Thesis submitted for the degree of Doctor of Philosophy,

University of Glasgow.

VIRUS   DISEASES   OF

BROCCOLI   AND   BRUSSELS   SPROUTS   (<u>BRASSICA OLERACEA</u>, L.)

AND   OF   STRAWBERRY   (<u>FRAGARIA</u> SPP.)

by

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November, 1944.

I certify that the work described in the paper "A mosaic disease of broccoli", <u>Ann. appl. Biol.</u>, 29, 1942, was performed by Ian W. Prentice and that approximately one half of the work described in each of the papers "The spread and effect of Broccoli Mosaic in the field", <u>Ann. appl. Biol.</u>, 29, 1942, and "An investigation into the stripe disease of narcissus, II.", <u>Ann. appl. Biol.</u>, 30, 1943, was performed by him under my supervision.

University College,

Exeter.   10th October, 1944.

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A. GENERAL INTRODUCTION - The Identification of Viruses.

Virus diseases of plants are known to have existed for centuries - "breaking" of tulips having been recorded by Carolus Clusius as early as 1576 - but it was not until 1892 that it was shown that the infective principle which caused the disease known as Tobacco Mosaic was able to pass through bacteria-proof filters (Ivanovski, 1892). This result was independently confirmed soon after (Beijerinck, 1898) and it is thus from the last decade of the 19th Century that our knowledge of filter-passing pathogens, or viruses, originates.

During the early years of the present century progress in the study of virus diseases was slow, but since 1920 increasing attention has been paid to this subject which now forms one of the most actively pursued branches of plant pathology. With increasing knowledge the unsuitability of filterability as a criterion for the classification of pathogens has been realised and infectious plant diseases of which no pathogen can be observed by examination with the ordinary optical microscope are now classified as virus diseases.

Naturally, much of the work of the last 20 years in this field has consisted of the description of hitherto unrecorded virus diseases, and a large number have been so described. It has now been shown that almost all cultivated plants are susceptible to one or more virus diseases which are, in many cases, of great economic importance.

In the past, different viruses have generally been identified by differences in host range and symptomatology, but variation in symptom picture (due to the effect of environment, existence of strains of the type virus, etc.) often renders identification on symptomatological grounds difficult.

For example, it has been shown that high temperatures often cause a reduction in severity or a masking of symptoms. In plants affected by Tobacco Mosaic, potato mosaics (Smith, 1933, p.41), Narcissus Stripe (Caldwell and Prentice, 1943), etc., high temperatures result in a reduction in intensity of symptoms, while in other cases high temperatures lead to the production of symptoms of increased severity. The type of symptom may also be affected by temperature conditions; e.g. Tobacco Mosaic virus produces small local necrotic lesions on Nicotiana glutinosa grown at low temperatures (circa 20°C.); these tend to increase in size and run together at temperatures above 28°C. while at over 35°C. no necrosis is caused and, instead, a systemic chlorotic spotting is produced (Bawden, 1939, p.19).

Other environmental factors similarly alter the type of symptom produced. Actively growing tobacco plants inoculated with Potato virus "X" become systemically infected while old or pot-bound plants develop local lesions only (Bawden, 1939, p.21).

It has also been shown that the symptoms of Tobacco Ringspot, etc., (Smith, 1933, p.43) and Strawberry Yellow-edge (King and Harris, 1942) are modified by conditions of light intensity and soil moisture respectively.

The existence of "strains" of viruses is another complicating factor in symptomatology since in many cases viruses are known which produce dissimilar symptoms although serological and other evidence shows them to be related strains. For example, strains of Tobacco Mosaic virus are known which cause a bright yellow mosaic or no symptoms at all on tobacco (McKinney, 1935; Holmes, 1934); one strain of Potato virus "X" causes top necrosis in Majestic potato while another strain causes chlorotic mottling (Bawden and Sheffield, 1944).

Thus it is evidently unwise to attempt to identify viruses solely on symptoms produced.

Johnson (1927), however, has shown that the resistance of viruses to different treatments in vitro can be used as an additional method of identification and, recently, characteristics such as resistance to ageing, resistance to chemicals, thermal inactivation point, etc., have been determined

for a number of viruses.

The virus of Tobacco Mosaic can withstand heating for ten minutes at 85°C., while Tomato Spotted-wilt virus is inactivated by heating to 45°C. for a similar time; expressed juice from a plant infected with Tobacco Mosaic is still infective after storing at room temperature for several years, while juice from a plant infected with Spotted-wilt loses its infectivity within a few hours (Smith, 1933, Chap.3). Some viruses, for example Tobacco Mosaic, are infective when juice from an infected plant is diluted 1:100,000 or more (Smith, 1933, p.57), while others, for example Lettuce Mosaic, lose their infectivity when diluted 1:100 (Ainsworth and Ogilvie, 1939). Mercuric chloride has little effect on Tobacco Mosaic virus while in 0.001 M. solution it immediately inactivates the Tomato Spotted-wilt virus (Best and Samuel, 1936).

Such criteria help in the identification of different viruses whose symptomatology is so similar as to cause confusion, but it is apparent that the estimation of characteristics such as temperature of inactivation in expressed sap can only be approximate owing to the magnitude of the experimental errors involved. As has been pointed out (Bawden, 1939, p.57) "inactivation is a gradual and not a sudden process, dilute virus preparations being inactivated by less heating or by shorter periods of storage than concentrated ones." The initial concentration of virus in an infected plant depends on a number

of factors including nitrogen supply (Spencer, 1942), age of leaf used in preparation of extract (Matsumoto, 1941) etc. and, as has been stated, the initial concentration of the extract in turn affects the experimental values obtained for thermal inactivation point, etc.

Even when purified preparations of viruses are used it is found that heating tests do not give a sharp "end-point". It has been shown that a partial loss of infectivity results when purified preparations of Tomato Bushy stunt virus are heated for ten minutes at temperatures between 45°C. and 80°C. and also that the pH of the preparation has an effect on the rapidity of inactivation (Bawden and Pirie, 1943).

Thus viruses cannot be distinguished by such means unless considerable differences in physical constants are detected.

In general such characteristics as filterability, and resistance to chemicals, ageing, heating and dilution have been determined only in the case of viruses transmissible by sap inoculation, but Severin and Swezy (1928), Carter (1927) and Bennett (1935) by the use of special insect-feeding techniques have obtained similar data for the virus of Sugar-beet. Curly-top disease and Storey (1932) has obtained data for the Maize Streak virus.

The difficulties briefly outlined above which are

inherent in any attempt to identify viruses according to their symptomatology, host range or properties have encouraged the study of the serology of viruses by means of which the inter-relationships of viruses and strains can be most readily established (vide Bawden, 1943, Chap.Vll.).   There are, however, difficulties in the way of serological study of viruses which are not sap transmissible.

As most plant viruses are transmitted in nature by insect vectors considerable attention has been paid to the study of vectors and virus-vector relationships.   The vectors of many viruses are known and although thrips, leafhoppers, white fly, beetles and mites have been shown to be the vectors of certain viruses, by far the commonest vectors are aphids.

Storey (1939) and Watson & Roberts (1939) have shown that many viruses with aphid vectors can be placed in one of two classes according to whether the vector remains infective for long periods or loses infectivity quickly.   These two groups of viruses have been described by Watson & Roberts as "persistent" and "non-persistent" viruses, respectively, (see also Section C, p.117et seq.), and such considerations of vector relationships may be an aid in distinguishing and identif ying viruses in cases where other methods cannot be readily applied.

The physiology of viruses, or the inter-relationship of virus and host plant, has also been investigated to some extent and such researches have supplied fundamental information

on the behaviour and nature of viruses.

Some viruses, e.g. Sugar-beet Curly-top virus, have been shown to be concentrated in the phloem (Bennett, 1934) while others are distributed throughout the plant. It has been shown, however, that Aucuba mosaic virus is not present in the xylem elements of infected tomato plants (Caldwell, 1930). Some workers have concluded from their experiments that, after the initial movement of virus from the point of inoculation through parenchymatous tissues to the phloem, the spread of virus through the plant takes place in the latter tissue (Holmes, 1930; Samuel, 1931; Bennett, 1939, etc.). Others consider that movement of virus is largely independent of the phloem (Caldwell 1931, 1934a).

The effect of virus infection on the carbohydrate/nitrogen ratio of a plant is often considerable, this ratio being sometimes increased or, with other viruses, decreased (Dunlap, 1930). The enzyme system of infected plants, too, is often profoundly affected (Caldwell 1934b; Wynd, 1942) and symptoms have been considered to be caused directly by products of a deranged metabolism (McKinney and Hills, 1941). A review of the often apparently contradictory work in this field has recently been published (Wynd, 1943).

By means of filtration and centrifugation techniques the probable particle size of many viruses has been calculated (Markham, Smith and Lea, 1942; Smith and McClement, 1940, 1941; McFarlane and Kekwick, 1938) and some of these determinations

of size and probable shape of particle have now been verified
by direct measurement by means of the electron microscope.
(Stanley and Anderson, 1941).   A review of the work of Kausche
and others in this field has appeared recently (Marton, 1943).

Work on the physico-chemical nature of viruses has
also been pursued in recent years.   In 1935 Stanley isolated a
"crystalline protein possessing the properties of Tobacco Mosaic
virus."   Although Stanley's "crystals" were later shown to lack
the three dimensional regularity of true crystals (Bernal and
Fankuchen, 1938), the virus of Tomato Bushy stunt has since been
isolated in truly crystalline form (Bawden and Pirie, 1938).

The classification of viruses has presented a
difficult problem which does not appear to have been satisfactorily
solved as yet though a variety of schemes (based on a consideration
of practically all aspects of virology) have been suggested.

Identification and classification are related topics
and enough has already been said regarding the difficulty of
identifying viruses on host range and symptoms to show that
systems of classification based on these factors are unlikely
to prove satisfactory.   Smith (1937) classifies viruses according
to host plant, the viruses infecting, for example, tobacco
being grouped together, while those infecting Brassica are put
in another class.   While such a classification supplies

the pathologist with a kind of artificial 'key to the plant viruses' it has been pointed out (Bawden, 1943, p.249) that it is fundamentally as unsound as would be a classification of fungi in which Ophiobolus graminis, Erysiphe graminis and Puccinia graminis were placed together because they all happen to infect Triticum.

Symptomatology is the chief basis of the classification of Holmes (1939), the viruses causing mosaics, ringspots, leaf curls, dwarfing diseases, etc., being placed in separate groups or "genera". The mosaic group, however, contains more than half of the viruses included in the scheme and the classification has been criticized (Valleau, 1940; Bawden, 1943) on the grounds that dissimilar and unrelated viruses are included in the same "genus".

Classification according to physical properties (temperature of inactivation, etc.) has been suggested by Johnson (1927) but such a system appears likely to bring together dissimilar viruses or to separate related ones according to the arbitrary range allotted to each class. Due perhaps to its seeming artificiality and to the difficulty of including viruses which are not mechanically transmissible this system has never gained wide acceptance.

Bawden (1943) while expressing the view that insufficient data are available for the formulation of a final system of classification suggests that an arrangement of a number

of viruses into groups might be made according to their
serological reactions. While serology has proved valuable
in indicating the relationship of strains of a type virus,
Bawden has himself pointed out the difficulty of forming larger
groupings on a serological basis (loc.cit.p.255); serology, so
to speak, indicates the relationship of the varieties of a
species but not the relationship of the genera of a family.
Another objection to a serological classification of viruses
in the opinion of the present writer is that it ignores the
fact that viruses are pathogens. Were it not for this fact
viruses would never have been discovered, and a non-infectious
virus appears to be a contradiction in terms. Yet viruses can
be so altered by heating, freezing, irradiation with ultraviolet
light or treatment with chemical substances that their infectiv
-ity is completely destroyed without their serological activity
being affected (Bawden and Pirie, 1938, 1943; Bawden and
Kleczowski, 1942). Thus serological techniques, which indicate
chemical similarities, do not differentiate between viruses and
non-viruses. A system of classification based on serology would
be a classification of proteins and a non-pathogenic substance
might be identified with a virus. At any rate such a pair of
similar substances would be considered as more closely related
than are two strains of a virus.

Attempts have been made to classify viruses accord-
ing to their vectors and vector relationships but these have, so
far, met with little success. Elze (1931) appears to have been the

first to suggest the possibility of such a classification and
the system of Holmes (1939) is based to some extent on a
consideration of the vectors of the various virus groups.
The limitations of any scheme of classification according to
the type of insect vector are, however, indicated by the work
of Bawden and Sheffield (1944) who find that Myzus persicae is
a vector of one strain of Potato virus 'Y' but is not a vector
of another strain of the same virus.

As has been stated, a consideration of virus-vector
relations allows of a division of aphid-transmitted viruses
into two classes but it does not at present seem likely that this
could form the basis of a system of classification owing to the
difficulty of further subdividing these two main groups.

Remarkably enough, no serious attempt appears to
have been made to classify viruses according to their host
relationships though such an approach to the problem seems a
reasonable one.

Probably a rational classification will not be
evolved until a larger number of viruses has been intensively
studied for, notwithstanding the great advances of research
along lines such as have been adumbrated above, it remains true
that the investigation of many plant virus diseases has been
restricted to a consideration of symptomatology and few or no
data on the viruses themselves have been obtained.

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**B.   VIRUS DISEASES OF BROCCOLI AND BRUSSELS SPROUTS
WITH SPECIAL REFERENCE TO BROCCOLI MOSAIC.**

I. Introduction

The first publications on virus diseases of the
Cruciferae appear to be by Gardner and Kendrick (1921) and
Schultz (1921) who independently reported a mosaic disease
of turnip. Many other papers have since been published but
until 1935 these are, in general, of a descriptive nature,
the symptoms produced on host plants being described and details
given of the transfer of infection to other plant species.
Some confusion, however, arises from the general similarity
of symptoms, and it is often difficult to decide when different
authors are, in fact, describing diseases caused by the same
virus. Indeed, in 1937, K.M.Smith wrote, "Considerable
uncertainty still exists concerning the number and identity of
the viruses which attack Cruciferous plants."

In more recent years, comprehensive studies on a
number of Crucifer virus diseases have been published in
America (Hoggan & Johnson, 1935; Tompkins et al., 1937-1939;
Larson & Walker, 1939, 1941) and it becomes increasingly evident
that a mere description of symptoms no longer suffices to
identify any particular virus of this group. Given, however,
the host range, symptoms on a number of test plants, methods

of transmission and physical characteristics (such as
resistance to ageing, to dilution and to heating), it then
becomes possible to identify virus agents with some degree
of certainty.

The relation of Broccoli Mosaic to the Crucifer
viruses described in the literature will be considered later.

II.   Broccoli Mosaic

(a) Occurrence

A mosaic disease of broccoli was observed in Devon and Cornwall by Dr. J. Caldwell in 1936.   Autumn, Winter and early Spring broccoli form an important crop in this area - according to the figures of the Ministry of Agriculture there were 3,400 acres in Cornwall alone in 1937 - and, as severely affected plants produce a small unmarketable 'curd', the disease is of considerable economic importance.   It has also been shown (Caldwell and Prentice, 1942b) that infected plants are more susceptible to frost damage than are uninfected plants so that additional indirect loss may result.   Fields containing over 60% of infected plants are frequently encountered.

Preliminary work on field spread and methods of transmission were begun by Caldwell and James[x] but, although it was suspected that the disease was caused by a virus, it was not found possible to transmit it artificially.   In 1938 the present writer continued the investigation and the results of the subsequent work are described below.

(b) Experimental

(i) Symptoms on Broccoli.

Symptoms on broccoli, whether resulting from

[x] Private communication.

natural infection in the field or from inoculation in the
glasshouse, consist of vein-clearing followed by vein-banding.
Vein-clearing first becomes evident 18 to 30 days after
inoculation - most usually in about 22 days.   It generally
begins at the base of the leaf on one side of the mid-rib
(Figs.1) and often spreads from there to the remainder of the
leaf.   The cleared veins often become yellowish in colour
and this condition may persist in the oldest leaves.   In the
younger leaves, however, it slowly passes to vein-banding,
the main veins being bordered by dark green areas and the
remainder of the leaf becoming chlorotic (Figs.2,3,4).
Pronounced vein-banding is generally apparent about six weeks
after inoculation.

In some cases small, irregular, necrotic lesions
develop subsequently in the chlorotic areas.   These lesions,
which are more frequent on the lower surface of the leaf than
on the upper, appear first as small, translucent or whitish
papillae and later become necrotic and cream, fawn or light
brown in colour (Fig.5).   Where vein-clearing is largely
restricted to one side of the mid-rib, the resultant uneven
growth causes curvature of the mid-rib and slight distortion
of the lamina (Fig.1).   Similarly curvature and distortion
are common in cases where necrotic spots are formed.

Occasional examples of light vein-banding have
been observed, the veins being bordered by light green bands
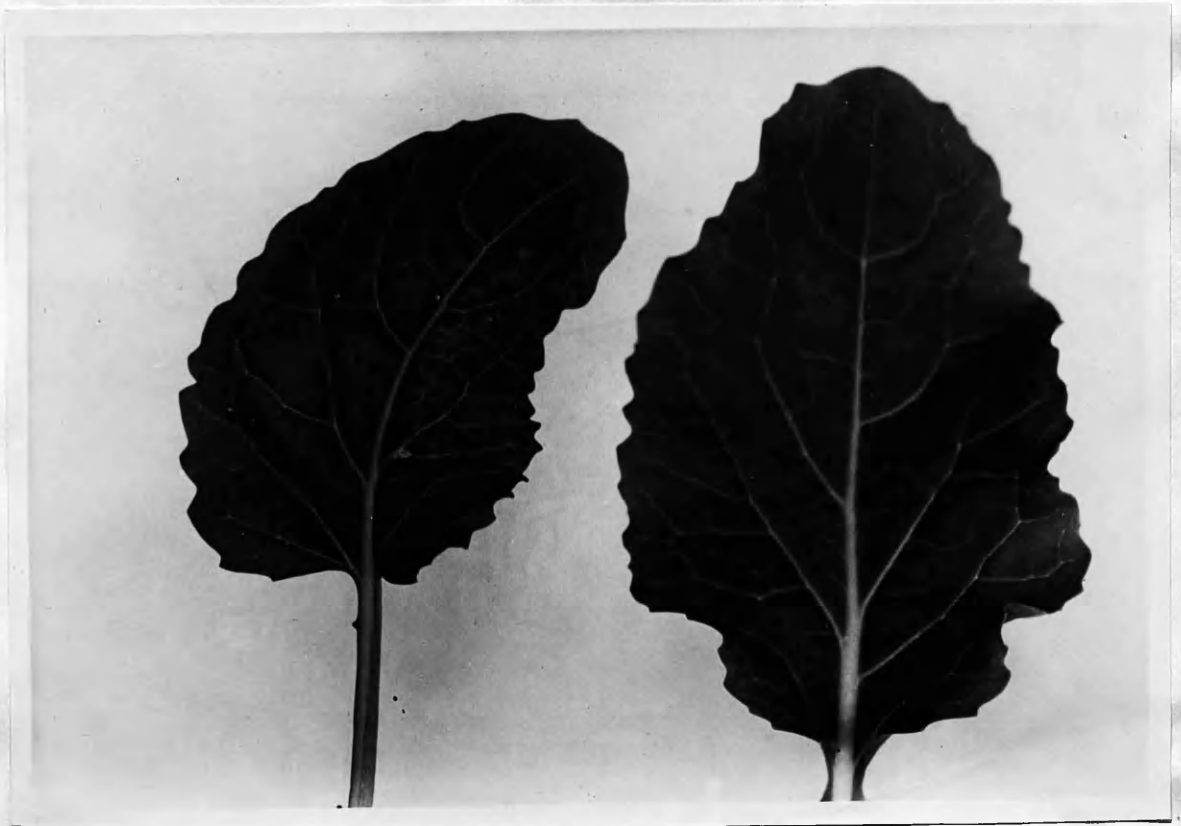
Figure 1.

Broccoli.
(Inoculated)

Left: Leaf showing vein-clearing and laminal distortion.
Right: Leaf showing incipient vein-clearing.

Figure 2.

Broccoli.
(Natural infection)

Leaf showing yellowing of veins and vein-banding.

Figure   3.

Broccoli.
(Inoculated)

Leaf showing vein-banding.

Figure 4.

Broccoli.
(Natural infection)

Leaf showing vein-banding.

Figure 5.

Broccoli.
(Inoculated)

Leaf showing vein-banding and necrotic spotting.

and the interveinal areas being dark green, i.e. a reversal
of the normal type.   In other cases the combination of a mild
type of vein-banding with pronounced yellowing of the vein
reticulum, chlorotic interveinal areas and areas of normal
colour gives to the leaf a more or less uniformly mottled
appearance.

Masking of symptoms is common.   Complete masking
usually occurs when the plants are grown at temperatures of
over 25°C., but partial masking - on the later-formed leaves -
occurs at lower temperatures in some varieties.   The factors
influencing this partial masking have not been investigated
fully but the phenomenon appears to be correlated with
environmental factors resulting in a slow rate of growth of
the infected plant.

It will thus be apparent that symptoms of infection
on broccoli are variable.   This is thought to be due mainly
to the effects of environmental conditions and to the multiplicity
of types included in each horticultural variety of broccoli
rather than to the existence of strains of the virus.

As a result, probably, of the reduced efficiency
of the leaves, infected plants produce smaller "curds" or
"flowers" than normal plants, the degree of reduction depending
on the severity of infection.   A plant infected at an early
stage develops symptoms on all its leaves and either fails

altogether to produce a curd or produces only a small "button"
4 or 5 cm. in diameter. Plants infected at a later stage of
development show symptoms on the subsequently formed (central)
leaves only, and may produce marketable curds of 15cm. or more
in diameter.

(ii) Preliminary Work.

1. Initial failure to transmit infection.

Between 30th January and 18th May, 1939, attempts
were made to transmit infection by inoculating small numbers
of young plants of broccoli, cabbage, red cabbage, kale, turnip,
swede, radish, rape, mustard, cress, stock and wallflower from
old and young leaves of naturally infected broccoli, Brussels
sprouts and Spring cabbage by hypodermic and carborundum
inoculation to cotyledons, hypocotyls and leaves. Attempts
were also made to transmit infection by means of white fly
(Aleurodes brassicae) taken from infected broccoli plants and
transferred to the species mentioned. In no case was any
symptom produced and plants were discarded on various dates
in June.

Seedlings of these species were also planted out
of doors in proximity to infected broocoli plants in the hope
that they might become infected by natural means. Such infection
did not occur.

During this period, the only infected broccoli
plants available as sources of inoculum were senescent plants

raised in the previous Spring.   Later work showed that such plants are less efficient as infectors than are young, freshly infected plants and the failure to obtain transmission in these early experiments is attributed to this fact.

The vector in the field was later shown to be Brevicoryne brassicae and as no aphis was observed on the infected broccoli plants at this period, the failure to transmit infection by natural means is readily explicable.

2. Effect of minor elements.

As no evidence of transmission of the disease had, as yet, been obtained, young leaves of a mosaic infected broccoli plant (growing out-of-doors) were injected on 28th April with solutions of salts of Boron, Manganese, Copper, Zinc and Magnesium to determine whether the mosaic symptoms were caused by a deficiency of one of these "minor elements". Injection, which was by means of a hypodermic needle, was repeated on 2nd May.   No visible effect was observed.

3. Successful transmission.

On 24th April an effort was made to transmit infection by grafting.   Small pieces of the "curd" from an infected plant were taken and the stalk cut as indicated in diagram 1(c).   The stem of a young broccoli plant (1a) was prepared (1b) to receive this scion and the stock and scion bound together with raffia.   The whole was then coated with paraffin wax.   Two such grafts (hereafter referred to as G1 and G2), were made and in both cases the tissues of stock
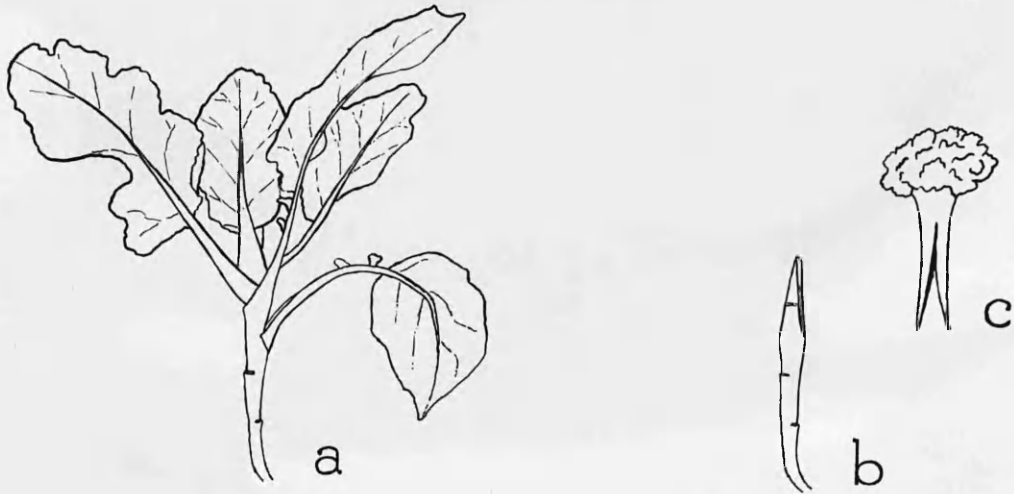
Diagram 1.

Broccoli – Grafting Technique.

a ... Young broccoli plant.
b ... The same, prepared for use as a grafting stock.
c ... Portion of 'curd' of broccoli, prepared for use as a
      scion to be grafted to 'b'.

and scion subsequently united satisfactorily. Two similar grafts, only one of which was successful (G3) were attempted on 28th April. On 24th May the stems of G1, G2 and G3 were incised with a scalpel and a wad of wet moss tied round the wound to encourage root production. On 3rd June roots had developed at the ends of the scion wedges and from the callus tissue of the grafts. The moss was removed, the stems cut off below the grafts and the rooted scions with the portions of stock united to them were potted up. One of them subsequently produced two basal vegetative shoots which showed mosaic symptoms. Shoots also arose from axillary buds on two of the stocks left after the removal of the rooted scions and by 7th July these shoots showed marked mosaic symptoms, indicating that infection had passed from the infected scions to the originally healthy stocks.

With later grafts, made on 12th May (two grafts of portions of curd to young broccoli plants - one successful, G4), 25th May (two grafts of shoot with flower buds to young broccoli plants - both successful, G5,G6) and 17th June (axillary shoot from G4 to young broccoli plant - successful, G7), no attempt was made to induce the scion to root. Instead, the scion was amputated above the graft as soon as it was seen that graft union had taken place. It was hoped that this would encourage the production of axillary shoots on the stock and in most cases this did occur.

G4, kept in the glasshouse, produced a sub-graft axillary shoot which showed mosaic symptoms by 14th June. G5 and G6 were put out-of-doors and the latter failed to produce an axillary shoot; G5, however, produced such a shoot and mosaic symptoms were evident on it by 14th July. The sub-graft axillary shoot on G7 exhibited symptoms in the glasshouse by 30th July.

In each case the symptoms were of the type already described - vein-clearing followed by vein-banding - but in the early stages, before experience was gained, plants were always retained until vein-banding was apparent, in order to confirm infection.

Six ungrafted control plants remained healthy.

These grafting experiments showed that the disease was of an infectious nature and in the absence of any visible pathogen it was concluded that broccoli mosaic was in fact a virus disease.

Five young broccoli plants were inoculated on 26th June using triturated leaves of G4 as inoculum and carborundum powder as an abrasive. By 9th September, two of the inoculated plants had developed vein-clearing and vein-banding, one developed doubtful symptoms and two were apparently healthy. Four uninoculated control plants all remained healthy.

Apterous cabbage aphides (<u>Brevicoryne</u> <u>brassicae</u>), collected from cabbage and broccoli plants were transferred on

24th June to one of the plants infected by grafting (G4).
The aphides were retransferred on 26th June to healthy
broccoli plants growing in an insect-proof cage in the glass-
house.   Four of the five plants receiving these aphides
became infected.

Aphides were transferred direct to three other
plants, without a period of feeding on the infected plant, and
while typical vein-clearing was not produced on these, the
leaves became somewhat distorted and whitish areas developed
on them.   These symptoms soon passed off, however, after the
removal of the aphides, and no symptoms of vein-banding
developed.   Eight young broccoli plants were inoculated from
leaves showing these white markings following aphis infestation,
and all remained healthy.   It was, therefore, concluded that
the distortion and whitening of portions of the leaves of plants
on which aphides had fed was directly due to aphis feeding,
and that these symptoms were probably non-infectious and had
no connection with infection by the broccoli mosaic virus.

Unidentified aphides collected from rosebuds were
similarly fed on the infected plant(G4) for two days and trans-
ferred to two plants of each of the broccoli varieties Sutton's
Early Roseoff, Sutton's Roseoff No.3, Scoble and Seale Hayne
DK7.   A similar number of plants received aphides direct from
the rose bush.   Two plants of each of these varieties were
inoculated from leaves of G4 by the carborundum method (described

in Section iii, p41), a similar number from / leaves of G3 and a similar number were left untreated as controls.

These transfers and inoculations were made on 5th July and the results visible on 31st August are shown in Table I.

TABLE I.

H = Healthy
I = Infected

TREATMENT	Early		No.3		Scoble		DK7		Total	
	H	I	H	I	H	I	H	I	H	I
Rose aphides fed on G4	1	1	1	1	0	2	0	2	2	6
Rose aphides - direct	2	0	2	0	2	0	2	0	8	0
Inoculated from G4	2	0	2	0	1	1	1	1	6	2
Inoculated from G3	1	1	1	1	0	2	0	2	2	6
Control uninoculated	2	0	2	0	2	0	2	0	8	0

The plants were retained until 26th September but no further symptoms developed.

As will be seen, six of the eight plants receiving rose aphides which had fed on an infected plant developed symptoms, while none of the plants receiving aphides direct from the rose did so. Eight of the 16 plants inoculated from infected plants developed symptoms and the controls remained healthy.

It was concluded firstly, that aphides other than
Brevicoryne brassicae are able to transmit infection; secondly
that the virus is transmissible by sap inoculation by the
carborundum method; and thirdly that several varieties of
broccoli are susceptible to infection.

On 7th July, 1939, ten seedling broccoli plants
(Sutton's Roscoff No.2) growing in the glasshouse were
mechanically inoculated from a naturally infected plant of
the same variety growing/the experimental garden, University
College, Exeter, and showing vein-clearing and vein-banding.
Three of these plants became infected and this culture was
maintained by serial transfers and used in all subsequent
experiments.

4. Comparison of inoculation techniques, etc.
Several experiments were performed in order to
ascertain the most reliable source of infectious inoculum and
the most efficient method of inoculation. It became apparent
that the carborundum abrasion method of inoculation produced
more infections than did inoculation by hypodermic needle,
veinal incision or leaf-rubbing without carborundum. The
carborundum method was therefore used in all later routine
inoculations. The effect of vigorous rubbing with carborundum
and of leaf-washing after inoculation was examined, but it
appeared that normal inoculation by light rubbing without
subsequent washing was at least as efficient as the alternative
methods. The addition of Sodium sulphite during the preparation

of inoculum (found to assist the transmission of the Tomato
Spotted-wilt virus from chrysanthemums - Ainsworth, 1936)
resulted in failure to transmit infection.

TABLE 2.   Comparison of methods of inoculation.

Method of Inoculation	Proportion of infections [x]
Normal carborundum inoculation ...	11/15
Vigorous " " ...	4/5
Normal inoculation followed by washing	2/10
Sodium sulphite added to inoculum.	0/12
Vein cutting	0/5
Control uninoculated	0/5

[x] Denominators show number of plants inoculated, numerator
                              number infected.

          A vein-banded leaf from an old, naturally-infected
plant was divided in half along the mid-rib.   One half was
triturated in a sterile mortar and used as inoculum.   The
other half was dissected into (a) dark bands along the veins
and (b) light green areas of the lamina and these were
triturated and used separately as inoculum.   A young, central

leaf of the same plant and a vein-cleared leaf of a young plant were also used as sources of inoculum at the same time. Five young plants (Sutton's Roscoff No.2) were inoculated from each source. The results (Table 3) indicated that the old plant was not a suitable source of inoculum.

TABLE 3. Comparison of sources of inoculum (a).

Source of Inoculum	Proportion of infections [x]
Vein-banded leaf .... (old plant)	0/5
Dark bands of vein-banded leaf..(" ")	1/5
Light areas " " " " ..(" ")	0/5
Young leaf .... (" ")	0/5
Vein-cleared leaf .... (young plant)	3/5
Control uninoculated	0/5

[x]   Denominator shows number of plants inoculated.

Vein-cleared leaves from a young plant were compared with leaves from the same plant showing vein-banding or necrotic spotting and, on the whole, it appeared that vein-cleared leaves were the most convenient source of

inoculum.   Leaves showing necrotic spotting also produced
highly infectious inoculum but, while infected plants always
produced vein-cleared leaves, they did not always produce
necrotic-spotted leaves of which it was thus difficult to
maintain a sufficient supply.   It was therefore decided to use
vein-cleared leaves from young, recently infected plants for
the preparation of routine inocula.

TABLE 4.   Comparison of sources of inoculum (b).

| Source of inoculum | Proportion of infections [x] | | | | |
	Trial 1	Trial 2	Trial 3	Trial 4	Total
Vein-cleared leaves [/]	2/3	5/5	0/4	4/6	11/18
Vein-banded " [/]	0/3	2/5	2/4	1/6	5/18
Necrotic-spotted " [/]	3/3	5/5	0/5	-	8/13
Control (uninoculated)	0/3	0/5	0/4	0/6	0/18

[/] Leaves of the three types were taken from the same
plant.

[x] Denominators show number of plants inoculated.

(iii) Materials and Methods.

As has been mentioned above (p. 37), a stock
culture of the broccoli mosaic virus was obtained in July 1939
from an infected broccoli plant (variety Sutton's Roscoff No.2)

showing vein-clearing and vein-banding and growing in the
experimental garden, University College, Exeter.   Infection
was transmitted by inoculation to seedling plants of the same
variety in the glasshouse; this culture was maintained by
serial transfers to young broccoli plants and used in all
experiments thereafter.   (Subsequently the disease was trans-
mitted by inoculation from mosaic broccoli plants taken from
fields at Stoke Canon and Pinhoe, Devon, and from Cornwall,
the symptoms produced on the inoculated plants being similar
to those induced by the stock culture.)

      To prepare inoculum, vein-cleared leaves from
young, recently infected plants were triturated in a sterile
mortar, a small quantity of carborundum powder being added
during the process.   The leaf to be inoculated was dusted
with carborundum powder and then, for the majority of inoculations,
rubbed lightly with a small piece of cotton wool dipped in this
pulped material.   In experiments to determine the inactivation
temperature, resistance to ageing and resistance to dilution,
the pulped material was transferred to a piece of washed muslin,
the juice expressed and, after appropriate treatment, used as
inoculum.

      In determining the inactivation temperature of
the virus, 0.5 ml. of the undiluted expressed juice was pipetted
into thin-walled, stoppered glass tubes which were placed in a
beaker containing water maintained at a desired temperature by

means of a gas jet.   After being held at this temperature for ten minutes, the tubes were rapidly cooled in a stream of tap-water.   For determinations of the dilution end-point, the leaf extract was diluted with sterile distilled water to the appropriate concentration.   Ageing tests were carried out using undiluted leaf extract stored in small stoppered tubes in an incubator maintained at $22^{\circ}C$.   In filterability tests the extract was first passed through a single layer of filter paper in a Buchner funnel and a portion of the filtrate was then passed through a series of Pasteur Chamberland filter candles of progressively finer grade.   The insect transmission studies were performed in a Cellophane-covered insect-proof cage in the glasshouse.   Broccoli plants were inoculated when they had from one to three leaves, that is, about one month after the seed was sown.

(iv)   Transmission.

The disease, as has been shown above, is readily transmitted to healthy broccoli seedlings by grafting and by mechanical inoculation, using carborundum powder as an abrasive. The virus is also readily transmitted by the mealy cabbage aphid (Brevicoryne brassicae) and by at least one other species of aphid;   it is thought that B.brassicae probably is the most important vector in the field.

To ascertain the time required by apterous aphides (Brevicoryne brassicae) to pick up infection from an infected

broccoli plant, aphides from an uninfected plant were transferred to an infected plant for periods of from ten minutes to twenty-four hours and then retransferred to an uninfected "indicator" broccoli plant (var. Sutton's Roscoff No.2) for twenty-four hours. Five aphides were transferred to each "indicator" plant and the number of indicators which subsequently developed symptoms is given in Table 5 (a).

TABLE 5 (a). Transmission of the Broccoli Mosaic virus by the cabbage aphid (Brevicoryne brassicae).

| Time for which aphides remained on infector. | PROPORTION OF SUBSEQUENT INFECTIONS[x] | | | |
	Trial 1 8/11/40	Trial 2 2/12/40	Trial 3 12/12/40	Total
10 min.	0/2	1/4	2/4	3/10
20 min.	0/2	0/4	3/4	3/10
30 min.	0/2	4/4	0/4	4/10
60 min.	2/2	5/5	0/3	7/10
24 hrs.	1/1	4/4	-	5/5
0 min. (Control)	0/2	0/4	0/4	0/10

[x] Denominator shows the number of plants receiving aphides.

It will be seen that some of the aphides picked up infection in 10 minutes (the shortest period investigated).

Experiments to establish the time required by infective aphides to transmit infection to healthy plants were also conducted. Apterous aphides (B.brassicae) were rendered infective by allowing them to feed on an infected plant for 24 hours. They were then transferred to uninfected indicator plants for periods of from 10 minutes to 24 hours, five aphides being transferred to each indicator. The number of indicators which developed symptoms of infection are given in Table 5 (b).

TABLE 5 (b).

Time for which infective aphides fed on uninfected indicator plants	PROPORTION OF INDICATORS INFECTED[x]		
	Trial 1 3/12/40	Trial 2 11/12/40	Total
10 min.	-	0/5	0/5
20 min.	0/1	3/4	5/5
30 min.	2/3	1/2	3/5
60 min.	3/3	2/2	5/5
24 hrs.	3/3	2/2	5/5
0 min. (Control)	0/3	0/2	0/5

[x] Denominator shows number of plants receiving aphides.

It will be seen that some of the plants on which infective aphides remained for 20 minutes developed symptoms but

that those on which aphides remained for 10 minutes did not. Owing to the small number of plants used in these trials it is doubtful whether this has any particular significance.

Using another isolate (believed to be identical with the Broccoli Mosaic virus) alate aphides (Brevicoryne brassicae) were also found to be vectors. Five alatae from a stock of aphides raised on an infected plant were transferred in 1943 to each of six test plants (var. All-the-year-Round) and allowed to feed there for 48 hours. All of the test plants developed symptoms of infection. Controls receiving no aphides remained symptomless.

White fly (Aleurodes brassicae) did not appear to be a vector of the Broccoli Mosaic virus. White fly collected from infected plants out-of-doors and white fly allowed to feed on infected plants in the glasshouse for two days did not transmit infection to young broccoli plants to which they were subsequently transferred.

To investigate the possibility that Broccoli Mosaic might be seed-transmitted, about 150 seedlings were raised in the glasshouse in 1940 from seed collected from an infected broccoli plant. None of the seedlings had developed symptoms of infection after two months growth. In a similar trial in 1941 about 500 seedlings were raised and all appeared healthy after three months growth.

Six of these plants were inoculated with broccoli mosaic virus when four weeks old, and four of the six developed symptoms of infection three weeks after inoculation. Thus it was apparent that these seedlings developed symptoms when infected and it was concluded that the uninoculated, symptomless plants were not infected with the mosaic virus. These experiments, therefore, gave no evidence that the virus is seed transmitted.

(v)   Varietal Susceptibility.

A number of varieties of cauliflower and broccoli (including all varieties of broccoli known to be grown commercially in Devon), were tested for susceptibility to infection by the virus by mechanical inoculation. These tests were carried out at various times from August 1939 to February 1941 and young plants were found to be susceptible to infection at all seasons of the year.

Varieties tested in August 1939 were Sutton's Roscoff Nos. 1 and 2; in October, Sutton's Roscoff No.3; in November, Tozer's September Giant, October Giant and November Giant, and Sutton's Roscoff No.4; in December, Sutton's Roscoff No.2.

In February 1940 the following varieties were tested;- Sutton's Roscoff No.2, Tozers November Giant, Early White, Satisfaction, Snow White, White Beauty, Whitsuntide, Winter White; in March, Sutton's Roscoff Extra Early, No.1,

No.2, No.3 and No.4, Knight's Protecting, Leamington, Veitch's Self-protecting and the Seale Hayne varieties AI, DK7 and BXS (Scoble); in April (in four trials), Sutton's Roscoff No.2, Tozers September, October and November Giants, All-the-year-round, Superlative and Veitch's Autumn Giant; in July (3 trials), Sutton's Roscoff No.2, Early White, Winter White and Seale Hayne B2, DK7 and DXS; in August (2 trials), Sutton's Roscoffs Extra Early, No.1, No.3 and No.4, Tozers September Giant, All-the-year-round, Knight's Protecting, Leamington, Satisfaction, Snow White, Superlative, Veitch's Autumn Giant and Self-protecting, White Beauty, Whitsuntide and Seale Hayne AI; in September (3 trials), Sutton's Roscoff No.2, White Beauty, Winter White, and Seale Hayne B2 and DXS; in December (3 trials), Sutton's Roscoffs No.2 and No.4, Early White, Snow White and Whitsuntide.

In January and February, 1941, the following were tested:- Majestic, May Blossom and Michaelmas White.

From the results tabulated below it will be seen that all varieties tested were highly susceptible to infection.

TABLE 6.   Varietal Susceptibility

(a) Broccoli.

VARIETY	PROPORTION INFECTED	
	Total[x]	Percentage
Early White	24/28	86
Knight's Protecting	18/27	67
Leamington	21/26	81
May Blossom	18/24	75
Michaelmas White	12/20	60
Satisfaction	20/25	80
Seale-Hayne: AI	17/25	68
B.2	22/25	88
DK7	16/20	80
DXS	23/27	85
Snow's Winter White	16/30	53
Snow White	16/25	64
Sutton's Roscoff: Extra Early	21/27	78
No.1	21/28	75
No.2	71/85	84
No.3	26/32	81
No.4	23/29	79
Veitch's Self-protecting	22/26	85
White Beauty	17/26	65
Whitsuntide	16/26	62

(b) Cauliflower.

VARIETY	Total	Percentage
All-the-year-round	24/27	89
Majestic	17/20	85
Sutton's Superlative	26/27	96
Tozer's September Giant	26/27	96
" October "	24/27	89
" November "	21/26	80
Veitch's Autumn Giant	19/25	76

x Denominator shows number of plants inoculated

(vi) Host Range and Symptoms.

The following additional species and varieties were found to be susceptible to infection by mechanical inoculation: Brussels sprouts, Cabbage, Colewort, Kale, Savoy, Sprouting broccoli, Kohl-rabi, Rape, Swede turnip and Charlock. In addition, Radish was infected by means of the vector <u>Brevicoryne brassicae</u> though not by mechanical inoculation (Fig.6). The percentage of successful infections (Table 7) was generally less than that obtained with cauliflower and broccoli.

TABLE 7. Host Range.

SPECIES		PROPORTION INFECTED	
		Total	Percentage
Brassica oleracea L.:			
var. gemmifera D.C.	(Brussels sprouts)	15/25	60
var. capitata L.	(Cabbage: Winningstadt)	6/10	60
" " "	(" : Spring)	6/10	60
" " "	(" : Red)	12/15	80
var viridis D.C.	(Colewort)	3/8	38
var. acephala D.C.	(Kale: Cottagers')	13/20	65
" " "	(" : Ormskirk)	4/10	40
var. bullata D.C.	(Savoy)	3/10	30
var. botrytis L.	(Sprouting broccoli: Early purple)	7/10	70
var. caulo-rapa D.C.	(Kohl-rabi)	3/20	15
B. napus L.	(Rape)	5/15	33[x]
" "	(")	12/15	80[x]
B. campestris L.			
var. napobrassica D.C.	(Swede turnip: Monkwood)	10/17	60
Raphanus sativus L.	(Radish: Early forcing)	0/20	0
" " "	(" " ")	6/18	33[x]
Sinapis arvensis L.	(Charlock)	5/15	33

x Transmission by means of <u>Brevicoryne brassicae</u>.
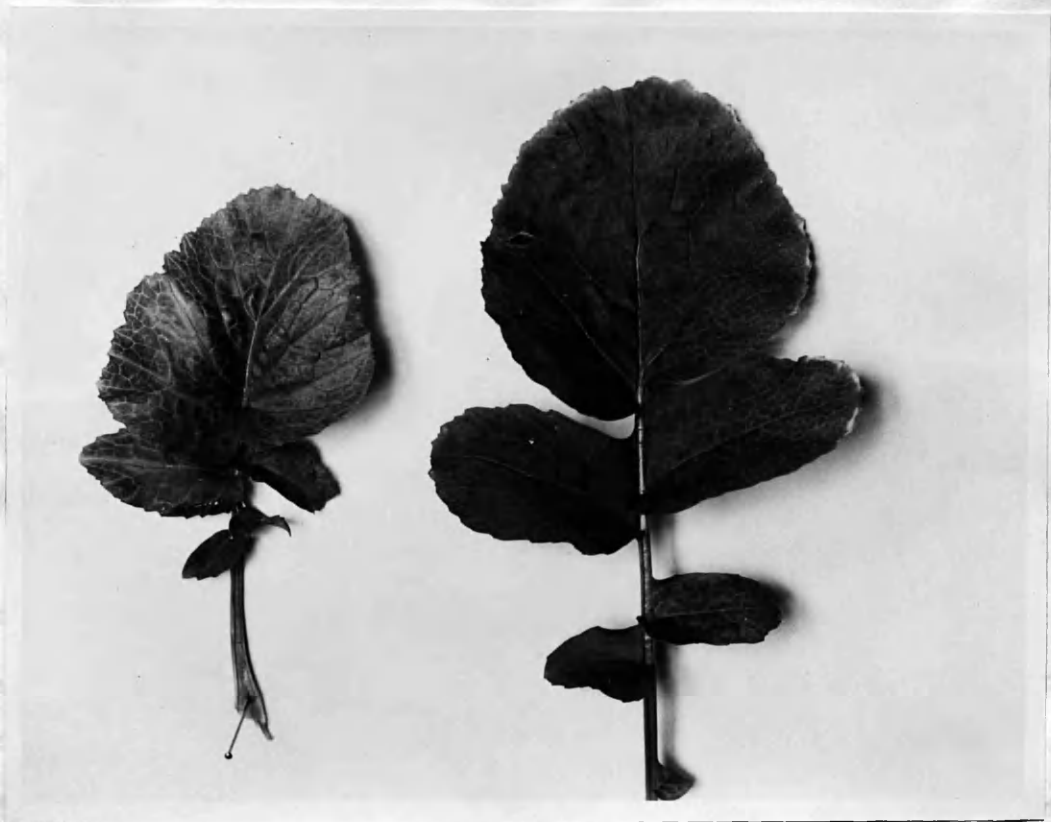
Figure 6.

Radish.
(Aphid inoculation)

Left: Leaf showing vein yellowing.
Right: Leaf showing vein-clearing on one side.

In each case the first symptom of infection was clearing of the veins of the leaves.   This, in the case of colewort, sprouting broccoli and swede turnip, developed about 20 days after inoculation while symptoms on Brussels sprouts, cabbage, kale, kohlrabi and rape (Fig.7) took about 25 to 30 days to develop, on radish about 40 days and on charlock about 60 days.   Infection of each host was confirmed by re-inoculation to broccoli and in the case of savoy (where vein-clearing is difficult to diagnose with certainty) all doubtful plants were tested by inoculation to broccoli.

Vein-clearing in Brussels sprouts, cabbage and sprouting broccoli was sometimes followed by vein-banding which was not, however, so pronounced as the typical vein-banding seen on broccoli.   Vein-banding of the reversed type (i.e. light bands along the veins and darker interveinal areas) was noted occasionally in sprouting broccoli.   Secondary symptoms in rape (Fig. 8) and swede turnip consisted of a mosaic chlorosis and "savoying" of the leaves with slight dwarfing of the plants.   Slight "savoying" (Fig.9) of the leaves was also noted in cottagers' kale/and charlock but in the other susceptible species and varieties no symptoms other than vein-clearing were noted.

After the initial vein-clearing stage, symptoms were often completely masked in Brussels sprouts, cabbage (all types), colewort, kale, kohlrabi, savoy and sprouting broccoli, but such "masked" plants remained infective, as was shown by
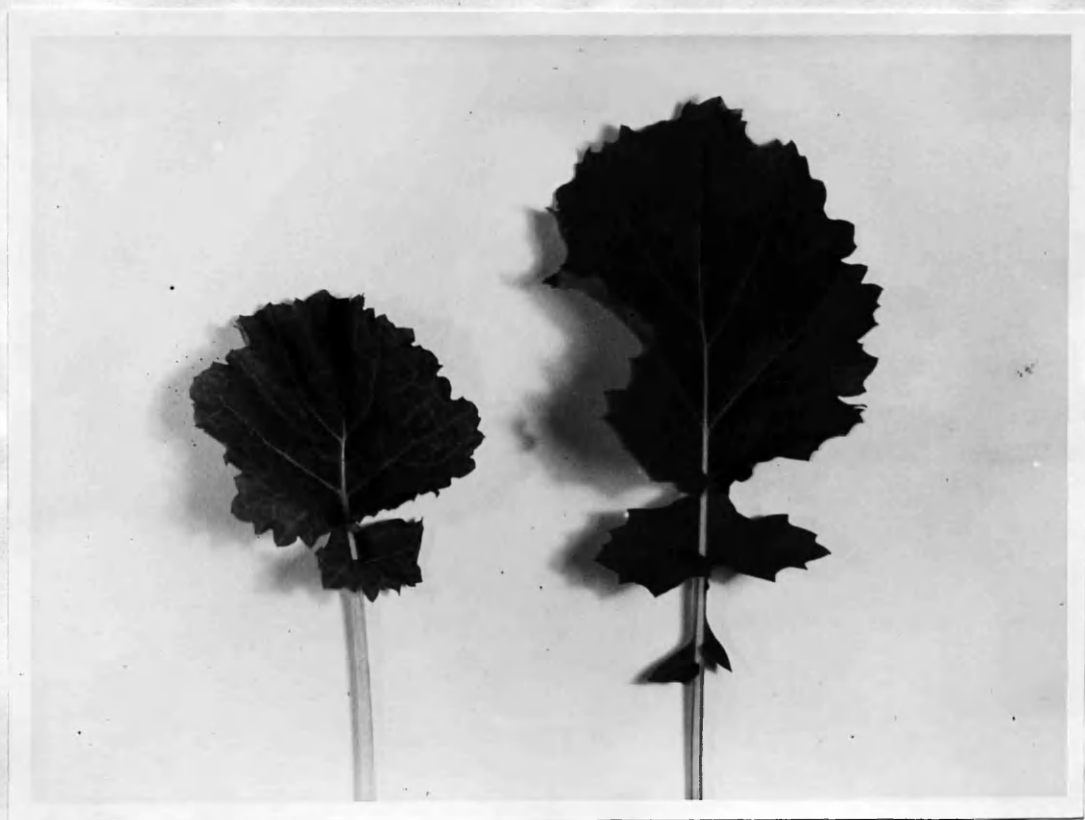
Figure 7.

Rape
(Inoculated)

Left: Leaf showing vein-clearing.
Right: Normal leaf (not inoculated).

Figure 8.

Rape
(Inoculated)

Left: Leaf showing vein yellowing and slight 'savoying'.
Right: Normal leaf (from uninoculated control).
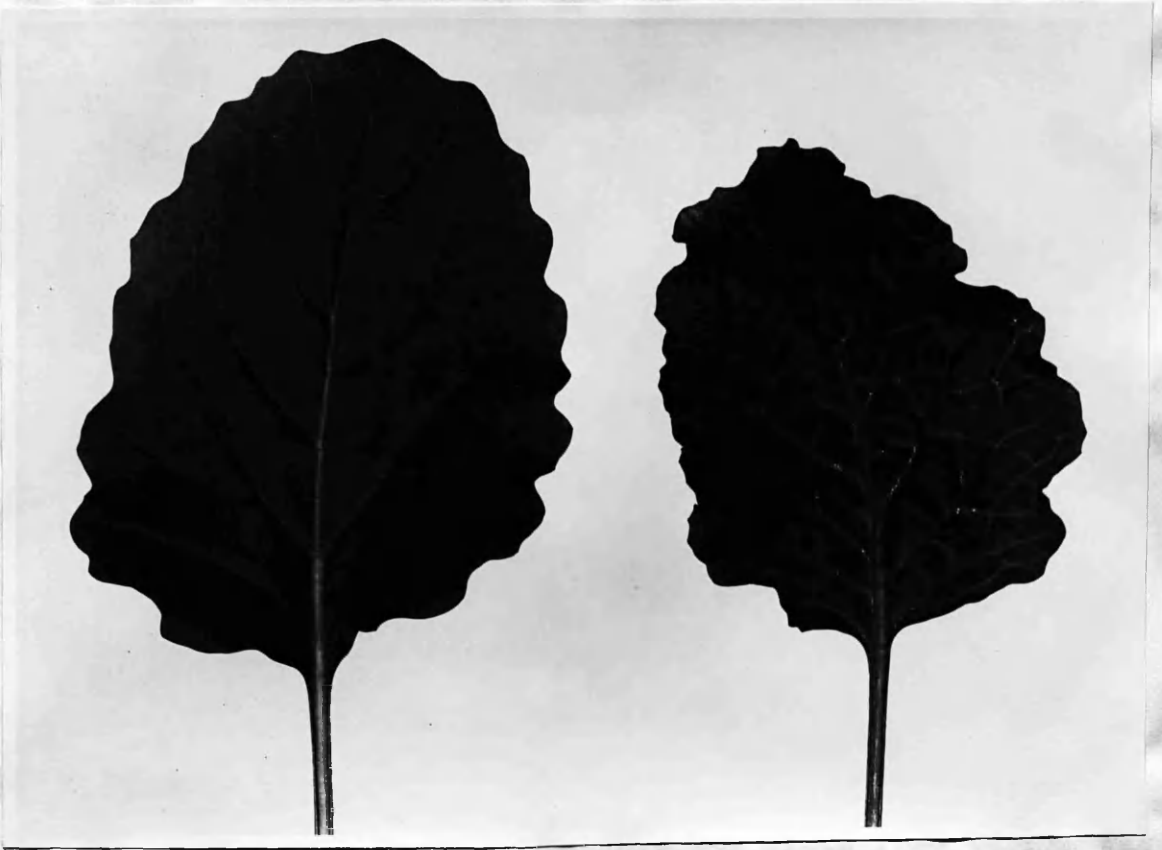
Figure 9.

Cottagers' Kale
(Inoculated)

Left: Normal leaf (from uninoculated control).
Right: Leaf showing vein yellowing and slight 'savoying'.

inoculation to broccoli.   Secondary symptoms were, in general, most pronounced when plants were grown at a low temperature (under 17°C.) but were never so conspicuous as on broccoli.

Natural infection of Brussels sprouts, Spring cabbage, cottagers' kale, marrowstem kale, sprouting broccoli (Figs.10-12) and swede turnip has been seen in the field in Devon and, in each case, inoculation to broccoli in the glasshouse has produced symptoms typical of infection by the Broccoli Mosaic virus.   Similar symptoms have been seen on Brussels sprouts, cauliflower, turnip, sprouting broccoli and swede turnip in Middlesex, on broccoli, Spring cabbage, cauliflower and kale in Kent, and on broccoli in Sussex, Hampshire and Worcestershire. Specimens of infected broccoli have been received from Yorkshire and Midlothian and it is believed that the virus is of common occurrence on cultivated Brassicae especially in the Southern counties of England.

An attempt to re-isolate the virus from turnip was unsuccessful.

(vii)   Non-suscepts.

Unsuccessful attempts were made to transmit infection to the following by mechanical inoculation:-

	Number inoculated
Nicotiana tabacum L. (var. White Burley)	10
(var. Turkish)	10
N. affinis, L.	10
N. glutinosa, L.	10
N. rustica, L. (var. Jamaicensis)	10
Lycopersicum esculentum, Mill (Tomato)	10

Figure 10.

Brussels Sprouts
(Natural   infection)

Part of leaf showing slight vein-banding.

Figure 11.

Sprouting Broccoli
(Natural infection)

Leaf showing vein-banding.

Figure 12.

Marrowstem Kale
(Natural infection)

Leaf showing vein yellowing and vein-banding.

Alliaria officinalis, D.C. (Jack-by-the-hedge)	10
Alyssum maritimum, Lam. (Sweet Alyssum)	20
A. saxatile, L. (var. compactum)	10
Capsella Bursa-pastoris (L.) Medik.. (Shepherd's Purse)	20
Cardamine hirsuta, L. (Hairy bitter-cress)	20
C. pratensis, L. (Lady's smock)	10
Cheiranthus allionii, L. (Siberian wallflower)	10
C. cheiri, L. (Wallflower)	10
Erysimum perofskianum Fisch. & Mey.	10
Hesperis matronalis, L. (Rocket)	10
Iberis amara, L. (Candytuft)	20
Isatis glauca, L.	10
Malcomia maritima, R.Br. (Virginia stock)	10
Matthiola incana, R.Br. var.annua Voss. (Ten-week stock)	10
M.incana, R.Br. (Brompton Stock)	10
Nasturtium officinale, R.Br. (Watercress)	10
N. palustre, D.C. (Marsh Yellow-cress)	10
Thlaspi arvense, L. (Penny cress)	20
Urtica dioica, L. (Nettle)	10
Brassica oleracea, L. var. acephela (Asparagus Kale)	10
B. rapa, L. (Turnip, var. White Milan)	20
B. rapa, L. (Turnip, var. Golden Ball)	15
Raphanus sativus, L. (Winter Radish)	10

Doubtful symptoms appeared on a few of the plants of Shepherd's-purse, Ten-week stock, Brompton stock and Nettle, but no symptoms were produced on broccoli inoculated from these with the exception of one plant inoculated from Brompton stock. This developed typical vein-clearing but efforts to repeat the infection of stock were unsuccessful and it was concluded that this one broccoli plant had become infected by accident and not from the stock. None of the other species mentioned showed any symptom of infection and broccoli inoculated from these plants

remained healthy.

Attempts to infect the following by means of the aphis vector (Brevicoryne brassicae) were unsuccessful also:- Shepherd's-purse, Bitter cress, Siberian wallflower, Erysimum, Honesty (Lunaria), Ten-week and Brompton stock, Watercress, Schizopetalon sp., Nettle, Turnip and Winter Radish.

Five plants of each species were tested except in the cases of Lunaria (3 plants) and turnip (10 plants); aphides were allowed to feed on an infected broccoli plant for a period of from 24 to 48 hours and from 3 to 5 aphides were then transferred to each test plant for 24 hours. A similar number of test plants received aphides from an uninfected broccoli plant.

(viii) Properties of the Virus.

1. Filterability.

Experiments were conducted at various dates to determine whether sap from infected leaves would retain its infectivity after passing through bacteria-proof filters. The results of these tests are shown in Table 8.

TABLE 8.   Filterability.

| Filter grade | Trial 1 18/10/39 | Trial 2 20/12/39 | Proportion of Infections [x] | | | | Total |
			3 1/2/40	4 13/8/40	5 5/10/40	6 24/5/41	
Unfiltered	1/2	-	3/5	2/5	4/5	2/5	12/22
Filter paper [ǂ]	0/8	2/5	-	0/5	0/5	0/5	2/28
P.C. [§] L I	0/8	0/5	0/8	-	-	-	0/21
" L 3	0/8	0/4	-	-	-	-	0/12
" L 5	-	0/5	-	-	-	-	0/5

[x] Denominator shows number of plants inoculated.
[ǂ] Whatman No. 3.
[§] Pasteur-Chamberland filter candle.

It will be seen that passage through filter paper reduced very considerably the infectivity of juice from infected leaves and that no infection was obtained after passage through an L I candle.   As the pores of both these filters are large enough to allow bacteria to pass, it is thought that the loss of infectivity is due to the absorption of the virus by the filters.

2. Inactivation temperature.

As noted above (p.41), 0.5ml. of the juice expressed from infected broccoli leaves was used in determination of the Inactivation Temperature of the virus.   This inoculum was maintained at the desired temperature for 10 minutes, cooled

rapidly and used immediately to inoculate young broccoli plants in the glasshouse. Such inoculations were carried out in November and December, 1940, and April, 1941, with the results shown in Table 9.

TABLE 9.   Temperature of Inactivation.

Temperature (°C.)	PROPORTION OF INFECTIONS [x]						
	Trial 1 5/11/40	2 12/11/40	3 13/11/40	4 14/12/40	5 2/4/41	6 28/4/41	Total
Unheated	4/5	3/5	2/5	3/5	2/5	3/5	17/30
60°	4/5	1/5	2/5	3/5	1/5	1/5	12/30
65°	4/5	0/5	2/5	1/5	0/5	0/5	7/30
70°	3/5	0/5	0/5	4/5	0/5	0/5	7/30
75°	1/5	0/5	0/5	1/5	0/5	0/5	2/30
80°	0/5	0/5	0/5	0/5	0/5	0/5	0/30
Uninoculated	0/5	0/5	0/5	0/5	0/5	0/5	0/30

[x] Denominator shows number of plants inoculated.

It will be seen that the expressed juice is perhaps less infective than the pulped leaf material used in the experiments previously described. Heating the juice to 65° or 70° markedly reduces the number of infections and no infections are produced by juice heated to 80° for 10 minutes. The thermal inactivation point of the virus, in the conditions

described, lies between 75° and 80°C.

3. Resistance to ageing in vitro.

As noted above (p.42), sap for ageing tests was stored in stoppered tubes in an incubator maintained at 22°C.

The results of experiments begun in October, 1940, and March and September, 1941, are shown in Table 10.

TABLE 10. · Resistance to Ageing.

Ageing period (days)	Proportion of Infections [*]					
	Trial 1 28/10/40	2 29/3/41	3 17/6/41	4 5/9/41	5 7/9/41	Total
0	9/10	5/5	2/10	1/5	1/5	17/35
1	3/10	2/5	1/10	0/5	1/5	7/35
2	6/10	0/5	0/10	2/5	1/5	9/35
3	0/10	0/5	1/10	2/5	0/5	3/35
4	3/10	0/5	0/10	1/5	0/5	4/35
5	0/10	0/5	-	1/5	0/5	1/25
6	0/10	0/5	-	0/5	0/5	0/25
7	0/10	0/5	-	1/5	-	1/20
8	0/10	0/5	-	0/5	-	0/20
10	0/10	0/5	-	0/5	-	0/20
12	0/10	0/5	-	0/5	-	0/20
14	0/10	0/5	-	0/5	-	0/20
17	0/10	-	-	0/5	-	0/10

[*] Denominator shows number of plants inoculated.

It will be seen that the longest period for which sap remained infective was 7 days.

4. Tolerance to dilution.

Expressed sap was diluted to the appropriate concentration with distilled water and used to inoculate young broccoli plants. It will be seen from the results shown in Table 11 that the leaf extract remains active after dilution to 1 in 2,000 but is inactivated by dilution to 1 in 3,000.

TABLE 11.   Tolerance to Dilution.

Dilution	Proportion of infections *										
	Trial 1 1/2/40	2 22/6	3 13/8	4 5/9	5 6/9	6 5/10	7 10/10	8 31/10	9 9/1/41	10 7/9	Total
Pulp	-	-	5/10	2/5	3/5	5/5	9/10	-	-	5/5	29/40
Undiluted sap	4/6	1/5	3/10	2/5	1/5	4/5	5/5	3/5	2/5	1/5	26/56
1:10	3/6	0/5	3/10	0/5	0/5	3/5	5/5	2/5	3/5	1/5	20/56
1:50	2/6	2/5	1/10	-	-	2/5	1/5	1/5	1/5	1/5	11/46
1:100	0/6	1/5	2/5	0/4	1/5	0/5	2/5	2/5	0/5	0/5	8/50
1:200	1/5	1/5	0/5	0/5	0/5	1/5	0/5	1/5	1/5	1/5	6/50
1:500	1/6	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	2/51
1:1,000	0/6	0/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5	2/51
1:2,000	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5	1/5	3/50
1:3,000	0/5	-	-	-	-	0/5	0/5	0/5	0/5	0/5	0/30
1:5,000	-	-	-	-	-	-	-	0/10	0/10	0/10	0/30
Distilled water	0/6	-	-	-	-	-	-	0/10	0/10	0/10	0/36
Control (uninoculated)	0/6	-	0/5	-	0/5	0/5	-	0/5	-	-	0/26
Undiluted sap‡	-	-	2/5	-	-	2/4	5/5	5/5	-	-	14/19

* Denominator shows number of plants inoculated.   ‡ One hour after extraction.

A total of 2 infections in 100 inoculated plants resulted from two dilution experiments conducted in April and July, 1941, and no infections occurred in an ageing experiment begun in July, 1941. No definite explanation can be given for these failures but it is possible that the muslin employed in the extraction of juice had not been washed sufficiently thoroughly before use and that the "filling" substance in the cloth had absorbed the virus. The reduction in the percentage of successful inoculations obtained with undiluted extracts as compared with those obtained when leaf pulp was used (e.g. 72% for pulped material and 46% for undiluted extract in the dilution experiments and a corresponding reduction in the Thermal inactivation and ageing tests, as compared with the Varietal susceptibility experiments, etc.) may also be due to partial adsorption of virus by the cloth used in extraction, or to retention of virus by the macerated leaf material.

(c)   Overwintering of the Virus.

As has been shown above (p.46), there is no evidence to suggest that infection of broccoli can be seed-borne so that the virus is presumably introduced afresh into each crop. The observations of Caldwell and Prentice (1942b) indicate that infection is brought in from the hedgerows by aphides.

Broccoli are generally sown in seed-beds in the field in March, planted out in June in the field in which the

seed-bed was situated and harvested from October to May depending on the season of maturity of the variety. As a result, the season of growth of one broccoli crop may overlap that of the following year and thus infection may be directly transmitted from crop to crop. Other brassicas, such as Savoys, Brussels sprouts, Sprouting broccoli and Spring cabbage (which have all been shown to be susceptible to infection), may also serve to carry infection over from one year to the next.

On the other hand infection is common in fields which have not been sown until a month or more after the harvesting of the brassica crop in adjacent fields. In such cases there are several possible explanations for the introduction of infection to the seed-bed. Aphides may have conveyed it from infected plants in fields and gardens situated some distance away; infection may have been brought by aphides from infected cruciferous weeds growing in the hedgerows or grass banks which commonly surround fields in Devon and Cornwall. As against the first hypothesis may be placed the fact that the percentage of infected plants in a seedbed is greatest near the hedges so that the vector apparently approaches via the nearest hedgerow. Thus if aphides bring infection from distant sources they must be considered as first migrating to the hedgerows and subsequently to the broccoli seedbed. As regards the other two hypotheses, no information is available regarding the period for which aphides remain infective when feeding on non-susceptible hosts and no weeds of perennial

or winter-annual habit have been found which are susceptible to infection.

It is not possible, therefore, to say precisely how overwintering occurs but it seems likely that there is some susceptible weed present in the hedgerows.

Methods of control of the disease are suggested by Caldwell and Prentice (1942b) including the placing of seed-beds as far as possible from hedgerows and the roguing of early infections.

III.   Necrotic-ring.

During the course of the work on Broccoli Mosaic,
specimens of diseased leaves of Brussels sprouts were received
from Mr. L. Ogilvie, Long Ashton.   These showed necrotic spots,
one to two millimetres in diameter, and minute necrotic dots
in irregular lines and groups forming an etch-line pattern on the
lower surface of the leaf.   Frequently the necrotic spots were
not "solid" but in the form of a ring or horse-shoe with living
but somewhat chlorotic tissue in the centre.

Infection was transmitted to broccoli by means
of the aphis _Brevicoryne_ _brassicae_ and by mechanical inoculation
by the carborundum method using as source of infection a Brussels
sprout leaf with necrotic spots.   The first symptoms of infection -
a mild chlorotic mottle on the youngest leaves - developed about
18 to 21 days after inoculation.   Subsequently formed leaves
showed small chlorotic spots on a background of normal colour.
These spots, which were at first about 1mm. in diameter, enlarged
and coalesced so that the leaf presented an appearance of islands
of normal green tissue on a chlorotic background, parts of which
later became necrotic.

The symptoms produced on broccoli by this virus are

thus quite distinct from those produced by the Broccoli Mosaic virus.

Brussels sprouts, cabbage, red cabbage and cauliflower all proved susceptible to infection with the necrotic ring virus by mechanical inoculation. Plants were inoculated on the first leaf when two leaves had been produced. No symptoms appeared on the first, inoculated leaf. In some cases a slight, general chlorosis developed at the base of the second leaf but in other cases there were no visible symptoms on this leaf. Chlorotic spots, which were more numerous towards the apex than at the base, developed on the third leaf and these enlarged and fused together in the manner described for broccoli. Necrotic spots appeared on these plants about two months after inoculation.

Another isolation, from a Brussels sprouts leaf with minute necrotic dots along the veins, produced, on broccoli, primary symptoms similar to those described above but no necrosis developed. This virus was at first thought to be different from the necrotic ring virus but subsequent work showed that the host range of the two isolates was similar and that the first isolate also sometimes failed to produce necrosis on broccoli. It was therefore concluded that the isolates were, at most, two strains of the same virus. The results of the host range trials are, however, shown separately in Table 12a, the first isolate (from the Brussels sprouts leaf with necrotic

spots) being distinguished as "Necrotic ring: severe" and the second isolate (from the leaf with minute necrotic dots) as Necrotic ring: mild".

TABLE 12a.   Susceptibility of Brassicae to infection by the Necrotic-ring Virus by mechanical inoculation.

| V a r i e t y | Proportion of infections | |
	Necrotic-ring: severe	Necrotic-ring:mild
Broccoli	8/13	13/22
Brussels sprouts	7/10	7/10
Cabbage	5/5	5/5
Cauliflower	4/4	-
Colewort	4/4	-
Kale (Asparagus)	0/10	0/5
" (Ormskirk)	2/5	3/4
Rape	2/5	1/5
Red Cabbage	4/5	5/5
Savoy	4/4	-
Sprouting broccoli	3/5	-
Swede turnip	4/5	2/5
Turnip	1/5	0/5

It will be seen that all the varieties of Brassicae
which were tested were susceptible to infection with the exception
of Asparagus Kale.   Infected plants of Colewort, Ormskirk Kale,
Savoy and Sprouting broccoli developed a mosaic mottle similar
to that described for Brussels sprouts and Cabbage;   on Rape,
Swede turnip and Turnip symptoms were chlorotic mottling and
distortion of the leaves, pronounced dwarfing of the plants and,
sometimes, death.

TABLE 12b.   Susceptibility of other Cruciferae to infection
by the Necrotic-ring Virus.

SPECIES	Proportion of Infections	
	Necrotic-ring:severe	Necrotic-ring:mild
Alyssum maritimum	0/5	-
Hesperis matronalis	5/5	-
Malcomia maritima	5/5	-
Matthiola bicornis	5/5	-
Raphanus sativus	0/4	0/4

Symptoms on Hesperis consisted of chlorotic spots
on the inoculated leaves;   on Malcomia, slight systemic mottling
of the leaves and severe stunting of the whole plant;   and on
Matthiola, mottling and stunting and finally the death of all the
inoculated plants.   No symptoms were produced on Alyssum or

radish.   No attempt was made to obtain proof of infection

(or of failure to cause infection) by reinoculating to Brassicae.

TABLE 12c.   Susceptibility of Nicotiana spp. and of Zinnia to
              infection by the Necrotic-ring Virus.

S P E C I E S	Proportion of infections	
	Necrotic-ring: severe	Necrotic-ring: mild
Nicotiana affinis	4/4	0/5
" glutinosa	14/14	9/14
" rustica	8/9	9/11
" sylvestris	1/2	0/2
" tabacum (White Burley)	6/8	8/10
" " (Turkish)	2/2	1/2
Zinnia elegans	3/5	0/5

On Nicotiana affinis the only symptom produced was a faint

chlorotic spotting of the inoculated leaves.

On N.glutinosa chlorotic spots appeared on the

inoculated leaves about 7 days after inoculation.   These spots

rapidly increased in size and became necrotic in the centre.

Large necrotic lesions were thus formed, dark brown or black in

colour, with tan coloured centres and margins.   Chlorotic spotting

then developed systemically on the uninoculated leaves and many

of these spots, too, became necrotic.   Finally the flowers showed

severe colour-breaking, with patches of white or cream on the

corolla.   In one experiment chlorotic and necrotic spotting

developed systemically on inoculated plants although no necrotic
lesions had appeared on the inoculated leaves.

Symptoms on N.rustica (var. Jamaicensis) were first
noted about 6 or 7 days after inoculation.   Chlorotic spots,
which were at first from 1 to 2 millimetres in diameter, spread
over the inoculated leaves until most of the lamina was chlorotic
and tiny, shining, sunken, necrotic spots appeared in these
chlorotic areas.   Systemic chlorotic spotting developed on the
other leaves about 20 days after inoculation.

Only one plant of N.sylvestris showed a reaction
to inoculation.   Small, local necrotic spots were produced on the
inoculated leaf.

On White Burley tobacco, chlorotic spots, about
1 millimetre in diameter, appeared on the inoculated leaves about
a week after inoculation.   The spots enlarged slightly and became
necrotic, having a light tan central spot about 0.25mm. in diameter
surrounded by a dark necrotic ring whose external diameter was 1
to $1\frac{1}{2}$ mm.

Symptoms on Turkish tobacco / were similar.

The virus was recovered from N.rustica by inoculation
to broccoli (Sutton's Roscoff No.2), but no attempt was made to
reisolate from other hosts.

A quantity of seed was obtained from plants in a
field of Brussels sprouts many of which were infected with the
necrotic-ring virus.   About two hundred seedlings were raised

from this seed and none developed symptoms of infection.   There

is thus no evidence that the virus is seed-borne.

In addition to the Necrotic-ring virus, a virus

producing vein-clearing on broccoli and believed to be the

Broccoli Mosaic virus was isolated from several naturally infected

Brussels sprouts plants showing necrotic spots, but the effect

of simultaneous infection with the viruses of Broccoli Mosaic and

Necrotic-ring was not studied.

IV. Discussion of Literature.

As already noted (Section I, p.20), some confusion exists concerning the number and identity of Crucifer viruses. The object of this discussion of the literature is to identity the Broccoli Mosaic and Necrotic-ring viruses with viruses already described and to collate data for the formulation of a system of identification of Crucifer viruses.

It may be said at the outset that as a result of the following examination of the literature and comparison of the Broccoli Mosaic and Necrotic-ring viruses with viruses described therein, it is concluded that the Broccoli Mosaic virus is synonymous with the Cauliflower Mosaic virus of Tompkins (1937) and that the Necrotic- ring virus is probably closely related to the Black ring virus of Tompkins, Gardner and Thomas (1938).

(a). Early Work (1921-1936)

Owing to the limited scope of the early papers on virus diseases of the Cruciferae it is impossible to identify the causal agents with the viruses of the present work; indeed as these early papers deal mainly with symptomatology, it is difficult to relate the viruses involved with any of the viruses which have

been fully described in recent years. They are, however, of
considerable historic interest and, for the sake of completeness,
they will be briefly mentioned.

The earliest published accounts of virus diseases
of the Cruciferae appear to be those of Gardner and Kendrick (1921)
and of Schultz (1921) who, almost simultaneously, described a
mosaic disease (or diseases) of turnip occurring in the U.S.A.
Gardner and Kendrick were able to transmit infection to healthy
turnips by mechanical inoculation while Schultz transmitted
infection by inoculation and also by means of the aphis Myzus
persicae to a number of Crucifers, including turnip.

It may well be that these workers were describing
the same disease (as was, in fact, assumed by them), but as several
viruses are now known to infect turnips, and as it is difficult
to distinguish these on purely symptomatological grounds, this
must apparently remain a matter of conjecture.

The first European record is a report by Gram (1925)
of a mosaic disease of Crucifers occurring in Denmark and trans-
missible by sap inoculation to turnip and a number of other Crucifers

Clayton (1930) reports an investigation of a mosaic
of Crucifers which he evidently believes to be that described by
the writers already mentioned. He extends the host range to
include additional species and varieties of Brassica and shows that
Brevicoryne brassicae (the cabbage aphis) is a vector. Dana and
McWhorter (1932) report a mosaic disease of horseradish trans-

trans-
missible by inoculation to turnip, etc.

In 1935 Hoggan and Johnson described a virus of
Crucifers more completely than had been attempted by earlier
workers. They showed the virus to be transmissible by sap
inoculation and by the aphid vectors Brevicoryne brassicae and
Myzus persicae and that it infects Nicotiana tabacum and N.glutinosa
in addition to Cruciferous plants. They determined the
inactivation temperature of the virus as 53 to 54° C., the tolerance
to dilution as 1:10,000 to 1:100,000 and the resistance to ageing
as 48 to 72 hours.

Apart from the above descriptive papers, records
of the occurrence of virus diseases on a number of crucifers in
various countries have been published.

Kaufmann (1936) reports a mosaic disease of rape
occurring in Germany. Mosaic diseases of Chinese cabbage have
been recorded from Japan, (Fukushi, 1932); the Philippines
(Ocfemia, 1924; Fajardo, 1934); Hawaii (Kunkel 1924); and the
U.S.A. (Weber 1932). Mosaic of turnip and swede has been recorded
in Australia (Samuel, 1931); New Zealand (Chamberlain, 1936);
South Africa (Van der Byl, 1931); U.S.A. (Weber 1932); and Germany
(Pape, 1935). A mosaic of cauliflower has been reported from
New South Wales (Noble et.al., 1934) and a mosaic of cabbage from
U.S.A. (Blank, 1935).

In general, these references are mere mentions of
the fact that such diseases have been observed, but they serve to

show that virus diseases of the <u>Cruciferae</u> are geographically

widely distributed

(b). <u>British Records.</u>

The literature under this heading, though scant,

is confused, and will be considered in some detail.

The first records of virus diseases of crucifers

in Britain appear to be by Ogilvie, Mulligan and Brian (1935)

and by Smith (1935a). The former, in a brief note, record the

occurrence of a mosaic disease of cabbage and other Brassicae in

the Bristol area, characterised by marked clearing and necrosis

of the leaf veins and dwarfing of the plants. Later, Ogilvie

and Hickman (1937) state that the disease is caused by the "Ringspot"

virus of Smith (1935a).

Smith (1935a) describes a disease of cabbage,

Brussels sprouts and other brassicas observed in the neighbourhood

of Cambridge. Symptoms on cabbage and Brussels sprouts "were of

a ringspot nature", the older leaves in particular being covered

with necrotic rings. The virus, which was transmissible by sap

inoculation, produced local necrotic lesions on cabbage, <u>Nicotiana</u>

<u>glutinosa</u>, <u>N.tabacum</u> and <u>N.langsdorfii</u> and later became systemic in

cabbage and <u>N.glutinosa</u>. Smith (1935b) in a later publication

states that the virus produced flower "breaking" in stock and

wallflower and mottling, distortion and vein-banding of broccoli.

In a third paper (Smith, 1936) he adds <u>Arabis</u> and <u>Hesperis</u> to the

list of susceptible plants. Describing symptoms on broccoli
and cauliflower he states, "On older plants there are two main
types of symptom, one is the presence of numbers of small black
rings on the older leaves and the other symptom is the development
of broad green bands following the midrib and larger veins." (loc.
cit. p.135).

Smith (1935a) contrasts the disease caused by the
Ringspot virus with the "Mosaic of crucifers" which he states
to be common on various brassicas in the Cambridge district.
The "mosaic" virus was transmissible by sap inoculation to brassicas
in which it produced vein-clearing followed by mottling and ros-
etting of the leaves, but it was not transmissible to N.glutinosa.
The ringspot virus of Smith (1935a) shows resemblances to the
Necrotic-ring virus, and the "Mosaic of crucifers" may be
synonymous with Broccoli Mosaic.

Ainsworth (1935) reports a mosaic of wallflowers
similar to that described by Smith. The virus, however, produced
local, non-systemic infection of N.glutinosa.

In the same note, Ainsworth (1935) records a mosaic
of watercress caused by the Cucumber Mosaic virus, while Salaman
and Wortley (1939) have stated that various crucifers, including
turnip and cabbage, are susceptible to infection with the Potato
Leaf-roll virus and with Potato virus "Y"; other workers, however,
have been unable to confirm the latter (Bawden and Sheffield, 1944).
or the former (Helson & Norris, 1943).
Ogilvie (1941, p.11) records the occurrence of mosaic on broccoli

and cauliflower and of virus ringspot on cabbage and presents data
from the work of Smith and of Tompkins and from the present
investigation.

Some of the data of the present investigation have
already been published (Caldwell and Prentice, 1942a) and
observations and experiments on the effect of Broccoli Mosaic
in the field have also been recorded (Caldwell and Prentice, 1942b).

(c)   Inter-relationships of the Viruses mentioned in (a)
       and (b) and their relationship to the Broccoli Mosaic
       and Necrotic-ring viruses.

It seems probable that the Turnip Mosaics of Gardner
and Kendrick, Schultz and Clayton are synonymous with Hoggan and
Johnson's.   It may be that all these viruses are identical.
The relation of Gram's virus is not clear as it is the only one
of this group which infects radish.

The Ringspot virus of Smith (with which Ogilvie's
Mosaic is probably identical) differs from Hoggan and Johnson's
virus and from Ainsworth's Wallflower Mosaic virus in producing
systemic infection of N.glutinosa.   The latter two viruses may be
identical.

As has been stated, it is impossible to identify
either Broccoli Mosaic or Necrotic-ring with any of the above
diseases.   The Broccoli Mosaic virus resembles Smith's Ringspot
virus in that it produces vein-banding on broccoli and cauliflower,
but differs from it in almost all other properties.   For example,

Broccoli Mosaic does not infect <u>Nicotiana</u> species or Wallflower, nor does it produce necrotic rings on cabbage.

The Broccoli Mosaic virus resembles Smith's Mosaic of Crucifers in causing vein-clearing of brassicas and in failing to infect <u>N</u>.glutinosa, but, as Smith gives no further details of the Mosaic virus, it is obviously impossible to establish its identity with that of Broccoli Mosaic.

Necrotic-ring shows greater similarities to Smith's Ringspot than to any of the other diseases so far considered. It resembles Ringspot in causing mottling and necrosis on cabbage, local necrotic lesions on <u>Nicotiana tabacum</u> and systemic infection of N.glutinosa.   It differs from Smith's Ringspot virus, however, in that it does not produce local necrosis on the inoculated leaves of cabbage (cf. Smith 1935a, p.240) or vein-banding on cauliflower and broccoli (cf. Smith 1935b, p.112; 1936, p.135).

It may be that the disease described by Smith (and by Ogilvie <u>et.al</u>.) is due to a simultaneous infection with a virus of the Brocooli Mosaic type (causing vein-clearing and vein-banding on brassicas) and one of the Necrotic-ring type (causing necrotic rings on cabbage and infecting <u>Nicotiana</u>). This would explain the appearance of vein-clearing (Ogilvie, Mulligan and Brian, 1935) or vein-banding (Smith 1935b, 1936) in association with necrotic spotting.

The occurrence of Cucumber Mosaic virus (Ainsworth, 1935) and of potato viruses (Salaman and Wortley, 1939) in cruciferous hosts is evidently unrelated to the diseases already

considered.   Other unrelated records, from the U.S.A., are Southern Celery Mosaic (Cucumber Mosaic) (Wellman, 1935), Sugar-beet Curly-top (Severin 1927, 1929), Aster Yellows (Kunkel 1926, 1931) and Potato Yellow-dwarf (Hansing, 1942) on a number of crucifers.

(d).   Recent Literature.

Since 1935 a number of more detailed papers on crucifer viruses have been published.

From the U.S.A. Tompkins and his co-workers have described virus agents causing diseases of cauliflower (Tompkins, 1937), Chinese cabbage (Tompkins and Thomas, 1938), turnip (Tompkins, 1938), cabbage (Tompkins, Gardner and Thomas, 1938), annual stock (Tompkins, 1939a) and radish (Tompkins, 1939b). Larson and Walker (1939, 1941) describe a mosaic disease and a Ring-necrosis of cabbage.

From New Zealand, Chamberlain (1936, 1939) describes a mosaic disease of turnip.

From China, Ling and Yang (1940) report a virus disease of rape.

From Germany, Moericke and Winter (1940) report a virus disease of cauliflower.

From Southern Rhodesia, Hopkins and Pardy (1942) describe a mosaic disease of cabbage and a dwarfing disease of cauliflower.

Of the viruses investigated by Tompkins and his
co-workers, all, with the exception of the two viruses of stock
(Tompkins, 1939a), infect cauliflower and all except the Cauliflower
Mosaic virus (Tompkins 1937) cause necrotic lesions on <u>Nicotiana</u>
<u>tabacum</u>.

Both of the viruses of Larson and Walker infect
cauliflower, the Cabbage Mosaic virus (Larson and Walker, 1939)
producing vein-banding on this host, and both produce local necrosis
on <u>N.tabacum</u>. Hopkins and Pardy (1942) give few details of their
Cabbage Mosaic but believe it to be identical with the Cabbage
Mosaic of Larson and Walker; they are unable to identify their
Dwarfing of Cauliflower with any other previously described
disease and consider that it may be caused by a mixture of viruses.

Chamberlain's Turnip Mosaic virus (Chamberlain, 1936,
1939) has many properties in common with the Black-ring virus of
Tompkins (1938) including the ability to infect cauliflower and
to produce local lesions on <u>N.tabacum</u> but is considered by
Chamberlain to be distinct from Tompkins' virus.

Ling and Yang's Rape Mosaic virus (Ling and Yang,
1940) does not infect cauliflower and information on its reaction
to <u>N.tabacum</u> is not available.

Moericke and Winter's Cauliflower virosis (Moericke
and Winter, 1940) produces vein-banding on cauliflower but, again,
these workers do not give the reaction of their virus to <u>N.tabacum</u>.

V.   Identities of the Viruses.

(a).   Broccoli Mosaic Virus.

Of the viruses for which more or less complete
data have been published only Tompkins' Cauliflower Mosaic virus
and Moericke and Winter's Cauliflower Virosis virus resemble
the Broccoli Mosaic virus in producing vein-banding on cauliflower.
Larson and Walker's Cauliflower Mosaic virus differs from
Tompkins' Cauliflower Mosaic virus and from the Broccoli Mosaic
virus in that it infects N.tabacum.   (Moericke and Winter have
not given information as to the reaction of their virus on
N.tabacum.).   All the other viruses mentioned in Section IV (d)
differ both in the symptoms produced on cauliflower and in the
reaction of N.tabacum.

Moericke and Winter's description of the Cauliflower
virosis is less complete than that given by Tompkins for
Cauliflower Mosaic and it is thus difficult to identify it
exactly.   The disease was seen by them in fields and gardens
in the Rhine valley between Cologne and Bonn and in some cases
as much as 90% of the cauliflower crop was infected.   Brussels
sprouts and Swede turnip were also found to be naturally infected -
the effect on, Swede turnip being severe.   The disease was

transmitted by mechanical inoculation to cauliflower, cabbage
and Brussels sprouts. Vein-clearing symptoms appeared on
cauliflower in 18 to 20 days and symptoms on other species were
"similar to those described by Tompkins" (for Cauliflower Mosaic).

They state, however, that the severity of the
injury caused to Brussels sprouts and red cabbage was noteworthy
("Bemerkenswert war die starke Schädingung von Rosenkohl und
Rotkohl") and included a marked reduction of the area of the
leaf lamina as compared with uninoculated controls; stems and
petioles developed normally. On these hosts leaf distortion
occurred too, but on white cabbage no marked symptoms were
produced apart from "mosaic spots" ("Mosaikflecken").

Thus Moericke and Winter's Cauliflower virosis
produces vein-clearing followed by vein-banding on cauliflower -
the vein-banding shown in their illustrations being, to all
appearances, identical with that produced by the Broccoli Mosaic
virus and with that illustrated by Tompkins for Cauliflower
Mosaic (with which Moericke and Winter consider their virosis
to be identical). Nevertheless, the symptoms described on
Brussels sprouts, red cabbage and white cabbage seem to differ
from those produced by the Broccoli Mosaic virus. The latter
produces only vein-clearing (sometimes followed by faint vein-
banding) on Brussels sprouts and cabbage. Very often these
symptoms become almost completely masked as the plants grow older
and in no case has any marked reduction in leaf area been noted.

Neither can the symptoms on cabbage be accurately described as mosaic "spots". (See above, p.51 for symptoms of Broccoli Mosaic on these hosts). Similarly Tompkins (1937) gives "vein-clearing" as the symptom produced by the Cauliflower Mosaic virus on these hosts, and makes no mention of reduction of laminal area, leaf distortion or mosaic spots.

It is, however, possible that the differences of reaction of Broccoli Mosaic, Cauliflower Mosaic and Moericke and Winter's Cauliflower Virosis are due to environmental causes and it is, therefore, unfortunate that Moericke and Winter have not given an extended host range or details of the physical properties of their virus.

It is possible that Moericke and Winter's virosis is related to the Cabbage Mosaic of Larson and Walker. The virus of the latter disease also causes vein-clearing and vein-banding on cauliflower although the vein-banding appears from Larson and Walker's illustrations to be of a slightly different type from that illustrated by Moericke and Winter. Knowledge of the reaction of Nicotiana species to Moericke and Winter's virus would assist in clearing up this point.

Some doubt has been cast on the validity of Larson and Walker's Cabbage Mosaic Virus by a note published by Walker et al. (1941). This states that Cabbage Mosaic (presumably the cabbage mosaic of Larson and Walker) is caused by simultaneous

infection by two viruses, one of which causes a mosaic mottle on cabbage and infects Nicotiana, etc., the other causes vein-clearing on cabbage and does not infect Nicotiana.

The similarity between the Cauliflower Mosaic virus of Tompkins and the Broccoli Mosaic virus is almost complete. Both viruses infect the following species and produce similar symptoms of infection:- Brassica oleracea: (Brussels sprouts, sprouting broccoli, white, red and Savoy cabbage, cauliflower, Kale and Kohlrabi); B.arvensis (Charlock); B.campestris (Swede turnip); B.napus (rape); Raphanus sativus (radish). The following are not susceptible to infection by either virus:- Alyssum saxatile; A.maritimum; Cheiranthus cheiri; Erysimum perofskianum; Hesperis matronalis; Malcomia maritima; Nasturtium officinale; Lycopersicum esculentem; Nicotiana glutinosa; N.tabacum. The following are susceptible to Cauliflower Mosaic but not to Broccoli Mosaic:- B.rapa (turnip); Capsella bursa-pastoris; Iberis amara; Matthiola incana.

The failure to obtain infection of turnip with the Broccoli Mosaic virus is difficult to explain, in that turnips apparently infected with this virus have been seen at various places in the South of England. Possibly further attempts, using an aphid vector as the means of transmission, would result in infection being transmitted to turnip.

Tompkins gives the incubation period before symptoms

appear on stock as 65 to 70 days as against 14 to 20 days for many
varieties of cauliflower, cabbage, kale, etc., and this may
perhaps explain the apparent failure to infect this species with
Broccoli Mosaic. An extended incubation period in conjunction
with possible symptom masking and the difficulty of reisolating
from senescent plants would render difficult the positive
identification of infection.

Whereas Tompkins obtains 100% of successful
transmissions with most host plants he obtains only 30% with
candytuft (Iberis amara). The failure of the writer to obtain
infection with Broccoli Mosaic may perhaps be ascribed to the
apparent resistance of this host.

No valid explanation can be offered of the failure
to transmit the Broccoli Mosaic virus to radish by mechanical
inoculation, since trials with aphides showed this species to be
susceptible. Perhaps a larger number of trials would have
resulted in success.

A comparison of the physical properties of
Tompkins' Cauliflower Mosaic virus and the Broccoli Mosaic virus
is given in Table 14.

TABLE 14. Comparison of Physical Properties.

(a). Resistance to Ageing at 22°C. in vitro.

Ageing Period (days)	Percentage of infections Cauliflower Mosaic ǂ	Broccoli Mosaic	Ageing Period (days)	Percentage of infections Cauliflower Mosaic ǂ	Broccoli Mosaic
0	100	48	9	100	-
1	100	20	10	80	0
2	100	26	11	64	-
3	100	9	12	60	0
4	100	13	13	24	-
5	100	4	14	12	0
6	100	0	15	0	-
7	100	5	16	0	-
8	100	0	17	-	0

(b) Inactivation Temperature (10 minutes)

Temperature (°C.)	Percentage of infections Cauliflower Mosaic ǂ	Broccoli Mosaic	Temperature (°C.)	Percentage of infections Cauliflower Mosaic ǂ	Broccoli Mosaic
Unheated	100	57	65	100	23
50	100	-	70	100	23
55	100	-	75	0	7
60	100	40	80	-	0

(c) Tolerance to Dilution

Dilution	Percentage of infections Cauliflower Mosaic ǂ	Broccoli Mosaic	Dilution	Percentage of infections Cauliflower Mosaic ǂ	Broccoli Mosaic
Undiluted	100	72	1:500	100	4
1:10	100	46	1:1,000	56	4
1:50	100	24	1:2,000	32	6
1:100	100	16	1:3,000	0	0
1:200	100	12	1:4,000	0	0[x]

[x] Dilution = 1:5,000   ǂ Figures calculated from Tompkins (1937).

It will be seen that the resistance to ageing of the Broccoli
Mosaic virus is less than that of the Cauliflower Mosaic virus,
that its inactivation temperature is slightly higher and that its
tolerance to dilution is similar.   Tompkins' figures, however,
show that the Cauliflower Mosaic virus is less affected by storing
for short periods, by heating at low temperatures and by moderate
dilution than is the Broccoli Mosaic virus.

It is concluded, as a result of the comparisons
made above, that the similarities in host range, symptomatology
and properties of the two viruses justify the view that the
Cauliflower Mosaic virus of Tompkins and the Broccoli Mosaic virus
of this investigation are the same.   The slight differences noted
between them could be explained on the assumption that they are two
different, but closely allied, strains of the same virus.

(b)   Necrotic-ring Virus.

The Necrotic-ring virus (described in Section III,
p.69) has been less fully studied than the Broccoli Mosaic virus,
and, owing to the incompleteness of the data, it is difficult to
identify it with certainty.   It is, however, probably related to
Tompkins' Black ring virus.

The salient points in the symptomatology of the
Necrotic-ring virus are the production of (1) a mosaic mottle and
necrotic rings on cabbage, Brussels sprouts, etc.,(2) local necrotic
lesions on tobacco and (3) local necrosis followed by systemic

chlorotic mottling and necrosis on Nicotiana glutinosa.

The virus thus differs from Tompkins' Stock Mosaic viruses and Ling and Yang's Rape Mosaic virus as these do not infect cabbage; from Tompkins' Turnip Mosaic which does not infect Brussels sprouts; Tompkins' Chinese Cabbage Mosaic virus and Tompkins' Radish Mosaic virus which do not produce necrosis on cabbage nor systemic infection of N.glutinosa; and from Tompkins' Cauliflower Mosaic and the Broccoli Mosaic already described, as these do not infect Nicotiana species.

Thus, only Larson and Walker's Cabbage Mosaic and Ring necrosis, Chamberlain's Turnip Mosaic and Tompkins' Black ring viruses remain to be considered.

Larson and Walker's Cabbage Mosaic resembles the Necrotic ring virus in many respects but causes vein-banding on cauliflower, produces no local symptoms on N.rustica and infects radish. As already indicated, however, this mosaic is probably caused by a complex of two viruses.

The host range and symptomatology of Larson and Walker's Ring necrosis resemble those of Tompkins' Black ring virus (and the Necrotic-ring virus) in general, but the Ring necrosis virus infects radish and its physical properties are different from those of the Black-ring virus.

Chamberlain's Turnip Mosaic virus resembles Tompkins' Black ring virus in host range and properties but does not produce

necrotic rings on cabbage.

Tompkins' Black ring virus seems to be identical with the Necrotic ring virus in all respects for which comparable data are available except that, while the Black ring virus does not infect N.rustica var. humulis, the Necrotic ring virus causes systemic infection of N.rustica var. Jamaicensis.

VI. Classification of Viruses of the Cruciferae.

Arising from the discussion of literature in the previous sections an attempt at classification of the viruses affecting plants of the family <u>Cruciferae</u> may be made. A primary classification into two groups might be made on the basis of the relations of the viruses to <u>Nicotiana</u> <u>tabacum</u>, viz. (1) those viruses infecting <u>N.tabacum</u> and (2) those not infecting <u>N.tabacum</u>.

A number of the viruses in the first class have many features in common, but they may be further subdivided into groups according to the host range of the viruses, their reaction on <u>N.glutinosa</u>, etc.

The validity of the criteria which have been used by various workers to differentiate these viruses is, however, open to question.

Such criteria as production or non-production of necrosis on cabbage are probably untrustworthy since, as has been pointed out above (Section A, p.4), the type of symptom produced by virus infection is affected by environmental conditions and in particular by temperature. Similarly it is doubtful whether a distinction can be made between viruses merely on the grounds that one produces erratic infection (i.e. local lesions followed by

systemic infection) while the other is directly systemic. For example, the Necrotic ring virus can produce either erratic or systemic infection of <u>N.glutinosa</u> (section III. p.73 above) and Smith seems to have obtained similar results with the Ringspot virus which he reports (Smith, 1935) as producing erratic infection and also (Smith, 1937, p.7) as producing directly systemic mosaic mottling on this species without initial local necrosis.   Similar phenomena have, of course, been observed with other viruses, e.g. the Tobacco Mosaic virus usually produces directly systemic infection of <u>N.tabacum</u> but under conditions of high temperature and high light intensity may first produce local chlorotic lesions (Smith, 1937, p.239).   This virus, too, (as mentioned above, Section A, p.4) can cause either local or systemic infection of <u>N.glutinosa</u> depending on environmental conditions.

It is also difficult to see how much reliance should be placed on minor discrepancies of host range for distinguishing brassica viruses, particularly when different varieties of the same species sometimes react differently. For example, Tompkins' Cauliflower Mosaic virus is found to infect four varieties of <u>Brassica juncea</u> but a fifth variety of <u>B.juncea</u>, from Japan, is not susceptible (Tompkins, 1937). Tompkins' Turnip Mosaic infects cabbage (<u>B.oleracea</u> var.<u>capitata</u>) but not Brussels sprouts (<u>B.oleracea</u> var. <u>gemmifera</u>);   Chinese radish (<u>Raphanus sativus</u> var. <u>longipinnatus</u>) but not radish

(<u>Raphamus</u> <u>sativus</u>). The Necrotic ring virus, appears to infect
some varieties of kale but not others (Section III, p.71) and
similar differences in varietal reaction probably occur in other
species in which they have not yet been noted.

Thus if viruses are to be distinguished on such
relatively minor differences as chlorotic spots becoming or not
becoming necrotic, by infection being erratic or truly systemic
or by minor differences of host range such as ability or inability
to infect radish, then it is necessary that standard environmental
conditions and standard varieties of test plants shall be used
by workers in this field. It is unlikely that this can ever be
achieved owing to the difficulty of exactly duplicating in England,
for example, the environmental conditions of California or New
Zealand and the difficulty of distinguishing a strain of <u>B.juncea</u>
susceptible to the Cauliflower Mosaic virus from a non-susceptible
strain (except by inoculation with the virus). The position is
further complicated by the fact that minor differences in
symptomatology and host range may be due merely to the existence
of strains of a type virus.

It is doubtful, too, whether slight differences in
physical characteristics of virus extracts justify their being
considered as separate entities since, as has been pointed out
above (Section A, p.6), the values obtained for resistance to
dilution, heating and ageing depend to some extent on experimental

conditions. Other sources of experimental error such as the adsorption of virus by coagulated plant proteins in heating tests, decomposition of components of the extract by bacterial action during storage, etc., are also likely to affect the results of such determinations.

Considerable differences in observed physical properties, however, probably indicate distinct viruses since such differences would not result from varietal differences between test plants, differences in the environment in which they are grown, or mere differences of virus "strain".

The thermal inactivation points, resistance to ageing and resistance to dilution of the viruses for which these data have been published are shown in Table 15.

TABLE 15.   Physical Characteristics of Crucifer Viruses.

Virus	Author	Inactivation Temperature	Resistance to ageing	Resistance to Dilution
Ring necrosis	Larson & Walker	$50^{\circ}C.$	48 hrs.	1:600
Turnip Mosaic	Hoggan & Johnston	54	72	1:100,000
Cabbage Mosaic	Larson & Walker	55	72	1:2,000
Black ring	Tompkins	57	48	1:1,000
Turnip Mosaic	Chamberlain	60	72	1:1,000
Stock Mosaic(mild)	Tompkins	60	144	1:5,000
" " (severe)	"	60	192	1:4,000
Turnip Mosaic	"	63	48	1:4,000
Radish Mosaic	"	68	384	1:15,000
Chinese Cabbage Mosaic	"	75	96	1:6,000
Cauliflower Mosaic	"	75	360	1:2,000
Broccoli Mosaic	Caldwell & Prentice	80	168	1:2,000

It would appear from these figures that the Black ring virus of Tompkins and the Turnip Mosaic virus of Chamberlain are either identical or are strains of the same virus; the similarity of their host ranges and symptoms supports this view. Similarly Tompkins' two Stock Mosaic viruses are probably strains of the same virus and the Cauliflower Mosaic virus of Tompkins and the Broccoli Mosaic virus appear to be either identical or strains of the same virus. Hoggan and Johnson used N.tabacum as a test plant so that their figures are not directly comparable with those of the other workers, all of whom have used Brassicae for this purpose. The differences between Tompkins' Turnip Mosaic and Black ring viruses are not great and these may prove to be related strains. As has been stated, the physical characteristics obtained by using crude sap preparations depend to some extent on the conditions of the experiment but the other viruses listed above seem to differ sufficiently, one from another, to warrant their acceptance as distinct viruses until serological and other studies indicate their inter-relationships, if any.

A classification and key to the identification of the viruses which have been found to infect crucifers may therefore be attempted according to the following scheme-:-
(Viruses which are synonymous in the opinion of the present writer are indicated by the equivalence sign ($\equiv$), while probable synonyms are bracketed.)

I. Infecting <u>N.tabacum</u>.

    (a)   Systemic infection of N.tabacum

            (1) Infecting cabbage or cauliflower:- Cucumber Mosaic virus, Sugar beet Curly top virus, etc.

            (2) Not infecting cabbage or cauliflower:- None recorded.

    (b)   Local (usually necrotic) lesions on <u>N.tabacum</u>.

            (1) Infecting cabbage or cauliflower:- Black ring virus of Tompkins
          = Ringspot virus of Smith.
          = Turnip Mosaic virus of Chamberlain.
          = Necrotic ring virus.
          (= Turnip Mosaic virus of Tompkins)
          (= Turnip Mosaic virus of Hoggan and Johnson)
          Ring necrosis virus of Larson and Walker.
          Cabbage Mosaic virus of Larson and Walker (in part).
          Radish Mosaic virus of Tompkins.
          Chinese Cabbage Mosaic virus of Tompkins.

            (2) Not infecting cabbage or cauliflower:-
          Stock Mosaic viruses of Tompkins.

II. Not infecting <u>N.tabacum</u>.

            (1) Infecting cabbage or cauliflower:-
          Cauliflower Mosaic virus of Tompkins.
          = Broccoli Mosaic virus
          (= Cabbage Mosaic virus of Larson and Walker
          (in part).

            (2) Not infecting cabbage or cauliflower:-
          None recorded.

III. Reaction of <u>N.tabacum</u> unknown.

            (1) Infecting cabbage or cauliflower:-
          Cauliflower virosis virus of Moericke & Winter.

            (2) Not infecting cabbage or cauliflower:-
          Rape Mosaic virus of Ling and Yang.

Viruses of Group III. will be transferred to Group I. or Group II. as the reaction of <u>N.tabacum</u> becomes known.

Final identification of viruses in the different groups can be achieved by consideration of their physical properties, etc.

VII. Summary of Section B (Virus Diseases of Broccoli and Brussels Sprouts).

1. The present confusion regarding the number and identity of viruses infecting plants of the family Cruciferae is indicated.

2. Broccoli Mosaic.

(a). The symptoms of a mosaic disease of broccoli in England are described, the latter consisting of vein-clearing followed by vein-banding and occasionally by necrotic spotting.

(b). The disease was transmitted by grafting, by sap inoculation and by aphides and, in view of its infectious nature and the absence of any visible pathogen was concluded to be of virus origin.

(c). Aphids (Brevicoryne brassicae) were found to become infective after feeding on an infected plant for ten minutes and infective aphids transmitted infection during a feeding period of twenty minutes. The alate form of B.brassicae was also shown to be a vector.

(d). No evidence of seed transmission of the virus could be obtained.

(e). Twenty horticultural varieties of broccoli and seven of cauliflower were tested and all were found to be highly

susceptible to infection by sap inoculation.

(f). Fourteen other plants (twelve varieties of Brassica and two other crucifers) were found to be susceptible to infection. In each case the first symptom of infection was clearing of the leaf veins.

(g). Masking of symptoms was found to be common.

(h). Unsuccessful attempts were made to infect twenty-seven other species (including twenty-one Cruciferae and five Solanaceae).

(i). It was found that sap from infected plants lost its infectivity during passage through porcelain filter candles of all grades, that it remained infective after heating for ten minutes at 75°C. but not at 80°C., that it was infective after storing at 22°c. for seven days but not after eight days and that it was infective after dilution with distilled water to a concentration of 1:2,000 but not after dilution to 1:3,000.

(j). The methods by which infection may be carried over from one season to the next are discussed, and it is considered that some susceptible cruciferous weed is involved.

3. Necrotic-ring Virus.

(a). Another virus disease of Cruciferae, causing necrotic rings on Brussels sprouts, is described.

(b). A list is given of twenty-one plants found to be susceptible (including fifteen Cruciferae and five species

of <u>Nicotiana</u>). Radish was found to be non-susceptible.

4. The literature dealing with virus diseases of the <u>Cruciferae</u> is summarised and discussed.

(a). It is concluded that the Broccoli Mosaic virus is identical with the Cauliflower Mosaic virus of Tompkins or that they are strains of the same virus.

(b). The data concerning the Necrotic-ring virus are not considered sufficient for accurate identification but it is thought to be closely allied to the Black ring virus of Tompkins.

5. After a consideration of the criteria for the identification of viruses a scheme for the classification of the viruses infecting <u>Cruciferae</u> is suggested, based on the reaction of <u>Nicotiana</u> <u>tabacum</u> and <u>Brassica</u> <u>oleracea</u> to infection.

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C. VIRUS DISEASES OF STRAWBERRY WITH SPECIAL REFERENCE

TO THE ANALYSIS OF VIRUS COMPLEXES.

I. Introduction.

(a). General.

A number of virus diseases of the strawberry have been described in the literature including Xanthosis (Horne, 1922; Plakidas, 1925, 1926), Witches' broom (Zeller, 1927), Dwarf (Plakidas, 1928), Crinkle (Zeller & Vaughan, 1932), Yellow-edge (Harris, 1933), Stunt (Zeller & Weaver, 1941) and Leaf roll (Berkeley and Plakidas, 1942).

Of these, only Crinkle (Ogilvie, Swarbrick and Thompson, 1934) and Yellow-edge (which closely resembles, and may be identical with Xanthosis) have been reported in Britain. Yellow-edge and Crinkle have also been reported from Holland (Banga, 1931), Canada (Conners, 1934), New Zealand (Chamberlain, 1934), France (Marcel, 1936), Tasmania (Raphael, 1937), Australia (Pugsley, 1938; Blackford, 1939) and Southern Rhodesia (Hopkins, 1939).

A considerable range of reaction to Yellow-edge is shown by different varieties and species. Some, such as Royal Sovereign and certain strains of _Fragaria virginiana_ show marked symptoms, others (for example Huxley's Giant and _Fragaria chiloensis_) are more or less perfect "carriers" (i.e. do not show visible

reaction to infection) and others such as Tardive de Leopold give
an intermediate reaction (Rogers, King & Massee, 1939).   There
appears also to be a seasonal fluctuation in symptom expression
in each variety and this has been studied by King and Harris (1942)
for the variety Royal Sovereign.   They find that symptoms are
most marked following on conditions of abundant soil moisture with
air temperatures of over $60^{o}F$. and that they regress rapidly in
conditions of drought or cold.   Conditions favourable to symptom
expression are generally met with in the South of England during
September and October and sometimes also in May and June.

Quite apart from varietal differences and seasonal
fluctuations in symptom expression infected plants exhibit a wide
range of symptom intensity and some show more severe symptoms than
others of the same variety (or even of the same clone) growing
under similar environmental conditions.   One possible explanation
of this phenomenon would be the existence of two or more strains
of the Yellow-edge virus, differing in virulence (as has been
found, for example, in the case of Potato virus 'X', Tobacco mosaic
virus, etc.).   King and Harris (1942), however, find that mild
types of Yellow-edge appear to give no protection against subsequent
infection by the virus (or viruses) causing severe Yellow-edge
symptoms (as might be expected if the different degrees of severity
of symptoms were due to infection with different strains).

Crinkle was first described by Zeller and Vaughan
(1932) and Zeller (1933).   Zeller (1933) stated, "Since some

plants show more severe symptoms than others under like environmental conditions we are lead to believe that there may be separable components of the virus, one producing more severe symptoms and another what might be termed a "mild crinkle"."

In its mildest form Crinkle produces on Royal Sovereign strawberry plants an inconspicuous chlorotic spotting of the leaves, most easily seen if they are viewed by transmitted light. In more severe cases the leaves become somewhat distorted and crinkled, the severity of the distortion depending on the number and size of the chlorotic spots. In yet severer cases, the size of the chlorotic spots is considerably increased and their centres become necrotic and of a reddish or purplish colour.

Harris and King (1942) found that Fragaria vesca, the woodland strawberry, is a sensitive indicator of mild crinkle. Royal Sovereign plants showing only the faintest Crinkle symptoms induce, when grafted to F.vesca, clearly defined symptoms on this species.

Massee has shown that, in England, the strawberry aphid, Capitophorus fragariae, Theob. (= Pentatrichopus fragariae, Theob. see Thomas and Jacob, 1940) is a vector of Yellow-edge (Massee 1935, 1936) and of Crinkle (Massee 1942). In America Vaughan (1933) has shown that Crinkle is transmitted by Myzus fragaefolii, Ckll. a species which is generally considered to be identical with C.fragariae but believed by some authorities (Hodson, 1937) to be distinct. Whitehead and Wood (1941) have

also transmitted Crinkle by means of another species, Pentatrichopus
(= Capitophorus) tetrarhodus, Walk.

Yellow-edge and Crinkle are of considerable economic
importance in various parts of the world, and are an important
factor in the so-called "degeneration" of the strawberry that has
been the subject of research during the last 25 years (Harris, 1934).

(b). Yellow-edge and Crinkle as Complexes.

As mentioned above, the variation in severity of
symptom expression in Yellow-edge infected Royal Sovereign plants
suggests that more than one virus or virus strain may be involved.
All grades of severity of infection, from a very mild type to a
very severe type, are found.   Furthermore, grafting of Yellow-edge
infected plants to F.vesca (which is a sensitive indicator of
Crinkle) has shown that Crinkle is very commonly associated with
Yellow-edge.   Indeed, according to Harris and King (1942, loc.cit.
p.232) "no case of Yellow-edge was noted that was free from Crinkle."
It is not known whether this association is fortuitous or whether
the Yellow-edge disease is caused by the interaction of a Crinkle
virus with some other virus - that is, by a virus complex.

Somewhat similarly, two diseases - Mild Crinkle and
Severe Crinkle - have been distinguished on symptomablogical grounds,
but the precise connection between them is not at all apparent
(Harris, 1938).

(c). <u>The Analysis of Virus Complexes</u>.

Obviously our knowledge of Yellow-edge, Crinkle and other virus diseases of the strawberry would be much extended were it possible to analyse the virus content of an infected plant and separate the different viruses involved. A variety of methods of analysing virus mixtures and complexes has been used by other workers or suggested in the literature and a number of these methods will now be discussed briefly in relation to the possibility of resolving hypothetical strawberry-virus complexes.

(1). Methods dependent on the Stability or Reaction of Virus
<div align="right">Extracts.</div>

In some cases, differences in physical constants of viruses in a mixture allow of their separation; for example, heating an extract which contains both the Cabbage Black-ring virus and the Cauliflower Mosaic virus to $60^{\circ}C$. for 10 minutes would inactivate the former virus but not the latter.

The components of virus mixtures have also been separated by chemical means. Tobacco plants inoculated with a mixture of Cucumber Mosaic virus and Potato virus X become infected with the former virus alone if the mixture is first treated with silver nitrate. If the mixture is treated instead with lithium carbonate, infection with virus X results (Allington, 1938).

Potato virus X can be separated from a complex of viruses X and Y by inoculation after altering the hydrogen ion concentration of the inoculum (Freeman, 1935).

The Tobacco Ringspot virus (Potato virus X) has been isolated from a mixture with Tobacco Mosaic virus by neutralizing the latter with antiserum (Stanley and Wyckoff, 1937).

(ii).   Methods dependent on Host Reaction.

Use has been made of differing host ranges and of differing physiological relations to a host plant to effect the separation of viruses.   For example, Datura stramonium is susceptible to Potato Virus X but immune to Virus Y and thus supplies a means of separating X from a mixture of X and Y (Smith, 1933, p.276).   This technique of "filterplants" can also be used to separate the Cabbage Black-ring virus which can infect wallflower from the Cauliflower Mosaic virus which cannot.

The virus of Green Mosaic of raspberries moves more rapidly in the plant than does that of Yellow Mosaic and it has been suggested that the former virus could be separated from a mixture of the two by a combined grafting and bark-ringing technique (Bennett, 1932).

Another method of separation depends on the ability of some viruses to cross graft unions before others.   For example, when a peach plant infected with Mosaic is grafted to a healthy peach, movement of virus to the healthy plant takes place with less delay than when the diseased plant is infected with Peach Rosette, Little Peach or Peach Yellows (Kunkel, 1938).   Differences in the time elapsing between grafting and transmission of infection

have also been shown to exist in the case of Cucumber Mosaic,
Tobacco Ringspot and Sugar-beet Curly-top and utilized to
separate Cucumber Mosaic and Ringspot from a mixture of these
three viruses (Bennett, 1943).

(iii). Methods dependent on Vector Relationships.

The specificity of insect vectors permits the ready
separation of viruses possessing different vectors.   For example,
the Sugar-beet Curly-top virus is transmitted by a leaf hopper and
the Sugar-beet Mosaic virus by an aphid.   There are several insect
vectors of Potato virus A but no vector of Potato virus X is known.
X, however, is readily sap-transmissible whereas A is only so
transmitted with difficulty.   Thus X can be separated from a
mixture of X and A by mechanical inoculation while A can be isolated
by means of a vector, e.g. *Myzus persicae*. (Smith, 1937, p.392.).

Even where viruses have a common vector, differences
in their relations to it can be employed in their separation.
Thus it is known that, in the case of certain viruses, aphides
which have fed on infected plants remain infective for long periods
after leaving the infected plant; with other viruses, however, the
ability to infect is quickly lost.   In some cases, too, an insect
is able to transmit infection immediately after leaving an infected
plant while in others a latent period (or incubation period) must
elapse before the insect is infective.   Such differences in the
times for which viruses remain active in the vector and differences

in the latent periods required by different viruses have been
utilized for the separation of viruses.

For instance, Potato virus Y has apparently no
latent period in the vector Myzus persicae and the latter normally
loses its power to infect within twenty-four hours of leaving the
source of infection (Smith, 1933, p.144). Potato Leaf-roll, on
the other hand, has a latent period of at least twenty-four hours
in M.persicae and the vector remains infective for at least seven
to ten days (Smith, 1931). Thus M.persicae feeding for a short
period on a potato plant infected with both virus Y and Leaf-roll
will at first transmit virus Y only but in later serial transfers
will transmit Leaf-roll alone. (Bawden, 1939, p.77).

It has been shown (Watson, 1936, 1938) that certain
viruses are more readily transmitted after short than after long
infection-feeding periods and that, for such viruses, the efficiency
of the vector is increased by preliminary fasting. To such
viruses the name "Non-persistent" has been given (Watson & Roberts,
1939; Watson, 1940). In the case of "Persistent" viruses, on the
other hand, the efficiency of the vector (i.e. the probability of
its transmitting infection) increases with increasing length of
infection-feed, preliminary fasting has no effect on the efficiency
of the vector and infectivity is usually maintained for some time
after leaving the source of infection.

Thus preliminary fasting followed by a short infection

feed will favour the transmission of "non-persistent" viruses and allow of their separation from complexes containing "persistent" viruses.   Longer infection feeds followed by consecutive transfers to a series of "indicator" plants will permit of the separation of the "persistent" viruses.   A system of serial transfers, too, will further subdivide both "persistent" and "non-persistent" viruses according to their actual persistence and length of latent period, if any.

This method of separation of the components of a complex or mixture, depending on the variation in efficiency of the vector with regard to the component viruses at different times after different infection-feeding periods has been employed successfully in the separation of Sugar-beet Mosaic virus (non-persistent) and Sugar-beet Yellow virus (persistent) (Watson, private communication).

(iv).   Applicability to Strawberry Viruses.

Turning now to the strawberry virus problem we find that, so far as the methods outlined in para.(ii) are concerned, we are limited by the fact that previous attempts to transmit the viruses of Yellow-edge and Crinkle by mechanical inoculation have been unsuccessful (Harris and King, 1940).   The present writer, too, has obtained negative results from such inoculations.   Neither is transmission by mechanical means likely to be successful in the future since it has been shown that extracts of various organs of

infected strawberry plants contain no detectable amount of protein (and therefore, presumably, no virus) and that such extracts inactivate preparations of other viruses (Bawden & Kleczkowski, communication in Press).   The absence of virus in plant extracts also renders separation by serological means impossible, which is in accordance with experimental evidence (Dumon & Swartele, 1937; Mushin, 1942; Bawden & Kleczkowski, in Press).

Because of the failure to transmit mechanically, most of the previous work on strawberry viruses has involved the transmission of all the viruses from one plant to another by some system of grafting, for example the stolon-inarching method of Harris (1932), Harris and Hildebrand (1937) and Harris and King (1942).   Such a grafting technique gives little scope for analysis of, or substraction from, any existing complex unless it is by the utilization of possible differences in the rates of movement of components (see para. (ii) p.115above).   The linking of numbers of plants by stolon-inarching offers possibilities but work begun by King (unpublished) along these lines has yielded negative results so far.

The small number of known alternate hosts of strawberry viruses (a few species of Fragaria and, possibly, one species of Potentilla) restricts the scope for any method of separation by 'filter plants' (para. (ii) above).   The method of analysis by means of alternative aphid vectors has been tried

for severe Crinkle (Wood, 1941) but has given negative results
and only two vectors of strawberry viruses, <u>Capitophorus</u> <u>fragariae</u>
and <u>C.tetrarhodus</u>, have been found.

The remaining method, i.e. selective feeding
(outlined in para.(iii), p.117), offers particular promise and early
experiments in analysis depending for success on possible differing
relations between the components of strawberry virus complexes and
the insect vector of Yellow-edge and Crinkle (<u>Capitophorus</u> <u>fragariae</u>)
forms the subject of the remaining section of the present thesis.

II. Experimental.

Attempted analysis of strawberry virus complexes by means of the vector Capitophorus (Pentatrichopus) fragariae.

(a). Materials and Methods.

As sources of infection, individual leaves removed from plants growing in an insect-proof glasshouse were used; all were of the variety Royal Sovereign.

The "Yellow-edge" plant which was used as a source of infectors showed marked symptoms typical of infection with this disease - i.e. great reduction in size of leaf lamina and length of petiole and pronounced marginal yellowing of the leaves (Fig.13). The "Severe Crinkle" plant showed large chlorotic spots and blotches some of which had necrotic centres and distortion of the leaf lamina (Fig.14). The Mild Crinkle plant was from the East Malling clone (M.35) and showed faint chlorotic spots on most of the leaves.

Indicators were of three types, young plants of a single clone ᵡ of Fragaria vesca, young Royal Sovereign plants producing/visible reaction when grafted to F.vesca and therefore presumably 'virus free' ("Malling 40" clone) and young Royal Sovereign plants infected with Mild Crinkle ("Malling 35" clone).

ᵡ Clone: A number of plants propagated vegetatively from one parent.

Approximate scale

0   1   2   3   4   5 cm.

Figure 13.

Royal Sovereign

Plant showing symptoms of Yellow-edge and used as source of infectors.

Approximate scale

| 0 | 2 | 4 | 6 | 8 | 10 | 12 cm. |

Figure 14.

Royal Sovereign

Plant showing symptoms of Severe Crinkle, used as source of infectors.

Aphides (<u>Capitophorus</u> - <u>Pentatrichopus</u> - <u>fragariae</u> Theob.) for the various experiments were taken from stocks raised on healthy <u>F.vesca</u> plants (of the same clone as the "indicator" plants) or on Royal Sovereign ("Malling 40" clone) in pots. These "aphid stock" plants were kept in the glasshouse in insect proof cages.

At the beginning of each experiment a suitable number of apterous aphides was transferred from the aphid stock to a half Petri dish by means of a fine camelhair brush. The Petri dish was first covered with tightly stretched "Cellophane" which was held in position by a rubber band passing round the rim of the dish. A small "window" was cut in the Cellophane and the aphides were introduced through this aperture. When the desired number of aphides had been transferred in this way, the window in the Cellophane was closed by means of a small, moistened Cellophane patch and the aphides left undisturbed for 17-18 hours (Fig14B). After this preliminary "starvation period", aphides were retransferred to leaves detached from the appropriate infected plant. These were maintained in a more or less natural position and prevented from wilting by embedding the petiole of each in moist sand. The leaf was then covered by an inverted glass tumbler the rim being pressed into the sand so as to prevent the escape of any wandering aphides. After being allowed to feed on the infected leaf for the appropriate time, aphides were transferred

Figure 14B.

Experimental Technique.

Left: Cellophane covered Petri dish employed in starvation
        of aphids.
Right: F.vesca plant enclosed by lamp chimney.

to test plants which were enclosed in Cellophane covered hurricane lamp chimneys to prevent escape of aphides during the transmission-feeding period.   Except where the effect of numbers of aphides was being studied, two aphides were transferred to each indicator plant.

All indicator plants were dipped in an insecticide wash (nicotine, derris and soft soap) within 24 hours of the removal of aphides at the end of the transmission-feeding period. In this way any young larvae which had been born during the period of the experiment and overlooked during the removal of the adult aphides were destroyed.   It has been shown (Wood, 1941) that newly born Capitophorus larvae are not infective even if produced by infective parents and that plants do not become infective for about 20 days after they have been fed upon by infective aphides.   Thus any newly born larvae on the test plants would not be viruliferous and could not become so in the few hours which elapsed before their destruction.   They would thus be unable to affect the results in any way even if overlooked during transfer of the parent aphides.

Most of the experiments were carried out in two parts with an interval of a few days between the parts so as to minimise the effect of variation in environmental conditions.   For convenience aphides were generally starved overnight and the first transfers were made at 07.00 or 08.00 hrs. G.M.T. so that the

remaining transfers occurred during hours of daylight. Similar
results would probably have been obtained, however, had the
feeding periods fallen at different times of the day, for Watson
(1938) has shown that, for Hyoscyamus virus 3 and the vector
Myzus persicae, the hour of feeding has no effect on vector
efficiency.

All plants were grown in pots in a glasshouse and
sprayed weekly with a nicotine soap insecticide except when
aphides were feeding on them.

(b).   Preliminary Experiments.

These two experiments were intended to give
information on the feeding period required for aphides to become
infective and the optimum number of aphides for transmission of
infection.

Experiment 1. (May, 1942).

Aphides were subjected to a preliminary fasting
treatment for 18 hours.   They were then allowed to feed on a
leaf from a Yellow-edge infected Royal Sovereign plant for a period
of 5 minutes, 1 hour or 24 hours when different numbers of aphides
(1, 2, 5 or 10) were transferred to young F.vesca plants for 24
hours.   The aphides were then removed, and the plants dipped in
insecticide as described above.   Treatments were duplicated except
in one case where the number of starved aphides was insufficient
to allow of this.

As will be seen from Table 1, none of the aphides which were fed for five minutes or one hour on the infected leaf transmitted infection to F.vesca. Of the 24 hour infection-feed series, one of the two plants receiving two aphides developed symptoms as did the three plants receiving more than two aphides. Control plants, which received aphides direct from the stock plant, remained healthy.

TABLE 1.

Infection feed	Proportion of infections[x]			
	1 aphid per plant	2 aphides per plant	5 aphides per plant	10 aphides per plant
5 min.	0/2	0/2	0/2	0/2
1 hr.	0/2	0/2	0/2	0/2
24 hrs.	0/2	1/2	2/2	1/1
0 min.(Control)	-	-	0/1	0/1

[x] Numerator shows no. of plants infected; Denominator, no. of plants receiving aphides.

Experiment 2. (June, 1942).

Aphides, after preliminary fasting for 17 hours, were fed on a detached leaf from the same Yellow-edge infected Royal Sovereign as in Experiment 1 for periods ranging from 2 minutes to 24 hours. Two aphides were then transferred to each

indicator plant (F.vesca) for 24 hours, each treatment being re-
plicated three times.   Observation showed that, as found by Watson
(1936) for Myzus persicae, aphides took about three minutes to
find a suitable place on the leaf and to assume the feeding position.
For a two minute feeding period, therefore, aphides were left on
the infected leaf for five minutes and for a ten minute feeding
period they were left for thirteen minutes.   For longer feeding
periods it was felt that the few minutes occupied by the aphides in
settling down to feed could be neglected.   There was, furthermore,
considerable variation in the behaviour of individuals in the
longer feeding periods.   Some aphides, after a short feed, wandered
about for long periods with short pauses during which they may have
been feeding;   others remained for long periods in the same spot and
seemed to be feeding almost continuously.   The time noted as
"feeding period" in this and following experiments is, therefore,
the actual time for which the insects were left on the plant leaf
except, as has been explained, for short (2 minute and 10 minute)
feeding periods.

The results of this experiment were as follows:-
aphides receiving infection feeds of 2 min., 10 min., 30 min., 1 hr.,
2 hr., or 4 hr. did not transmit infection;   of the three plants
receiving aphides fed on the source of infection for 8 hours, one
became infected;   of those receiving aphides fed for 24 hours, two
were infected;   controls remained uninfected.

(c). Experiments in Analysis.

The experiments to be described below were designed to test the assumption that the virus transmitted in the previous experiments was part of a virus complex.

(i). Description of type experiment.

All experiments described in this section were of the same general plan and the transfers of aphides in a typical experiment are indicated graphically in Diagram **2.**

Aphides from a stock raised on a symptomless F.vesca or Royal Sovereign ("Malling 40" clone) strawberry plant were subjected to a preliminary fasting period in a Petri dish as described above (Sect. (a), p.**121**). Pre-fasting took place over-night and was of 17 or 18 hours duration (except in part of Experiment 4 where the fast was for $4\frac{1}{2}$ hours). Some of these aphides were then placed on a leaf from the appropriate infected plant and allowed to remain there for five minutes (equivalent to a two-minute feeding period). Two of them were then transferred to an indicator plant (1A in diag.**2**). After aten minute feeding period (i.e. 13 minutes) on this first indicator, the two aphides were transferred to a second, similar indicator plant (1B) for two hours, thence to a third indicator (1C) for twenty-four hours and, finally, to a fourth indicator (1D) for a further twenty-four hours.

Other fasted aphides were treated similarly, but with infection feeds of longer duration, i.e. one hour (plants 2A-2D) or twenty-four hours (plants 3A - 3D).

Diagram 2.

Representation of the transfers of aphids in a typical experiment. (Most experiments consisted of five such sets of plants).

Experiments **were** carried out in two parts, the treatments being duplicated in one part and triplicated in the other so that five plants in all received each treatment. In addition there were ten control plants, five receiving aphides direct from the stock and five not receiving aphides.

Two parallel series of three experiments on the above plan were made comparing Fragaria vesca (Expts. 3, 4 & 5) and Royal Sovereign (M.40) (Expts. 6, 7 & 8) respectively as indicators. The variant within these groups was the source of infection, namely, Royal Sovereign leaves showing Severe Yellow-edge (Expts. 3 & 6), Severe Crinkle (Expts. 4 & 7) and Mild Crinkle (Expts. 5 & 8), respectively. The Yellow-edge leaves were from the same clone as that used in Expts. 1 & 2 and the source of Mild Crinkle was the "Malling 35" clone.

(ii). Experiments using F.vesca as indicator.

These experiments (Expts. 3, 4 & 5) were carried out at various dates in May, June and July, 1942 and all plants were kept under observation for at least six months after inoculation.

In no instance were any symptoms detected in the control plants/or in the indicator plants of the two-minute infection feed series (Series 1, diag.2). In the one-hour series, one plant only, in the first (10 minute) serial transfer of Experiment 4, developed symptoms. (Expt. 4, group 2A). On the other hand, a number of plants in the twenty-four hour infection-feed series

(Series 3) developed symptoms twenty to twenty-four days after inoculation, and the results are summarized in Table 2.

TABLE 2.   F.vesca indicators infected by aphides receiving an infection-feed of 24 hours.

Expt. No.	Source of infection	No. of indicators developing symptoms [I]			
		1st transfer (10min) 3A[x]	2nd transfer (2 hr.) 3B[x]	3rd transfer (24 hr.) 3C[x]	4th transfer (24 hr.) 3D[x]
3	Yellow edge	2	3	1	0
4	Severe Crinkle	2	5	3	0
5	Mild Crinkle	0	4	1	0
Total		4	12	5	0

[x]   See diag. 2.
[I]   Two aphides per plant, five "indicators" per treatment.

(iii). Experiments using "virus-free" Royal Sovereign strawberry plants ("Malling 40" clone) as indicators.

These experiments (Expts. 6, 7 & 8) were carried out during June and July, 1942, and the plants were kept under observation for twelve months after inoculation.

No visible symptoms appeared on any of the indicator plants in any of the three experiments during 1942, apart from

faint chlorotic spots which developed on some of the inoculated
plants and also on some of the controls.   These spots were
noticeable on the young leaves of the plants and especially on
'runners' produced by them, but the chlorosis varied in intensity
and tended to disappear after a time.   No correlation of this
chlorotic spotting with treatments could be seen;   it was observed
in a few plants of each treatment and of the control series and
may have been associated with unusually warm conditions in the
glasshouse.

By June, 1943, a number of the plants showed slight
chlorotic leaf spotting.   In some cases this was typical of Mild
Crinkle as seen in the "Malling 35" clone of Royal Sovereign but
in others it was less distinct and on some plants only a few
indistinct isolated chlorotic spots appeared.   Other plants
remained symptomless.   This gradation from typical "Mild Crinkle"
plants to completely symptomless plants made it impossible to
diagnose infection with any degree of certainty and it was decided
to graft a number of the Royal Sovereign indicators to plants of
F.vesca.

Six plants from Expt. 6, twenty-nine from Expt. 7
and four from Expt. 8 were therefore grafted to F.vesca during
June and July, 1943, by the stolon inarching method.   At the time
of grafting, four of the plants showed symptoms typical of infection
with Mild Crinkle and all of these produced symptoms on the F.vesca

to which they were grafted.   Of four plants which showed less
distinct chlorotic spotting, two produced symptoms on F.vesca and
of three plants with a few isolated chlorotic spots, one produced
symptoms on F.vesca.   Ten plants from Series 3 (24 hour infection
feed) showed no symptoms but four of these produced symptoms on
F.vesca.   Seventeen plants from Series 1 and 2 and one control
plant showed no symptoms and produced no symptoms on F.vesca.

It thus appeared that it was difficult, if not
impossible, to diagnose the infection obtained on Royal Sovereign
since some infected plants showed no obvious visible symptoms.

(iv).   Experiment using Mild Crinkle infected Royal Sovereign
        strawberry plants (Malling 35 clone) as indicators.

The design of this experiment (Expt. 9) was the
same as that of Expts. 3 - 8 except that treatments were replicated
four times instead of five.   The source of infection was leaves
from a Royal Sovereign plant showing Severe Yellow-edge (as in
Expts. 1,2,3 & 6).

At the commencement of the experiment (August 1942)
most of the indicator plants and controls showed the chlorotic
spotting typical of Mild Crinkle infection.   By June, 1943, all
the plants showed chlorotic spotting which was more severe in some
cases than in others, but no symptoms of Yellow-edge had developed.

(v).   Additional experiment.

This experiment (Expt. 10) was of a different pattern from those previously described and was intended to investigate the effect which would be produced by longer feeding periods.

Aphides were transferred without pre-starving to a Royal Sovereign plant infected with Severe Crinkle.   They were allowed to feed for eight days when ten of them were transferred to a single F.vesca plant for 24 hours.   From this they were transferred to a second F.vesca plant and thence to a third. The transference was repeated at intervals of 24 hours until the aphides had fed on ten successive F.vesca indicators.   During the period of the transfers (10 days) some of the aphides died and there were only four of the original ten aphides left at the end of the experiment.

Symptoms of infection developed on the first indicator plant but the others of the series were still without visible symptoms two months after inoculation.

(d).   Symptoms produced on F.vesca.

The symptoms produced on F.vesca plants by direct aphid transfers in Experiments 1 to 5 and 10 and by grafting to Royal Sovereign indicator plants of Experiments 6,7 and 8 were all of the same general type.

The earliest symptom observed was a slight clearing
of the leaf-veins of some infected plants, though this was not noted
in all cases.   Later symptoms, appearing on all infected plants,
consisted of angular, yellowish, chlorotic spots or flecks on the
leaves, puckering and distortion or "blistering" of the leaf and
reduction in size of the lamina. (Figs. 16 & 17).

The chlorotic spots, which were linear or star-shaped,
varied both in frequency and size, distortion of leaves and leaflets
being most marked where the chlorotic spots were large and numerous.
The severity of the puckering of the leaflets and the degree of
reduction of laminal area showed considerable variation also.
In some cases the leaves of infected plants were almost normal in
size but in others they were much reduced and the whole plant
appeared dwarfed in comparison with the controls.   This variation
in symptom intensity occurred in each experiment but it seemed
to be greater among plants infected from Yellow-edge infectors.

A similar range in the severity of symptoms has
been noted by Wood, 1941, in F.vesca plants infected from a single
Severe Crinkle plant.
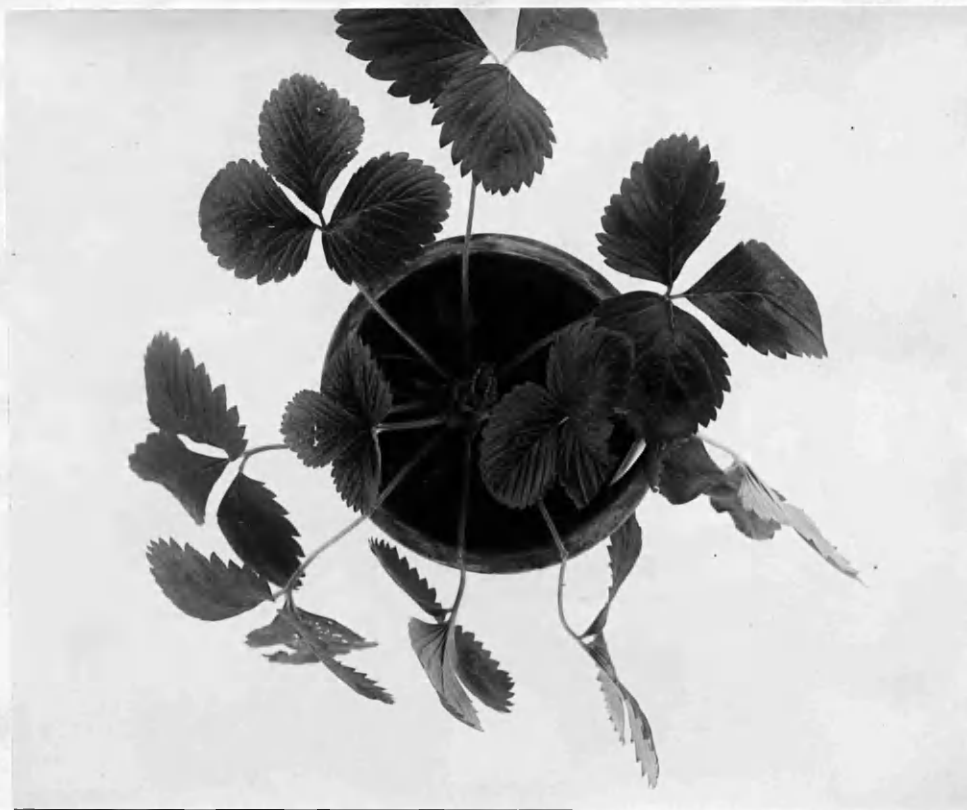
Approximate scale

0   2   4   6   8   10 12 cm.

Figure 15.

Fragaria _vesca_
(Aphid inoculated, 1)

Normal plant, (uninoculated control)
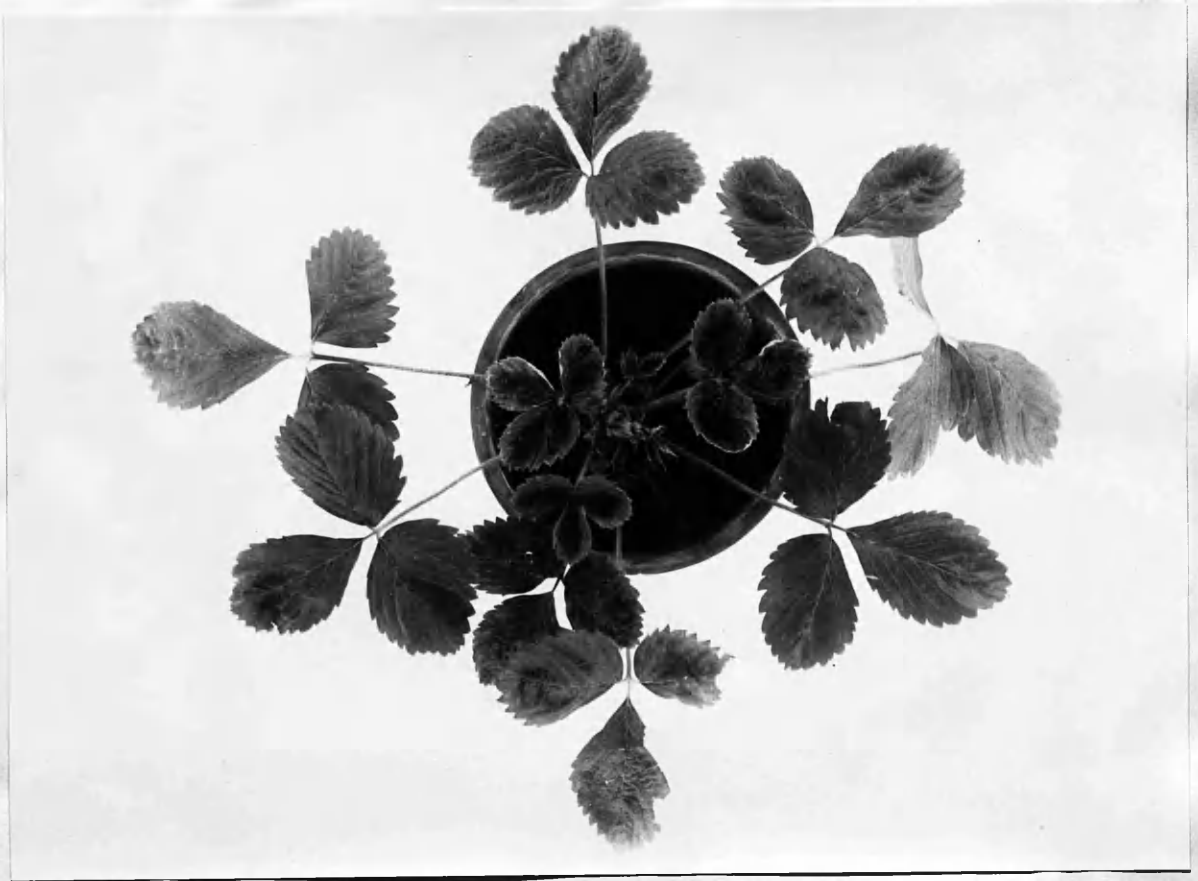
Scale:- Same as Fig. 15.

Figure 16.

Fragaria vesca
(Aphid inoculated, 2)

Plant showing mild symptoms. Source of infection, Royal
Sovereign infected with Mild Crinkle.

Scale:-   Same as Fig. 15.

Figure 17.

Fragaria vesca
(Aphid inoculated, 3)

Plant showing severe symptoms.   Source of infection, Royal
Sovereign infected with Yellow-edge.

III. Discussion of Results.

As the transmission of "non-persistent" viruses is favoured by preliminary fasting and short infection-feeding (see Sect. I(c), p.117), such viruses would be more likely to be transmitted to plants of Series I and 2 (diag.2) than to plants of Series 3.   Moreover, such viruses do not persist in the vector so that they are unlikely to appear in plants C and D of these series.

On the other hand, as the transmission of "persistent" viruses is not affected by preliminary fasting but favoured by long feeding periods, any virus of this type would be expected to be transmitted in Series 3 or 2 rather than in Series I. Long transmission-feeding times also favour the transmission of such viruses so that, in these series, they are most likely to occur in plants C or D (depending upon the degree of persistence of the transmitted virus).   Viruses with "latent" periods may be transmitted to plants 2C or 2D, depending on the length of the latent period, or to plants of Series 3.

Any resultant separation of viruses will not, of course, be absolute or occur at every trial, but the probability of infections occurring as indicated will be greater and a

sufficient number of replicates of the type experiment should
result in the isolation of different viruses in some of the
indicators.

If the results of Experiments 3,4 and 5 (F.vesca
indicators) are now considered (see p.132 and table 2) it will be
seen that all but one of the infections occurred in Series 3 and
that they are confined to the first three transfers.   Thus the
virus or viruses transmitted in these experiments have behaved in
a manner expected for a "persistent" virus.   In each experiment,
too, more infections occur in Group B than in Groups A or C so that,
if more than one virus has been transmitted, the individual viruses
have similar vector relationships.   The absence of infections in
plants of the fourth transfer (Group D) shows that, under the
conditions of the experiments, the viruses each persist for less
than about twenty-four hours.   As has been noted, the time required
for development of symptoms (20-24 days) and the symptoms themselves
were also similar in each of the experiments and the similarity
of type, vector relations, persistence, symptoms and time required
for the development of the latter, suggests that the same virus
has been transmitted in each of these experiments whether the
sources of infection showed symptoms of Yellow-edge, Severe Crinkle
or Mild Crinkle.   On the other hand, the variation in the severity
of the symptoms produced on F.vesca may be due to infection by
more than one virus and further separation of the transmitted

viruses may be possible. Nevertheless, for the purposes of further discussion it will be assumed that the symptoms described on F.vesca are all due to one virus.

As regards Experiments 6, 7 and 8 (Royal Sovereign indicators), the symptoms produced were found, as has been stated above, to be too vague for diagnostic use. Nevertheless, the grafting tests (above, p.134) showed that some of the indicators in these experiments had been infected and, as the infections occurred in Series 3 and produced symptoms on F.vesca similar to those obtained by direct aphid transfer in Experiments 3, 4 and 5, it is concluded that the same virus has been transmitted in Expts. 6, 7 and 8 as in Expts. 3, 4 and 5.

It is obvious from the symptoms produced on Royal Sovereign that the diseases Yellow-edge and Severe Crinkle have not been transmitted as such and it is concluded that one virus common to all three sources of infection has been isolated. The symptoms produced by this virus on F.vesca and Royal Sovereign resemble those of Mild Crinkle and the simplest hypothesis is that the isolated virus is the Mild Crinkle virus.

Massee (1935, 1936, 1937, 1942), however, has shown that Capitophorus fragariae, the aphid used in the present experiments, is able to transmit Yellow-edge and Severe Crinkle. Various explanations for the apparent failure to transmit these diseases in the present experiments can be suggested.

In the first instance, other viruses may have been transmitted without producing symptoms on either F.vesca or Royal Sovereign.   For example, if Yellow-edge is caused by simultaneous infection with viruses A, B and C, A being the component isolated in the above experiments, and if all these fractions have been transmitted separately then viruses B and C are each "carried" by both F.vesca and Royal Sovereign.   Mild Crinkle infected plants, however, contain virus A, (as has been shown by the isolation of this virus in Expt. 5), and therefore the transfer of virus B and virus C to different indicators infected with Mild Crinkle (in Expt. 9) would result in the formation of the complexes A + B, and A + C.   As no change in the appearance of the indicators in Expt. 9 was noted, it would follow that B and C do not modify the symptoms produced by A.

Thus the assumption that all fractions of the Yellow-edge complex have been transmitted in the above experiments leads to the conclusion that virus B and virus C produce no symptoms on Royal Sovereign, A, A + B and A + C each produces identical symptoms of a mild type, and yet, from the hypothesis, A + B + C produces symptoms of Yellow-edge.   Such a condition of affairs is highly improbable and it is therefore considered most unlikely that all fractions of Yellow-edge were transmitted in the experiments described.

As Expt. 9 was not repeated using leaves showing

Severe Crinkle symptoms as infectors, it is not possible to apply this argument in its entirety to the hypothetical transmission of Severe Crinkle but it is considered unlikely too that transmission of all the components of this disease occurred.

To test this hypothesis in the case of Severe Crinkle, however, five aphides were allowed to feed on each indicator plant of Expt.4 so that any virus which had been transmitted would be picked up again by the aphides. Aphides were allowed to feed on plants of Series 1 of Expt.4 for two minutes and were then transferred to a Royal Sovereign ("Malling 40" clone) plant for 48 hours. Similarly aphides were allowed to feed for one hour and twenty-four hours on the plants of Series 2 and Series 3, respectively, and were then transferred to two other "Malling 40" plants. The aphid transfers were carried out in May 1943 and in July a "runner" was taken from each of the three plants and the three young "daughter" plants were grafted together. No symptoms of Severe Crinkle had developed on the grafted plants by October 1943 and it was concluded that the indicators of Expt.4 did not contain all the fractions of the Severe Crinkle complex.

This first hypothesis was also tested as regards the transmission of Yellow-edge. The plants of Series 1 and 2 and the symptomless plants of Series 3 in one part of Expt.3 were grafted together so as to recombine any fractions of the Yellow-edge complex which might be "carried" in these plants. No symptoms

developed.   One of the grafted plants was regrafted to a plant
of Series 3 which showed symptoms of infection, so as to recombine
all the viruses transmitted in Expt.3.   No alteration in the
symptoms exhibited by the already infected plant took place and
it was concluded that no other fractions of the Yellow-edge
complex had been transmitted in Expt. 3.

A second, more probable, explanation of the 1942
experimental results is that all the constituent viruses of the
complexes have not been transmitted.

Thus strains of C.fragariae inefficient as vectors
of some fraction of Yellow-edge or Severe Crinkle may exist, just
as Storey (1932) has demonstrated the existence of "active" and
"inactive" races of Cicadulina mbila the vector of Maize Streak
and Black (1943), the existence of "active" and "inactive" races
of the Clover leaf-hopper (the vector of Potato Yellow-dwarf).

Again the feeding periods used in the experiments
may have been unsuitable, as a brief survey of the experiments of
other workers in this field will show.   Massee in his work on
transmission of Yellow-edge (Massee, 1935, 1936 and 1937) and of
Mild and Severe Crinkle (Massee, 1942) by C.fragariae, used aphides
raised on the appropriate infectors.   The aphid stocks were
allowed to breed on infected plants for periods of six months or
more and the infection feeding periods were thus limited only by
the length of life of the aphid.   Such aphides were then

transferred to indicator plants and generally allowed to remain there for one month.   Symptoms developed in about 8 weeks in experiments with Yellow-edge and in 17-36 days in the case of Crinkle.   From 5 to 35 aphids per indicator were used in different experiments.

Chamberlain (1934) transmitted Yellow-edge by means of Capitophorus fragariae using 20 to 100 aphides per plant. About 30% of the test plants became infected and symptoms developed 3 to 4 months after the transfer of aphides.   The aphides used were "from a virus-infected plant" and had presumably fed there for an indefinite period.

Plakidas (1927) also used aphides from infected plants and again it is probable that they had fed there for a considerable time.   Ten to twenty aphides were transferred to each indicator plant and allowed to feed there for ten days. Symptoms of Xanthosis (probably identical with Yellow-edge) appeared in three weeks.

Vaughan (1933) used aphides colonized on Crinkle-infected plants and allowed to feed there for an unspecified period.   Twenty aphides were then transferred to each of fifty healthy plants and allowed to feed there for one week.   Forty two of the plants developed symptoms but the symptoms disappeared later from twenty-three of them.

Hopkins (1941) reports that he has transmitted

the Severe Crinkle virus from diseased Royal Sovereign plants
to healthy seedlings by means of the strawberry aphid.   Eight
out of fifteen plants developed symptoms but the symptoms
disappeared later from four of these.   The four others continued
to show symptoms although the disease was not in severe form.
Details of feeding periods are not given.

Thus, while earlier workers have not always
specified the lengths of feeding periods employed in successful
transmissions, such periods have probably been longer than those
employed in the present investigation.   It is difficult to see
why a lengthy infection-feeding period should be necessary but
perhaps the proportion of successful transmissions after short
infection feeds is so small as to be undetected in experiments
with comparatively few indicators to which small numbers of aphides
are transferred.   Alternatively, if the virus has a long
"incubation period" in the aphid, a short infection-feed followed
by a short post-infection feed would fail to transmit infection.

It is possible, therefore, that failure to transmit
Yellow-edge or Severe Crinkle in the present experiments was due
to the infection-feed or post-infection feed being too short.
As the normal life of an individual Capitophorus fragariae is in
the region of three to four weeks, it is reasonable to infer that
the sum of the infection feed and post-infection feed necessary to
achieve transmission does not exceed this period.

In Expt. 10 (above), the infection feeding time on a "Severe Crinkle" Royal Sovereign plant was eight days and the sum of the infection feeding and post-infection feeding times was ten days, yet no transmission of Severe Crinkle resulted. If this failure was due to the shortness of feeding periods then either an infection feeding period of more than eight days would be required to achieve transmission of Severe Crinkle or the virus has an "incubation" or "latent" period of several days in the vector. The failure to transmit Yellow-edge could be similarly explained.

If, however, such virus-vector relations do exist for some of the fractions of the strawberry virus complexes then such fractions should be readily separable, by means of the aphid vector, from the virus already isolated and an extension of the selective-feeding-period and serial transfer schedule of the present thesis should lead to the complete analysis of strawberry virus complexes by the aphid vector.

The relationship of the virus isolated in the experiments described to the diseases Yellow-edge and Severe Crinkle is not clear. As has been stated, the virus isolated has many similarities to the virus of Mild Crinkle and it cannot, at present, be said whether the presence of Mild Crinkle in plants infected with Severe Crinkle and Yellow-edge is purely fortuitous or not. The widespread occurrence of this disease among

strawberries means that such an hypothesis is tenable but the
alternative explanation, that Mild Crinkle is an integral
component of Yellow-edge and Severe Crinkle seems equally feasible.
A final decision on this point must await the isolation of other
viruses from plants infected with Yellow-edge and Severe Crinkle
and the resynthesis of complexes causing these diseases.

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Summary of Section C. (Virus diseases of the Strawberry).

1.   An introductory survey of the virus diseases of strawberries is made and possible causes of the variation in severity of Yellow-edge and Crinkle symptoms are considered.

2.   It is suggested that Crinkle and Yellow-edge may be caused by virus complexes.

3.   Possible methods of resolving complexes into their constituent viruses are discussed and a method employing the aphid vector is favoured for the analysis of strawberry virus complexes.

4.   Experiments are described in which aphides (Capito-phorus fragariae) are fed for different periods on leaves from plants infected with Mild Crinkle, Severe Crinkle or Yellow-edge and then transferred to series of consecutive indicator plants.

5.   The results show that a virus or viruses was trans-mitted by aphides allowed to feed on infected leaves for 24 hours but not by aphides allowed to feed for 1 hour or 2 minutes.   In one experiment infection was transmitted after an infection feeding period of eight hours but not after one of four hours.

6.   The experiments also show that aphides lose their infectivity within less than approximately twenty-four hours of

leaving the source of infection.

7.   Symptoms produced on <u>Fragaria</u> <u>vesca</u> consist of angular chlorotic leaf spotting, and puckering, blistering and dwarfing of the leaf. Symptoms on Royal Sovereign consist of slight, indistinct, chlorotic leaf spotting.

8.   The similarity in symptoms, incubation period in <u>F</u>.<u>vesca</u> and vector relationships leads to the conclusion that the same virus has been isolated from all types of infector.

9.   It is concluded that the transmitted virus is the virus of Mild Crinkle.

10.   Reasons for the apparent failure to transmit other viruses from Yellow-edge and Severe Crinkle infectors are considered.

11.   It is believed that feeding periods employed were probably too short and that an extension of the methods employed will result in the complete analysis of strawberry virus complexes.

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