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Cucumber Green Mottle Mosaic Virus, its Effect on Yield and its Control in the Lea Valley, England

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SUMMARY

Cucumber green mottle mosaic virus was very widespread in cucumber crops in the Lea Valley in 1964. Experiments on the effect of this virus on yield indicated that losses of approximately 15 per cent occurred with early infection. Later infection had little effect on yield.

Dry heat treatment of infected cucumber seeds at 70°C for more than one day was sufficient to inactivate the virus and these results were also confirmed using virus-impregnated filter paper squares. Cucumber seeds were found to tolerate dry heat treatment at temperatures of 75°C and 80°C with little adverse effect but, at 80°C, germination was delayed and the cotyledon leaves were distorted.

In a commercial trial, over 45,000 cucumber plants free from symptoms of the virus were raised from infected seeds which had previously been heat treated at 70°C for three days.

INTRODUCTION

A virus disease of cucumbers whose symptoms agreed with those of cucumber green mottle mosaic was recorded in the Lea Valley by Bewley (1923). Cucumber viruses were later investigated by Ainsworth (1935) at the Cheshunt Experimental Station and he described a disease which produced a mild mosaic on cucumbers which he called cucumber green mottle mosaic. Work in Holland by van Koot and van Dorst (1959) showed this virus to be seed-borne and that the highest incidence of seedling infection resulted when freshly harvested seed was used. Recently there have been other reports of the disease in glasshouse-grown cucumbers in various countries (Geś, 1965; Jaeger, 1965; Yakovleva, 1965; and Inouye *et al.*, 1967) and Geś reported that yield losses of up to 10–15 per cent occurred.

There are few records of the occurrence and distribution of the virus in England. From 1960 to 1963 the disease was found in the Lea Valley on three, four, ten and four nurseries respectively, but the vast majority of the crops examined at the end of the 1964 season showed symptoms. At this time practically all the cucumbers in the Lea Valley were of the cv. Butcher's Disease Resister. Most of the seed was produced locally by two growers, and the majority of the crops were from one particular source. It has since been established that the 1963 seed crop of this source was infected and this appears to be one of the main reasons for the sudden increase in cucumber green mottle mosaic virus (CGMMV) in 1964.

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Experimental work was started in 1964 to investigate the effects of the virus on yield and quality and also to try to find a satisfactory seed treatment for its control.

EFFECTS ON YIELD AND QUALITY

Experiments were made in 1965 and 1966 at the Lee Valley Experimental Horticulture Station. The crops were grown on straw bales with a manure and peat bed on top. Because of the difficulty of screening this house to keep pollinating insects out, it was necessary to use the gynoecious cultivar Simex. The cordon system of crop culture was used in both years.

TABLE 1

Effect of CGMMV on yields and quality
(mean plot figures)

Treatment	Total		Marketable grades				
	Wt. (lb)	No. of fruits	Large per cent	Medium per cent	Small per cent	Bent fruit Wt. (lb)	Bent fruit No.
Inoculated cotyledons	2855·7	232·7	11	35·0	53·8	580·3	52·0
Inoculated when first fruit cut	3693·7	273·3	19·4	35·6	44·2	754·3	62·0
Naturally infected	3789·3	279·7	15·5	40·8	43·5	637·0	54·0
Naturally infected	3917·3	292·0	15·2	36·5	47·5	656·7	53·0
S.E.	101·0	9·06	3·04	3·06	1·91	224·6	6·6

1 lb = 0·4536 kg.

In 1965 there were four treatments, each replicated three times with 10 plants per plot. A polythene partition was constructed between each plot in an attempt to prevent accidental spread. A local isolate of CGMMV was used and in the first treatment plants were inoculated six days after seed sowing by rubbing the cotyledon leaves with a sap/celite mixture. In the second treatment inoculation was done ten weeks after sowing, shortly after the first fruits were harvested. Two young but fully expanded leaves per plant were rubbed with a sap/celite mixture and the leaves were then washed with distilled water. In the other two treatments the plants were allowed to become naturally infected later in the growth of the crop: one of these treatments had been intended as a third inoculation treatment but the onset of natural infection prevented this.

All inoculated plants showed symptoms within three weeks of inoculation. Eight weeks after inoculation in the second treatment all the plants in the uninoculated treatments were showing symptoms. Yield and quality records were made during a four-month harvesting period (Table 1).

The yield results per month are shown in Table 2.

TABLE 2

Monthly yields of cucumbers infected by CGMMV
(mean plot figures)

Treatment	1st month		2nd month		3rd month		4th month	
	Wt. (lb)	No.	Wt. (lb)	No.	Wt. (lb)	No.	Wt. (lb)	No.
Inoculated cotyledons	785·7	59·7	716·0	52·3	684·0	48·3	670·0	72·3
Inoculated when first fruit cut	1134·7	83·3	1197·3	73·3	677·0	47·0	684·7	69·7
Naturally infected	1080·3	76·7	1181·0	78·0	863·3	58·3	664·7	66·7
Naturally infected	1142·7	82·3	1217·0	76·3	845·7	57·7	712·0	75·7
S.E.	199·6	4·22	138·9	3·67	148·5	3·76	244·7	6·75

1 lb = 0·4536 kg.

In this experiment the earliest inoculation significantly reduced yield and quality during the first and second month of harvest and, to a lesser extent, during the third.

In a similar experiment in 1966 there were three inoculation treatments each replicated four times. Plants were inoculated either at planting (i.e., six weeks from seed sowing) or six or twelve weeks after planting. The same isolate of CGMMV was used as in 1965 and the plants were inoculated by rubbing the two youngest expanded leaves with a sap/celite mixture. The leaves were then washed with distilled water. Symptoms were seen on all inoculated plants within three weeks of inoculation. The yield and quality records (Table 3) show a trend towards lower yields in weight and number and smaller fruit with the earliest inoculation treatments, but the differences are not quite significant at the 5 per cent level. However, uninfected plants of the same cultivar grown in an identical way in the adjoining compartment of the same glasshouse yielded 26 per cent more fruit than those in the earliest inoculation treatment. This difference is comparable with that obtained in the first experiment between the first inoculated and the naturally infected treatments.

TABLE 3

The effect of CGMMV on yield and quality
(mean plot figures)

Treatment	Yield		Marketable grades							
	Wt. (lb)	No. of fruits	+10	10	12	14	16	18	18—	Bent fruit Wt. (lb)
Inoculated at planting	259·74	308·7	0·7	3·0	6·7	13·3	20·8	22·2	20·1	13·3
Inoculated six weeks after planting	301·90	349·7	3·1	2·7	7·9	14·1	22·7	17·1	18·2	14·2
Inoculated twelve weeks after planting	322·89	372·5	3·0	3·3	6·3	16·4	19·4	16·6	20·5	14·5
S.E.	39·96	43·9	1·1	1·3	2·2	2·8	2·9	3·2	4·7	1·9

1 lb = 0·4536 kg.

The numbered marketable grades refer to the numbers of straight fruit packed in a market tray. +10 and 18— refer to very large and small fruit respectively.

EFFECT OF EARLY INOCULATION ON ROOT GROWTH

There were very noticeable effects on growth of the early inoculated treatments described in the previous experiments (Plate I, 1). In order to investigate the effects of this early infection on root growth, cucumber seedlings cv. Butcher's Disease Resister (BDR) were inoculated soon after germination and plants were sampled at weekly intervals in order to record the amount of root growth. A similar batch of uninoculated plants was also sampled. The compost was carefully washed from the roots and the whole of the root system and the stem up to the cotyledon leaf node was weighed fresh and then dried and reweighed. Weights of six plants were recorded at 1, 2, 3 and 4 weeks after inoculation (Table 4).

After four weeks the root systems of the healthy cucumbers weighed approximately twice as much as those of the infected plants.

TABLE 4

Fresh and dry weights of root systems of healthy and infected cucumber plants

Days from inoculation when plants were sampled	Mean weight in g of six plants			
	Fresh weight		Dry weight	
	Infected	Healthy	Infected	Healthy
7	3·53	2·85	0·41	0·23
14	5·60	5·16	0·26	0·24
21	5·89	7·72	0·42	0·51
28	6·36	11·45	0·32	0·68

CONTROL BY SEED TREATMENTS

Because of the similarities between CGMMV and tomato mosaic virus it was decided to try a number of the treatments which Howles (1961) and Broadbent (1965) had found to be the most satisfactory with tomato seeds and TMV. In the first experiment, treatments with various chemicals and heat were compared on disease-free seed in order to ascertain their effects on the appearance of the seeds, the percentage germination and subsequent growth. The chemicals used were: 10 per cent trisodium orthophosphate applied as a 20-min soak and air-dried immediately after treatment; 10 per cent Teepol applied as a 2-hr soak and air dried immediately after treatment; and 70°C dry heat for five days in a thermostatically controlled oven. These treatments were compared with untreated seeds. There were four replicates of each treatment with 25 seeds per replicate. The seeds were sown in J.I. Seed Compost and germinated at approximately 27°C. A final germination count was made 10 days after sowing and records made of growth abnormalities. The mean percentage germination was 98, 97, 99 and 100 for the Teepol, trisodium orthophosphate, heat and untreated, respectively, and 5, 4, 7 and 1 per cent minor cotyledon leaf distortion was recorded. From this experiment it appeared that all three treatments were satisfactory in that they did not adversely affect germination. However, both chemical treatments caused considerable discoloration of the testas and the trisodium orthophosphate treated seeds tended to stick together. For this reason and because of the added difficulty of drying soaked seed the chemical treatments were discarded.

The effects of the dry heat treatment on infected seeds were determined by treating freshly harvested BDR seeds taken from CGMMV infected plants. The seeds were divided into batches of 10 and placed in envelopes. Five samples of 10 were removed from the oven at 70°C each day for five days. These seeds were tested for CGMMV by grinding them in 2 ml of phosphate buffer pH 7·1 with a small quantity of celite. The paste was then rubbed onto the cotyledon leaves of two cucumber seedlings. The heat-treated seeds were compared with a similar sized sample which had not been heat treated but were virus tested in the same way. Infected sap-inoculated controls were also included. In this experiment all cucumber indicator plants showed CGMMV symptoms from the untreated infected seed samples and the sap inoculated controls, but none of the heat-treated samples was positive. It appeared from this that one day at 70°C was sufficient to inactivate the virus. Similar results were obtained when clean seed was soaked in sap containing CGMMV for 24 hr then dried and treated. The seed samples were treated for one to six days, and five samples of the seeds removed per day and tested for CGMMV in the same way as in the first experiment. All the heat-treated seeds when tested gave negative results and the untreated controls were positive.

After 1965 and subsequent years it was difficult to obtain freshly harvested seeds infected with CGMMV and further work on the effects of dry heat on the virus was done by soaking filter paper squares in a sap extract, drying and treating these. Ten 2 × 2 in. (50·8 × 50·8 mm) Whatman No. 1 squares were used and each placed individually in envelopes for each temperature/time treatment. The filter paper was tested for viable CGMMV by adding 2 ml of phosphate buffer pH 7·1 to each square, allowing it to soak for a few minutes and then squeezing the liquid out. This liquid was then rubbed onto the cotyledon leaves of cucumber seedlings which had previously been dusted with celite. The results of various experiments on the effect of dry heat treatment are shown in Table 5.

TABLE 5

Effect of dry heat treatments

Treatment	Number of test plants out of 10 with CGMMV symptoms		
	60°C	65°C	70°C
1 day	2	5	3
2 days	2	0	0
3 days	2	0	0
4 days	0	0	0
Untreated	10	8	10
Sap inoculated	10	10	10
Control uninoculated	0	0	0

In a further experiment, impregnated filter paper squares were treated at 70°C for 1, 3, 6, 18, 24, 48 and 72 hr and tested for viable CGMMV in the same way as previously described. The number of test plants, out of five used for each treatment, which showed CGMMV symptoms was 5, 2, 3, 0, 0, 0, 0, respectively.

TABLE 6

The effects of dry heat treatment on the germination of cucumber seeds

Days from sowing	Percentage germination (50 seeds per treatment)																Unheated control
	Days at 70°C				Days at 75°C				Days at 80°C				Days at 85°C				
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
3	96	98	98	100	98	96	96	100	98	92	88	64	86	76	42	2	96
4	100	100	98	100	98	98	96	100	100	96	98	92	100	94	82	74	98
5	100	100	98	100	98	100	98	100	100	100	98	98	100	100	82	92	100
7	100	100	98	100	98	100	100	100	100	100	100	98	100	100	82	94	100

From these various experiments it appeared that treatment with dry heat at 70°C for a period of time in excess of 24 hr was satisfactory for the inactivation of CGMMV. The first experiment indicated that cucumber seeds would withstand treatment at this temperature for up to five days. To obtain further information on the heat tolerance of the seed, various temperatures and time treatments were done and the viability of the seeds tested. Seed of the cv. B.D.R. harvested in 1965, was heat treated in January 1968 at 70°, 75°, 80° and 85°C for 1, 2, 3 and 4 days respectively. There were five replicates per treatment and ten seeds per replicate. The seed was germinated in Levington Seed Compost at approximately 27°C. The results of this experiment are shown in Table 6.

The seeds treated at 85°C showed considerable distortion of the cotyledon leaves all of which had very wavy edges. A chlorotic blotch was present along the main veins of these leaves extending almost to the distal and proximal ends of the leaves. The seedlings were also considerably stunted (Plate I, 2). A few seedlings from seed treated at 80°C also showed similar symptoms. The seedlings from the 85°C treated seeds produced normal true leaves and the chlorosis of the cotyledon leaves decreased and eventually disappeared.

In a similar experiment, B.D.R. seeds were treated at 70°C from one to six days with no apparent effects on germination.

The results from these experiments suggest that cucumber seeds survive dry heat treatments at temperatures below 80°C for up to four days with little or no distortion or adverse effect on percentage germination.

COMMERCIAL TRIALS OF HEAT TREATMENT OF CUCUMBER SEEDS

In 1965 a complete stock of Butcher's Disease Resister was heat treated at 70°C for three days. This stock was sampled before heat treatment, and tests for the presence of CGMMV were all positive. The seeds were taken from a 1965 crop and just over 1,000 oz (28·35 kg) were heat treated. The treated seeds were used in 1966 to produce plants for 10 acres of cucumbers (approximately 45,000 plants) and the crops were inspected at intervals during propagation and cropping. There was a high percentage germination of all treated seeds (approximately 98 per cent) and no plants infected with CGMMV were found at any stage.

In 1966, 506 oz (14·34 kg) and in 1967, 403 oz (11·43 kg) of seed of various cultivars were treated. The virus status of these samples was not known but a check was made on the effects of the treatment on germination and growth abnormalities. In every instance the percentage germination was very good (above 95 per cent) and there was no apparent increase in the amount of leaf distortion or other abnormalities. Cultivars treated included Bestseller, Sporu and Topscore, in addition to three strains of B.D.R.

The majority of the commercial seed samples were treated in 1-oz (28·35-g) packets or in 1,000 seed lots (there are 800–1,000 cucumber seeds per oz). This quantity of seed is a convenient amount for treatment, and on a commercial scale heat treatment could be done after packaging. Usually cucumber seeds are sold in 1-oz packets and an experiment was carried out to investigate weight loss as a result of heat treatment. Sixteen 1-oz (28·35-g) packets of B.D.R. were heat treated at 70°C for five days and reweighed immediately after heat treatment. The average loss in weight of the 16 packets was 1·602 g, i.e. 5·6 per cent. When the packets were reweighed after 14 days storage at room temperature the weight loss was approximately 0·14 g per packet, i.e. less than 1 per cent; and after 40 days there was a mean weight gained of 0·4 g per packet, i.e. approximately 1 per cent.

These results indicate that 1-oz (28·35-g) samples of seed stored for 14 days after treatment at room temperature would return to approximately their original weight.

DISCUSSION

The decreasing number of cucumber seed producers in the Lea Valley during the past 10 years has resulted in a large proportion of the acreage being grown

from two strains of Butcher's Disease Resister. When in 1963 one of the seed strains was contaminated by CGMMV much of the cucumber acreage in the area was infected by this virus. Crop yields were low during the 1964 season and on many nurseries were down by about 15 to 20 per cent. Much of this crop loss was attributed by growers to infection by CGMMV. The experiments on the effect of the virus on yield and quality reported here, indicate that a loss in yield of approximately 15 per cent can occur when plants are infected before or at planting, but if the infection is delayed for six weeks the loss in yield may be far less. The yield losses in these experiments are comparable to those reported by Geś (1965). The infectious nature of the disease made it difficult to compare infected and healthy plants within the same glasshouse, and it is probable that a yield loss of 15 per cent is an underestimate for the comparison of early infected plants with healthy. The rate of spread of the disease is very rapid on commercial nurseries. In a number of instances spread took place throughout a large glasshouse (150 ft × 30 ft (45·7 m × 9·1 m)) in less than six weeks from a small number of infected plants found shortly after planting. A loss in yield of 15 per cent would not be surprising in these circumstances. In the experiments on heat treatment of virus-contaminated seeds a temperature of 70°C for more than one day was sufficient to inactivate the virus. This result was confirmed with filter paper impregnated with CGMMV. Because of the apparent tolerance of the seeds to longer treatments at this temperature, commercial trials were done with a treatment time of three days. This length of treatment at 65°C had inactivated the virus on filter paper squares so that a slight drop in temperature from 70°C during the treatment period would probably not affect the result. Similarly an increase in temperature of 5°C does not appear to affect the germination of the seeds adversely. A treatment time of three days at 70°C would, therefore, allow a fairly wide range of tolerance both for the inactivation of the virus and the satisfactory germination of the seeds.

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Cucumber Green Mottle Mosaic Virus (*see p.* 16)

1. Effect of cucumber green mottle mosaic virus infection on cucumber plants five weeks after inoculation of cotyledon leaves (*left*) compared with uninoculated plant (*right*).

2. Distortion of cucumber seedlings following treatment at 85°C for four days (*right*).

PLATE I