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STUDIES ON THE SEED TRANSMISSION OF PLANT VIRUS DISEASES

by

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The embryoculture investigations and the results of the dissection of tomato spotted wilt virus infected seeds described in Section B were presented in my M.Sc. thesis in 1953. They are again included here for the sake of completion.

THE SEED TRANSMISSION OF PLANT VIRUS DISEASES

C O N T E N T S

SUMMARY

Investigations made to find the reason for the rarity of seed transmission of plant virus diseases have demonstrated that:-

1. it is not commonly due to sterility through virus induced abortion of embryos;
2. it is not due to the presence of virus-"inactivators" in the seeds;
3. even though highly infectious viruses are able to invade the testa and endosperm of nearly all the seeds of an infected plant they are unable to invade the embryo developing in the infected endosperm.

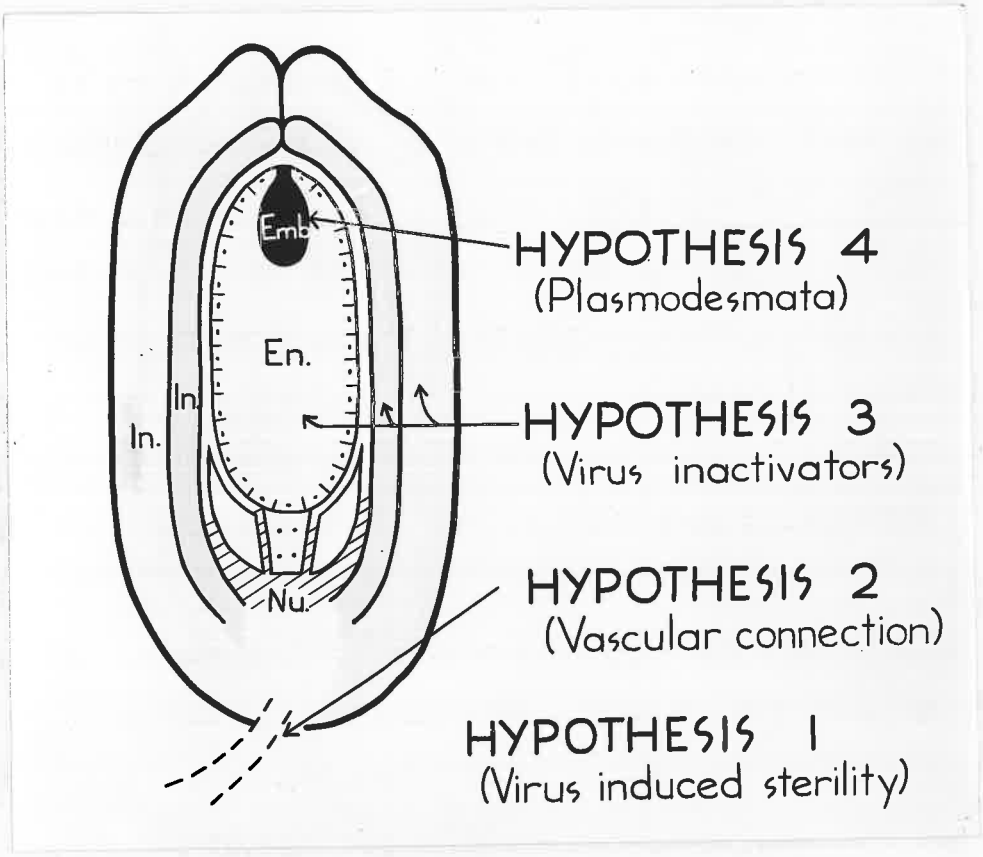
It is concluded that the rarity of seed transmission is due to the inability of most viruses to infect the mega- or microspore mother-cells of infected plants, together with the inability of viruses to infect the developing embryo because of the lack of plasmodesmatal connection with the endosperm.

I. INTRODUCTION

Of several hundred plant virus diseases that have now been described only 45 are reported to be seed transmitted. These are tabulated in Appendix 2. Several points can be seen from a study of the information tabulated in this Appendix:-

1. The transmission of plant viruses through seeds is not, as has often been claimed, more common in the Leguminosae than in some other families.
2. In only four cases does the percentage transmission exceed 50.
3. The property of transmissibility is not a property of any virus, nor of any host, but is clearly an interaction of the two, virus and host.

The site of operation of three hypotheses that have been put forward to explain the rarity of seed transmission are diagrammatically illustrated in Figure 1.



In.	Integuments
Nu.	Nucellus
En.	Endosperm
Emb.	Embryo

Fig. 1. Seed transmission; hypotheses.

Hypothesis 1. Allard (1915) suggested that virus infection "may disturb the normal relations of stamens and pistils to such an extent as to cause sterility". In 1952 Caldwell demonstrated that in the Asperny disease of tomato "the presence of the virus in the microspore-mother-cell results in a complete interference with the normal stages of meiosis....". This hypothesis adequately explains cases of non-transmission

such as that of tomato aspermy disease, but cannot be extended to explain why seed transmission of disease is so limited, or even absent, in other virus diseases where infected plants produce large quantities of seed. This hypothesis also fails completely to explain how embryos can develop to maturity in a virus infected endosperm without themselves becoming infected.

Hypothesis 2. Bennett (1936) suggested that the lack of vascular connection between the embryo and its parent plant prevents the seed transmission of those virus diseases which are largely limited to the vascular tissues. This theory adequately explains the complete absence of seed transmission of all virus diseases of this type, but like hypothesis 1 is not capable of extension to other virus diseases.

Hypothesis 3. Dugger (1930) suggested that the seed transmission of those highly infectious viruses which are almost ubiquitous in their distribution within a plant might be prevented by the inactivating action of some "specific protein or other specific material" in the seeds. This hypothesis was elaborated by Kausche (1940), but neither of these workers distinguished between virus inactivators and virus inhibitors in seeds. Until their 'inactivators' are proved not to be inhibitors their conclusions are unjustified.

Hypothesis 4. Bennett's second theory is the most plausible. He suggested that the lack of plasmodesmatal connection between the embryo and the parent plant prevents the seed transmission of even the most infectious virus diseases. This suggestion rests on two assumptions, and neither can be tested experimentally: (1) that the micro, and megaspore-

mother-cell either escape infection, or are unable to support virus multiplication (2) that the only path of intracellular virus movement is via the plasmodesmata.

The investigations described here have been carried out to determine:-

1. whether virus-induced sterility could be the reason for the rarity of seed transmission of some of the more infectious plant virus diseases;
2. what tissues of the seed are infected by these viruses;
3. whether seeds do contain virus inactivators;
4. whether plant embryos themselves inactivate viruses during their development;
5. whether seed transmitted viruses infect the embryos of their hosts before fertilization, or after fertilization.

II. EXPERIMENTAL

A. THE EFFECT OF VIRUS INFECTION ON POLLEN AND SEED PRODUCTION

An essential requirement for investigations associated with the seed transmission of plant virus diseases is a knowledge of whether viruses are able to infect microspores or macrospores, and if this infection will interfere with the normal behaviour of these cells.

Caldwell (1955) showed that with tomato asperity disease seed transmission is made impossible by virus-induced abortion. He suggested that this may be the usual consequence of infection of microspores or macrospores by a virus, and that in cases where virus infection does not induce abortion seed transmission will occur. There is evidence which seems to support Caldwell's suggestion. Tobacco ringspot virus induces no sterility in soybean in which it is seed transmitted (Desjardins 1954), but does induce sterility in both petunia and tobacco where it is not seed transmitted (Henderson 1931, and Valleau 1941). Similarly, a non-seed-transmitted mosaic disease of Dolichos biflorus was found by Uppal (1931) to reduce fertility greatly, and a graft transmissible disease of tobacco tested by Kostoff (1933) induced complete sterility of infected plants. However, these results do not establish whether in these instances the reduced fertility of the infected plants results from the stunting and impairment of vigor induced by the virus, or from a specific effect of the virus on microspores or macrospores.

The results of Blakeslee's (1921) investigations with the

Quercina disease of Datura stramonium would seem to be at variance with Caldwell's suggestion, for Blakeslee found that although virus infection induced a high degree of sterility, 100% of the pollen produced on infected plants was infected. In this case, at least, virus infection does not invariably lead to the abortion of pollen mother-cells. Unfortunately there is no evidence to indicate whether the sterility induced by this virus results from an interference with the meiotic divisions, or from a physiological disturbance of floral development.

If Caldwell's suggestion is assumed true the rarity of reports of virus induced sterility is surprising. It is particularly surprising in the case of the highly infectious systemic virus diseases such as tomato spotted wilt, cucumber mosaic or tobacco mosaic viruses. It has been estimated recently (Nixon 1956) that the number of particles of tobacco mosaic virus per cell of tobacco leaf tissue is of the order of 6×10^7. If their concentration in floral tissues is anything approaching this magnitude there should be a significant reduction in fertility, unless some mechanism prevents the infection of the gametophytic tissues, or the survival of viruses in them. Allard (1915) has reported a reduction in the seeds produced by mosaic infected tobacco plants, but there are no reports of any effect of this virus on the seed production of any of its other hosts.

Few critical investigations have been carried out on this aspect of the effect of plant viruses on their hosts; the effects, therefore, of four viruses on the fertility of their hosts have been studied in the present work. The viruses used were bean yellow mosaic, bean mosaic,

tobacco mosaic, and cucumber mosaic.

(1) The effect of bean mosaic and bean yellow mosaic viruses on the fertility of bean.

The two viruses of bean were used because the yellow mosaic virus is not seed transmitted, and the common mosaic virus is transmitted through both seed and pollen. An obvious conclusion is that if the primary cause of the lack of pollen infection by bean yellow mosaic virus is the abortion of the microspores then the virus should have a marked effect on fertility. The bean mosaic virus was provided by Mr. J. Johnson of the Queensland Department of Agriculture and Stock. The virus was not infectious to peas or clovers and in beans it induced symptoms closely corresponding to those described by Reddick and Steward (1919). The bean yellow mosaic virus used was isolated from some naturally infected bean plants at Mount Gambier and was infectious to bean, pea, Trifolium incarnatum L., and T. hybridum L. In all of these hosts the symptoms corresponded closely to those described by Pierce (1934).

Two experiments were carried out with these viruses. The first was begun early in the summer of 1955. It was repeated early in 1956, but with all plants raised in a cooled glasshouse so that the symptoms of the diseases would not be masked by the high temperatures. Thirtythree Canadian wonder bean seedlings were used in the experiments. Plants were selected at random and allocated to each treatment. Eleven plants were inoculated with bean mosaic virus, eleven with bean yellow mosaic virus, and eleven were left uninoculated. Throughout the growing period of the plants, records were kept of the number of flowers appearing daily on

each plant, of the number of pods produced, and after harvest, of the number of seeds per pod in each of the three treatments. The results of these experiments are tabulated in Table 1.

TABLE 1

THE EFFECT OF VIRUS INFECTION ON THE FERTILITY OF BEAN PLANTS

Virus	Experiment 1				Experiment 2
	Flowers per plant	Pods per plant	Seeds per pod	Seeds per flower	Seeds per flower
None	28	10	3.8	1.4	1.1
Bean mosaic	33	10	4.1	1.3	0.4
Bean Yellow mosaic	25	8	3.2	1.0	0.7

The best estimate of plant fertility is, I think, the number of seeds produced per flower. On this basis a slight reduction in fertility was produced by both viruses. In the first experiment the bean yellow mosaic virus had the greater effect. In the second experiment the bean mosaic virus had the greater effect. The effect of both viruses on flower production was negligible although it is interesting that bean mosaic virus appeared to stimulate flowering. From these results the conclusion is justified that virus induced sterility is not a sufficient explanation for the prevention of seed transmission of bean yellow mosaic virus.

(2) The effect of tobacco mosaic and cucumber mosaic viruses on the fertility of pungent pepper

A more satisfactory test of the hypothesis of virus induced sterility could be carried out by the use of viruses which reach very high concentrations in their hosts, and can move rapidly between cells. If sterility does result from the infection of the megaspores and pollen grains by viruses, then those viruses which are in highest concentration should induce most sterility. Tobacco mosaic and cucumber mosaic viruses were chosen as suitable for this investigation. The tobacco mosaic virus was isolated from pungent pepper seed (Capsicum frutescens) obtained from Dr. H.H. McKinney, who reported 22% seed transmission to occur in this host (McKinney 1952). The cucumber mosaic virus was provided by Mr. L.L. Stubbs, and originated from naturally infected cucumbers. The virus was infectious to Cucumis sativa, C. melo, Cucurbita pepo, Citrullis vulgaris, Nicotiana tabacum, Lycopersicum esculentum, Datura stramonium, and Vigna sinensis. On all these hosts the symptoms produced were consistent with those described by Doolittle (1920).

My original intention was to determine the effect of two strains of tobacco mosaic virus on the fertility of pepper, in which only one strain was seed transmitted. However, pungent peppers, inoculated with the ordinary strain of tobacco mosaic virus produced only local lesions on the inoculated leaves, and systemic symptoms did not develop. It was also found that although seed transmission of the pepper strain of this virus does occur in pungent pepper the embryos are not infected, but become infected by contamination from the infected testa during germination.

A similar occurrence with the "tomato streak" strain of tobacco mosaic virus was described by Chamberlain (1946) in tomato.

These results thus prevented the investigation of this aspect of the problem using these two strains of a single virus on C. frutescens. Instead, the effect of the two viruses, tobacco mosaic and cucumber mosaic, on the fertility of pungent peppers, was investigated. Experiments were carried out as described earlier with bean. Eight plants were used in each treatment.

TABLE 2

THE EFFECT OF TOBACCO MOSAIC AND CUCUMBER MOSAIC VIRUSES ON THE FERTILITY OF PUNGENT PEPPER PLANTS

Experiment 1

Virus	Flowers per plant	Fruits per plant	Seeds per fruit	Seeds per flower
None	82	18.6	134	30.4
Cucumber mosaic	82	5.1	91	5.7
Tobacco mosaic	89	16.9	135	25.7
		Experiment 2		
None	70	7.0	112	11.1
Cucumber mosaic	44	2.2	43	2.2
Tobacco mosaic	43	7.2	88	15.0

We can see from these results that tobacco mosaic virus did not affect the fertility of pungent pepper plants, while cucumber mosaic virus reduced their fertility by between 50% and 80%. Comparisons of the pollen grains from healthy, and cucumber mosaic infected plants, did not

reveal the presence of any abnormal pollen. Examinations of anthesis at an earlier stage gave no indication of any virus induced disturbance of meiosis, and tetrads of microspores were regularly found in young anthers of infected plants. I do not think it possible that a 50% reduction in the fertility of plants could be produced by interference with the normal process of meiosis, without some microscopically visible effect being produced on both anthesis and pollen production. On the other hand, cucumber mosaic virus greatly upsets the normal growth of pepper plants. Infected plants are stunted; flower production is disturbed; infected plants produce fewer flowers over a much longer period; fruits are greatly distorted. The most reasonable conclusion consistent with these results is that cucumber mosaic virus reduces the seed production of pungent pepper plants primarily by upsetting the normal hormonal control of plant growth. As a result of this, fruit growth is abnormal and stunted and seed production is inhibited.

It is concluded that although virus-induced sterility may be an adequate explanation for the lack of seed transmission of tomato asperny disease, it does not appear to be a contributing factor to the lack of seed transmission of the viruses investigated. There is no evidence that these viruses interfere with the meiotic divisions of their hosts, and as neither of the viruses used are pollen transmitted, it would seem that some other mechanism must exist to prevent the infection of microspores and macrospores.

B. THE LOCATION OF VIRUSES IN THE SEEDS OF THEIR HOSTS

As the viruses studied did not induce abortion of the microspores or macrospores it was concluded that either

1. infection of these cells does not kill them, or
2. viruses are excluded from gametophytic tissues.

Assuming the first true, embryos must be infected early in their development and seed transmission then prevented by the inactivation of virus in the embryo during seed maturation. If the second alternative is true then embryos are not infected initially and seed transmission can still occur unless infection of the developing embryo is prevented.

The facts of the distribution of viruses in seeds are remarkably few. Different workers have reached conclusions which are conflicting; they have stated that in tomato seeds tobacco mosaic virus is:- mainly a superficial contaminant (Chamberlain 1950); present in the testa only (Ainsworth 1934); capable of infecting testes and two thirds of the embryos (Berkley and Madden 1932). (However, the seeds used by Berkley and Madden had already begun to germinate, and hence the possibility of contamination from the infected testa makes their results valueless). The distribution of only three other non-seed-transmitted viruses has been investigated. Bennett (1936) showed the curly top virus of sugar beet to be present in all the tissues of the seed except the embryo. Sheffield (1941) obtained evidence from a study of virus inclusion bodies that "severe etch" virus infects the testa, but not the endosperm or embryo of the seeds of Hyocyamus niger. Cheo (1955) reported the infection of

100% of both embryos and testas of bean by "southern mosaic" virus. He suggested that seed transmission of this virus was prevented by the inactivation of the virus during maturation and storage of the seed. A similar suggestion was made by Gold (1956) to explain the fact that although barley mosaic infected 100% of the developing embryos only 50-90% seed transmission occurred. In summarising - the results of Ainsworth and Sheffield seem to support the hypothesis that viruses are unable to infect embryos; while the results of Cheo and Gold support the hypothesis that viruses do infect embryos but are inactivated before the seed germinates.

Investigations were carried out to determine whether embryo infection does take place, and whether there is any virus inactivation associated with the maturation of the seed. Two viruses of bean were used, of which only one was seed transmitted. Three other viruses were chosen such that with each, one host could be used in which seed transmission did occur, and a second host in which seed transmission had not been reported. The virus-host combinations were:

Bean mosaic virus (43% transmission; Archibald 1921) and bean yellow mosaic virus in bean.

Tomato spotted wilt virus in cineraria (96% transmission; Jones 1944) and tomato.

Cucumber mosaic virus in wild cucumber, Echinocystus lobata (22% transmission; Doolittle 1919) and cucumber.

Tobacco mosaic virus in pungent pepper (22% transmission; McKinney 1952) and tomato.

(1) Seed dissection and inoculation techniques

No special techniques were necessary for the dissection of bean, cucumber or wild cucumber seeds. The tissues were washed in water, ground in neutral composite buffer and inoculated onto test hosts. All tests for the presence of bean viruses were carried out by inoculation to five 10-14 day old bean seedlings of the variety Canadian Wonder. This variety was used as it was found more susceptible than Kentucky Wonder. Cucumber mosaic virus was inoculated to the cotyledons of five young "Long Green" cucumber seedlings. The dissection of seeds of cineraria, tomato and pepper was simply accomplished if the seeds were first soaked for one or two hours. In dissecting cineraria seeds, the embryo was removed by making a small cut at the bottom of the fruit through which the embryo was ejected by gently pressing with a flat pointed needle. Similarly young seeds of tomato and pungent pepper can be separated into testa, endosperm and embryo. N. tabacum was used in all inoculations for the detection of tomato spotted wilt virus because it was found to be more susceptible than N. glutinosa.

The "tomato spotted wilt" infected tomato seeds were all obtained from naturally infected fruits from the field. The infected cineraria seeds were obtained from both naturally infected field grown plants, and from glasshouse plants infected with one field strain and four pure strains of tomato spotted wilt virus. The pure strains were provided by Dr. R.J. Best. All other viruses used have been described above. The seeds from tobacco mosaic infected fruits were dissected in the same manner as the cineraria seeds, and the tissues soaked in 10%

'Teepol' for several hours. This inactivated any virus particles that were superficially contaminating the tissues, particularly those of young seeds in which the endosperm is gelatinous and invariably contaminates the other tissues. 'Teepol' was found to be the only satisfactory surface sterilising agent capable of inactivating superficial virus contamination without significantly affecting the infectivity of the tissues. It was shown that embryos dipped in a concentrated tobacco mosaic virus preparation could be freed of contaminating virus by rinsing in 'Teepol'. The results in Table 3 show that its effect on the infectivity of tissues is negligible.

TABLE 3

EFFECT OF SOAKING TESTAS OF PUNGENT PEPPERS FOR 1 HOUR IN A 20% SOLUTION OF 'TEEPOL'

	Mean No. of Lesions/Half Leaf	
	Undiluted	Diluted $^{1}/_{10}$
'Teepol' soaked	5	20
Water soaked	58	23

Nicotiana glutinosa was used in all inoculations to detect tobacco mosaic virus.

Tobacco mosaic and cucumber mosaic virus are so highly infectious that their detection by these methods presents no problem. The tissues used with the viruses of bean were large enough to ensure their detection if infected. However, tomato spotted wilt virus is not

highly infectious and the tissues used were, in many cases, very small. These factors could prevent the detection of the virus in the embryos of either tomato or cineraria.

The results of all the dissection investigations are presented in the diagram on the following page.

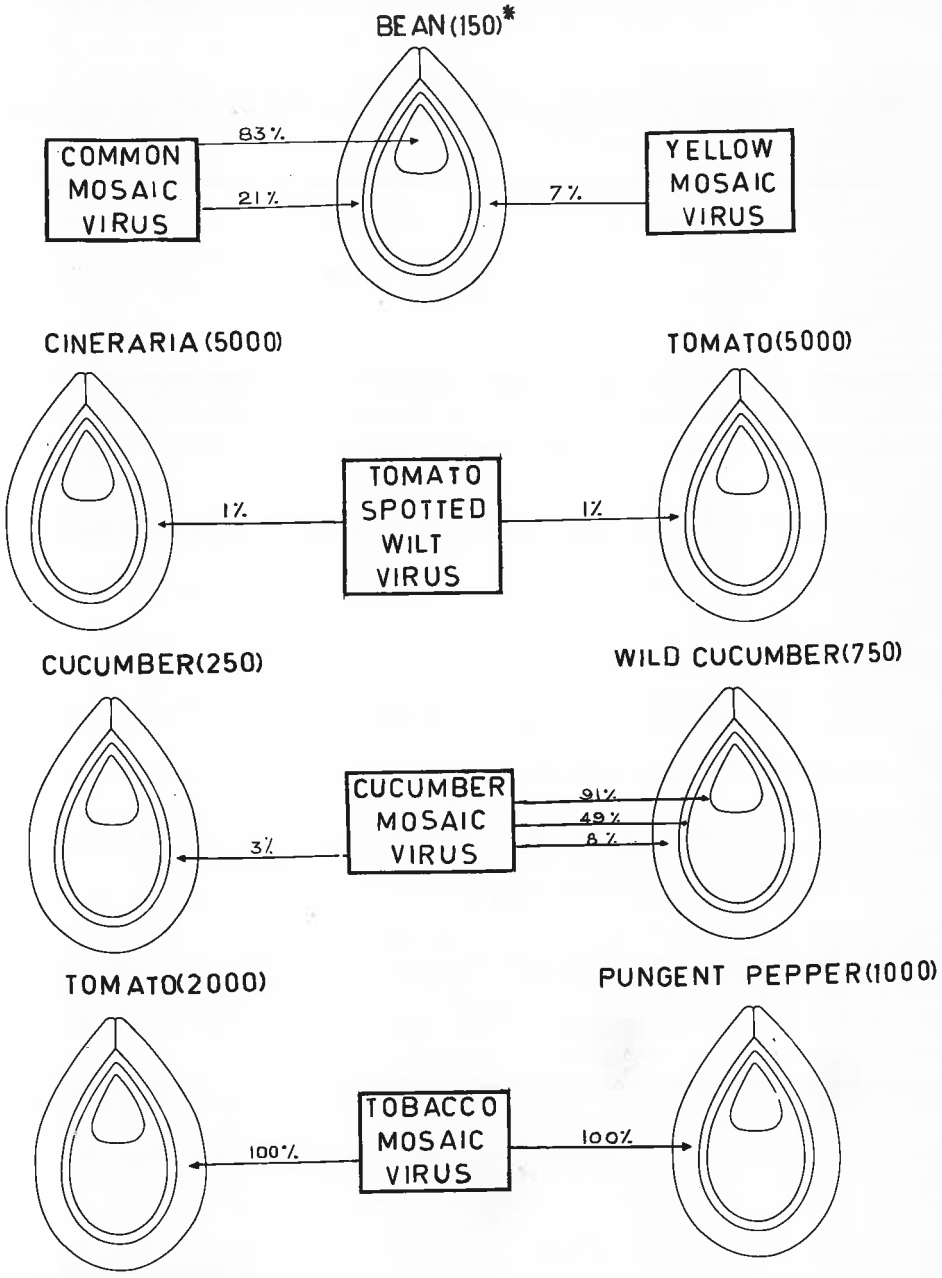
(2) Embryoculture investigations

To eliminate the possibility that the technique was not adequate to detect low concentrations of tomato spotted wilt virus 900 tomato embryos were grown in sterile embryo-culture on solid "White's Medium" containing coconut milk (White 1943, van Overbeek 1941). The technique used is illustrated in Figure 2. During four to six weeks the tomato embryos developed into small seedlings at the four or five leaf stage. The amount of development and active photosynthesis which had taken place by this stage would have been sufficient to allow any viruses present to multiply to easily detectable concentrations. The embryo-seedlings were next ground and inoculated to tobacco. None was infected. Three hundred and forty cineraria embryos were also grown in embryo-culture for six to eight weeks and then ground and tested. Again none was infected. It was concluded that tomato spotted wilt virus does not infect the embryos of either of these hosts.

(3) Seed transmission

The obvious difficulty is to understand how seed transmission can occur in cineraria, wild cucumber and pungent pepper without the embryos of these seeds being infected. Several trials were carried out

DISTRIBUTION OF VIRUSES IN SEEDS



* NUMBER OF SEEDS DISSECTED

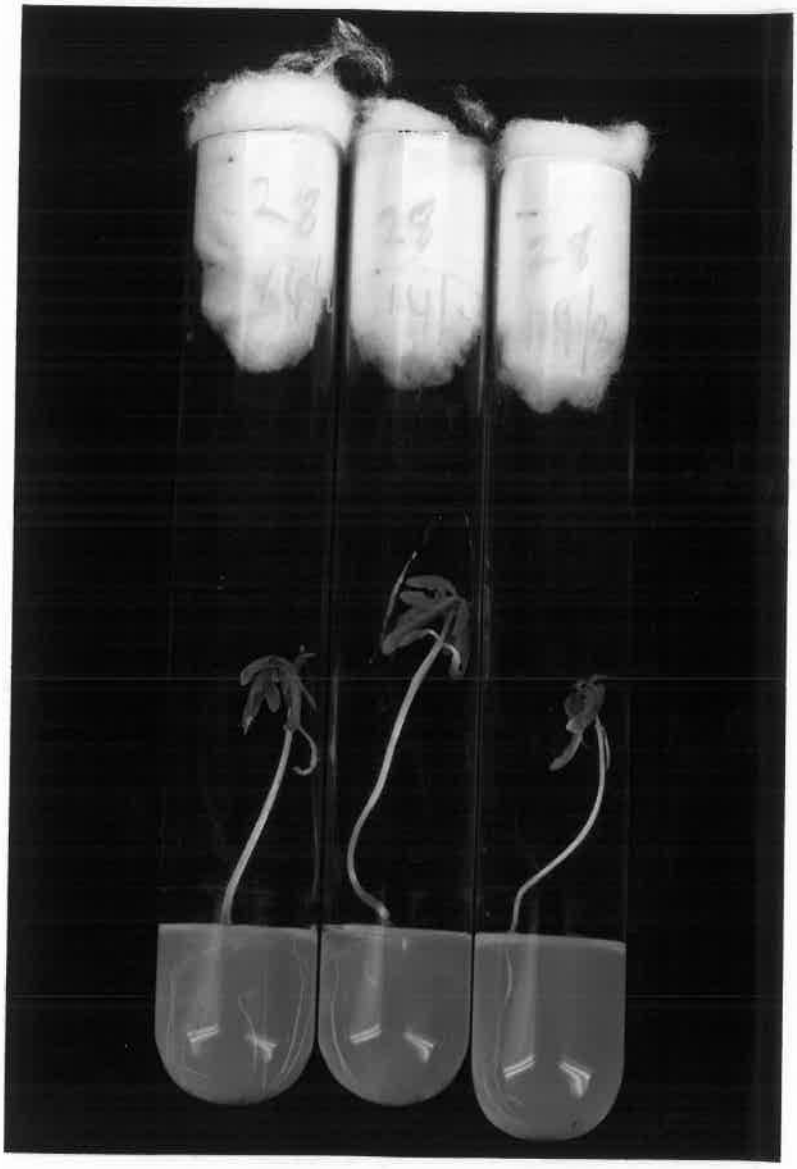


Fig. 2. Tomato embryoculture (2 weeks old) on "White's Medium" in 42 cm test tubes

to determine whether seed-transmission did, in fact, occur in these hosts. More than 5,000 cineraria were raised from seed of tomato spotted wilt infected plants. Not one infected seedling was discovered. Thus,

the 9% seed transmission reported by Jones must have been obtained either with a most unusual strain of tomato spotted wilt virus, or with a different species of cineraria. Four hundred wild cucumber seedlings were raised from the seed of cucumber mosaic virus infected plants. Only one infected seedling was observed. With this virus also, there seems no alternative to the assumption that the strain of cucumber mosaic used was not (or only very rarely) seed transmitted under the conditions of these trials. In pungent pepper, the percentage seed transmission of tobacco mosaic virus varied between 15% and 30%. However, if neither embryo nor endosperm of pungent pepper is infected by this virus, seed transmission must result from the contamination of the germinating embryo by the infected testa.

This conclusion was confirmed experimentally. A sample of freshly harvested pepper seeds was divided into halves. One was sown immediately; the other after the testa had been removed. Twentyfive percent of the seedlings of the first lot were found to be infected when inoculated to N. glutinosa, but not one infected seedling was detected amongst the dissected (testa removed) seed.

(4) Pollen transmission

As a further check on the conclusions that tobacco mosaic and bean yellow mosaic viruses do not infect the embryos of their hosts, tests of the pollen transmission of these viruses were carried out. For if pollen transmission occurs, embryo infection must occur. Healthy pepper plants were pollinated with pollen from infected plants, and of the seeds

produced, some were dissected and some sown. No infected tissues were detected in these seeds, and none of the seedlings produced was infected. Healthy bean plants were pollinated with pollen from mosaic, and yellow mosaic infected plants. The seeds were harvested when about three-quarters mature, the embryo removed, ground, and inoculated to bean seedlings. Fifty percent of the embryos from the seeds pollinated with common mosaic infected pollen were infected, but not one bean yellow mosaic infected embryo was detected. This is in marked contrast to Cheo's finding that southern bean mosaic virus infects 100% of the embryos of bean. At present southern bean mosaic virus is the only one known where seed transmission is prevented by some virus inactivating process that takes place during seed maturation (Cheo 1955).

(5) Changes during maturation

Investigations have shown that neither tobacco mosaic nor tomato spotted wilt virus infects the embryos of their hosts at any stage, and no change in the proportion of testas infected by either of these viruses could be detected as the seeds matured. With these two viruses, therefore, the results illustrate virus distribution in seeds at all stages of development. However, the distribution of cucumber mosaic virus in the seeds of wild cucumber altered significantly during their maturation. Table 4 presents the results of one trial in which a total of 235 seeds were dissected at all stages of maturity.

TABLE 4.

DISTRIBUTION OF CUCUMBER MOSAIC VIRUS IN THE SEED OF WILD CUCUMBER

	Percentage infected			
	Testa	Perisperm	Endosperm	Embryo
Immature	91	49	8	6
Mature	27	0	-	0.7

To calculate these results, seeds were classed as immature in which the embryo was less than half its mature size. Those in which the embryo had attained its full size and the testa had coloured were classed as mature. As the testa achieves its full size at a very early stage in seed development this arbitrary classification was easily carried out.

As no very young embryos and only one mature embryo was infected, a mechanism in the developing embryo must prevent its infection by viruses present in the endosperm or perisperm. The lack of infected endosperms in mature seeds is because this tissue is absorbed by the developing embryo. The decline in the percentage of testas infected could be caused by the inactivation of the virus or the production of inhibitors in the maturing testa.

The results obtained with all five viruses show that these non-seed transmitted viruses are unable to infect the developing embryos of their hosts, and indicate that seed transmission depends on the ability of a virus to infect the embryo even if, as in the case of pungent pepper seeds, embryo infection occurs during germination. The next step in these

investigations was to discover the means by which embryo infection is prevented.

C. THE EFFECT OF SEED EXTRACTS ON PLANT VIRUSES

It is clear from the investigations detailed above that some mechanism operates to prevent the infection of embryos during their development. Some embryos, such as those of wild cucumber, are developing within an endosperm that is often infected, and yet, even in this case, embryo infection practically never occurs. One of the most easily investigated mechanisms is that suggested by Dugger (1930): that "by some specific protein or other specific material" virus inactivation occurs and seed transmission is prevented. Kausche published results in 1940 to demonstrate the ability of seed extracts to reduce the infectivity of the virus by as much as 50%. The work described here has been carried out to determine whether inhibitors, or inactivators, of plant viruses do exist in seeds, the nature of their action, and their bearing on the problem of seed transmission.

(1) The effect of seed extracts on plant viruses

Two viruses, tobacco mosaic and cucumber mosaic, were selected for this work; tobacco mosaic virus because it had been used for similar work by Kausche, and because of all the plant viruses known the rarity of seed transmission of this virus is possibly the most puzzling; cucumber mosaic virus because it provided an example of a highly infectious plant virus disease reported to be seed transmitted in one host (wild cucumber)

and not seed transmitted in another (cucumber). If Duggar's theory is correct, that seed transmission is prevented by virus inactivators, then the virus inactivators present in the seeds of cucumbers and wild cucumbers must differ greatly. A comparison of the relative inactivation produced by seed extracts of these two hosts should enable a critical test of Duggar's theory. These tests have been carried out and the results are presented in Tables 5 and 6.

The technique used was very similar to Duggar's. Extracts of the tissues of cucumber or wild cucumber were prepared by grinding 1 g. of tissue in 20 ml. of distilled water and filtering through muslin. Five mls of this extract were added to 5 mls of inoculum, and changes produced in the infectivity of the virus were detected by inoculation to cowpea. Similar samples of inoculum to which 5 mls of distilled water had been added were used as control treatments. Three dilutions of the extract were used to enable a more precise estimation of its effect. The technique of raising and inoculating cowpeas has been described previously (Crowley 1954). Four treatments were involved in these comparisons and their infectivity was compared by the half-leaf technique using 16 replicates of each treatment randomized so that all treatments occurred on every plant, and occurred an equal number of times in each leaf position. The cucumber mosaic virus inoculum used was a $^{1}/_{25}$ buffered dilution (pH 7) of the sap of three week old cucumber seedlings. Extracts of tobacco seed used to obtain the results presented in Table 6 were prepared by grinding 2 g. of Blue Prior tobacco seed in 10 mls of phosphate buffer at pH 7 over a period of three to

five hours, and then filtering through muslin. After I had found the inhibitor in tobacco seed to be heat stable, its partial purification was effected by heating for 10 minutes in boiling water and again filtering. Portions of this extract were then added to equal portions of virus inoculum. The inoculum used was a $^{1}/_{1000}$ dilution of a purified virus preparation. N. glutinosa was used as the test plant. Again the treatments were compared by the half-leaf technique, and randomized so that all treatments occurred equally often on left and right halves. At least twenty replicates were used.

TABLE 5

EFFECT OF SEVERAL DILUTIONS OF AQUEOUS CUCUMBER EMBRYO EXTRACTS ON LESION PRODUCTION BY CUCUMBER MOSAIC VIRUS ON CONTRA

Extract from	Mean Number of Lesions per Half leaf			
	Material added to Inoculum			
	Extract	Extract/10	Extract/100	Control Dist. Water
Cucumber embryos	0***	8***	71***	176
Cucumber testas	0***	6***	90**	138
Wild cucumber embryos	2***	136*	171	182
Wild cucumber testas	55***	152	147	152

* Difference from control significant at P = 0.05
** Difference from control significant at P = 0.01
*** Difference from control significant at P = 0.001

TABLE 6

EFFECT OF BUFFERED AQUEOUS EXTRACT OF TOBACCO SEED ON LESION PRODUCTION BY TOBACCO MOSAIC VIRUS

Treatment	Number of lesions per half leaf
Inoculum + buffer	141
Inoculum + extract	37***

*** Difference significant at P = 0.001

The results in tables 5 and 6 demonstrate the existence of some constituent in seeds which can greatly reduce the number of lesions produced by both cucumber mosaic and tobacco mosaic viruses on their respective hosts.

In the case of cucumber mosaic virus, embryo extracts were much more effective than testa extracts in reducing the number of lesions produced on cowpea. Cucumber extracts were regularly found to be more effective than wild cucumber extracts.

With tobacco mosaic virus, whole seeds were used as the small size of seeds did not permit the dissection of large numbers. The addition of tobacco seed extract to tobacco mosaic virus inoculum consistently resulted in a significant reduction in the number of lesions produced; but the extracts used here were four times as concentrated as those reported by Kausche to produce a similar effect. This is attributed to the fact that Kausche used a different variety of tobacco seed, and slightly different materials and methods. At first sight the results in

Tables 5 and 6 would appear to support Dugger's theory. However, there remains the possibility that the effect of the seed extracts on the virus may not in fact be that of a "virus inactivator" which actively breaks down virus particles and makes them non-infectious. Instead, its effect could be merely that of inhibiting the infection of N. glutinosa, without having any effect on the virus itself. Investigations were therefore carried out to determine the manner in which seed extracts alter the infectivity of virus inocula.

(2) The nature of the action

It is impossible to tell in any simple manner whether a treatment which reduces the number of lesions produced by a given virus inoculum produces its effect by an effect on the host used for measuring infectivity, or by inactivating the virus. The two processes can be distinguished in several ways suggested by Caldwell 1935; Siegle et al. 1952; Bawden and Freeman 1952. Inhibitors, whose effect is primarily to affect the susceptibility of the host used, can be distinguished from inactivators by possessing the following characteristics: they are capable of instantaneous effect; their effect is dependend on the host used; they have greater effect on concentrated inocula; the effect is diminished by dilution; they have an effect when applied prior to inoculation or when applied to the under-surface of leaves.

The experiments described below, using all of these methods, were carried out to determine the nature of the action of aqueous extracts both from cucumber embryos and tobacco seed.

(3) Reaction time

The results in Tables 7 and 8 show that the effect of both of the extracts used is just as great immediately (within 30 seconds) after addition to the inoculum as it is several hours later. The decline in the infectivity of the treatments was consistently found to be of the same order as the decline in the infectivity of the controls, and was not more than normally occurs through a decline with age in the infectivity of inoculum. This occurrence is not consistent with the conclusion that a virus inactivator is present in the extracts, for the effect of a virus inactivator will increase with the time of contact of the virus and inactivator, whereas the effect of an inhibitor will be maximal immediately after its addition to the virus inoculum.

TABLE 7

INSTANTANEOUS EFFECT OF CUCUMBER EMBRYO EXTRACT IN INHIBITING LESION FORMATION BY CUCUMBER MOSAIC VIRUS ON COWPEA

Inoculation time	Mean number of lesions per half leaf	
	Extract	Control
Immediately after mixing	9.8	24.4
4 hours after mixing	4.8	24.6

TABLE 8

INSTANTANEOUS EFFECT OF TOBACCO SEED EXTRACT IN INHIBITING LESION FORMATION BY TOBACCO MOSAIC VIRUS ON N. GLUTINOSA

Inoculation time	Mean number of lesions per half leaf	
	Extract	Control
Immediately after mixing	26.9	85.1
5 hours after mixing	26.2	84.7
Immediately after mixing	26.4	94.9
24 hours after mixing	20.7	89.1

(4) Effect of host used

The results of an experiment in which four different hosts were used to measure the infectivity of the inocula are set out in Table 9,

TABLE 9

INFECTIVITY OF MIXTURES OF CUCUMBER MOSAIC VIRUS AND CUCUMBER EMBRYO EXTRACTS TO DIFFERENT HOSTS

	Host			
	Cowpea	Cucumber	N. glutinosa	Tobacco
	Mean lesion No.	Proportion of plants infected		
Treatment	0	$^{20}/_{20}$	$^{2}/_{2}$	$^{1}/_{2}$
Control	69	$^{20}/_{20}$	$^{2}/_{2}$	$^{2}/_{2}$

and show that the effect of cucumber embryo extract is dependent on the host used for infectivity measurements. These differences in the

infectivity of a single inoculum could not be due to differences in the susceptibility of the different hosts, as in trials carried out concurrently with this work the susceptibility of cowpeas and cucumbers to cucumber mosaic virus was found to be of the same order. The dilution end point of inoculum was found to be 1 in 10,000 regardless of whether cucumber or cowpea was used for the test. On the contrary, although the mixture is not infectious to cowpea, the infectivity of the virus per se is unimpaired.

Similar trials could not be carried out with tobacco mosaic virus because no local lesion host other than N. glutinosa was available and even on this host it was impossible to completely inhibit local lesion production. Hence the use of any host producing systemic symptoms on infection would be futile as there would be no difference in the systemic reaction of a plant to a weak or a strong inoculum.

(5) Dilution of extract-inoculum mixtures

Gupta and Price (1950) in studying the nature of the effect of fungal extracts on plant viruses showed that non-infective mixtures could be made infective simply by dilution and they concluded that the fungal extracts did not inactivate the virus but that "either the inhibitory agent enters into a reversible combination with the virus or that it alters host susceptibility". The results of several experiments carried out with two viruses using a series of dilutions of an extract-inoculum mixture are graphed in Figures 3 and 4. The experimental points in these graphs are each means of 24 replicates.

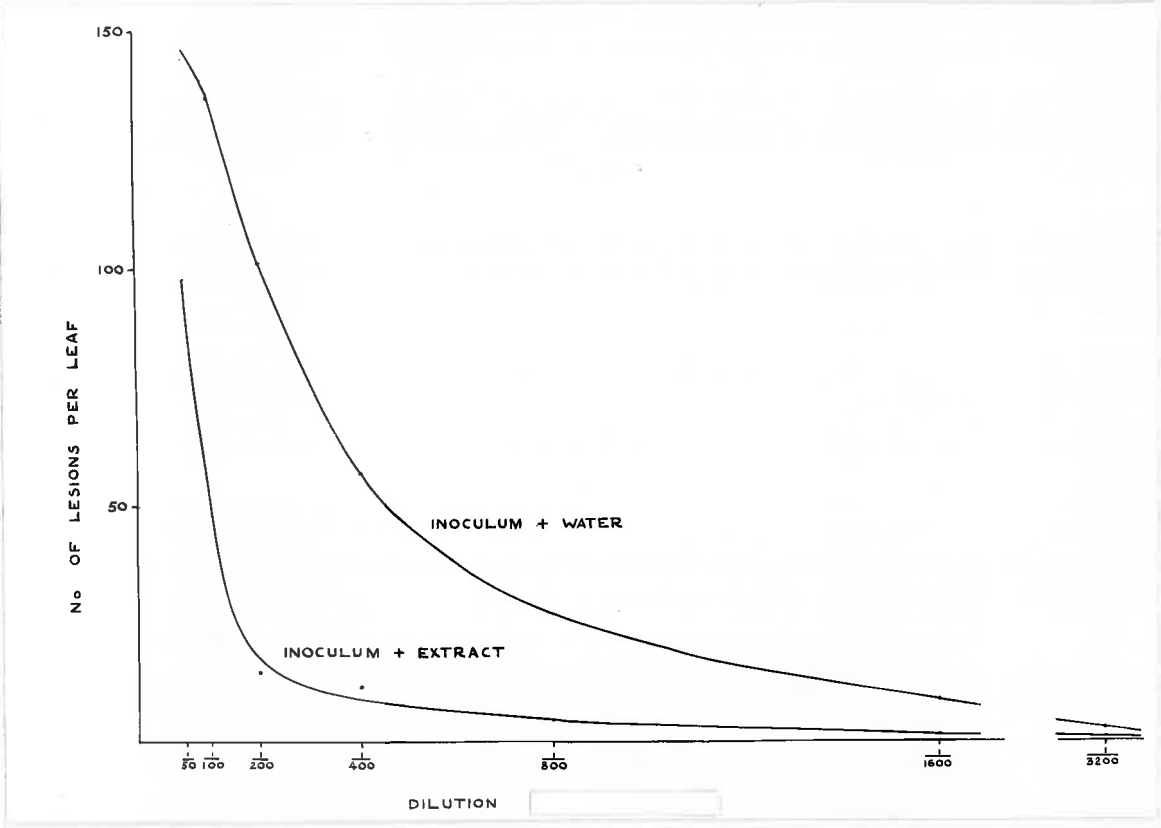


Fig. 3. Effect of diluting a mixture of cucumber mosaic virus inoculum and cucumber embryo extract with buffer, on its infectivity to cowpea.

The ratio of extract concentration to inoculum concentration does not alter throughout the series of dilutions, yet the reduction in lesion production induced by both inhibitors becomes progressively less as the mixture is diluted. In fact the converging nature of the dilution curves indicates that theoretically it would be possible to overcome completely the effect of either inhibitor simply by sufficiently diluting a mixture of inoculum and extract. Thus, either the inhibiting constituents of the extracts are combined with the virus in some way that is readily dissociated by dilution, or their effect is on the host

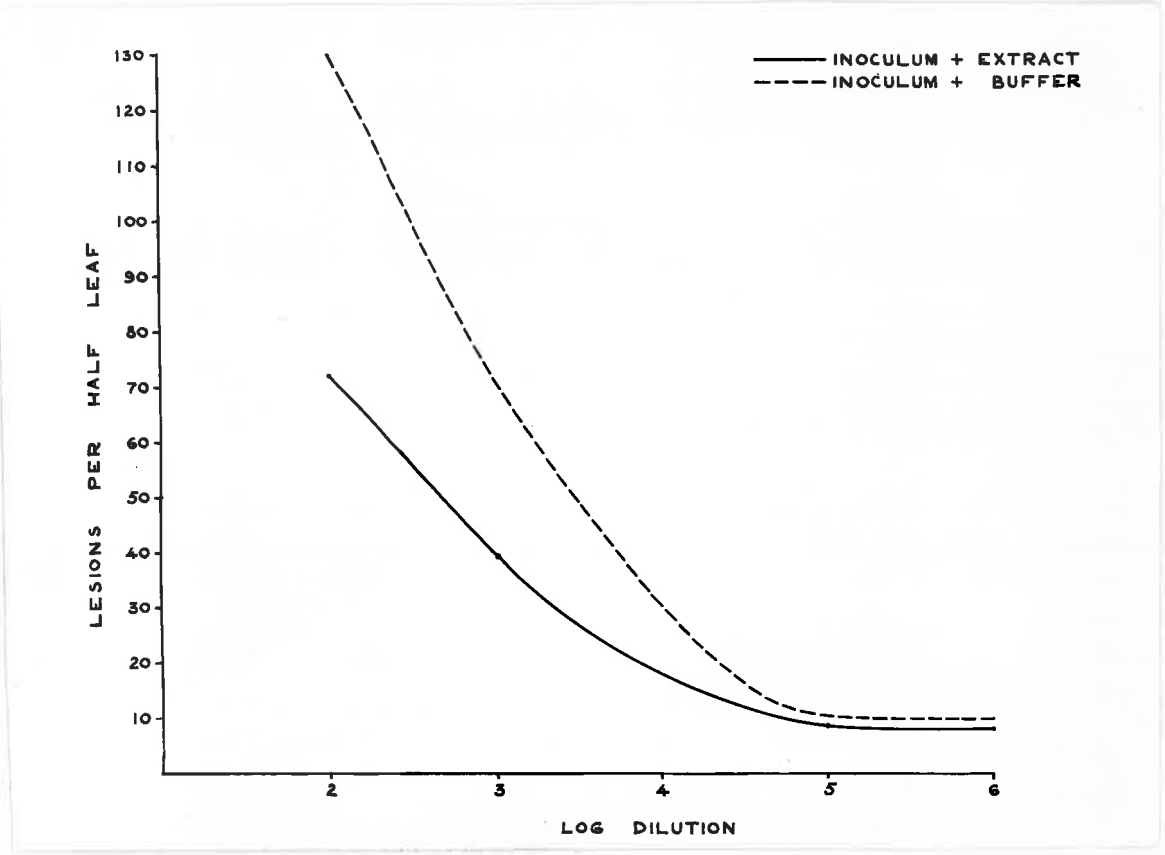


Fig. 4. Effect of diluting a mixture of tobacco mosaic virus inoculum and tobacco seed extract with buffer, on its infectivity to N. glutinosa.

used for the infectivity tests and their tolerance of dilution is less than that of the virus.

(6) Effect of inoculum concentration

Caldwell (1935) showed that substances inhibiting infection by a virus could be distinguished from substances inactivating a virus because inhibiters have their greatest effect on concentrated inocula, whereas inactivators have their greatest effect on diluted inocula. Figures 5 and 6 show the results of experiments where constant amounts of extracts were added to a series of dilutions of cucumber and tobacco mosaic virus

inocula. With both viruses the reduction in lesion numbers was consistently found to be greatest with concentrated inocula and it is concluded that the action of both seed extracts used is purely that of an inhibitor of virus infectivity.

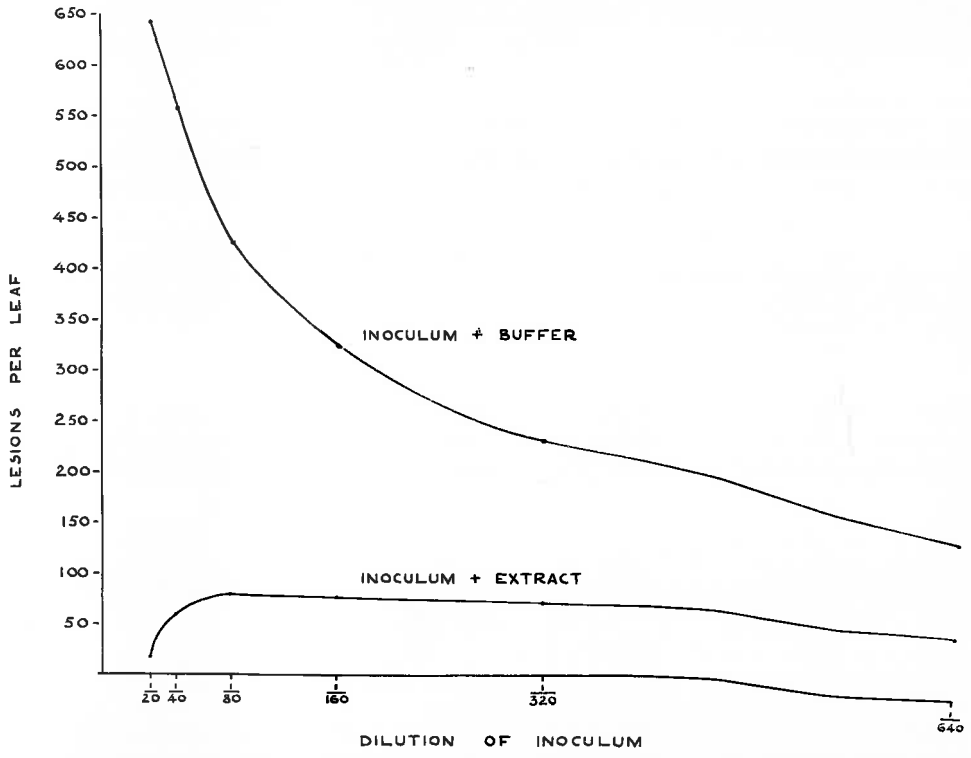


Fig. 5. Effect of a constant amount of cucumber embryo extract on infectivity of several dilutions of cucumber mosaic virus to cowpea.

(7) Separate applications of extract and inoculum

Two attempts were made to determine whether the action of the extracts was primarily on the host, or on the virus, by inoculating plants with the extract and the virus inoculum separately. The first

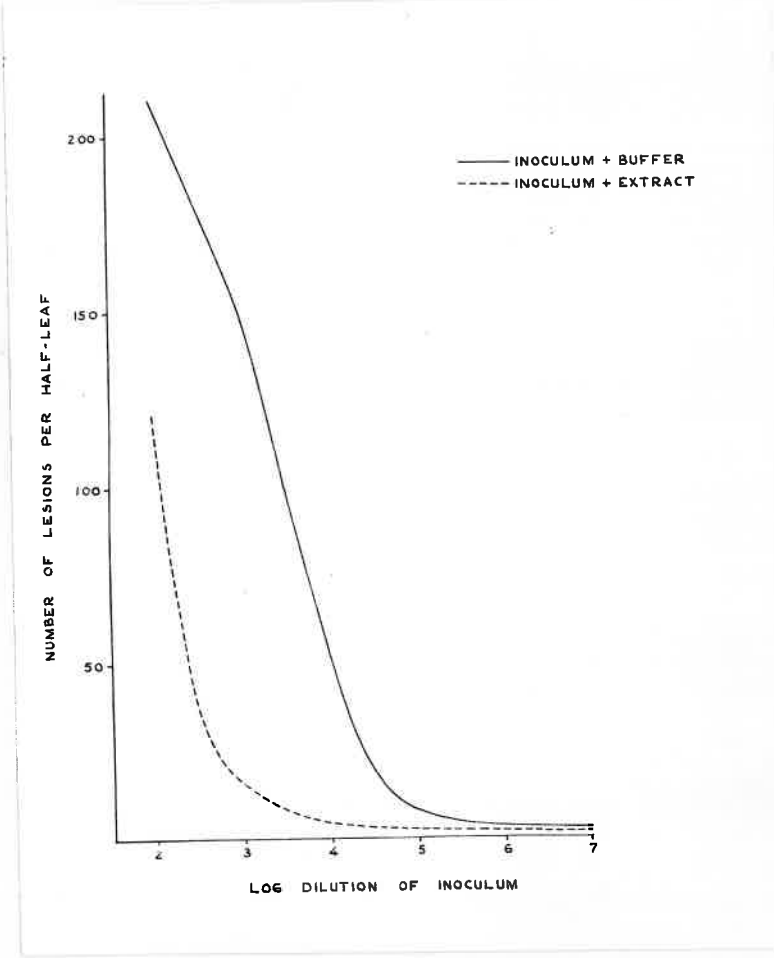


Fig. 6. Effect of a constant amount of tobacco seed extract on infectivity of several dilutions of tobacco mosaic virus to N. glutinosa.

method was to treat the cowpea and N. glutinosa plants with the extract at various periods before and after inoculation with the virus inoculum. The results in Table 11 are means of 40 replicates of each treatment, the treatments being randomized in five 8 x 8 latin squares.

TABLE 10

EFFECT OF CUCUMBER SEED EXTRACT ON LESION PRODUCTION BY COWPEA WHEN APPLIED BEFORE OR AFTER APPLICATION OF VIRUS INOCULUM

Time of application of treatment relative to time of application of inoculum	Mean No. of lesions per half leaf with:	
	Cucumber seed extract	Distilled water
-48 hours	1.1***	145
-24 hours	0***	60
+24 hours	191	194

*** Differences significant at P = 0.001

TABLE 11

EFFECT OF TOBACCO SEED EXTRACT ON LESION PRODUCTION BY N. GLUTINOSA WHEN APPLIED BEFORE OR AFTER APPLICATION OF VIRUS INOCULUM

Time of application of treatment relative to time of application of inoculum	Mean No. of lesions per half leaf with:	
	Tobacco seed extract	Distilled water
-48 hours	27.2***	63.5
-24 hours	25.7***	73.3
Mixed with inoculum	3.8***	62.4
+24 hours	56.0	58.6

*** Differences significant at P = 0.01

The results in Tables 10 and 11 show that both seed extracts used were able to induce a highly significant reduction in the number of local lesions produced by their respective hosts when applied one or two days prior to the application of the virus inoculum. Both inhibitors, therefore, are capable only of interfering with infection by their respective viruses. They are incapable of interfering with virus multiplication because both are incapable of producing any effect when applied after infection has taken place, a property always found associated with substances capable of inhibiting virus multiplication (Matthews 1951; Bawden and Kassanis 1954).

The second method of separate application of the extracts and inocula was to apply the extract to the under-surface of the leaves before applying the virus inoculum to the upper-surface. The under-surface of the leaves was dusted with carborundum, and the extract applied with a cotton wool swab.

TABLE 12

EFFECT OF UNDER-SURFACE PRE-TREATMENT WITH CUCUMBER EMBRYO EXTRACT ON LESION FORMATION BY CUCUMBER MOSAIC VIRUS ON COWPEA

Leaf under-surface inoculated with:	Mean number of lesions per leaf		
	Dilution of extract		
	Undiluted	$^1/_{10}$	$^1/_{100}$
Extract	23*	27*	32
Distilled water	35	33	29

* Difference significant at P = 0.05

TABLE 13

EFFECT OF UNDER-SURFACE TREATMENT WITH CUCUMBER EMBRYO EXTRACT BEFORE AND AFTER INOCULATION WITH CUCUMBER MOSAIC VIRUS

Time of application of extract relative to time of application of inoculum	Mean number of lesions per leaf			
	Under-surface treated with:			
	Embryo extract	Untreated	Water	Untreated
- 36 hours	9.5	15.5	22.3	30.0
- 1 hour	13.3	22.3	21.6	19.5
+ 24 hours	34.3	30.3	23.6	21.6

The results in Tables 12 and 13 show the cucumber embryo extract able to reduce the susceptibility of cowpea leaves to infection by cucumber mosaic virus, when applied to the undersurface of the leaves, up to 36 hours before inoculation. In fact the results in Table 13 show that when the extract is applied to the undersurface of only one leaf of a plant prior to inoculation it inhibits lesion production by both leaves on that plant. These results refute the suggestion that the effect of the extract is to inactivate the virus particles, or to render them non-infective, because there is no reason to suppose that the extract even comes in contact with virus particles when applied in this manner. The active constituent of the seed extract has been found to be a protein, and there is little likelihood that such a large molecule could diffuse throughout the leaf within a few hours of being applied to the under surface even though it is able to enter the leaf epidermis in much the same way as virus particles. However, it is possible that the presence

of this foreign protein in the leaf alters the metabolism of the whole leaf in a manner that reduces its susceptibility to virus infection.

In similar experiments carried out with tobacco mosaic virus a significant reduction in lesion numbers could not be induced by applying tobacco seed extracts to the under-surface of N. glutinosa leaves. This is attributed to the fact that tobacco seed extracts were always far less effective than cucumber embryo extracts, and also to the hairy nature of the under-surface of N. glutinosa leaves making them almost impossible to wet. Hence the uptake of seed extract by the under-surface of N. glutinosa leaves would be negligible, and its effect would similarly be negligible.

(8) The nature of the inhibitors

Investigations were carried out to determine the nature of the inhibitors in cucumber and tobacco seed.

The inhibitor from cucumber embryos was found to be heat-labile, non dialysible, and was precipitated from solution by alcohol or half-saturated ammonium sulphate. The inhibitor from tobacco seed was heat-stable, non dialysible, and was precipitated from solution by 60% alcohol, but not by ½ saturated ammonium sulphate. In tests kindly carried out by Dr. R.J. Swaby of the Soils Division of C.S.I.R.O., both substances were identified as proteins by their electrophoretic mobility, and their staining reaction with bram phenol blue (Swaby, unpublished data). This is at variance with the conclusion reached by Kausche that the virus inhibiting substance present in tobacco seeds belongs to the amino alcohol group, but he presented no evidence to support his claim.

(9) Conclusion

The presence of an inhibitor of cucumber mosaic virus in the seeds of cucumber and wild cucumber and one of tobacco mosaic virus in the seeds of tobacco has been demonstrated. The following evidence is considered to show conclusively that the action of these substances is to inhibit infection of the host rather than to inactivate the virus: 1. The substances are capable of instantaneous effect. 2. Their effect is dependent upon the host used. 3. Non-infective mixtures of inhibitor and inoculum can be made infectious by dilution. 4. Their effect is greatest on concentrated inocula. 5. They will affect lesion production when applied up to two days before inoculation. 6. The inhibitor from cucumber seeds affects lesion production when applied to the under-surface of cowpea leaves.

The results (Table 1) show the inhibitors of cucumber mosaic virus to be presented at an almost ten-fold higher concentration in cucumber embryos than in wild cucumber embryos. However, as seed transmission does not occur to any extent in either of these hosts, this indicates a difference in the protein composition, or concentration, of these two seeds, and has no significant bearing on the problem of seed transmission of cucumber mosaic virus. It is also significant to note that the inhibitor is present in approximately equal concentrations in both testas and embryos of wild cucumber although 92% of the testas and only 0.7% of the embryos were infected.

The presence of these inhibitors in the embryos of seeds cannot prevent the infection of embryos by plant viruses, for similar inhibitors

are also present in leaf tissues, which undoubtedly can be infected. Sill and Walker (1952) reported the presence of an inhibitor (possibly the same one as that described here) of cucumber mosaic virus in all of the tissues except the ovules of cucumber plants. Yet no one would dispute the fact that the virus can infect cucumber leaves and multiply in them. It is far more likely that the action of the inhibitors is upon the local lesion host used for measuring virus infectivity. Its action could be either to attach itself to the receptor sites in the cells at which virus multiplication is presumed to commence; or, more likely, as suggested by Bawden and Freeman (1952) for an inhibitor from the fungus Trichothecium roseum to "so alter the physiology of the host cells that they no longer support virus multiplication". It might seem possible that inactivators could be present in the embryos of seeds in addition to the inhibitors described here, but if such were the case it should be possible to demonstrate their presence by incubating seed extracts with viruses for some hours. Their presence would then be manifested by a greater decline in infectivity than would occur through normal aging of the inoculum. This was found not to be the case. (Tables 3 and 4).

It would seem that Dugger's theory that inactivators in seeds prevent the seed transmission of plant viruses must be abandoned as an explanation for the rarity of seed transmission of plant virus diseases, unless the inactivator is some transitory product of the metabolism of embryos. Such a transitory product could be an enzyme or group of enzymes which breaks down virus particles, together with the other proteins of the endosperm, prior to their absorption by the developing embryo; and

re-synthesis into embryo proteins. Inactivators of such a nature could inactivate viruses without ever accumulating to such an extent that their presence could be detectable by the usual infectivity techniques. This possibility has been investigated, and the results are presented in the following section.

D. THE EFFECT OF DEVELOPING EMBRYOS ON VIRUSES

Three facts have been established: 1. that the infection of the testa is a common phenomenon in all cases studied; 2. that infection of the endosperm does occur; 3. that the embryo is able to develop within this infected endosperm without itself becoming infected. Ignoring the reasons for the lack of microspore or macrospore infection, some mechanism must exist to prevent infection of the embryo during its development. Two mechanisms are possible; that the lack of plasmodesmatal connections prevents infection; that viruses are inactivated in the endosperm, or at the interface of the embryo and endosperm.

There is general agreement amongst embryologists and anatomists that the lack of plasmodesmatal connections to embryos is almost universal, and amongst virologists that the only likely path of between-cell movement of virus particles is via the plasmodesmata. However, as all other tissues of plants are connected by plasmodesmata it is impossible to detect experimentally any other means of between-cell movement of virus particles. The second suggestion that viruses are inactivated in the endosperm seems a reasonable alternative. For if a pathogenic virus is able to multiply actively in the endosperm, and if the endosperm is largely (and in some cases totally) absorbed by the developing embryo, some explanation for the lack of embryo infection is necessary. All other metabolites are absorbed by the embryo as small molecules, presumably following the breakdown of larger molecules by enzymes excreted by the embryo. It would, therefore, seem possible for viruses in the endosperm

to be similarly broken down. This theory can be investigated, if only indirectly. If embryos do, in fact, prevent their own infection by breaking down viruses present in the surrounding medium, then embryos growing in culture medium containing virus should break down at least a portion of this virus. It is also necessary that if such a mechanism does operate it must do so throughout the entire period of the development of the embryo. Hence the stage of development of the embryo used should be unimportant in experimental work.

The assumption is made that the technique is capable of detecting this virus inactivating effect. I believe this assumption to be a reasonable one for two reasons. The volume of culture medium used per embryo was, in many experiments, far less than that surrounding a naturally developing embryo, and the virus concentration in the medium was, in nearly all cases, less than that of naturally infected tissues. The embryos would, therefore, have less virus to inactivate when cultured in this way than under natural conditions.

The technique used was to grow tomato embryos on "White's Medium" containing tobacco mosaic virus. After periods of 24 hours to 12 days, the infectivity of the medium was compared with the infectivity of similar samples containing the same number of dead* embryos, and with similarly treated samples of medium containing no embryos. All infectivity assays were carried out in a randomised block design on N. glutinosa. The tobacco mosaic virus used in all experiments was

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* The embryos were killed by boiling them for five minutes.

diluted from a purified preparation kindly provided by Dr. R.J. Best. It was sterilized by filtration through a Lloyd's sintered glass filter (Lloyd 1945). On several occasions the infectivity of the embryos cultured in the virus solution was tested by washing them in water, rinsing in 'Teepol', again washing and inoculating (in lots of 20 or more) to N. glutinosa. None was found to be infected. In this respect the cultured embryos behave as they do in vivo. Apparently the damage sustained by the embryos in dissection is not adequate for their infection under embryoculture conditions.

In the first experiments carried out, tomato embryos were placed, 10 per tube, in 0.5 ml. of a culture medium containing a $^{1}/_{1000}$ dilution of tobacco mosaic virus. They were incubated for 14 days at 25°C, and assayed. The results in Table 14 of three trials provided no evidence of any reduction by the growing embryos of the infectivity of the medium. In two experiments there was an insignificant increase in the infectivity of the medium. This is attributed to the fact that the embryos used in these experiments were almost mature and in the course of their growth absorbed water from the medium, leaving the virus contained in the remainder relatively more concentrated.

TABLE 14

EFFECT OF TOMATO EMBRYOS ON INFECTIVITY OF TOBACCO MOSAIC VIRUS IN THE MEDIUM IN WHICH THEY ARE GROWING

Treatment	Mean number of lesions per leaf			
	Experiments			
	1	2	3	Mean
Live embryos	53	37	76	55
Dead embryos	55	35	63	51
Without embryos	57	28	51	45

(1) Aerobic embryoculture

Some workers have suggested that under the relatively anaerobic conditions of complete immersion embryos do not develop normally but tend to germinate immediately, whatever their state of development. This change, if it does occur, could significantly alter the metabolism of the embryos, including their effect on virus particles present in the surrounding medium. Two techniques were used to overcome this difficulty. The first was to shake the culture tubes either constantly or intermittently throughout the experiments. The results of these experiments were in every way similar to those of experiments in which the culture tubes were unshaken. The second technique was to place the embryos on strips fo filter paper that extended into the medium, rather than placing them directly in the medium. Using this technique the embryos developed quite normally. Germination in culture occurred only when mature embryos were used. The technique is illustrated in Figure 7.

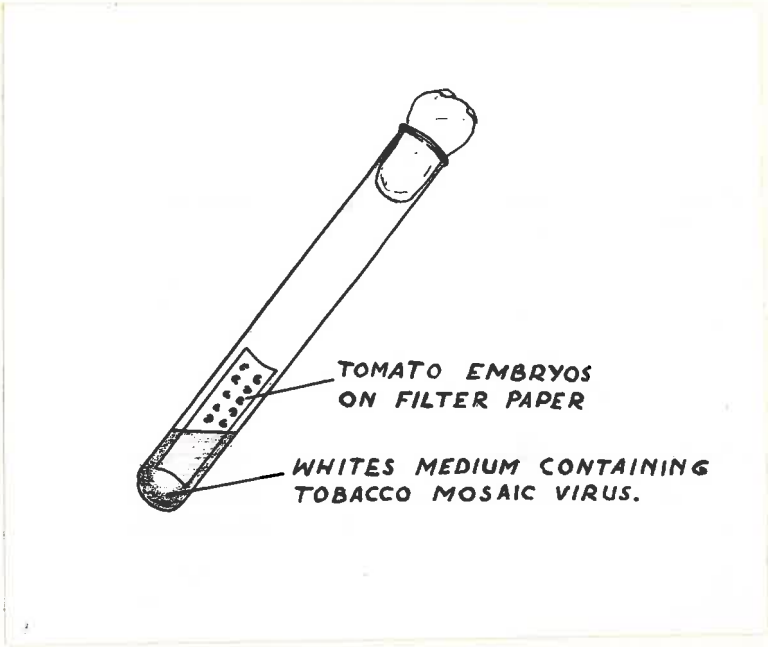


Fig. 7. Embryoculture technique.

The results in Table 15 are of one experiment in which this technique was used. Three dilutions of the virus in the medium were used, to enable the more certain detection of any inactivation caused by the growing embryos, for if the virus is being inactivated, the lower the concentration of the virus, the greater the proportion of the virus that must be inactivated. The results of this and several similar experiments did not give any indication of virus inactivation.

TABLE 15

EFFECT OF TOMATO EMBRYOS ON THE INFECTIVITY OF TOBACCO MOSAIC VIRUS

Treatment	Mean number of lesions per half leaf (20 replicates)		
	Dilution of virus preparation in medium		
	1/100	1/1,000	1/10,000
Live embryos	15.6	18.8	2.4
Without embryos	17.2	13.5	1.0

(2) Variations in the composition of the medium

The physical and chemical properties of the culture medium also influence, to some extent, the metabolism of the embryos, and could conceivably affect the results of these experiments. The following variations were tried:-

1. The sugar concentration of the medium was varied from 2% to 8%, because this factor has been reported by Rappaport (1954) to have a pronounced effect on the development of embryos in culture.
2. Experiments were carried out in which the acidity of the medium was adjusted to pH 4, 5, 6, 7 and 8. This factor could have a great effect on both development of the embryo, and the infectivity of the virus.
3. Coconut milk, both sterile and un-sterile, was added to the medium. This was found by van Overbeek et al. (1942) to contain excellent growth stimulators.

In none of these experiments was evidence of virus inactivation detected, nor were any of these modifications of the technique found to have a significant effect on the development of the embryo. The medium used finally contained 2% sucrose, and was adjusted to pH 7. Coconut milk was not added to the medium.

A further modification at this stage was to use embryos of all ages. Some experiments were carried out using the youngest embryos that could be dissected, others with half mature or mature embryos. No differences were detected in the effects of embryos of different ages and this age factor is not considered important; any mechanism preventing embryo infection must operate throughout its development. For most of the experiments described the embryos used were between one-half and two-thirds mature, because at this stage they are growing rapidly and are easily dissected and handled.

(3) Combined effect of embryo and endosperm

A further possibility worthy of investigation was that the inactivating mechanism could be of an adaptive nature requiring the presence of the endosperm for its operation. To test this both embryo and endosperm were cultured as a whole by using very immature tomato seeds in which the endosperm was still in an almost gelatinous state and could be removed from the seed intact. These endosperms, together with the embryo developing within them, were then cultured in the usual manner. The results of one such experiment, shown in Table 16, provide no evidence of any inactivation of the virus under these conditions.

TABLE 16

THE EFFECT OF TOMATO ENDOSPERMS AND EMBRYOS

ON TOBACCO MOSAIC VIRUS

Treatment	Mean number of lesions per half leaf (12 replicates)	
	Dilution of virus in medium	
	1/100	1/10,000
Live endosperms and embryos	29	7.6
Boiled endosperms and embryos	41	8.5
Without endosperms or embryos	23	3.2

In a number of these experiments the addition of dead embryos to the medium appeared to increase the infectivity of the medium. This difference could not be attributed to the absorption of water from the medium by the embryos because the embryos, having been boiled in sterile distilled water, are far from dry when cultured. As they do not grow, they would not take up more water. Both treatments contain pieces of filter paper of the same size; thus absorption of virus particles onto the filter paper is not the cause. The most probable explanation is that some substances are lost into the medium from the embryos, which through being boiled are presumably "leaky". This could also account for the fact that the live embryos did not increase the infectivity of the medium as much as the dead embryos. In fact it is possible that the live embryos do equally increase the infectivity of the virus but the increase is masked by a simultaneous virus inactivating process. The

result being that neither process is detectable. Experiments were therefore carried out in which this difference between "leaky" (boiled), and "non-leaky" (live) embryos was eliminated by using only live embryos. They were cultured at two temperatures, 3°C and 25°C. The effects of a virus inactivating mechanism should be detectable by a comparison of the infectivity of the media as the virus inactivation being a physiological reaction would be accelerated by a factor of four times for a 20°C rise in temperature. The results of one such experiment are tabulated in Table 17.

TABLE 17

EFFECT OF DEVELOPING EMBRYOS ON TOBACCO MOSAIC VIRUS AT DIFFERENT INCUBATION TEMPERATURES

Virus Concentration in medium	Incubation temperature	Mean number of lesions per half leaf		
		Incubation Time		
		1 day	3 days	6 days
1/100	3°	176	201	181
	25°	179	138	154
1/1,000	3°	37	27	31
	25°	35	29	21
1/10,000	3°	4.7	5.2	5.0
	25°	4.5	3.5	3.5

From these results it is clear that at 3°C the infectivity of the medium remained relatively constant for the duration of the experiment at all three virus concentrations. At 25°C there appears to be a reduction

in the infectivity of the virus at the $^{1}/_{1,000}$ dilution, but such a conclusion is quite inconsistent with the results of the lowest dilution where the same number of embryos did not significantly reduce the infectivity of $^{1}/_{10}$ of the amount of virus. Because of this, and the fact that at the lowest virus concentration the change in infectivity did not continue throughout the experiment, the apparent drop in infectivity is thought not to be real.

(4) The effect of repeated additions of embryos to the medium

One further possibility by which the technique used could fail to demonstrate the presence of a virus inactivating mechanism, is that such a mechanism might not continue to operate in artificial embryoculture. Although no reason is known why this should be so, an attempt was made to obtain evidence that would eliminate even this possibility. In several experiments 10 embryos were added to each tube daily. Previously, they had been added only at the beginning of the experiment. In this way any inactivation should be detectable, even if it does not continue for long after the embryos have been cultured, and though the extent of virus inactivation be extraordinarily slight, for after four days the concentration of embryos in the medium was 80 per millilitre. The results of one experiment carried out in this manner are shown in Table 18.

The experimental error is high because the technique involves unavoidable sampling errors, diluting errors, and slight variations in the treatment. In all cases the inoculum in which embryos had been growing at 25°C was less infective than inoculum in which they had been growing at 3°C. This is attributed to the secretion, by the growing

TABLE 18

EFFECT OF REPEATED ADDITIONS OF TOMATO EMBRYOS

ON THE INFECTIVITY OF TOBACCO MOSAIC VIRUS

Dilution of virus	Incubation temperature °C	Lesions per half leaf		
		Incubation time		
		1 day	2 days	4 days
1/50	3°	37*	45	41
	25°	28	36	36
1/500	3°	3.2	5.5	5.5
	25°	2.1	3.0	4.7
1/5,000	3°	0.6	0.8	1.0
	25°	0.3	0.8	0.4

* No treatment differences significant at P = 0.05

embryo, of some substances that inhibit the infectivity of the virus. It is not thought to be due to inactivation of the virus by the embryos, because the reduction in infectivity is as great, or greater, in the most concentrated inocula as in the most dilute, and the reduction in infectivity does not in any case increase with increasing incubation time. For these reasons I think that these results give no indication of any virus inactivating activity of the developing embryos.

(5) The effect of developing embryos on cucumber mosaic virus

Because of the comparatively short in vitro life of cucumber mosaic virus, and because of the difficulty in obtaining sterile infectious preparations of the virus, similar embryoculture experiments could not be carried out. Instead, the presence of virus inactivators was investigated

by adding extracts of the endosperms, or embryos, of wild cucumbers to cucumber mosaic virus inoculum. Tissues of wild cucumber were used primarily because they provided an excellent example of a case where the endosperm is frequently infected, yet the embryo growing within the endosperm is not infected. Secondly, the endosperm and embryos of wild cucumbers are large and readily provide sufficient material for experiments. An extract was made from the endosperms of more than 30 wild cucumber seeds by chopping them finely in 10 mls of neutral buffer and filtering through muslin. Five millilitres of this extract were then added to 5 mls of six different dilutions of cucumber mosaic virus. After standing for several hours their infectivity was compared by half-leaf comparisons on composs with similar samples to which 5 mls of neutral buffer had been added. The results in Table 19 give no indication of any virus inactivating activity of this extract. Similar results were obtained in several repetitions of this experiment and in experiments where an extract was prepared similarly from wild cucumber embryos instead of endosperms.

TABLE 19

EFFECT OF WILD CUCUMBER ENDOSPERM EXTRACT ON CUCUMBER MOSAIC VIRUS

Treatment	Mean number of lesions per half-leaf					
	Dilution of inoculum					
	1/50	1/100	1/400	1/800	1/1,600	1/3,200
Inoculum & buffer	223	141	40	16	4	1.2
Inoculum & extract	250	151	30	16	5	1.5

A second experiment was carried out to determine whether the endosperm extract could have any influence on the inoculum over a longer period of time. In this experiment a more concentrated endosperm extract was used, and it had an immediate inhibiting effect when added to the inoculum. When tested again after two and four hours, the results in Table 20 show that the decline in infectivity of the treatments to which the extract was added, was no greater than the decline in infectivity of the treatment to which buffer alone was added.

TABLE 20

EFFECT OF WILD CUCUMBER ENDOSPERM EXTRACT ON CUCUMBER MOSAIC VIRUS

Treatment	Mean number of lesions per half leaf		
	Incubation Period		
	0 hours	2 hours	4 hours
Inoculum & buffer	285	217	125
Inoculum & extract	9.6	5.4	5.1
Inoculum & extract/10	176	126	79
Inoculum & extract/100	319	210	186

From the results of both these experiments it is concluded that no inactivating mechanism could be detected in the embryos or endosperms of wild cucumber.

(6) Conclusion

It was postulated that the mechanism whereby embryos may prevent their infection could be by inactivating virus particles in their

surrounding medium. Experiments carried out with both tobacco mosaic virus and cucumber mosaic virus produced no evidence in support of the hypothesis. This lack of evidence could possibly be due to the inadequacy of the technique, rather than the absence of the mechanism, hence experiments were carried out to investigate any modifications which could affect the metabolism of plant embryos. Virus inactivation did not occur under either aerobic or anaerobic conditions. Metabolic stimulants such as coconut milk, or alterations in the pH, or sugar concentration of the medium also failed to detect any virus inactivating mechanism. Similar results were obtained when both endosperm and embryo were cultured together. The possibility that the virus inactivation was rendered undetectable by the effect of some substances from the embryos increasing the infectivity of the medium to N. glutinosa was also eliminated by the results of experiments in which two incubation temperatures were used. Finally, the possibility that the virus inactivating activity of the embryos might not continue in embryoculture was eliminated by experiments in which embryos were added daily to the medium for up to six days. The only remaining possibilities are either that the postulated mechanism does not exist or that its effect is so slight as to be undetectable by the techniques used. In view of the fact that the concentration of virus in infective tissues is much less than that used in the experiments and considering the great numbers of embryos that were used in some experiments, the possibility of the mechanism being undetectable seems slight. It is concluded that virus inactivators do not prevent the infection of embryos by viruses. Some other explanation must be sought,

and the only remaining alternative is Bennett's suggestion that viruses are unable to infect the developing embryo because of its lack of plasmodesmatal connection with other tissues.

E. THE EFFECT OF ENVIRONMENTAL FACTORS ON SEED TRANSMISSION

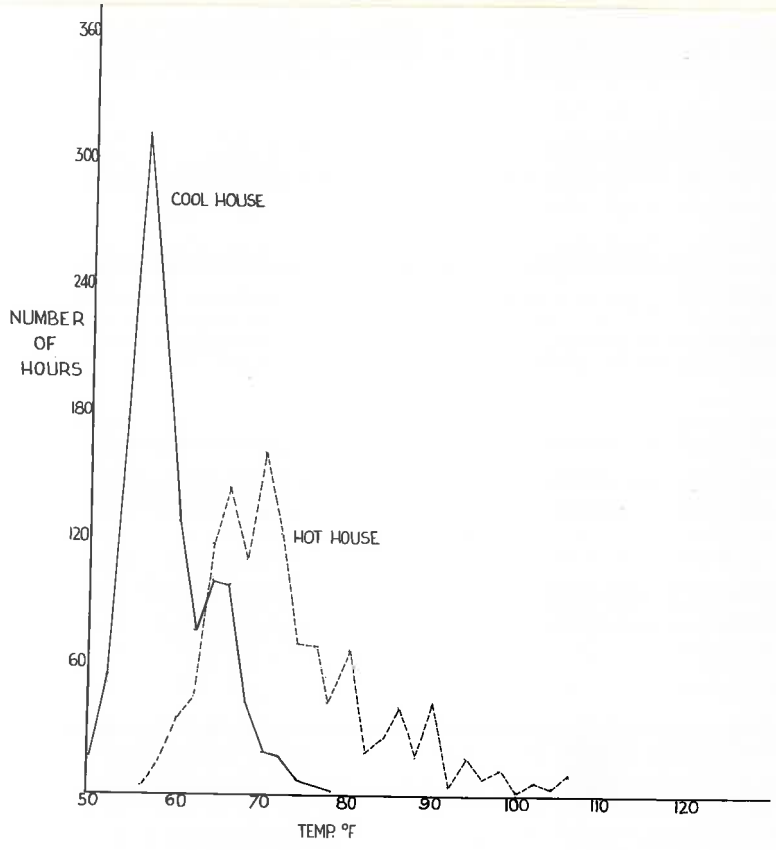
All of the evidence that has been put forward strongly suggests that the lack of seed transmission of the highly infectious plant viruses is not explicable in terms of the three hypotheses that have been advanced. It has been shown that the viruses studied do not induce sufficient sterility to account for their lack of seed transmission; that the seeds of the hosts used do not contain any virus inactivating substances; that the developing embryos do not inactivate virus particles in the medium surrounding them. The remaining hypothesis that viruses are not seed transmitted because they are unable to infect or survive in the gametophytic cells of their hosts, and are unable to infect developing embryos because of the lack of plasmodesmatal connections between embryo and endosperm, cannot be investigated directly. However, it is possible to investigate a conclusion that can be drawn from this hypothesis. Those viruses which are pollen-transmitted must be able to infect gametophytic cells. Most probably all viruses which are seed transmitted must also be able to infect gametes, for otherwise they must infect the developing embryo despite the lack of plasmodesmata. If infection of the developing embryo actually occurs, the percentage of infected embryos must increase during embryo development. This has been studied with bean mosaic virus, and the percentage of infected mature embryos was no different from the percentage of those infected, but immature. However, it was impossible to test very young embryos and a more satisfactory method was sought.

The percentage seed transmission of bean mosaic virus varies

from 20% to 60% (Harrison 1935). The environment could be one of the factors responsible for this variation, because of its great effect on virus concentration in plants and through this on the percentage of infected gametes. Temperature is probably the most important environmental factor affecting the virus concentration of bean mosaic virus in beans (Fajardo 1930), and as it is one of the most easily controlled factors, experiments have been carried out to determine whether the percentage seed transmission of bean mosaic virus is affected by the temperature before fertilization or the temperature after fertilization. This information should indicate when embryo infection takes place before fertilization, (by way of the gametes) or after fertilization (during embryo development), or both.

An experiment was carried out to obtain this information. Two glass-houses were used. One was insulated by double glass walls and refrigerated to a temperature usually below 62°F, never above 68°F. The other was equipped with thermostatically controlled heaters which kept the minimum temperature from falling below 68°F. The day-temperature varied between 80°F and 90°F. The frequency distribution of the temperatures in the two glasshouses for the entire period of the experiment are graphed in Figure 8.

The beans used were of the variety Canadian Wonder and were inoculated with common bean mosaic virus at the primary leaf stage. All plants were raised in the heated glass-house until about two weeks before flowering. Then half of the plants were selected at random and placed in the cooled glass-house. The plants were hand-pollinated with pollen



FREQUENCY DISTRIBUTION OF TEMPERATURES IN HOT HOUSE AND COOL HOUSE

taken from healthy Canadian Wonder plants of the same age. This was done to eliminate any variation caused by the use of infected pollen, of which a variable percentage of the grains would be infected. As an additional treatment some plants were pollinated immediately after emasculation and others 24 hours after. This exposed the plants to the different temperatures for a longer time during the critical period before fertilization. The complete experiment involved the following eight treatments:-

(a) Plants kept in hot-houses;

(b) Plants kept in hot-houses, but moved to cool-house immediately after emasculation and pollination;

(c) Plants kept in cool-house;

(d) Plants kept in cool-house, but moved to hot-house immediately after emasculation and pollination;

(e) Plants kept in hot-house, but a 24 hour gap between emasculation and pollination;

(f) Plants kept in hot-house, pollinated and moved to cool-house 24 hours after emasculation;

(g) Plants kept in cool-house, but a 24 hour gap between emasculation and pollination;

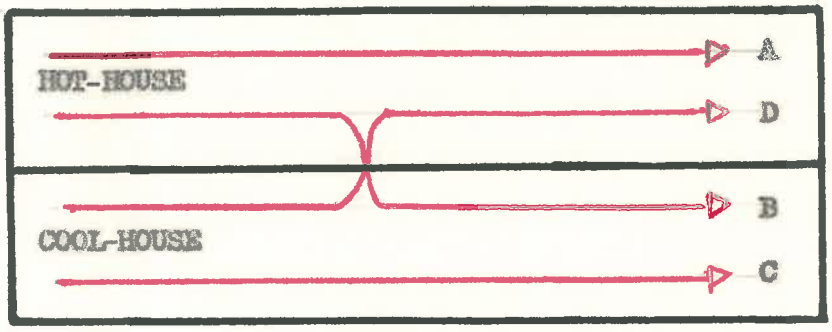
(h) Plants kept in cool-house, pollinated and moved to hot-house 24 hours after emasculation.

There were 11 replicates of each treatment. All the plants were numbered from 1-88 and random numbered plants were allotted to each treatment. The seeds were harvested from each treatment and the percentage seed transmission determined. The results are set out overleaf.

The number of seeds harvested in this experiment was far less than expected, partly because of a considerable number of aborted seeds, and partly because it was rarely possible to set more than three pods on any one plant. The aborted seeds were produced almost exclusively on plants maturing in the cool-house and it is tentatively concluded that under these conditions virus infected embryos rarely develop to maturity.

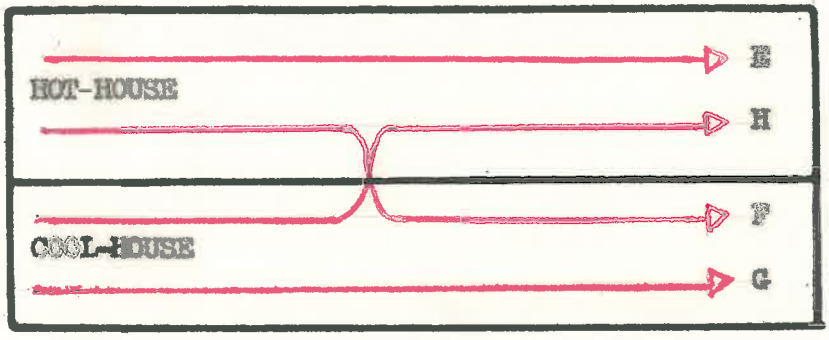
Emasculated and pollinated

Proportion of infected seed



5/51
4/55
1/25
0/15

Emasculated Pollinated
24 hours



2/38
0/28
1/18
0/20

SEED TRANSMISSION

	Before Fertilization		After Fertilization	
	Hot	Cold	Hot	Cold
Total Effect	9/132 A+B+E+F	4/118 C+D+G+H	11/172 A+D+E+H	2/78 B+C+F+D
When pollination immediately follows emasculation	6/76 A+B	4/70 C+D	9/106 A+D	1/40 B+C
When pollinated 24 hours after emasculation	3/56 E+F	0/48 G+H	2/66 E+H	1/38 F+G

Far fewer seeds were harvested from the plants in the cool-house, and of these, only two infected seeds were detected amongst the 78 harvested. The average percentage seed transmission in the experiment was slightly less than 3%. This is the lowest that has ever been recorded and is much lower than obtained in several previous trials with the same material. One other factor contributing to the low percentage transmission is the fact that all pollinations were carried out with flowers from healthy plants. It is difficult to draw more than tentative conclusions from results of such a small number of seeds, and where the percentage seed transmission is so low. However, the results do indicate that the percentage seed transmission of bean mosaic virus is increased by high temperature regardless of whether it is applied before or after fertilisation. The effect of the temperature treatment when applied after fertilization is, I believe, not due to any increase in the number of embryos infected in the heat treated plants, but simply due to the abortion of the infected embryos on the cold treated plants. This conclusion must be substantiated, or rejected by further experimental results. Amongst those pods matured in the hot-house, there were very few aborted embryos and the results do indicate that the warmer environment before fertilization did result in a higher percentage seed transmission. Presumably the higher temperature was closer to the optimum for virus multiplication, or movement, and under these conditions more embryo-sacs were infected.

It is tentatively concluded that even when the possibility of pollen transmission is eliminated, embryo infection does take place prior

to fertilization, but evidence was not obtained to show whether infection of the embryo after its fertilization is possible or impossible. At their face value the results would indicate that post-fertilization infection is possible.

III. GENERAL DISCUSSION

It is now clear that the rarity of seed transmission of plant virus diseases cannot be attributed to the same cause in all cases. Seed transmission is impossible for the many viruses that fall into one of the following four classes:-

1. Those which kill their hosts, e.g. the necrotic strain of tomato spotted wilt and all similar necrotic virus diseases.
2. Those which prevent flower formation, e.g. tomato big bud, lucerne witches brooms, etc.
3. Those which are limited in their distribution within their host:- viruses such as tobacco necrosis, lettuce big vein, and peach yellows, apparently limited to the roots of their hosts; Pierce's disease of the vine, sugar beet curly top and many others limited to the vascular tissue of their hosts. Infection of the embryo by these viruses is clearly impossible.
4. Those which are unable to tolerate the changes that take place in the seed during its maturation and desiccation, e.g. southern bean mosaic virus. A number of the less stable viruses such as tomato spotted wilt, may also be included in this group, but the tolerance of dessication by a virus in living tissues may bear little relationship to its tolerance of desiccation in vitro.

However, these four classes include less than 10% of the known

plant viruses and there must be yet other reasons for the lack of seed transmission of the remaining 90%. Several theories have been put forward and each of these is now examined in relation to the evidence obtained. The theories that have been advanced fall naturally into two classes: those dealing with the infection of microspores and macrospores, and those dealing with the infection of the developing embryo. A synthesis of these is necessary to provide a complete explanation for the rarity of seed transmission.

Only two explanations have been suggested for the failure of viruses to infect microspores and macrospores. The first, in order of time, was Allard's suggestion that virus infection in some cases induces abortion. It was left to Caldwell in 1952 to show in what manner a virus can induce abortion, and the extent of the resulting infertility. He has established that at least in the case of tomato asperny disease, the infection of the microspores or macrospores results in a "breakdown in the normal process of meiosis". In this way the only surviving cells are those which have escaped infection. This explains the absence of seed transmission of tomato asperny disease, and because virus infection is sometimes associated with reduced seed production it is possible that virus-induced abortion might be a common cause of the lack of seed transmission. However, the results presented have shown that this explanation is quite inapplicable to any of the three viruses investigated. Although cucumber mosaic infected pepper plants were found to produce far less seed than healthy plants, no virus-induced abortion, or breakdown in meiosis, could be detected, and the failure of infected plants to produce

normal quantities of seed is attributed to the general impairment of plant growth and the particular stunting and malformation of fruit growth that results from infection with this virus. It appears that virus infection can reduce the seed production of a plant in either of two ways; by causing the abortion of the microspores or macrospores, or by impairing the growth of plants. The latter probably occurs to some extent in all virus infected plants, and is in no way associated with the occurrence or absence of seed transmission. The former does act as a method of preventing the survival of virus infected pollen grains and egg cells, although tomato aspermy disease is the only case where this mechanism has been shown to operate.

The second explanation for the rarity of microspore or macrospore infection is Bennett's suggestion that "The improbability of embryo infection by direct passage of virus from adjacent cells, together with the apparent correlation between pollen infection and seed transmission, indicate that seed transmission may hinge on the ability of a virus to enter the megaspore, microspore or embryo sac and maintain themselves in these structures through the successive developmental stages". The results obtained are consistent with this explanation. It is suggested that viruses are unable (in most instances) to survive in these cells which differ from all other cells of the plant essentially in the fact that they are haploid. A still more fundamental question with regard to seed transmission is, therefore, whether viruses are in fact able to infect and survive in haploid cells or whether they are excluded from these cells before the reduction divisions take place.

In this regard Lindstrom (1941) has reported a haploid line of tomato to be susceptible to an aucuba strain of tobacco mosaic virus, although only very slight symptoms were produced. Later, however, he reported "the haploid seems to have developed an immunity to the natural infection which was constantly affecting other tomato varieties in the same glass-house". It seems then that Lindstrom's lines of haploid tomatoes did possess, if not an immunity, at least a very high degree of resistance to infection by tobacco mosaic virus, and even when infected, they do not support virus multiplication as readily as the diploid. If this resistance to virus infection is a normal characteristic of haploid cells the rarity of pollen and seed transmission is only to be expected. One test of the validity of this conclusion would be to determine whether a virus is seed transmitted in an autotetraploid host. The gametes of such a plant would be diploid, and therefore presumably susceptible. Findlay (personal communication) informed me that seed transmission did not occur in either diploid or autotetraploid potatoes infected with both potato viruses X and Y. However, both Sprav (1951) and Reddick (1936) have reported the seed transmission of potato virus Y, and some investigations of the seed transmission of viruses in autotetraploid plants are at present in progress.

It is concluded that, in general, the rarity of microspore or macrospore infection by viruses results from the inability of viruses to infect these cells. This may be associated with the fact that these cells are haploid. Caldwell's theory that viruses do infect microspores and macrospores, and that seed transmission is prevented by the abortion

of all infected cells seems to be of only very limited application.

The investigations described in Section B have established that although all five of the viruses studied infect the testas of the seeds of their hosts, and at least one of them also commonly infects the endosperm, the embryo is able to develop to maturity within these infected tissues without ever being infected. In fact, the only virus found capable of infecting the embryos of its host was the seed-transmitted bean mosaic virus. The report of Chao (1955) that southern bean mosaic virus does infect a high proportion of the embryos of bean plants is the only record of embryo infection by a non-seed-transmitted virus. In this case, the absence of seed transmission results from the inability of the virus to survive the changes that take place in the seed during maturation, not to the inability of the virus to infect the embryo. However, the results obtained with bean yellow mosaic, cucumber mosaic, tobacco mosaic and tomato spotted wilt viruses in seven different host plants indicate that in most cases the absence of seed transmission can be attributed to the inability of viruses to infect embryos. Three theories have been advanced to explain how virus-free embryos can develop within virus infected tissues.

Duggar's theory that seed transmission is prevented by the action of virus inactivators in seeds is not supported by any of the evidence obtained. In fact no evidence for the existence of virus inactivators in seeds could be obtained and the substances reported by Duggar to be inactivators have been shown to be inhibitors which would be capable of no <u>in vivo</u> effect, and could have no connection with the

mechanism whereby infection of the developing embryo is prevented. Dugger made no attempt to eliminate this possibility. Similar, if not identical, inhibitors are present in most plant tissues and do not influence the susceptibility of such tissues in any way.

However, the absence of a detectable virus inactivator in mature seeds in no way proves that immature developing embryos do not have some virus inactivating mechanism. Developing embryos do have the ability to break down other proteins in the endosperm, and the possibility that virus particles in the medium surrounding them could be broken down by a similar process has been investigated.

A large number of embryo-culture experiments, using many different techniques, gave no evidence of any virus inactivating ability of developing embryos. It is, of course, impossible to prove that virus inactivators are not present in seeds, but the evidence obtained indicates that the existence of such substances is highly improbable.

Bennett's theory that the lack of plasmodesmatal connections into the embryo makes its infection impossible is the only theory consistent with the results. If this theory is generally applicable, seed-transmitted viruses must all be able to infect the microspores, megaspores, or embryo sacs of their host. This is apparent from the evidence that the only viruses recorded to be transmitted in a high percentage of seeds (bean mosaic 50%, barley false stripe 50-100%, and Q disease of Datura stramonium) have also been recorded to be pollen transmitted. In Section E it is shown that the percentage seed transmission of bean mosaic virus can be altered by modifying the

environment of the plants prior to fertilization. This supports the hypothesis that the infection of the embryo by this virus is achieved at, or prior to, fertilization, not later. Finally, the fact that only those viruses which are seed-transmitted (with the single known exception of southern bean mosaic) infect the embryos of their hosts, is also consistent with Bennett's explanation.

The only explanation for the rarity of seed transmission which is not at variance with any of the available evidence is that most viruses are unable to survive in the microspores of macrospores, and apparently all viruses are unable to infect developing embryos because of their lack of plasmodesmatal connections with other seed tissues. A virus will be seed transmitted only if it is able to survive in the microspores or macrospores without disrupting their normal metabolism. This theory aligns the prevention of seed transmission with the more basic problem of resistance of plants (or in this case of individual cells) to viruses, and with the genetic control of resistance. The genetic control of seed transmission is emphasized by the findings of Couch (1955) that the genotype of the host is the major factor determining the percentage seed transmission of lettuce mosaic virus. Similarly, the results of Cation's experiments (1952) well illustrate the complex nature of the interaction and the importance of the genotype of the virus. He reported that in the seed harvested from a single cherry tree infected with two seed transmitted viruses, the percentage transmission of one of them (cherry ringspot) was four times as great as the percentage transmission of the other (cherry yellows). If seed transmission depends on

the ability of a virus to survive in haploid gametophytic cells, it is to be expected that the genotype of both host and virus would be of major importance, and that different results would be expected when different strains of a virus or different varieties of a host are used.

The low percentage seed transmission in those cases where it does occur is difficult to explain completely. The only reason that can be suggested is that viruses are not present in the nucellar, and amber tissues in sufficient concentration to achieve 100% infection. The rarity of seed transmission of plant viruses has usually been regarded as a remarkable phenomenon. However, it is obvious that natural selection would tend to eliminate any line of plants in which the seed transmission of a severe virus disease was common. And if we consider that in order to be seed-transmitted a virus must be able to systemically invade its host; must be able to infect and survive in the haploid gametophytic cells; and must be able to survive in the embryo throughout its development, maturation, storage and germination, it is, I think, remarkable that seed transmission should occur at all.

IV APPENDIX I

SEED TRANSMITTED VIRUSES

Host	Virus	% Transmission	Author
CANNABINACEAE			
Humulus lupulus	Chlorotic disease of hop	27	Salmon and Ware (1935).
CHENOPODIACEAE			
Beta vulgaris	Beet yellows	30	Clinch and McLoughane (1948).
COMPOSITEAE			
Cineraria sp.	Tomato spotted wilt *	96	Jones (1944).
Helianthus annus	Unnamed	17-43	Traversi (1949).
Lactuca sativa	Lettuce mosaic	6	Ainsworth and Ogilvie (1939).
" "	" "	3	Newall (1923).
" "	" "	10	Ogilvie et al. (1934).
" "	Mosaic	8	Gregan, R. G. (1950).
" "	Yellow mosaic virus	30	Vasudeva et al. (1948).
Senecio vulgaris	Lettuce mosaic	0.5	Ainsworth and Ogilvie (1939).
CONVOLVULACEAE			
Cuscuta campestris	Dodder latent mosaic	4.8	Bennett (1944).
CUNCURBITACEAE			
Cucumis melo	Muskmelon mosaic	28-94	Rader et al. (1947).
" "	Cucumber mosaic	2	Hendrick (1934).
" "	" "	16	Mahoney (1935).
Cucumis pepo	Muskmelon mosaic		Rader et al. (1947).

Host	Virus	% Trans-mission	Author
Cucumis sativa	Cucumber mosaic	1.4	McClintock (1916).
Cucurbita pepo	Squash mosaic	0.96	Middleton (1944).
" "	Cucumber mosaic	1.5	Chamberlain (1939).
Cucumis moschata	Muskmelon mosaic	26-94	Rader (1947).
Echinocystus lobata	Cucumber mosaic	* 22	Doolittle (1919).
GRAMINEAE			
Hordeum vulgare	False stripe	P50-100	Gold et al. (1954).
" "	" "	86	Hagborg (1954).
Triticum vulgare	" "	71	Hagborg (1954).
Hordeum vulgare	" "	58	McKinney (1951).
LEGUMINOSEAE			
Dolichos biflorus	D. biflorus mosaic	25-40	Uppal (1931).
Glycine soja	Soybean mosaic	10-25	Kendrick and Gardner (1921).
" "	Tobacco ring spot	54-78	Desjardins et al. (1954).
" "	Tomato ring spot	76	Kahn (1956).
Lathyrus odoratus	Pea mosaic		Dickson (1922b).
Phaseolus limensis	Lima bean mosaic	25	McClintock (1917).
Phaseolus vulgaris	Bean mosaic	P	Reddick and Stewart (1919).
" "	" "	43	Archibald (1921).
" "	" "	20-59	Harrison (1935).
" "	" "	10-30	Fajardo.
" "	Red Node	27	Thomas and Graham (1951).
Pisum sativum	Pea mosaic	0.5	Dickson (1922b).

Host	Virus	% Transmission	Author
Trifolium hybridum	Pea mosaic	0.5	Dickson (1922a).
Trifolium pratense	" "	47	Dickson (1922a).
Vicia faba	Mosaic	1	Quantz (1953).
Vigna sesquipedalis	Asparagus bean mosaic	37	Snyder (1942).
Vigna sinensis	Cowpea mosaic	14	Gardner (1927).
" "	" "	7	McLean (1941).
" "	" "	11	Yu (1946).
RUTACEAE			
Citrus aurantifolia	Xyloporosis	66	Childs (1956).
MALVACEAE			
Abutilon sp.	Abutilon mosaic	< 1	Keur (1933).
ROSACEAE			
Prunus avium var. mazzard	Cherry ringspot virus	5	Cochran (1946).
" " "	" " "	56	Cation (1952).
Prunus cerasus	" " "	30	Cation (1949).
Prunus mahaleb	Cherry yellows	7.8	Cation (1949).
" "	Cherry yellows	41	Cation (1952).
" "	Cherry ringspot	10	Cation (1952).
SOLANACEAE			
Capsicum frutescens	Tobacco mosaic *	22	McKinney (1952).
Capsicum annum	Mosaic P		Ikeno (1930).
Datura stramonium	Q disease P	100	Blakeslee (1921).

Host	Virus	% Transmission	Author
Lycopersicum esculentum	Tomato streak	66	Berkeley and Maddon (1932).
" "	Tobacco mosaic		" " "
" "	" "	2	Doolittle and Beecher (1937).
" "	Cucumber mosaic	0.2	Van Koot (1949).
Nicotiana tabacum	Tobacco ringspot	17	Valleau (1941).
Petunia sp.	" "	20	Henderson (1931).
Physalis peruviana	Tomato bunchy top	29	McLean (1948).
Solanum tuberosum	Virus Y.	16	Sprav (1951).
" "	" "	14	Reddick (1936).
Solanum uncanum	Tomato bunchy top	53	McLean (1948).
URTICACEAE			
Ulmus campestris	Elm mosaic	3	Bretz (1950).

/ Names of viruses used throughout are those tabulated in the Review of Applied Mycology 24 : 515-556, 1945.

* Results with viruses marked thus have not been confirmed in the work described here.

P Viruses marked thus have also been recorded to be pollen transmitted.

V. ACKNOWLEDGEMENTS

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