% of the plants

Natural infection of guar

Explanations to Plate I

A. Comparison of a virus diseased guar plant with a healthy plant. Note curled bud leaves and absence of older leaves.

B. Four guar plants in different stages of the virus disease. Note the black necrotic stem of the plant on the left and the curled bud leaves and acutely bent stem tip of the plant on the right.

Plate 1

A.

B.

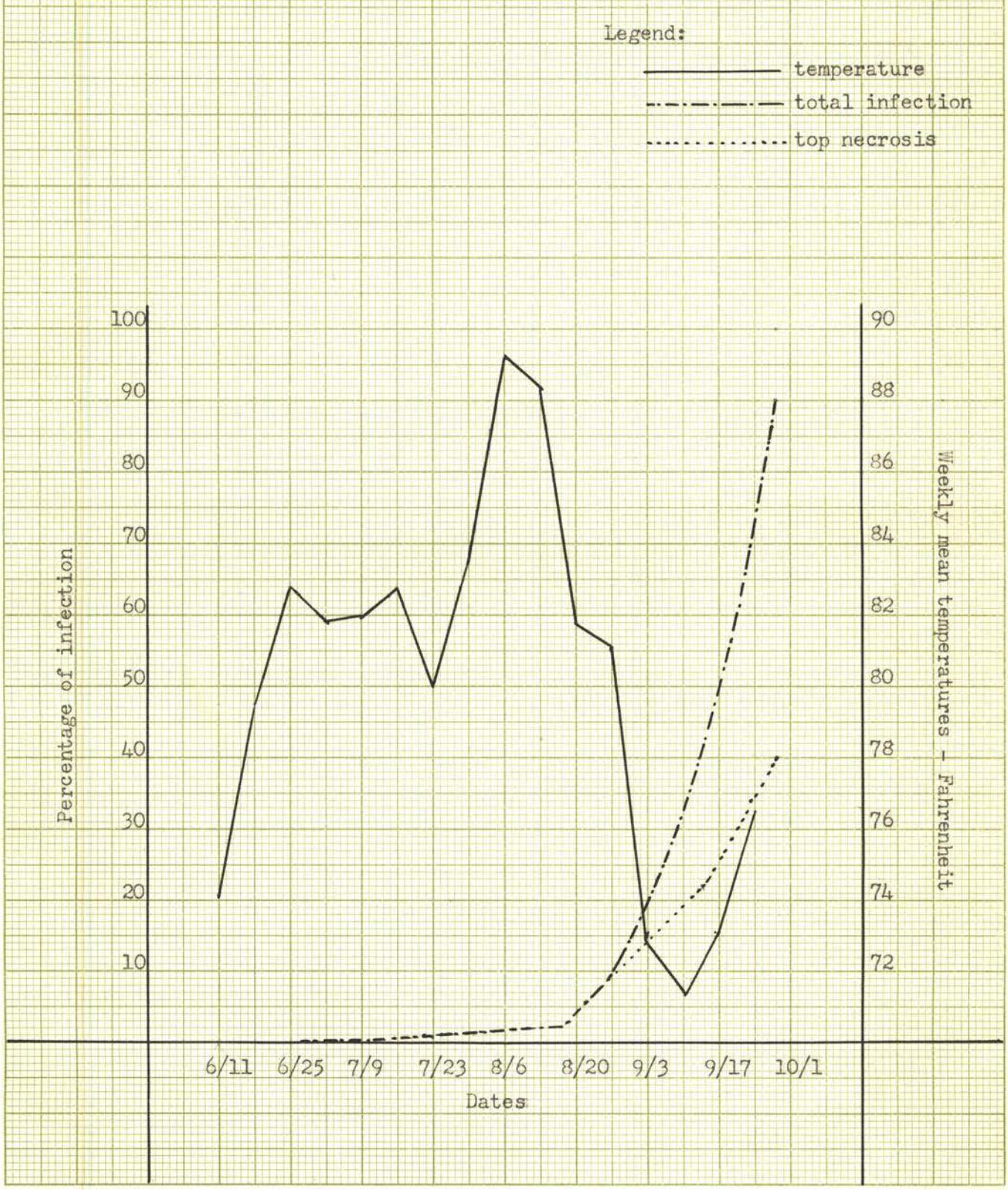
were showing severe top necrosis (Plate 1). As previously stated, this rapid increase in the percentage of infected plants may have been partially due to the expression of symptoms on plants that had been carrying the virus in a masked condition. However, it is also possible that it was due to an increase in transmission by an insect vector.

Further counts on September 26 gave evidence of a continued rapid spread of infection with approximately 40% of the plants severely infected. At this time only about 10% of the plants retained all their foliage, and the majority of these were dwarfed and chlorotic.

A correlation between the virus infection and root-decay was observed in the field; the latter may have been due to fungal pathogens attacking the weakened plants. This phase of the disease needs further study.

Another expression of the disease was observed on plants from which the tops had been removed: a definite light chlorotic mottle of the leaves of the axillary shoots. These symptoms rarely occurred on plants in which the top died, but was not removed. This difference may be due to the removal of the tissue containing the highest virus concentration, which otherwise might inhibit further growth by killing the developing axillary buds. Both positive and negative results were obtained in trying to recover the virus from these axillary branches when grown under winter greenhouse conditions.

Figure 1. Relationship of mean weekly temperature and the percentage of field infection of guar with the guar virus, 1944



SYMPTOMS OF THE DISEASE ON VARIOUS SUSCEPTS

The following descriptions apply to the disease on several species of plants artificially inoculated, usually under greenhouse conditions. Particular attention is given to those plants usually used in diagnosis of viruses.

The rubbing method of inoculation was used almost exclusively in making these inoculations. The virus was applied by swabs consisting of a fold of soft cotton cloth, which was kept moist with sap expressed from macerated infected tissues.

GUAR

Guar, Cyamopsis psoraloides DC., plants artificially inoculated in the field and in the greenhouse gave symptoms similar to those on plants naturally infected. However, symptoms produced in the greenhouse were usually more severe.

On July 12, Chester inoculated 100 plants, 10 each in 10 randomized locations in the field planting. After nine days some plants were infected, showing very small chlorotic dots on the young developing leaves. On the 12th day, 75% of the inoculated plants showed a stipple necrosis. Necrosis began in the depressed chlorotic areas observed on the ninth day. No symptoms were seen on the inoculated leaves at this time.

After thirteen days, there was 98% infection evident, with some withering of the infected leaves. Local lesions had now become evident. There was 100% infection by the fifteenth day. By the 76th day 31% of the plants were dead in the top, while the remainder were more or less severely infected.

The mean temperatures at the time of the above studies, as recorded by a weather station approximately three-fourths of a mile from the guar plantings, were 82.7° F. for the week ending July 16 and 80.0° F. for the week ending July 23 (Fig. 1). However, the maxima recorded during this period ranged from 80.4° to 100° F. and the field maximum temperatures would have been above this.

Inoculations of guar plants, growing in plots outdoors in the shade, on August 16 showed well developed local lesions on 15% of the plants within four days. The systemic symptoms were similar to those produced in the field, but the incubation period was only 6 days with a mean temperature of 82° F. However, recall that these plants were growing in the shade.

Several different inoculation tests in the greenhouse gave results almost identical with those just described, but not always with 100% infection, although usually with a very high percentage of infection.

TOBACCO

Tobacco, Nicotiana tabacum var. Turkish. Eight young healthy tobacco plants growing in 5-inch pots were inoculated on September 21, and placed outdoors in the shade. Four of these plants developed from 1 to 3 local lesions each. The lesion consisted of a thin necrotic ring enclosing slightly chlorotic tissue. Some of these "green islands" finally became necrotic, forming uniformly necrotic lesions. The initial diameter of the lesions ranged from 3 to 5 mm.

Under normal greenhouse conditions a total of 25 plants inoculated at different times failed to produce any symptoms and sub-inocu-

lations into Phaseolus vulgaris var. Giant Stringless Green Pod failed to show the virus to be present in the tissue. However by placing some freshly inoculated plants under a moist sheet, under which the temperature averaged about 68° F., symptoms similar to those that had formed on the plants outdoors developed in about eight days. In these plants the first noticeable symptom was water-soaked rings, which soon became necrotic, leaving the green centers, some of which slowly became necrotic throughout. No systemic symptoms developed, nor were the leaves formed after inoculation carrying the virus as shown by sub-inoculations to cowpea, var. Black Eye.

It should be noticed at this point that several factors, i.e., humidity, light, and temperature, were changed when the plants were placed under the sheet, any one of which may have influenced the expression of the symptoms.

Three of four tobacco plants growing in six-inch pots and inoculated on March 1, developed systemic symptoms. No local lesions were observed, and this probably was due to leaf injury while inoculating, as a fine sand was used as an abrasive. This systemic infection was overlooked until March 27.

On March 10 twelve rank, succulent plants growing in a bed in the floor of the greenhouse were topped back to a height of about 18 inches and inoculated. By March 20 local lesions were observed on five of the twelve plants; 4 days later, systemic lesions were evident on two plants, and by March 27, 11 of the 12 had developed systemic as well as local lesions.

A comparable inoculation made March 11, using tobacco ring spot

virus to inoculate plants similar to those inoculated with the guar virus, also resulted in severe symptoms.

It should be noted that the temperatures ranged between average daily maximum of 93.5° F. and average daily minimum of 71.8° F. with a mean temperature of 80.6° F. when these infections occurred. (See Table 1). Probably the vigorous growth of the plants, and the fact that they were topped overcame the inhibitory effects of the high temperatures (Valleau, 1932).

Results of further inoculation of tobacco are given in Table 2. It will be observed that in all of the older inoculations, a small percentage of the plants had developed systemic symptoms. However, none of these plants possessed the vigor of those in the previous experiment.

When the plants inoculated March 1 and showing systemic infection were placed in dense cool shade, a very severe necrosis developed, with necrotic rings and lines several times as wide as those on the plants growing under normal greenhouse conditions.

Table I. Daily temperatures in greenhouse during the period of comparable inoculation test of guar virus and tobacco ring spot virus on N. tabacum, as recorded by thermograph

Date	Daily Maximum	Daily Minimum	Daily Mean*
March 10	97	77	83
11	90	74	81
12	91	71	79
13	97	75	83
14	84	76	81
15	94	74	83
16	96	78	84
17	94	67	78
18	93	71	77
19	93	70	75
20	92	66	77
21	90	71	79
22	97	67	80
23	101	73	84
24	94	73	83
25	90	72	79
26	94	68	82
27	97	70	82
Average	93.5	71.8	80.6

*The mean of temperature readings taken every two hours.

Table 2. Further inoculations of N. tabacum with guar virus

Date	Number of plants Inoculated	Local infection	Systemic infection	Notes
3/17	14	6	3	Topped when inoculated Kept in shade of bench
4/4	6	5	1	Left on bench unshaded
4/27	5	3	0	Left on bench unshaded

JIMSON WEED

Jimson Weed, Datura stramonium L.; six days after inoculation, very small, indefinitely outlined chlorotic areas appeared on the inoculated leaves. Two days later, these had developed into necrotic rings, occasionally broken, or lesions, with diameters .5 - 1.0 mm. By the 15th day all the ring-lesions had become necrotic throughout. The systemic infection consisted of chlorotic or occasionally necrotic rings or lesions, which by enlargement of the individual lesions, developed a mosaic-like mottle. The virus was recovered from D. stramonium in two out of ten healthy bean plants inoculated. Symptoms were typical. However in later studies with other Datura plants, the virus was not recovered when cowpea seedlings were inoculated with infected Datura tissue.

COWPEA

Cowpea, Vigna sinensis Endl. and V. catjang (Brum.) Walp. The first cowpea inoculation test was on September 21 when 42 plants of the Clay variety were inoculated with sap from infected guar plants growing in the field. From this inoculation test only five local red necrotic lesions with water soaked outlines developed. (Plate 2).

In an attempt to confirm the above and at the same time find a local lesion host suitable for quantitative studies of the virus, five varieties of Vigna sinensis, namely: Clay, Black Eye, New Era, Brown Sugar Crowder, and Chinese Red, and V. catjang var. Catjang, were inoculated February 9. A very fine washed sand was used as an abrasive in this test. Seed of the above varieties were furnished by Professor Robert Stratton, Curator of the Oklahoma A. and M. College Herbarium.

Symptoms of guar virus

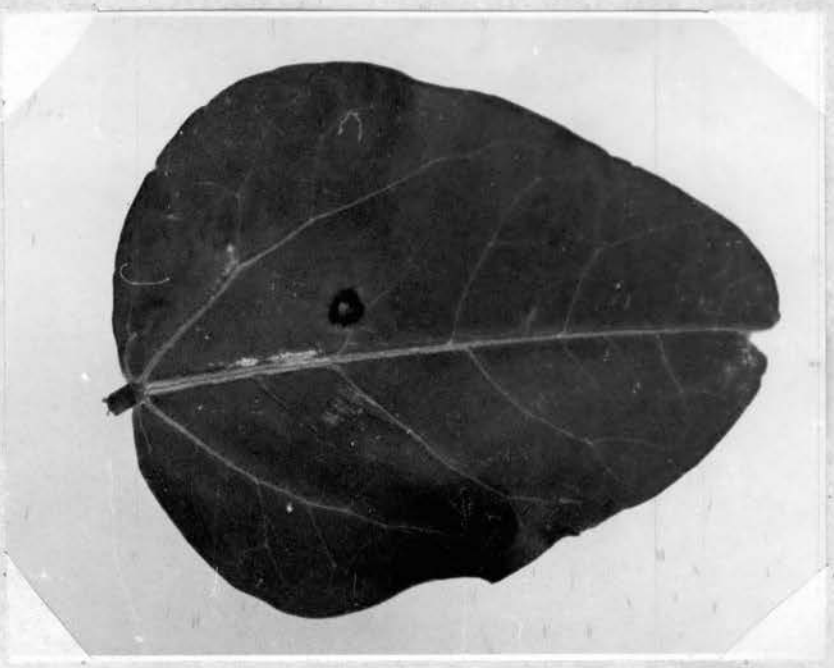
Explanations to Plate 2

A. Jimson weed, the leaf on the right has local lesions of guar virus, whereas the leaf on the left has early systemic lesions. Most of these sytemic lesions will later become necrotic. (x 1.)

B. Cowpea. A leaf with a single local lesions of guar virus. Note the slightly chlorotic center.

Plate 2

A.



B.

The infections of the varieties Clay, Black Eye, and Catjang (Table 3) were in the form of continuous red lesions 1/2 - 4 mm. in diameter, five days after inoculation. The varieties New Era, Brown Sugar Crowder, and Chinese Red formed reddish necrotic rings with more or less normal centers, the diameter varying somewhat with the varieties, from .5 - 2 mm. for Clay to 2 - 5 mm. for New Era.

Except in the variety Clay, the lesions rapidly enlarged, becoming less distinct, necrosis developing faster along the veins, with intercostal tissue soon becoming chlorotic. The leaves drooped at the pulvinus on the 6-7th day, and this was rapidly followed by petiole and stem necrosis resulting in death of the plant in 9-12 days after inoculation.

Cross sections of the stem just below the point of attachment of the first pair of leaves, when it is just beginning to show external necrosis, show discoloration and disorganization of some cells in pericyclic fibers, phloem and xylem parenchyma, and some scattered pith cells. Thus it would appear that the advance of the necrosis is not limited to the conductive tissue.

It might be well to note here that even though the plant is killed, the infection is not systemic, as in most varieties of Phaseolus vulgaris, but is, instead, a progressive necrosis of the tissue from the point of infection.

Table 3. Results of comparative inoculations on several cowpea varieties

Variety	Plants Inoculated	Number of Leaves	Local Lesions	Average Lesions/Leaf	No. Plants killed (13 days)
Clay	12	24	19	0.8	0**
New Era	15	29	118	4.1	14
Brown Sugar Crowder	7	14	56	4.0	7
Catjang*	7	14	116	8.3	7
Black Eye	10	19	415	21.8	10
Chinese Red	10	20	195	9.8	9

* Catjang is of the species Vigna catjang; the others are varieties of the common cultivated species V. sinensis.
**After three weeks no plants of the Clay variety had developed veinal necrosis.

BEANS

Beans, Phaseolus vulgaris L. Several varieties were tested and all proved susceptible in some form. However the majority developed only systemic symptoms. Preliminary inoculations showed that certain bean varieties are susceptible to the virus, and for some of the studies the variety Giant Stringless Green Pod was used as an indicator of the presence of the virus in inoculated plants, as well as in early studies of virus concentration and thermal inactivation point. Thirty varieties of bean were furnished by the Corneli Seed Company, St. Louis, Missouri, and twenty-five by the Asgrow Seed Company, Memphis, Tennessee. Results of inoculating these are shown in Table 4.

Due to lack of, or questionable, symptoms some varieties were inoculated two or even three times, always with a check variety, Plentiful, which gave typical symptoms in the first trial. Some of the failures in the original inoculation were due to the inferior

Plate 3

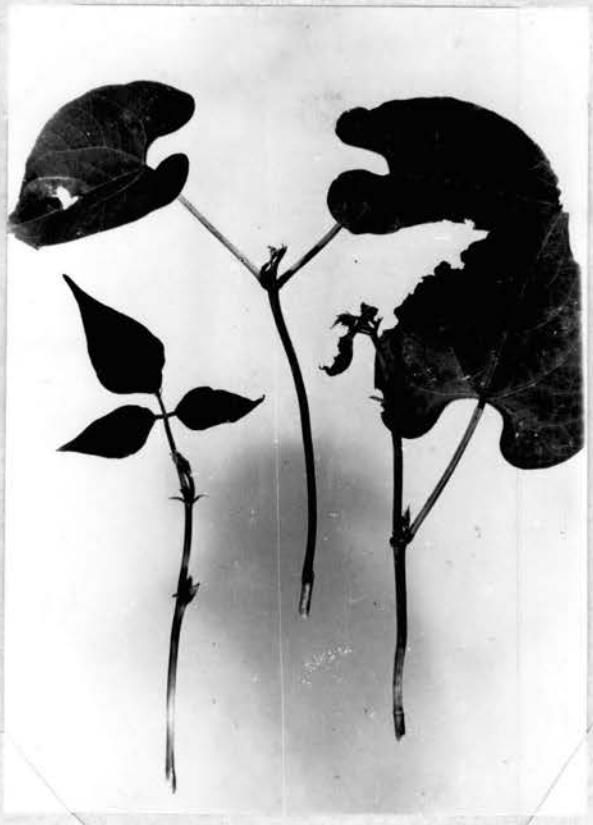
Guar virus symptoms on bean

Explanations to Plate 3

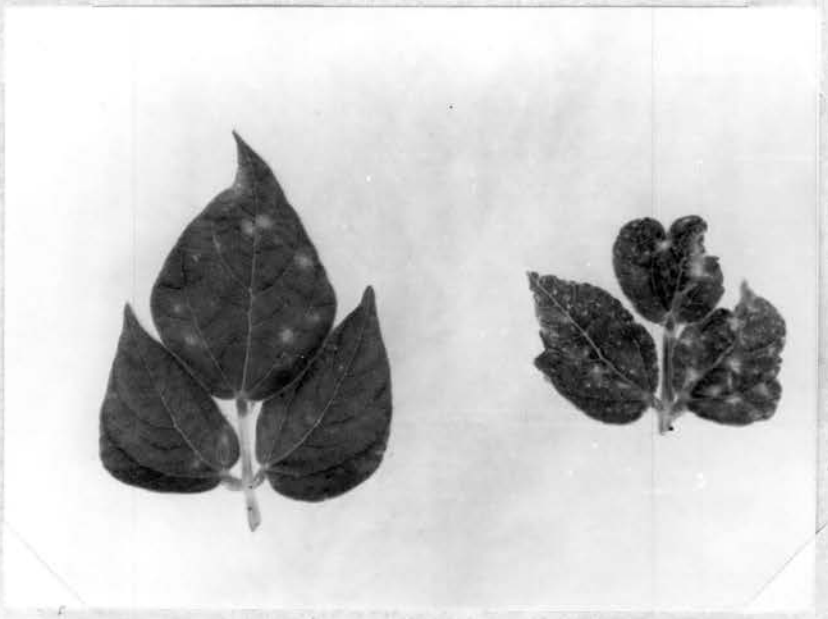
A. Systemic symptoms of guar virus in Giant Stringless Green Pod bean, showing necrosis of growing point.

B. Systemic symptoms of guar virus in bean produced after tops have been removed.

Plate 3



A.



B.

inoculum used on one date as evidenced by symptoms produced on check plants.

Very few of the varieties appeared to be 100 per cent susceptible as is shown by the ratio; i.e. the number of infected plants divided by the number inoculated.

In Table 4, the disease ratings are based upon the condition of the infected plants and not upon the percentage of infection. Factors considered were amount of necrosis of leaf and growing tip and abscission of leaves. Thus "+" indicates very mild and localized necrosis or even a chlorotic stipple of the leaves, usually occurring along the margins and causing deformation of the leaves, as for example in the Kentucky Wonder selections tested. The group rated as "++" show a marked necrotic stippling of the young leaflets which may or may not absciss, sometimes with killing of a few of the growing tips, especially in plants inoculated before the secondary leaves have begun to develop. Fulmeasure will typify this group. The third group is similar to the above, but show more necrosis and abscission; therefore in these bean varieties the virus is more lethal. There is no marked distinction between this group and the preceding, other than comparative severity.

The time necessary for symptoms to develop after inoculation varies from 4 to 10 days, apparently depending upon the age of the plants, the type of symptoms produced, and the stage of infection in the plant tissues used as source of inoculum.

The local infections consist of necrotic veins occurring on the inoculated leaves within 4 to 6 days, soon followed by chlorosis

of surrounding tissue. Necrosis rapidly spreads down the veins, becoming externally evident in the petioles within one to several days, depending upon varietal differences. Although the percentage of plants infected in some varieties is low, this necrosis may continue spreading to enter the stem whereupon it causes death of the entire plant. Necrosis of the petiole usually results in a drooping of the leaves at the pulvinus or a downward curvature of the entire petiole.

Some of the varieties developing local symptoms also developed typical systemic stipple necrosis, for example, the variety White Creaseback.

Two varieties, Ideal Market and Genuine Cornfield, developed a light reddish brown ring at the point of infection, which appeared at about the same time as veinal necrosis began.

Another bean variety, the Yard Long bean, Vigna sesquipedalis (L.) W. F. Wright, botanically belonging to the cowpea group, gives symptoms very similar to the other members of this group studied (See cowpea, page 10 ff.).

Sources of inoculum for this series of tests were infected bean plants.

Table 4. Reaction of bean varieties to guar virus.
(Explanation in text)

Variety	Source	Systemic Ratio	Systemic Rating	Local Ratio	Local Rating	Notes
Burpee's Stringless Green Pod	A	7/8	++			
London Horticultural	A	3/4	+++			
Fulmeasure	A	9/10	++			
Fulmeasure	C	8/10	++			
Florida Belle	A	17/21	++			
Florida Belle	C	10/10	++			
Red Valentine	A	3/10	+++			
Red Valentine	C	4/10	++			
Red Valentine Stringless	C	8/10	++			
Asgrow Stringless Green Pod	A	9/10	++			
Streamliner	A	5/9	++			
Streamliner	C	7/9	+			
Dark Red Kidney	A	4/10	+			
Bountiful	A	5/10	++			
Bountiful	C	2/10	+++			
McCaslan	A	8/10	+++			
Blue Lake Stringless	A	7/17	++			
Kentucky Wonder	A	13/19	+			
Kentucky Wonder	C	11/19	+			
Kentucky Wonder Wax	A	11/20	+			
Kentucky Wonder Rust Resistant	C	14/20	+			
Pencil Pod Black Wax	C	22/22	++			
Dwarf Horticultural	C	4/10	+++			
Tendergreen	C	4/10	++			
Davis Stringless Wax	C	14/28	++			
Brittle Wax	C	17/24	+++			
Golden Wax Topnotch	C	8/10	++			
Golden Wax Improved	C	9/9	++			
Fordhook Favorite	C	5/7	++			
Commodore	C	2/10	++			
Alabama Pole #1	C	10/20	+			
Keystonian	C	7/9	+++			
Black Stringless Creaseback	C	10/10	++			
Tendergreen, New Style	C	7/10	++			
Plentiful	A	10/10	+++			
Plentiful	C	8/8	+++			
Asgrow Stringless Black Valentine	A	9/10	++			
Stringless Black Valentine	C	10/10	+++			
Landreth Stringless Green Pod	C	9/10	++			
Giant Stringless	C	5/9	++			
Ideal Market	A	14/31	++	10/31		Ringed
Tennessee Wonder	A	6/20	+	20/20		
Genuine Cornfield	A			21/32		Ringed

Table 4 - Continued

Variety	Source	Systemic Ratio	Systemic Rating	Local Ratio	Local Rating	Notes
Potomac	A	4/12	+	4/12		
Decatur	A	5/10	++	5/10		
Great Northern	A			12/21		
White Creaseback	C	20/30	++	18/30		
U. S. Refugee #5	A			6/18)		Occasional at-
U. S. Refugee #5	C			4/31)		tacks on stem only
Idaho Refugee	C			3/18		
Yard Long	A			7/21		

* "A" refers to Asgrow Seed Company; "C" refers to Corneli Seed Company.

LIMA BEANS

Lima beans, Phaseolus lunatus L. Twelve Baby Potato lima bean plants were inoculated and placed out of doors in the shade. Eight local lesions appeared as reddish-brown, irregularly-outlined necrotic areas. There was no apparent systemic infection. Other attempts at inoculating this variety of lima beans were made, but gave entirely negative results.

Three varieties of lima beans were included in the seed lot furnished by the Corneli Seed Company to be tested for resistance. These were inoculated on January 16, the result being a mild infection on only one out of ten plants of each variety. In order to confirm the above results, plants of these varieties were inoculated on March 1. This time the inoculation gave 100% local infection of the Early Market Bush and the Dixie White varieties, with the first infection appearing about seven days after inoculation. In the former, numerous irregularly defined reddish-brown areas developed. These rapidly spread along the veins. However some of the inoculated leaves were still partially green a month after inoculation.

The plants were severely stunted with very little apical growth. In the latter variety small yellow to necrotic spots spread very rapidly along the veins in the form of a brown necrosis. The entire leaf yellowed and drooped. The infections finally resulted in death of the plants. The other variety, Henderson's Bush, developed symptoms very similar to Early Market Bush, but not as many lesions per leaf and only 40% of the plants became infected. The leaves drooped at the pulvinus. One of ten inoculated plants was killed by stem necrosis.

TALL BENE

Tall Bene, Sesame orientale (indicum) L. Ten plants were inoculated on October 17. Nine of these plants developed large local necrotic lesions that rapidly spread throughout the leaf. However before the leaves were entirely necrotic, systemic necrosis began to develop in the stems and the growing tip. Within five days after the first symptoms appeared, all the plants were dead. The results were confirmed by further inoculations.

It is of interest to note that a single row of this crop was growing adjacent to the field planting of guar. No localization of the disease of the guar along this side of the field was apparent; however, there were definitely fewer infections in a small guar planting separated from the main planting of guar by a 20-foot terrace that was planted to cowpeas. The Tall Bene plants were all dead by the middle of September; however their death could have been due to some cause other than the guar virus.

There is a possibility that the disease was introduced into the field in Tall Bene. Studies of seed transmission of both guar

and Tall Bene will be necessary to determine this. Such work was not possible during the winter months as neither of the crops is well adapted to greenhouse culture.

OTHER SUSCEPTS

Petunia, Petunia hybrida Vilm. vars. Balcony and Rose of Heaven developed local and systemic infections. The local symptoms consist of small necrotic rings while systemic symptoms are necrotic rings and lines causing a severe distortion of the leaves (Plate 4A). The virus was recovered from both locally and systemically infected petunia tissues.

Husk tomato, Physalis pubescens, L. developed small necrotic rings on inoculated leaves. Although no systemic symptoms were observed, the virus could be readily recovered from the young leaves as well as the inoculated leaves.

Tobacco, Nicotiana glutinosa L. No symptoms have been obtained on this species, although many plants have been inoculated and carefully watched for symptoms. By sub-inoculations into cowpeas, variety Black Eye, one lesion was obtained on 20 inoculated leaves, using sap from the inoculated N. glutinosa leaf and five lesions on 20 inoculated leaves when the young N. glutinosa leaves were used as source of virus. N. glutinosa is evidently a symptomless carrier of the virus.

Green Mung Bean, Phaseolus aureus Roxb. On this plant the virus produced a necrosis of the young leaves and growing point. Only 2 out of 28 inoculated plants became infected. These were plants growing in a flat placed out of doors in the shade.

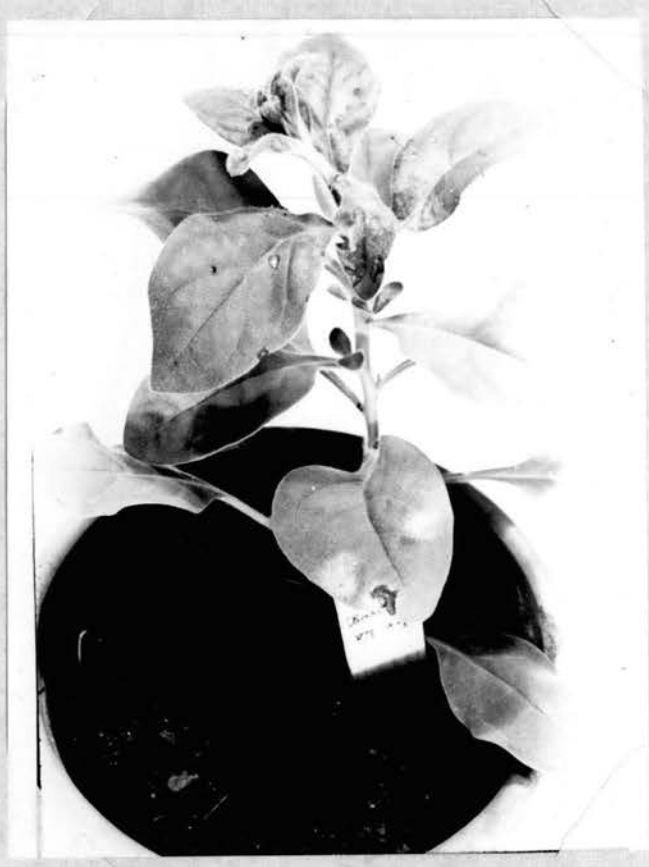
Symptoms of Guar Virus

Explanations to Plate 4

A. Petunia infected with guar virus. Note two chlorotic local lesions on leaf in lower foreground, and systemic distortion and necrosis of the terminal leaves.

B. Soybean infected with the guar virus, showing systemic stipple necrosis on the young leaves.

Plate 4



A.

B.

Soybean, Soja max (L.) Piper, var. 5-100. The virus produced a systemic stipple necrosis of the newly forming leaves and also a necrosis of the growing point (Plate 4B). Only 4 of 17 inoculated plants became infected.

NON-SUSCEPTS

No symptoms were evident in the following plants when inoculated with the guar virus. Unsuccessful attempts were made to recover the virus from those species checked with an asterisk.

LEGUMINOSAE:

Peanut, Arachis hypogaea L.

Sesbania, Sesbania macrocarpa, Muhl.

Urd bean, Phaseolus mungo L.

Pea, Pisum sativum L.

Adzuka bean, Phaseolus angularis (Wild.) W. F. Wright

Hairy Vetch, Vicia villosa Roth.

Crotolaria, Crotolaria intermedia Kotschy.

SOLANACEAE:

Pepper, Capsicum frutescens L. Var. Ruby King*

Jimson Weed, Datura meteloides L.* (But note symptoms on D. stramonium, page 10)

Tomato, Lycopersicon esculentum Mill. var. Bonny Best

COMPOSITAE:

Zinnias, Zinnia elegans Jacq. var. Giant* and Come and Cut Again

Sunflower, Helianthus annus L.

AMARANTHACEAE:

Globe Amaranth, Gomphrena globosa L.*

PHYSICAL PROPERTIES OF THE VIRUS

RESISTANCE TO DILUTION

Inoculation was made on Phaseolus vulgaris var. Stringless Green Pod on October 10, with sap expressed from freshly gathered, moistened guar leaves and stem tips. The leaves were moistened in order to extract the virus, as the plant tissue contained a very low percentage of water due to arid field conditions. In the plants that developed symptoms from inoculation with virus at the higher dilutions, the symptoms were much milder and slower to become evident. Plants infected with virus at dilutions of 1:300 and 1:1000 abscissed very few leaves and in no case died.

Due to the large number of plants necessary to conduct dilution studies where only systemic infection occurs, search was made for a local lesion host. This was found in Vigna sinensis var. Black Eye.

In the first determination using these plants, only a few dilutions were used because of the limited number of plants available. Inoculum was obtained by macerating the leaves of bean plants that were just beginning to develop symptoms. The expressed sap (stock solution) was termed 100% plant sap. Inoculations and results are shown in Table 5.

For the second test, using cowpeas, the inoculum consisted of 100% plant sap from the secondary leaves and stem tips of several bean varieties. It is possible that the stems did not contain as high concentration of the virus as the leaves, resulting in a more dilute inoculum to begin with.

Note the increase in infection with increasing dilution up to 1:10, with dilutions greater than that resulting in a proportional

decrease of infectivity. This phenomenon, evidently due to virus-inhibitory substances in concentrated plant sap, has also been observed with other viruses, such as that of tobacco mosaic.

Considering the results of the three tests as shown in Table 5, the virus may be considered to be non-infective at dilutions greater than 1:1000.

RESISTANCE TO HEATING

In attempts to determine the thermal inactivation point of the virus before discovering that cowpeas could be used as local lesion hosts, the systemic infection of Giant Stringless Green Pod beans was used to determine infectivity of the sap.

Expressed sap was put into small test tubes which were attached to thermometers. These were then immersed in large beakers of water at the given temperatures and remained there at constant temperature for 10.5 minutes. At the end of this period the tubes were removed and plunged into cool water to stop the effect of heating. Inoculations were by the swab method, beginning with the tube exposed to the highest temperature and progressing to that exposed to the lowest. The above procedure was used in all thermal inactivation point studies.

A further study was made using cowpea as host, since it is more sensitive to changes in infectivity. Results of both tests are given in Tables 6 and 7.

From these data it is evident that the thermal inactivation point is just above 67° C.

Table 5. Dilution Studies

Test plant / Dilutions	Beans[1] Plants Inoc.	Inf.	%	Black Eye Cowpeas Leaves[2] Inoc.	Lesions	Aver.	[3] Inoc.	Lesions	Aver.
1:1	39	38	97.5	17	1040[4]	61.3	40	150	3.8
1:3	18	16	88.9	-	-	-	37	287	7.8
1:10	13	13	100.0	-	-	-	42	431	10.3
1:30	15	15	100.0	-	-	-	40	246	6.2
1:100	19	12	63.2	14	176	12.6	38	52	1.4
1:300	18	10	55.5	16	30	1.9	40	13	0.3
1:1,000	21	4	19.0	18	10	0.6	40	0	0.0
1:3,000	15	0	0.0	23	0	0.0	40	0	0.0
1:10,000	15	0	0.0	23	0	0.0	40	0	0.0
1:30,000	-	-	-	24	0	0.0	-	-	-
1:100,000	16	0	0.0	28	0	0.0	-	-	-
1:1,000,000	19	0	0.0	-	-	-	-	-	-

1 Giant Stringless Green Pod beans inoculated on October 24; inoculum from guar plants in field.

2 Black Eye cowpeas inoculated on March 10; inoculum from newly-infected bean leaves.

3 Black Eye cowpeas inoculated on April 10; inoculum from leaves and stem-tips of several bean varieties

4 Lesions numerous and overlapping; count approximate.

Table 6. Thermal inactivation point. Giant Stringless Green Pod beans as Test plant

Temperature °C	Ratio*	Percentage Infected
40	27/27	100
45	30/30	100
50	28/28	100
55	30/32	94
60	21/34	62
65	1/31	3
70	0/40	0
80	0/28	0
90	0/34	0
95	0/34	0
Check	68/69	99

*Infected plants/inoculated plants

Table 7. Thermal inactivation point. Black Eye cowpea as test plant

Temperature ° C	Number leaves inoculated	Number lesions	Average
57	38	984	25.9
59	42	683	16.3
61	42	874	20.8
63	38	556	14.6
65	40	210	5.2
67	38	44	1.2
Check	40	759	19.0

RESISTANCE TO COLD STORAGE

Two procedures were used in this study, viz. keeping the macerated tissues at refrigerator temperatures above 5° C., and keeping them frozen. The tissue consisted of guar leaves and tops that were definitely showing symptoms of the disease. These were put through a food chopper and enough distilled water added to make a fairly light paste of the material. This was then pressed into 12 mm. 8-inch test tubes, and these were plugged with cotton and put into a refrigerator or into the freezing unit of same. The results of these studies are shown in Table 8.

There does not appear to be much difference in the keeping qualities of the virus at the two temperatures, i.e. just above and just below freezing.

RESISTANCE TO DRYING

The resistance of the virus to drying was determined from dried, well-infected bean leaves. Inoculation of cowpeas with expressed sap from some of this fresh material gave evidence of its virulence. After drying in the greenhouse for 40 hours the material was fairly crisp. Some of the dried material was moistened, macerated, and the expressed solution was used to inoculate 18 cowpea plants. Not a single lesion developed, indicating that the leaves had completely lost their infectivity during drying.

Table 8. Storage qualities of virus *in vitro*

Time	Ratio*	Symptoms
Refrigerated Plant Material		
15 days	18/19	Symptoms typical for fresh virus
55 days	1/16	Symptoms mild
83 days	1/14	Symptoms mild
130 days	0/12	No infection**
201 days	0/12	No infection**
Frozen Plant Material		
15 days	17/20	Not quite so severe as with fresh concentrated virus; more like those from inoculations with diluted sap
41 days	8/12	As above
69 days	5/22	Symptoms milder than preceding. No leaf abscission
116 days	0/16	No infection**
187 days	0/12	No infection**

* Plants infected/plants inoculated

**Cowpeas were used in last tests, Burpee's Stringless Green Pod beans were used in the preceding tests.

COMPARISON TO SOME KNOWN VIRUS DISEASES

COMPARISON OF BEAN MOSAIC VIRUSES 4 AND 4A WITH GUAR VIRUS

Guar virus produces systemic symptoms on certain bean varieties that are almost identical with those described by Zaumeyer and Harter (1943) for the bean mosaic viruses 4 and 4A. However, other varieties differentiate between the two viruses. Some of these differences are shown in Table 9. Other outstanding differences as dilution end-points and thermal inactivation points are included.

Table 9. Some differences between bean mosaic viruses 4 and 4A and the guar virus

Host or other characteristic	Guar Virus	Bean Mosaic 4 and 4A*
Alabama Pole #1	Systemic	Local
Kentucky Wonder	"	"
McCaslan	"	"
Ideal Market	Local & Systemic	"
Vigna sinensis	Dark red rings; veinal necrosis	No infection
N. tabacum	Local necrotic rings; systemic ring spot	No infection
Soja max	Systemic leaf and stem necrosis	No infection
Thermal inactivation point	67+° C.	95° C.
Dilution end point	1:1,000	1:100,000

*From Zaumeyer and Harter (1943).

The above differences are regarded as conclusive evidence that the guar virus is distinct from these bean viruses.

COMPARISON OF THE CUCUMBER MOSAIC VIRUSES AND THE GUAR VIRUS

Although there is not much similarity between symptoms produced by the cucumber or cucurbit viruses and the guar virus, it is well to point out certain differences, especially since both viruses produce local lesions on cowpea, and there are many strains of the cucumber mosaic viruses known.

Holmes (1939) recognizes two strains of the cucurbit mosaic virus, Marmor astrictum Holmes. However, neither strain is known to affect any plants other than cucurbits. He also recognizes seven strains or varieties of cucumber mosaic virus. Of these seven strains, all except the cowpea mottle strain (Price, 1934) produce dark red necrotic lesions 1-2 mm. in diameter on cowpea. These lesions are necrotic throughout and are not ringed. They do not become systemic, though the disease is sometimes lethal.

The cowpea mottling strain, M. cucumeris var. vignae Holmes, produces local chlorotic lesions which are usually surrounded by necrotic rings. This strain also causes a systemic greenish-yellow mottling, and the infection is not lethal (Price, 1934).

The local lesions produced by guar virus infection in cowpeas are either ringed from the outset, or soon become so, with the center of the ring somewhat chlorotic. There is no true systemic infection, although the virus is usually lethal to the plant.

The following strains of cucumber mosaic virus all infect zinnia, and may be recovered from this plant by sub-inoculation to cowpea: typical strain, M. cucumeris Holmes, var. vulgare; southern celery mosaic strain, M. cucumeris var. commelinag lima bean mosaic strain, M. cucumeris var. phaseoli; lily mosaic strain M. cucumeris var. lilii;

indicator strain, M. cucumeris var. judicis. Guar virus failed to develop any symptoms in zinnia, nor could it be recovered from the inoculated leaves by sub-inoculations into cowpeas.

Only one strain of the cucumber mosaic viruses, the potato veinbinding strain, M. cucumeris var. upsilon, Holmes, has not been eliminated from possible similarity to guar virus. This virus has a thermal inactivation point of only 52° C. as compared to 67+° C. for the guar virus.

These strains of cucumber mosaic virus produce varied symptoms in tobacco, N. tabacum var. Turkish. None of them give the clear-cut necrotic local lesion or systemic pattern that the guar virus produces.

COMPARISON OF A POTATO VIRUS WITH THE GUAR VIRUS

This virus was obtained from a potato plant found growing in a local garden. The symptoms were indicative of potato aucuba mosaic. However, when N. tabacum var. Turkish was inoculated with it and placed under a moist sheet in the greenhouse, in order to obtain lower temperatures (about 20° C.), wide necrotic rings about .5 cm. in diameter developed locally. The center of the circle remained about normal color, usually becoming lighter with age. Occasionally the ring slowly necrosed both inwardly and outwardly such that the lesion finally became necrotic throughout. Systemic lesions may be circular if formed in the intercostal region, but may be very irregular if formed near a vein of the leaf. In both instances the lesions may consist of concentric rings. Later the infection forms a definite oak leaf pattern, likewise composed of parallel necrotic lines sepa-

rating the lighter tip portion from the darker basal portion of the leaf.

March 17 comparative inoculations of the potato virus and the guar virus were made, inoculating 9 N. tabacum plants with the former virus and 14 with the latter. All these plants were placed under a cool shaded bench. Within two weeks all those plants inoculated with the potato virus had developed severe local and systemic symptoms, whereas only four of those inoculated with the guar virus developed local symptoms. Four weeks after inoculation, four plants inoculated with guar virus were observed to have mild systemic symptoms.

According to Salaman (1938) only the "S" strain of potato virus "X" produces ring spot symptoms on tobacco. In potato the "S" strain of potato virus "X" is usually masked. However the chlorosis of this potato plant may have been due to a virus complex. This was further suggested by the fact that the virus inoculated to cowpea formed numerous red, necrotic, local lesions. The potato "Y" virus would cause such lesions, and occurs in this locale (Harding, 1941). A complex of the potato "Y" virus, Marmor cucumeris var. upsilon, Holmes, and a strain of the potato "X" virus, Marmor dubium Holmes, causes a destructive virus complex known as rugose mosaic (Holmes, 1939). Rugose mosaic symptoms are quite different from those of aucuba mosaic. Whether or not this potato virus is an abnormal form of rugose mosaic needs further experimental study, but such an assumption would explain the symptoms produced on tobacco and cowpea.

November 4, 18 guar plants were inoculated with sap from the original potato plant. Six days later 14 of these had developed numerous necrotic lesions on the younger trifoliate leaves. The lesions were about 1 mm. in diameter. It should be noted here that these lesions were considerably larger than the newly formed systemic guar virus lesions. There were no definite local lesions; however, some of the inoculated leaves became necrotic as if due to injury while inoculating.

Continuing with this lead on November 16, twenty-six bean plants Phaseolus vulgaris var. Giant Stringless Green Pod, were inoculated with the potato virus, and for comparison, 50 plants were inoculated with guar virus. The potato virus infected 100% of the plants, while the guar virus infected 98%. The symptoms were very similar. However, the stipple necrosis was not nearly so pronounced in plants inoculated with the potato virus, but many of the leaves curled upward and inward and abscissed without apparent lesions. Even though the leaf abscission was greater in these than in the plants infected with guar virus, there were very few of the plants killed as compared with those infected with the guar virus. With both viruses, the leaves developing on plants surviving the initial infection showed large, 3-6 mm., depressed, irregularly outlined, chlorotic lesions. Especially was this true if the tops were removed, as was done to secure virus inoculum.

The above comparative inoculations were repeated January 13 using Burpee's Stringless Green Pod beans and keeping the plants under a moist cool sheet. Here numerous very small local necrotic lesions developed on the potato virus infected plants. However the

systemic infection of each virus proved lethal under these conditions.

Attempts to use Datura stramonium as a differential host proved unsuccessful since the symptoms produced by the two viruses inoculated December 20 to four plants each were indistinguishable.

Comparative inoculations of the two viruses were made to two Nicotiana glutinosa plants each. These were also kept under the moist sheet. One local necrotic lesion appeared on a plant inoculated with the potato virus and neither of the plants inoculated attained the size of those inoculated with the guar virus. No systemic symptoms other than the stunting developed on any of the plants inoculated with either of the two viruses.

Considering only the symptoms produced on these plants, the differences between these two viruses are small. Some of the differences are the regularity with which the potato virus infection becomes systemic in tobacco as compared to that of guar virus. The local lesions that the potato virus produces on beans occur at lower temperatures. Likewise the lesions that the potato virus produces on cowpeas are smaller than guar virus lesions and usually necrotic throughout.

A COMPARISON OF TOBACCO RING SPOT AND THE GUAR VIRUS

Tobacco ring spot virus causes a systemic necrosis of beans, as does the guar virus, and a comparative study of the two viruses was therefore indicated. In order the facilitate this comparison W. C. Price kindly furnished a source of the ring spot virus that Wingard described (1928). This inoculum was received February 22 and in order to increase the supply, four Nicotiana tabacum plants

and two N. glutinosa plants were inoculated. The N. tabacum all developed typical symptoms. The N. glutinosa inoculated leaves slowly yellowed and died. The tops were very much stunted, never getting more than about 10 inches high.

Comparative inoculations of the two viruses were made on several hosts. On guar, inoculated March 31, the following results were recorded.

Guar virus; 7 plants inoculated:

4/10 No infection after 10 days

4/12 Six plants systemically infected. A fine chlorotic to necrotic stipple with many of the young leaves abscissing; inoculated leaves not infected and not drooping at pulvinus.

Ring spot virus; 8 plants inoculated:

4/4 Watersoaked local lesions

4/10 No much change; lesions slightly larger and more necrotic

4/12 Local lesions with dark necrotic centers; lighter necrotic periphery, indefinite outline. No systemic infection observed on any plant. 11 of the 16 inoculated leaves abscissed. Two plants showed stem necrosis. Growing point not necrotic.

4/17 Stem necrosis definitely beginning at point of petiole attachment to the stem and developing both upward and downward. No systemic infection.

Similar inoculations to Tall Bene, Sesame orientale L.

Guar virus; 3 plants inoculated March 31:

4/4 Local watersoaked lesions

4/10 2 plants dying - others with necrotic lesions on stem at point of petiole attachment

4/12 All dead

Ring spot virus - inoculated 4 plants March 31:

4/4 Local watersoaked lesions, 2-4 mm. diameter

4/8 All plants dead from stem necrosis beginning at peticle.

Similar inoculations on cowpea, variety Black Eye:

Guar virus; 24 leaves inoculated March 30:

4/4 Red lesions, red rings with light green centers which enlarged as the rings enlarged

4/5 2.5 lesions per leaf

4/10 9 plants showed stem necrosis; inoculated leaves chlorotic; lesions very irregularly outlined; 4.7 mm. in diameter; now a necrotic center surrounded by a chlorotic ring, then by a necrotic ring.

4/12 8 plants dead with stem necrosis. All eventually died.

Ring spot virus; 20 leaves inoculated March 31:

4/4 Many reddish brown lesions much smaller than above

4/5 17 lesions per leaf

4/10 5 plants showed stem necrosis. Some inoculated leaves chlorotic. Most lesions continuous, others ringed as above.

4/12 4 plants dead; 4 others with severe stem necrosis

It should be noted here that local lesions usually form on the inoculated leaves of guar when the plants are inoculated with the guar virus. This is true whether the first true leaves or the secondary leaves are inoculated. In the above comparative studies the first true, unifoliate leaves were inoculated.

However in guar the petiole-stem necrosis of the ring spot virus is very different from the symptoms caused by the guar virus, and the absence of the systemic stipple necrosis is a marked difference.

In tobacco, Nicotiana tabacum, the only comparative infectivity test consisted of the inoculation with the guar virus of 12 tobacco plants on March 10 as already described. For symptoms of guar virus on tobacco, see page 6 ff.

On March 11, 5 other plants of the same group were inoculated with the tobacco virus.

Plate 5 is a photograph of guar virus ("A") and ring spot ("B") local lesions taken 44 days after the plants were inoculated. It will be readily noticed that the guar lesions are very diffuse, apparently as though the camera was out of focus. However, this is not the case, as all the local lesions of this inoculation were diffuse. However they were more or less definite when first formed. At the time these photographs were made, the plants were from 7 to 10 feet high and very rank. On plants growing in pots and less vigorous, the local lesions may remain definite or necrose both outwardly and more slowly toward the center of the lesion.

Plate 6, systemic symptoms of guar, and plate 7, systemic symptoms of ring spot, show that there are few qualitative differences between the symptoms of the two viruses. The demarcations between the systemic and recovered portions of the ring spot infected leaves appear to be greater than those between similar areas of guar virus infected leaves. The systemic symptoms tend to develop on guar virus infected plants for a longer period, and the necrotic lines are usually much finer.

Note that leaves C and B in plates 6 and 7 respectively are very necrotic and deformed. This is a typical symptom of ring spot on suckers developing on plants that are topped back after systemic infection is well evident. Symptoms of similar definiteness developed on three potted Nicotiana tabacum plants showing systemic infection of the guar virus that were placed under a shaded bench and kept cool with day temperature above the benches of about 25° C.

Further inoculations with the guar virus gave only a low percentage of systemic infection with symptoms similar to those in the other potted plants. (See guar virus symptoms on N. tabacum, page 7 ff.)

When diseased bean tissue is used as a source of inoculum, guar virus is inactivated at dilutions greater than 1:1000, whereas the tobacco ring spot virus is inactivated at 1:10,000 (Wingard, 1928).

Valleau (1932) stated that plants affected with tobacco ring spot produce a reduced amount of seed and the pollen grain size is much reduced, i.e. there is a large percentage of pollen grains much below the average size of those produced by a healthy plant.

PLATES 5, 6, 7

Comparison of Guar Virus and Ring Spot

Explanations to Plate 5

A. Local lesions of guar virus on tobacco. Note the diffuse indefinite lesions. Photograph was made 45 days after inoculation. (x 1/3).

B. Local lesions of tobacco ring spot virus on tobacco. Photograph made 44 days after inoculation. (x 1/4).

Explanations to Plate 6

A. Systemic symptoms of guar virus on tobacco leaves. Observe the fineness of the necrotic lines, and lack of color differences between the areas inside and outside the oak-leaf outline. (x 1/2).

B. Systemic infection of guar virus in tobacco. This was one of the first leaves on the plant to show systemic infection. Observe the many concentric lines. (x 1/3).

C. Systemic symptoms of guar virus on tobacco suckers heavily shaded by larger plants. (x 1/3).

Explanations to Plate 7

A. Systemic symptoms of tobacco ring spot in tobacco. Note the difference in shading of the areas inside and outside the oak-leaf outline. Compare with 6A. (x 1/3).

B. Systemic symptoms of tobacco ring spot in a sucker of a tobacco plant topped after systemic infection became evident. (x 1/2).

Plate 5

A.

B.

Plate 6

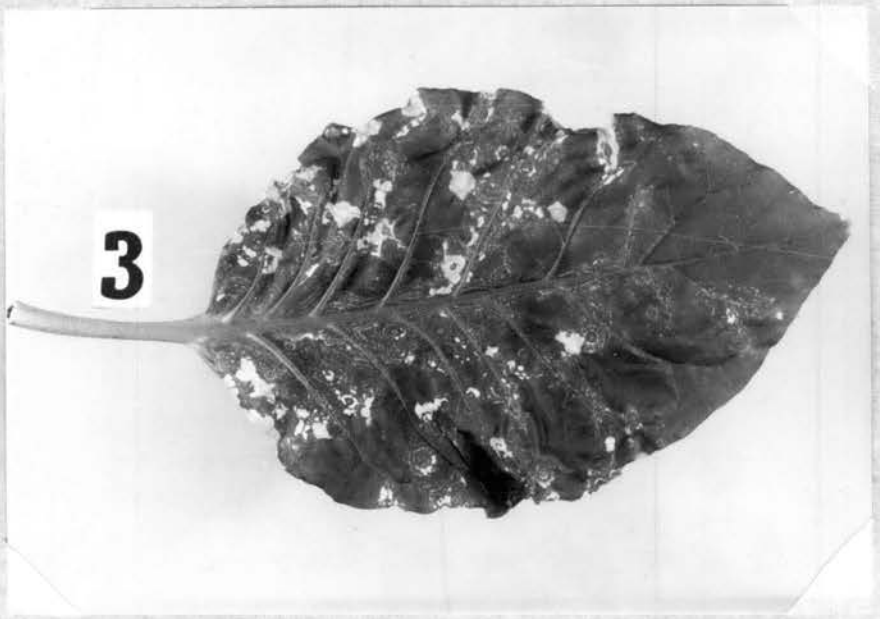
A.

B.

C.

Plate 7

A.



B.

He considers that even though the vegetative symptoms may be masked, the disease has invaded the embryonic region of the plant. Valleau further states (1941) that the six strains of tobacco ring spot which he studied all caused pollen sterility, which greatly reduced the amount of seed produced in each capsule.

Comparative measurements were made of pollen grains from healthy tobacco plants and those locally and systemically infected with the guar virus and also from plants infected with tobacco ring spot. These data are shown graphically in Figure 2. However no marked differences can be shown even for ring spot. This may have been due to the fact that the measurement classes, with steps of 3.21μ , were too broad, although they were less than one and one-half times as large as those used by Valleau. Therefore another collection of flowers was made from two plants systemically infected with the guar virus and a similar collection from two plants infected with ring spot virus. The pollen grains from both plants of each infection were mixed and were mounted in lacto-phenol with acid fuchsin as a stain. Both mounts were warmed by rapidly passing them through the flame while holding them in the same hand. The measurements were made using an oil-immersion objective. The unit spaces of the micrometer were equivalent to 1.37μ . Plasmolyzed grains were not measured, but a record was kept of the number of those in each field as an indication of the comparative turgidity of the two lots of pollen grain.

The results of the measurements are given in Table 10 and shown graphically in Figure 3. The mean size of the pollen grains was

27.59 μ for the ring spot and 27.38 μ for those infected with the guar virus. There is a very close similarity between the two groups. However, due to the limited number of plants included, and absence of check plants, only limited emphasis can be placed on this similarity. At this time there were no healthy plants flowering that would compare in vigor to the diseased one sampled.

Since no definite symptoms could be detected in Nicotiana glutinosa, not even appreciable stunting as with comparable ring spot inoculations, several attempts were made to recover the guar virus by inoculation of cowpea with macerated N. glutinosa tissue. Tests were made using either the inoculated leaf or the younger leaves. The results of these studies are shown in Table 11.

From these data it is evident that N. glutinosa is not always carrying the guar virus in masked condition, but may carry it in small quantities in part of the plants inoculated. Ring spot was recovered in all similar attempts, however in rather dilute form as compared with infected N. tabacum, especially with tissues showing necrotic symptoms.

Figure 2. Measurement of Nicotiana tabacum pollen grains from virus-infected and healthy plants

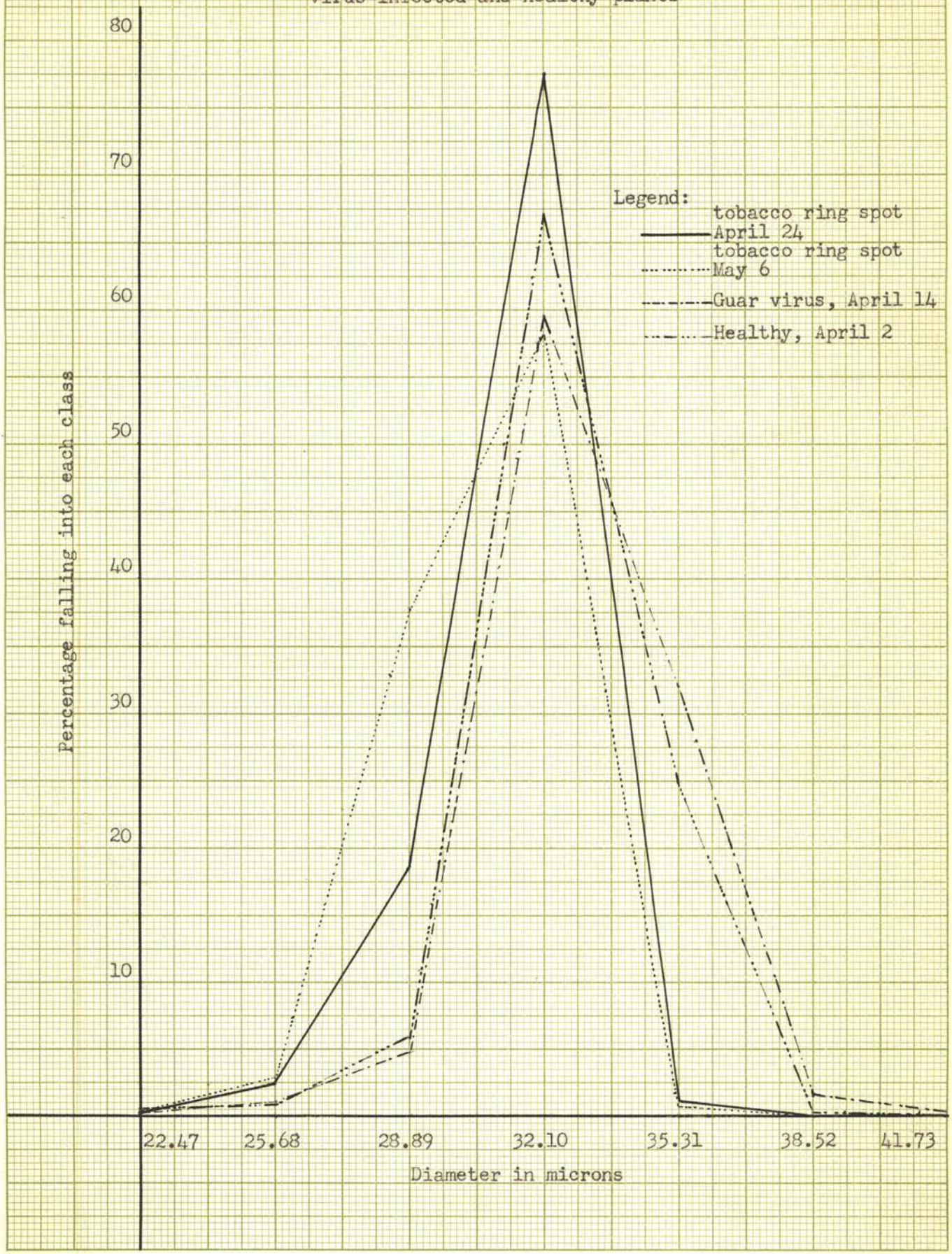


Table 10. Comparative measurement of Nicotiana tabacum pollen grains from plants infected with tobacco ring spot and guar viruses[1]

Diameter	Ring Spot		Guar Virus	
	Frequency	Percentage[2]	Frequency	Percentage[2]
20.55	1	.38	4	1.51
21.92	5	1.88	7	2.64
23.29	7	2.63	18	6.79
24.66	22	8.27	35	13.21
26.03	34	12.73	42	15.85
27.40	77	28.95	53	20.00
28.77	83	31.20	53	20.00
30.14	33	12.48	35	13.21
31.51	4	1.50	11	4.15
32.88			3	1.13
34.25			2	.75
35.62			1	.38
36.99			1	.38
Total	266		265	
Plasmolyzed[2]	71		64	

[1] Measurements May 13, 1945

[2] The plasmolyzed grains were not included in calculation of percentages.

Figure 3. Comparative measurement of Nicotiana tabacum pollen grains from plants infected with tobacco ring spot and guar virus

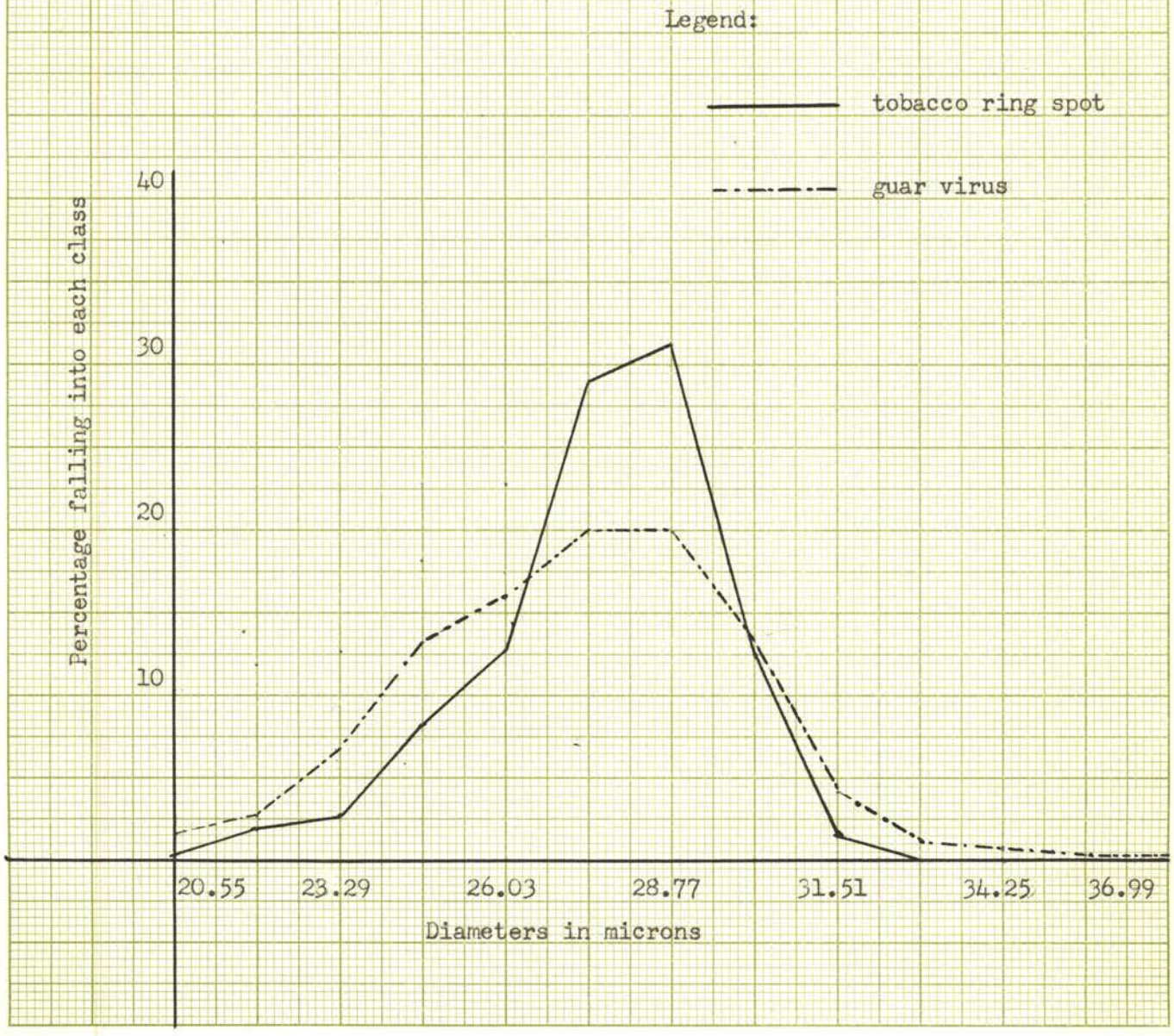


Table 11. Comparison of the recovery of guar and tobacco ring spot viruses from Nicotiana glutinosa using cowpea as indicator

Virus	Date N. glutinosa inoc.	Date of Recovery	Source of inoculum	No. of leaves inoc.	No. of lesions formed	Average lesions per leaf
Guar	3/17	5/6	Local	24	0	0
			Systemic	20	0	0
"	4/4	4/28	Local	20	1	.05
			Systemic	20	5	.25
"	4/4	5/6	Local	22	0	0
			Systemic	24	0	0
"	4/10*	5/6	Local	18	2	.11
			Systemic	16	11	.70
Tobacco Ring spot	2/22	5/6	Local	—	—	—
			Systemic	22	27	1.37
"	4/11*	5/6	Local	20	5	.25
			Systemic	20	7	.35

* Plants very vigorous growing in floorbed of greenhouse. They showed no symptoms of either disease.

CONCLUSIONS

The physical properties of the virus, its host range, and its symptom expression on the different suscepts differ in some respects from those of any previously described virus. Considering the data presented here, the possibility of its being a strain of one of the known mosaics of tobacco, cucumber, or legumes is readily eliminated.

The unidentified potato virus gave symptoms on guar, beans, and tobacco somewhat similar to those produced by the guar virus infection. However the more common of the potato viruses, that is, "X" and "Y", both develop severe symptoms when inoculated on pepper, Capsicum frutescens, whereas guar virus does not infect this plant. Only potato virus "Y" causes local lesions on cowpea, and it is inactivated at 52° C. for 10 minutes, while the guar virus resists heating up to 67+° C.

This could not be the spotted wilt virus or one of its strains, since these have a thermal inactivation point of 42° C.

There are many points of similarity between the ordinary tobacco ring spot virus and the guar virus; however, definite differences do exist between the latter and any of the strains of tobacco ring spot thus far described. (Wingard, 1928; Valleau, 1932, 1941; Price, 1935-1936b; and Smith, 1944).

Some of these differences will be listed briefly: 33 of 44 varieties of bean tested with guar virus failed to produce local lesion symptoms as does tobacco ring spot. Likewise guar virus did not infect zinnia, nor could it be recovered from inoculated zinnia. The ring spot of Wingard failed to infect guar systemic-

ally in the manner that the guar virus did. The guar virus symptoms are milder in tobacco. The ring spot virus and guar virus show marked difference of symptoms with variation in environment. Preliminary studies indicate that the guar virus reduces the size and uniformity of tobacco pollen grains as does ring spot (Valleau, 1932).

Considering the symptom expressions on the different suscepts, and the host range in comparison with that of tobacco ring spot virus, the differences are believed to be such as to warrant considering the guar virus a new variety of the tobacco ring spot virus, for which the name Annulus tabaci Holmes var. oklahomensis is proposed.

SUMMARY

An evidently undescribed virus was found naturally causing nearly a total loss in guar (Cyamopsis psoraloides DC. = C. tetragonalobus (L.) Taub.). The rate of the spread of the infection in the field was from less than 0.1 per cent to better than 90 per cent in two and one-half months.

The virus is sap transmissible to bean, lima bean, soybean, cowpea, green mung bean, tobacco, Nicotiana glutinosa, husk tomato, petunia, Jimson weed, and tall bene. It did not infect peanut, sesbania, urd bean, garden pea, adzuka bean, hairy vetch, crotolaria, pepper, Datura meteloides, L., tomato, zinnia, sunflower, and globe amaranth.

The guar virus was found to be systemically infective to 33 varieties of beans, however, not always with 100% infection. Five varieties showed both local and systemic infection, and six varieties developed only local veinal necrosis. Eight varieties of cowpeas inoculated developed local lesions. These were used in studies of virus concentration. The disease was usually lethal in seven of these varieties.

On the basis of preliminary measurements, there are indications that the guar virus, like tobacco ring spot virus, reduces the size and uniformity of the pollen grains on infected tobacco plants.

The thermal inactivation point of the virus is slightly higher than 67° C. (10 minute exposure). The dilution end point is 1:1000, and the virus is inactivated by drying the infected tissue for 40 hours.

When infected macerated plant material is kept frozen, the virus retains some of its original infectivity for 69 days. It is inactivated in 116 days under these conditions. When viruliferous sap is stored at refrigerator temperature, about 5° C., it retains full infectivity for 15 days and a trace of infectivity after 83 days.

Guar was found to be susceptible to tobacco ring spot. The virus is evidently a variety of tobacco ring spot for which the name Annulus tabaci Holmes var. oklahomensis is proposed.

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