SOME FACTORS AFFECTING LOCALIZED AND SYSTEMIC NECROTIC REACTIONS TO VIRUS Y IN THE POTATO

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(Plates 1-2)

Summary

Factors influencing hypersensitive and necrotic reactions to virus Y in potato seedlings, developed from common varieties like Katahdin and Snowflake, have been studied mainly by means of hand inoculation in the greenhouse. This method provides more accurate and detailed information than either graft inoculation or aphid inoculation. The virus dosages applied by aphid inoculation have not been sufficient to assess the hypersensitivity of seedlings accurately.

The strain of virus Y used in inoculation is an important factor determining whether a seedling will give a localized reaction or not. A seedling hypersensitive to one strain has a one in two chance of being hypersensitive to a different strain. Seedlings hypersensitive to all strains of Y tested have been developed. Strains of virus Y differed in the virus concentrations which they developed in tobacco, but this did not appear to be a factor influencing the relationship between strain of Y and the hypersensitive reaction of seedlings.

In a number of the seedlings, lethal and localized necrotic reactions were interchangeable, depending on plant vigour at the time of inoculation. Young actively growing plants of hypersensitive seedlings give reactions localized in the inoculated leaves, while old plants or plants with an apparently lowered metabolism give a diffused type of necrotic reaction which results in the inactivation of the virus in tissues distant from the inoculation sites. This is possible, because the stem tissue of such seedlings can react hypersensitively to virus Y. Histological studies confirmed these findings, and showed a correlation between the extent of necrosis in stem sections and the degree of sensitivity to virus Y.

I. Introduction

Immunity in potatoes to a potato virus was obtained for the first time with the American seedling U.S.D.A. 41956 (Schultz *et al.* 1934) which is immune to all strains of virus X, and which transmits this factor to hybrid progeny (Stevenson, Schultz, and Clark 1939). Up to the present, immunity or non-susceptibility to the other potato viruses has not been found. It was discovered, however, that some varieties and their seedlings were resistant to the mosaic viruses A and X (Cockerham 1943) under field conditions due to the extreme susceptibility of their tissues. This hypersensitivity, which is heritable (Cadman 1942), prevents the spread of the virus because of the rapidity of tissue death around the infection points of inoculated leaves. Consequent upon these findings, the elimination of viruses A and X from potato stocks has been made possible by the development of new varieties possessing these genetic factors.

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The development of field immunity to virus Y, the other important mosaic disease, has proved a difficult problem. Necrotic reactions to this virus, resulting in high field resistance, had been observed in the potato variety Katahdin and its progeny in the United States (Jones and Vincent 1937; Jones, Vincent, and Burk 1940), and in Snowflake in Australia (Bald and Pugsley 1941). Work in progress at Canberra (Hutton and Bald 1945; Hutton 1945, 1946) has had as its objective the development of seedlings hypersensitive to virus Y, using as basic parental material the necrotic reacting Katahdin and Snowflake varieties. The reactions of the seedlings to hand inoculation with virus Y in the greenhouse have been described, as well as the inheritance of the various types of necrotic reaction obtained (Hutton 1945, 1946).

Cockerham (1945) and Cockerham and M'Ghee (1947) found clones of three wild *Solanum* species with hypersensitive reactions to virus Y, and in tests with progenies from two of them, S. *demissum* and S. *simplicifolium*, observed the reactions to be heritable and to result in immunity to virus Y under field conditions. It is apparent from this work and the research on virus Y resistance previously quoted (Hutton 1945, 1946) that varieties field immune to this virus are in sight.

Recently, Bawden and Kassanis (1946), Roland (1946), and Schultz, Stevenson, and Akeley (1947) have examined the bases for differences in susceptibility to virus Y found among common potato varieties. Bawden and Kassanis (1946) found that a variety like Majestic, reacting with leaf-drop streak, developed a lower concentration of virus Y and was as resistant as Arran Banner, which reacted with a mosaic and developed a high concentration of this virus. They found the reaction of Katahdin to virus Y to approximate hypersensitivity. Roland (1946) concluded that defective diffusion of virus Y rather than sensitivity was the reason for the tuber progeny of infected plants being free from this virus. Schultz, Stevenson, and Akeley (1947) found a severe necrotic reaction and field resistance to virus Y to be correlated, and emphasized the effect that a heavy aphid infestation giving the chance for repeated plant inoculation has on the degree of infection in varieties.

The work described in this paper was done with the object of finding what factors, apart from genetic, influenced or modified the expression of the localized and other necrotic reactions in the potato seedlings developed at Canberra. These seedlings have all been selected from crossbred progenies involving common potato varieties as parents, so that it is possible that a different gene complex from that present in Cockerham and M'Ghee's (1947) hybrids with wild species is operating to produce the necrotic reactions obtained. The Canberra progenies contained seedlings with individual reactions to virus Y ranging through complete tolerance, leaf-drop streak, and severe systemic necrosis to hypersensitivity. Greenhouse tests over several years have shown a number of the localized reactors to be more sensitive than Katahdin under most conditions, whereas a proportion of them appear to have inherited a similar reaction to Katahdin. Limited field tests have largely supported the greenhouse tests, but it is apparent that the level of necrotic reaction required for field immunity is dependent on the density of

the aphid population present. With relatively low aphid populations, the Katahdin type of reaction results in near field immunity under Australian conditions, and it is only when seasonal conditions optimal for aphid development are obtained, that a noticeable infection occurs in Katahdin and seedlings with a similar reaction to virus Y.

II. MATERIALS AND METHODS

In this study, the 342 seedlings used were those selected in the 1945-46 season as having the best agronomic characters in the Dickson Experiment Station field plots containing hybrid progenies bred for virus Y resistance. As they were selected initially on an agronomic basis, they included types giving localized, lethal necrotic, systemic necrotic, and mottle reactions to virus Y in the greenhouse. With respect to the latter two classifications, a systemic necrotic seedling usually showed some mottling, and a seedling classed as a mottle type often had some systemic necrosis. For convenience, seedlings with leaf-drop streak were classified as having systemic necrosis.

In the preliminary stages of this study all the field selected seedlings were involved, but during the later stages of the work on the effect of strain on resistance, 50, and then 13, seedlings were selected. The varieties Factor and Katahdin were used as checks throughout, and in some instances Sequoia was used as well.

Most of the greenhouse inoculations were done during the spring and summer period from October to March when growth of potatoes is active and relatively normal. For comparison, however, some inoculations were made at other times of the year when vegetative growth was restricted due to the shorter photoperiod and lowered temperatures (Werner 1934). The varieties and seedlings were hand inoculated at various stages of growth with a ground glass spatula after the leaves to be inoculated were tagged and dusted lightly with an "extra fine" grade of carborundum. The inoculum was prepared in a mortar by grinding virus Y infected tobacco leaves (showing vein banding) with a phosphate buffer at pH7 in the constant proportion of 1:20 (w/v). Aphid inoculations were done with *Myzus persicae*, using the procedure of Watson and Roberts (1939).

For the 1945-46 and 1946-47 seasons the strain of virus Y used was that found in the Carman potato variety in the Ballarat district of Victoria. In the 1947-48 season, the work on the effect of the strain of virus Y on hypersensitivity was made possible through the generosity of F. C. Bawden who supplied the cultures of a highly virulent strain, Rothamsted, a strain of medium virulence known as Sheffield, and a mild strain designated 18.2 from the Rothamsted Experimental Station, England. Virus C had been obtained previously from Dr. J. G. Bald (Bald and Norris 1945), and this had been kept in Brown's River free from all other viruses. As a result, comparisons of the effects of Victorian Y, the three Rothamsted strains of Y, and virus C were made on selected seedlings.

When not being used for inoculation, the strains of virus Y, with the exception of C, were kept in the variety Epicure which, due to its hypersensitive reactions, remains free from viruses A, C, and X. For inoculation purposes, the virus Y strains including C (Bawden and Sheffield 1944) were transferred by hand

inoculation to young tobacco plants. In order to maintain the strains in high concentration they were transferred each month to a fresh batch of young tobacco plants.

III. CORRESPONDENCE BETWEEN THE RESULTS OBTAINED WITH THE SAME SEEDLINGS IN TWO DIFFERENT SEASONS

Duplicate samples of the 342 field selected seedlings were hand inoculated in the greenhouse with Victorian Y in both the 1945-46 and 1946-47 seasons. In the 1945-46 season, the seedlings were inoculated before flowering in November, while in the 1946-47 season this inoculation was done at approximately the same time, but those with localized reactions were given a second inoculation after flowering. In both seasons the tubers from the localized reactors were kept, and when these were grown on, the plants were healthy and gave typical localized necrotic reactions on reinoculation with virus Y.

A fair correspondence between the results from the seedlings in two different seasons at the pre-flowering inoculation is shown in Table 1. Of the localized reactors in the 1945-46 season, 72 per cent. gave the same reaction in the 1946-47 season, the rest developing systemic necrosis. Twelve of this latter group were early types which did not flower, and were inoculated at the equivalent of their post-flowering stage in the 1945-46 season, so that their quick maturation had localized the virus. In the 1946-47 season, all seedlings were inoculated at what was judged to be their pre-flowering growth stage, so that rapid maturation of early types was allowed for, and the results generally were thus more accurate.

TABLE 1

TWOFOLD FREQUENCY DISTRIBUTION OF 342 POTATO SEEDLINGS, CLASSIFIED ON PLANT REACTION AFTER HAND INOCULATION AT THE PRE-FLOWERING STAGE WITH VICTORIAN Y IN THE 1945-46 AND 1946-47 SEASONS

Plant Reactions 1945-46 Season	Plant Reactions 1946-47 Season				
	Localized	Lethal Necrosis	Systemic Necrosis	Mottle	Total
Localized	49	0	19	0	68
Lethal necrosis	40	11	90	0	141
Systemic necrosis	0	0	35	10	45
Mottle	0	0	33	55	88

In Table 1, of the seedlings which were lethally necrotic to Victorian Y in the 1945-46 season, 8 per cent. gave the same reaction in the 1946-47 season and 28 per cent. localized this virus, the rest developing systemic necrosis after inoculation. These results indicated that lethal and localized necrosis can be interchangeable in the same seedling, and that the separation of true lethal from systemic necrotic seedlings is influenced by some factor like plant vigour. In the 1945-46 season, the plants came from small tubers raised in pots in the greenhouse from first year seedlings, and were on the average less vigorous than the 1946-47 duplicates from field grown tubers.

Correspondence between systemic necrotic and mottle types in the two seasons was close, when due allowance is made for the fact that the one reaction is usually accompanied by the other to a greater or lesser extent.

(a) The Effect of Plant Vigour on the Reaction to Virus Y in Potato Seedlings

In order to investigate whether plant vigour could influence the final reaction to virus Y, vigour ratings recorded in the 1944-45 season were examined, and the results for the 1,074 necrotic reacting seedlings analysed. The vigour rating was made at inoculation, which was carried out at the pre-flowering stage. Those seedlings which were weak and spindly at inoculation were given a rating of 1, those which were strong growing and vigorous, a rating of 5, and the rest appropriate ratings intermediate between these two categories.

Table 2 shows a decided fall in the number of lethal necrotic seedlings, and less marked rises in the numbers of systemic necrotic and localized reactors to virus Y, passing from the least vigorous to the most vigorous plant classes. These results make it clear that plant vigour can markedly influence the virus Y reaction in some seedlings. It has been observed repeatedly, that if weakly growing

TABLE 2

PERCENTAGES OF SEEDLINGS OF DIFFERENT VIGOUR RATINGS AT INOCULATION IN RELATION TO THEIR FINAL Y REACTION CLASSES

Vigour Rating	Percentage of Seedlings in the Different Reaction Classes			Number of Seedlings
	Systemic Necrosis	Lethal Necrosis	Localized Necrosis	
1	58.1	36.0	5.9	86
2	60.4	35.2	4.4	270
3	69.2	24.2	6.6	335
4	77.0	16.1	6.9	204
5	84.3	6.7	9.0	179

seedlings develop lethal necrosis after inoculation, this may not be their normal reaction, as strongly growing duplicate material will give either a localized or systemic necrotic reaction. This explains the inconsistencies of Table 1 with the lethal necrotic class, since the 1946-47 material was on the average much more vigorous than that grown the previous season. The results in Table 2 indicate that to obtain the most reproducible results from hand inoculation with virus Y in the greenhouse, it is necessary to plant tubers which will give vigorous plants.

IV. THE RELATION BETWEEN STAGE OF GROWTH OF SEEDLINGS AT INOCULATION AND LOCALIZED REACTION TO VIRUS Y

The 89 seedlings shown in Table 1, as giving in the 1946-47 season a localized reaction to virus Y when inoculated at the pre-flowering stage, were given a second inoculation 14 days after the commencement of flowering. At this stage a large proportion of the seedlings had completed flowering, although some of the later types were still producing a few flowers.

The results in Table 3 show that a number of the seedlings with localized reactions after the pre-flowering inoculation developed systemic necrosis following the post-flowering inoculation. In a few of the seedlings, the systemic necrosis was severe and almost lethal in the maturing plants, whereas in the remainder it was light, being evident as a few small veinal lesions on 3 to 6 leaflets in different positions on the plant. The Factor checks developed leaf-drop streak after the first inoculation, while the Katahdin checks localized the virus, but developed systemic necrosis after the second inoculation.

TABLE 3

THE EFFECT OF STAGE OF GROWTH AT INOCULATION ON THE SUBSEQUENT REACTION OF 89 POTATO
SEEDLINGS TO VIRUS Y

Number Localized After 1st Pre-flowering Inoculation	Result of 2nd Post-flowering Inoculation	
	Number Localized	Number of Systemic Necrosis
89	44	45

It appeared from the results in Table 3, that the physiological plant response which causes localization of virus Y, was not rapid enough in half of the seedlings at the second inoculation to prevent systemic invasion of tissues away from the inoculation sites. In contrast, the tendency in very early type seedlings, as mentioned previously, is for the virus to be localized owing to the rapid maturation.

The results of Table 3 made it necessary to find to what extent the hypersensitive reaction had failed to prevent systemic infection in the seedlings in which localization was incomplete at the second inoculation. Further, it was of importance to know whether the systemic necrosis resulting after the post-flowering inoculation was of the same type as that previously obtained after the pre-flowering inoculation in many seedlings. In order to investigate these points, tubers from some of the seedlings of both groups in Table 3 were kept and planted in May 1947.

TABLE 4

EMERGENCE RESULTS FROM THE TUBERS OF SEEDLINGS INOCULATED PREVIOUSLY WITH VICTORIAN Y
AT PRE- AND POST-FLOWERING GROWTH STAGES

Results of Hand Inoculation November and December 1946		Symptoms in Plants from 2 Tubers of Each Seedling			
Pre-flowering	Post-flowering	Both free of Y	One Plant free of Y, one with Necrosis	Both Plants with Necrosis	Total Seedlings
Localized	Localized	12	0	0	12
Localized	Systemic necrosis	8	3* + 1	4* + 4	20
Localized	Lethal necrosis	1	0	1*	2

Note.—Seedlings marked with an asterisk produced one or two plants with lethal necrosis. The other plants developed a severe necrosis which was almost lethal. Freedom from virus Y was proved by inoculation to 11-84 a hypersensitive seedling used as an indicator plant.

Table 4 gives the results obtained from the tuber progeny of some of the plants from the groups in Table 3. It is apparent that seedlings localizing virus

Y after the two inoculations, were hypersensitive at both growth stages. The results from seedlings which developed systemic necrosis after the second inoculation are interesting, as they show that this reaction is another phase of localization, and that there is a possibility that nutritional factors caused the variation in the hypersensitive response. The fact that 2 tubers from each of 8 seedlings produced healthy plants indicated that the tissues away from the inoculation sites were unfavourable for the extensive development of virus Y. Further, where extensive invasion of tissues including the tubers had occurred, some tubers or "eyes" were free of virus, the rest developing lethal necrosis which appears to be interchangeable with localization. In Table 4, the lethally necrotic plants (see Plate 1, Fig. 1) grew 4 to 6 in. high and died within 21 days of emergence without forming tubers. Those plants shown which were almost lethally necrotic (see Plate 1, Fig. 2) grew 12 in. high and formed tubers before death within 6 weeks of emergence. Plate 1, Figure 2, also illustrates the fact that one tuber from a previously infected plant can produce a healthy plant, while the other produces an almost lethally necrotic one. The reactions in the Katahdin controls (see Plate 1, Fig. 3) were not as severe as in the two former necrotic types. For comparison, Plate 1, Figure 4, shows the tuber progeny from a seedling which was hypersensitive after both inoculations. The tuber progeny of the Factor controls which developed leaf-drop streak after the first inoculation, produced typical secondary symptoms of rugose mosaic.

These results tend to disprove Roland's (1946) hypothesis that the irregular distribution of virus Y in the tuber progeny of infected plants is due to defective diffusion of the virus and not to a reaction of the host tissues. It appears from the results of the Canberra work that if a young vigorously growing plant gives a localized reaction to a relatively heavy dosage of virus Y by hand inoculation, this is a hypersensitive reaction, even if the localization appears to break down at later growth stages, or at periods such as in autumn and winter when the plant is not growing normally.

(a) The Distribution of Virus Y in Seedlings developing Systemic Necrosis after the Post-flowering Inoculation

A number of the plants of seedlings shown in Table 3 as giving a localized reaction at pre-flowering and systemic necrosis following inoculation after flowering were kept. At this stage, although tuber formation was advanced, the plants were still making new growth. Samples consisting of two leaflets with, and two leaflets without, necrotic lesions were taken from 22 of the seedlings, and together with leaflets from Factor and Katahdin check plants used to inoculate half leaves of the seedling 11-84. The two leaflets of every sample were each inoculated at random to a half leaf of seedling 11-84. A potato leaf is considered to be divided longitudinally in halves by the petiole and midrib, so that half the terminal leaflet and the next leaflet on the same side constitute a half leaf. In another experiment, 10 half leaves of 11-84 were inoculated from a seedling with a severe Y mottle, the opposite halves being inoculated from another with a severe systemic necrosis. The results of these two experiments are given in Table 5. Seedling 11-84 is a good indicator plant for virus Y, as it is

immune to virus X, and quickly produces small, well-defined, and easily counted localized lesions after inoculation with Y infected material (see Plate 2, Fig. 2).

The results set out in Table 5 are interesting, as they indicate that in a number of the seedlings which developed systemic necrosis after the second inoculation, virus Y was either in very low concentration or absent when both lesion free and necrotic leaflets were considered. Apparently, in these seedlings, the type of systemic necrosis developed was another phase of the localized

TABLE 5

A NUMBER OF POTATO SEEDLINGS, FACTOR AND KATAHDIN, CLASSIFIED ON THE BASIS OF THE REACTIONS OBTAINED AFTER INOCULATION OF THEIR LEAFLETS TO 11-84

| Variety | Number of Lesions per Half Leaf on 11-84 after Inoculation from: | |
	Leaflets free of Necrosis	Leaflets with Necrosis
Factor	–	15
Katahdin	5	4
15 seedlings	0	0
7 seedlings	2 to 5	0
Mottled seedling	14	–
Necrotic seedling	–	6

reaction. The effect of the necrotic reaction in reducing virus concentration is shown with the 7 seedlings, which had a medium concentration of virus Y in lesion free leaflets and a very low concentration or absence of this virus in necrotic leaflets. This is further borne out by the results with Factor, Katahdin, and the mottled and necrotic seedlings shown in Table 5. In Factor, which develops a strong mottle as well as necrosis, and in the mottled seedling, virus Y concentration appears to be almost double that in the necrotic reacting Katahdin and the necrotic seedling when 11-84 is used as the indicator of concentration. These results are in agreement with those of Bawden and Kassanis (1946) who showed serologically that mottle reacting types had a higher virus concentration than those giving necrotic reactions.

TABLE 6

NUMBERS OF SEEDLINGS WITH SYSTEMIC NECROSIS CLASSIFIED INTO GROUPS ON THE BASIS OF THE RESULTS OBTAINED AFTER REINOCULATION OF TWO OF THEIR LESION FREE LEAFLETS WITH VIRUS Y

	No Reaction on either Leaflet	Reaction on both Leaflets	Reaction on one Leaflet	Total
Seedlings	9	24	5	38
Factor	4	0	0	4
Katahdin	2	0	0	2

Note.—The number of lesions developing on reinoculated leaflets varied from 10 to 20.

In order to investigate these results further, the seedlings which were kept following the development of systemic necrosis after the post-flowering inoculation, were reinoculated with virus Y on selected lesion free leaflets. Two leaflets were inoculated on each seedling, one on a leaf with all leaflets free of systemic necrosis, and one opposite a leaflet with systemic necrosis. Factor and Katahdin checks used in these experiments were inoculated similarly.

The results obtained in this experiment are given in Table 6, and bear out those in Tables 4 and 5, since it is apparent that the tissues of seedlings hyper-sensitive at pre-flowering, are also unfavourable to the extensive development of virus Y when inoculated after flowering, even though some systemic invasion occurs at this latter stage. The fact that two-thirds of the seedlings shown in Table 6 developed necrotic reactions on lesion free leaflets on reinoculation indicated absence of virus Y in them, and the inference is that where systemic necrosis resulted, the virus had developed and become localized. In Factor and Katahdin virus Y multiplication was more general.

V. INTERACTION BETWEEN STAGE OF GROWTH AND STRAIN OF VIRUS Y IN RELATION TO THE NECROTIC REACTION OF SEEDLINGS

It is apparent from the results presented so far, that localization of virus Y in the potato seedlings which have been developed is not a clear-cut reaction, and although dependent primarily on certain gene frequencies, the expression of this reaction is influenced by plant metabolism at the time of inoculation. In turn, plant metabolism is influenced by environmental factors like photoperiod and temperature which are varying entities in the ordinary greenhouse during the year. Another factor which can influence the localized reaction is the strain of virus Y used. Recently, Bawden and Kassanis (1947) have demonstrated the existence of strains of virus Y differing in virulence.

(a) The Effect of Strain on the Concentration of Virus Y in Tobacco Plants

For the experiments concerning the relation between strains of virus Y and the localized reaction of potato seedlings, the following strains of Y were used: Rothamsted (R), Sheffield (S), 18.2, Victorian Y, and virus C. As explained previously, these were transferred each month to a new batch of young tobacco plants. Effects in this host were observed to vary according to the strain, R and S producing relatively severe symptoms, 18.2 and Victorian Y mild symptoms, and virus C causing hardly discernible symptoms. This variation of symptom expression in tobacco due to the strain of virus Y could result from differences in virus concentration. To test this hypothesis, 22 plants of the seedling indicator 11-84 at the flowering stage and as uniform as possible were selected. Three leaves midway on every plant were chosen and 5 of the 6 half leaves each inoculated

TABLE 7

EFFECT OF STRAIN OF Y ON THE VIRUS CONCENTRATION IN TOBACCO PLANTS, SHOWN BY THE NUMBER OF LESIONS ON HALF LEAVES OF THE POTATO SEEDLING INDICATOR 11-84

Strains of Virus Y	R	S	18.2	Vict. Y	C
Mean number lesions/half leaf	36.60	37.41	59.77	101.27	20.50
Mean transforms	5.93	5.99	7.51	9.85	4.35

Minimum difference in mean transforms for significance at 5% = 0.8029.

at random to one of the 5 strains of virus Y. The tobacco plants from which the strains of Y were taken were of similar size, and had all been inoculated at the same time a month previously. A standardized method of applying the inoculum

with the spatula was used throughout, and the inoculum was prepared by grinding 2 g. of infected tobacco leaf in 40 ml. of phosphate buffer at pH 7. The lesions were counted 10 days after inoculation and the results are given in Table 7.

Under the conditions of this experiment, it can be seen from Table 7, that the more severe R and S strains of Y were in significantly less concentration than the relatively mild 18.2 and Victorian Y. Virus C was in significantly less concentration, and Victorian Y in significanfly greater concentration, than the other strains. Such differences in concentration could explain in part the relation between virus Y and the hypersensitive reaction of potato seedlings discussed later. It is apparent that severity of symptoms and virus concentration are not necessarily correlated in tobacco, although virus C with its hardly discernible symptoms in this host was in lowest concentration.

(b) Relation between Strain of Virus Y and the Localized Reaction in 50 Selected Seedlings

For this experiment, 50 of the potato seedlings used in the previous experiment were selected. Of these, 24 had localized reactions after pre- and post-flowering inoculations with Victorian Y in the 1946-47 season, and 26 had reacted with systemic necrosis after the pre-flowering inoculation (see Tables 1 and 3). The R, S, 18.2, Victorian Y strains, and a mixture of the first three, were each inoculated separately into different plants of every seedling. The first inoculation was carried out in October 1947 as soon as the first two leaves had expanded sufficiently after emergence. Inoculation at this early growth stage did not decrease the percentage of seedlings with a localized reaction. Those with localized reactions were given a second inoculation at the post-flowering growth stage some 6 to 8 weeks later. The localized reactors from this experiment were harvested and planted again early in March 1948, when they emerged free from virus Y. When 9 to 12 in. high, they were reinoculated with the strain or mixture of strains used on them in the previous October inoculation. A second inoculation was given the plants a month later. It was difficult to separate the different results of the two last inoculations as the plants, although green, grew slowly and tended to mature quickly owing to the shortening photoperiod of autumn, and Y reactions appeared slowly. The results are given in Table 8.

In columns A and B, where one of the plants was late in emerging or weak growing, the result from the more normal duplicate was recorded. It was noticeable that weak plants tended to react with lethal necrosis where the duplicate gave a localized reaction, which is in accord with the previous results. In column C, a seedling was recorded if it had one or more plants which localized virus Y. No seedling at the autumn inoculation gave a localized reaction in all of the 2-5 replicates, showing that the hypersensitive seedlings discussed in this paper will develop a type of systemic necrosis if given the right conditions. The plants which developed systemic necrosis at the autumn inoculation did not have typical symptoms involving the leaves, as the necrosis was confined mainly to the stem.

The results shown in Table 8 do not detract from the general principle of virus Y hypersensitivity propounded in this and previous papers (Hutton and Bald 1945; Hutton 1945, 1946), as seedlings which have reacted hypersensitively at the pre-flowering stage when growing normally in the early summer, will, in the majority of cases give a localized reaction or a light systemic necrosis after flowering. The latter reaction, which can also occur in hypersensitive seedlings inoculated under unfavourable environmental conditions, results, as shown previously, in most of the tuber progeny being healthy or lethally necrotic.

TABLE 8

NUMBER OF SEEDLINGS IN A GROUP OF FIFTY WHICH GAVE LOCALIZED REACTIONS AFTER INOCULATION IN THE SUMMER AND AUTUMN WITH DIFFERENT STRAINS OF VIRUS Y

| Strain of Y | Summer 1947-48 Number of Seedlings in Batch of 50 with Localized Reactions | | | Autumn 1948 Number of Seedlings from B with Localized Reacting Plants |
	A Inoculation at Emergence	B Inoculation at Post-flowering	Differ- ence	C Inoculations March and April 1948
R	36	32	4	13
S	28	18	10	6
18.2	25	18	7	11
Victorian	21	20	1	4
R + S + 18.2	34	28	6	11

Note.—Columns A and B result from inoculation of duplicate plants of each seedling, column C from 2-5 plants of each.

The influence of strain of Y on the numbers of seedlings giving a localized reaction is clearly shown in Table 8. The tendency for R, the most virulent of the strains, to give a localized reaction in the greatest number of seedlings is interesting. This influence is also apparent where the mixture of R, S, and 18.2 was used, the R strain appearing to have eliminated in some way the effects of the S and 18.2 strains, so that the number of localized reactors for the mixture tends to follow the R pattern. The numbers of seedlings localizing the other strains are much the same, although it is of interest that only one seedling developed systemic necrosis at post-flowering with the Victorian strain. This latter fact may be of significance as the 20 seedlings hypersensitive to Victorian Y, after the two summer inoculations, came from the group shown in Table 3 as giving localized reactions in the 1945-46 and 1946-47 seasons after pre- and post-flowering inoculations. It is difficult to correlate the results of Table 8 with those of Table 7. Apparently, other biochemical characteristics, apart from concentration differences, determine the relationship between strain of virus Y and hypersensitivity in potato seedlings.

In order to examine the strain effect more closely, the results from the 20 seedlings which localized Victorian Y in the summer on both occasions, as shown in Table 8, were compared with those from plants of the same seedlings inoculated with the other strains of Y.

It can be seen from Table 9 that the number of seedlings previously found hypersensitive to Victorian Y and localizing the other strains in this experiment varied, the R strain being localized by the greatest number. Almost half the seedlings of the Group in Table 9 gave localized reactions to the other three strains of Y, whereas only one seedling did not have a localized reaction to any of the other three. It is apparent that strain is an important factor determining whether a seedling will be hypersensitive or not, and that a seedling localizing one strain has about a one in two chance of localizing a different strain. The check plants of the varieties Factor and Katahdin used in the experiment of

TABLE 9

NUMBERS OF SEEDLINGS FROM THE GROUP OF TWENTY, WHICH LOCALIZED VICTORIAN Y AT BOTH SUMMER INOCULATIONS, LOCALIZING THE OTHER STRAINS OF VIRUS Y

Localizing R, S, & 18.2		Localizing R		Localizing S		Localizing 18.2		Not Localizing R, S, & 18.2	
1st Inocn.	2nd Inocn.	1st Inocn.	2nd Inocn.	1st Inocn.	2nd Inocn.	1st Inocn.	2nd Inocn.	1st Inocn.	2nd Inocn.
10	6	17	17	12	7	15.	11	1	1

Note.—Localizing R, S, and 18.2 in the first and last columns refers to each separately, and not the mixture.

Table 8 gave symptoms depending largely on the strain of Y used. The R and Victorian strains caused the development of leaf-drop streak after the first inoculation in Factor, whereas Katahdin localized these strains. After the second inoculation, Katahdin developed systemic necrosis with the Victorian strain, but localized the R strain. With the S and 18.2 strains, Factor and Katahdin developed after the first inoculation a strong mottle which was more pronounced with the S strain.

(c) Second Experiment on the Relation between Strain of Virus Y and the Hypersensitivity of Potato Seedlings

In view of the influence of the strain of virus Y on the localized reaction of seedlings, a second experiment involving fewer seedlings and more treatments was made during the same season as the previous experiment. Thirteen of the seedlings from the 50 used in the previous experiment, and Katahdin, Sequoia, and Factor plants were hand inoculated with the 4 strains of Y which were used separately, and as a mixture. In addition, virus C was used on its own, and side grafting with Epicures containing R and 18.2 Y respectively was done. The mixture of Y strains was obtained by grinding together leaves from different tobacco plants, each carrying one of the strains.

The seedlings selected for this experiment were those which gave a localized reaction the greatest number of times in the previous experiment quoted in Table 8 where each seedling had a chance of giving a localized reaction on 10 occasions, since each seedling was represented by 5 plants which were inoculated twice.

Table 10 gives the numbers of localized reacting seedlings on the different numbers of occasions. The 13 seedlings selected for the second experiment came from the 14 which gave a localized reaction on 8, 9, or 10 occasions in Table 10.

TABLE 10

THE NUMBER OF LOCALIZED REACTING SEEDLINGS ON THE DIFFERENT NUMBERS OF OCCASIONS IN TABLE 8

					Number of Localized Reactions out of 10							Total
	0	1	2	3	4	5	6	7	8	9	10	
No. of seedlings	7	1	3	5	6	3	4	7	5	4	5	50

The results of the experiment are given in Table 11, where the symptoms following the second or post-flowering inoculation and grafting are given, as well as the symptoms in the vegetative progeny of the tubers harvested from the inoculated and grafted plants.

At the first or pre-flowering inoculation, the seedlings Katahdin and Sequoia gave a localized reaction except where lethal necrosis occurred, as indicated in Table 10. Factor gave the usual leaf-drop streak or mottle after the first inoculation with the virus Y strains, and a localized reaction at both inoculations to virus C. After the post-flowering inoculation, Katahdin and Sequoia developed lethal necrosis, systemic necrosis, or leaf-drop streak, depending on the strain of virus Y used.

At the second inoculation, the seedlings with few exceptions gave a localized reaction, lethal necrosis, or a light systemic necrosis, and these reactions resulted in the tuber progeny being healthy or lethally necrotic, showing as before (Table 4) that these reactions were different phases of hypersensitivity. The results in Table 11 from the tuber progeny follow from the planting of 2 tubers from each inoculated plant, and where the results from the two differed it is indicated in the table. In the R, 18.2, Victorian Y, and 4 mixed strain series, 27-41 and 30-39, 27-41, 24-10 and 30-39, and 27-41 and 28-86, respectively, produced healthy plants from the tuber progeny of lethally necrotic plants. Seedling 11-76 and Sequoia are interesting in the R series, as one of the tuber progeny was healthy, and the other lethally necrotic, showing that the apparently localized reaction after the second inoculation allowed the virus to travel down to the tubers. At planting, it was interesting to note that rotting due to necrosis occurred only in tuber progeny of the R series.

When the results of Table 11 were compared with those obtained earlier the same season with the same seedlings, the tendency was for fewer localized reactions at the second inoculation, and more lethal and light systemic necrotic reactions indicating that the level of plant metabolism in the late summer was less conducive to a completely localized reaction. With some seedlings too, like 11-76, 11-84, and 11-158 in the S series, an apparently non-hypersensitive type of reaction was obtained. In most of these instances, the type of leaf-drop streak was more severe than that obtained in Y infected Factor. This has happened before with

TABLE 11

RESULTS FROM THE SECOND OR POST-FLOWERING INOCULATION, AND GRAFTING OF THIRTEEN SELECTED SEEDLINGS AND THREE VARIETIES WITH VARIOUS STRAINS OF VIRUS Y, AND THE SYMPTOMS OBTAINED IN THEIR TUBER PROGENY

Hybrid	R		S		18.2		Vict. Y		4 Mixed		C		R		18.2	
	2nd Incon.	Tuber Progeny	2nd Incon.	Tuber Progeny	2nd Incon.	Tuber Progeny	2nd Incon.	Tuber Progeny	2nd Incon.	Tuber Progeny	2nd Incon.	Tuber Progeny	Graft	Tuber Progeny	Graft	Tuber Progeny
2-404	L	H	Leth	-	LSN	H	L	H	LSN	H	L	H	Leth	-	Leth	Leth
11-76	L	H, Leth[2]	LDS	LDS	LDS	LDS	Leth	Leth[2]	LDS	LDS	Leth[1]	Leth[2]	Leth	-	Leth	Leth
11-84	L	H	LDS	LDS	Leth[1]	Leth	Leth	Leth[2]	Leth	Leth	LSN	H	Leth	H	Leth	Leth[2]
11-158	Leth	Leth	LDS	LDS	Leth	Leth	Leth[1]	Leth	Leth	Leth	L	H	Leth	-	Leth	Leth
13-4	LSN	H	L	H	Leth[1]	Leth	Leth[1]	-	L	H	Leth[1]	Leth[2]	Leth	-	Leth	-
19-1	LSN	H	LSN	H	LSN	H	LSN	H	LSN	H	LSN	-	Leth	-	LSN	H
23-9	L	H	L	H	L	H	L	H	L	H	L	H	Leth	LDS	Leth	LDS
24-10	LSN	H	LSN	H	LSN	H	Leth	H	Leth	Leth	Leth	-	Leth	Leth[2]	Leth	Leth
27-41	Leth	H	LSN	H	Leth	H	Leth	Leth	Leth[1]	H	Leth	Leth	Leth	-	Leth	Leth
28-86	LSN	H	SN	Mottle	LDS	LDS	LDS	LDS	Leth	H	L	H	Leth	H	LDS	LDS
30-39	Leth	H	SN	-	SN	SN	Leth	H	Leth	Leth	L	H	-	-	Leth	-
32-164	LSN	H	SN	Mottle	L	H	LSN	Leth[2]	L	H	L	H	Leth	H	Leth	-
34-86	Leth	-	Leth[1]	Leth	Leth[1]	Leth	Leth	Leth	Leth[1]	Leth	L	H	Leth	-	Leth	Leth
Katahdin	Leth	Leth	SN	Mottle	SN	Mottle	Leth	H, LDS	Leth[1]	Leth	SN	Rugose Mottle	Leth	Leth	Leth	LDS
Sequoia	Leth	H, Leth	SN	LDS	SN	Mottle	LDS	LDS	LDS	LDS	SN	Rugose Mottle	LDS	LDS	Leth	-
Factor	LDS	Mottle	Mottle	Mottle	LDS	-	LDS	Mottle	LDS	LDS	L	H	LDS	LDS	LDS	LDS

H = healthy; L = localized necrotic reaction; Leth = lethal necrotic reaction. Seedlings marked Leth[1] reacted with lethal necrosis after the first or pre-flowering inoculation, those marked Leth[2] did not emerge owing to rotting of the tuber or death of all the "eyes."

SN = systemic necrosis of most of leaflets without leaf drop; LSN = light systemic necrosis; LDS = typical leaf-drop streak.

seedlings which are usually hypersensitive, and the indications are that if hypersensitive seedlings are tested repeatedly at different times and under a wide enough range of conditions, their hypersensitivity occasionally appears to break down. Katahdin frequently reacts with severe leaf-drop streak, being less sensitive than most of the selected seedlings.

The results with virus C in Table 11 paralleled those obtained with the other virus Y strains. When the graft reactions are examined, there is little difference between the results from the R and 18.2 strains, and generally hypersensitivity of seedlings has been expressed by lethal necrosis (see Plate 2, Fig. 1), and where there were tuber progeny, some have emerged healthy. Grafting has not provided more information than hand inoculation, in fact hand inoculation at different growth stages followed by an examination of the tuber progeny will give a more accurate assessment of hypersensitivity to Y than grafting.

As the seedlings shown in Table 11 were specially selected, this experiment shows in a more limited fashion than the earlier, the effect of strain on hypersensitivity. However, it emphasizes how seedlings with hypersensitive reactions to all strains of Y can be developed from basic material like Katahdin.

VI. THE RELATION BETWEEN TYPE OF INOCULATION AND REACTION TO VIRUS Y IN SEEDLINGS

(a) Hand Inoculation of Leaves compared with Hand Inoculation of Stems

In the field, aphids, particularly Macrosiphum gei, often feed on the young stem tissue at the tips of stems. In view of this, and the fact that comparisons between the Y sensitivity of leaf and stem tissues had not been made, an experiment was done in the 1947-48 season to determine what effect hand inoculation of stems and leaves had on the respective final plant reactions to virus Y.

TABLE 12

COMPARISON BETWEEN THE REACTIONS FOLLOWING HAND INOCULATION OF STEMS AND LEAVES OF POTATO SEEDLINGS WITH VIRUS Y

Reaction of Inoculated Tissue	Total Number of Plants		Final Plant Reaction	Total Number of Plants	
	Stem Inoculation	Leaf Inoculation		Stem Inoculation	Leaf Inoculation
No necrosis	25	0	Free of Y	17	16
Light necrosis	14	8	Systemic* necrosis	27	28
Medium necrosis	3	17			
Heavy necrosis	2	19			

* Plants with systemic necrosis were also showing a mottle in a number of instances.

Duplicate plants of a number of seedlings were inoculated with one of the following: R, S, 18.2 strains of Y, or the three strains mixed. The leaves of one plant were hand inoculated in the usual way, and the stem of the other plant, after dusting with carborundum, was inoculated by rubbing between the thumb and

forefinger dipped in inoculum. The seedlings came from a batch of 20, including both localized and systemic necrotic reactors, the duplicates of which were allocated to the different strain treatments according to the number of plants of each available. The results showed that the strain of Y used had no effect on the comparisons between stem and leaf inoculation, so the results were summated and are given in Table 12.

Table 12 shows that over half the plants did not give a visible necrosis after stem inoculation, whereas all the duplicates reacted necrotically on inoculated leaves. It is doubtful whether this indicates that stem tissue is less sensitive than leaf tissue, as the final plant reactions were much the same irrespective of the method of inoculation.

(b) Hand Inoculation compared with Aphid Inoculation

In the 1946-47 season, duplicate plants of 100 seedlings which had been found to include localized, lethal necrotic, systemic necrotic, and mottle reactors to hand inoculation with Victorian Y in the greenhouse, were selected for aphid inoculation. Factor and Katahdin plants were included as checks. The plants were aphid inoculated in the greenhouse when they were at the flowering stage, using *Myzus persicae* under the optimal conditions described by Watson and Roberts (1939). A leaflet on each plant was tagged and inoculated with 4 aphids which had fed for 4 minutes on the underside of a tobacco leaf infected with Victorian Y. Aphid inoculations were carried out in the morning, and the aphids were killed by fumigation in the late afternoon some 5 hours later.

Each week, the tagged leaflets and the plants were examined for virus Y symptoms. After 8 weeks, the 47 plants which exhibited no leaf or plant symptoms were discarded, and the results from those which reacted were analysed and are given in Table 13. Four out of six Factor checks developed leaf-drop streak, and three of the six Katahdin checks reacted, two with a localized reaction, and one with systemic necrosis.

TABLE 13

COMPARISON BETWEEN THE HAND AND APHID INOCULATION RESULTS FROM DUPLICATES OF THE SAME SEEDLINGS

Reaction to Hand Inoculation	Number of Hybrids	Results from Aphid Inoculation			
		Localized	Lethal Necrosis	Systemic Necrosis	Mottle
Localized	19	17	0	2	0
Lethal necrosis	2	1	1	0	0
Systemic necrosis	25	10	0	15	0
Mottle	7	0	0	0	7

Table 13 shows a fair correspondence between hand and aphid inoculation results. That 40 per cent. of the systemic necrotic reactors to hand inoculation gave localized reactions after aphid inoculation suggests that with the lower virus dosages applied in the latter method the virus tends to become inactivated by the necrotic reaction of the inoculated tissues. With the more concentrated virus dosages applied in hand inoculation, only the more complete and rapid localized

reactions are capable of preventing systemic invasion by virus Y. It is possible that the resistance to initial infection of the variety Katahdin to virus Y, as shown by Bawden and Kassanis (1946), is due to a balance between virus dosage and the necrotic reaction of this variety.

The general tendency as shown throughout this study is for the necrotic reaction to inhibit the development of virus Y, and Table 13 indicates the effect that virus dosage has on this relationship. This has already been observed by Schultz, Stevenson, and Akeley (1947) in relation to the field infection of varieties. It is apparent that the level of necrosis required for field resistance will vary according to the density of virus-infected aphids present.

In the experiment of Table 13 it is possible that a number of the 47 seedlings which apparently escaped aphid infection had necrotic reactions which were so minute as to escape detection. Where visible necrotic reactions resulted, these were often no larger than a pin's head and were difficult to find. In view of this, and the possibility of virus dosages influencing the field immunity of a variety or seedling, it appears of greater value to study the development of hypersensitivity in seedling material by means of hand inoculation in the greenhouse. Under these conditions, a high and relatively constant dosage of virus Y can be applied, so that a more accurate assessment of the true hypersensitivity of any particular seedling can be made.

VII. Histological Changes in the Stems of Virus Y Infected Plants

Quanjer (1931) and Bawden (1932) examined microscopically the stems of potato plants affected with acropetal necrosis or leaf-drop streak caused by virus Y, and found in the collenchyma necrotic areas which sometimes extended into the rest of the cortex but not to the vascular bundles. Bawden (1932) found that the epidermis and periderm occasionally showed necrotic areas, and that the internal necrosis characteristic of virus Y was usually present immediately below nodes from which leaves had fallen. He found that acropetal necrosis spread perpendicularly in the stem and produced dark streaks visible below the epidermis. Bawden also found a correlation between external and internal symptoms, varieties like Up to Date (Factor) reacting with leaf-drop streak having internal necrosis, whereas Epicure and varieties reacting with a mottle having no internal necrosis, thus showing the necrotic reaction to be dependent on variety.

The work described in this paper confirms Bawden's (1932) findings and presents further results on the distribution of internal necrotic areas, particularly in hypersensitive reactors to virus Y. It was made possible by using a modification of the method used by Sheffield (1943) in the diagnosis of potato leaf roll. Wilson (1948) has pointed out that phloem necrosis in leaf-roll affected potato plants is more easily distinguished if concentrated hydrochloric acid is used in Sheffield's method. Work at Canberra has shown that 40 per cent. sulphuric acid (v/v) used in conjunction with 2 per cent. phloroglucinol (w/v) in 50 per cent. alcohol (v/v) is a reliable combination for distinguishing necrotic areas in hand cut transverse stem sections, whether they be due to virus Y or leaf roll. With this

modification, necrotic areas due to virus Y stain a bright red colour, and those due to leaf roll a pink colour.

When the stem sections of plants which have reacted necrotically to virus Y are treated as described and examined microscopically, a bright red staining of cell walls in the necrotic region is visible. Usually the collenchyma with the thickening at cell corners is characteristically outlined and often the staining of cell walls continues into the rest of the cortex (see Plate 2, Fig. 3), and in severely affected plants phloem tissue is involved as well. Cell distortion often occurs when the thin walled cortical cells and the phloem are affected. In lethal necrotic types, cell walls often in large areas of the stem are stained from the epidermis to the pith, involving secondary phloem as well as the internal and external primary phloem. With such plants, there is little likelihood of confusing the reaction with leaf roll, as the phloem is only involved in Y necrosis when other tissues are heavily infected. It was not possible to determine whether the xylem was affected by virus Y as it showed no distortion and stained red in healthy plants.

Most of the observations on plants reacting necrotically to virus Y were made on material grown in the greenhouse during 1948. One group of plants examined was grown from tubers of seedlings and the varieties Factor, Katahdin, and Sequoia infected the year previously with the various strains of virus Y. On emergence, the plants of this group were either healthy or had mottle, leaf-drop streak, or lethal necrotic symptoms. Those free of virus Y symptoms had no internal necrotic areas in transverse sections hand cut progressively from the base to the tip. Thirty-three plants of the group had necrotic areas in various portions of the stem, the distribution and extent of necrosis being dependent on the severity of the symptoms. Table 14 gives the different distributions of the plants within the mottle, leaf-drop streak, and lethal necrotic groups respectively, according to the stain reactions found in the base, mid, and tip stem sections. Factor plants occurred in the mottle group, as the secondary Y symptoms in this variety were a rugose mottle which was also the secondary symptom in the other varieties with some of the virus Y strains used.

In Table 14, when basal stem sections are considered, the majority of the plants in the mottle and leaf-drop streak groups had a light staining in the collenchyma and the rest of the cortex, whereas in the lethal necrotic group most of the plants were heavily stained in this region, and in over one-third of them staining extended into the pith. Where staining was intense, phloem tissue was involved in the stem sector affected. The results for the mid-stem sections show a similar gradation, half of the plants of the mottle group being unstained and the rest lightly stained, whereas in the leaf-drop streak group over half were heavily stained, and in the lethal necrotic plants the majority were stained extensively from the epidermis to the pith. In the stem tip sections staining was absent in the mottle group, and absent or light in the leaf-drop streak plants, whereas the majority were stained in the lethal necrotic group, half being intensively stained.

These results are an index of the varying sensitivity to virus Y found among varieties and seedlings. There appears to be a clear distinction between the mottle group on the one hand and the lethal necrotic group on the other, the leaf-drop streak plants having internal necrotic symptoms intermediate between these two. Table 14 also indicates that in the lethally necrotic plants the tissue is so sensitive that a systemic invasion of the virus results in necrosis and death wherever the virus develops, whereas in the tolerant mottle types virus development can occur in various sites without observable necrosis of the tissue.

TABLE 14

NUMBERS OF PLANTS WITH MOTTLE, LEAF-DROP STREAK, AND LETHAL NECROSIS CLASSIFIED ON THE BASIS OF THE INTERNAL STAINING REACTION OF THEIR STEMS TO VIRUS Y

Type and Extent of Staining* observed in Stem Sections	Mottle Sections at:			Leaf-drop Streak Sections at:			Lethal Necrosis Sections at:		
	Base	Mid	Tip	Base	Mid	Tip	Base	Mid	Tip
No staining	3	5	10	0	0	5	0	0	2
Light staining collenchyma and cortex	6	4	0	7	4	5	3	1	4
Heavy staining collenchyma and cortex	1	0	0	3	3	0	6	3	1
Heavy staining from epidermis to pith	0	1	0	0	3	0	4	9	6
Total plants	10			10			13		

* The observations on staining refer to tissues other than the xylem, which is always stained with the method used. "No staining" refers to absence of stained areas in tissues other than the xylem; "light staining" means relatively small lightly stained areas; and "heavy staining" refers to extensive deeply stained areas.

A further set of observations was made on a group of seedlings, the plants of which had given a localized reaction after hand inoculation of leaves in some, and stems in others with the various strains of virus Y. The numbers of localized reacting plants examined varied owing to the effect of strain, and also owing to the number of duplicates of the seedlings available initially for inoculation. Stem sections were taken from just above to just below the point of attachment of the inoculated leaf, and through the inoculation site where the stem was inoculated. The results are summarized in Table 15.

All the plants of the seedlings shown in Table 15 were subsequently proved free of virus Y when the tubers harvested from them were grown on in the greenhouse. The fact that a staining reaction occurred in stem sections of a varying proportion of seedlings which gave an apparently localized reaction to virus Y in inoculated leaves is interesting. The necrotic areas in the stem were stained

relatively lightly, but were clearly defined, and in approximatly half the plants, occurred in the collenchyma and rest of the cortex, and in the others extended from the collenchyma to the pith. These results indicate that the localizing reaction is not always completed in the inoculated leaf, but in stem tissue. The extent to which this occurs is probably dependent on the nutritional status of the plant at the time of inoculation. That staining, and hence necrotic tissue, can occur away from the inoculation site in localized reactors further substantiates the findings of Tables 4 and 11, in which it was shown that a light systemic necrosis after the post-flowering inoculation, in localized reacting seedlings at pre-flowering, was a variation of the localized reaction.

TABLE 15

NUMBERS OF LOCALIZED REACTING SEEDLINGS CLASSIFIED ACCORDING TO INTERNAL STAINING IN THE REGION OF INOCULATION AND IN RELATION TO THE Y STRAINS USED

| Strain of Virus Y Used | Leaf Inoculation Number of Seedlings with: | | Stem Inoculation Number of Seedlings with: | |
	No Staining in the Region of Inoculation	Staining of Collenchyma and Cortex or Collenchyma to the Pith	No Staining in the Region of Inoculation	Extensive Staining from the Epidermis to the Pith in the Region of Stem Necrosis
R	15	9	5	5
S	8	8	3	4
18.2	19	2	5	1
R + S + 18.2	9	7	6	5

Although not highly significant, it is interesting that the mildest Y strain, 18.2, gave the highest proportion of the seedlings free of stained necrotic areas away from the inoculated leaves. In the stem inoculation results, those seedlings which had no internal staining were free of stem necroses, so it is probable that the localization sites were not easily discernible in the sections examined microscopically. Where stem necroses were visible externally, internal staining was intense and extended from the epidermis to the pith and involved the phloem tissue as well. It is apparent that stem tissue as well as leaf tissue can react in a hypersensitive fashion. In the instances where internal necrotic areas followed stem inoculation, staining was restricted to the inoculation sites and the virus must have been localized in these necrotic areas as the tuber progeny of the plants were healthy.

The staining results give a clearer understanding of the type of localized reaction which occurs in many of the seedlings developed. Since stem tissue, as well as leaf tissue, can react hypersensitively it is apparent that if the virus travels from the inoculated leaf it can still be localized in the stem before it reaches the tuber. In lethal necrotic types, which form tubers from which healthy plants are at times produced, it is probable that the virus does not diffuse to all the "eyes." When the virus reaches the "eyes," they are either killed or produce lethally necrotic plants. In view of the staining results, it is suggested that the use of the staining technique in conjunction with hand inoculation methods would be

useful in the selection of seedlings with promising reactions to virus Y from hybrid progenies.

VIII. DISCUSSION

This study has demonstrated how hypersensitivity to virus Y and its strains can be developed from common varieties with a severe necrotic reaction to this virus. It has been made possible by the heterozygous nature of the potato, which has allowed the accumulation by the selection methods used, of genes conditioning necrosis of seedlings to virus Y. This has resulted in seedlings with gene frequencies high enough to produce hypersensitivity to a range of virus Y strains. Even though new strains of virus Y may arise to which such seedlings are no longer hypersensitive, this work has indicated how such a problem could be overcome.

In view of these results, hypersensitivity to virus Y can now be added to the characteristics of new potato varieties. That this attribute can be combined with other desirable features has been demonstrated, as 1-2 per cent. of the seedlings from the progenies of carefully planned potato crosses had high yield, desirable tuber characters, immunity to virus X, and hypersensitivity to viruses A and Y. Since relatively low and fluctuating aphid populations have occurred in all the field trials made, it has not been possible accurately to assess the value of the new seedlings in comparison with varieties like Katahdin and Snowflake. Virus dosage is a varying entity under these conditions, so comparisons have been made in the greenhouse where a greater degree of control can be exercised over this and other factors.

It is apparent from this study that the type of sensitive reaction to virus Y present in the seedlings is not similar to the hypersensitivity of Epicure and other varieties to virus X, as this latter reaction is not recorded as being influenced by nutritional or other conditions. Further, it has been shown (Hutton 1945) that virus Y sensitivity in the seedlings is inherited as a recessive character. Cockerham and M'Ghee (1947) have shown that the hypersensitivity to virus Y discovered in *Solanum simplicifolium* is due to a unit dominant gene, so this reaction is apparently governed by different factors from those reported in this study.

In tolerant types reacting with a mottle to virus Y, this virus can move freely and build up a high concentration, whereas if the tissues of a variety react necrotically, the virus, if it gains entrance, is restricted in its movement and development. With tolerant types, infection and kind of reaction do not appear to be greatly influenced by environmental and other factors. On the other hand, in hypersensitive seedlings, the expression of the localized reaction is influenced by growth conditions. The localized reaction is rapid and confined to the inoculation site when plants are young and actively growing. Conditions which lower the metabolic activity of such plants can allow the ingress of the virus into tissues distant from the inoculation site, but since stem as well as leaf tissue is sensitive, the virus is usually localized before it reaches the tubers. Reactions like these give some understanding of the physiological basis for intolerance to viruses. Intolerance to viruses offers most promise for the solution of current virus problems in the potato crop.

IX. Acknowledgments

X. References

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EXPLANATION OF PLATES 1-2

PLATE 1

Fig. 1.—Lethally necrotic plants raised from two tubers of a seedling which previously gave a localized reaction to Victorian Y after the pre-flowering inoculation, but systemic necrosis following the post-flowering inoculation.

Fig. 2.—A plant with almost lethal necrosis and a healthy plant raised from tubers of a seedling which previously gave a localized reaction to Victorian Y after the pre-flowering inoculation, but systemic necrosis following the post-flowering inoculation.

Fig. 3.—Plants with a severe leaf-drop streak raised from two tubers of a Katahdin plant which gave a localized reaction to Victorian Y after the pre-flowering inoculation, but systemic necrosis following the post-flowering inoculation.

Fig. 4.—Healthy plants from the two tubers of a seedling which gave a localized reaction after both pre- and post-flowering inoculations with Victorian Y.

PLATE 2

Fig. 1.—The lethally necrotic reaction in a hypersensitive potato seedling, following a side graft with a Y infected Epicure scion.

Fig. 2.—Type of localized necrotic reaction in a leaf of the potato seedling indicator plant 11-84 a week after inoculation with virus Y.

Fig. 3.—Photomicrograph (x 100) of hand cut transverse stem section of a potato plant reacting with systemic necrosis to virus Y, showing the staining reaction in the collenchyma (A) and the rest of the cortex (B) following treatment with 40 per cent. sulphuric acid and 2 per cent. phloroglucinol solution.
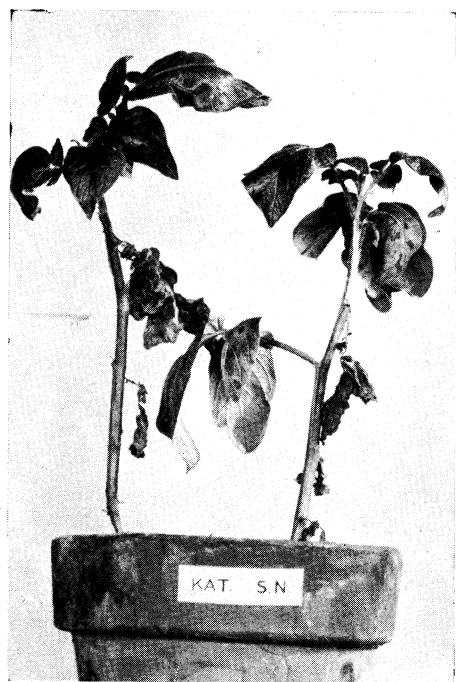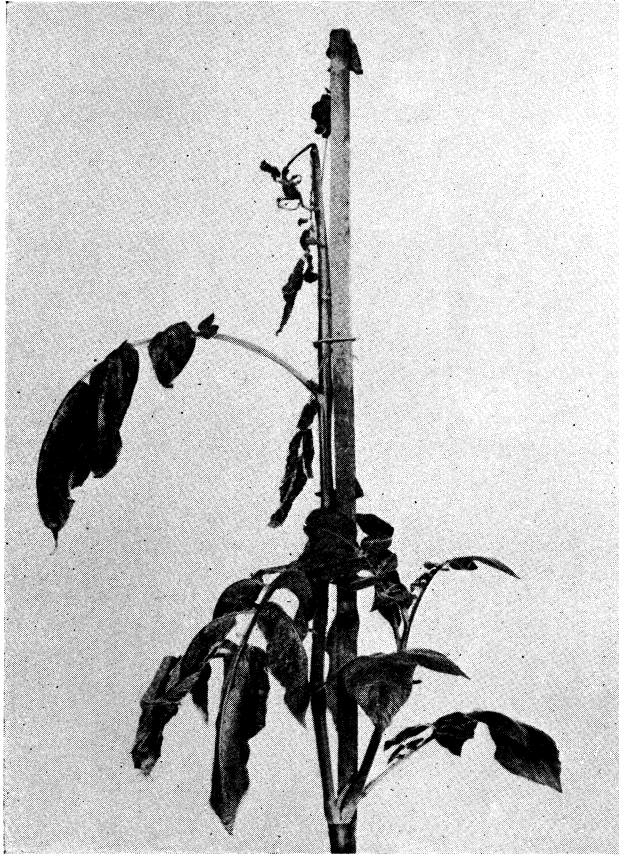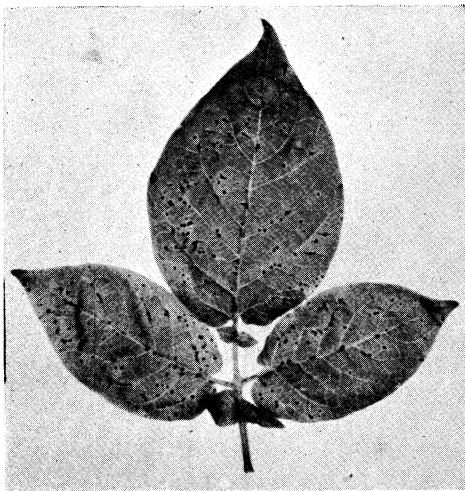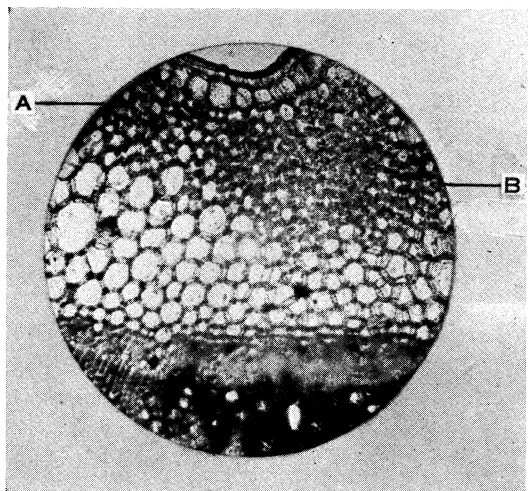
Fig. 1

Fig. 2

Fig. 3

Fig. 4

HUTTON.—SOME FACTORS AFFECTING LOCALIZED AND SYSTEMIC NECROTIC REACTIONS TO VIRUS Y IN THE POTATO

Fig. 1

Fig. 2

Fig. 3

HUTTON.—SOME FACTORS AFFECTING LOCALIZED AND SYSTEMIC NECROTIC REACTIONS TO VIRUS Y IN THE POTATO