# THE POTATO VIRUS "X": ITS STRAINS AND REACTIONS

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(*Received* 27 *November* 1937)

[Plates 18–25]

CONTENTS



## INTRODUCTION

The virus, the subject-matter of the following study, is known under several names: the $X$ virus of Dr Kenneth M. Smith (1931), the common mosaic of Quanjer (1923), the healthy potato virus of Johnson (1925), which he also called tobacco ringspot (a

name now given to a quite distinct virus, viz. that described by Wingard (1928) and others (Thung, 1936), and potato virus 1, the name now given by Smith (1937).

The extensive work which Smith, the writer and his colleagues, have done on this virus during the last four years has led to the appellation of the *X* virus being generally adopted as the most convenient, as well as the least committal, of its synonyms, and it is the one which will be adhered to in this paper.

Smith drew attention to the variation in virulence of the symptoms exhibited by the virus infection he then called tobacco ringspot, induced by the inoculation of the juice of a potato suffering from what appeared to be simple mosaic.

Smith (1929*a*) records that when transmitting "virulent ringspot" by needle to tobacco, as many as one plant in six might develop the "non-virulent" form of ringspot, that passage of this altered virus through several generations of tobaccos did not further alter its character, and that he regarded this as a definite attenuation of the virus resulting from the original passage. In the light of Smith's later work the correct explanation is to be found in the fact that in certain needle transmissions one of the components of the original complex, viz. *Y*, which together with *X* produced what he then termed the virulent form of ringspot, had dropped out, leaving the *X* virus to infect the plant, and that it was this virus which was conveyed unaltered by further needle inoculation.

Smith (1929*b*) further observed an alteration of symptoms resulting from progressive passage of "ringspot or intensified mosaic" in the opposite direction, viz. that of an intensification of virulence. In this case he was undoubtedly dealing with a mixture of *X* and *Y* and was unconsciously selecting in favour of the complex which contained one of the more virulent forms of the *X* virus shortly to be described.

Later, Smith (1933) recognized that the *X* virus alone might exhibit a variation in respect to its symptoms on tobacco, thus: "the rings may be large or small, single or concentric, and even half double rings, and again it has been found that, while the standard *X* virus will not infect *Petunia* sp., or only does so with great difficulty, other strains will infect the same plant very easily...."

This latter variation of behaviour is now known to be a function of the *Petunia* and based probably on a difference of genetic behaviour and not on the character of the *X* virus strain used.

It should be pointed out that when the *X* virus was first transmitted to the tobacco plant it did not generally produce necrotic concentric rings. The commonest symptom was a vague mosaic mottling associated with darkening of the surrounding green tissue; continued passage through tobacco, however, sooner or later led to the production of necrotic rings. It was occasionally as long as two years in its passage through tobacco before the systemic symptoms took the form of necrotic rings. The change when it did occur was more or less sudden.

Murphy (1932) is inclined to regard the virus forms induced in tobacco after prolonged passage of inoculum from potato "as distinct entities, no longer the same either

individually or in their sum, as those which produced the original disease in the potato".

This view, we shall see, is no longer tenable, and the distinction between the effects on the tobacco and the potato respectively is capable of a simple explanation.

The position prior to the work about to be described is reflected by Smith (1933) who says: "There are slight differences [in their reactions] which seem sufficient to allow of the suggestion that these various *X* types are variants of one virus and are probably not permanent mutations", a view no longer tenable.

The discovery of protective inoculation, of which a short note was published in 1933 (Salaman 1933), is discussed in the latter part of this paper (p. 198). Inasmuch as this phenomenon has in the meanwhile become an important epistemological weapon in the hands of virus workers, it has been necessary to assume previous knowledge of its occurrence in dealing with many of the problems dealt with in the earlier portion of this paper.

## Recognition of specific strains of the virus

Attracted by the problem of ring formation on leaves, so common an effect of virus infection in general and that of the "*X*" virus in particular, the writer in 1932 attempted to secure minute quantities of tissue from various parts of such rings and study their virus content. This proved to be a more difficult task than had been anticipated, and the simpler one of isolating small samples of the yellow or green areas from "*X*" infected tobacco leaves, with a view to discovering their respective virus contents, was adopted.

The source of the virus to which much of the present work relates was found in 1932 in a plant of a clone of Arran Victory potatoes hitherto considered free of any virus, and which since 1926 had been examined regularly and maintained in an insect-proof glasshouse.

In 1933 the virus was transferred to *Datura stramonium*, in which it produced a mild but characteristic reaction, viz. an interveinal mottle with some yellow spotting.

From the *Datura* the infection was carried through a series of five tobaccos of the variety White Burley whose symptoms are detailed below.

Table I

| | | | |
|---|---|---|---|
| *Experimental Group IV. | No. 26 | 1st tobacco passage | Spotty mottle with half rings which were not necrotic |
| ,, | ,, 36 | 2nd ,, ,, | Local necrotic ring lesions followed by a severe systemic yellow mottle and later large double necrotic rings |
| ,, | ,, 37 | 3rd ,, ,, | A mottle with a few definite rings, much less severe than No. 36 |
| ,, | ,, 38 | 4th ,, ,, | Symptoms much the same as in first passage, No. 26 |
| ,, | ,, 39 | 5th ,, ,, | Mild vein-banding and yellow mottle |

* See note p. 143.

It will be observed that in culture No. 36 there is a sudden intensification of symptoms with the development of local lesions, and that in the three subsequent passages this as suddenly dies down.

It was from culture No. 39 that the first selections were made. The plants had been inoculated on 1 October 1932: on 30 November the mottle was composed of rather irregularly disposed areas of green and yellow on leaves in which green vein-banding was still a prominent feature. Circular disks of 4 mm. diameter* were removed by a punch from the green and yellow areas respectively, ground up in small agate mortars with tap water and inoculated into further plants. The subsequent histories of these two sources is recorded in Experimental Groups I and IV. From the first the two series were sharply differentiated, and it will be convenient if we speak of them as representing the "*G*" and "*L*" strains of the virus respectively, although at this stage neither were in a pure state.

At an early stage it was evident that besides these two strains there must be another strain responsible for the necrotic lesions so frequently reported. Had such been due to mere quantitative concentration of virus, necrotic lesions should have appeared in some of the plants derived in later and unselected passage from the two extracts. But that was not the case, the strain $X^S$ producing such was isolated later. In all, six distinct strains of the virus have been recognized.

These six strains of the "*X*" virus will be described in the order of their virulence on tobacco and *Datura*, excepting strain "$X^D$", which will be considered after "$X^S$" and before "$X^N$" with which it has certain affinities.

## The masked or $X^H$ strain

Whilst work was in progress during 1936 on the $X^N$ strain (see p. 155) it was discovered that the "virus-free" stocks of Arran Consul and Up-to-date in the possession of the Research Station were immune to infection with this intensely virulent strain. The sap from such healthy plants prior to their attempted infection had been inoculated to both tobaccos and *Daturas*, none of which had shown any reaction. These same plants with the necessary controls were reinoculated with the $X^N$ strain and again showed no evidence of disease. It was evident that something derived from the potato plants and communicated to the tobacco and *Daturas* had induced in them a complete protection against a virus to which they are otherwise extremely susceptible. It was assumed on the analogy of the protection which $X^G$ had previously been found to confer against $X^S$ that there must be present a silent or masked strain of the virus, to which the name $X^H$ was given. As evidence for its presence at that time depended on its capacity to induce protection against more virulent strains it was necessary to repeat the experiment every time its presence was suspected. In this manner the existence of $X^H$ has been

* In all later work a punch of 1 mm. diameter was employed.

demonstrated more than one hundred times and on over a thousand plants. It has lately been found that $X^H$ produces a definite reaction on *Capsicum annuum* which facilitates its recognition.

### *$X^H$ on potato varieties*

The only varieties which have been found to react to infection with the $X^H$ strain are the following:

Arran Crest: inoculation from a source in *Datura* killed the plant with top necrosis in 6 weeks. Grafted with an infected *Datura* scion, the same variety was killed in a month.

Epicure: inoculated with juice of an infected *Datura* was killed with top necrosis in 6 weeks, and when grafted with an infected *S. nodiflorum* scion died within a month.

King Edward: $X^H$ was inoculated to King Edward and produced a completely localized top necrosis; the rest of the plant grew vigorously and remained healthy, and no virus could be found in the unaffected portion when taken to *Datura* by inoculation. King Edward was infected by grafts, once with a *Datura*, a second time with an infected *S. nigrum* scion: in both death ensued from top necrosis. In one case symptoms appeared after 2 months and the plant died at the end of the third. In the other they appeared after 6 weeks and the plant died 2 weeks later. Two further grafts failed to infect.

The relative retardation of symptoms following inoculation with the masked strain in King Edward is common in this variety when infected with other *X* strains, as is the difficulty of infection by inoculation. The problem is further discussed on p. 161.

Eclipse, Up-to-date and President: on these varieties $X^H$ is carried without any visible reaction. The behaviour of the first two of these varieties is discussed on pp. 158 and 163.

Spooner and Bawden (1935) have shown that the *X* virus acts as an antigen in the rabbit, and the presence of strains is readily demonstrated by precipitin and complement fixation tests. They have independently examined sap from plants which, though perfectly healthy in appearance, were suspected of being infected with the $X^H$ strain and were able to prove its presence by the precipitin reaction.

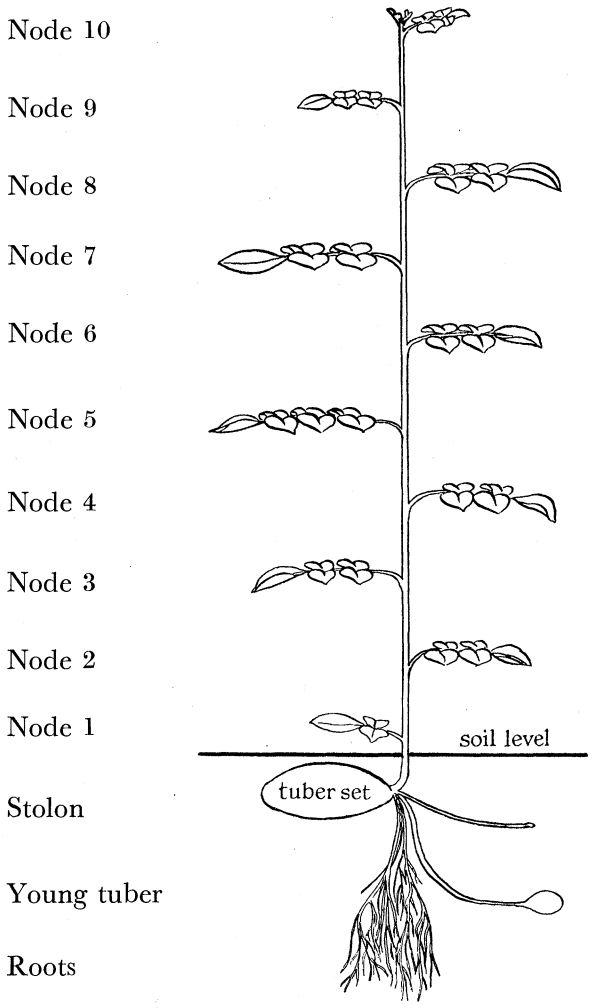
Bawden has lately called the writer's attention to the fact that sap from $X^H$ infected tobacco plants which in Cambridge were completely symptomless, used as inoculum on healthy tobacco plants raised in the experimental insect-free glasshouses of Rothamsted has induced on some plants a slight but just visible mottle. This may more reasonably be explained on the basis of difference of environment and genetic constitution of the test plants used, than on any real change in the nature of the virus strain itself.

The reactions of this and the remaining strains on various members of the Solanaceae are recorded in Table II, p. 144.

The physical reactions are to be found in Table V, p. 176.

### Distribution of the $X^H$ strain in an Up-to-date plant

When it was found that plants of the variety Up-to-date owed their freedom from systemic infection by the $X^N$ strain to the fact that they were carriers of the non-pathogenic $X^H$ strain, it seemed possible that the occasional local reaction or localized systemic infections which did occur might be due to unequal quantitative distribution of the protecting strain or its temporary absence from some portion of the plant. To test this an examination of tissue for its virus content was made from several levels of the same Up-to-date plant. Three weeks later the same plant was re-examined.




| | Relative amount of $X^H$ present on 8 June 1936 | On 26 June 1936 |
|---|---|---|
| Node 10 | not expanded | $\frac{7}{8}$ |
| Node 9 | not expanded | $\frac{7}{8}$ |
| Node 8 | $\frac{7}{8}$ | $\frac{7}{8}$ |
| Node 7 | $\frac{6}{8}$ | $\frac{8}{8}$ |
| Node 6 | $\frac{8}{8}$ | $\frac{8}{8}$ |
| Node 5 | $\frac{6}{8}$ | $\frac{8}{8}$ |
| Node 4 | $\frac{7}{8}$ | $\frac{8}{8}$ |
| Node 3 | $\frac{6}{8}$ | no leaves available |
| Node 2 | $\frac{8}{8}$ | no leaves available |
| Node 1 | $\frac{8}{8}$ | no leaves available |
| Stolon | $\frac{0}{8}$ | $\frac{1}{8}$ |
| Young tuber | — | $\frac{1}{8}$ |
| Roots | $\frac{0}{8}$ | $\frac{2}{7}$ |

Fig. 1. Diagram of Up-to-date plant examined for the presence of $X^H$.

The method adopted was to test small portions of old roots, young secondary rootlets, developing tubers not more than 1 in. in diameter, the stolon on which they were borne, and leaf tissue from each node numbered from the base to the apex.

The material from each locus was ground up and inoculated into:

(*a*) Three tobacco plants, two of which were subsequently reinoculated with $X^N$.

(*b*) Three *Datura* plants, two of which were subsequently reinoculated with $X^N$.

(*c*) Three tobacco plants after mixture with *Y* virus—a double reaction being taken as evidence of the presence of the "*X*" virus.

As $X^H$ produces no lesions in tobacco (cf. fig. 2, Plate 18) or *Datura*, the results are read as follows: the number of failures to reinfect tobacco with $X^N$+the number of failures to reinfect the *Datura*+the number of double reactions induced with *Y*, divided by the total number of plants so treated, was taken as giving a rough estimate of the amount of $X^H$ present at any one locus.

The first examination was made on 8 June, when the plant had developed eight nodes above the ground line, and was repeated on 26 June when there were ten nodes.

It appears from Table II that the distribution of $X^H$ is not uniform, the virus being absent or but feebly developed in the roots, the young stolon and its growing tuber. In the leaflets springing from each node there is some evidence of variation in virus content which, however, is noticeably less at the second examination. It seems likely that a more detailed examination might disclose definite areas in the plant which the virus has failed to reach and which would be susceptible to infection by other strains of the *X* virus.

Doubtless a plant which has no inherent resistance to the virus will eventually be infected in all or nearly all its parts; though not necessarily in the same concentration throughout. Before the complete dissemination of the virus has taken place, however, reinfection with another strain is obviously possible. Such, in fact, does occur and is referred to on p. 163 (see $X^N$).

It will be observed that whilst the concentration of $X^H$ is very low in the recently formed stolons and tubers, it is abundantly present at the base of the stem from which they are growing. Hence it may be said that the concentration of the virus is a function of the relative age of the plant organs rather than of their spatial relationship.

### The mild or $X^G$ strain

The *G* strain originated from the green tissue selection of the culture represented as No. 39 in the Experimental Group No. IV* where it makes its first appearance in culture

* By an experimental group is understood a collection of experiments designed to elucidate one or other aspect of the problems under investigation such as separation, purification, or conversion of individual strains. The culture number refers to a collection of not less than 3 and often 6 of either tobacco or *Datura* plants, as well as individual potato plants, and occasional groups of 3 or more individuals of other species of plants. Twelve such experimental groups each containing from 100 to 400 such cultures form the experimental data from which most of the deductions drawn in this paper are derived. Each group with the behaviour of each plant in each culture contained therein has been represented diagrammatically; their reproduction in full has, however, proved impractical. Certain extracts illustrating the more important points will be found in Figs. 48–51.

TABLE II. DIFFERENT STRAINS OF *X*.

| Test plant | $X^H$ | $X^G$ | $X^L$ | $X^S$ | $X^D$ | $X^N$ |
|---|---|---|---|---|---|---|
| *Browallia speciosa* | Carried | No local lesions; systemic lesions, faint interveinal mottle | No local lesions; systemic lesions, bright vein-banding mottle | Local lesions, few lightly necrotic rings; systemic lesions, bright interveinal mottle with pale necroses, some leaf drop | No local lesions; systemic lesions, interveinal mottle | Local lesions, small necrotic; systemic lesions; interveinal mottle with brown necrotic blotches, some leaf drop |
| *Capsicum annuum* | Very faint or no local lesions; later systemic etched figures, some necroses and rugosity | Same as $X^H$ | Generally faint local necrotic lesions; systemic lesions rather more severe than with $X^H$ | Severe necrotic local lesions killing the leaf; systemic lesions, severe necrosis | Same as $X^H$ | Same as $X^S$ |
| *Datura stramonium* | Carried, no local or systemic lesions | No local lesions; systemic mottle similar in type to that induced by $X^L$ but much less severe | No local lesions; systemic tortoise-shell-like vein-banding mottle | Both local and systemic lesions similar to but less severe than those caused by $X^N$ | No local lesions; faint systemic mottle milder than that induced by $X^G$ | Severe local necrotic lesions; severe systemic necroses which may coalesce and destroy leaf |
| *Hyoscyamus niger* | Carried | Carried | No local lesions; trace of systemic mottle | Necrotic local lesions; very bright interveinal systemic mottle with abundant necroses which coalesce and destroy the leaves | Carried | Necrotic local lesions which, photographed through a green filter, appear to have a dark outline; systemic interveinal mottle with numerous small round foliar necroses |
| *Lycopersicum esculentum* and *L. racemigerum* | Carried | No local lesions; trace of systemic mottle or carried | No local lesions; trace of systemic mottle or carried | Faint local lesions; bright spotty systemic interveinal mottle with few small necroses | No local lesions; trace of mottle or carried | Severe local lesions; systemic mottle with abundant necroses leading to collapse |

| | $X^H$ | $X^G$ | $X^L$ | $X^S$ | $X^D$ | $X^N$ |
|---|---|---|---|---|---|---|
| *Nicotiana alata* | Carried | No local lesions; mild interveinal mosaic | Similar to $X^G$ | Local lesions, faint rings; systemic lesions, similar to those on *N. tabacum* | Similar to $X^G$ | Local lesions, white, papery with brown margins; systemic lesions similar to those on *N. tabacum* |
| *N. angustifolia* | Carried | Carried | No local lesions; trace of mottle of vein-banding type | Local lesions, rings; bright non-necrotic systemic mottle of vein-banding type | Carried | Local lesions, rings; few lightly etched systemic figures followed by mottle, vein-band type |
| *N. langsdorfii* | Carried | No local lesions; trace of systemic mottle | No local lesions; systemic interveinal mottle and well-developed vein-banding | Similar to $X^N$ | Carried | Small grey local lesions; systemic mottle with figures as on tobacco |
| *N. tabacum* | No local or systemic lesions; carried | No local lesions; very faint systemic mottle | No local lesions; systemic tortoise-shell, vein-banding mottle | Similar to but less severe than that caused by $X^N$ | No local lesions; systemic mottle similar to that caused by $X^G$ | Severe local necrotic lesions; severe systemic ring spot necrosis |
| *Physalis pubescens* | Carried | Carried | No local lesions; trace of mottle | Similar to $X^N$ | Carried | No local lesions; systemic mottle with etched rings as on tobacco |
| *P. Francheti* | — | — | — | — | — | Carried |
| *Phytolacca* sp. | Immune to all strains | | | | | |
| *Salpiglossis sinuata* | Carried | No local lesions; trace of interveinal systemic mottle | No local lesions; bright vein-banding systemic mottle | Local lesions, pale green rings; yellow systemic mottle with few necroses | No local lesions; faint systemic mottle | Local lesions, faint local rings; etched systemic rings and figures |
| *S. variabilis* | Carried | Carried | No local lesions; bright systemic vein-banding mottle | No local lesions; yellow systemic mottle with few necroses | Carried | Local lesions; small brown necrotic spots; large systemic necrotic figure covering leaf |

TABLE II (*continued*).

| Test plant | $X^H$ | $X^G$ | $X^L$ | $X^S$ | $X^D$ | $X^N$ |
|---|---|---|---|---|---|---|
| *Solanum capsicastrum* | Apparently resistant to all strains; no local or systemic lesions have been caused by any of the strains and no virus has been recovered from the young growth of the inoculated plants | | | | | |
| *S. dulcamara* | Carried | Carried | Carried | Pale green local lesions. Trace of systemic mottle and a few pale brown rings | Carried | Lightly etched local lesions. Vague systemic interveinal mottle with few scattered small dark brown etched rings |
| *S. melongena* | Carried | Carried | Carried | Very faint local lesions; conspicuous interveinal mottle | Carried | Faint green local lesions; spotty systemic interveinal mottle with lightly etched necrotic lines along veins |
| *S. nigrum* | Carried | Carried | Carried | Local lesions as in $X^N$. No systemic symptoms observed | Carried | Small local lesions with green halos; no rings, systemic bright blotchy interveinal mottle with necrotic specks and slight ruffling |
| *S. nodiflorum* | Carried | Carried | No local lesions; Veinal mottle | Similar to $X^N$ | Carried | Small necrotic local lesions; severe systemic yellow veinal mottle with waving, ruffling and deformity |
| *S. tuberosum*, variety President* | Carried; no local lesions | No local lesions; no systemic lesions or a passing and faint interveinal mottle | No local lesions; mild interveinal systemic mottle which tends to fade | Local lesions, occasional small necrotic lesions; a bright interveinal systemic mottle which often fades | Local lesions, occasional; interveinal systemic necroses on intermediate leaves which shrink and drop. The disease spreads upwards, resembles $X^N$ infections | Local lesions, extensive and necrotic; bright systemic interveinal mottle with numerous interveinal necroses which tend to coalesce; leaf-drop of middle and lower leaves |

* For the reaction of other varieties see text.

No. 48. The strain has been carried in tobacco through some thirty consecutive passages involving over 250 experiments and more than 1000 plants. In addition, the strain was subjected to a further analysis and purification, involving about twenty consecutive passages and 500 plants, whilst over 3000 *G* infected plants have been employed in relation to protective inoculation, and in research on antisera in rabbits.

It may be said that in at least 4000 plants belonging to over thirty consecutive passages derived by the inoculation of *unselected* leaf portions of successive plants infected from the original isolation, the symptoms induced in tobacco and other Solanaceae have remained constant. The characteristic reactions of this *G* strain, for which we propose to employ the symbol $X^G$, in tobacco and *Datura* are as follows:

*In tobacco, variety White Burley.* No local lesions are formed. In the glasshouse at a temperature of about 70° F., some 9–12 days after inoculation a faint systemic mottle appears in which the smaller interveinal areas some 2–3 mm. in diameter are of a yellowish green, whilst the surrounding tissue is of normal colour. Occasionally symptoms may be delayed for more than 15 days. The mottle is generally less readily seen towards the base of the leaf (see fig. 3, Plate 18). If the virus strain is strictly pure there will be neither isolated yellow spots, rings, nor conspicuous vein-banding. As the plant ages, the leaves may appear healthy, but if allowed to become pot-bound, the mottle may reappear and be intensified. Occasionally the mottling of the leaf may be so slight as to escape observation. Should there be a small quantity of the $X^S$ strain present, then a few yellow spots will occur superimposed on the general faint mottle as seen in fig. 4 (Plate 18).

The presence of the $X^G$ strain of the virus has a slight dwarfing effect on the plant, greater than that exerted by the $X^H$ strain, but far less than that induced by infections with $X^L$, $X^S$ or $X^N$.

*In Datura.* There are no local lesions. A very faint systemic mottle appears on the younger leaves between the eighth and eleventh day, disposed in small areas as in tobacco, and demarcated by the more pronounced green banding of the smaller veins (fig. 5, Plate 18). Under favourable conditions this may be preceded by a brightening of the finer veins; such may be seen towards the base of the leaf in fig. 5. When this strain of the virus is more or less invisible on tobacco, it is always possible to obtain some reaction on *Datura*. Under glasshouse conditions this reaction may become very faint, yet it has always proved adequate for the distinction between $X^G$ and $X^H$, even with aged plants, a fact which makes *Datura* in this respect a more serviceable test plant than *Nicotiana tabacum*. Infection with the $X^G$ strain virus has only a slight inhibitory effect on the growth of the plant.

*In potatoes.* The $X^G$ strain does not readily pass by inoculation to the potato, but such results as have been obtained are the same as those induced by grafting. Thus in Arran Victory there usually occurs a very faint interveinal mottle which does not always show in the young top growth. In other cases no symptoms develop, though the presence of the virus can be demonstrated by inoculation back to *Datura*. In

President the $X^G$ strain of the virus may give rise to a faint and transient interveinal mottle; more generally it produces no symptoms. In Epicure and in Arran Crest, if introduced by graft or inoculation, it produces a fatal top necrosis. In Irish Chieftain it combines with the *A* virus latent in this variety to produce a very mild crinkle, from which the plant may ultimately grow free.

For reactions on other members of the family Solanaceae see Table II, p. 144. For physical reactions see Table V, p. 176.

### The medium or $X^L$ strain

At the same time as the *G* strain was obtained by selective inoculation from the green portion of the leaf tissue of an *X*-infected tobacco plant, the *L* strain was obtained by selection from the yellow portion of the same leaf. It will be shown that neither selection was, in fact, at first pure, but each was so largely composed of the *G* or *L* strains respectively as effectively to mask the presence of others.

The *L* strain of the virus, for which it is proposed to employ the symbol $X^L$, when pure is characterized as follows:

*In tobacco, variety White Burley.* No local lesions are formed; systemic infection may show itself under glasshouse conditions on the seventh day, though more usually about the tenth. The first symptom is the clearing of the veins on the leaves next in succession to those on which the inoculation was made. The main and secondary veins become chlorotic and the smaller ones to a lesser degree (fig. 7, Plate 18). Later, the chlorosis fades and a yellow mottle appears at the apex of the leaf. The yellow areas, which are small and more or less rectangular, are bounded by dark green bands alongside the veins. As the yellow clearing of the veins fades, the tissue of the lamina on either side assumes a dark green colour resulting in a striking and characteristic vein-banding which may be described as a tortoiseshell pattern, a term first used in this relation by Köhler (fig. 8, Plate 19). As the plant grows older and the larger leaves begin to pale, the smaller vein-banding areas fade leaving a coarser and more regularly disposed vein-banding enclosing larger and still roughly rectangular areas. The younger leaves of these older plants display more often an irregular mottle whose vein-banding character still persists. In plants 3 months old this latter becomes less pronounced and a rather vague mottle supervenes, as is shown in fig. 10 (Plate 19). In still older plants, except for some degree of chlorosis which may be but slight, there is nothing to indicate infection. Plants infected with the $X^L$ strain are, however, never so vigorous as those infected with $X^G$.

*In Datura.* Local lesions do not occur, but if there is but a small admixture of $X^S$ then small faint rings may be formed on the inoculated leaves. Systemic symptoms begin about the eighth day as a clearing of the veins in the younger leaves; this is followed by a very pronounced yellow mottle bordered by an equally striking dark green vein-banding which tends to obscure the earlier chlorosis of the veins (fig. 9, Plate 19).

The yellow mottle which $X^L$ produces in *Datura* is generally far more vivid than that produced by the same strain in White Burley tobacco plants. Rarely *Datura* may suffer some deformity of the leaf, which disappears as the plant grows older; the rectangular patterned mottle, however, persists. It is doubtful whether any actual necrosis of leaf tissue occurs when the virus is really pure for the *L* strain.

*$X^L$ in potatoes.* The $X^L$ strain of the virus was communicated to both President and Arran Victory, three times by grafting and eight times by inoculation. Subsequent inoculation to tobacco showed the virus to be present in all. Two of the five Presidents developed no symptoms; in the remaining plants an interveinal mottle developed, brighter in Arran Victory than in President. In both varieties the mottle tended to fade as the plant became older. In subsequent years, the infected tubers have given rise to strong plants in which a patchy interveinal mottle can always be seen in the earlier part of the season. In the field, plants which are infected with either $X^G$ or $X^L$ are well grown and strong and show but a very mild and generally transient mottle.

The several attempts to infect Epicure by inoculation or direct graft failed. When, however, an infected scion of *Solanum dulcamara* was grafted to Epicure, the latter succumbed to top necrosis. King Edward, when infected by graft with $X^L$, died of top necrosis.

For the reaction of this strain on other members of the family Solanaceae see Table II, p. 144. For physical properties of the strain see Table V, p. 176.

### The severe or $X^S$ strain

It is this strain of the *X* virus which has been usually described by virus workers as the typical potato mosaic virus. That its reactions on standard plants varied somewhat at times was recognized, but that the variation was due to admixture with other strains was not.

The stock of the *X* virus which originated from the isolation by Dr Kenneth Smith some ten years ago, and which has passed under the stock number 723, has been carried through several hundreds of passages as exhibited in tens of thousands of tobacco and *Datura* plants. It was never selected as a strain, severe or otherwise, but such parts of a plant as showed outstanding symptoms were automatically used as the inoculum for the next passage. In consequence of this unconscious and prolonged selection a type of reaction in the test plants has now been attained and become standardized which is, in fact, identical with that produced by a purified *S* strain.

Even after all these years this particular 723 stock is not a pure strain of $X^S$ but contains traces of the *G* strain as will be shown later. Although the original source of the virus was an Up-to-date potato and was carrying the characteristic Up-to-date streak or *B* virus as well as the *X* virus Bawden has shown that some of the derivative lines retained this latter whilst others lost it on passage. The strain with which this investigation is concerned is one from which the *B* virus has dropped out. It should be

explained that whilst the *X* virus is readily sap inoculable, the *B* streak virus is far less so to tobacco or *Datura* and fails altogether to enter the potato by direct inoculation.

The writer has worked with several different strains of the $X^S$ virus derived from different sources; one from an Up-to-date potato in which it was in association with Up-to-date streak; another isolated from the *G* strain cultures in which it was not so associated; a third strain was isolated from a potato plant in the open, and a fourth from an American source of potato ringspot kindly sent to the writer by Professor James Johnson. Whatever the source of the strain, its reactions have been identical.

*In tobacco, variety White Burley.* In the glasshouse young plants exhibit local lesions on or about the fifth day. These take the form of rings with small necrotic centres. After a few days the rings become better defined and are often multiple. Two or three concentric lightly necrotic white lines cover an area with a diameter of about 4 mm. and enclose a central brown spot. The lesions may subsequently take on an elaborate configuration in relation to the veins consequent on the spread of the virus to the phloem. This latter development is very variable and seems to depend on the size of the infected plant and the conditions under which it is growing. It is never seen on the inoculated leaves of plants which are old at the time of inoculation, i.e. with a leaf area of over 4 sq. in., and is not often observed when the plants are growing rapidly.

On the eighth to tenth day after inoculation systemic symptoms make their appearance on the younger leaves, commencing with a bright yellow clearing of the larger veins which rapidly assumes a necrotic character (fig. 11, Plate 19). This is followed by a deposit of bright yellow spots at the apical portion of the younger leaves at the points of dichotomy of the smaller veins. As the leaves grow these tend both to spread and coalesce.

The progress of the systemic changes which occur in a single tobacco leaf consequent on an infection with $X^S$ has been recorded by means of tracings on cellophane taken at various intervals. Chlorosis and the accompanying necrotic changes occur first at the apex of the leaf and gradually extend towards the base. Similar changes are seen to begin independently in each interveinal area nearer to but within the margin of the leaf (see fig. 12).

If the inoculated plant is very young when infected, the necrosis will often destroy the first affected leaf and very occasionally the whole plant. The plant, however, generally survives this first onslaught, and the next stage, the formation of rings of relatively broad bands of the similar yellow necrotic tissue to that as originally disposed in spots, follows. These rings are frequently incomplete and their surface is roughened owing to the necrosis of the upper layers of the leaf tissue (fig. 13, Plate 19); often they coalesce and in consequence considerable areas of the leaf may collapse.

If the *S* strain is mixed with some $X^L$, even if the latter is but relatively small in amount, lesions frequently take the form of rings and figures of diverse shape, outlined by fine parallel lines of necrotic tissue—the complexity of the pattern seems to vary

with the relative proportions of the two strains in the mixture—but on any one plant it is more or less constant (fig. 14).

As the plant grows, the several clinical stages of the infection may be seen on the same plant. Local lesions with or without figures occur on the lowest leaves; higher up are massed necrotic spots covering the entire surface of the leaf, or, alternatively,

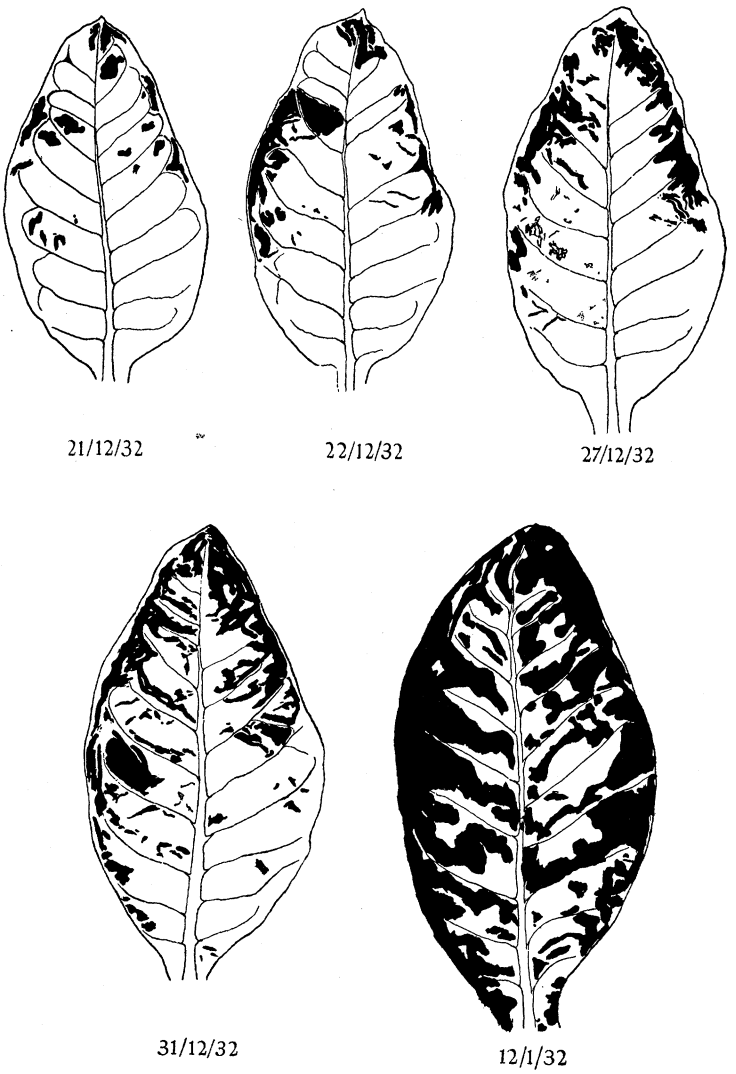



FIG. 12. Tracings of a single leaf of an $X^S$ infected tobacco plant inoculated 1 October 1932 and taken at intervals of 4, 5, 10, 14 and 26 days after systemic symptoms had become visible. The black portions represent the yellow areas.

more or less incomplete rings which are neither so clearly demarcated as the figuration on the lower leaves, nor so deeply necrotic. On the quite young leaves there may be nothing but a few bright yellow spots and streaks situated directly on the veins, usually the smaller ones. When the plant is over 2 months old it outgrows nearly all its symptoms, and when it is 4–6 months old all that may be seen is a rather diffuse

chlorosis on the younger leaves. Notwithstanding the absence of symptoms, the virus, unchanged in character, is still present.

The growth of an $X^S$-infected tobacco plant is much hampered, compared with one infected with the *L*, and still more with one infected with the *G* strain. The leaf area during the first 2 months is probably less than one-quarter of the latter and about one-third that of one infected with the *L* strain.

It is an interesting fact that old tobacco plants infected with the *L* strain frequently display a more chlorotic mottle than do those which harbour the more virulent *S* strain. In fact the character of the strain cannot be gauged by the clinical appearance of the plants which harbour it after a stay of 2–3 months in the glasshouse.

*On Datura.* The *S* strain differs in its reaction from that produced by either $X^G$ or $X^L$ by invariably producing local lesions which are often very severe and may in young tender plants occasionally coalesce and destroy the major parts of the inoculated leaf. The systemic reaction in the older plants is similar to that called forth by the *L* strain, only more severe. In young seedlings it may induce large areas of necrosis which bring about the collapse of the leaf (fig. 35, Plate 22).

*In potatoes.* In both President and Arran Victory the $X^S$ strain produces an interveinal mosaic similar to but more pronounced than that induced by $X^L$, but in contradistinction to the former it persists throughout the season. In addition a few scattered fine necrotic spots may develop on the younger leaves; they do not coalesce or produce any serious change.

In Eclipse the interveinal mottle is less, and in Kerr's Pink rather more pronounced than that seen on President. In Epicure, Arran Crest and King Edward a rapid and fatal top necrosis is set up.

It is only these latter varieties which exhibit lesions in the tuber when infected with any of the *X* strains, and then only such plants as have actually succumbed to top necrosis. The affected tuber may show no sign of damage on the outside, or there may be nothing more than a sinking in of the tissue in the neighbourhood of an "eye". More commonly the damage is extensive and obvious involving the destruction of most or all of the "eyes" (fig. 45, Plate 25). Such tubers when cut open (fig. 46, Plate 25) are seen to contain numerous scattered foci of brown corky matter which stand in no immediate relation to the vascular ring.

Microscopical examination shows that the necrosis commences in the fine phloem strands and spreads to the surrounding parenchyma, the walls of which swell up to about three times their normal width and become infiltrated with a brown substance which makes its first appearance at the angles of the cell.

The cell contents become granular and in unstained sections are clearly visible, whilst the nucleus takes on a brown colour, and the starch grains disappear. The whole focus of degeneration soon becomes hemmed in by a many layered phellogen which is visible to the naked eye as a rough corky deposit.

### The virus of Foliar Necrosis or $X^D$

In 1934 my former colleague, Mr F. C. Bawden (Bawden 1934), described a disease of the potato to which he gave the name Foliar Necrosis, ascribing it to a virus he called *D*, and called attention to the fact that many of the reactions induced by this virus were identical with, and others akin to, those produced by the *X* virus. Evidence will be adduced for the view that the virus *D* conforms more closely to the status of a strain of the *X* virus than to that of an independent species. For that reason it appears both more correct and convenient to designate it as the $X^D$ strain of the *X* virus. Whilst Foliar Necrosis was being studied the writer was working on another virus disease in the potato which presented many similarities to it. This virus which, in the writer's opinion, is yet another strain of the *X* virus group will be here described as the $X^N$ virus.

The essential characters of Bawden's Foliar Necrosis and its causative virus may be stated as follows:

(1) The disease in the potato takes the form of a widespread multiple necrotic spotting of the leaves, inducing leaf-drop and a palm-tree effect. It affects a great many varieties in like manner but a few, including Epicure, succumb with top necrosis. The disease in the potato is very similar to that resulting from an infection by $X^N$; the divergences are given in Table III, p. 165.

(2) Previous infection with a weak strain of the *X* virus protects the potato against infection with Foliar Necrosis.

(3) Sap inoculation to *Nicotiana tabacum* and *Datura stramonium* produces a reaction identical with that of $X^G$.

(4) Tobaccos or *Daturas* infected with $X^G$ are protected against Bawden's Foliar Necrosis and those infected with virus *D* are protected against $X^S$ and $X^L$.

(5) The virus of Foliar Necrosis and the various strains of *X* are antigenically similar and display complete cross-immunity.

(6) The virus *D* of Foliar Necrosis can be purified by exactly the same process as the $X^S$ and $X^G$ strains.

The physical properties (see Table V, p. 176) of the virus of Foliar Necrosis are similar to those of the *X* strains.

### The virus of interveinal necrosis or $X^N$

This new strain was found in certain plants of the variety Majestic, tubers of which were given to the writer in 1932 by Mr A. Millar of the Department of Agriculture for Scotland. These particular tubers were derived from plants which, late in the previous season, were said to have developed large moist necrotic areas on the leaves, an appearance to which Mr Millar gave the name of "Watery Streak". The symptoms observed in Cambridge in the following year, where the plants were raised under glass,

were, however, declared by Mr Millar to be distinct from those previously observed by him. Although the large scale necrosis which this virus may produce in the potato renders the name "Watery Streak" by no means unsuitable, it was decided that in order to avoid confusion this name should be allowed to lapse.

The characteristic clinical feature of the disease in the potato, both in the first season no less than in the second in plants derived from infected tubers, is a necrosis of the leaf which is largely, though not entirely, interveinal. It is suggested in consequence that the disease may be known as Interveinal Necrosis and the causative virus as the *N* strain of the *X* virus, or $X^N$.

### *The $X^N$ virus on tobacco and Datura*

In both plants the local lesions and systemic effects are of the same general character as those induced by the $X^S$ strain, except that the $X^N$ lesions are invariably more severe. Young plants of either species often succumb, whilst it is common even in fair-sized *Daturas* for large portions of the leaf to become necrotic *en masse*.

Mr Bawden has kindly examined the serological reaction of $X^N$ and finds it to be the same as that of $X^S$ and $X^G$. The physical reactions, which are similar to those of the other *X* strains, are given in Table V, p. 176.

The question of the purity of the various strains is considered on p. 173, but that of $X^N$ may be best dealt with here. The systemic infection of a tobacco plant infected with $X^N$ results in a severe necrotic mottle with intervening green areas. When any of the less virulent strains are coexistent experience has shown that they are to be found in the green tissue of the lamina.

A gradacol filtered sap of $X^N$ was inoculated to three tobacco plants and samples of the green areas removed with a 1 mm. punch and inoculated to three tobacco plants. All displayed a full $X^N$ reaction. In a similar experiment in which unfiltered juice was extracted from another $X^N$ source and inoculated to three plants, at the end of 10 days one plant gave an early and full $X^N$ reaction; another a single local lesion, and the third nothing. After a further week both the latter plants displayed the full $X^N$ symptoms. In this case it was evident that although the inoculation from the green portion of the leaf contained extremely little $X^N$ virus, it was not charged with any of the lower strains, for, had they been present, they would have suffused the test plants in the first week and thus have prevented the ultimate $X^N$ reaction.

In a further experiment, juice from the fine punched green areas was diluted 1 in 200 and inoculated to three test plants but produced no reaction. These plants when reinoculated 10 days later with the $X^N$ virus developed the normal necrotic mottle. Had any of the strains $X^H$, $X^G$ or $X^D$ been present they might have induced little or no immediate expression of symptoms, but they would have completely protected the plant against the later infection with the $X^N$ strain (see p. 198).

A similar experiment in which the inoculum was derived from a necrotic area and diluted to 1 in 200 produced an early and full $X^N$ reaction in all plants.

Still more recent trials with undiluted juice have confirmed the view that whilst the green areas in the leaves of infected tobacco plants may contain a small quantity of $X^N$, it is not unusual for them to be entirely free of any virus.

From these experiments it appears that our $X^N$ sources, originally derived from three separate plants of the Majestic potato from the field, are not mixed with any of the less virulent strains. It is not possible, however, to exclude the presence of $X^S$, for that being necrotic in its action would presumably occupy the same leaf areas as the $X^N$ strain. The differential reactions obtained between the two strains (p. 144) make it unlikely that any appreciable quantity of this strain is in fact admixed with the $X^N$ source under observation.

Why the $X^N$ strain, originally derived from potatoes in the field, should be free from admixture with $X^G$ or $X^L$ so commonly present, is an interesting problem to which at the moment there seems no obvious explanation.

### *$X^N$ strain in varieties of the potato*

The disease is readily communicated by means of sap inoculation, and less so by means of grafting. In the latter case we may find the following:

(*a*) Failure of union between scion and stock.

(*b*) Partial union followed by rotting of the stock below the union.

(*c*) Good union but no communication of the disease.

(*d*) Union and full development of the disease.

Failure under (*a*) and (*b*) may be due to the high virulence of the virus producing a rapid local necrosis of the host tissues.

It is difficult to account for the failures under (*c*), for they occurred even when the stocks belonged to such highly susceptible varieties as Majestic and President, and in grafts made in May and June, the most favourable period of the year. Sap inoculations made at the same time were completely successful.

It has already been stated that both the $X^L$ and $X^S$ strains of the virus, when introduced to the different varieties of the potato induce a rather dull, blotchy interveinal mosaic which either disappears or loses its intensity as the plant approaches maturity, except in the cases of Arran Crest, Epicure and King Edward, in all of which a top necrosis develops. The outstanding feature of infection with the $X^N$ strain, on the other hand, is the production of necroses which, so far from abating as the plant grows older, tend to involve increasingly larger leaf areas till the death or dropping of the leaves brings growth to an end. A further difference between the two strains $X^S$ and $X^N$, though it be of degree only, is that the $X^N$ strain is readily communicated to the potato by inoculation; the $X^S$ strain only with difficulty. This difference may be due to a character recently acquired by the $X^S$ strain. The history of the $X^S$ strain employed may throw some light on this point. Originally won from an infected potato it was at first readily inoculable to other potatoes; after eight years of passage through tobacco it has largely lost this faculty. The $X^N$ strain has been maintained for four

seasons, both in the potato and in tobacco; from the former it is rather more readily inoculable than from the latter. It may be that after prolonged cultivation in tobacco it may become as intractable as has the $X^S$ strain.

The potato varieties which have been infected with the virus $X^N$ may be subdivided into groups according to their reaction:

*Group I.* Contains the majority of varieties tested, the reaction is essentially that of an interveinal necrosis followed by leaf-drop.

*Group II.* Contains varieties which may develop either an interveinal mottle with some ruffling, or a necrotic disease similar to, but less severe than that exhibited by, the members of Group I.

*Group III.* Contains varieties which react with a relatively mild interveinal mottle.

*Group IV.* Contains a variety which may behave as a carrier, or alternatively develop an interveinal mottle.

*Group V.* Contains varieties which (*a*) develop acute top necrosis, (*b*) behave as carriers or alternatively develop top necrosis.

*Group VI.* Contains a variety which is highly resistant or immune to the virus.

*Group I.*

A description of the progress of this disease in Arran Victory will serve as a picture of the clinical reactions common to the other varieties in this group.

If inoculations take place in the early summer, necrotic local lesions, more or less circular spots of about 2–4 mm. in diameter, develop on the inoculated leaves, within 14–20 days. In a single case local lesions were observed on the eighth day; later in the season the incubation period tends to be much prolonged and may be over 50 days. In the variety Arran Comrade local lesions were inconspicuous.

Systemic lesions generally follow the local ones in about 14–21 days, i.e. 28–40 days after inoculation. The interval which elapses between the two stages varies with the season, and may be as long as 40 days. The leaves just below the apical growth display the earliest symptoms, viz. an interveinal blotchy mosaic replaced sometimes, particularly when infection is by graft, by a veinal mottle. In either case the mottle is accompanied by the appearance of numerous fine necroses which occur first as minute punctate lesions on the finest veins within the main interveinal areas of the leaf (fig. 16, Plate 20). The necrotic lesions tend to elongate and at the same time extend downwards through the lamina to the lower surface. As the leaves grow older and come to occupy an intermediate position on the stem, the necrotic lesions which were at first small and widely spread, tend to enlarge and coalesce. Necroses now commonly appear on the under surface of the leaves along the greater veins.

The diseased condition now makes rapid progress, the greater part of the leaf becomes involved in a massive necrosis culminating in its collapse; at the same time necrotic streaks appear on the stem and less commonly on the petiole, and before long the leaf hangs vertically downwards by its petiole from the stem, from which it may

eventually drop. Whilst this progressive destruction is proceeding on the intermediate and older leaves, new leaves appear above and develop a more or less severe crinkle with some interveinal mottle and fine interveinal necroses (fig. 15, Plate 20).

Occasionally the lowest leaves escape involvement and remain relatively healthy. Infected plants grow to about two-thirds of the size attained by normal plants under glasshouse conditions. Plants infected by grafting, except for the absence of local lesions, behave as do those which have been inoculated. The necroses are particularly well shown by infra-red photography (fig. 16, Plate 20).

The clinical picture differs from that induced in Epicure by the other *X* strains in two ways. Epicure is destroyed by a wave of necrosis starting at the apex and proceeding rapidly to the base. The reaction of $X^N$ on varieties of Group I begins as a necrotic disease affecting leaves at the junction of the upper and middle thirds and then proceeds in both directions and, in the group of varieties we are considering, never kills the plant. The progress of the disease downwards is more rapid and the lesions on the leaves more extensive than those on leaves nearer the apex.

The second season symptoms of this group may be illustrated by the behaviour of Majestic. Infected tubers may give rise either to quite healthy plants, fully infected ones, or fail to sprout. A similar behaviour was observed by the writer (Salaman 1930) in infections with the two streaks *A* and *B*, and has since been recognized as occurring in tubers of Epicure when infected with the *X* virus. Bawden (1936) has described this phenomenon in some detail. If the shoots are infected they are completely so, and show no amelioration of symptoms such as has been described by Botjes (1934). This doubtless is due to the fact that the original infection is the result of a single virus and not a complex. From a single tuber it is not uncommon to find one shoot healthy, and a second from another eye diseased.

Infected plants are invariably stunted in their growth; the leaves, as soon as they unfold, display innumerable necrotic foci which appear under the upper epidermis and at first do not penetrate throughout the thickness of the lamella. As the plant grows, similar lesions appear on the new leaves, whilst in the older ones the necroses fuse, bringing about a collapse of the leaf (fig. 15, Plate 20), streaks appear in the petiole and stem, and the collapsed leaves begin to hang and then drop. Before long all the lower part of the plant is stripped bare, the young leaves are mottled, spotted with necrotic spots, and may be considerably deformed by contractions which occur in the yellow interveinal areas.

In neither seasonal nor secondary infection have any lesions been observed in the tubers.

The following varieties react to the $X^N$ virus in the manner already described. (The numbers following each name refer as to the numerator to the successful infections, as to the denominator to the number of experiments; the letters I and G refer to infections by inoculation and graft respectively.)

Abundance (I $\frac{2}{2}$, G $\frac{0}{1}$), Arran Chief (I $\frac{2}{3}$, G $\frac{1}{1}$), Arran Comrade (I $\frac{2}{3}$, G $\frac{0}{1}$), Arran

Consul (I $\frac{1}{2}$, G $\frac{0}{1}$), Arran Pilot (I $\frac{1}{1}$), Arran Scout (I $\frac{1}{1}$), Arran Victory (I $\frac{8}{8}$, G $\frac{1}{5}$), British Queen (I $\frac{2}{3}$), Catriona (I $\frac{1}{1}$), Di Vernon (I $\frac{2}{3}$, G $\frac{0}{1}$), Great Scot (I $\frac{3}{3}$, G $\frac{0}{1}$), International Kidney (I $\frac{2}{3}$), Kerr's Pink (I $\frac{4}{5}$), Majestic (I $\frac{5}{5}$, G $\frac{2}{4}$), May Queen (I $\frac{1}{1}$), President (I $\frac{10}{10}$, G $\frac{1}{5}$), Rhoderick Dhu (I $\frac{1}{2}$), Sharpe's Express (I $\frac{2}{5}$).

*Group II.*

The varieties Arran Cairn I $\frac{2}{3}$, G $\frac{0}{1}$, and Champion I $\frac{2}{3}$, G $\frac{0}{1}$, react to $X^N$ sometimes with a mild crinkle and interveinal mottle; at other times with an interveinal necrosis and leaf-drop similar to but less severe than that exhibited by the members of Group I.

*Group III.*

The varieties Arran Banner I $\frac{0}{1}$, G $\frac{1}{1}$, Ballydoon G $\frac{1}{1}$, Doon Star G $\frac{1}{1}$ react to $X^N$ with a relatively mild interveinal mottle.

*Group IV.*

*Eclipse.* Thirteen examples of Eclipse were infected with $X^N$. All belonged to the same clone and were from glasshouse stocks supposedly free from all virus infection, in fact these stocks have been tested each year and have been found free of any known virus. Inasmuch as $X^H$ was only discovered in 1936 it is not possible to be certain as to whether they were free of it prior to this date; six were specifically examined for the presence of the masked strain or $X^H$ prior to the experiment. This was done by inoculating the sap to *Datura* and reinoculating the latter 10 days later with $X^N$. In four of these the examination was negative; in the fifth the possibility of a trace of $X^H$ could not be excluded. In this latter case on the reinoculation of the *Datura* with $X^N$ there resulted a few faintly necrotic local lesions and a systemic infection more like that produced by $X^L$ than $X^N$. In the sixth example the *Daturas* developed similar local lesions to the last but no systemic infection. Both results indicate that there must have been present a certain amount of a weaker strain in the original potato, but that it was so small in quantity that it had not had time to multiply sufficiently to infect the entire *Datura* before the reinoculation with $X^N$ took place.

It may therefore be concluded that whilst some plants of our Eclipse stock are carrying $X^H$ virus, unevenly distributed, presumably, in their tissues, the remaining plants are probably free of any virus.

If now we examine our results from this standpoint, in the virus-free group we find one plant in which the virus never entered, though the graft was good; in the remaining three the plants developed a mild interveinal mottle. In the two examples of Eclipse which we have reason to believe contained some $X^H$ prior to inoculation with $X^N$, both developed a similar mild interveinal mottle.

Of the six plants of which we know nothing as to their possible $X^H$ content, three showed no sign of infection, two exhibited a mottle similar to that already recorded, and one developed a few interveinal necroses, interveinal mottle and leaf-drop,

producing an effect similar to but less advanced than that which occurs in the variety President.

If now we study the virus when extracted from obviously infected Eclipse plants we find

(*a*) that its distribution is irregular,

(*b*) that the character of the virus may be altered.

The behaviour of the Eclipse plant, 119 L, will illustrate the peculiar and apparently haphazard manner in which the $X^N$ strain may be localized within it.

This plant was examined for the presence of $X^H$ prior to infection with $X^N$ in the manner already described, the result indicated that some $X^H$ was present, sufficient to prevent systemic infection but not the formation of local lesions in the reinoculated *Daturas*. The Eclipse potato plant was then grafted with an $X^N$ infected President and examined at various times and at different loci with a view to determining what strains of the *X* virus were present. The test plants employed were tobacco. Details of the experiment are set out below:

| | | Infected plant under examination Eclipse, No. 119 L | Test for the presence of *X* virus strains |
|---|---|---|---|
| 1 | 16. v. 36 | Top of Eclipse prior to grafting | $X^H$ present |
| 2 | 16. v. 36 | Eclipse 119 L grafted with an $X^N$ infected President potato scion | $X^N$ shown to be present in the President at an earlier date |
| 3 | 3. vi. 36 | The President scion now firmly united tested for $X^N$ | No $X^N$ found |
| 4 | 3. vi. 36 | The leaves of Eclipse 119 L near the scion tested for $X^N$ | No $X^N$ found |
| 5 | 13. vii. 36 | Eclipse plant shows a blotchy interveinal mottle observed on second shoot below scion | |
| 6 | 13. vii. 36 | The President scion tested for $X^N$ | $X^N$ present |
| 7 | 13. vii. 36 | The top of plant 119 L removed at time of the grafting and rooted, examined for $X^N$ | No $X^N$ found |
| 8 | 13. vii. 36 | The leaves of 119 L from shoot near the scion examined for $X^N$ | $X^N$ found |
| 9 | 9. iii. 37 | First sprout from a tuber of 119 L examined for $X^N$ | No $X^N$ found |
| 10 | 8. iv. 37 | Second sprout from same tuber of 119 L examined for $X^N$ | A mixture of $X^G$ and $X^N$ (or possibly $X^S$) found |
| 11 | 4. v. 37 | Second year plants 6 in. high slight interveinal mottle and few necroses | |
| 12 | 10. vi. 37 | Same second year plant 3 ft. high looks healthy | $X^G$ and ? $X^N$ mixture found |

It will be observed that the President scion may at one time be found to contain $X^N$, whilst at another none is detected. Yet between these two observations enough $X^N$ has been developed to infect the stock. That no $X^N$ is demonstrated in the stock plant 18 days after grafting, yet is present in the same position 39 days later is not surprising, but that in one and the same tuber harvested 3 months after grafting and examined

after 7–8 months' storage no $X^N$ should be found in one sprout and a mixture of $X^G$ and $X^N$ (or $X^S$) in another, indicates how irregular may be the distribution of the $X^N$ virus within the potato plant.

Further evidence demonstrating the peculiar distribution of $X^N$ when in the presence of $X^H$ is shown by the behaviour of the Eclipse plant $X^N$ 13:

| | | Infected plant under examination Eclipse, No. 13 | Tests for the presence of *X* virus strains |
|---|---|---|---|
| 1 | 17. iii. 36 | Eclipse No. 13 inoculated with $X^N$ sap mixed with carborundum | No sign of infection at any time |
| 2 | 1. v. 36 | Green leaf from near apex tested for $X^N$ | No $X^N$ found |
| 3 | 4. v. 36 | ,, ,, | $X^H$ and possibly some $X^G$ found |
| 4 | 8. v. 36 | ,, ,, | $X^H$ found |
| 5 | 15. v. 36 | ,, ,, | Mixture of $X^N$ and $X^H$ found |
| 6 | 9. iii. 37 | First sprout of tuber examined | $X^N$ found |
| 7 | 8. iv. 37 | Second sprout of same tuber examined | No $X^N$ found |
| 8 | 28. iv. 37 | Second year plant 5 in. high showing mild patchy veinal mottle | Not tested for $X^N$; though this strain was evidently the cause of the symptoms |
| 9 | 10. vi. 37 | Same plant 3 ft. high fairly well developed with slight veinal mottle and ruffling | ,, ,, |
| 10 | 11. viii. 37 | Ruffling increased, mottle still present | ,, ,, |

On 21 June 1937 an Eclipse plant was grafted with a *Datura* scion infected with $X^N$. Throughout the season the only symptoms developed were those of a bright interveinal mottle and some ruffling, but no necroses.

An Eclipse plant was inoculated on 12 March 1934 with sap of $X^N$ purified by MacClement's (1934) $CO_2$ method. No examination of the plant for the presence of $X^H$ prior to inoculation was made, as this strain was not then recognized. From the sequence of events $X^H$ was probably not present. The plant developed no reaction, but when on 28 April 1934 grafts were made to Arran Victory and President potatoes both reacted with a bright interveinal mottle but with no necroses. Some 18 days prior to this, sap from the Eclipse had been inoculated to tobaccos which responded with a necrotic mottle which was described as rather less severe than the normal reaction with $X^N$. The three reactions correspond exactly to that which normally ensues from an $X^S$ infection. It is not unreasonable therefore to assume that during its passage through the Eclipse, the $X^N$ strain has been converted to $X^S$.

In another Eclipse ($X^N$ 12) treated in the same manner on the same date, although the plant itself showed no lesions at all, every extraction was found to contain enough $X^N$ to infect with the utmost virulence all the test plants used. This plant was not tested prior to infection for $X^H$, but it is presumably one which was entirely free of the same. In the second year the plants were slightly stunted, leaves ruffled, waved and showed a light interveinal mottle and scattered necrotic spots.

It thus appears that some plants of the Eclipse clone contain $X^H$ and others none; in the former group the $X^H$ is not evenly distributed throughout the plant, so that when $X^N$ gains an entry into some non-protected portions of the plant, the two strains are neither distributed in the plant in equal quantities, nor do they attain the same localization, nor do they travel at the same rates. This behaviour presents a sharp contrast to that of the Up-to-date variety towards the $X^N$ strain. Here there is much greater uniformity which is consistent with the more uniform spread of the $X^H$ strain throughout the plant tissues in this variety.

We may conclude that the Potato variety Eclipse, even when itself free from any prior infection with the $X^H$ strain, may either react to $X^N$ by the production of a mild interveinal mottle occasionally accompanied by a slight ruffling of the leaf surface, or may behave as a carrier.

*Group V A.*

Another type of reaction is displayed by the following varieties:

Arran Crest I $\frac{2}{3}$, G $\frac{1}{1}$; Epicure I $\frac{6}{7}$, G $\frac{2}{3}$.

In these varieties the reaction is a typical top necrosis which rapidly destroys the plant from above downwards. In one plant of Arran Crest inoculation resulted in severe local lesions but no further spread of disease. This variety is extremely susceptible to the common form of the *X* virus and it may be that occasionally the local reaction is so severe and death of the tissues follows so rapidly that spread of the virus is inhibited. It was found that the healthy non-inoculated leaves of this plant showed no evidence of $X^H$ or any other virus, so that its immunity cannot be ascribed to a protective reaction. Nor do we find any support for Loughnane and Clinch's claim (1935) that the *X* virus does not survive in Arran Crest, for not only is this variety killed right out even by $X^H$, but the virus can be recovered from the affected plant. In one plant of Epicure a localized outbreak of necrotic spots occurred on leaves just below the apex and spread no further. In this plant, as in the Arran Crest above, no form of the *X* virus was found when it was examined prior to inoculation, indeed, in both varieties $X^H$ is quite as virulent in its action as any of those strains which produce symptoms in tobacco.

*Group V B.*

*King Edward.* It was fully expected that this variety would be found ranged alongside the above in its behaviour towards the $X^N$ virus, seeing how susceptible it is to the *H*, *G*, *L*, *S* and *D* strains of the virus, but such was not the case. Seven attempts to infect by inoculation, including two in which carborundum was added to the inoculum, failed to elicit any visible reaction, whilst of five attempts by graft, two only succeeded, bringing about a rapidly fatal top necrosis in each case.

In three cases of attempted infection by inoculation no $X^N$ was found in the leaves though the same were examined on the 52nd and 82nd day after inoculation; two

sprouts from a tuber of each examined in the following spring at intervals of 4 weeks likewise contained no $X^N$ nor indeed any evidence of a protecting strain such as $X^H$.

Two other inoculated plants behaved as carriers. In one of these latter, $X^N$ 15, sap taken from apparently healthy leaves six weeks after inoculation with $X^N$, infected one out of three tobacco plants with $X^N$, whilst the tuber sprouts in the following season were found to be free of $X^N$. Here then a small quantity of virus had entered the plant but had not increased. In the second plant, $X^N$ 14, inoculated 17 March 1936, the $N$ strain was obtained twice from leaves growing on different parts of the stem, and again in the following spring from two sprouts of a tuber. Here infected King Edwards behaved as perfect carriers during a period of over 6 months. In the following season the plants behaved differently: the first remained symptomless and when tested on *Datura* was found to be free of any $X$ strain of virus; the second, when grown in 1937, produced a feeble ill-developed plant with small ruffled leaves but no necrosis. No longer was it a carrier. The sap tested out on *Capsicum* and tobacco was found to contain the $X^N$ strain.

In one of the King Edward plants ($X^N$ 19), a lethal top necrosis developed after being grafted with an $X^N$ infected potato scion; the leaves of this same plant, examined when the earliest symptoms of top necrosis became evident, were found free of $X^N$, whilst a week later the symptoms having advanced rapidly, a leaf taken from near the necrotic top yielded $X^L$ and apparently no $X^N$. The scion examined but 7 days earlier contained $X^N$. The sprouts of the tubers formed in 1937 were found to contain no $X$ of any kind.

In 1937 two King Edward plants were grafted with $X^N$ infected scions from Arran Cairn and President respectively. The first plant died rapidly from top necrosis, the second developed no top necrosis, but 6 weeks after grafting necrotic spots appeared on the middle leaves causing local deformity; 10 weeks later five of the middle leaves were dead and hanging and a spotty necrosis appeared on the leaves immediately above; the uppermost leaves remained healthy throughout.

Extractions from affected leaves to tobacco made 6 August 1937 demonstrated the presence of $X^N$.

The peculiar behaviour of this variety is difficult to understand. Its apparent resistance to the $X^N$ strain of the virus is not likely to be due to protection afforded by a prior infection with $X^H$, for not only is the variety particularly susceptible to this strain, but in six of the plants, including the two in which symptoms developed, no evidence of its presence was found. Whether the existence of the virus of paracrinkle, always present in King Edward, has any effect, it is not possible to determine, as no King Edward plant is known in which the latter is absent. It is, however, an interesting fact that whilst paracrinkle in Arran Victory is a severe disease, paracrinkle when accompanied by $X^S$, or where induced by a King Edward carrying $X^N$ ($X^N$ 14), is considerably less so. This is discussed later (see p. 203).

Apart from the co-existence of the paracrinkle virus, it may be that there is a physical

barrier preventing the passage of the $X^N$ virus—at least by graft—to King Edward, as only when the scions grew vigorously did infection take place.

As with Eclipse, there is some evidence that the King Edward variety of potato is able to convert $X^N$ into a strain of lower virulence. The following is an example: a King Edward plant ($X^N$ 19) was grafted on 4 May 1936 with a scion of an $X^N$ infected President 384, the sap of which was frequently tested throughout the season 1936 for $X^N$ and found to be positive. On 1 June 1936 the scion, having made a good union, was again tested on tobacco and found to contain $X^N$; on 10 June 1936 the King Edward plant began to develop top necrosis and was dead 14 days later. Sap from leaves which showed a mottle near the apical growth was inoculated to three *Datura* plants, two only of which became infected, both developing a full *L* reaction with no necrosis. It is interesting that sprouts from the tubers of this King Edward plant contained no $X^N$ or other strain in two samples taken 9 March 1937 and 8 April 1937, nor did its tuber display any necrosis such as occurs in varieties infected with Up-to-date streak. In this case the virus was not only depressed in virulence but later disappeared, i.e. died out within the tissues of the plant.

*Group VI.*

The potato variety Up-to-date was inoculated with $X^N$ sap ten times and grafted with $X^N$ infected scions eleven times. Of the twenty-one Up-to-date plants experimented with, 14 were tested for the presence of $X^H$ and in all except one about to be mentioned it was found to be present. From previous work it was known that the remaining seven did not contain any of the other *X* strains though it may be assumed that they did contain the $X^H$ strain. In only one case ($X^N$ 122R) was there a reaction and that began in the apical growth above the graft as a necrosis of veins and then ceased. Notwithstanding that the scion, viz. President ($X^N$ 384), grew vigorously, the rest of the plant remained normal. The sap of leaves from the upper portion of this plant on the day of grafting was tested for $X^H$ or other strain with negative result. This, however, must not be taken as evidence that $X^H$ was absent from all other parts of the plant. When a month later a scion from the affected portion of this plant was put to President, the latter developed an interveinal mosaic and not the typical interveinal necrosis. Exactly the same occurred in those cases where $X^N$ infected Eclipse had been grafted to President. The sprouts of tubers from all the inoculated and grafted Up-to-date plants were examined in the following year and were found to contain $X^H$ but no $X^N$. There seems good reason to believe that the Up-to-date at the time of grafting was infected with $X^H$, but that the virus had not attained a footing in the young apical growth. Lower down the tissues were sufficiently impregnated with the masked strain to withstand any aggression on the part of the $X^N$ which had gained a foothold in its younger apical portion. The explanation of the fact that such infected portions, whether in Up-to-date or Eclipse, when grafted back to President and Arran Victory, produce only an interveinal mottle and not the normal $X^N$ reaction, would seem to be

as follows: The scion which showed evidence of $X^N$ is, by the time it is removed from the mother plant, also rich in $X^H$; the sap which passes into the healthy stock contains therefore a mixture of strains and as such does not produce the characteristic necrotic symptoms of $X^N$ but a simple mottle. In tobaccos we have found in hundreds of cases that a mixture in which the weaker virus is present in equal or slightly greater proportions than the virulent type results in a much reduced reaction. It is noteworthy that when in the following spring the sprouts of the President tubers were tested, they were found to contain $X^N$. In this case we have an example of change of strain behaviour induced by mixture, in contrast to that just described in relation to King Edward where a mutation may reasonably be assumed.

The clinical picture of an infection with the $X^N$ virus in those varieties of the potato in which a full reaction occurs, presents close affinities to that induced by Bawden's virus *D*. The size and disposition of the necrotic lesions on the older leaves, the mottle and fine stippled necroses on the apical growth, the leaf-drop and subsequent palm-tree appearance, the identity of behaviour of the lesions to infra-red photography, and their similar action on protected plants, all suggest a close relation between their respective causative agents. The main points of semblance and distinction between the clinical expressions and reactions of the two virus strains which induce the two diseases Interveinal Necrosis and Foliar Necrosis respectively are set out in Table III, p. 165.

## The reactions of the *X* strains on various species of the Solanaceae

Although these reactions are set out in tabular form (Table II), those of *Capsicum*, *Hyoscyamus* and *Lycopersicum* call for special remark.

The reaction of the *X* strains on varieties of *Capsicum* are of particular interest inasmuch as they allow of the detection of $X^H$. In other respects they follow, in the main, the same lines as those on tobacco (White Burley) and on *Datura*. That is to say, $X^N$ and $X^S$ call forth severe necrotic local lesions ending in the destruction of the inoculated leaf, whilst $X^L$ may produce faint lightly necrotic local lesions but more often fails to do so. The strains $X^G$, $X^D$ and $X^H$ may produce very faint lightly etched local lesions, or may fail to produce any. If a series of young plants be inoculated at the same time with all six strains, an examination at the end of the first 15 days allows of a sharp division into two groups:

(1) Plants in which the inoculated leaf and adjacent stem have died back and new green shoots have appeared in the lower axils (fig. 17, Plate 20). Such plants are those infected by $X^N$ and $X^S$, the former being the more seriously affected.

(2) Those plants in which the inoculated leaves are damaged but little if at all, and the plant remains virtually intact and almost normal. This will include plants infected with $X^H$, $X^G$, $X^L$ or $X^D$.

This latter group, whilst clearly differentiated by the absence of any serious local

TABLE III. DIFFERENTIAL DIAGNOSIS OF STRAINS $X^D$ and $X^N$.

| | Tobacco and *Nicotiana glutinosa* | *Datura stramonium* | *Capsicum annuum* | *Hyoscyamus niger* | *Lycopersicum* | *Solanum tuberosum* | Physical properties |
|---|---|---|---|---|---|---|---|
| $X^D$ | No local lesions | No local lesions | Fine etched local lesions forming late, or none | No local lesions | No local lesions | Varieties: Arran Banner, Champion, Di Vernon, and Arran Scout behave as carriers | Withstands dilution up to 1 in 5000 |
| | Mild systemic mottle similar to that induced by $X^G$ | Mild systemic mottle | Systemic symptoms, occasional streaks on stem, and later yellow mottle and crinkle with necrotic spots | No systemic lesions | No systemic lesions | No lesions are developed on the stem in President, Arran Victory and other susceptible varieties | |
| $X^N$ | Numerous necrotic local lesions | Very extensive necrotic local lesions | Necrotic local lesions | Severe necrotic local lesions | Severely necrotic local rings and figures | Arran Banner develops an interveinal mosaic | Withstands dilution up to 1 in 100,000 |
| | Necrotic systemic infection similar to but more severe than that induced by $X^S$ | Necrotic systemic infection similar to but more severe than that induced by $X^S$ | Necrotic systemic infection which may kill the plant or destroy all older leaves and stem apex; new shoots arise from lower nodes | Slight veinal mottle and occasional necrotic spots: later growth normal in appearance | Systemic infection; necrosis of interveinal areas in young leaves | Arran Scout, Di Vernon and Champion develop interveinal necrosis and leaf-drop streak. Necrotic lesions are common on the veins of leaves and on the stem of varieties which develop interveinal necrosis | |

damage, may include plants in which there occur necrotic streaks in the stem but this, except in plants which are very immature when inoculated, does not lead to collapse.

If the plants be examined about 6 weeks after infection, a further differentiation will be observed. On the $X^N$ and $X^S$ plants, the inoculated leaves will be dead and hanging, and a new growth will have appeared from the axillary bud nearest to the basal end of the necrotic portion of the upper stem as already described. This new growth may be occasionally quite normal in appearance, or may develop an interveinal mottle and necrosis. In the former case it will be found to be free of virus (fig. 17, Plate 20).

The $X^H$, $X^G$, $X^L$ and $X^D$ plants develop in the young upper leaves etched figures more or less closely following the veins (fig. 18, Plate 20). These neither destroy nor seriously damage the leaves. Later the new apical growth becomes brightly mottled and the interveinal areas of the leaf are thrown into folds giving rise to a "crinkle-like" appearance. At the base of the leaves necroses develop, becoming more numerous as the plant ages (fig. 19, Plate 20).

Of the varieties of *Capsicum* used, viz. Cardinal, Red Cluster, Elephant's Trunk and Golden Dawn, the latter was found to give the most consistent and distinctive reactions.

*Capsicum* is not only valuable as distinguishing between $X^N$, $X^S$ and the remaining strains, but is the only plant so far experimented with which allows of the detection of $X^H$. It has a further advantage in that it distinguishes to some extent between $X^G$ and $X^D$, the latter producing a less destructive reaction than that induced by the former or by $X^H$.

### *Hyoscyamus niger*

*Hyoscyamus* develops local lesions when infected with $X^S$ or $X^N$ but not with other strains, they are at first sharply defined circular necrotic spots and no difference is observed between those produced by either strain. Later those induced by $X^N$ may appear rather more sharply outlined against the green background of the leaf but such affords no critical distinction. When, however, they are photographed through a green screen the two sets of lesions become differentiated; those following infection with $X^N$ are outlined by a black necrotic band enclosing the white necrotic spot originally observed (fig. 21, Plate 20) which is absent in the case of $X^S$ (fig. 20, Plate 20).

No systemic symptoms are developed on plants infected with $X^H$, $X^G$, $X^L$ or $X^D$; with $X^S$ and $X^N$, however, it is otherwise. The strain $X^N$ causes a veinal chlorosis with semi-necrotic spots in the younger leaves with but little damage; symptoms may be absent in the younger leaves. Infections with the $X^S$ strain result in a mass necrosis of the interveinal areas leading to wilting and a complete collapse of the younger leaves, a process which is reproduced in the new apical leaves as they unfold (fig. 23, Plate 21).

In *Hyoscyamus*, therefore, we have a plant which allows not only of a broad distinction being made between the group of the $X$ virus strains composed of $X^H$, $X^G$, $X^L$ and $X^D$,

all of which are of the mottle type on tobacco, and that consisting of $X^S$ and $X^N$ examples of a ringspot type, but it also affords some aid in the differentiation between the two members of the latter group.

The writer's thanks are due to his colleague, Dr R. W. G. Dennis, for the observations on the reactions of *Hyoscyamus*.

### *Lycopersicum*

On the tomato, variety Kondine Red, neither local nor systemic lesions are aroused by infection with $X^H$, $X^G$, $X^L$ or $X^D$. All these strains are carried in the tomato. The two virus strains $X^S$ and $X^N$, however, cause both local and systemic lesions and their respective reactions allow of a differentation between the two.

Infection with $X^S$ induces faint local and lightly necrotic lesions followed by a spotty systemic and generally non-necrotic interveinal mottle. The strain $X^N$ induces severely necrotic local ring lesions with fine black edges, and similarly outlined figures along the veins appear on the inoculated leaves (fig. 22, Plate 20). This is followed by interveinal necroses on the young leaves which fuse and bring about their collapse (fig. 24, Plate 21). For the reactions on these and other species of the family Solanaceae see Table II, p. 144.

The reactions enumerated above allow of a differentiation of the various strains. Thus $X^G$ and $X^L$ can be identified on tobacco or *Datura*, and $X^H$, masked on all species other than *Capsicum*, by the complete protection it affords to all plants against infection with any of the other strains. The strains $X^G$ and $X^D$ which behave similarly on the above plants are readily separated by their totally unlike behaviour on President, Arran Victory, Majestic and many other varieties. In the same manner $X^S$ and $X^N$ are distinguished by their reaction on potato, though an easier and more rapid differentiation is obtained by their respective reactions on *Hyoscyamus* and tomato. The identical reaction of $X^D$ and $X^N$ on the potato varieties President and Arran Victory commonly used as test plants, calls for more attention, and a table of differential reactions is given above (Table III).

## Inclusion or *X* bodies

In 1924 Kenneth Smith described inclusion bodies in the leaf of a potato suffering from mosaic disease. At that time the virus responsible was not known, but there can be but little doubt that the plant under examination was infected with the *X* virus now recognized as the commonest cause of simple mosaic mottling in the potato. In 1932 Salaman and Hurst made an examination of a large series of potato plants infected with the viruses *X*, *Y*, *Z*, paracrinkle and leaf roll. It was in the former only that cell inclusions were found. The *X* virus used to infect in these experiments was from the same source as that from which $X^S$ was later isolated. It is known now that this source contains together with $X^S$ some, probably a small proportion of $X^G$. As the

various strains were then unrecognized, and as some of these behaved so differently to the common stock of *X* virus generally used, it was thought advisable to examine fresh plants specifically infected with each strain. This work was undertaken for the writer by Miss F. M. Roberts, and the microphotographs were taken by his colleague Mr J. P. Doncaster; to both the writer conveys his thanks.

Infected plants of tobacco and *Datura* were employed for each strain. Miss Roberts' conclusions may be summed up as follows:

(*a*) Inclusion bodies were only observed with difficulty in fresh unfixed leaf tissue, hence all tissues were fixed in formalin-chrom-acetic under reduced pressure, after a momentary immersion in absolute alcohol. Sections were cut at 14$\mu$ and stained with gentian violet, methylene blue, safranine and light green, Heidenhain's iron haematoxylin or Flemming's triple stain. The first three were found to be the most satisfactory.

(*b*) In all the strains, viz. $X^H$, $X^G$, $X^L$, $X^S$, $X^D$ and $X^N$, inclusion bodies were found.

(*c*) In all strains the characters of the inclusions were the same.

(*d*) The size of the inclusion body is commonly greater than that of the nucleus of the cell, and may be nearly twice as big.

(*e*) The majority of the bodies were granular and deeply staining, though others with vacuoles were observed (fig. 36, Plate 23); in these latter it was not uncommon to find one or more deeply staining granules similar in appearance to nucleoli (fig. 36). In fixed material the bodies are so clearly outlined as occasionally to suggest the presence of a limiting membrane: this is probably an artefact.

(*f*) As between tobacco and *Datura* there was a slight difference in the localization of the inclusion bodies: in the former they were most frequently in the pallisade but were also found in the spongy parenchymatous tissue, the upper epidermis, and more rarely in the hair cells; in the latter they were found in the pallisade and upper epidermis. Those occurring in the pallisade tissue of *Datura* are usually elongated. In plants infected with $X^H$, however, none were seen in the latter layer. In neither species were inclusion bodies found in the guard cells. In tobacco *X* bodies were found in the hairs of the upper epidermis in infections with $X^D$, $X^S$ and $X^N$.

(*g*) Of the six strains of *X*, the two which are accompanied in the host plant by the most inclusions are $X^H$ and $X^L$. In $X^D$ infections they are rare, but those seen do not differ in type from those occurring in plants infected with the other strains.

(*h*) In $X^H$, and to a lesser degree in $X^L$, infections, groups of cells were found, each of which contained an inclusion body. In the case of $X^L$ such groups were found mainly in the pallisade and upper epidermis; in $X^H$ they were found only in the pallisade tissue.

(*i*) In infected plants, no matter by which strain, only one *X* body in any one cell was observed. In tobacco plants infected with the $X^N$ strain two nuclei were not infrequently found occupying a single cell, but no evidence of recent mitosis (fig. 37) was seen.

### Strains of the *X* virus recovered from the field

The incidence of the *X* virus and its strains in the common potato fields is a problem of considerable interest. At the writer's suggestion, Mr H. R. Hansen, a visiting post-graduate worker in 1934, examined eight plants culled direct from the field and two from the glasshouse: of the latter one had in past years shown itself virus-free but in 1934 developed a slight mottle; the other came originally from America and appeared to be healthy. It must be pointed out, however, that at this time we did not know of $X^H$, nor had the virus of Watery Streak been identified as the strain $X^N$ of the *X* group. The results of the investigation are summarized in Table IV. In this table an attempt is made to indicate the amount of each strain present. This is based on the following:

(*a*) The character of the infection resulting in the tobaccos from the first inoculation of potato material.

(*b*) The ease with which the types are separated.

(*c*) The virulence or otherwise of the types separated and their behaviour in further selective passage.

Table IV

| Lab. no. | Plant no. | Variety | Origin | Clinical conditions | Strains of *X* virus found: *G* | *L* | *S* | Other viruses |
|---|---|---|---|---|---|---|---|---|
| 5 | 1 | Arran Banner | Field, Cambridge | Healthy | × × × × | — | — | |
| 6 | 2 | Arran Banner | Field, Cambridge | Faint mottle slight rugosity of leaves | × × × | — | × | |
| 3 | 3 | Arran Banner | Field, Cambridge | Bright mottling, ruffling and rugosity of leaves. No stunting | — | × × × | × | |
| 2 | 4 | Arran Banner | Field, Cambridge | Faint mottling slight rugosity and a little stunting | — | × × | × × | |
| 7 | 5 | Majestic | Field, Cambridge | Apparently healthy | × × × | — | — | × ? new strain of *X* |
| 8 | 6 | Majestic | Field, Cambridge | Apparently healthy | × × × | — | × | |
| 4 | 7 | Majestic | Field, Cambridge | Bright mosaic, rugosity and ruffling of leaves and some stunting | × | × | × × | |
| 10 | 8 | Seedling of Arran Crest | Field, Arran | Top necrosis | — | — | × × × ×* | |
| 1 | 9 | Burbank | P.V.R.S. glasshouse | Healthy | × × | × × | × | |
| 9 | 10 | Arran Victory | P.V.R.S. glasshouse | Faint mottle | × × × | — | × | |

×, × ×, etc. is a rough indication of the amount of each type present.

* The virus recovered by Hansen from this plant would, from his description of symptoms, suggest the strain $X^N$.

It is further assumed that at any given moment in its life a plant is only capable of containing a definite amount of the *X* virus, no matter whether one or more strains are present. This total quantity has been represented by $\times\times\times\times$; the reason for this assumption will be found on p. 175.

It will be seen that in the case of Arran Banner, as well as in that of Majestic, the clinical reaction in the potato bears a distinct relation to the nature and the estimated quantity of particular virus strains present, and that the two more virulent forms of the virus, viz. *L* and *S* determine the character of the pathological changes exhibited by the plant.

The virus discovered in plant No. 7 has not been identified, but inasmuch as a preliminary infection of the *G* strain prevented its ingress to tobacco plants, it may possibly be yet another strain of the *X* virus.

Mr Hansen made a comprehensive study of the protective capacity of seven of the various *G* strains he isolated, as against the nine different $X^S$ strains which he had recovered from the plants under examination. Of the possible sixty-three cross-experiments, he performed forty-three and in each case the $X^G$ strains protected against each and all the $X^S$ strains. We may therefore conclude that in the field the $X^S$ and $X^G$ strains are identical with those we have encountered in the glasshouse and behave towards each other in a similar manner.

Hansen's work shows that both in the field and the glasshouse the *X* virus content of most naturally infected potato plants is rarely confined to a single strain, and subsequent work at Cambridge goes to confirm this. Natural infections, however, with either of the two extremes of the series $X^H$ and $X^N$ respectively, often appear to be pure.

In potato plants suffering from interveinal necrosis in the field only the $X^N$ strain has been found, though when a stock is carried on by tuber in the glasshouse it is not rare to find that in subsequent years in one or other plant of a clone a more or less complete clinical recovery has taken place. In such a case only $X^G$ may be found. It would, however, be quite unjustifiable to assert that the $X^N$ was in fact unmixed at the start without more careful analysis. The virulence of $X^N$ is so far in excess of that of $X^L$, $X^G$ and still more of $X^H$, that even were such to be present, they would need to be in very considerable concentration before their presence would be detected. If for some reason $X^N$ died out, then another strain, even if present in the smallest quantity, would have a chance of overrunning the plant. At the other end of the series we have found $X^H$ apparently unassociated with any other strain in all the otherwise healthy Up-to-date plants examined.

From experimental work we know that healthy potato plants infected with either $X^H$, $X^G$ or $X^L$ cannot in subsequent seasons be further infected by sap inoculation with $X^S$ or $X^N$. These facts should inspire caution before invoking mutation within the tissues of the potato as a general explanation of the mixed infections found in nature. It would seem more probable that potato plants in the field suffering from an infection with mixed strains had received such mixture either simultaneously, in a single

inoculation, or in two or more individual infections at short intervals of each other. Whatever the vector in nature is, it would seem to exert no selective action in the process of infection, though it is not impossible that the plant may do so.

## Strains of the *X* virus in the literature

That the potato virus known under the generic title *X* was pleomorphic has been recognized for many years both on the Continent and in America. The recognition, however, of specifically distinct strains is more recent and not so general.

In America Johnson (1925) distinguished between mottle and ringspot viruses and found that either might exist in an apparently healthy potato stock.

Koch (1933) clearly distinguished between mottle and ringspot; from his plates, however, it is evident that the one corresponds with a mixture of the writer's $X^G$ and $X^L$, and the other with his $X^S$. Koch does not suggest that they are sister strains but regards them as specific viruses. In a later paper with Johnson (1935) the same attitude is maintained.

Chester (1936) recognized a strain of the *X* virus which he describes as a masked potato mottle because it gave no reaction on tobacco or *Datura*: this is evidently the same as the writer's $X^H$ strain.

Putnam (1937) has recently described three viruses, all of which he has found in the potato and which he regards as members of the *X* group. They are: potato mottle virus, which would seem to be a mixture of $X^G$ and $X^L$; potato ringspot virus, the reactions of which on *Datura*, tobacco and tomato suggest that it might be equivalent to the writer's $X^N$. It is however quite uncertain whether it is pure, because when transferred by Putnam to various potato varieties it was "carried" in a symptomless condition, a result only to be expected seeing that none of his potato plants are virus *X* free. On extraction from these carriers a mild ringspot and/or a typical mottle was secured. From the plates submitted it would seem likely that Putnam's Ringspot virus is a mixture of $X^S$ and $X^N$. Putnam's potato yellow-mottle virus has some characteristics in common with the writer's $X^L$, but is differentiated from it, and indeed from all other forms of the *X* virus, by the bright yellow mottle induced in tomato and a thermal death-point of 73° C. It may be either a distinct specific virus or yet another strain of the *X* virus. The writer is inclined to accept the former alternative until its serological reactions are determined.

Köhler (1936) has distinguished several clinical types of *X* derived from one or other potato variety but has not proceeded to the detailed analysis which is necessary to disentangle the mixture of strains present. From the illustrations given, it is obvious that most of his types, derived as they are from field potatoes, are in reality mixed, and our experience with extracts of the *X* virus from the field would confirm this. There is little doubt however that he is dealing with mixtures of the same strains which have been described, more especially with $X^G$, $X^L$ and $X^S$, and that the type described by

him is determined by which of them is present in the greatest quantity and hence dominates the clinical expression. One of his types, Cs 35 (Köhler 1937), is identical in its effect on tobacco with the dissected *L* type described on p. 180 which has been definitely shown to be due to a mixture of *S* and *L*, a combination which requires many passages of selective inoculation to dissolve into its two components. Köhler divides the strains of the *X* virus into a mottle and a ringspot group (Köhler 1935*b*, 1937) and states that they have slightly different thermal death-points, and that they do not "protect" one against the other when inoculated into tobacco. The writer's experience (see p. 198) is directly opposed to this latter conclusion, whilst the evidence for the former cannot be regarded as statistically significant.

The tendency to form rings on tobacco, though not the exclusive property of the *X* virus, nevertheless distinguishes that virus and some of its strains from the entire family of tobacco mosaic and its seventy odd modifications, none of which calls forth ring formation. We have found, however, that when the "mottle" strains of the *X* virus are mixed *in vitro* with the virus of common tobacco mosaic and inoculated to tobacco, systemic rings develop as a result of the combination with the mottle strains $X^H$, $X^G$, $X^D$, and $X^L$. The demarcation of the rings by outspoken necrotic outlines varies but little with the virulence of the series of strains $X^H$ to $X^N$. This would seem to be further evidence in support of the view that there is no fundamental difference between the mottle and ringspot group of the *X* strain.

Böhme (1933*a*, *b*) also recognizes the existence of different varieties of the *X* virus, but as all of his produce a top necrosis on the President variety of potato, and hence contain the Up-to-date streak virus, it is obvious that he is not dealing with a single virus group. Like Köhler (1934) Böhme is of opinion that *X* variations undergo change within the potato plant of the nature of *Dauermodifikationem*, but as he has not evaluated the clinical effects of quantitative mixtures of really pure types and their mutual neutralizing interaction, such conclusions do not seem to be warranted by the evidence adduced. In Cambridge evidence suggesting such a change in two potato varieties is described on p. 160 and p. 163.

Hamilton (1932) described a virus she called Hy IV, which she found in a field plant of *Hyoscyamus niger* grown for commercial purposes and which she distinguished from the *X* virus by the fact that it would not infect the potato varieties Arran Victory, Arran Chief, President or Epicure. Miss Hamilton kindly gave the author material, and in 1933 it was successfully transferred to President and Epicure, killing the latter with top necrosis and calling forth a mottle in the former. It was found that prior inoculation of tobaccos with $X^G$ completely protected against Hy IV.

The symptoms in tobacco and *Datura* indicate that Hy IV is merely a mixture of *X* strains, and the suggestion is made that Miss Hamilton's difficulty in transferring the virus to potato was probably due to the lengthy period during which it had been acclimatized in *Hyoscyamus*.

### The purity of strains

The various strains of the *X* virus which have been isolated and described are defined by their reactions on a variety of Solanaceous plants. It should be pointed out that the reactions claimed to be constant are those observed within the limits of a specified environment, a temperature range of between 50 and 70° F., a well-lighted glasshouse and a high degree of humidity. The chief diagnostic plants used are the tobacco variety White Burley and *Datura stramonium*. As both of these are raised as seedlings a source of variation in response is to be found in any genetic heterogeneity there may be in the two stocks. The seedlings of White Burley have over a long period of years exhibited definite morphological variations such as broad versus narrow leaf, short versus high growing plants. The *Datura* plants present a picture of complete phenotypic similarity which presumably has its genetic counterpart in a more or less perfect homozygosity.

It is not surprising, therefore, that we find a certain amount of variation within any one strain in its behaviour on tobacco, and very little in its behaviour on *Datura*. The occurrence of a very faint mottle in some tobacco plants infected with $X^H$ at Rothamsted is probably to be explained on the basis of different environment including a possible genetic difference in the nature of the test plants which are there employed. Nevertheless, it must not be supposed that the strains are so fixed that they have not each a limited range of variation about a mean which has here been described as the norm. The difficulty is illustrated by the behaviour of the strain $X^G$; sometimes out of a group of plants inoculated at the same time one plant may be almost symptomless and another exhibit a very faint tortoiseshell-like mottle. It would be possible to explain this as partial mutations towards $X^H$ and $X^L$ respectively, but it is difficult to see how and why the mutated particles should congregate in such a manner as to reach one plant rather than another of a given series. It seems more likely that such differences of expression are in the main due to host environment. On the other hand, as already described, our tobacco cultures of $X^L$ after three years of isolation and continuous passage in tobacco became in 1937 more and more $X^G$ like, although their counterpart held for the same length of time in potato remained true to type. In this case we are forced to regard the change as occurring in the virus itself, and to be of the nature of a mutation of increasing quantities of the virus from $X^L$ to $X^G$.

If the virus particles we are considering are in reality highly complex fixed and definite molecular structures, then in a controlled environment it is to be presumed that their reactions will be absolutely constant. If they are living units a considerable range of variation in behaviour is to be expected. The relative constancy of the reactions exhibited by the strains here described lends some colour to the former hypothesis, as does the recent work on the isolation of certain specific nucleoproteins from virus infected plant juices.

### The protein base of the *X* virus

Bawden and Pirie (1937*b*), following on the work of Stanley and his co-workers as well as their own on the isolation of nucleoproteins of high molecular weight from the juices of plants infected with tobacco mosaic, have lately demonstrated the existence of similar protein bodies in the sap of plants of various species infected with the $X^H$ and $X^S$ strains of the *X* virus respectively.

All these workers are at one in considering that there is every reason to regard the bodies so derived from the tobacco mosaic group of infections as identical with the virus agent itself. Bawden and Pirie hold the same view in regard to the proteins they have isolated from the *X* strain infections. It is of much interest that they find no significant differences between the yield of such proteins from the juices of plants infected with the writer's masked strain $X^H$ and that from the virulent $X^S$.

The protein which these workers have isolated from *X* virus infected plant juices differs in its properties from those derived from tobacco mosaic infections, as widely and in the same directions as do the respective raw juices. The nucleoprotein of *X* virus is far less stable *in vitro* than that derived from tobacco mosaic, it is denatured at 70° C., reacts serologically with anti-*X* and not with anti-tobacco mosaic sera, and unlike tobacco mosaic it is destroyed by trypsin and hydrolysed at *p*H 3. Its solutions when inoculated to plants reproduce the same type of *X* infection as that which the plant exhibited from which it was derived. The infectivity can be destroyed by nitrous acid, formaldehyde, or ultra-violet rays without affecting its serological activity or its anisotropy of flow.

Anisotropy of flow, the capacity to make liquid crystalline solutions, and on concentration to form birefringent jellies, are properties shared by the *X* virus proteins with those derived from tobacco mosaic and its strains. On the other hand, no crystals or paracrystals, as Bawden and Pirie prefer to call the needle shaped crystal-like structure observed in tobacco mosaic preparations, have been secured from *X* virus infections. Instead of crystal-like structures an amorphous deposit is obtained on separation and purification of *X* juices with acid or ammonium sulphate. The purified product like that obtained from tobacco mosaic juice is far less readily filtered, and it is suggested that in the process of purification an aggregation of small particles into chains has taken place. Characteristic of the *X* virus product is its extreme isotropy, a feature present in a lesser degree in the corresponding preparation from tobacco mosaic.

The workers in this new field of plant virus disease investigation have emphasized the fact that the peculiar protein body they isolate from infected juices (*a*) represents almost all the soluble protein present in the juice; (*b*) that it contains 95% and over of the total virus present; (*c*) that both in the case of tobacco plants infected with mosaic virus and of those infected with the *X* virus, a given quantity of raw infective juice in each series always yields an equivalent weight of the protein; (*d*) that there is every reason to regard the protein and the virus agent as one and the same.

These conclusions constitute a great advance in our knowledge of viruses and their importance cannot as yet be fully gauged. One deduction made by the writer several years back from other and different reasons and referred to on p. 170 would seem to receive confirmation, namely, that a plant infected with one or more than one strain of any one virus contains at any given moment in a given environment the maximum number of virus particles, irrespective of the number of strains involved.

### The serological reactions of the strains of the *X* virus

The writer's colleague, Mr F. C. Bawden (Spooner and Bawden 1935, Bawden 1935) has reported on the serological reactions obtained with the strains $X^G$, $X^L$, $X^S$ and $X^D$. He found that all of them induce precipitin and neutralization reactions with an anti-$X^G$ or an anti-$X^S$ rabbit serum and observed no difference between their behaviour in these respects.

Both Bawden and Spooner on behalf of the writer have kindly tested the reactions of the $X^N$ and $X^H$ strains; they find that, as with the former group both the $X^N$ and $X^H$ strains produce precipitin reactions with either an anti-$X^G$ or anti-$X^S$ rabbit serum, the masked variety $X^H$ being particularly rich in antigen. The neutralization of $X^H$ by antisera was not attempted because the absence of symptoms in infection with this strain would render any quantitative assessment of the relation of antigen to antibody practically impossible. The neutralization of the virulent $X^N$ strain has been undertaken for the writer by Mr Bawden. The following is the protocol of his experiment.

Expressed sap was diluted 1 in 5 and added to equal volumes of saline, anti-tobacco mosaic serum and specific anti-$X^G$ serum, thus producing a dilution of 1 : 10, mixed and allowed to stand for 2 hr., after which it was inoculated to tobacco and the lesions counted and expressed as the average number per leaf:

| | |
|---|---|
| $X^N$ + saline | > 600 lesions per leaf. |
| $X^N$ + tobacco mosaic anti-serum diluted 1 : 5 | > 400 lesions per leaf. |
| $X^N$ + tobacco mosaic anti-serum diluted 1 : 25 | > 520 lesions per leaf. |
| $X^N + X^G$ anti-serum diluted 1 : 5 | > 75 lesions per leaf. |
| $X^N + X^G$ anti-serum diluted 1 : 25 | > 225 lesions per leaf. |

The $X^N$ sap though diluted 1 in 5 is still excessively virulent, a greater dilution would show still more clearly the neutralizing effect of the anti-$X^G$ serum.

It has been suggested that the *X* virus possesses certain antigens in common and others peculiar to each strain. So far no work has been reported on these lines, but now that we have pure lines of each strain there should be no difficulty in putting this theory to the test by absorption experiments. Chester (1936) demonstrated the existence of antigens common to the strains of ordinary and aucuba mosaic of tobacco, as well as of such peculiar to each. Bawden and Pirie (1937*c*) have further enlarged our

knowledge of the antigens of the tobacco mosaic group by the inclusion of enation mosaic, which they show has certain antigens in common and others peculiar to itself. Of great interest is their comparison of the serological behaviour of Ainsworth's cucumber viruses Nos. 3 and 4. Whilst finding that these have certain antigens in common with the tobacco mosaic group and others presumably peculiar to themselves, they see reason to believe that those which are held in common may be present in widely different quantities in the two groups. Such an explanation might apply to a yet unidentified antigen responsible for virulence. Strains such as $X^H$ and $X^N$ would then differ from one another in proportion to their respective content of the antigen in question.

## Physical characters

The physical characters of the *X* virus strains so far ascertained are given in Table V. For the particle size I am indebted to Dr Kenneth Smith: the figure given by him, considerably higher than the estimates of 75 m$\mu$ made in earlier years, is derived from filtration end-point experiments with gradacol membranes.

Table V

| | Physical properties | | | | | |
|---|---|---|---|---|---|---|
| | $X^H$ | $X^G$ | $X^D$ | $X^L$ | $X^S$ | $X^N$ |
| Longevity in raw unclarified juice at room temperature | 4 months | 5 months | 4 months | 5 months | 4 months | 4 months |
| Thermal death-point ° C. | 68 | 68 | 68 | 68 | 68 | 70 |
| Dilution end-point | 1 in 3000 | 1 in 10,000 | 1 in 5000 | 1 in 10,000 | 1 in 10,000 | 1 in 100,000 |
| Particle size (in m$\mu$) | 113 | 113 | 113 | 113 | 113 | 113 |

## The separation and purification of the three strains of the *X* virus

It has been stated that the $X^G$ form of the virus was obtained at once by the punching out of a green area in an *X* infected tobacco. The strain has been carried on in tobacco for over forty generations with only slight variation of symptoms. In one line we have twenty-four consecutive generations in which no selection of inoculum in relation to symptom expression of the parent plant was made, and the end product proved to be as good a type of $X^G$ as did the original source. We may state therefore that passage *per se* has no effect in altering the virulence—as expressed by symptom development—of the $X^G$ strain of the *X* virus.

When the cultures were more closely examined, it was seen that they were not all strictly alike. In a number of plants a greater or lesser number of small bright yellow spots were found superimposed on the normal $X^G$ mottle. In those cases where the inoculum was obtained exclusively from these yellow spots the inoculated plants exhibited an increasingly more virulent type of reaction. These facts led to the view

that although the $X^G$ strain reacted generally in a uniform manner, the cultures were nevertheless contaminated with a more severe type of virus.

It was natural to suppose that inasmuch as the *L* type had been recovered from another spot on the same leaf of the same plant as that from which the *G* strain was derived, we should find that the virus strain which was acting as a contaminant would be the *L* type. Curiously enough, the $X^L$ type was only recovered twice in the $X^G$ series even when it was put to the most severe analysis. The first occasion was early in the history of the strain, viz. in its third passage, when the *L* type appeared without any directive selection. The second appearance was in culture occurring in the fifth passage after filtration through an L3 candle and as a result of direct selection from the yellow spots on an $X^G$ mottled plant.

## Separation of $X^S$ from $X^G$

In order to test out the nature of the virus in the yellow spots, specimens of the same, as well as of green unmottled areas, were punched out with a fine punch of rather less than 1 mm. diameter, made by grinding down a subcutaneous injection needle,* and used as inoculum. As the yellow punched inoculum gave rise to local lesions these were often punched out and used as inoculum and frequently gave an immediate and complete $X^S$ reaction. In other cases, more particularly where the yellow systemic spots were selected, a readily recognized mixture of $X^G$ and $X^S$ symptoms was obtained and further selective punchings were necessary before the pure *S* type was isolated.

Although local lesions on the White Burley variety of tobacco are produced by the $X^S$ and $X^N$ strains alone, it does not follow that the local lesions resulting from a mixed infection will contain only the $X^S$ or $X^N$ strains to the exclusion of others. Indeed, mixtures of strains in which $X^S$ is predominant and $X^G$ or $X^L$ are in association have been frequently obtained throughout the work from carefully punched out local lesions. On the other hand, isolation of the $X^S$ strain by local lesion puncture is a practical and valuable method if the virus sap be previously diluted to about 1 in 10,000. In such cases a separation of *G* and *S* has been effected at one stroke.

A culture of $X^G$, which had been carried through fourteen unselected cultures without appreciable variation of symptoms, was subjected to sustained selective analysis. The inoculum was obtained by means of the 1 mm. punch from either the yellow or green areas of the systemic mottles or from the local lesion tissues as required. The $X^S$ strain was readily recovered from an apparently pure $X^G$, and in some cases the $X^S$ was unmixed with any other strain as shown by the fact that inoculum from the green areas induced in some cases a completely negative result, in others, an $X^S$ infection fully as severe as if the inoculum had been derived from a yellow necrotic area. The former is taken as evidence of the absence of any less virulent strain, whilst the latter is due to the fact that $X^S$ had begun to invade the green tissue, and there being no $X^G$ to

* In all the subsequent work selections were made with this punch.

hide it, showed itself at its full strength in the inoculated plant. Examples of both types of reaction have occurred frequently throughout the cultures and are represented in diagram I.

### Separation of $X^G$ from $X^S$

The $X^S$ culture used was that already referred to as No. 723 and is the direct descendant of the *X* virus stock isolated by Dr Kenneth Smith in 1928 from an Up-to-date plant. This culture has been carried through many hundreds of passages since that time without any *intentional* selection, but as has already been explained with an inevitable although unconscious direction towards the attainment of utmost virulence. For the last six years at least there has been no departure in the character of the reaction.

The method used for separation was a combination of dilution and selection. Inoculation of a 1 in 10,000 diluted juice into four *N. glutinosa* plants resulted in two developing local lesions only from which pure $X^S$ strains were obtained, in one which exhibited a full $X^S$ reaction and finally one which displayed an $X^G$ reaction with a single yellow spot on one leaf; isolation of the yellow spot led eventually to a separation of pure $X^S$ and pure $X^G$. Selection from the faintly mottled *G* areas led to cultures which exhibited the $X^G$ character with some slight evidence of $X^S$, Nos. 24 and 25.* Further selective inoculation yielded a pure $X^G$.

The pure $X^S$ line has been maintained through a large number of passages, in all of which inoculum punched out from the green tissue has either given an $X^S$ or a completely negative reaction.

### Separation of $X^G$ from $X^L$

Separation of the two strains in a condition of approximate purity was both easy and rapid; the complete isolation of $X^L$ from all admixture of $X^G$ is not so. The weaker strain $X^G$ has appeared without specific selection after the fifth passage on an apparently pure line of $X^L$, and again in the 21st passage. Reference has already been made to the fact that during the season 1937 it was found difficult to maintain $X^L$ at its normal standard of reaction in tobacco, the line apparently reverting to a weaker and more *G*-like type.

The $X^L$ type, with the exceptions noted above, assumed a stable and characteristic type of reaction which remained unaffected throughout some thirty generations of unselected culture. During this period seven selections were made from the green vein-banding areas and the central yellow ones respectively, with the object of separating any $X^G$ strain which might be present. The resultant infections, however, showed so little departure from the normal that it was assumed that there was no appreciable quantity of $X^G$ present in the $X^L$ cultures.

* These numbers refer to cultures in Experimental Group No. III, the diagrammatic representation of which has not been reproduced.

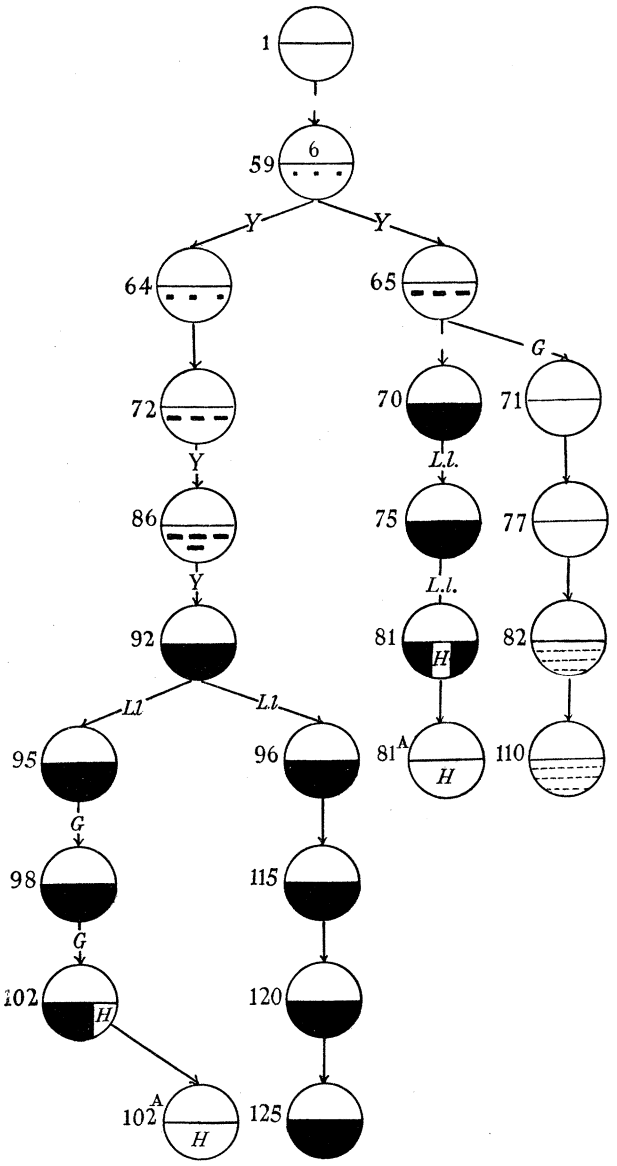



DIAGRAM I. Being an extract from Experimental Group No. II; shows how selection applied to an apparently pure culture of $X^G$ in tobacco resulted in the extraction of a pure culture of $X^S$. Each circle represents a minimum of three plants; if more are involved, the number is inserted in the centre of the circle. If not otherwise stated, all are tobacco seedlings. An all-white area in the lower hemisphere represents the faint mottle characterizing an $X^G$ infection; black blocks within the same, represent in proportion to their size, the presence of necrotic or yellow spots coexistent with the $X^G$ mottle. Horizontal hatching on a white background represents the tortoiseshell marking characterising an $X^L$ reaction; a broken line indicates that the pattern is only poorly developed. An all-black lower hemisphere represents a fully developed $X^S$ reaction. Where part of the lower hemisphere is white and marked with an *H*, it represents one or more plants which are free of any infection; the actual area represents the number of plants involved. *G* = inoculum removed from a green area of the leaf by means of a 1 mm. punch. *Y* = inoculum removed in a similar manner from a yellow spot or mottle. *L.l.* = inoculum derived from a punched out local lesion. Where there is no letter, the inoculum was obtained from a portion of the leaf containing both yellow and green areas.

### Separation of $X^S$ from $X^L$

The possibility of extracting $X^S$ from $X^L$ cultures, remarkable for their consistent uniformity, was explored.

Continued selection of inoculum from yellow and necrotic areas was carried out through nineteen generations. After four generations a certain superficial necrosis of the epidermis overlying the yellow areas of the leaf became a common feature producing a silvery etched appearance. This "etching" at first appeared in the centre of the yellow areas enclosed within the green vein-banding (fig. 25, Plate 21), or sometimes in the same plant it might take the form of fine rings or irregular figures which persist after the typical vein-banding has faded (fig. 26). This was a considerable advance on the occasional etched spot which had previously been observed both in the selected and unselected early cultures represented in diagram IV, Nos. 57, 97, 100, etc. Continuing such selection, "etching" became a general feature throughout the cultures, invading increasingly larger portions of the yellow areas of the younger leaves, until a stage was reached when the yellow etched areas tended to coalesce at the expense of the green vein-banding, the remains of which were to be seen lying along the major and secondary veins (fig. 27, Plate 21).

The appearances just described are best seen in the leaves immediately succeeding those which were inoculated; leaves developing later on the same plant often tend to revert to the more normal $X^L$ pattern (see figs. 28 and 29, Plate 21). In all the mixed types, as the plants age their newer leaves tend to become alike in that they approach the normal and almost unmottled condition after attaining an age of about four months.

As further selection continued, a new form differing sharply in degree rather than in type from that already described, made its appearance rather suddenly in the 9th passage.

The new features are an intensification of the "etching" so that it more nearly approximates to the necrosis of an $X^S$ infection, and at the same time a subdivision of the vein-banded areas characteristic of the $L$ strain into ever smaller mosaic patterns which creates a recognizable clinical picture which will be referred to as the dissected $X^L$ pattern.

The dissected $X^L$ pattern occasionally exhibits a new and distinct feature inasmuch as the central portions of the small yellow heavily etched areas bounded by the dark green vein-bands are replaced by green tissue, so that the etched area itself becomes a band lying between two green lines of tissue. Such a condition is seen in fig. 30, (Plate 21). The significance of the change in terms of the relative quantities of the two strains present has not been determined.

Continued selection may lead to further intensification of the dissected $X^L$ pattern as is seen in fig. 31, Plate 22, a condition which may persist unchanged through several more passages till it quite suddenly passes over to that normally produced by the $X^S$

strain. This last change remains fixed. In diagram II is shown the progressive change in reaction resulting from selective inoculation during thirty-two successive passages.

The distinctive character of the dissected $X^L$ pattern and its persistence, unchanged often through as many as nine selective passages, led to the supposition that it might be evoked by yet another and independent strain of the virus. Experience shows that these lines eventually end in the $X^S$ strain pattern, or that the $X^S$ strain can be extracted from a local lesion or a severely etched portion of the leaf, whilst inoculum from the green vein-banding still yields the compound type from which the $X^S$ strain can eventually be won by further selective inoculation.

In order to examine the nature of the dissected $X^L$ pattern, experiments with graduated mixtures of $X^L$ and $X^S$ were made in the following proportions and inoculated to batches of three tobacco plants, viz. $L:S::99:1$; $L:S::49:1$; $L:S::19:1$; $L:S::3:1$; $L:S::1:1$; $S:L::9:1$; $S:L::19:1$; $S:L::49:1$. In no case did the infected plants exhibit the dissected $L$ pattern; subsequent selective inoculation, however, caused it to appear in all the groups. Its appearance following infection with a selection of the green tissue of the $X^L$ clinical reaction resulting from the inoculation $X^L$ 49 : $X^S$ 1 indicated the lowest dilution of $X^S$ required to produce the simplest form of the dissected $L$ reaction. Obviously, but a very small portion of $X^S$ is necessary for the conversion under the conditions of this particular experiment. That a much higher proportion is necessary in a long-standing natural infection would appear from the work on the established $X^L$ cultures as represented in diagram II. Subsequent separation of the components by selective inoculation led to the appearance of the dissected $X^L$ type. It proved, however, much easier to separate out the $X^S$ clinical pattern from the artificial mixture series than it did in the natural $X^L$ series. Whether this is due to the difference in the original proportions of the two strains present, or to the longer association of the two in the latter, is not clear, but it was observed that when the proportion of $X^S$ in the original mixture reached $1\,X^S : 3\,X^L$ and over, the separation of the full $X^S$ type is readily effected.

We may conclude that the dissected $X^L$ pattern is due to a mixture of the $X^L$ and $X^S$ strains in which the latter preponderates, and not to an independent strain of the virus as Köhler (1935*b*) and some others have thought.

### Reactions induced by mixtures *in vitro* of pure strains of the *X* virus

Holmes (1929, 1930), Price (1930), Bald (1937) and others have shown that in the case of viruses which produce local lesions on suitable test plants, the number of such lesions gives an accurate measure of the relative strength of the virus in two or more diluted suspensions when the experiment is conducted on a reliable statistical basis. Best (1937), however, has proved that both with tobacco mosaic and tomato spotted wilt such proportionality occurs only over a limited range of low concentration. This method might be a suitable one for estimating the proportion of $X^S$ or $X^N$ in a mixture

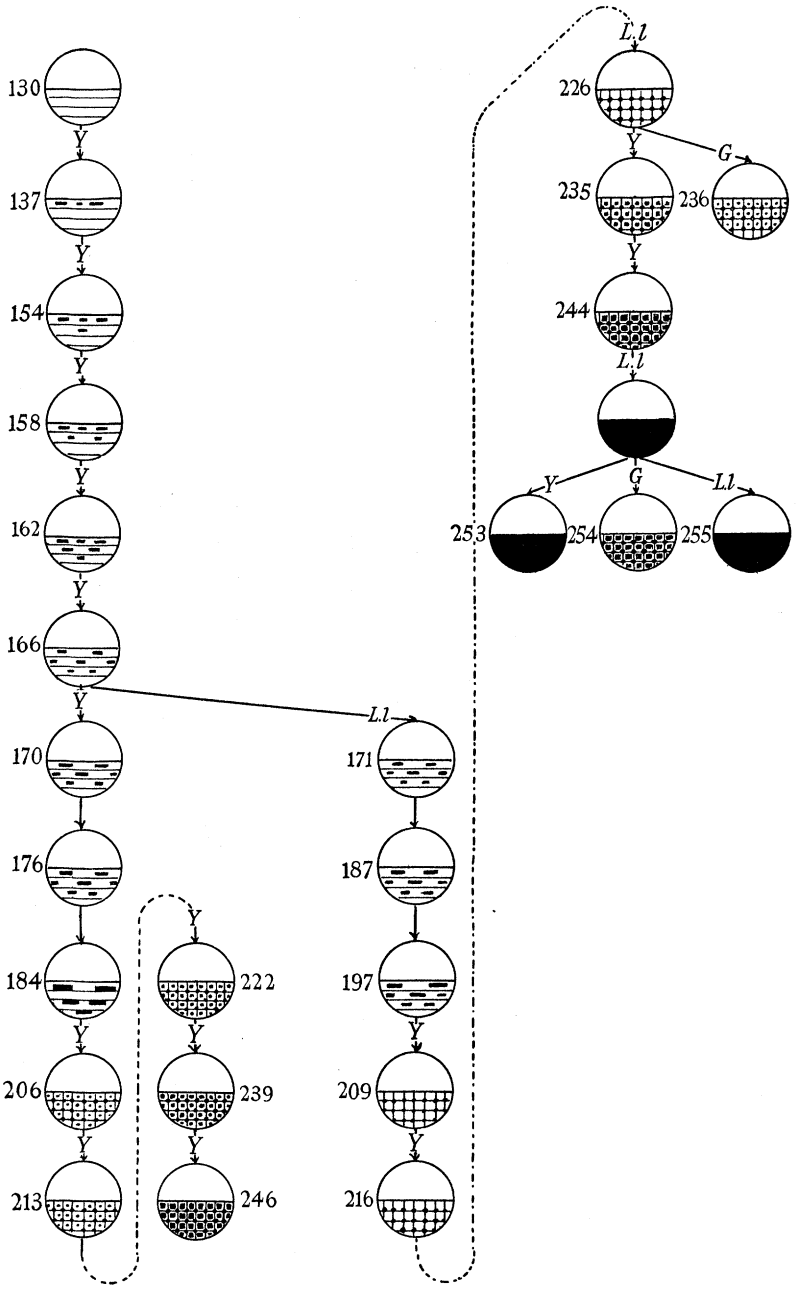



DIAGRAM II. Being an extract from Experimental Groups IV and V, shows how selection of yellow areas in an apparently pure culture of $X^L$ has resulted in the appearance, first of an etched form of the $L$ pattern, then in a new clinical type, the dissected $L$ pattern and, finally, in a typical $X^S$ pattern. $G$ = inoculum removed from a green area of the leaf by means of a 1 mm. punch. $Y$ = inoculum removed in a similar manner from the yellow or, later in the series, from the etched yellow areas. *L.l.* = inoculum derived from local lesions. Each circle represents a minimum of three tobacco plants. The plain horizontal hatching of the lower hemisphere indicates the normal tortoiseshell pattern of an $X^L$ infection. The small black areas interposed between the horizontal lines indicate the amount of light necrotic etching present. The portcullis pattern indicates the dissected $L$ pattern and the thickening of the junctions of the lines and the size of the black centres of the small intersected areas indicate the increasing complexity of the leaf pattern and the attendant necrosis of tissue. A full black pigmentation of the lower hemisphere indicates the normal pattern following an $X^S$ infection. Culture No. 130 is the 17th successive passage from the first plant in which the typical $X^L$ tortoiseshell pattern was observed. During the period prior to the 17th passage three selective inoculations were made with "$Y$" inoculum without change of reaction.

of strains were the whole sufficiently diluted, but for the following difficulty. It was found that when raw juices containing the $X^S$ or $X^N$ strains were used the lesion counts were greatly reduced and that the admixture of sap, whether of normal plants or of those infected with one of the non-local-lesion producing strains, inhibited the formation of the lesions which the $X^S$ or $X^N$ strains might have been expected to have produced were they alone. For this reason the effect on healthy plants of mixtures of strain was expressed in terms of the systemic reaction brought about and the quantities of each constituent strain in the mixture as the relative volume of the juice employed.

It soon became clear that the presence of a strain of low virulence actually protected the plant against one of higher virulence, and that in a degree roughly proportionate to the relative quantities of the two strains present in the inoculum. Although the occurrence of such protective action is obvious enough, the interest in its occurrence was overshadowed by the problem of protective inoculation, which was being attacked from a different angle. This subject is considered in a later section (p. 198).

Various mixtures of the strains have been made *in vitro* and the effect tried out on tobaccos and *Daturas*. The method employed in most of the experiments has been to mix the expressed juices of tobacco plants infected with the respective strains and after two hours to inoculate the same to tobaccos, *Daturas*, or *Nicotiana glutinosa*. The results are shown below in Table VI.

Before discussing them it is necessary to consider what effect as registered by the clinical reaction of the inoculated plants is due to the admixture of plant sap as such on the reaction produced by the more virulent strains $X^L$ or $X^S$.

Experiments were made in which both the $X^S$ and $X^L$ strain were diluted with the sap of healthy plants and the reactions compared with that following dilution with similar quantities of $X^G$ infected sap.

The $X^L$ strain mixed with untreated healthy juice of tobacco or *Datura* produces a somewhat less virulent result than when unmixed or diluted with a similar volume of water or saline; on the other hand, when the sap of healthy plants was centrifugalized and filtered through kieselguhr and used as a diluent, such reduction of virulence was not observed. If plant sap containing $X^G$ strain replaces that of healthy plants at the same dilution, the resultant effect is consistently less virulent.

It is preferable, therefore, when studying the interaction of virus strains to employ clarified sap rather than the fresh expressed juices.

The dilution of the $X^S$ strain with purified healthy tobacco sap had no effect on the systemic symptoms evolved, but dilution with tobacco sap containing the $X^G$ strain whether purified or not reduced in a very marked degree the clinical effect of the $X^S$ strain.

Turning to Table VI it will be seen that the $X^G$ strain in the presence of the $X^L$ dominates the clinical picture if it is in a ratio of about 9 : 1 and successfully masks the $X^S$ strain at a dilution of about 5 : 1.

Inasmuch as we do not know the number of virus particles present in any given

plant juice extract, nor whether two plants of the same size grown under like conditions and inoculated with the same or different strains of the virus contain the same quantity of virus, it might be expected that the resultant effects of mixtures made *in vitro* would vary considerably. This, however, is not the case. Inoculation with the mixture $9G:1L$ has been repeated at different times with remarkably constant results. This agreement suggests the view held by the writer that at any given moment a tobacco plant which is systemically infected with either one or a mixture of the strains of the *X* virus contains the maximum number of virus particles it is capable of holding under the existing conditions, and that that number is a function of the plant and the virus and is independent of the relative quantities of the virus strains present.

TABLE VI

| Strains of *X* virus present | Proportion of infected juices present | No of* experiments | Resulting clinical type and range of variations as seen in White Burley tobacco or/and *Datura* plants |
|---|---|---|---|
| $X^G : X^L$ | 1 : 1 | 1 | *L* Normal tortoiseshell pattern |
| | 2 : 1 | 2 | *L* ,, ,, ,, |
| | 3 : 1 | 1 | *L* ,, ,, ,, |
| | 4 : 1 | 1 | *L* ,, ,, ,, |
| | 9 : 1 | 8 | *G* An occasional plant may show a weakly developed *L* pattern |
| | 19 : 1 | 1 | *G* With an occasional dull yellow spot |
| | 49 : 1 | 1 | *G* Passing in some to a weak *L* with dull vein-banding |
| | 99 : 1 | 1 | *G* Similar to above |
| | 249 : 1 | 1 | *G* Mild *G* |
| | 499 : 1 | 1 | *G* Very mild *G* |
| $X^G : X^S$ | 79 : 1 | 1 | *G* Mild with occasional yellow spot |
| | 39 : 1 | 1 | *G* ,, ,, ,, |
| | 19 : 1 | 1 | *G* ,, ,, ,, |
| | 15 : 1 | 2 | *G* With several yellow spots |
| | 9 : 1 | 2 | *G* With many yellow spots |
| | 4 : 1 | 2 | *G* With bright spots suggestive of dissected *L* pattern |
| | 3 : 1 | 1 | *G* ,, ,, ,, ,, ,, |
| | 2 : 1 | 1 | *S* Not fully developed |
| | 1 : 1 | 3 | *S* Almost fully developed |
| | 1 : 1·5 | 1 | *S* ,, ,, ,, |
| | 1 : 2 | 1 | *S* ,, ,, ,, |
| | 1 : 3 | 2† | *S* ,, ,, ,, |
| | 1 : 7 | 1 | *S* ,, ,, ,, |
| | 1 : 15 | 1 | *S* Fully developed |
| | 1 : 31 | 1 | *S* ,, ,, |
| | 1 : 63 | 1 | *S* ,, ,, |
| $X^L : X^S$ | 3 : 1 | 1† | *L* Dissected type, with considerable necrosis |
| | 1 : 1 | 1† | *L* With severe necrotic mottle |
| | 1 : 3 | 1† | *S* Almost pure but with some definite vein-banding |

* In each experiment either 3 plants of tobacco or 5 of *Datura* or 3 of *Nicotiana glutinosa* were used. Most of these trials were conducted in the early months of 1933.

† Further experiments in each of these classes carried out with plant juice, centrifugalized and filtered through kieselguhr yielded identical results.

A further series of mixtures *S* and *G* were made in 1935, viz. $1S:4G$; $3S:7G$; $2S:3G$; $1S:1G$; $3S:2G$; $7S:3G$; $4S:1G$; and $9S:1G$; and inoculation made on

to four *Nicotiana glutinosa* plants in each case; the resultant infections were in general agreement with those recorded above except that the $X^G$ damps down the $X^S$ more effectively in *N. glutinosa* than in either tobacco or *Datura*.

## Double reactions

### *Mixture of the Y virus and the X strains on tobacco*

Kenneth Smith showed that when the *X* and *Y* viruses infected tobacco plants in conjunction, the vein clearing normally induced by the *Y* virus was exaggerated. The veins took on a bright yellow colour and became necrotic, whilst the subsequent mottling was replaced by a deeply necrotic spotting. The condition thus induced corresponds to the spot necrosis of earlier observers.

With the separation of a number of strains of the *X* virus differing in virulence it became desirable to observe whether their respective combinations with the *Y* virus induced different clinical pictures to those originally described by Smith.

It was found that the strains $X^S$ and $X^N$ which, acting alone produce local lesions on tobacco, in combination with *Y* do likewise; whilst the strains $X^G$, $X^L$ and $X^D$, which do not bring about such when alone, are still without that power when in combination with the "*Y*" virus.

As regards vein clearing, the reactions of the double infections vary in intensity in exact relation to their own individual virulence (fig. 38, Plate 24). Thus $X^H$ and *Y* produce but little intensification of the normal *Y* reaction; $X^G$ and *Y* bring about a considerable brightening of the veins with a light necrosis at various points. A similar but much more definitely necrotic condition is induced by $X^L$ and *Y*.

The mixture $X^S$ and *Y* induces a still more severe and necrotic vein-banding which may cripple the leaf if the plant be very young; when $X^N$ is substituted for $X^S$ in the mixture, however, the results are invariably more severe and end in a pronouncedly necrotic vein-clearing, leading to the collapse of the whole leaf.

The subsequent systemic reactions of the infected plants intergrade in a precisely similar manner, the necrotic spotting being but slight when $X^H$ is present, increasing in intensity with $X^L$, and still more so with $X^S$. The combination of *Y* and $X^N$ is so severe as to be generally fatal to all the earlier formed leaves and frequently leads to the death of the plant. All of these results are based on three, and in some cases, four trials for each combination in which 3 test plants for each mixture were used.

It may be said that when the strain of the *X* virus is constant and that of the *Y* varied, a similar graded set of reactions results. These will be referred to in detail in a later publication.

### *Mixture of the "A" virus of Murphy and the X strains on tobacco*

As is well known, the combination of $A+X$ produces a crinkle in most potato varieties; efforts to effect a combination with the various strains in plants of ten different

varieties of potato all failed because the "$A$" virus obtained no entry by inoculation although carborundum was used. A series of mixtures on tobacco plants was more successful. In this plant $A+X$ produces a reaction similar in character to that induced by $Y+X$ but of much less intensity. Such at least is true of the combinations $A+G$, and $A+X^L$; the combinations $A+X^S$ and $A+X^N$ are not to be distinguished from uncomplicated infections of $X^S$ and $X^N$ respectively, fig. 44, Plate 25.

*Mixtures of common aucuba and masked tobacco mosaic strains virus and those of the X virus*

These reactions (fig. 39, Plate 24) which have been studied in close co-operation with my colleague Dr Dennis, have brought to light new facts of importance. Infection with any of these mixtures on tobacco is heralded by the formation of local lesions which do not occur as a result of simple infections with either $X^H$, $X^G$, $X^L$ or any of the three tobacco mosaics when operating alone. They appear in five days when $X^S$ or $X^N$ are present with either of the three tobacco virus strains, and in 11–15 days when any of the remaining $X$ strains take their place. The lesions are fully necrotic and in the case of either $X^H$, $X^G$, $X^D$ or $X^L$ are similar both in number and in the destruction of leaf surface involved. When $X^S$, and to a still greater extent when $X^N$ is the partner, the local lesions are so rapidly formed, so deeply necrotic and extensive, that they cause the inoculated leaves to wither and drop.

The systemic lesions also fall into two groups: those occurring when $X^H$, $X^G$, $X^D$ and $X^L$ are partners, and those when $X^S$ or $X^N$ take their place. In the first group the reaction is remarkably uniform and is accompanied by a considerable amount of rather superficial necrotic destruction of tissue. When $X^L$ was the partner of any of the three tobacco strains the reaction was somewhat less severe than that produced by any of the others not excluding $X^H$ (see fig. 40). This fact affords further evidence for the substantive nature of this strain. In appearance the reaction consists of the well-known mottle of pale and dark green areas common to simple tobacco mosaic infection, but on the pale areas are superimposed lightly necrotic rings. When the aucuba strain is present the plants later exhibit brighter yellow areas which distinguish them from plants infected with mixtures in which the two other tobacco mosaic strains are present. The leaves are narrower, reduced in size, the surface slightly rugose and the contour irregular, but the actual destruction of tissue does not involve the whole thickness of the leaf. In the second group, when $X^S$ and $X^N$ are partners, necroses are greatly increased both in extent and severity; often the damage is so great that the earlier formed leaves succumb, and if very young plants have been used, they generally die. The $X^N$ mixtures always produce more severe symptoms.

A discussion of the problem raised by these reactions and an hypothesis designed to explain it will be found on p. 207. The graph (fig. 40) records the variations in respect to the virulence of the reaction produced on young seedling tobacco plants when infected with each of the six strains of the $X$ virus alone, with the masked, common and aucuba strains of tobacco mosaic, and the mixtures of each of these latter with each of

the *X* virus strains. The degrees of virulence are designed so that non-necrotic mottles fall below 5, whilst 10 represents a lethal result.

It was found that the strain $X^H$ alone produced no symptoms on tomato, but the combination of the two viruses called forth a bright interveinal mottle on the upper leaves, some ruffling of the intermediate ones, in addition to a mild interveinal mottle. On the lower older leaves a few necrotic spots may develop. There is no streak of the stem, nor any impairment of growth in the plant.

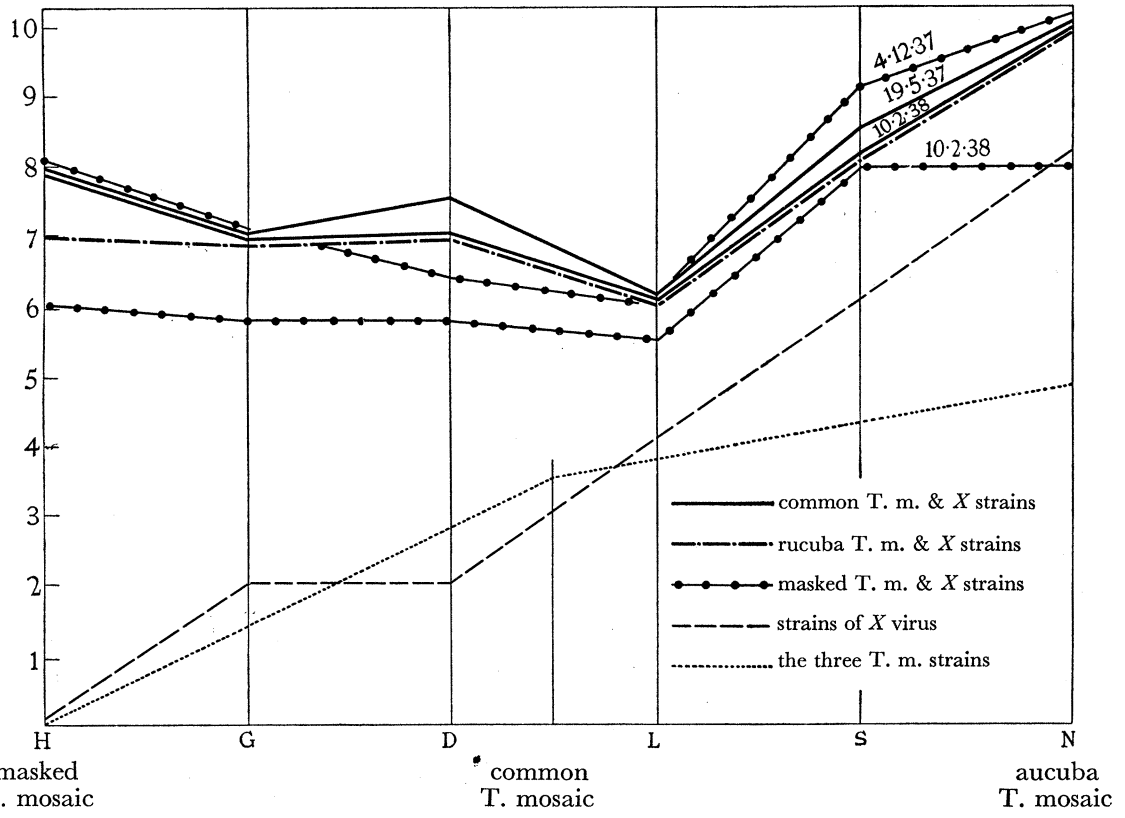



Fig. 40. 1–10 = Degrees of virulence: 1–5 being mottles; 5–10 mottles with necrosis of tissue. H–N = Strains of the *X* virus.

The strain $X^G$ induces a mild interveinal mottle on the younger and some yellow spotting on the older leaves; the addition of tobacco mosaic intensifies the mottle but is not followed by any streak of stem. There is again no noticeable check to the plant's development.

The strain $X^L$ on tomato has a similar effect to that produced by $X^G$, except that the mottle is brighter when tobacco mosaic is added; in addition there is much ruffling, a coarse interveinal mottle with bright vein-banding, but no necrosis of leaf or streak in stem (fig. 43, Plate 25).

It is the strain $X^S$ in combination with tobacco mosaic which reproduces the experimental streak of the commercial glasshouse, characterized by interveinal necrotic

flecks on the leaves and streaks on the stems. The plants, although often seriously handicapped, continue to grow (see fig. 43).

When $X^N$ replaces $X^S$ the ensuing disease is of the utmost virulence and rapidly kills the plant (see figs. 42 and 43, Plate 25), where two plants of the same age and originally of the same size are shown suffering from infections of $X^S$ + tobacco mosaic virus, and $X^N$ + tobacco mosaic virus respectively.

When the masked strain of tobacco mosaic replaces the common in a series of mixtures with the $X$ strains, the results on young tomato plants are practically identical (see fig. 41). The reactions of both tomato and tobacco plants to these mixtures approximate so closely that there is reason to think that the explanation offered on p. 207 will in its main features hold true for both.

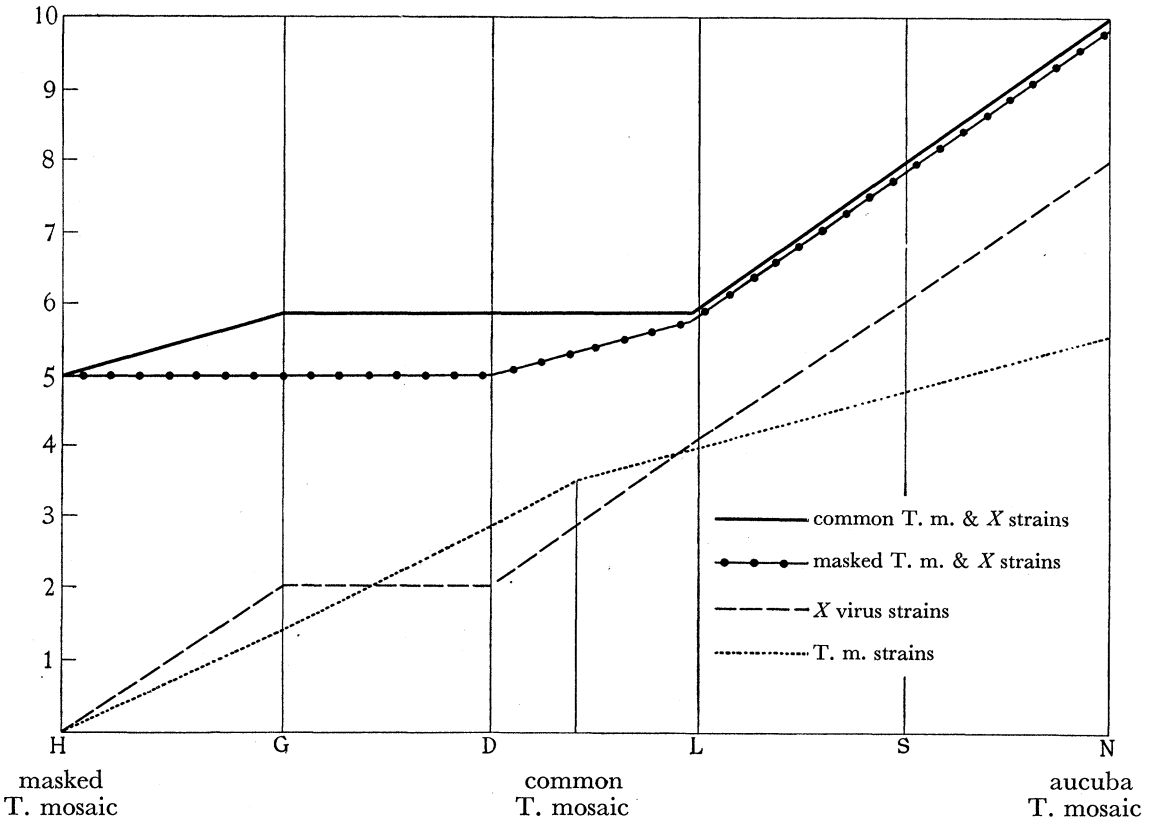



FIG. 41. 1–10 Degrees of virulence. 1–5 = Mottles; 5–10 = Necrosis and mottle. H–N = Strains of the $X$ virus.

## THE CONVERSION OF STRAINS

The possibility that one strain of the $X$ virus might be converted into another has been alluded to earlier and evidence suggestive of such recorded in relation to the behaviour of the $X^N$ strain in the varieties Eclipse and King Edward.

Throughout the 7–8 years during which the $X$ stock 723 has been used by the writer

and his colleagues, spasmodic cases have occurred in which definite changes of type have occurred in a single passage independently of any conscious selection. It is impossible to give the exact number of such occurrences, as only those observed by the writer have been recorded, but the number of tobacco plants inoculated from this source must be in the neighbourhood of 6000–7000, and the total number of such changes probably not more than ten.

The conversions which have been observed and studied are recorded below. It should be stated that prior to this, analysis of the 723 stock had shown it to be an almost pure strain of $X^S$ with a slight admixture of $X^G$. No trace of $X^L$ had at that time, or since, been recovered from it by selection. Later work has failed to show the presence of any of the other strains in the 723 stock, though one is not justified in assuming that none is present. In one respect these spontaneous conversions differ from those to be described later in that in respect to these latter we are concerned with a source of virus which there is good reason to believe contains but one strain.

### *A. Changes in the unpurified $X^S$ stock* 723

*Conversion No.* 1, 30 *January* 1934. Juice from young plants infected with the sap of the *X* source 723 was mixed with the juice of healthy plants and some anti-$X^S$ rabbit serum; the mixture was inoculated to four plants:

| | |
|---|---|
| Plant 1 | remained healthy. |
| Plants 2 and 3 | gave an $X^L$ reaction. |
| Plant 4 | gave an $X^S$ reaction. |

Plants 2 and 3 were carried on through five generations without change of symptoms. Fresh lines were started from the original plant 3 at different intervals as shown below:

(*a*) After 100 days: This was carried through six generations. In the first three the $X^L$ type of reaction persisted, in the fourth it had become feeble, and in the fifth and sixth it had developed into a pure $X^G$ type.

(*b*) After 270 days: an inoculation was made to three tobacco plants with the result that one developed a weak *L* and the rest remained healthy. These latter may have been examples of $X^H$ which were not then known and hence not tested for.

(*c*) After 420 days: a further inoculation to six tobacco plants resulted in an $X^G$ reaction in all. Further passages through *Datura* and tobacco showed that the *L* strain had not entirely disappeared. This case of conversion is the nearest approximation to a complete change of type which has been observed without change of host species.

In all fifty-one cultures containing 179 plants were inoculated from the original mutated source and none showed evidence of the presence of $X^S$. Two cultures were made from the specially punched out yellow portions of the mottle, but they likewise failed to exhibit any sign of $X^S$.

*Conversion No.* 2. On 20 May 1934 some 100 c.c. of sap from the 723 source was purified by my colleague Mr Bawden by means of the $CO_2$ process of MacClement (1934). Of this:

(*a*) A portion was diluted 1 in 10 and inoculated to three *Nicotiana glutinosa* plants: reaction = $X^S$.

(*b*) A portion was mixed with normal rabbit serum and inoculated to five *Datura* plants: reaction = $X^S$.

(*c*) A portion of the mixture (*b*) inoculated to one *Datura* plant: reaction = $X^L$.

(*d*) A portion was diluted 1 in 100 and inoculated to two *Nicotiana glutinosa* plants: reaction = $X^S$.

(*e*) A portion of the same mixture (*d*) was inoculated to one *N. glutinosa* plant: reaction = $X^L$.

(*f*) A portion was diluted 1 in 1000 and inoculated to five *N. glutinosa* plants: reaction = $X^S$.

(*g*) A portion of the same mixture (*f*) was inoculated to one *N. glutinosa* plant: reaction = $X^L$.

Lines (*c*), (*e*) and (*g*) were carried on and their behaviour will now be reviewed.

*Line c*: This was carried through seven passage generations and the $X^L$ character grew progressively weaker and more $X^G$-like.

A fresh passage from line *c* was started 131 days later; the reaction was that of $X^L$.

*Line e*: This was carried through seven passages. In the second passage six plants were infected, four reacted as $X^G$ and two as weak $X^L$. One of the latter carried on through four further generations, maintained the $X^L$ type but showed some evidence of admixture with $X^S$. The final passage resulted in two plants which exhibited a weak $X^L$ pattern, doubtless due to the combined presence of $X^G$, and a third plant in which the $X^L$ pattern was modified by the presence of $X^S$.

*Line g*: This was carried on through five generations, but became weaker and more like $X^G$ towards the end; in the fourth passage, however, there were some necrotic spots indicating the presence of some $X^S$ as well as $X^G$.

### *B. Conversion within a pure strain*

The various strains of the virus already described are here regarded as pure so far as the methods of separation at our disposal allow. Some of them have been carried on for over 30 passages, the oldest, i.e. the first separated, being those of $X^G$ and $X^L$. Of the former we have no dramatic change to record; of the latter, after the extraction of the "dissected type" shown to be a mixture of the $X^L$ and $X^S$ strains, the residuary $X^L$ strain was carried on in tobacco from a plant of the 20th passage. In the last twelve months the tobacco plants descended from this source have tended to display less characteristically *L* symptoms and to-day their reactions are almost indistinguishable from $X^G$ type plants. Efforts to recover the strain by selective inoculation from the faint yellow marking of the mottled leaf have not led to the restoration of the normal

$X^L$ pattern. The true $X^L$ type of reaction in tobacco has, however, been regained by sap inoculation from potato plants which were infected in the previous year with the pure $X^L$ strain.

The author having recently found evidence for the belief that a change of strain in the *Y* virus might be effected by treating the roots of infected plants in sterile culture media at various temperatures, an attempt was made to bring about similar changes with some of the *X* strains.

The two pure strains $X^S$ and $X^N$ were chosen for experiment and the root fibres were put up in small flasks containing nutrient fluid under sterile conditions at the following temperatures: 1·5°, 3° C., room temperature, 25° and 40° C. The flasks were maintained at these temperatures for 4, 9 and 17 days respectively. At the end of each period samples were removed, ground up in water, and each used as an inoculum to batches of three *Datura* plants. Controls were taken at the same time from non-incubated roots of infected plants and inoculated to batches of healthy *Datura* plants all of which developed either the $X^S$ or an $X^N$ reaction or remained healthy. The plants which remained healthy were reinoculated with virulent $X^N$ or $X^S$ and in all cases a normal reaction ensued, proving that they were not carrying $X^H$, but were virus-free having received no virus from the particular piece of root employed.

Of the thirty incubated root samples inoculated to ninety plants, all but two proved completely free from virus, i.e. the treatment had destroyed the virus where such was present.

The two exceptions were:

(1) $X^S$ maintained for 9 days at 25° C.: Two out of the three plants infected responded with an $X^G$ reaction which showed evidence of some slight admixture with $X^S$, whilst the third contained no virus.

(2) $X^S$ maintained for 17 days at room temperature: Here the plants failed to respond for three weeks, when a normal $X^S$ reaction with local and systemic lesions supervened.

In the first of these two cases we have a definite conversion of $X^S$ to $X^G$; in the second it is probable that nearly all the virus originally present was killed off but that just enough reached the *Datura* plants to cause a multiplication of the same within the host.

In contrast to the behaviour of *X* infected tobacco roots is that reported by Samuel (1934) of tomato stems containing insufficient tobacco mosaic virus to induce an immediate infection in healthy plants, but in which, when maintained under sterile conditions at room temperature, a rapid increase of unconverted virus takes place.

### *Conversion of types by passage through unrelated plants*

Carsner (1925), Carsner and Lackey (1928) and Lackey (1931), showed that curly-top of sugar beet was reduced in virulence by passing it through carrier varieties of sugar beet, and again restored by passage through the unrelated plant *Stellaria media*. Against this,

however, are the almost completely negative results obtained by Johnson and Grant (1932) with tobacco, cucumber and Wingard's ringspot viruses respectively when passed through a variety of solanaceous plants. Some attenuation of virulence when tobacco mosaic was passed through *Martynia louisiana* was, however, observed. In 1934–6 experiments of a similar character were made on the *X* virus. The $X^G$ strain was passed through a number of related and unrelated plants and later extracted and tested on tobacco.

Pure $X^G$ in tobacco was passed through the following solanaceous species and later recovered without recognizable change:

*Capsicum annuum* (four varieties),
*Datura stramonium*,
*Hyoscyamus niger*,
*Lycopersicum esculentum*,
*Nicandra physaloides*,
*Nicotiana alata*,
*Nicotiana angustifolia*,
*N. culata*,
*N. langsdorfii*,
*N. noctiflora*,
*N. Sanderae*,
*Physalis pubescens*,
*Salpiglossis sinuata*,
*Salpiglossis variabilis*,
*Solanum capsicastrum*,
*S. dulcamara*,
*S. melongena*,
*S. nigrum*,
*S. nodiflorum*.

The same $X^G$ strain was recovered unaltered from the *inoculated* leaves of:

Chenopodiaceae: Red beetroot.
Compositae: Groundsell.
Leguminosae: Cow-pea.
Scrophulariaceae: Mimulus; Speedwell.

In the following species $X^G$ became systemic and was recovered unchanged from the young growth:

Compositae: Chrysanthemum.
Scrophulariaceae: *Browallia speciosa*.

Attempts to infect members of the following families with $X^G$ all failed:

Boragineae: Houndstongue.
Caryophyllaceae: Chickweed, Gypsophylla.
Chenopodiaceae: Sugar beet.
Compositae: Aster, marigold, lettuce, scabious, Michaelmas daisy, coreopsis.
Cruciferae: Brussels sprouts, cabbage, cauliflower, Matthiola, shepherd's purse.
Labiatae: Dead-nettle.
Leguminosae: Sweet-pea.
Plumbagineae: Statice.
Scrophulariaceae: Pentstemon, speedwell, antirrhinum.
Rubiaceae: Goose-grass.
Umbelliferae: Celery.

Having failed to convert $X^G$ into a more virulent strain, attempts were made to convert the virulent $X^S$ strain into a weaker form by passage both through related and unrelated plants. To this end a purified stock of $X^S$ was used in which repeated efforts to extract either $X^G$ or $X^L$ by selective inoculation from the green parts of infected plants had failed. The plant species selected were:

Solanaceae: plants of the same species as those employed for the attempted conversion of $X^G$ were also used for that of $X^S$. In all cases infection was successful, but on

extraction and return to tobacco it was found that no change in type had been effected.

The unrelated species used belonged to the following families:

Chenopodiaceae: Red beet, sugar beet.
Cruciferae: Brussels sprouts, cabbage.
Geraniaceae: Pelargonium.
Leguminosae: Broad bean, cow pea, horse bean.

Of these, the following species alone effected any change of type, viz. the beans and the beets. In no other was there any evidence of infection.

*$X^S$ on Horse Beans*

No true local lesions developed, nor was there any evidence of systemic infection. On the inoculated leaves wherever the epidermis had been wounded, small superficial areas of necrosis developed, these were cut out, extracted with water and used as inoculum. On *Datura* plants they produced a reaction such as would result from a mixture of $X^G$ and $X^S$ in which the former was in excess. Further passage to tobacco produced a group of plants, one of which indicated a mixture of equal parts of $X^G$ and $X^S$; a second plant in which $X^G$ was largely in excess of $X^S$; and a third which appeared to be an infection of pure $X^G$.

*$X^S$ on Red Beet*

Infection by gently rubbing infected tobacco juice on the leaves of young seedling beets produced small lightly necrotic rings around which there is an intensification of the anthocyanin pigment. No systemic infection occurred. The local lesions were excised 12 days later, ground up in water and used as an inoculum to groups of red beet seedlings, tobaccos and *Daturas*. The two latter developed an apparently normal $X^S$ reaction; the red beets responded with local lesions only. These latter were extracted one month later and inoculated to five *Daturas* all of which developed a mild $X^G$ mottle. No evidence of $X^S$ was found. Three weeks later material from the local lesions of the second generation of beet was again inoculated to *Daturas* but with negative results.

*$X^S$ on Sugar Beet*

Sugar beet, like red beet, responds to inoculation with the formation of well formed finely etched local rings of about 2 mm. diameter. Occasionally in plants which have been infected when young, and in which the inoculated leaf has persisted for about 6 weeks, the individual lesions may expand into a collection of numerous concentric rings (fig. 32, Plate 22) covering an area of more than a square cm. No evidence of systemic infection was found.

Thirteen separate experiments were made in each of which sap from $X^S$ infected tobacco plants was inoculated to three sugar beet plants. After an interval of at least 14 days the local lesions from each plant of the group, or failing the formation of such,

those portions of the inoculated leaves which had been abraded, were ground up and passed to tobaccos or *Daturas*. In seven of the thirteen experiments evidence of change of *X* type was found in this first passage; in two others it was obtained in the second or third passage by means of selective inoculation; in four cases no infection occurred.

The changes observed in the first passage varied very considerably: in only two cases did the reaction record a complete change of type. In one of them the local lesions were passed to five *Datura* plants, four of which gave a more or less full $X^S$, whilst the fifth responded with what seemed to be a pure $X^G$ reaction. Analysis showed (diagram III, Exp. 1), that the conversion from $X^S$ to $X^G$ was almost complete though a trace of $X^S$ still remained.

In the second case of immediate conversion (diagram III, Exp. 2), the analysis was carried over five passages by selective inoculation in two directions. Lines almost pure for the $X^G$ type, as well as those with an increasing admixture of $X^S$, were isolated. In the course of these experiments the reaction of a few plants suggested the presence of some $X^L$ (diagram III, Exp. 2). Further selection yielded a more definite $X^L$ strain of reaction. This was the only occasion on which such occurred.

Whilst there was no reason to believe that any $X^L$ was present in the $X^S$ inoculum used, yet as in each of the natural conversions previously referred to $X^S$ was changed to $X^L$, the suggestion that this is a further example of such a change cannot be lightly dismissed. It will be remembered that at a certain stage in the extraction of $X^S$ from the $X^G$ line (diagram I), $X^L$-like forms did occur. However, in this case $X^L$ was abundantly present in the same plant as that from which $X^G$ was originally isolated and hence might be regarded as a contamination.

The $X^S$ strain, having been inoculated to sugar beet and extracted therefrom in a much altered $X^G$-like condition (diagram III, Exps. 3 and 4), was passed afresh to sugar beet. From the local lesions it was once more extracted, and passed to *Daturas* and tobaccos. The virus was now found to be more consistently pure to $X^G$, a purity which was further exhibited in later cultures.

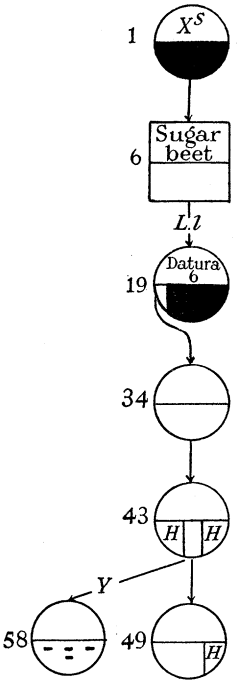
On four occasions, an almost completely pure $X^G$ derived by previous conversion in sugar beet of $X^S$ and subsequent selective inoculation was passed again to sugar beet and the local abrasions subsequently extracted and passed to tobaccos: in all four no virus was recovered.

This result is one which would be expected from previous experience (p. 92) when attempts to infect sugar beets with a pure line of $X^G$ failed completely.

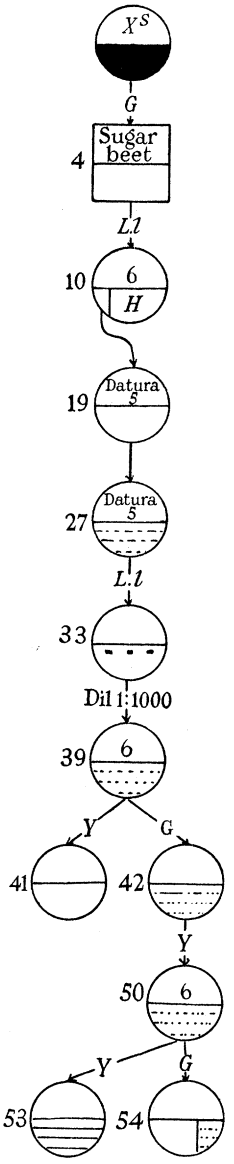
That a mixture of $X^G$ and $X^S$ should succeed, implies that the $X^S$ constituent obtained an entry and brought with it its partner $X^G$ which by itself could not have obtained access; the $X^S$ was then converted within the beet tissue so that the resultant inoculum contained an almost pure $X^G$ strain.

Seeing that the virulent strain $X^S$ suffers conversion to the less virulent form $X^G$ within the tissues of the sugar beet, it seemed desirable to examine whether such might

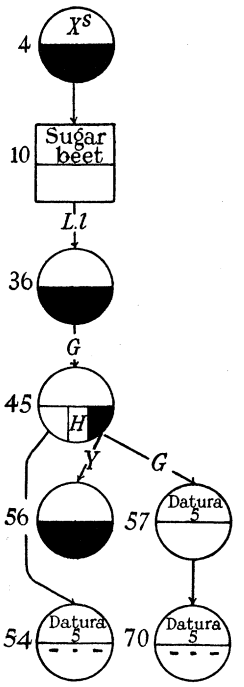
H. 1934. Experiment 1




H.S. 1935. Experiment 2




H. 1934. Experiment 3




H.S. 2, 1935. Experiment 4

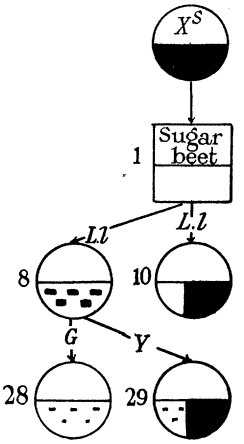



DIAGRAM III. Being extracts from Experimental Groups No. VII and No. VIII; in these a pure strain of $X^S$ was inoculated into sugar beets and the local lesions passed on to tobacco seedlings. For the explanation of symbols, see diagram I. In Exp. H. 1934, only a small part of the $X^S$ inoculum had undergone change to $X^G$ but the extraction of the mutant form was readily effected. In Exp. H.S. 1935, the $X^S$ inoculum was from a green portion of the infected tobacco, and hence only a very small quantity of virus entered the sugar beet. The extraction of the mutant $X^G$ proved easy but was complicated later by the appearance of some $X^L$ which, diluted by $X^G$ in cultures 39 and 42, was later extracted in an almost pure form in culture 53. In Exp. H. 1934, so little of the $X^S$ has been converted to $X^G$ that the latter does not make itself obvious till selective inoculations bring it into prominence. In Exp. H.S. 2, 1935, the conversion of $X^S$ was immediate and obviously in considerable quantity.

also occur *in vitro*. A mixture of equal parts of expressed juice from a tobacco plant infected with a pure $X^S$ strain and sugar beet juice from healthy seedling leaves was mixed *in vitro*, well shaken, and allowed to stand for 20 hr. when it was inoculated to a group of six tobacco plants (see diagram IV).

The result was an $X^S$-like infection of five of the plants and absence of any infection in the sixth plant. Subsequent selective inoculation differentiated a mixed $X^S$–$X^G$ and a pure $X^S$ infection in the first passage (see diagram IV, Nos. 21 and 22). Further selective inoculation brought about the production of a pure $X^G$ culture in the second subsequent passage (No. 96).

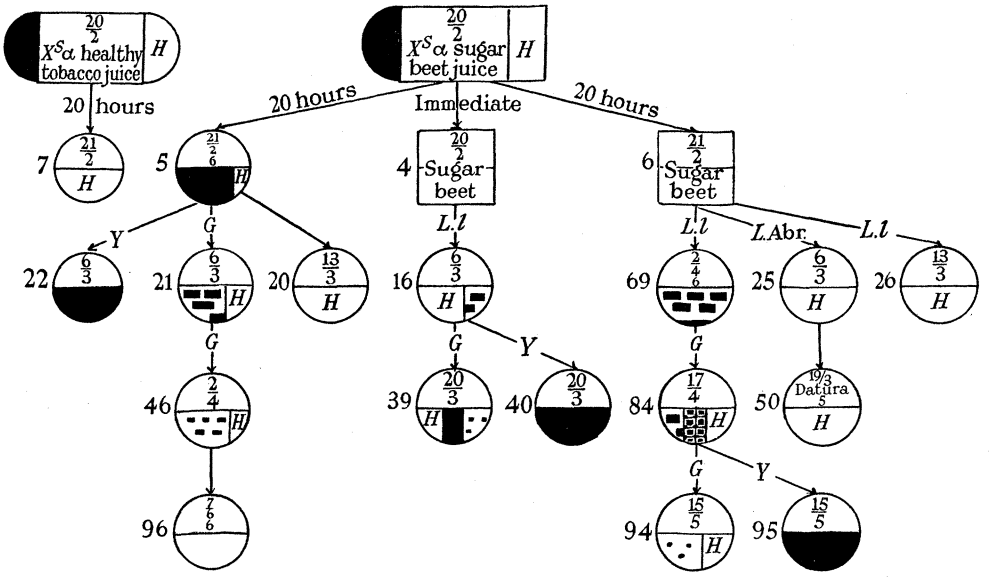



DIAGRAM IV. Being an extract from Experimental Group No. IX, shows how the admixture of the juice of sugar beet effects a change in a pure culture of the $X^S$ virus, and how the change is accelerated if the mixed juices be further inoculated to healthy sugar beets and re-extracted to tobacco. For an explanation of symbols see diagram I. In this diagram the dates on which each passage was made are indicated in the upper hemispheres, the whole taking place in the year 1935. *L.Abr.* = the wounds caused by abrasions at the seat of inoculation on sugar beet seedlings where no true local lesions developed.

The same mixture was inoculated to a group of sugar beet seedlings immediately after mixing (diagram IV, No. 4), resulting in local lesions from which a more or less pure $X^G$ culture as well as pure $X^S$ were secured by selective inoculation (diagram IV, Nos. 39 and 40). A further attempt to infect sugar beet and tobacco seedlings with mixtures after standing 20 hr. failed to induce any true local or systemic lesions in either. Fifteen and twenty-one days later (diagram IV, Nos. 25 and 26) when extracts were made from the local abrasions and from such spots as were suggestive of faint local lesions on the sugar beets, passage to tobaccos and *Daturas* failed to demonstrate the presence of any virus. It should be noted that the existence of $X^H$ was not then known and hence the healthy looking plants in the first and second passage were not tested for its presence.

We now know, however, that $X^H$ alone is unable to infect sugar beet. These same infected sugar beets (diagram IV, No. 6) re-examined 41 days after the original inoculation were found to have developed some local lesions. Extractions from these and subsequent selective inoculation secured in the third passage (diagram IV, Nos. 69, 84, 94, 95) almost pure $X^G$ and $X^S$ cultures.

It seems probable that after 20 hr. standing *in vitro* the mixture of $X^S$ tobacco and healthy sugar beet juice has undergone so considerable a conversion that there is insufficient $X^S$ present to induce local lesions on sugar beet seedlings within 15 days. Nevertheless, in the course of the next 6 weeks such $X^S$ as did gain an entry multiplied sufficiently to bring about local lesions and at the same time convert itself in part to $X^G$.

A mixture of equal parts of tobacco juice from an $X^S$ infected plant and of the raw juice of a healthy tobacco plant, when allowed to stand for 20 hours and inoculated to tobacco plants, produced no reaction. Whether this is a case of neutralization or inhibition remains to be studied. Diagram IV, No. 7.

These experiments show that conversion of $X^S$ to $X^G$ can take place *in vitro* in the presence of healthy sugar beet juice. Although the two juices were not purified and plant débris was present, it is reasonable to assume that the process occurred independently of cellular action, including mitosis, within the sugar beet. Sheffield (1936) has shown that mitosis takes place in the local lesions in *Nicotiana glutinosa* following inoculation with aucuba mosaic. Whether such occurs in the sugar beet local lesions has not been established, but the type of conversion we are considering in these *in vitro* mixtures is obviously independent of mitotic activity.

The facts so far recorded relating to the conversion of the virus *X* strains allow of the recognition of certain definite types of behaviour.

(*a*) Within the environment of a single species host such as tobacco growing under more or less constant conditions, sudden change of strain is very rare.

(*b*) In the majority of cases where change has taken place, interference with the *p*H and other physical conditions of the sap inoculum has taken place, e.g. in the process of purification. Such changes have been far commoner than in passages arising from raw juice.

(*c*) Passage of the *X* virus through solanaceous plants other than certain varieties of the potato has failed to bring about any change.

(*d*) Passages through horse bean and beet have effected an immediate change of type of virulence in at least part of the virus which gained an entry and multiplied within the local lesions induced.

(*e*) Conversion has been effected *in vitro* by mixing $X^S$ infected tobacco juice with that of healthy sugar beet.

(*f*) Every example of strain conversion occurring spontaneously or artificially induced has been from a higher to a lower virulence.

## Acquired immunity

In April 1933 the writer (Salaman 1933) published a note in which the phenomenon of protective inoculation was described. At the time the discovery was made, viz. the autumn of 1932, he was unaware that a somewhat similar phenomenon had been described by Thung (1931). In an investigation on the nature of viruses and the distribution of two or more infecting a plant simultaneously, Thung developed the theory that one virus present in a cell might exert an antagonistic action against a second invading virus so that the latter is refused entrance by the cell. In a paragraph of which the following is a translation from the original Dutch sanctioned by himself, Thung states: "That this is indeed the fact is shown when the virus of the common mosaic (of tobacco) is inoculated into a plant (tobacco) already suffering from white mosaic and the symptoms of the common mosaic fail to appear. Inoculations with the sap of the twice infected plant reproduce only the white mosaic in healthy individuals, showing that the virus of the common type is not present, even in a latent condition. Similarly, plants affected by common mosaic fail to develop the white type on inoculation. This, however, is not an invariable result, for one does meet cases where inoculations with white mosaic into plants already suffering from common mosaic, produced the mixed pattern consequent on the action of the two viruses."

These observations, which escaped general notice at the time, disclose fundamentally the same phenomenon as that observed by the writer, hence to Thung belongs priority. There is, however, a certain difference between the two observations: in Thung's case one outspoken pathological condition prevented the appearance of another equally obvious one. In the writer's case, a virus strain of a virulence so low that infected plants are not infrequently mistaken for normal ones was isolated from a mixture. Infection with this low strain afforded a complete protection to a previously healthy plant against the most virulent strains of the same virus.

Price (1932) observed that tobacco plants which had been infected with Wingard's ringspot virus and which in due course developed the usual symptoms of the disease, as they grew older produced leaves which appeared entirely normal, and that all attempts to reinoculate them with the same virus failed. He found, however, that these apparently normal leaves contained the virus in full strength, and that inoculation from them to healthy plants induced the normal symptoms of the disease. He describes the phenomenon as one of acquired immunity but this is clearly a misnomer. They have no immunity against the parent or any other virus; they are carriers of a particular virus, and in that they illustrate a phenomenon distinct to that described by Thung or the writer.

Since the publication of the note referred to, several other workers have described similar phenomena. Bawden (1934) found that the writer's *G* strain protected tobacco and potato plants against the virus of foliar necrosis, $X^D$. Ainsworth (1934) has demonstrated the protective action of the *G* strain of the *X* against the virulent strain in

tomatoes. Köhler (1935*a*), working on the *X* virus, reported the occurrence of protection which was incomplete: reference will be made to this investigator's results later in this section. Kunkel (1934), working with tobacco mosaic, showed that ordinary mosaic protects against the aucuba type and that heat attenuated strains of the latter protect against the virulent form of the same. Caldwell (1934) showed that the green strains of tobacco mosaic No. 6 (aucuba) protected against the yellow. Price (1935) demonstrated a similar relation between common cucumber mosaic and a necrotic strain of the virus. Whilst most of the work on the protective reaction of one strain against another has been carried out on *Nicotiana* or some closely allied species, Kunkel (1936) has shown that a similar relation exists between Little Peach and Peach Yellows in peach trees. Salaman (1936, 1937*a*) has shown that a protection against the potato virus *Y* may be obtained by prior infection with an attenuated stock of the same.

The conclusions here presented on protective inoculation have been derived from experiments in which many thousands of plants have been used and in which each observation has been repeated scores of times. This superfluity of experimentation is to be explained by the fact that as soon as the principle was firmly established, the writer and his colleagues made use of the phenomenon as a routine method for testing for the presence of non-virulent *X* strains in the potato or other plant under investigation, as well as for replacing a severe strain by a mild one in any given mixture of viruses which can be communicated by sap inoculation or graft. A further use was made in Cambridge of the reaction as a criterion for the assignment of any given virus to the *X* group. In all these respects it has proved of very real value.

As several workers in the same and other fields of virus research have recorded results in full accord with those of the writer, it has seemed more desirable to epitomize the results whilst enlarging on any deviations from the normal, rather than to present a long list of protocols of the various experiments performed.

Experiments on protective inoculation with the strains of the *X* virus were carried out on *Datura stramonium*, *Nicotiana tabacum*, *N. glutinosa* and potatoes, the great majority being on the two first named.

The normal procedure has been to select eighteen plants of tobacco or *Datura* of the same age and size: twelve are inoculated with $X^G$ or $X^H$ and six of them left as controls, after an interval of 10–14 days, six are reinoculated with one or other of the virulent strains, viz. $X^L$, $X^S$, $X^N$, or $X^D$; at the same time, the third set of six plants hitherto uninoculated are now infected with the strain under examination and remain as controls. After 8 days the latter controls begin to show the reaction characteristic of the second strain, whilst the protected plants remain indistinguishable from the $X^G$ or $X^H$ inoculated controls (see figs. 33, 34, 35, pl. 22). It is important to select young plants for a demonstration experiment because the virulent strains when introduced into older plants usually produce local lesions and only a restricted systemic reaction. For the same reason it is not advisable to leave an interval of more than 14 days

between the two inoculations. It is not that the plant is less protected, but only that the contrast between it and the control is less striking.

When the $X^H$ strain was isolated in 1935 this was substituted for $X^G$. Notwithstanding the fact that it is symptomless on the host plants used, its powers of protection against subsequent infection with $X$ strains are not only equal to but perhaps more consistently perfect than those of $X^G$.

The degree of protection depends in the main on two factors:

(1) *The age of the plants employed.* This applies especially to tobacco; with *Datura*, successful protection may be readily secured in older plants. The plants, especially tobaccos, must be in active growth, so that the protective strain becomes disseminated through the plant before the advent of the virulent one. In the winter months it is well to assist the plants with artificial light and heat. It is noticeable that even in September tobacco plants infected with the $X^G$ strain may show an imperfect protection against the severe strains, because of the delayed dispersion of the $X^G$ virus.

(2) *The length of interval between the two inoculations.* Experiments were made on tobaccos during March under glasshouse conditions in which the second inoculation, i.e. that of the virulent strain, was introduced at 24 hourly intervals from the end of the first to the end of the eighth day. The resultant protection varies with the length of the interval up to the fifth day; after that it is well advanced, and on the eighth day it is complete. In this experiment $X^G$ was the protecting strain and $X^S$ the virulent one.

The clinical picture in this series varies from one of an almost completely $X^S$ type when the interval is but one or two days, to one which is composed of a more or less uniform mottled $X^G$ background on which numerous bright yellow spots occur, some of which may be necrotic.

So long as the precautions outlined above are observed, the result of a preliminary infection with $X^H$ or $X^G$ is to afford the plant in question protection against infection by any of the other five strains, and the protection does not vary in degree with the virulence of the particular strains.

The specificity of the protective action induced by inoculation with the $X^H$ or $X^G$ strain of the $X$ virus is shown by the complete absence of any protection against subsequent inoculation with the $Y$ virus,* the virus of spotted wilt,† the virus of Wingard's ringspot,‡ the virus of tobacco mosaic,§ and that of cucumber mosaic.‖

The $X^L$ strain of the $X$ virus protects against the $X^S$ strain as well as does $X^G$; far fewer experiments have been made with this combination and these only in tobacco, but they are unequivocal in their result.

Protection by a lesser against a more virulent strain of the same virus being established, it remains to be seen how far such protection is complete, i.e. in force all over

* *Solanum* virus 2, according to K. M. Smith, 1937.
† *Lycopersicum* virus 3, according to K. M. Smith, 1937.
‡ *Nicotiana* virus 12, according to K. M. Smith, 1937.
§ *Nicotiana* virus 1, according to K. M. Smith, 1937.
‖ *Cucumis* virus 1, according to K. M. Smith, 1937.

the plant, and how far it is absolute, i.e. proof against repeated inoculation, or against infection effected by other means.

In many cases in tobacco where protection has seemed to be complete against a virulent strain a careful survey of each individual leaf reveals the presence of small yellow foci—in a few leaves such spots have been necrotic. If such foci are cut out with a fine 1 mm. punch, rubbed up and inoculated to young healthy tobaccos, a typical infection with the unmixed virulent strain usually results, at other times a certain amount of the weaker strain will be found to have been included in the punched out area.

In the earlier experiments, when the $X^S$ strain was used for reinfection, it was quite common to find a few local lesions on the inoculated leaves of the protected plant, usually finely necrotic rings considerably fainter and far less numerous than those on the corresponding leaves of the controls, and this though no subsequent systemic infection with the *S* strain supervened other than an occasional yellow spot.

In later experiments such local lesions are the exception, and the appearance of yellow systemic spots, except in the autumn, considerably rarer. The reason lies doubtless in the method of inoculation employed; in the earlier work the sap was introduced by placing a few drops on the surface and then abrading the leaf with a sterile needle. This procedure not only breaks the surface hairs but wounds the leaves and here and there must lay open the fine terminals of the phloem bundles into some of which the virus laden sap presumably gains an entrance.

For rather more than two years inoculation has been effected with Samuel's glass spatulae which when carefully used only break the surface hairs, not otherwise injuring the tissues of the leaf, and thus direct infection of the phloem is avoided.

Where the protective virus has been introduced by inoculation it may be said that a plant is rendered, in nine cases out of ten, completely immune to a later infection with a virulent strain however often repeated, and in the tenth case the latter only gains a footing on strictly isolated spots and does not become generalized. If instead of tobacco and *Daturas*, *Nicotiana glutinosa* be used, the results are somewhat different; the degree of immunity is strictly dependent on the length of time between the two inoculations, the interval necessary for protection being longer than in the case of *Nicotiana tabacum*, and the result less certain. The growth of the plant, which is very seriously affected by infection with the virus $X^S$, is closely related to the immunity attained. That complete immunity can be attained is shown in fig. 33. Bawden (1934) also found that the virus of foliar necrosis when in *N. glutinosa* was less effective in its protective action against the $X^S$ virus than it was when tobacco plants were employed.

A number of protection experiments have been conducted on potato; the results are the same. Most varieties of potato plants when infected with the $X^G$ strain display but a very mild interveinal mottle and may often pass as normal. Later inoculation with the $X^L$ or $X^S$ strain has no further influence on them, and on subsequent extraction neither strain is found.

Reference has been made to the failure to infect Up-to-date, Duke of York, and King George varieties with $X^N$, and the cause has been found to be due to the protective action exerted by a latent $X^H$ or $X^G$ infection. In the field it is suggested that a similar state of affairs exists. This view is supported by the independent work of both Bawden and the writer who have repeatedly shown that potatoes experimentally infected with $X^G$ or $X^L$ remain immune to either $X^D$ or $X^N$.

In another section it has been shown that in the field infection with one or other strain or a mixture of the same is common, and it is doubtless because of this that both foliar necrosis and interveinal necrosis are very rare diseases, notwithstanding the high infectivity of their sap, and this rarity is the direct consequence of the protection afforded by the less virulent strains of $X$, the lesser evil protecting against the greater.

Köhler (1935*a*) states that when the more virulent $X$ strain is introduced by means of a graft to a potato stock itself infected with a weaker strain, protection is incomplete; this he considers is due to the fact that the virulent virus reaches the new growing tissues by means of the phloem. Whilst this would seem to be probable, the writer has met with but very few such cases. It should, however, be stated that the number of graft infections effected is a matter of about one hundred compared to the thousands of plants in which reinfection has been attempted by inoculation. Bawden's (1934) experience with grafting as a means of introducing foliar necrosis into $X^G$ infected plants resembles to some extent Köhler's; he found that the superimposed disease was both limited in regard to the extent of the plant parts affected and much reduced in its destructive effect.

Köhler as we have seen divides the $X$ virus strains into two groups, potato mottle and potato ringspot, and states that "mottle" strains only protect against "mottle" and "ringspot" strains against "ringspot". The writer's experience lends no support to this conclusion; any of the strains of the mottle group $X^G$, $X^L$, or $X^D$, no less than the completely masked type $X^H$ protects equally and effectively against the necrotic ringspot types $X^S$ and $X^N$.

As regards the nature of the immunity with which we are dealing, it is obvious that the protected tissue is itself infected with the primary inoculum to the exclusion of the secondary, and that on those spots where protection fails, a pure culture of the secondary virus can generally be obtained, the protective strain being absent. The varying degree of protection attained by reducing the time interval between the protective and the final inoculation, points in the same direction, for those portions of the tissue which are uninfected by the second strain contain the primary virus only. When the individual strains are extracted and mixed the strong with the weak *in vitro* there is no neutralization or destruction of the former as would be expected were anti-bodies concerned.

These facts suggest that when a living cell has set up an association with one strain of a given virus it is unable to repeat the process with a closely similar one, which implies that some irreversible union takes place between some protein constituent of the cell

and the virus particle. Although this combination may give rise to no pathological reaction, as is the case with $X^H$, yet the nature of the combination must be essentially similar so far as its attachment to the protein molecule is concerned, to that which results when a highly virulent strain is involved with its consequent morbid reaction. Hence we must assume that the attachment of the particle and its capacity for inducing morbid change are distinct. This being so, a virulent virus molecule must contain some other group which by its interaction on the cell protein impairs the latter's activity. If now a second but unrelated virus is introduced into the plant cell, we know that it will not interfere with the attachment mechanism of the first, but it is not difficult to picture it as modifying by mutual interaction the effect on the cell protein molecule, which otherwise would have produced a morbid condition. This leads to the further view that we should expect to find that a plant virus particle is possessed of more than one—indeed several antigens. It was from considerations such as these and the absence of any evidence of the formation of anti-bodies in plants, that the writer originally characterized the process as one which might be described as "First come, first served".

It has been already stated that the protection afforded by the prior inoculation of a weak strain has been found to be specific: at least no exceptions have so far been recorded, but inasmuch as one of the criteria for a strain is that it should exhibit cross immunity, one is apt to argue in a circle. The work of Ainsworth (1935) and Bawden and Pirie (1937*b*) on the behaviour of cucumber viruses III and IV and tobacco mosaic shows how viruses which from their serological reactions must be regarded as related strains, from another point of view, viz. the capacity to infect a common host and there exhibit cross immunity, are unrelated inasmuch as the cucumber strain will not infect tobacco, nor the tobacco the cucumber.

## Acquired immunity and the carrier problem

In 1932 Salaman (1932) put forward evidence in support of the suggestion that "carriers" might be created artificially by the co-existence of more than one or two unrelated viruses within the plant, each of which might be individually pathogenic. This view would at first sight appear to be contradicted by the fact established independently by Murphy and McKay (1931) and Salaman (1932), viz. that two viruses each of which acting alone was relatively harmless, might when present together produce a serious disease. In the one, union determines pathogenicity, in the other neutralization of symptoms, the immediate environment in either case being similar. As an example of the first class of phenomena may be cited Murphy's experiment where the union of the viruses *A* and *X*, and here $X^G$ may be used, neither of which acting independently calls forth any but the mildest symptoms in the potato variety President, yet together produce a serious disease known as "Crinkle A". Another example is that described by the writer in which virus *Z*, derived from paracrinkle, a

virus carried by President; when in union with *X* reproduces a picture similar to that of "Crinkle A". An example of the second class is the repeatedly observed fact that whereas paracrinkle virus carried by the King Edward potato produces a severe crinkle in the variety Arran Victory, if the virus *X* be added to it the resulting morbid condition is much reduced in intensity. A further stage of what may be the same process was obtained when the writer's potato Streak B (Salaman 1930) associated with virus *X* was introduced together with paracrinkle to Arran Victory by means of grafts which grew vigorously. In this case the following reactions would be expected from each virus if acting singly:

Paracrinkle on Arran Victory would produce a severe deforming crinkle.

Streak B on Arran Victory would produce a mosaic with some ruffling.

*X* on Arran Victory would produce a mild interveinal mottle.

Acting together, an accumulative effect might have been expected, instead of which only a transient veinal clearing resulted, the plant subsequently appearing quite healthy.

Thung (1936) records observations of a somewhat similar nature. We quote verbatim:

"Immunity against a third infection.

"(1) Accumulation of immunizing effects.

"Most of the mosaic diseases applied in the writer's experiments give no immunity against infections with the white mosaic or the ringspot necrosis. But after a second infection with the ordinary mosaic the plants with e.g. the severe mosaic or the distorting mosaic will not attract the other viruses on a third infection. The explanation is to be found in the fact that the ordinary mosaic will enter into all parts of these plants. . . . We have to suppose that the plant cell with different viruses keeps the immunizing effect of each of these. By the following data it appears probable that these immunizing effects of two viruses in one plant strengthen each other. Plants with a combination of the severe mosaic and the ringspot necrosis as also plants with the distorting mosaic and the ringspot necrosis are not susceptible to the white mosaic, whereas none of the three itself affords immunity against the white mosaic. So an accumulation of the several immunizing effects in the mentioned combinations of viruses may be probable, reaching at least such a strength that a complete immunity against the white mosaic has come into being."

It is not easy to follow Thung's line of thought, but it seems to be as follows: Each virus on entering a plant stimulates the cells to produce an "averting principle" against other viruses; a strong virus, i.e. one which in a simultaneous mixed infection dominates the other, produces more of such substances. It is further supposed that such substances can penetrate into non-infected cells of a plant and then set up a protection against a second invading virus.

Infection or immunity therefore becomes a matter of the ultimate equilibrium between blocking elements and the "strength" of virus. The fact that such a theory

demands the existence of neutralizing substances in the sap, for which there is no evidence, is acknowledged by Thung. This objection, as well as the localization of one or other virus in definite areas in mixed infections militates against the possibility of humoral immunity in plants.

One cannot altogether evade the thought that in some cases discussed by Thung the first two invading viruses have, so to speak, exhausted the clinical capacity for morbid reaction of the plant, and that a further virus, which acting alone would call forth a severe necrosis in its host, has now no stage on which to display its capacity for damage.

### Immunity to the "*X*" virus in potatoes in nature

The discovery of the masked $X^H$ strain in certain potato stocks which had not only been maintained under strict insect-free conditions, but had been examined annually over several years and thought to be virus-free, naturally suggests that this strain may be present in other apparently healthy stocks.

The complete protection afforded by its presence against such destructive and readily communicated diseases as Foliar necrosis $X^D$ and Interveinal necrosis $X^N$ leads one to believe that the rarity of these two diseases in the field may be due to antecedent infection of $X^H$ conveyed through successive tuber generations.

We have repeatedly shown that apparently healthy potato plants in the field may harbour the $X^G$, $X^L$ and even the $X^S$ strains without serious ill effect; the most luxuriantly healthy ones may carry $X^H$. The writer is convinced that in the field this type of protection has been at work for an indefinite period, possibly since the potato was first cultivated as a field crop in Europe. However this may be, it is not the only type of immunity to be found in the potato plant.

A recent examination by Dr R. Dennis of a collection of some 60 varieties and cultivated potato species which reached the Station in 1938 from Puño on Lake Titicaca in Peru, has demonstrated the presence of weak strains of the *X* virus in over 50 of them.

The Department of Agriculture of the United States of America has brought out a potato variety numbered 41956 which it is claimed will withstand infection with the *X* virus. In 1935 Professor Dykstra kindly supplied the writer with a couple of tubers, and in 1936 various trials with the material were made.

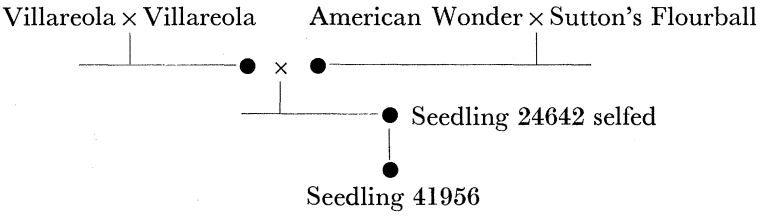
Seven of the "41956" plants were examined, sap from each was tested by inoculation to sets of tobacco and *Datura*, none of which showed any reaction and all of which, on reinoculation with $X^N$, developed full and characteristic symptoms. No masked strain of *X* was, therefore, present.

Plants of "41956" were inoculated with the $X^G$, the $X^L$ and the $X^S$ strains of the virus, but no evidence of any infection was observed, nor did the sap from such plants produce any reaction on tobacco or *Datura* or protect the same from subsequent inoculation with $X^N$. Three "41956" plants were grafted with President scions containing

$X^S$, and one with $X^L$; there was good union in all cases. The leaflets from the stock were examined for the presence of the *X* virus 32 and 54 days after grafting, but no trace of any *X* virus strain including $X^H$ was found, nor could Bawden obtain any reaction between the sap expressed from the leaves of these plants and anti-*X* sera.

It would thus appear that the variety "41956" owes its very real immunity to the *X* virus to some mechanism distinct from that which we have hitherto studied. That such is not a mechanical one causing difficulty of ingress to the virus is shown by the fact that simple inoculation, inoculation with carborundum, and grafting, all failed to bring about infection. One can only assume that the basis of the immunity is of the kind found in the case of such diseases as Wart disease of the potato (*Synchytrium endobioticum*), and that it may be genetic in origin. No evidence for genetic resistance in the domestic varieties has been observed by the writer, though a claim of this nature is made for the varieties Katahdin and Chipewa, whose ancestry is fully domestic. Experiments with the former have failed to demonstrate any immunity to the *X* strains.

The seedling 41956 was raised by Dr C. F. Clark and the pedigree is as follows:




The Villareola potato is a native cultivated variety grown in Chiloe. In this connexion it is of interest to recall the claim made by Bukasov (1930, 1933) and his co-workers that our domestic potatoes were derived from Chiloe, a claim disputed by the author (Salaman 1937*b*). If the contention of the former is correct it is both noteworthy and peculiar that in none of our domestic varieties is there any suggestion of a like resistance. Indeed extreme susceptibility to infection with the *X* virus is common to every known variety of European potato.

## Discussion on some of the problems raised in this investigation

To-day any consideration of the behaviour of viruses either when within or removed from the host plants must take cognizance of the recent work of Stanley (1937) and his colleagues in America, and of Bawden and Pirie (1937*a*, *b*, *c*) and Bernal and Fankuchen (1937) in this country. These workers have shown that the active substance, i.e. the virus, can be neither separated nor distinguished from a protein body isolated from the plant, or animal infected. The *X* virus of potatoes is one of the plant viruses from which such has been won. These bodies have a definite molecular structure with a para-crystalline or, as in the case of the virus Tomato Bushy Stunt, a truly crystalline structure. They have been shown to be nuclear proteins of very large molecular

size and complex structure, and to be infectious in dilutions of $10^{-8}$ to $10^{-10}$. There seems to be good reason for identifying them with the virus itself.

Bernal and Fankuchen claim that these protein molecules won from the different strains or mutations of common tobacco mosaic so far examined can be recognized by differences of structure as exhibited by X-ray examination; these differences one presumes are based on changes affecting certain groups or radicles in the virus molecule itself. The various strains of the *X* virus here recorded may all be regarded as of this nature.

Protective inoculation demonstrates that a non-virulent strain affords complete protection against the most virulent, and that such protection is specific. If such protection is due, as is suggested, to the fact that the prior attachment of the virus molecule to the plant protein prevents the subsequent attachment of a second related strain, then the radicle by which such attachment is attained must be distinct from that producing virulence. We may designate this as "*A*" in the *X* virus molecule.

Strains $X^H$, $X^G$, $X^D$, and $X^L$ of the *X* virus produce either no visible symptoms under the Cambridge conditions, as in the case of $X^H$, or various types of mottle, as in the case of the others; but none of the four strains induce necroses local or systemic. The strains $X^S$ and $X^N$ produce both necrotic local and systemic lesions. Here then is a definite differentiation between the two groups which makes itself very apparent not only when they are the only infective agents present but also when either of these two groups of strains are in association with unrelated viruses such as that of tobacco mosaic.

It would seen probable therefore that we have both in the *X* virus molecule and the virus of the common tobacco mosaic at least two types of radicle responsible for the evocation of symptoms in the host: the first, or *M* type, responsible for mottle in tobacco and other hosts. This *M* radicle is itself capable of a limited range of modification as exemplified in the intensity of its reactions in the host plant. Whether these modifications are qualitative or, as is more likely, quantitative, must be left open, but such must be held responsible for the clinical pictures on tobacco already described as peculiar to infections with the strains $X^G$, $X^D$, and $X^L$. The second radicle, *N*, induces necroses local and systemic and is entirely responsible for the extensive destruction of tissue brought about by the $X^N$ strain; the $X^S$ strain which also produces necrotic reactions is regarded as being furnished with the *M* and the *N* radicle, for reasons which will be made clear directly.

The "masked" strain $X^H$ we must regard as either having no *M* radicle or, on the hypothesis that the *M* radicles of the various strains differ quantitatively, as possessing less of *M* than do the others. The latter is the view adopted here. These different values of *M* might be represented by giving the radicle such values as *M* of $X^H = 1$, of $X^G = 3$, of $X^D = 3$, of $X^L = 5$, in which case the effect of 1 *M* is regarded as being just below the threshold of clinical observation.

The difference between $X^S$ and $X^N$, as that between $X^L$ and $X^S$, is thus a qualitative

one, for $X^S$ is represented (fig. 47, p. 209), as possessing both $M$ and $N$ radicles, whilst $X^N$ (fig. 47), possesses only $N$, and $X^L$ only $M$. It might be thought that as $X^N$ produces a more virulent and destructive effect on tobacco, and even more so on potato, than does $X^S$, that it is illogical to suppose that $X^S$ is equipped with both $M$ and $N$, and $X^N$ with $N$ alone. There are, however, arguments of weight in favour of such a view. It has been shown that it is generally possible to extract a mottle form such as $X^G$ from an $X^S$ infected plant, and that such forms have not infrequently arisen as mutations; further, that $X^S$ on most potatoes produces a mottle only, all of which point to the presence of the $M$ radicle. On the other hand, all attempts to extract a mottle form from $X^N$ infected plants by selective inoculation have failed, which is to be expected if the $M$ radicle is absent. As regards the greater virulence of the $X^N$ compared with the $X^S$ strain which is furnished with both the $M$ and $N$ radicle, this is in fact what should be expected.

It has been shown that the resultant effect on the host plant of a mixture of two strains, one of greater and one of lesser virulence, is not a summation of virulences but a dilution effect, and if this happens when the radicles are in different molecules it is not unreasonable to suppose that it should happen when both are situated on the same molecule.

It is, of course, equally possible to regard the difference between $X^S$ and $X^N$ as quantitative rather than qualitative. Thus if $X^N$ is possessed of an $M$ radicle with a virulence value of, say 10, rather than 5 attributed to $X^S$, and further if this particular type of $M$ radicle is more stable than those of lower value, then the fact that $X^N$ does not mutate in tobacco cultures would be explained. On the other hand, there is good reason to believe that $X^N$, when present in certain potato varieties (pp. 160 and 163), does undergo change and under such conditions it would be necessary to assume that its high value $M$ radicle mutated to ones of lower value. If, however, there be no $M$ radicle present in $X^N$, then such mutation would presumably be consequent on the breakdown of the radicle $N$ to a low $M$ form, which leads to the conclusion that there is no essential difference between the two views.

A comparison of the two strains $X^D$ and $X^N$ presents an interesting problem. On the potato both have a clear-cut and similar destructively necrotic effect; on tobacco the former is indistinguishable from $X^G$, whilst the latter is severely necrotic in its action and resembles $X^S$. We can explain this by assuming that the $X^D$ differs from the $X^G$ strain in the possession of a radicle $P$ which only comes into action in a potato host plant (fig. 47), and that $X^N$ is similarly furnished.

The experiments recording the effect of inoculating mixtures of each of the six strains of $X$ with three distinct strains of tobacco mosaic respectively, indicate that both the $X$ virus and the tobacco mosaic virus possess a further distinct radicle. Alone the radicle is without effect, but when acting in unison with its fellow of the second virus, they together produce a distinct lightly necrotic reaction in healthy tobacco plants. This radicle is referred to as $C$. The position is represented diagrammatically in

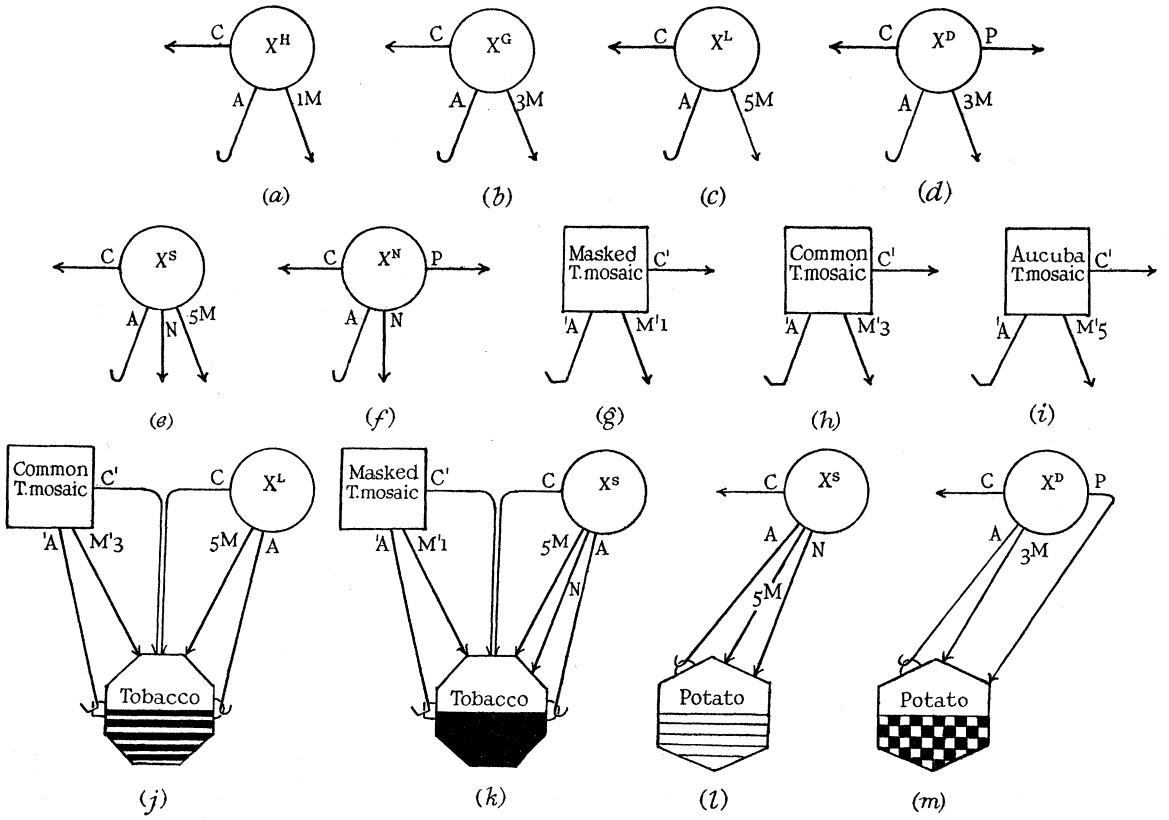



FIG. 47. (*a*) Represents the masked strain $X^H$.

(*b*) Represents the mild strain $X^G$.

(*c*) Represents the tortoiseshell pattern strain $X^L$.

(*d*) Represents the strain $X^D$ which produces the same effect on tobacco as $X^G$ and foliar necrosis on potato, a disease very similar to that produced by $X^N$.

(*e*) Represents the severe strain $X^S$ producing necroses and mottle on tobacco and a mottle on potato.

(*f*) Represents the necrotic strain $X^N$ producing a very severe necrosis on tobacco and an interveinal necrosis on potato.

(*g*) Represents masked tobacco mosaic.

(*h*) Represents common tobacco mosaic.

(*i*) Represents Aucuba tobacco mosaic.

(*j*) Represents the interaction of common tobacco mosaic and the $X^L$ strain. The necrotic result is in the main due to the united action of $C$ and $C'$. An exactly similar reaction ensues when $X^H$, $X^G$ or $X^D$ is substituted for $X^L$. The shaded portion of the tobacco figure represents their common reaction.

(*k*) Represents the interaction of masked tobacco mosaic and the $X^S$ strain. The intensely necrotic and commonly lethal effect is due to the $N$ radicle added to that produced by $C$ and $C'$. The blackened portion of the tobacco figure represents the lethal necrosis.

(*l*) Represents the action of the severe strain $X^S$ on a President potato plant—a mild mottle. The lightly shaded portion of the potato figure represents the slight and often transient mottle produced by either $X^G$, $X^L$, or $X^S$.

(*m*) Represents the action of the strain $X^D$ on President potato—a severe interveinal or foliar necrosis produced by the radicle $P$. A similar result ensues when $X^N$ is substituted for $X^D$. The chequered pattern on the lower half of the "potato" figure represents the scattered punctate necroses of the foliage peculiar to infection with these two strains.

fig. 47. Here we see that the clinical result produced on the host by the combination of a masked *X* strain and a masked tobacco mosaic strain each possessing a subliminal dose of the *M* radicle is practically the same as that resulting when sister strains in which a larger and more effective doses of *M* is substituted for $X^H$.

In the case of the tobacco mosaic virus, the substitution of either the common or the aucuba strain for the masked one makes no difference to the necrotic reaction, but in the latter case the bright yellow mottling appears as a late symptom. It is permissible to speak of the radicle responsible in the *X* virus as "*C*", and that in the tobacco mosaic virus as "*C'*".

In other words, the united action of *C* and *C'* is sufficient to produce the major part of the pathological picture. When, however, the strains $X^S$ or $X^N$ enter into the combination the effect of the radicle *N* converts the pathological picture into one of intense necrosis, ending in the death of the earlier leaves invaded and often that of the whole plant.

It will be remembered that the strains $X^H$, $X^G$, $X^D$ and $X^L$ produce identical reactions on *Capsicum*, and that the two strains $X^S$ and $X^N$ although they evoke a more severe and different type of symptom cannot themselves be differentiated on this plant. It was further observed that all strains brought about the development of local lesions. If we attempt to explain the *Capsicum* reactions on the basis of the assumptions already made, we may say that the radicle responsible for the $X^H$ to $X^L$ effects cannot be "*M*" because these do not show the quantitative differences which would be expected with different values of "*M*"; nor is it likely that "*M*" would cause local lesions. The identity of the $X^S$ and $X^N$ reactions is a further argument against the interaction of "*M*". It is the radicle "*C*" one would associate with this reaction, seeing that tobacco mosaic virus and its strains induce a similar effect on *Capsicum* As with the tobacco mosaic mixtures, the addition of the "*N*" radicle gives an intensely necrotic character to the original effect.

We have spoken of distinct radicles in the protein molecule as responsible for certain specific reactions; it is a logical step to relate them to antigens and to turn to the serological data to see what support they may give to the idea. So far, but little analytical work on serological lines has been done with plant viruses. A common agglutinin has been demonstrated for all the *X* strains and shown to be present in at least as great a quantity in $X^H$ as in any of the others. We shall probably not be wrong in considering this antigen as the equivalent of the radicle *A*, supported as it is by the fact that $X^H$, $X^D$, and $X^L$ all equally protect against $X^S$ and $X^N$. Bawden (1935) showed that formalin destroyed the infectivity of the *X* virus strains but left the flocculation intact, i.e. the radicle *M* is destroyed and *A* survives. He did not work with $X^N$. Spooner and Bawden (1935) demonstrated that when anti $X^G$ serum was mixed with $X^S$ juice, 87 % of the subsequent inoculations failed, but when mixed with $X^G$ or $X^L$ juices 100 % failed. Again anti-$X^S$ serum when mixed with $X^G$ juice neutralized 89 %, and with $X^L$ 95 % of the plants, the dilution and procedure in all cases being

the same. This may be taken as showing that although $X^S$ is more virulent than $X^G$ its anti-serum does not react on $X^G$ as effectively as does an anti-$X^G$ serum itself. In other words, the antigens responsible for the clinical effect brought about by the different strains are not identical though they may be very closely related, a relation which it is suggested is a quantitative one. Similar experiments recorded on p. 175, show that $X^N$ is not readily neutralized by anti-$X^G$ serum. There would seem to be little doubt that further study of the antigen constitution of the different strains of the virus cannot fail to throw more light on the structure of the virus molecule.

Five distinct radicles in the *X* virus molecule and its strains are recognized:

Radicle *A*: responsible for attachment of the virus to the plant protoplast, and is specific for virus *X*.

Radicle *M*: responsible for the induction of non-necrotic mottle in tobacco plants.

Radicle *N*: responsible for the induction of necrotic lesions in tobacco plants.

Radicle *P*: responsible for necrotic foliar lesions in the potato.

Radicle *C*: responsible in union with a radicle present in each of the three strains of tobacco mosaic under review, for the evocation of a distinct and lightly necrotic mottle in tobacco.

## The frequency of virus mutation

Mutation rates have been studied intensively in *Drosophila* and, according to Müller (1930), in a normal environment mutation is unlikely to occur in a gene more than once in one hundred thousand to a million individuals of a given generation. It has however been shown that this rate can be much accelerated by subjecting *Drosophila* to the action of X-rays or radium. Others have induced an increased mutation rate through the application of high temperatures to the germ cells.

The best ascertained plant virus mutations, viz. those of common tobacco mosaic to yellow mosaic, can be observed under normal conditions of temperature and the like in every infected plant if kept long enough. There is no means of even guessing the frequency of such mutation in terms of virus particles, but McKinney's investigations suggest that it cannot be a very rare occurrence. There is good evidence that a plant virus with appropriate treatment may be induced to mutate at an increased pace. Such acceleration we have seen happen when *X* is introduced into a member of the family Chenopodiaceae or, in the case of the *Y* virus, when it is passed through *Schizanthus* and possibly also when certain treatment is applied to root fibres of infected plants.

It should be observed, however, that although virus mutations must be accepted as facts, it does not follow that a mutation has taken place every time a change of reaction is observed in an apparently pure line. A change, even a sudden change in reaction, may be due to selective agency operating on a mixture of strains. We have seen that an artificial mixture in which $X^G$ and $X^S$ are mixed in proportions of 9 : 1 induces a

predominantly $X^G$-like picture. It is easy to see that any factors which hinder the development of $X^G$ and encourage that of $X^S$ in such a mixture will bring about a more or less rapid change in the clinical picture from $X^G$ to $X^S$. Any factor which induced an aggregation of like particles would still further hasten a change over a clinical reaction; such indeed probably accounts for the greater frequency of mutations in highly purified virus suspensions where we know such aggregation does, in fact, take place.

The difficulty, however, is to decide which is a case of selection from a pre-existing mixture and which a mutation. McKinney (1935), who has paid much attention to this problem, is strongly of the opinion that in the case of the variants arising from the common mosaic of tobacco we are dealing with true mutations. It cannot be said that the evidence is conclusive; in the nature of the problem it is unlikely to be so, but the following experiments of McKinney are strongly in favour of a mutation occurring under the conditions described.

When *Nicotiana glauca* is infected with common tobacco mosaic the virus becomes systemic; when infected with yellow mosaic it does not do so. If a mixture of the two strains be applied simultaneously to a leaf of a healthy *N. glauca*, the yellow remains localized whilst the common mosaic spreads everywhere; when an extract of sap is made from the young green top growth and communicated to healthy tobacco plants, these develop the common green mosaic and eventually all of them produce yellow spots from which a pure yellow mosaic can be extracted. A similar experiment in which mild dark green mosaic is substituted for common mosaic supports the view that the yellow mosaic which appeared in the young leaves in the former experiment is a genuine mutation and has not migrated from the original mixture. Mild dark green mosaic has not been observed to give rise to yellow mutants and extracts from the young top growth reproduces in healthy plants the mild dark green virus only, no spots of yellow mosaic occurring. Till we can isolate virus particles and cultivate them *in vitro*, a more decisive answer is not likely to be found along these lines.

Jensen (1936), working with both tobacco mosaic and cucumber mosaic, has come to similar conclusions. He emphasizes the instability of the virus particle of tobacco mosaic inasmuch as he not only has isolated some score of yellow mosaics from the common type, but finds that they themselves when isolated give rise to still further strains.

If, however, virus particles are not themselves capable of reproduction but induce the creation of their like in the living cell of their host in which they find themselves, it is not unreasonable to suppose that mutated particles might also induce a change affecting the character and the quantity of the particles produced or about to be liberated from the host cells. Something of this kind seems to be indicated by the conversion of $X^S$ to $X^G$ brought about *in vitro* by contact with sugar beet juice, containing fragments of living plant tissue.

We may conclude that virus strains are not to be regarded as the result of the

experimental sorting out of an age-long mixture, but rather as forms arising *de novo* in our experimental plants and in nature, from pre-existing virus stocks.

Findlay (1936), in an exhaustive account of variation of viruses affecting animals, and again in an unpublished account of those affecting plants which he has kindly allowed the writer to see, is inclined to look on virus mutations as *Dauermodifikationem*. Jollos (1932), who is responsible for this view, held that apart from other considerations, bacteria and the like are incapable of true mutation because they are devoid of the necessary nuclear apparatus. Without pursuing this aspect of the problem further it must be admitted that changes in bacteria and virus particles may be brought about by a different mechanism to that at work in higher forms of life, but that nothing seems to be gained at present by using a different term to describe the new forms.

The writer is not aware of any case where a mutant has been reported to have reverted to the common tobacco mosaic, a substantial argument in favour of mutation rather than selection from pre-existing mixtures. McKinney (1937) remarks also on the absence of reversion.

Price (1934), in the course of his work, attained a new strain of cucumber mosaic which developed yellow primary lesions instead of necrotic ones in the cow pea, and which became systemic instead of being localized to the primary lesion. This new form must have arisen within a local lesion and in this respect resembles the formation of $X^G$ in a local lesion in sugar beet produced by what is to the best of our knowledge a pure $X^S$ strain.

Another consideration of importance is the size of the minimal dose of particles required to bring about infection. If, as is now generally supposed, this ranges round some figure such as $10^9$ particles, then a mere mutation of a single particle cannot induce a recognizable mutation effect. Either a large number of particles must mutate simultaneously in the same direction, or the environmental conditions must be particularly favourable to the rapid multiplication of the single mutated particle. One such condition would be that which allowed the progeny of a mutated particle to aggregate in such a manner that it would acquire a reasonable chance of inducing an individual infection.

The occurrence of mutations in protein molecules of definite structure which compared with the living cell of plant or animal are minute, inevitably incites a comparison with like variations in more complex living organisms. The comparison which suggests itself is between the virus particle and the gene on the one hand, and the host plant and the somatic structure of plant or animal on the other. Such a view has recently been discussed by McKinney (1935, 1937). The objections to such an analogy, so long as we are only considering the nuclear genes, are obvious: the virus particle can be isolated from its host and when returned to its appropriate environment exert its normal reaction. The gene is known only as part of a fixed structure within the cell nucleus acting invariably in relation with its fellows; it is reproduced in an orderly manner in company with other cell structures, whilst the virus particles multiply in

vast quantities in a manner as yet unknown. These objections lose much of their weight, and the analogy gains in strength, if it is made between the smaller virus particles and the free genes of the cytoplasm, a suggestion made to the writer by Professor J. B. S. Haldane. The gene, it is generally assumed, has only one specific activity, the virus particle may have several, as has been shown to be the case with the *X* virus. If, however, we regard the changes to which both virus particle and gene are prone, we find one character, that of mutability, which is especially striking when we consider the phenomenon of multiple allelomorphs, such as those controlling eye colour in *Drosophila* or albinism in the coats of rodents. Here a series of ordered mutations of the parent gene produce specific modifications in a step-like manner. In such a series there is a tendency for the mutations to occur in a definite direction, generally in a descending scale, that is, from one inducing greater colour to one inducing a lesser. Changes in the reverse direction are, however, recorded. In regard to the changes observed in the *X* virus we have a close parallel: the strains present definite steps from a higher to a lower virulence, and so far as has been observed they take place in one direction only. The basis of a mutation probably lies in some relatively slight modification of the original gene, possibly the loss of a radicle, or its replacement by another. It is just such changes as may be suspected of being the underlying cause of change in the virus particle. Of less moment, but of considerable interest, is the close similarity of effect between the action of certain viruses and specific genes of which the enation strain of tobacco mosaic on tomato and the shoe-lace genetic mutation in the same plant is an example. Recent researches on the *X* and other viruses have shown that no hard and fast line can be drawn between what we have been accustomed to regard as living as opposed to non-living matter. All we may perhaps permit ourselves to say to-day is that some viruses, like the *X* virus, are complex organic particles endowed with but a few of the characters encountered in the simplest known living organisms. Such viruses fit into neither of the accepted categories of the living and non-living. The facts are there: we must adjust our concepts to them.

The writer desires to express his deep gratitude to his former colleague Mr F. C. Bawden and to his present colleague Dr R. Dennis for the unstinted assistance they have rendered him throughout the whole course of these investigations. It is not too much to say that without their generous co-operation this work could not have been brought to a conclusion.

## Summary

The *X* virus is a term which should be applied to a group of strains, rather than to a single specific pathogen with fixed and constant reactions. It is shown that at least six strains are to be found distributed amongst the diseased potato plants of this country, and that most of them are represented in Germany, Canada and the U.S.A.

In the open field a potato plant is usually infected by two or more strains simultaneously, but in the case of the milder $X^H$ and the more virulent $X^N$ pure single strain infections do occur.

The strains range in respect to their virulence, as judged by their behaviour in tobacco and *Datura*, from the creation of a symptomless carrier to an intensely virulent necrosis endangering the continued existence of the host. The reaction of the strains is described on a large range of plants.

The strain $X^N$ is shown to be the pathogen responsible for a distinctive morbid condition in the potato, here called "Interveinal necrosis". The disease and the reactions of the strain are described for the first time.

Infection by any of the strains brings about the formation of inclusion bodies within the host tissues. Such inclusions do not vary in character or frequency in relation to the virulence of the strain. There is a certain variety in their appearance which may be recognized, whichever strain is infecting the host.

All the strains are made up of similar sized particles and possess certain identical serological reactions.

From plants infected by each strain the same type of nucleoprotein has been recovered, which in solution exhibits anisotropy of flow.

The physical properties of the strains are similar except for the degree of dilution which they will withstand.

The clinical results obtained by mixture of pure strains has been studied, evidence is given that a tenfold excess of a weak over a strong may lead to the clinical masking of the latter in the host plant; a lesser relation of strong to weak has the opposite result.

The double reactions that ensue from mixtures of the different $X$ strains with the $Y$ virus, the $A$ virus and tobacco mosaic virus respectively, conform to the actual differences in virulence of the different strains themselves.

The reactions resulting from mixtures of the various strains of the $X$ virus with three distinct variants of tobacco mosaic virus disclose the fact that the strains $X^H$ $X^G$ $X^L$ and $X^D$ all behave in a similar manner in the presence of the tobacco strains. An explanation is offered.

Conversion of one strain to another has been studied both in material which is relatively pure to strain as well as in that which we have good reason to believe is in reality pure. In both conversions have taken place. Conversions have been effected by passage through certain unrelated plants.

All conversions, whether purposely designed or not, have been from the more to the less virulent strain.

The nature of these conversions is discussed and the conclusion reached that they should be regarded as true mutations.

The phenomenon of acquired immunity as exhibited by the potato and other solanaceous plants in respect to the $X$ virus is described, as well as its occurrence in

nature, and the possible relation of the same to the occurrence of certain types of carriers.

The structure of the virus particle in terms of active specific radicles and their relations to antigens is discussed. An hypothesis to account for the differences between the strains is suggested.

A comparison between the virus particles and the gene is considered and the suggestion made that the analogy should be with the free genes of the cytoplasm rather than with those of the nucleus.

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## Description of Plates 18–25

### Plate 18

Fig. 2. *Nicotiana tabacum.* Healthy leaf: one infected with $X^H$ would be indistinguishable from it.

Fig. 3. *Nicotiana tabacum.* Infected with $X^G$: in which the mottle is more evident at the apex of the leaf.

Fig. 4. *Nicotiana tabacum.* Infected with $X^G$: in which the presence of a trace of $X^S$ is shown by the presence of small bright yellow spots.

Fig. 5. *Datura stramonium.* Infected with $X^G$: note the very fine vein-banding.

Fig. 6. *Datura stramonium.* Infected with $X^D$: the symptoms are essentially the same as those evoked by $X^G$.

Fig. 7. *Nicotiana tabacum.* Infected with $X^L$: showing early vein clearing.

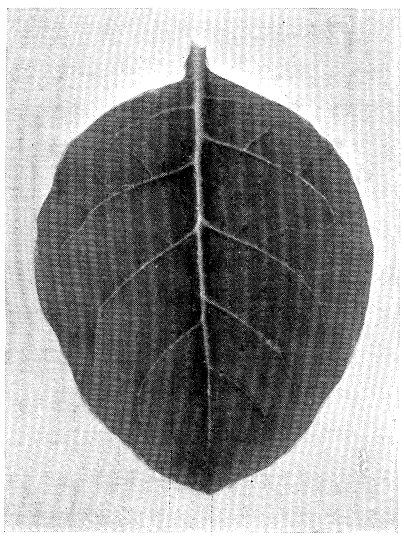


FIG. 2

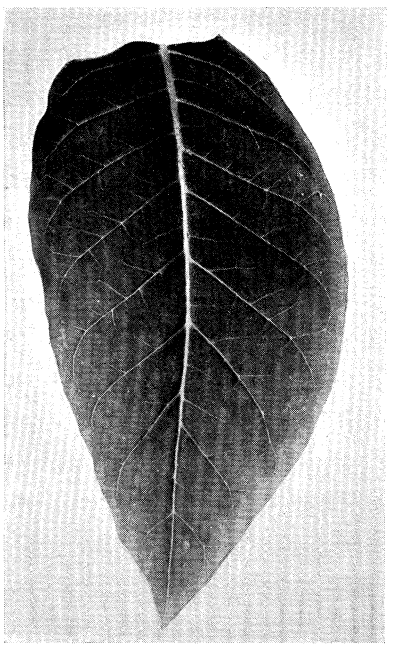


FIG. 3

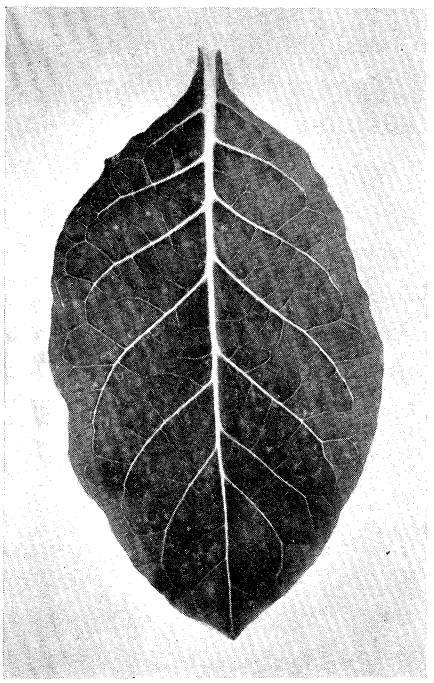


FIG. 4

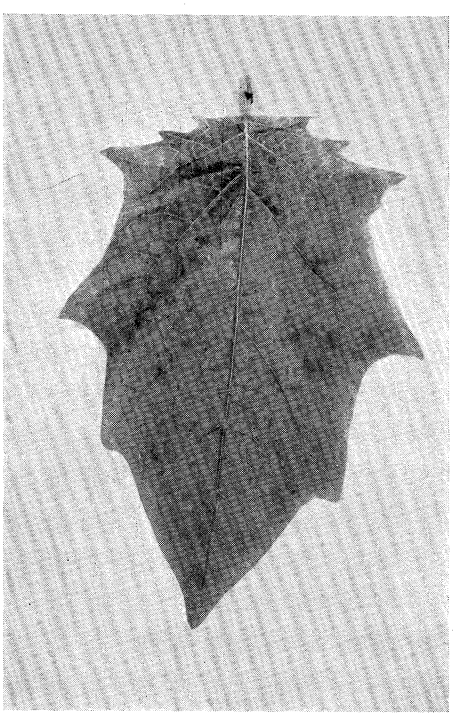


FIG. 5

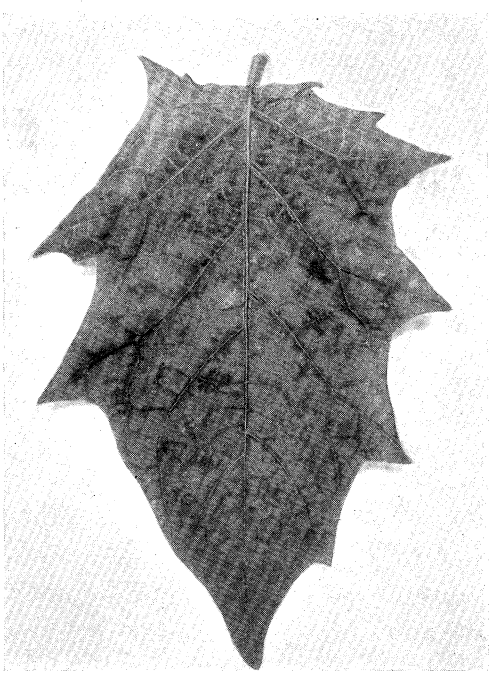


FIG. 6

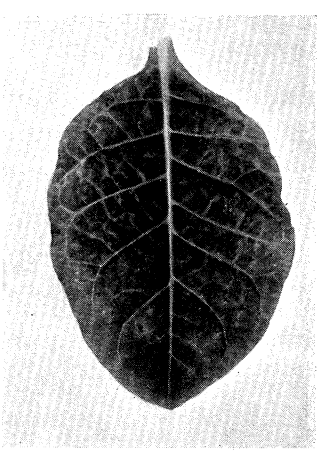


FIG. 7

## PLATE 19

FIG. 8. *Nicotiana tabacum.* Infected with $X^L$: showing the characteristic tortoiseshell pattern.

FIG. 9. *Datura stramonium.* Infected with $X^L$: displaying a brilliant yellow mottle with green vein-banding.

FIG. 10. *Nicotiana tabacum.* Infected with $X^L$: showing symptoms after 10 weeks when the tortoiseshell pattern has been replaced by an irregular mottle.

FIG. 11. *Nicotiana tabacum.* Infected with $X^S$: showing early necrotic vein clearing.

FIG. 13. *Nicotiana tabacum.* Infected with $X^S$: showing systemic symptoms, necrotic spotting and rings.

FIG. 14. *Nicotiana tabacum.* Infected with a mixture of $X^L$ and $X^S$: showing necrotic rings and figures modelled on an essentially $X^L$ type of mottle.

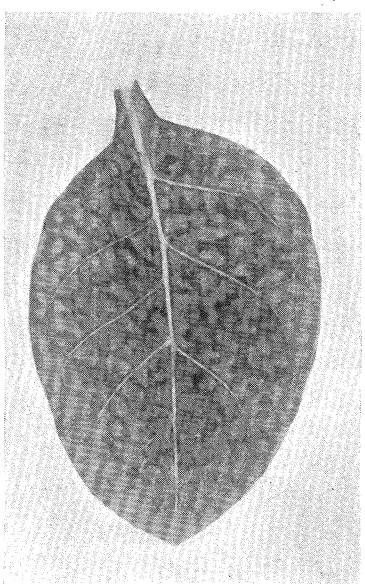


FIG. 8

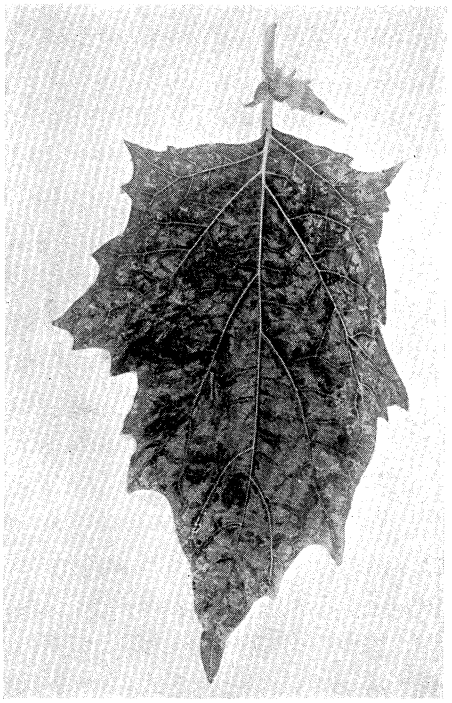


FIG. 9

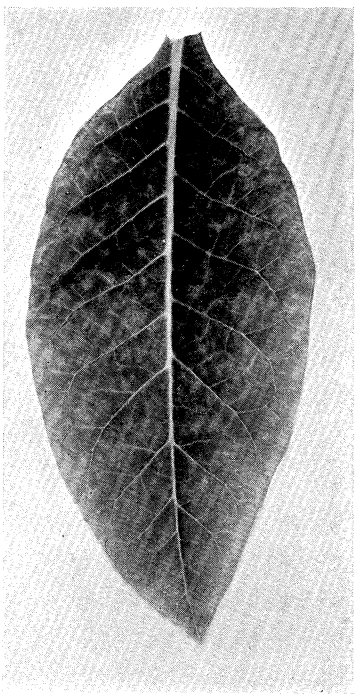


FIG. 10

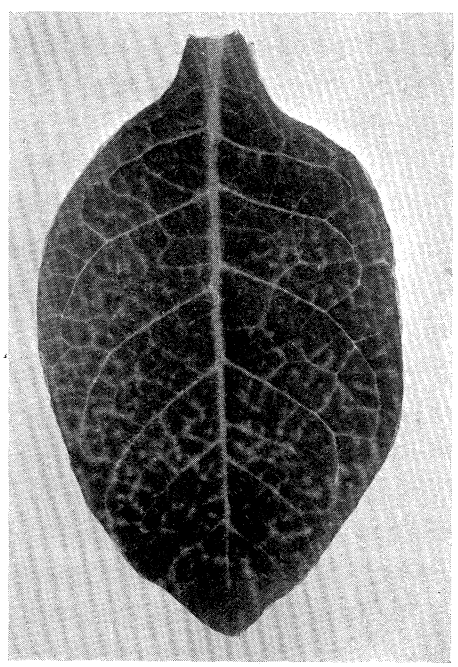


FIG. 11

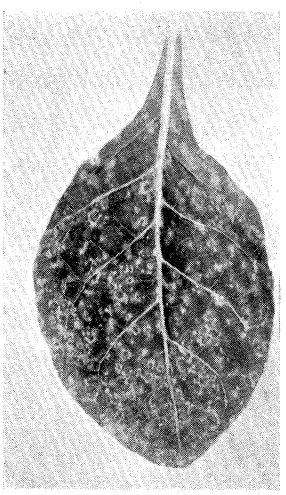


FIG. 13

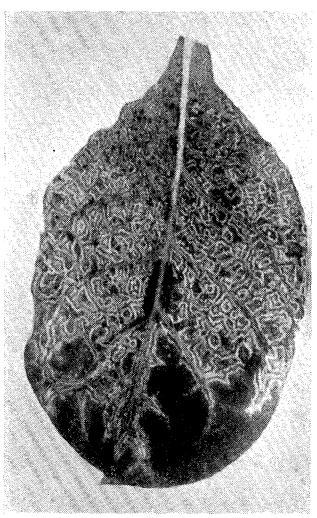


FIG. 14

## PLATE 20

FIG. 15. Potato, variety Majestic. Infected with $X^N$: second year symptoms showing interveinal necroses and leaf-drop.

FIG. 16. Potato, variety Arran Victory. Infected with $X^N$: showing early interveinal necroses. Infra-red photograph.

FIG. 17. *Capsicum annuum.* Infected 10 weeks previously with $X^S$. The upper leaves and stem have been destroyed, new growth appearing from lower node; at first healthy, later diseased.

Fig. 18. *Capsicum annuum.* Infected with $X^H$: young leaf showing early systemic etching, lesser in degree but similar in character to that which follows infections with $X^G$, $X^D$, and $X^L$.

FIG. 19. *Capsicum annuum.* Infected with $X^D$: showing later systemic interveinal necroses and crinkle, common to infections with this strain and $X^H$, $X^G$, and $X^L$.

FIG. 20. *Hyoscyamus niger.* Infected with $X^S$: local necrotic lesions, photograph through green screen.

FIG. 21. *Hyoscyamus niger.* Infected with $X^N$: local necrotic lesions, the dark outlines are rendered visible by a green screen.

FIG. 22. *Lycopersicum esculentum,* variety Kondine Red. Infected with $X^N$: local necrotic lesions.

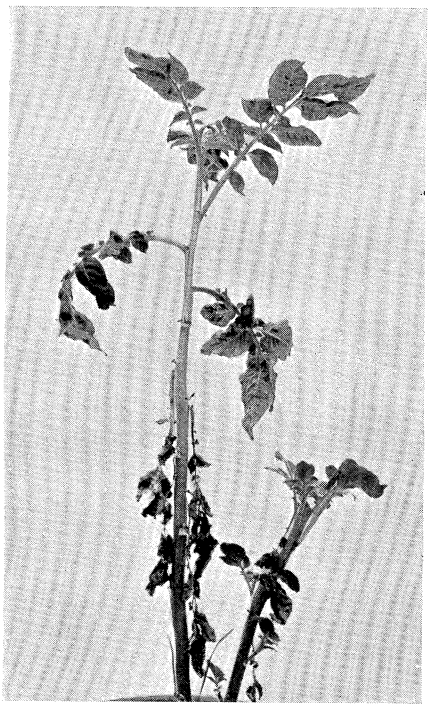


FIG. 15

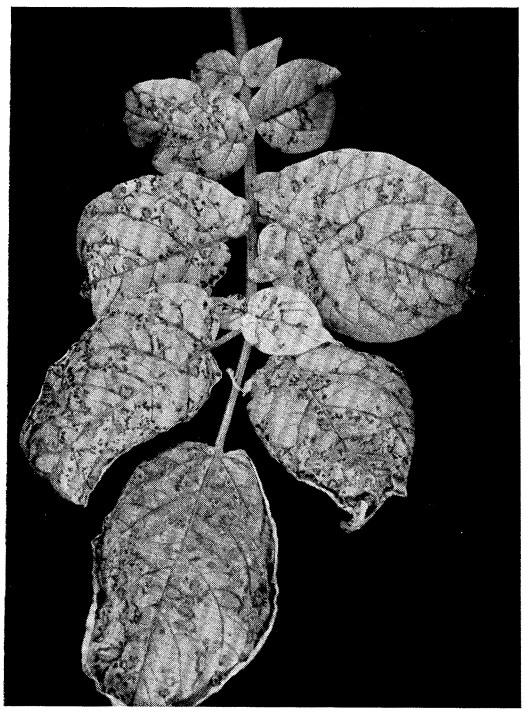


FIG. 16

FIG. 18

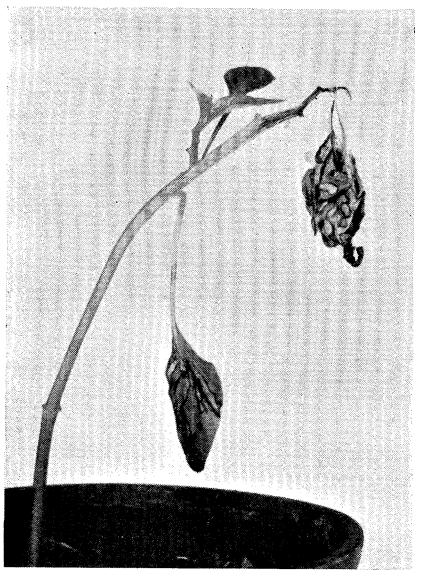


FIG. 17

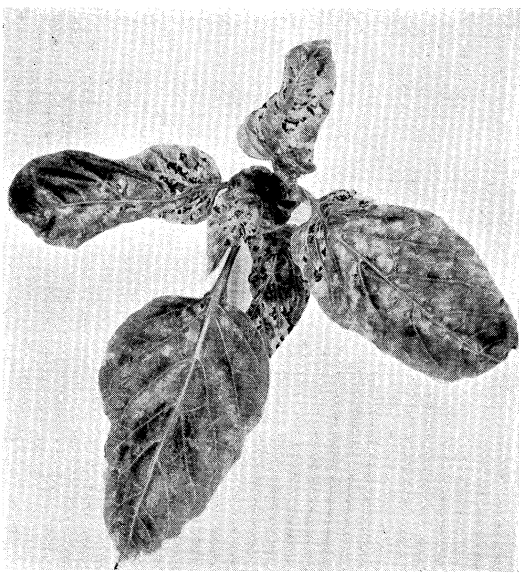


FIG. 19

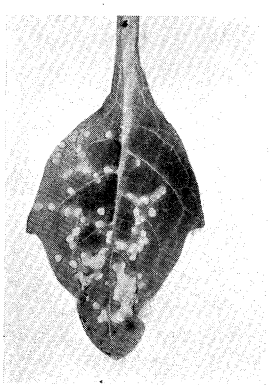


FIG. 20

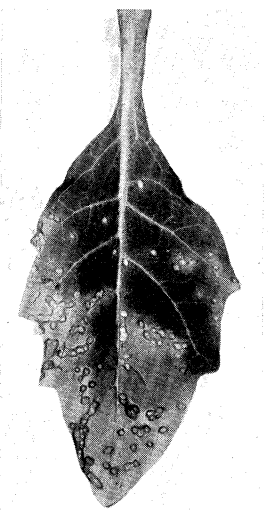


FIG. 21

FIG. 22

## PLATE 21

FIG. 23. *Hyoscyamus niger*, seedlings. Infected with $X^S$ and $X^N$, with healthy control. $X^S$ induces mass necrosis and collapse of leaf; $X^N$ induces scattered discrete necrotic spots.

FIG. 24. *Lycopersicum esculentum*, variety Kondine Red. Infected with $X^N$: severe systemic symptoms: wilting of leaves and stunting of plant.

FIGS. 25–31. *Nicotiana tabacum.* Progressive stages in a gradual concentration of $X^S$ during a separation of the same, by selective inoculation, from a mixture of $X^L$ and $X^S$.

FIG. 25. *Nicotiana tabacum.* An essentially $X^L$ type pattern in which the yellow areas are lightly etched.

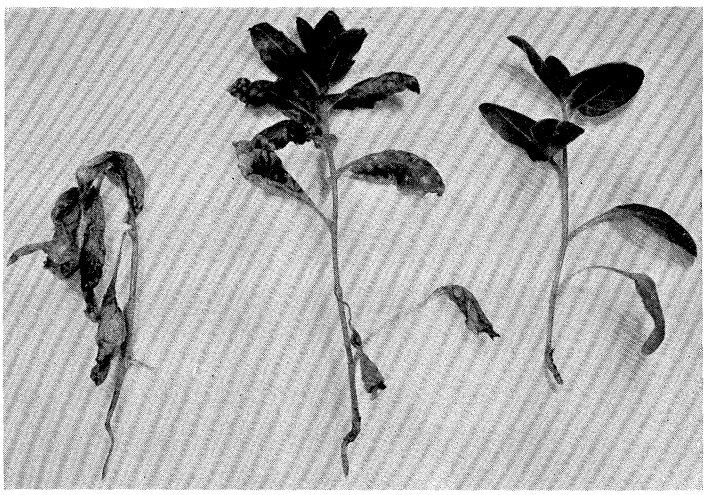
FIG. 26. *Nicotiana tabacum.* An older leaf of the above plant in which finer etching and figures are superimposed on the basic $X^L$ pattern.

FIG. 27. *Nicotiana tabacum.* A further stage in $X^S$ concentration, in which the vein-banding pattern is almost confined to the major veins, the intervening areas being lightly necrotic.

FIG. 28. *Nicotiana tabacum.* The $X^L$ pattern is retained but the etching is more severe.

FIG. 29. *Nicotiana tabacum.* A younger leaf of the same plant in which a relatively normal $X^L$ pattern is developing.

FIG. 30. *Nicotiana tabacum.* An exceptional phase in the development of the "dissected $X^L$ pattern" in which the central interveinal areas remain green and the tissues nearer to the veins are pale and necrosed.



$X^S$ $X^N$ Healthy

FIG. 23

FIG. 24

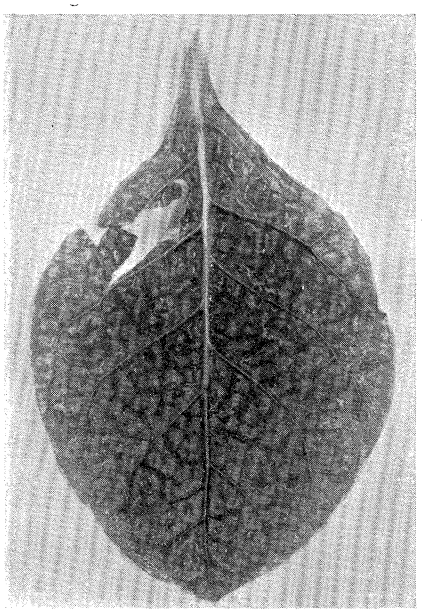


FIG. 25

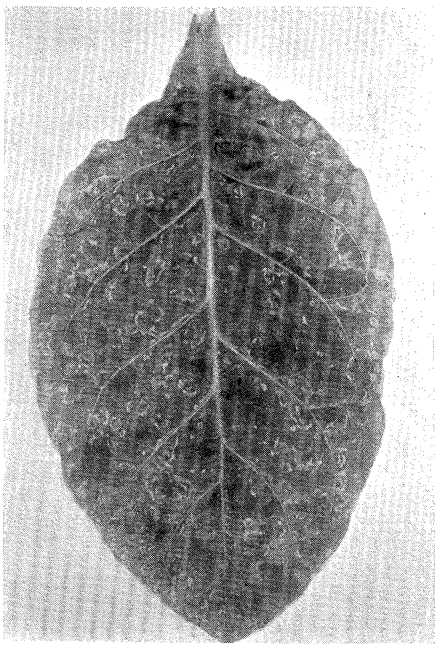


FIG. 26

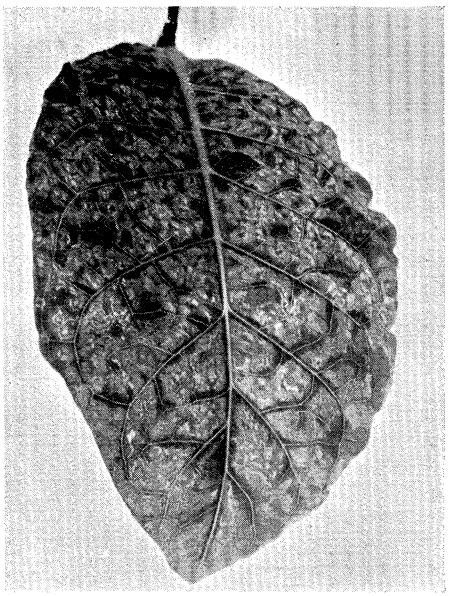


FIG. 27

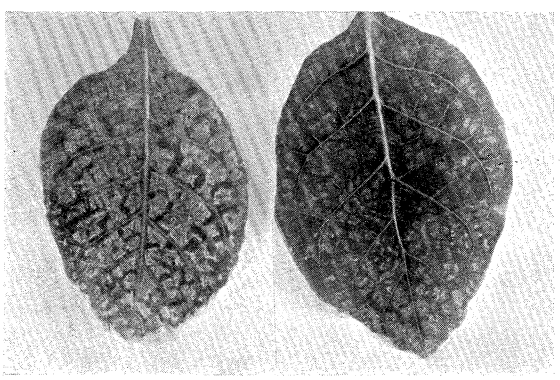


FIG. 28

FIG. 29

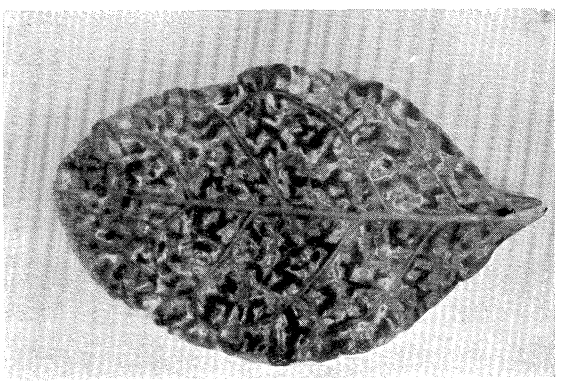


FIG. 30

## PLATE 22

FIG. 31. *Nicotiana tabacum.* Full "dissected $X^L$ pattern" is reached after ten generations of selective inoculation.

FIG. 32. *Beta vulgaris*, common sugar beet. Infected with $X^S$: maximum development of local lesions, consisting of concentric necrotic lines.

FIG. 33. *Nicotiana glutinosa.* Acquired immunity. Left: plants protected by prior inoculation with $X^G$ against infection by $X^S$ eight days later; right: control plants infected with $X^S$ on same date.

FIG. 34. *Nicotiana tabacum.* Acquired immunity. Right: plant protected by prior inoculation with $X^G$ against infection with $X^S$ fourteen days later; left: control plant inoculated with $X^S$ on same date.

FIG. 35. *Datura stramonium.* Acquired immunity. Right: protected by $X^G$; left: control infected with $X^S$.

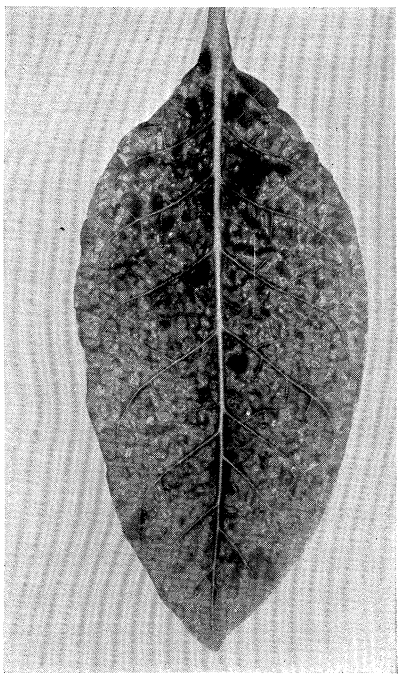


FIG. 31

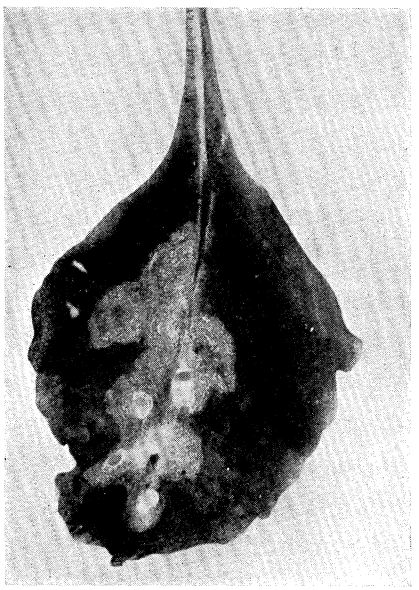


FIG. 32

FIG. 33

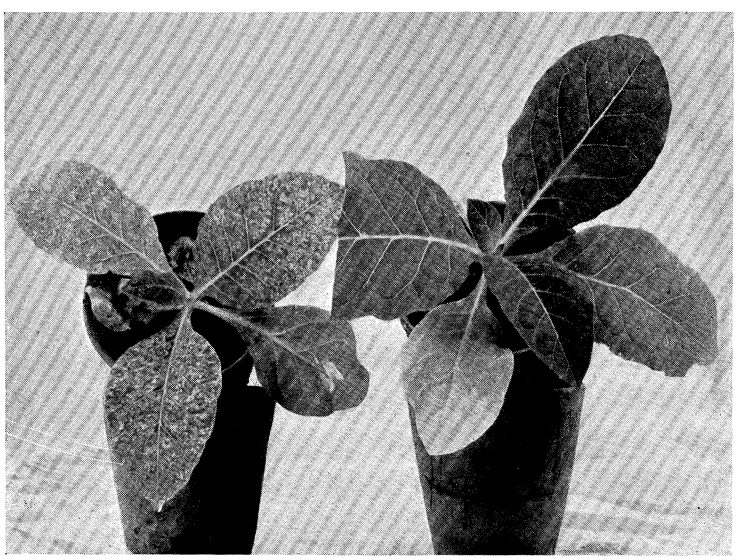


FIG. 34

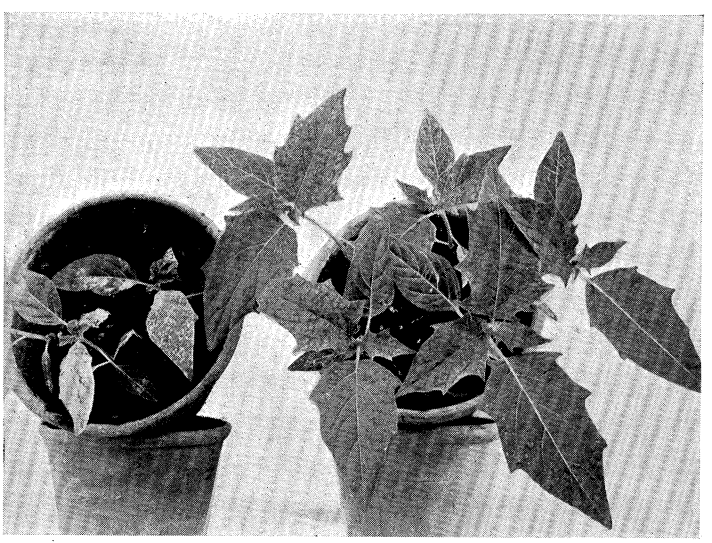


FIG. 35

## PLATE 23

FIG. 36. *Nicotiana tabacum.* Intracellular inclusions or *X* bodies in epidermal cells of leaf infected with $X^L$.

FIG. 37. *Nicotiana tabacum.* Two nuclei in a single cell in leaf infected with $X^N$.

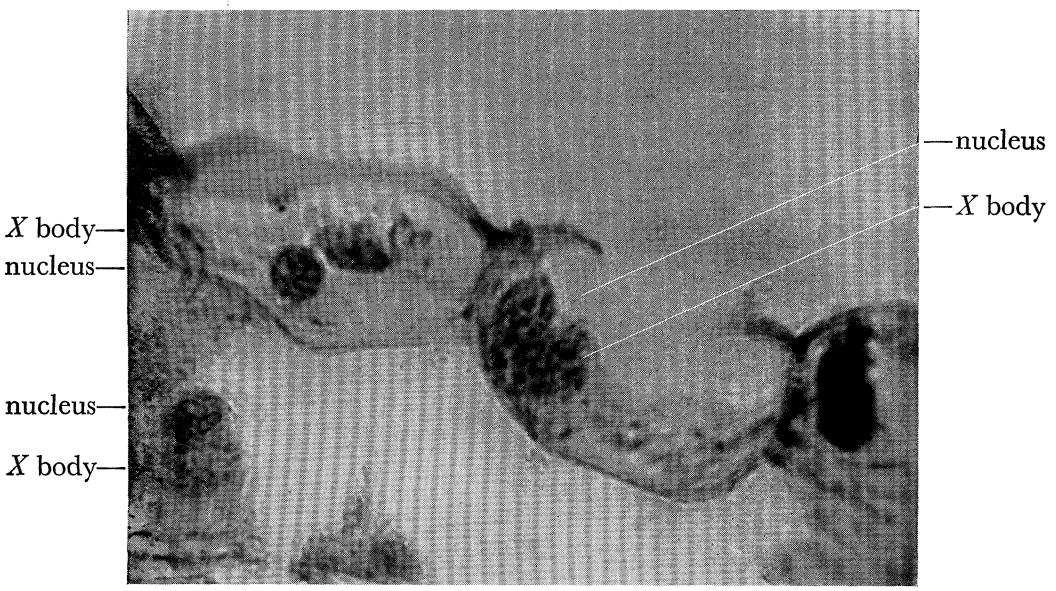

X body
nucleus
nucleus
X body
nucleus
X body

FIG. 36

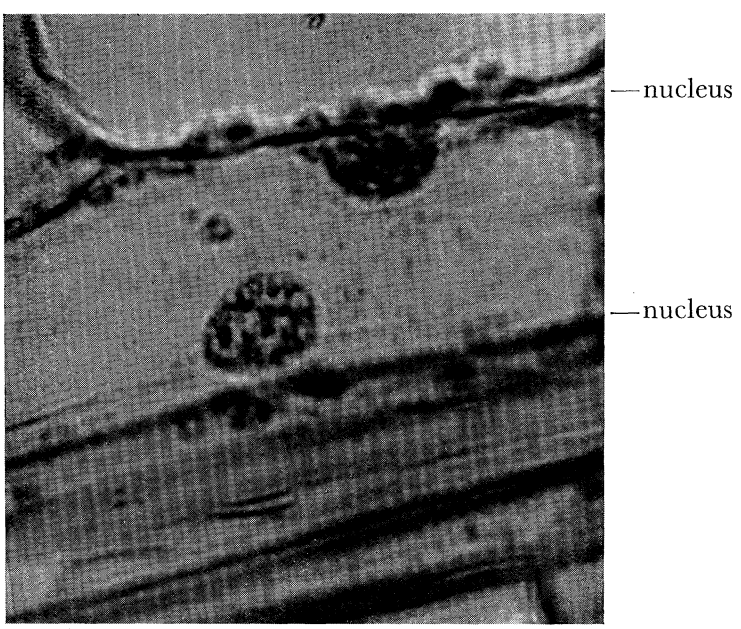
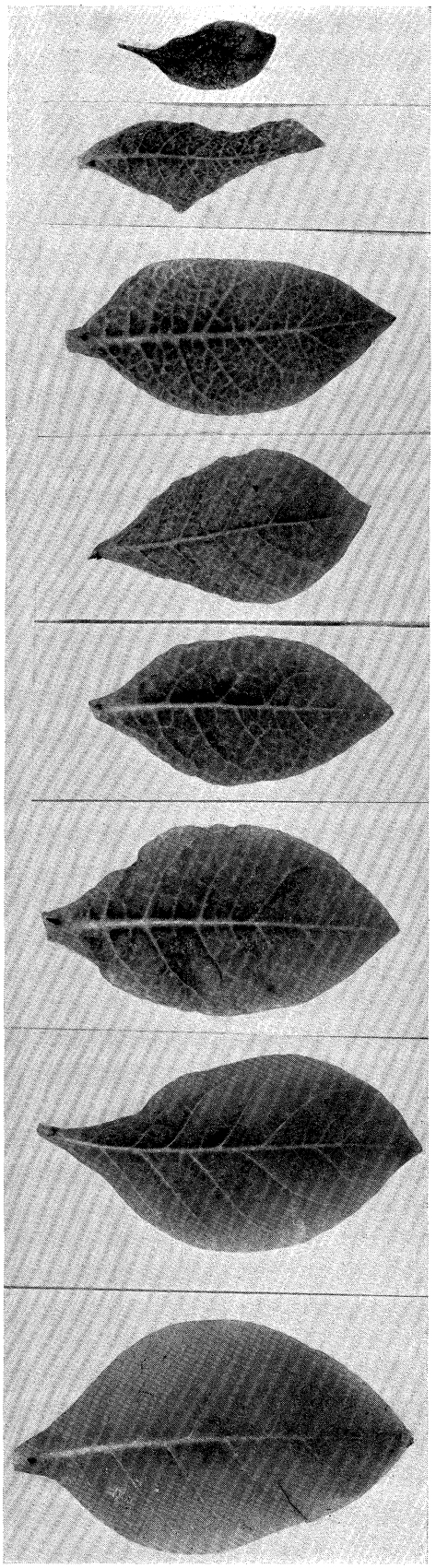

nucleus
nucleus

FIG. 37

## PLATE 24

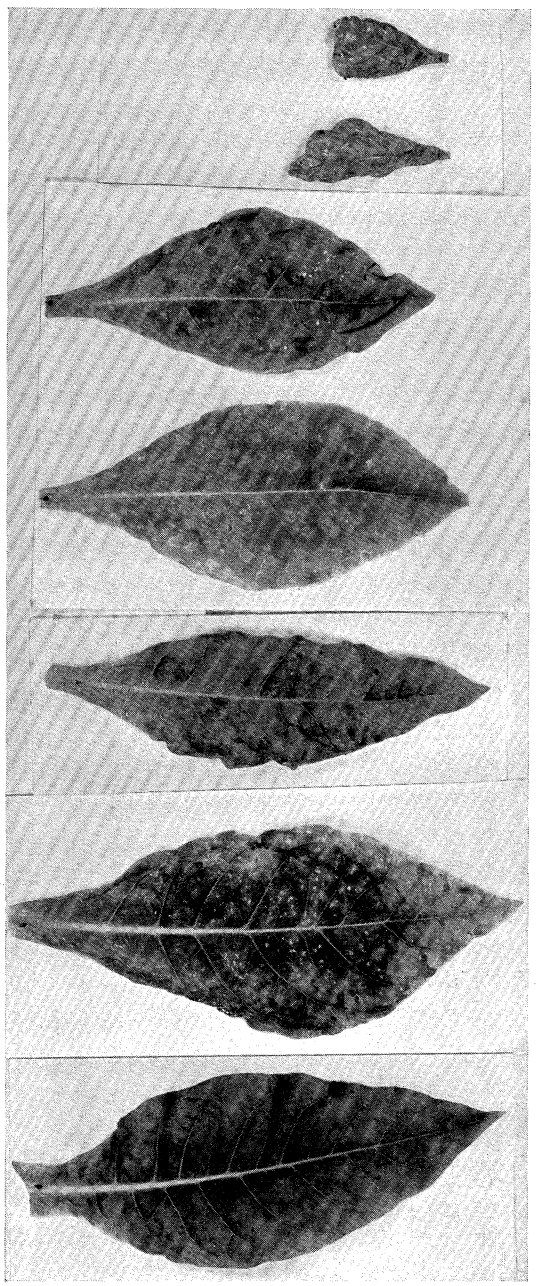
FIG. 38. *Nicotiana tabacum.* Series of leaves showing: healthy control, "*Y*" infected control, and series of leaves from plants infected with *in vitro* mixtures of the *Y* virus and the six strains of the *X* virus respectively.

FIG. 39. *Nicotiana tabacum.* Series of leaves showing: common tobacco mosaic, and series from plants infected with *in vitro* mixtures of tobacco mosaic and the six strains of the *X* virus respectively.



control $Y$ $X^H+Y$ $X^D+Y$ $X^G+Y$ $X^L+Y$ $X^S+Y$ $X^N+Y$

FIG. 38



common T. mosaic $X^H$ + T. m. $X^D$ + T. m. $X^G$ + T. m. $X^L$ + T. m. $X^S$ + T. m. $X^N$ + T. m.

FIG. 39

## PLATE 25

FIG. 42. *Lycopersicum esculentum*, variety Kondine Red. Right: mixed infection with common tobacco mosaic and $X^S$, constituting the experimental streak of the glasshouse; left: mixed infection of tobacco mosaic and $X^N$ with early lethal effect.

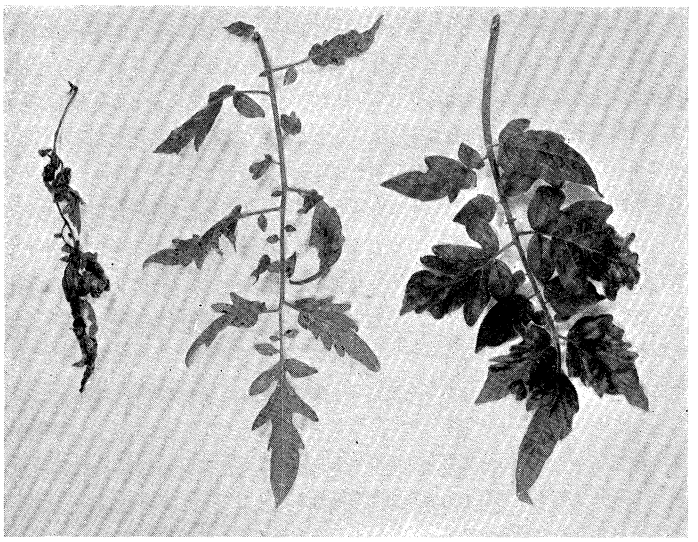
FIG. 43. *Lycopersicum esculentum.* Leaves of tomato plants infected with mixed infection of common tobacco mosaic and $X^N$, $X^S$, and $X^L$ respectively.

FIG. 44. *Nicotiana tabacum.* Left: infection with Murphy's virus *A*; centre: infection with *in vitro* mixture of virus *A* and $X^G$; right: same with $X^L$.

FIG. 45. Epicure potato. A tuber of a plant which has died of an infection by $X^S$. The eyes are necrotic and obliterated.

FIG. 46. Epicure potato. The same, showing necrosis within the tuber, and extending to the eyes.

FIG. 42



$X^N$+T. m. $X^S$+T. m. $X^L$+T. m.

FIG. 43

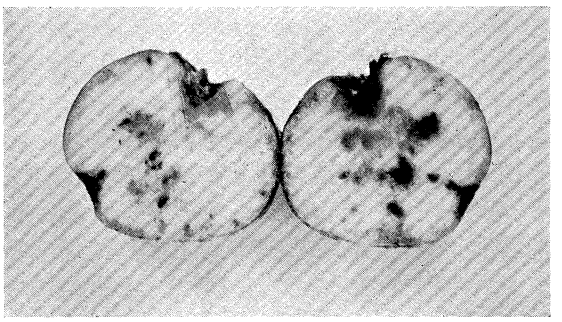
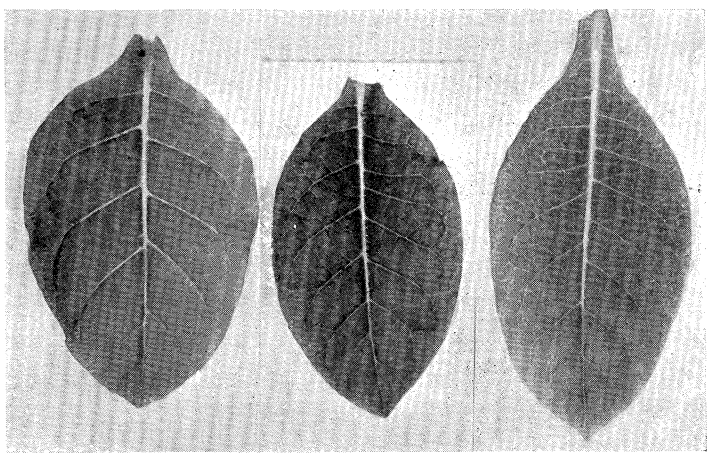


FIG. 46



'*A*' '*A*'+$X^G$ '*A*'+$X^L$

FIG. 44

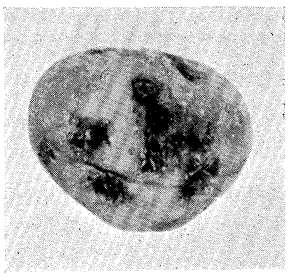


FIG. 45

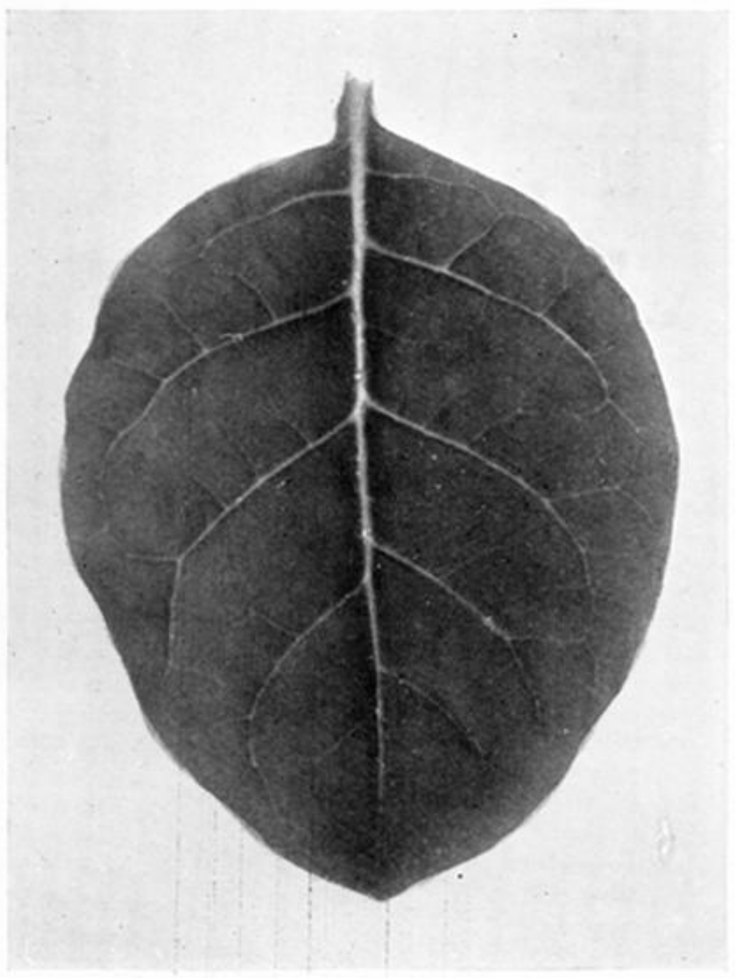


FIG. 2

FIG. 3

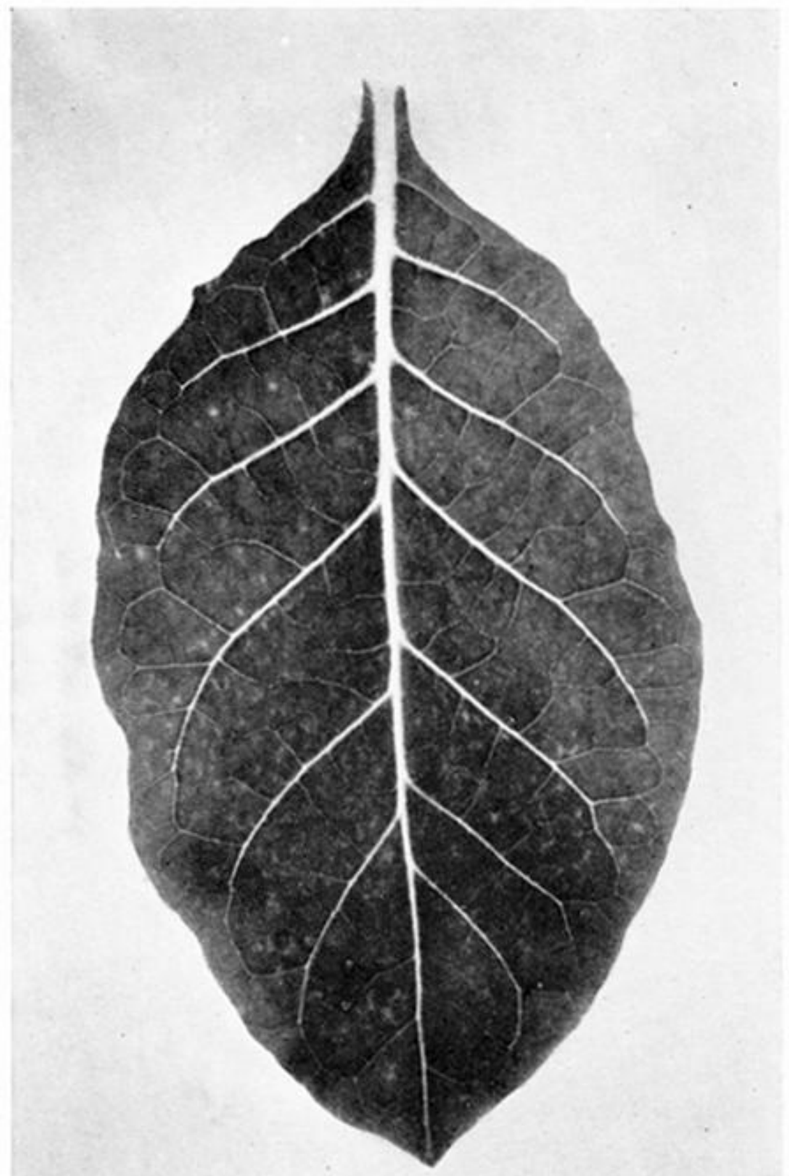


FIG. 4

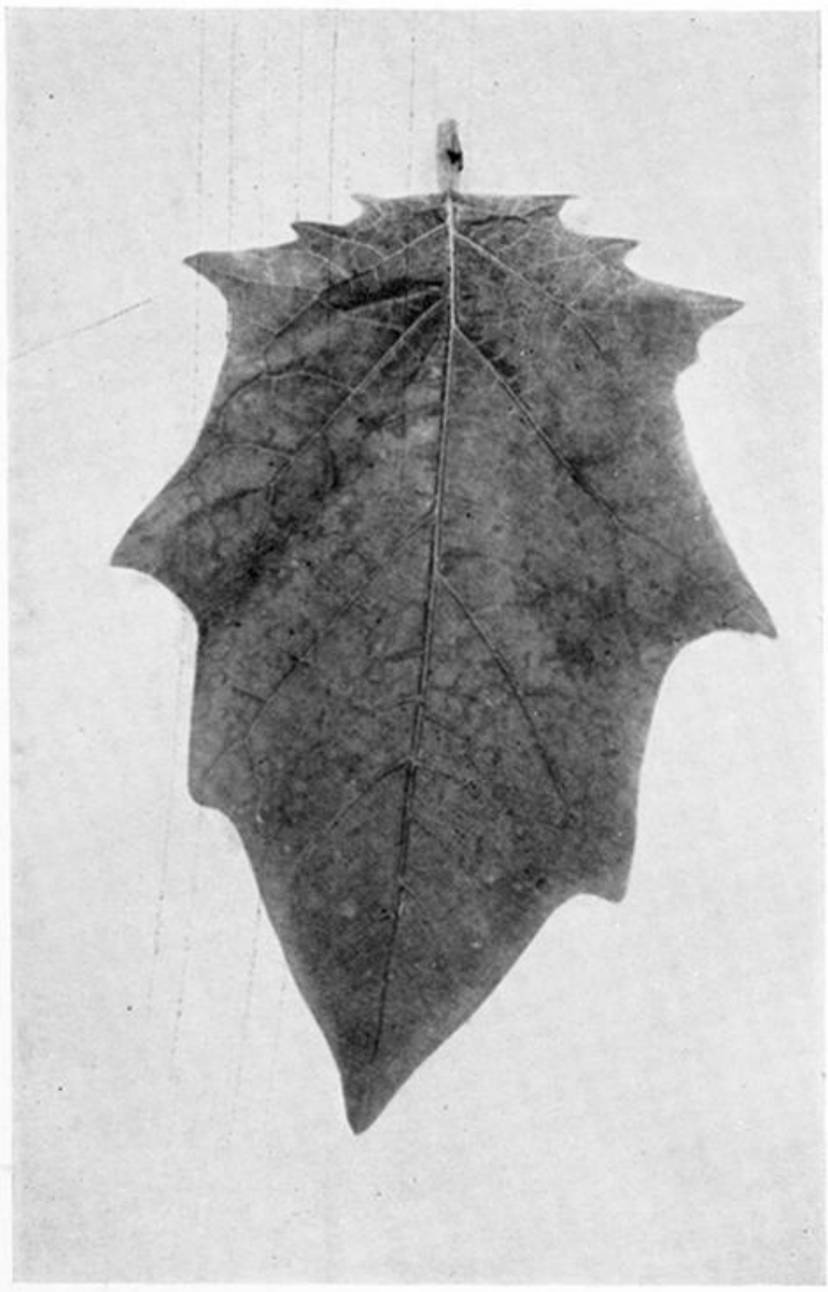


FIG. 5

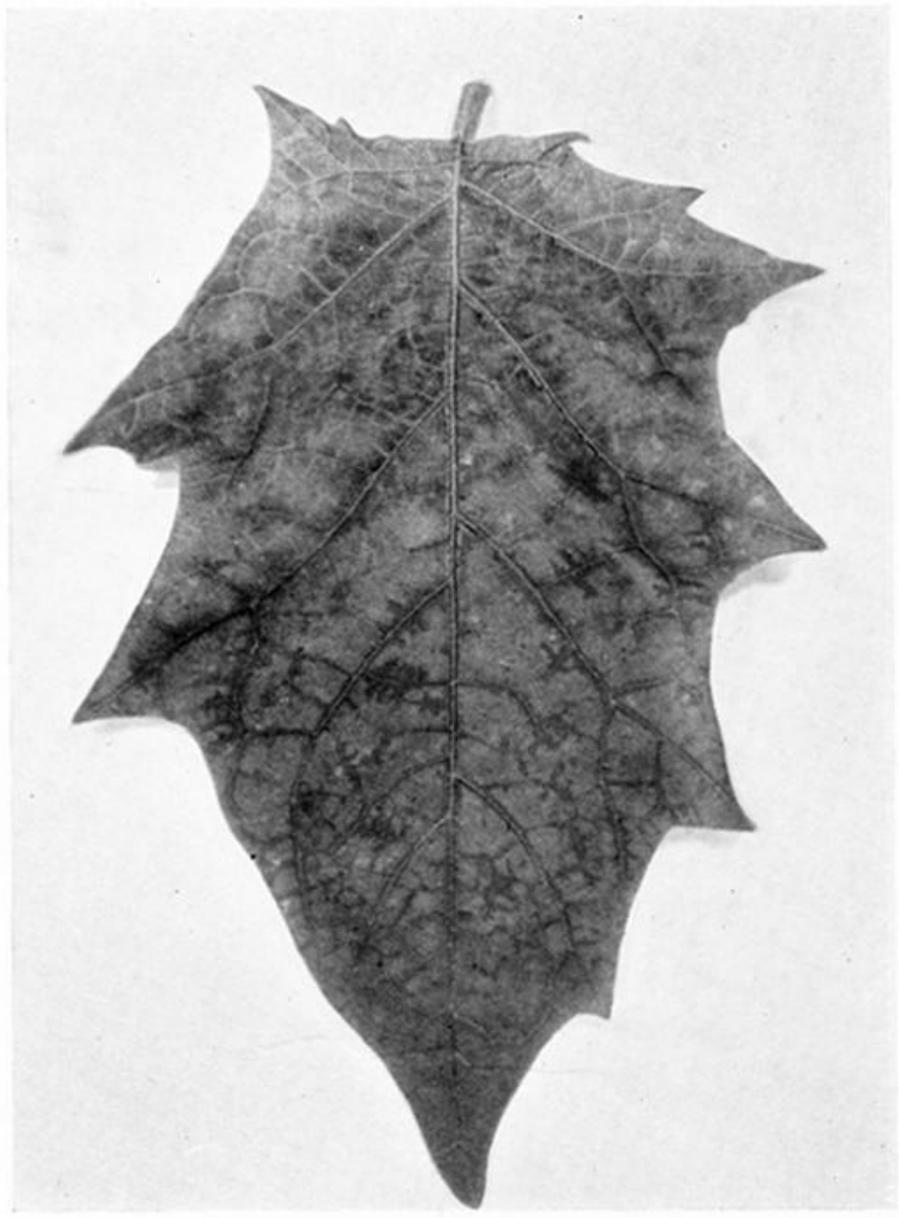


FIG. 6

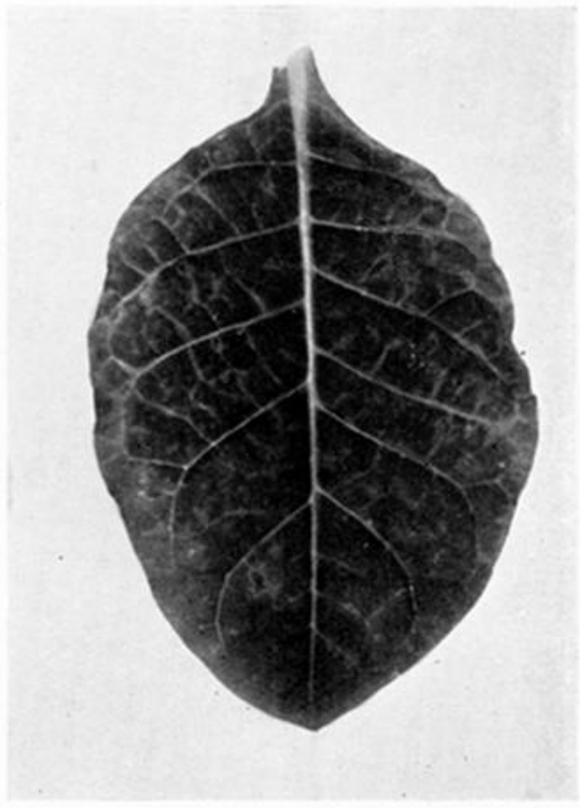


FIG. 7

## PLATE 18

FIG. 2. *Nicotiana tabacum.* Healthy leaf: one infected with $X^H$ would be indistinguishable from it.

FIG. 3. *Nicotiana tabacum.* Infected with $X^G$: in which the mottle is more evident at the apex of the leaf.

FIG. 4. *Nicotiana tabacum.* Infected with $X^G$: in which the presence of a trace of $X^S$ is shown by the presence of small bright yellow spots.

FIG. 5. *Datura stramonium.* Infected with $X^G$: note the very fine vein-banding.

FIG. 6. *Datura stramonium.* Infected with $X^D$: the symptoms are essentially the same as those evoked by $X^G$.

FIG. 7. *Nicotiana tabacum.* Infected with $X^L$: showing early vein clearing.

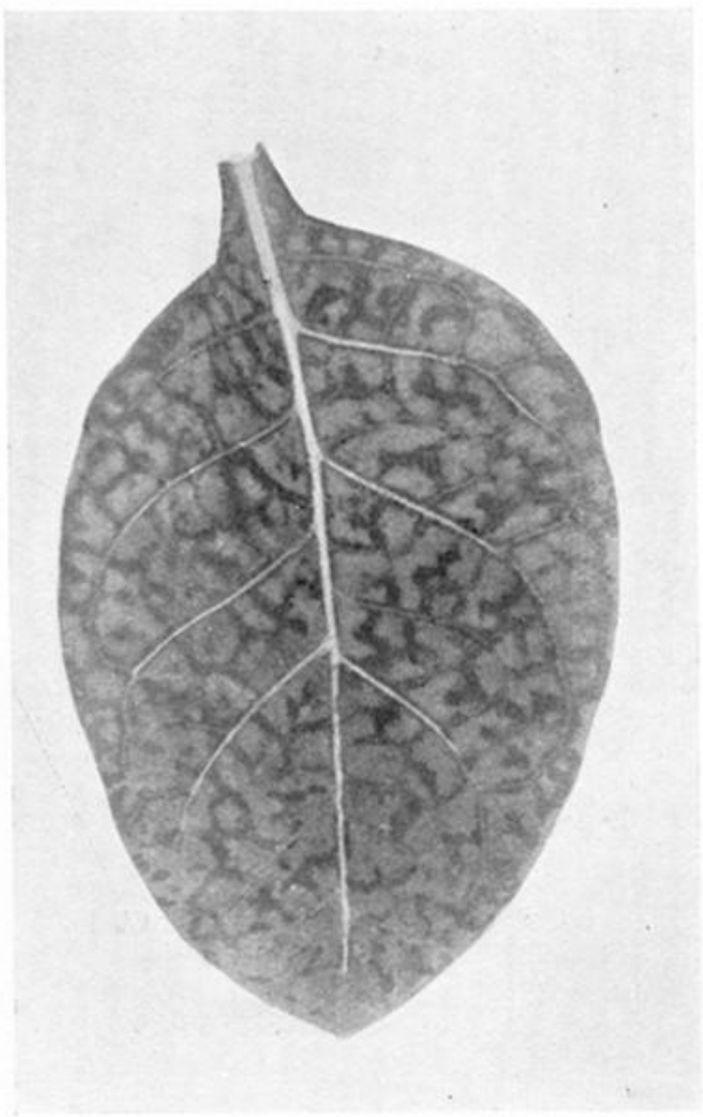


FIG. 8

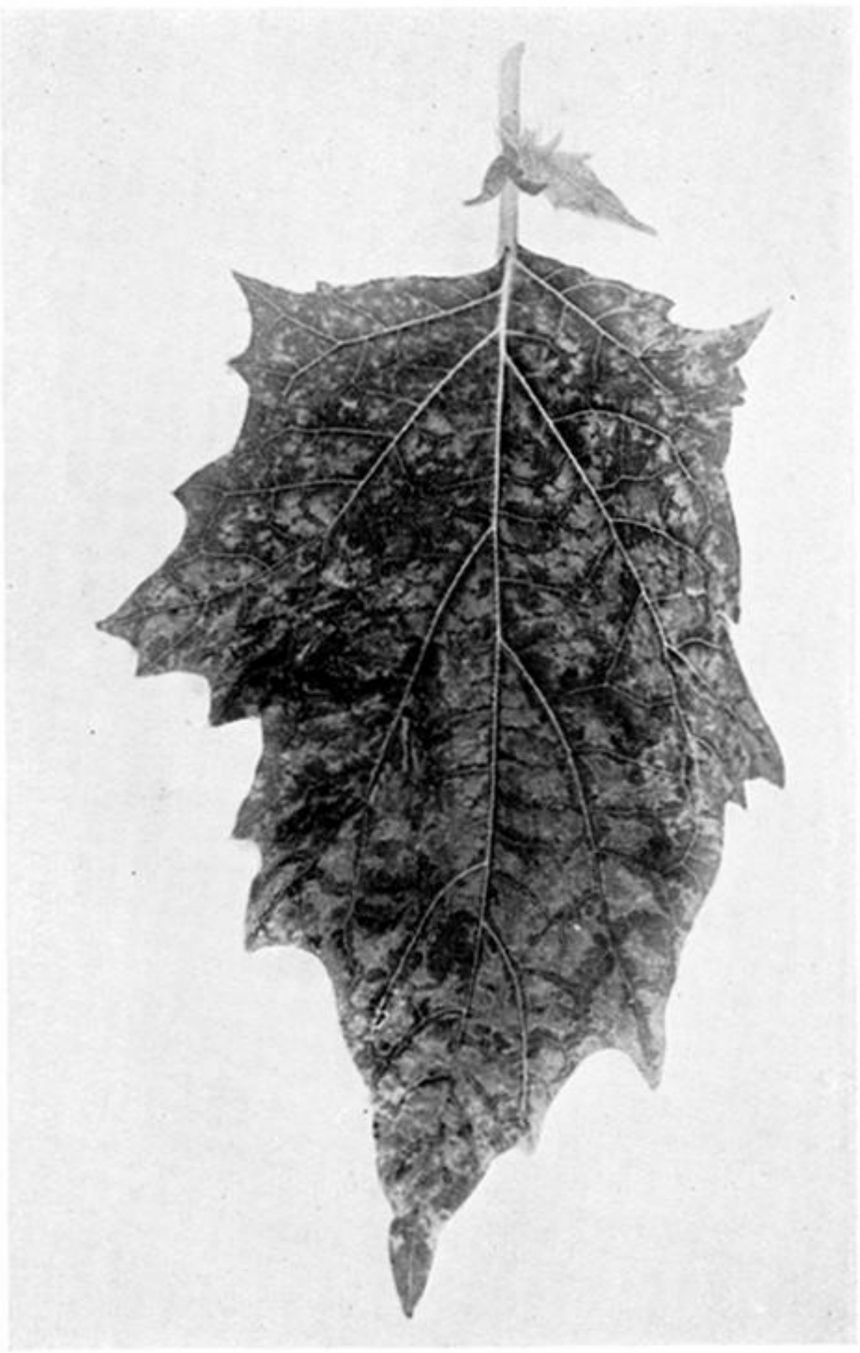


FIG. 9

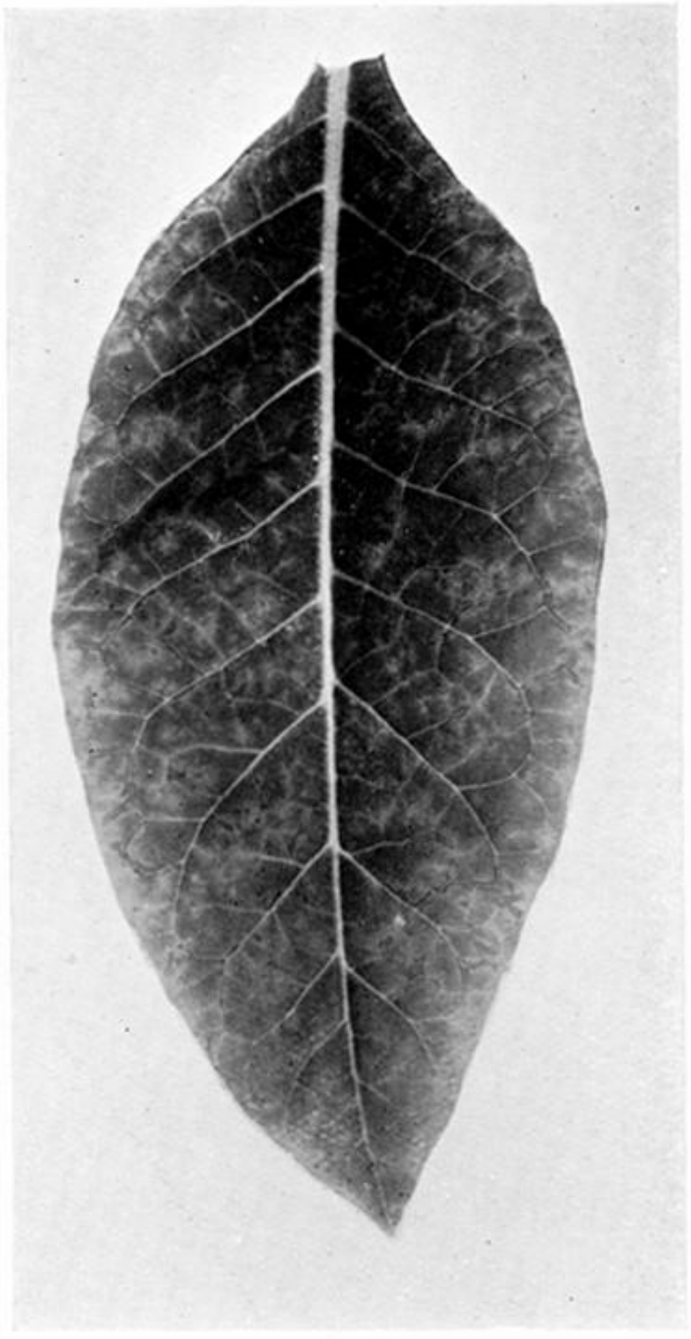


FIG. 10

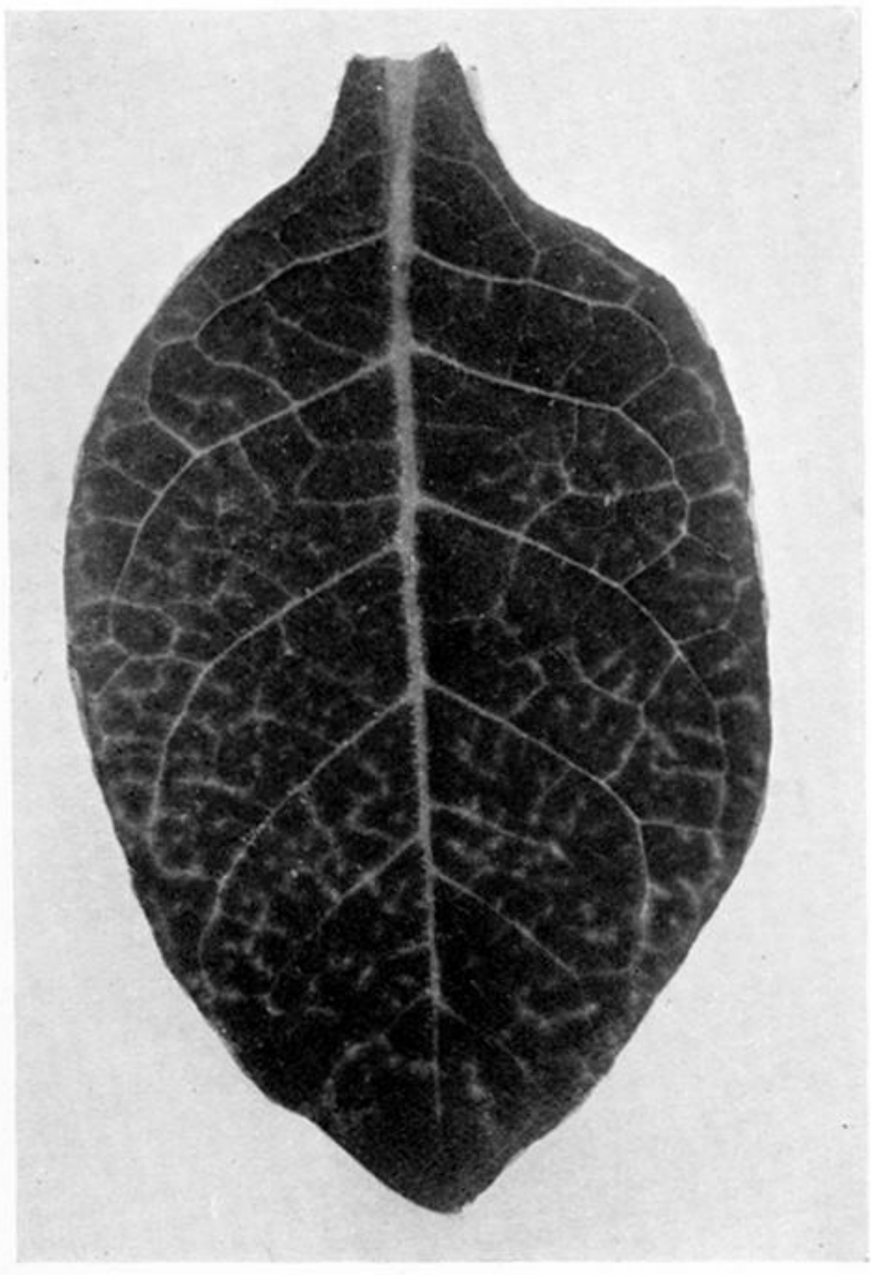


FIG. 11

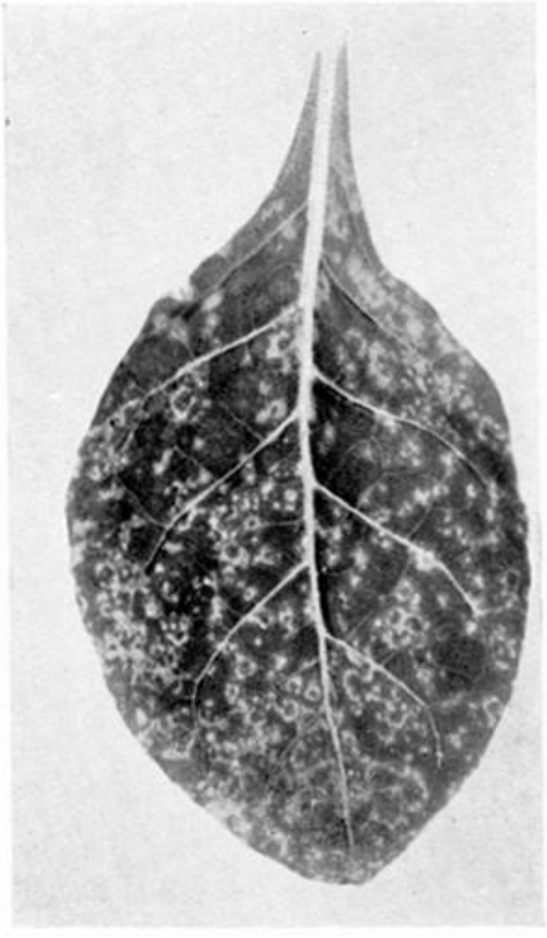


FIG. 13

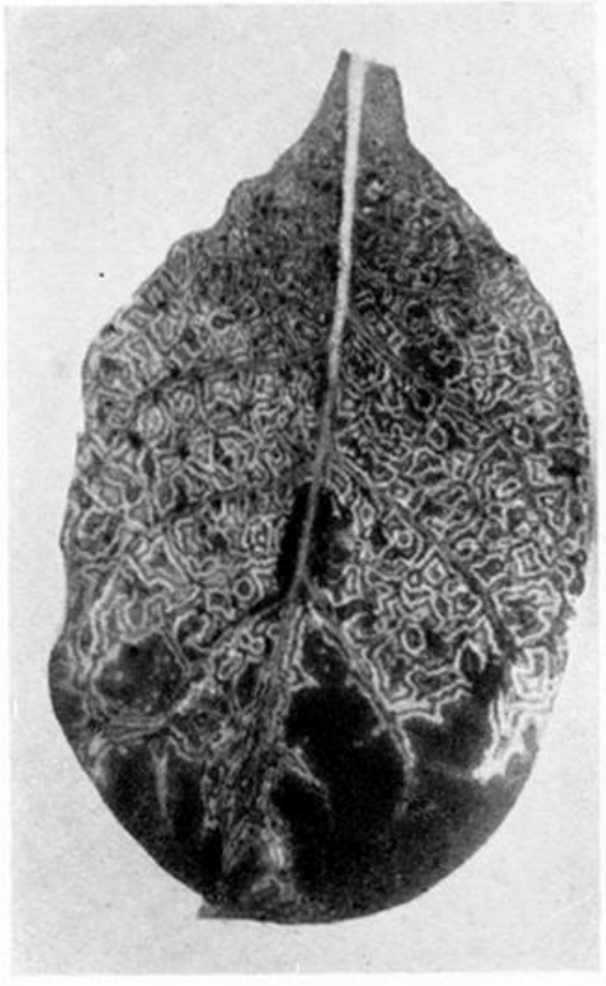


FIG. 14

## PLATE 19

FIG. 8. *Nicotiana tabacum.* Infected with $X^L$: showing the characteristic tortoiseshell pattern.

FIG. 9. *Datura stramonium.* Infected with $X^L$: displaying a brilliant yellow mottle with green vein-banding.

FIG. 10. *Nicotiana tabacum.* Infected with $X^L$: showing symptoms after 10 weeks when the tortoiseshell pattern has been replaced by an irregular mottle.

FIG. 11. *Nicotiana tabacum.* Infected with $X^S$: showing early necrotic vein clearing.

FIG. 13. *Nicotiana tabacum.* Infected with $X^S$: showing systemic symptoms, necrotic spotting and rings.

FIG. 14. *Nicotiana tabacum.* Infected with a mixture of $X^L$ and $X^S$: showing necrotic rings and figures modelled on an essentially $X^L$ type of mottle.

FIG. 15

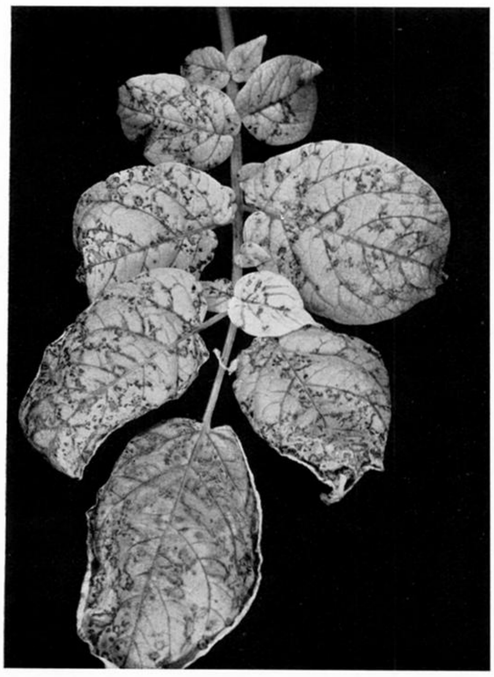


FIG. 16

FIG. 18

FIG. 17

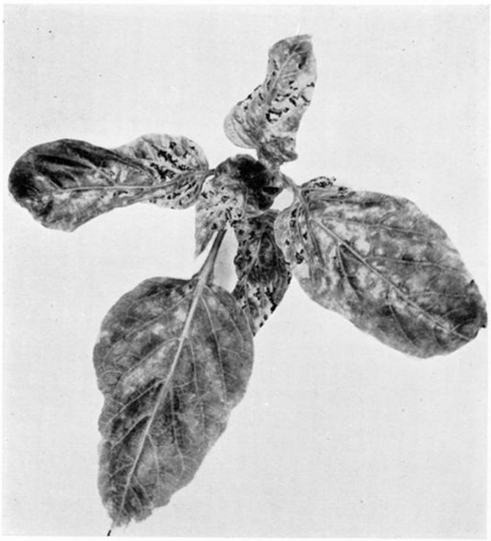


FIG. 19

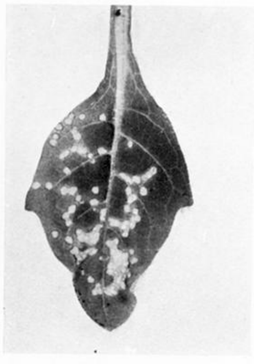


FIG. 20

FIG. 21

FIG. 22

## PLATE 20

FIG. 15. Potato, variety Majestic. Infected with $X^N$: second year symptoms showing interveinal necroses and leaf-drop.

FIG. 16. Potato, variety Arran Victory. Infected with $X^N$: showing early interveinal necroses. Infra-red photograph.

FIG. 17. *Capsicum annuum*. Infected 10 weeks previously with $X^S$. The upper leaves and stem have been destroyed, new growth appearing from lower node; at first healthy, later diseased.

Fig. 18. *Capsicum annuum*. Infected with $X^H$: young leaf showing early systemic etching, lesser in degree but similar in character to that which follows infections with $X^G$, $X^D$, and $X^L$.

FIG. 19. *Capsicum annuum*. Infected with $X^D$: showing later systemic interveinal necroses and crinkle, common to infections with this strain and $X^H$, $X^G$, and $X^L$.

FIG. 20. *Hyoscyamus niger*. Infected with $X^S$: local necrotic lesions, photograph through green screen.

FIG. 21. *Hyoscyamus niger*. Infected with $X^N$: local necrotic lesions, the dark outlines are rendered visible by a green screen.

FIG. 22. *Lycopersicum esculentum*, variety Kondine Red. Infected with $X^N$: local necrotic lesions.

$X^S$ $X^N$ Healthy

FIG. 23

FIG. 24

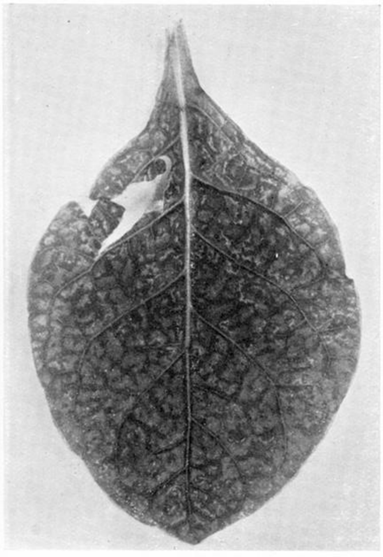


FIG. 25

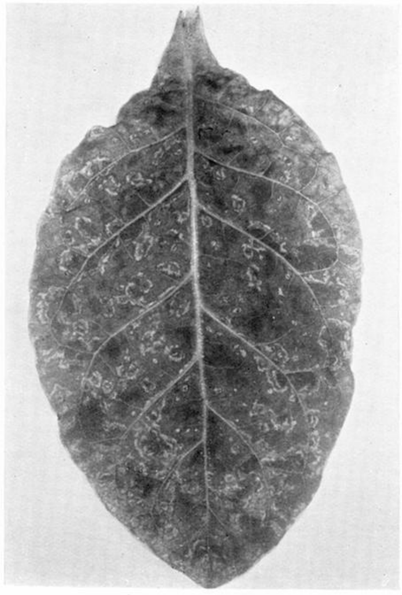


FIG. 26

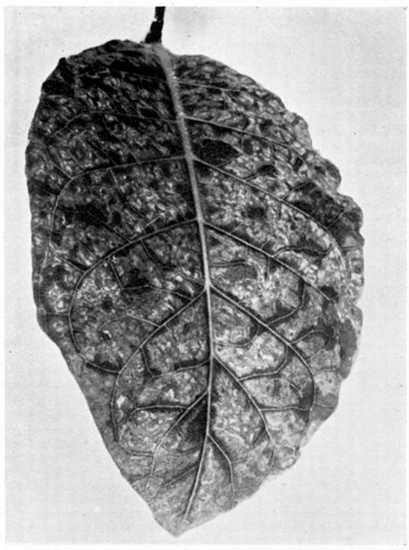


FIG. 27

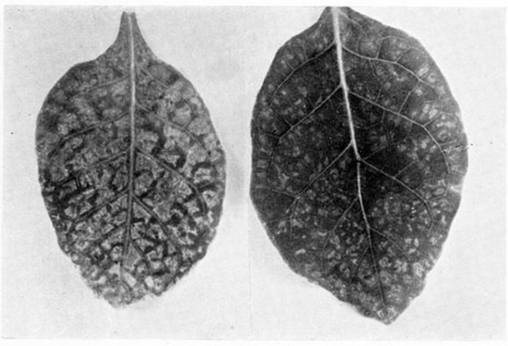


FIG. 28

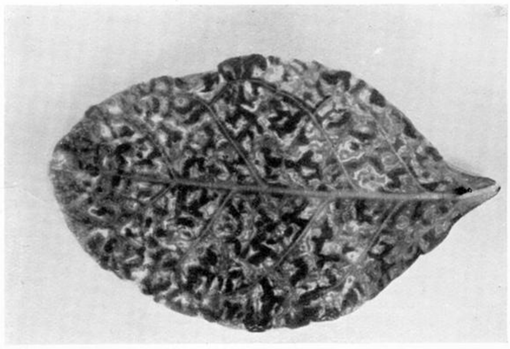


FIG. 29 FIG. 30

## PLATE 21

FIG. 23. *Hyoscyamus niger*, seedlings. Infected with $X^S$ and $X^N$, with healthy control. $X^S$ induces mass necrosis and collapse of leaf; $X^N$ induces scattered discrete necrotic spots.

FIG. 24. *Lycopersicum esculentum*, variety Kondine Red. Infected with $X^N$: severe systemic symptoms: wilting of leaves and stunting of plant.

FIGS. 25–31. *Nicotiana tabacum*. Progressive stages in a gradual concentration of $X^S$ during a separation of the same, by selective inoculation, from a mixture of $X^L$ and $X^S$.

FIG. 25. *Nicotiana tabacum*. An essentially $X^L$ type pattern in which the yellow areas are lightly etched.

FIG. 26. *Nicotiana tabacum*. An older leaf of the above plant in which finer etching and figures are superimposed on the basic $X^L$ pattern.

FIG. 27. *Nicotiana tabacum*. A further stage in $X^S$ concentration, in which the vein-banding pattern is almost confined to the major veins, the intervening areas being lightly necrotic.

FIG. 28. *Nicotiana tabacum*. The $X^L$ pattern is retained but the etching is more severe.

FIG. 29. *Nicotiana tabacum*. A younger leaf of the same plant in which a relatively normal $X^L$ pattern is developing.

FIG. 30. *Nicotiana tabacum*. An exceptional phase in the development of the "dissected $X^L$ pattern" in which the central interveinal areas remain green and the tissues nearer to the veins are pale and necrosed.

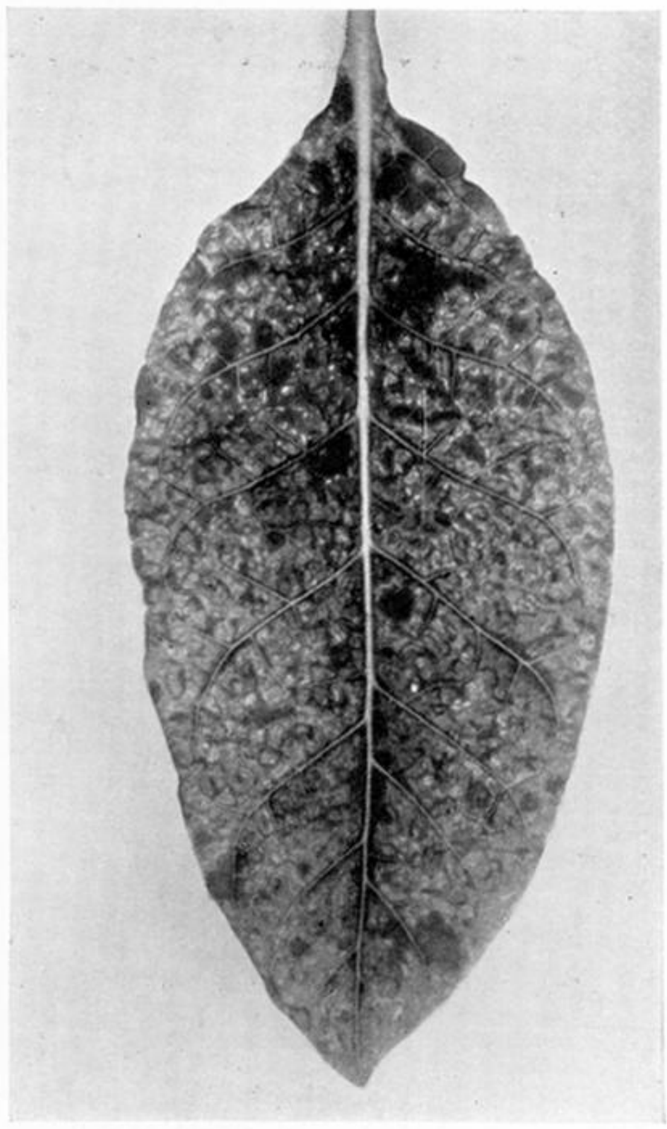


FIG. 31

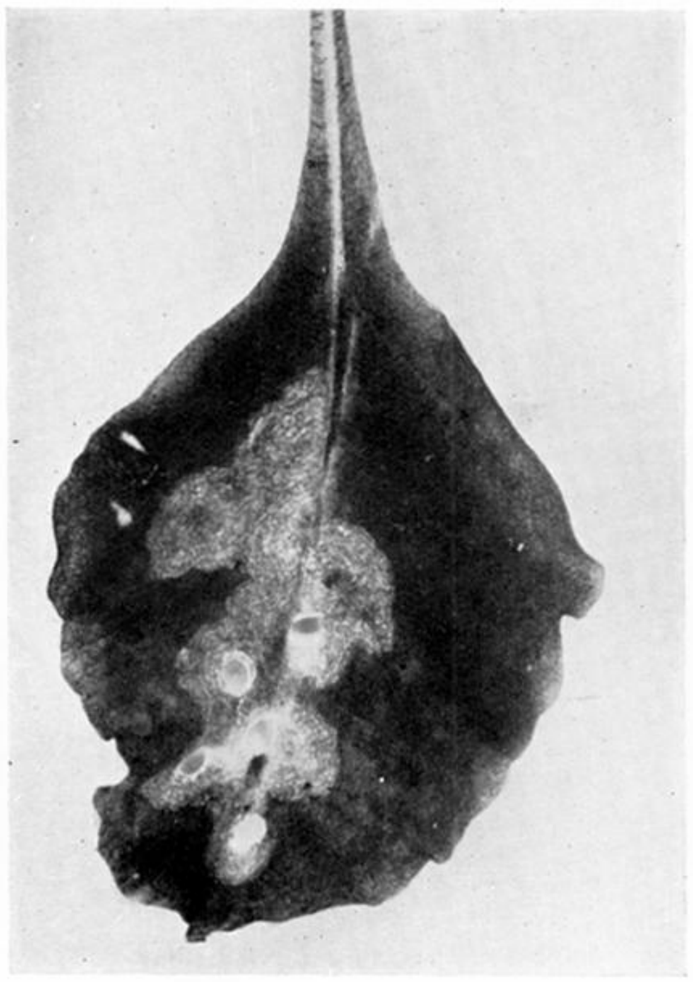


FIG. 32

FIG. 33

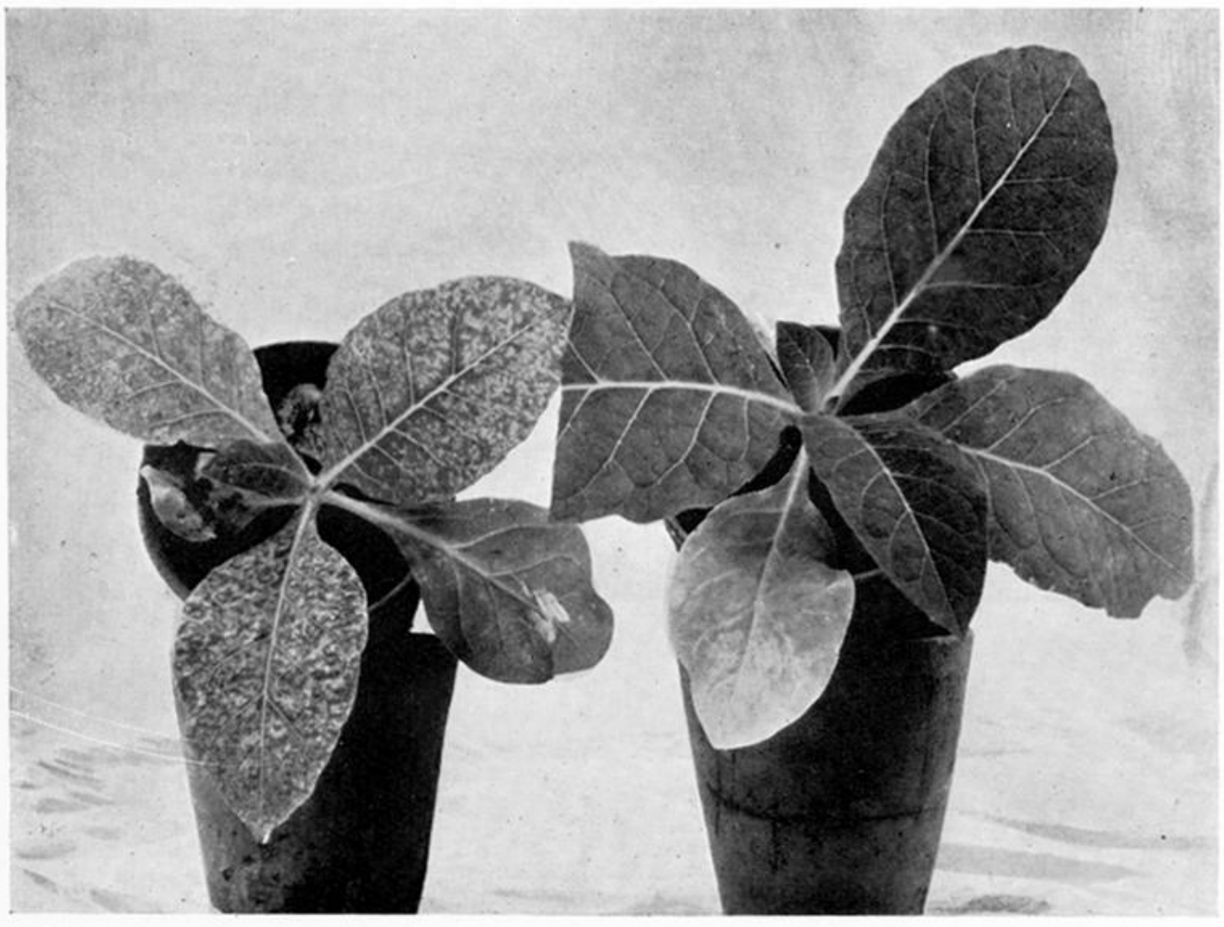


FIG. 34

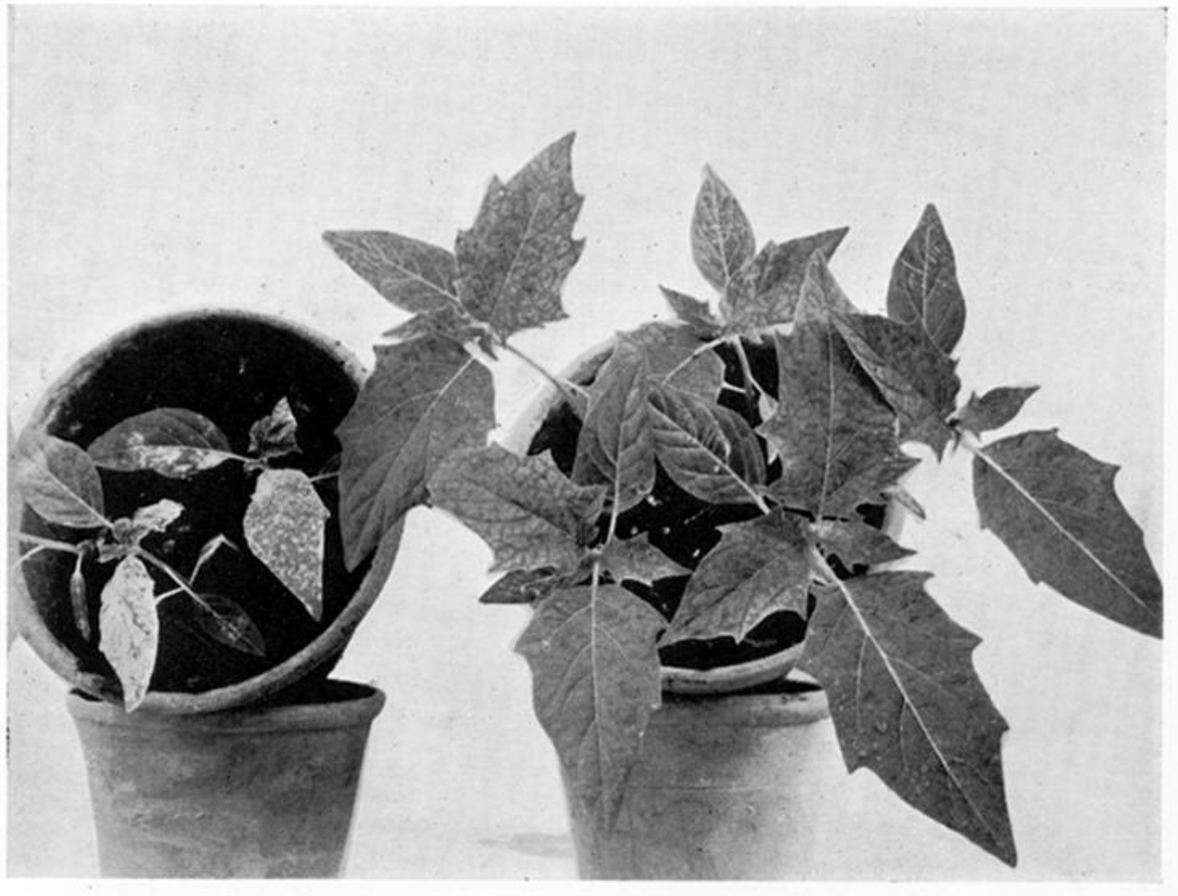


FIG. 35

## PLATE 22

FIG. 31. *Nicotiana tabacum.* Full "dissected $X^L$ pattern" is reached after ten generations of selective inoculation.

FIG. 32. *Beta vulgaris*, common sugar beet. Infected with $X^S$: maximum development of local lesions, consisting of concentric necrotic lines.

FIG. 33. *Nicotiana glutinosa.* Acquired immunity. Left: plants protected by prior inoculation with $X^G$ against infection by $X^S$ eight days later; right: control plants infected with $X^S$ on same date.

FIG. 34. *Nicotiana tabacum.* Acquired immunity. Right: plant protected by prior inoculation with $X^G$ against infection with $X^S$ fourteen days later; left: control plant inoculated with $X^S$ on same date.

FIG. 35. *Datura stramonium.* Acquired immunity. Right: protected by $X^G$; left: control infected with $X^S$.

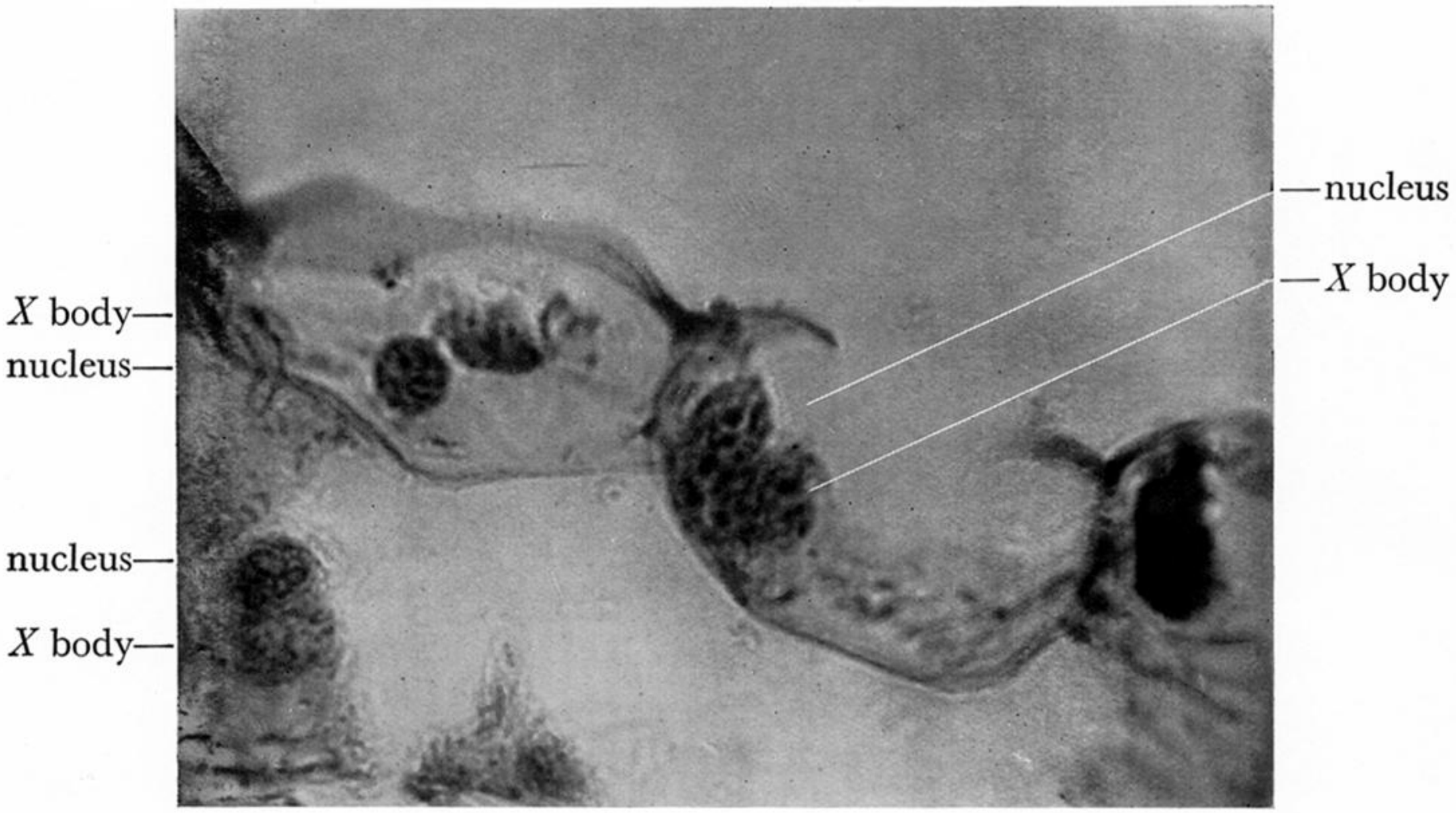



FIG. 36

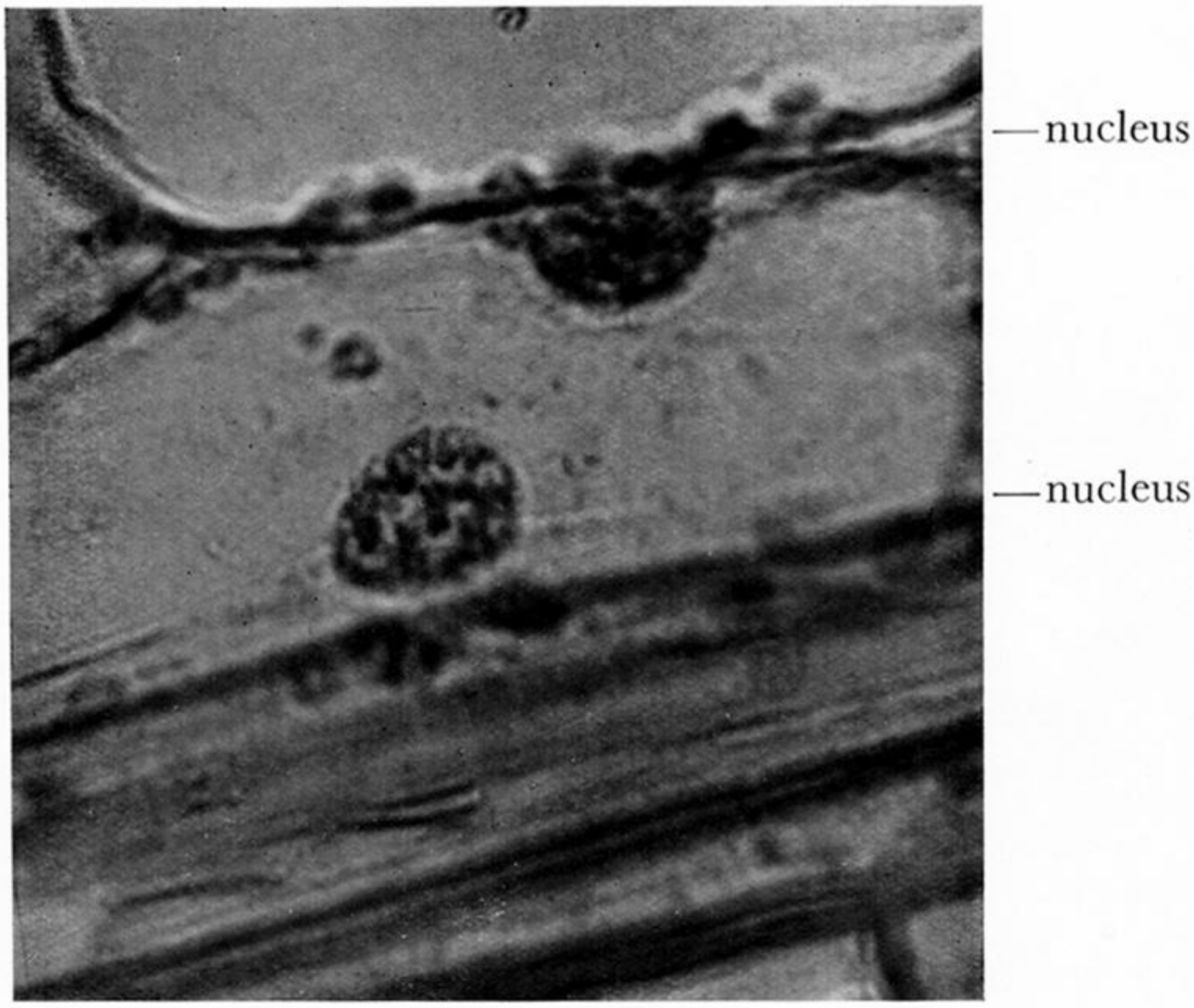
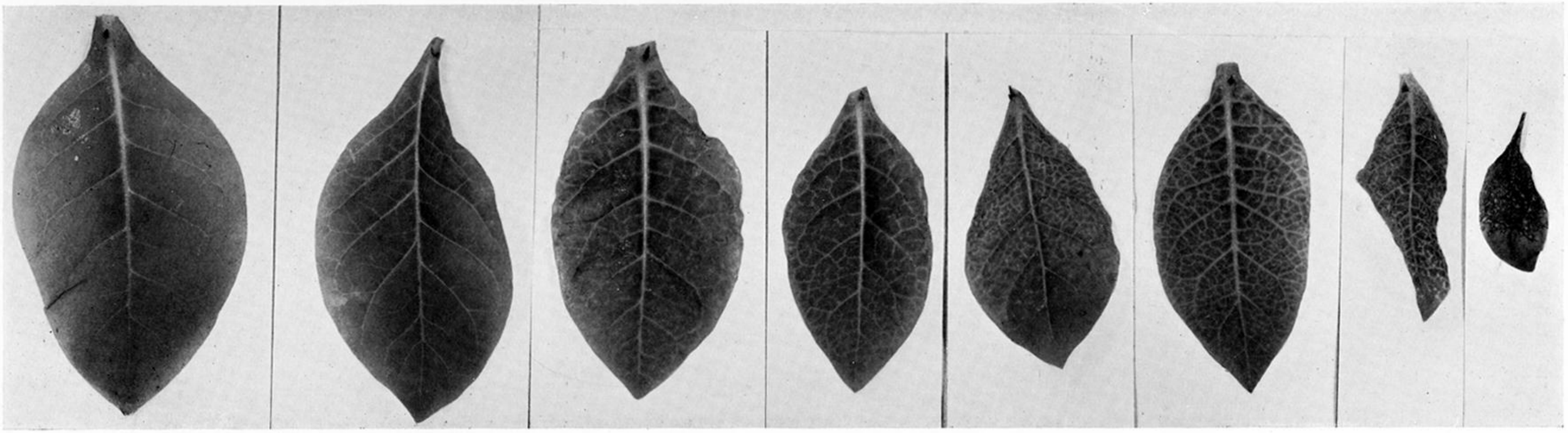



FIG. 37

## PLATE 23

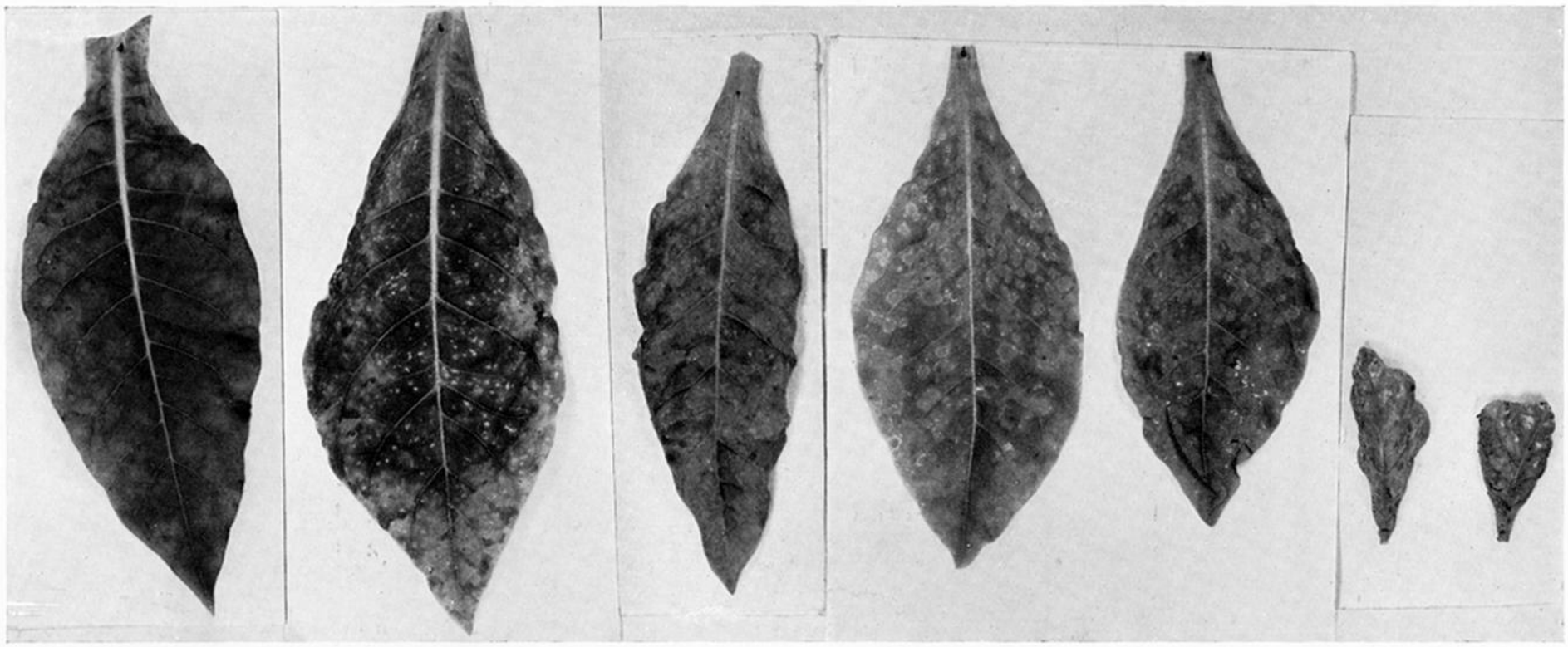
FIG. 36. *Nicotiana tabacum*. Intracellular inclusions or *X* bodies in epidermal cells of leaf infected with $X^L$.

FIG. 37. *Nicotiana tabacum*. Two nuclei in a single cell in leaf infected with $X^N$.



control | $Y$ | $X^H + Y$ | $X^D + Y$ | $X^G + Y$ | $X^L + Y$ | $X^S + Y$ | $X^N + Y$

FIG. 38



common T. mosaic | $X^H$ + T. m. | $X^D$ + T. m. | $X^G$ + T. m. | $X^L$ + T. m. | $X^S$ + T. m. | $X^N$ + T. m.

FIG. 39

## PLATE 24

FIG. 38. *Nicotiana tabacum.* Series of leaves showing: healthy control, "*Y*" infected control, and series of leaves from plants infected with *in vitro* mixtures of the *Y* virus and the six strains of the *X* virus respectively.

FIG. 39. *Nicotiana tabacum.* Series of leaves showing: common tobacco mosaic, and series from plants infected with *in vitro* mixtures of tobacco mosaic and the six strains of the *X* virus respectively.

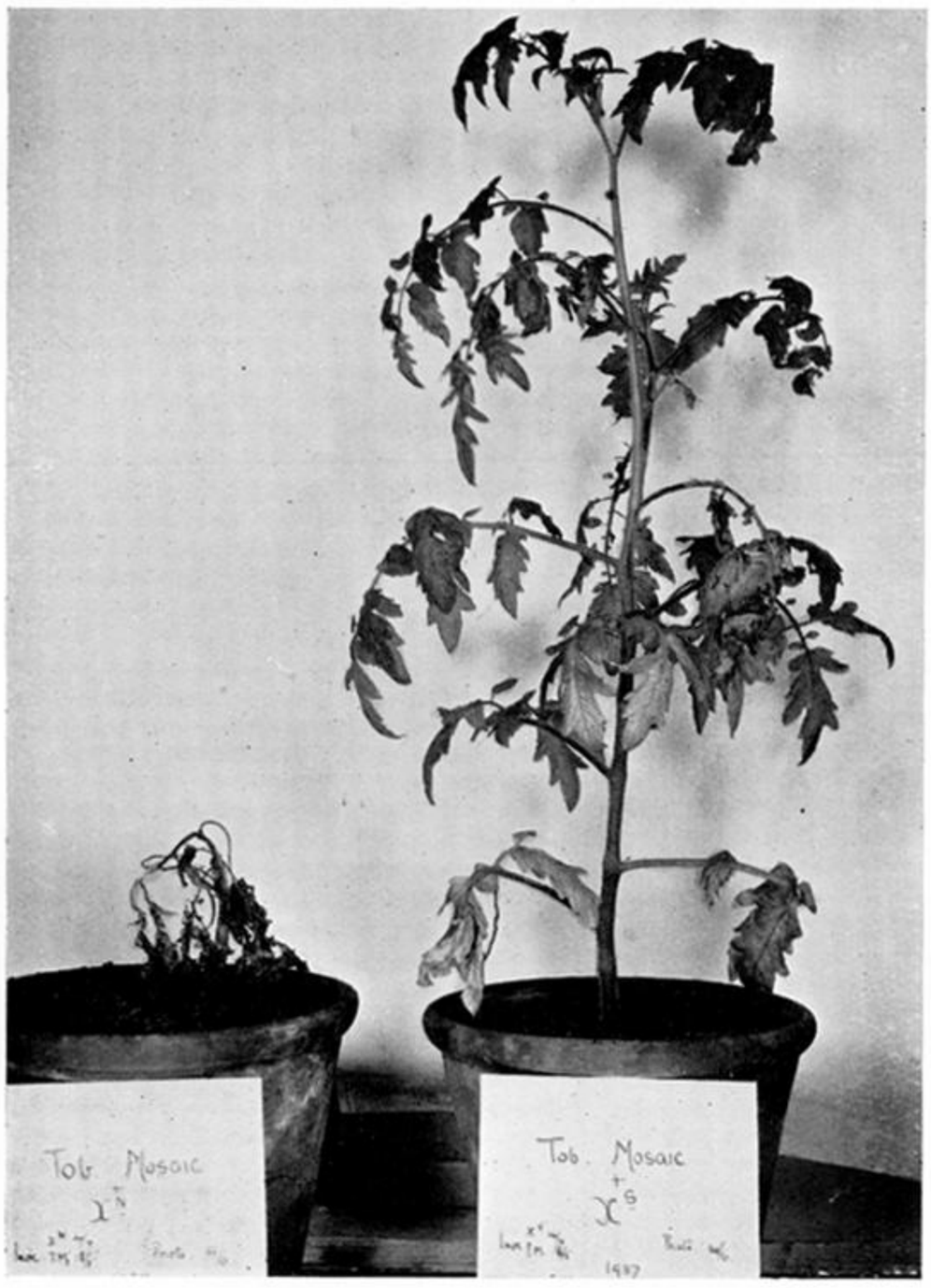
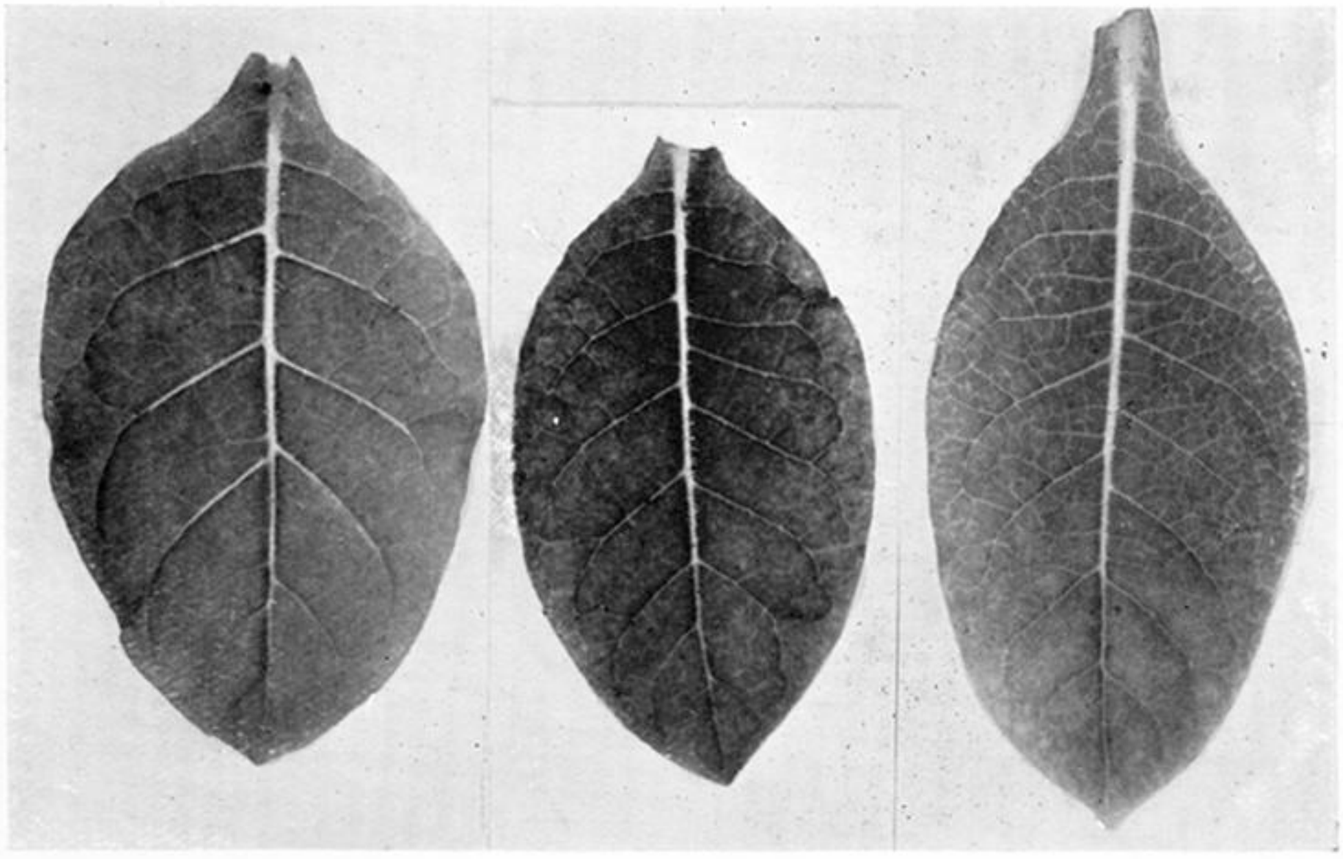



FIG. 42

$X^N$ + T. m. $X^S$ + T. m. $X^L$ + T. m.

FIG. 43

FIG. 46



'A' 'A' + $X^G$ 'A' + $X^L$

FIG. 44

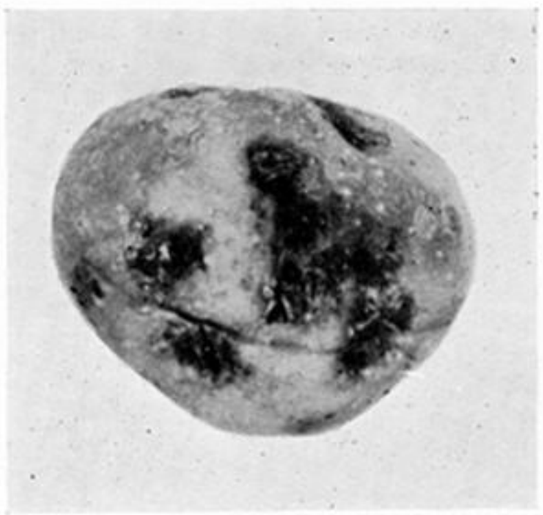


FIG. 45

PLATE 25

FIG. 42. *Lycopersicum esculentum*, variety Kondine Red. Right: mixed infection with common tobacco mosaic and $X^S$, constituting the experimental streak of the glasshouse; left: mixed infection of tobacco mosaic and $X^N$ with early lethal effect.

FIG. 43. *Lycopersicum esculentum.* Leaves of tomato plants infected with mixed infection of common tobacco mosaic and $X^N$, $X^S$, and $X^L$ respectively.

FIG. 44. *Nicotiana tabacum.* Left: infection with Murphy's virus *A*; centre: infection with *in vitro* mixture of virus *A* and $X^G$; right: same with $X^L$.

FIG. 45. Epicure potato. A tuber of a plant which has died of an infection by $X^S$. The eyes are necrotic and obliterated.

FIG. 46. Epicure potato. The same, showing necrosis within the tuber, and extending to the eyes.