STUDIES ON THE NATURE OF RESISTANCE TO VIRUS X IN POTATO (*SOLANUM TUBEROSUM* L.)

by

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AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
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Isolation of virus X from "immune" varieties of potato (Solanum tuberosum L.)

This work was undertaken to determine the nature of the immune type of resistance in potato (Solanum tuberosum L.) to virus X. Approach grafts were made to varieties of potato considered to be immune to virus X (USDA potato seedling clone S. 41956, Saco, and Tawa). To these stocks, scions were grafted using Datura tatula (L.) Torr. and Erlaine selfed seedling clones free of virus X or Irish Cobbler infected with virus X. Inoculation to scions free of virus X were made 14 days after grafting at which time grafts were cut to produce plants with a single scion carrying virus X on an immune stock. Isolation of virus X was attempted from: (1) the complete root system, (2) 4-6 mm. sections cut from below the graft union and at a distance of 1.5 to 2.5 mm. away from the graft, (3) a stem section at the soil line, (4) necrotic tubers when present.

Symptom expression was erratic requiring incubation periods of 5 to 23 days before symptoms were expressed in the Datura tatula test plants. Virus X was isolated in low frequency from all 4 locations. Isolations were more frequent and consistent from the variety Tawa indicating possibly that this variety was more susceptible than others tested.

Time of symptom response in Datura tatula L. (Torr.) to virus X as a function of virus concentration

Under normal greenhouse conditions, D. tatula ordinarily requires 5 to 9 days for full symptom expression, after inoculation with virus X. Incubation periods in D. tatula were determined by serial dilutions of both clarified and highly purified preparations of virus X, strain X 5. Tests were also prepared using a constant concentration of virus and limiting factors of leaf area and inoculum volume.

Plants inoculated with high concentrations of the virus preparations developed symptoms within 5 to 7 days. As the virus concentration was decreased, incubation periods progressively increased. At the lowest concentration before reaching the dilution end point for the virus preparations, plants occasionally required up to 25 days for symptom expression. It was considered that incubation periods were a function of initial virus concentration.

Certain physiological and morphological reactions of potato seedling clone S. 41956 (Solanum tuberosum L.)

Studies were made of reactions of plants grafted either with virus X carrying scions, or scions free of the virus and later inoculated with virus X after the grafts had become established. Plants responded in the following ways: (1) scions and stocks became completely necrotic. (2) scions became necrotic, an apical bud proliferated producing a side

branch either above or below the graft union. (3) plants remained stunted and weak. (4) plants with bifurcated tops remained healthy and vigorous. Scions and stocks of grafted plants were cut longitudinally and tested for the presence of starch. Accumulation of starch in virus X carrying scions was observed when grafts were made to immune (S. 41956) and hypersensitive (Epicure) stocks. Starch accumulated up to 3 cm. below the graft as well as in susceptible scions when inoculation of the virus was delayed until after the graft had become established.

Relative carbohydrate deficiencies in the presence of virus X were demonstrated in roots of immune and hypersensitive stocks when grafted with virus X inoculated, susceptible scions.

Evidence is presented suggesting that immunity to virus X in potato, was similar to the hypersensitive reaction to virus X.

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by

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Isolation of virus X from "immune" varieties of potato (Solanum tuberosum L.)

Introduction and review of literature

The identification of a very high type of resistance to virus X in potato (Solanum tuberosum L.) by Schultz and Raleigh (1933) was subsequently described as immunity in the seedling S.41956 (Raleigh, 1936). Attempts to recover the virus from above ground portions of S. 41956 inoculated with virus X either by grafting or by mechanical means have in the past been unsuccessful (Salaman, 1937; Schultz et al, 1937; Schultz and Raleigh, 1933; Raleigh, 1936; Clinch, 1942; Anonymous, 1952; Cockerham, 1943). These reports of attempts to isolate virus X from this seedling are often brief and lack detailed descriptions of methods, or listings of plant parts from which isolations were attempted. It is probable that isolations were attempted from either leaves or tubers rather than stem or root tissues. It has been shown in twice grafted plants with a susceptible stock and scion, and an intermediate scion of resistant S. 41956, that virus X was transmitted readily downward through the intermediate scion to infect the susceptible stock (Clinch, 1944). The upward movement of the virus was extremely slow when the stock was carrying virus X. This condition suggested that possibly stems and roots of resistant stocks of plants grafted with a virus X carrying scion might possibly yield that virus upon

isolation. The purpose of this research was to determine if virus X could be isolated from immune types of potato which had been graft inoculated with virus X.

Materials and methods

Isolations were attempted from potato varieties highly resistant to virus X, USDA potato seedling clone S.41956 (Schultz and Raleigh, 1933), Saco (Akely et al, 1955), and Tawa (Peterson and Hooker, 1959). These were graft inoculated using approach grafts to scions which were susceptible to virus X. At the time of grafting, scions were either free of virus X, or were infected with virus X depending upon the particular inoculation treatment. Grafts were wrapped with Sealtex latex bandage. The variety S.41956 carries virus S in a latent condition. This virus, however, could not interfere with the isolation studies since the test plants used are considered to be immune from virus S.

At intervals following virus X inoculation, attempts were made to isolate the virus from grafted stocks of highly resistant varieties. Plant parts from which isolations were attempted were: (1) the complete root system; (2) 4-6 mm. stem sections cut from below the graft union and at least 1.5 to 2.5 mm. away from the graft; (3) a stem section immediately at and below the soil line; and (4) necrotic tubers grown on stocks of grafted plants. Necrotic tubers were occasionally produced on plants grafted with virus X carrying scions.

Whole tubers or excised necrotic tissues from such tubers were ground in a mortar and pestle and used for inoculating Datura tatula (L.) Torr.

Plant parts to be tested were handled with sterile forceps and parts were excised with sterile razor blades. Portions for isolation were ground using a steamed mortar and pestle. Only enough water was added to the triturated material to provide sufficient inoculum for transfer to an indicator plant. Carborundum,400 mesh was mixed with the inoculum.

Tests for presence of virus X were made by mechanical inoculation of 2 plants of Datura tatula (L.) Torr. Leaves were sprayed with water, dusted heavily with 400 mesh carborundum, and inoculated with a glass spatula. After rubbing, inoculum was allowed to dry on the leaves and later thoroughly washed with water.

In order to evaluate the possibility of chance contamination or of visually unidentifiable incipient infection with virus X in the 2 Datura test plants, a third control plant of D. tatula was inoculated using one leaf from each of the 2 test plants. Thus, if either test plant were infected with virus X but not showing symptoms at the time of inoculation with potato juice, symptoms would have been expressed on the control plant. There was no evidence of uncontrolled infection with virus X in any of the Datura plants used in these tests.

When virus X was recovered from tissues of highly resistant plants it was identified as such by each of the following tests: (1) cross protection in D. tatula previously inoculated with a mild strain of virus X, X8 (Timian et al.1955); (2) serological chloroplast agglutination test with virus X antiserum (antiserum developed against strain X5); (3) synergistic reaction with virus Y in Nicotiana tabacum L.; and (4) inoculation to Gomphrena globosum L. Plants in the trials were grown in sand and watered twice a week with a production type nutrient solution[1]. Greenhouse temperatures during the period of observation were approximately 20° C.

Scions grafted to resistant stocks were of 3 types: (1) Irish Cobbler or Green Mountain naturally infected with virus X; (2) D. tatula plants free of virus X; and (3) susceptible potato plants (S. tuberosum) free of virus X. It should be pointed out that all stocks of Cobbler and Green Mountain are naturally infected with virus X. Susceptible plants free of virus X were grown from true selfed seed of the variety Erlaine. Individual seedling plants were subsequently grown as clonal lines. Before being grafted these scions were demonstrated to be free from virus X using the serological chloroplast agglutination test.

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[1]The following compounds were dissolved in 1 gallon of water:

1.39 gr. $Ca(NO_3)_2 \cdot 4 H_2O$	0.69 gr. NH_4Cl
0.35 gr. KH_2PO_4	0.69 gr. $NH_4H_2PO_4$
0.87 gr. KNO_3	1.39 gr. $MgSO_4 \cdot 7 H_2O$
1.73 gr. K Cl	

Virus X free Erlaine selfed scions and D. stramonium scions were mechanically inoculated 14 days after grafting with a severe strain of virus X which had been subcultured from isolate X5 (Timian et al 1955). Standard trituration inoculation procedures were followed. Several plants grafted as described were not inoculated and held as controls free of virus X.

Approach grafted plants were cut 14 days after the initial graft producing a plant with a single scion and a single stock. Susceptible scions were inoculated at the time of cutting.

Experimental results

Mechanical Isolations from growing plants.

In attempts to isolate virus X from 20 grafted stocks of S.41956 (Table 1), the virus was successfully isolated from portions of 11 plants. Virus X was obtained from roots, from subterranean stems, and from above ground stems 1.5 to 2.5 mm. below the graft union. Isolations from plants grafted with clones of Erlaine selfed inoculated with virus X were slightly more successful than from plants grafted with D. tatula. Virus X was isolated from all 3 test portions of only one grafted plant of S.41956. It was isolated from both roots and stems below the graft union in only 2 instances, and from both roots and subterranean stems in 3 instances. Of the 11 plants from which virus X was isolated the virus was obtained

Table 1. Isolation of virus X from grafted stocks of USDA potato seedling clone S.41956

Virus X carrying scion		Source of tissue for isolation: root (days 1/)		subterranean stem (days 1/)		above ground stem below graft union (days 1/)	
Erlaine	selfed	-	-	-	-	6	14
"	"	-	-	-	-	12	-
"	"	21	23	12	-	9	-
"	"	9	9	-	-	8	8
"	"	-	-	-	-	7	7
"	"	10	11	-	-	-	-
		No virus X obtained from 3 plants					
Datura tatula		11	-	-	-	7	7
"	"	22	-	-	-	-	-
"	"	-	-	-	-	8	11
"	"	8	-	11	-	-	-
"	"	-	-	8	-	-	-
		No virus X obtained from 6 plants					

1/ Days required for development of virus X symptoms following rub inoculation of 2 D. tatula plants.

from only 1 of the test positions in 8 plants. Of the 16 successful isolations, only one of the 2 D. tatula test plants was infected in 8 instances and both of the D. tatula test plants were infected in 8 instances. Virus X was isolated in greatest frequency from the area below the graft union, and least frequently from the subterranean stem. Furthermore, both test plants were infected in 5 of the 7 successful isolations. This suggests that the virus concentration at the former location was considerably higher than in other portions tested.

Tissues of S.41956 were examined macroscopically before being ground for inoculum. There were no differentiating symptoms nor unusual conditions associated with tissues from which the virus was successfully isolated. At the time isolations were attempted roots of resistant stocks had become somewhat necrotic after grafting with virus X infected scions. Again the presence or absence of virus X could not be associated with macroscopic differences in root appearance.

Isolation attempts were made with 27 grafted Saco plants (Table 2). Virus X was obtained from only 1 of the 3 isolation locations in each of 10 plants. It was not obtained from 2 locations within a single plant. Isolations were not successful in 17 plants attempted. Both D. tatula test plants were infected in only 3 of the 10 successful

Table 2. Isolation of virus X from grafted stocks of the Saco variety

Virus X carrying scion	Source of tissue for isolation: root (days 1/)		subterranean stem (days 1/)		above ground stem below graft union (days 1/)	
Erlaine selfed	-	-	-	-	23	-
" "	6	6	-	-	-	-
" "	20	-	-	-	-	-
" "	14	-	-	-	-	-
" "	-	-	19	-	-	-
" "	-	-	-	-	19	-
" "	13	-	-	-	-	-
" "	17	-	-	-	-	-
	No virus X obtained from 11 plants					
Datura tatula	-	-	-	-	5	5
" "	-	-	6	13	-	-
	No virus X obtained from 6 plants					

1/ Days required for development of virus X symptoms following rub inoculation of 2 D. tatula plants.

isolations. Roots of grafted stocks again tended to be slightly necrotic.

Virus X was isolated in 10 of 19 attempts with grafted plants of the Tawa variety (Table 3). Results of this series were in general agreement with the 2 previous trials except that the variety appeared to be somewhat more susceptible than S.41956 and Saco as the frequency of successful isolations was somewhat higher with Tawa. Another difference which may be of some importance is that virus X was recovered from roots in 7 of the 10 successful isolation attempts. In no instance was virus X recovered from the subterranean stem. It was isolated in only 4 instances from the stem portion below the graft union. The virus was isolated in 2 locations in only one plant. Furthermore there was considerably more uniformity of infection in both D. tatula test plants than was observed in isolations from either S. 41956 or Saco.

Isolations from tubers

Unsuccessful attempts to isolate virus X from tubers of plants which had been grafted with scions carrying the virus have been reported (Clinch, 1942; Anonymous, 1952). Isolations were attempted from 22 tubers of S.41956 harvested from plants with grafts described in the previous experiments. Of these, 6 tubers exhibited a diffuse, fleck type necrosis (Fig. 1A) which was generally distributed throughout the tuber. Usually, only a small percentage of tubers exhibited this

Table 3. Isolation of virus X from grafted stocks of the Tawa variety

Virus X carrying scion	Source of tissue for inoculation: root (days) 1/		subterranean stem (days) 1/		above ground stem below graft union (days) 1/	
Erlaine selfed	-	-	-	-	9	9
" "	-	-	-	-	7	7
	No virus X obtained from 1 plant					
Datura tatula	10	10	-	-	-	-
" "	21	22	-	-	-	-
" "	10	11	-	-	-	-
" "	18	-	-	-	-	-
" "	19	21	-	-	-	-
" "	10	13	-	-	7	7
" "	17	19	-	-	-	-
" "	-	-	-	-	8	9
	No virus X obtained from 8 plants					

1/ Days required for development of virus X symptoms following rub inoculation of 2 D. tatula plants.

limited necrosis. Isolation of virus X was successful from only 1 of 6 necrotic tubers. The presence of virus X in this instance was demonstrated by inoculation to D. tatula and subsequent tests which were previously described for the identity of the virus. Virus X could not be isolated from 16 tubers which did not exhibit necrosis.

In tests determining resistance in potato to virus A virus X was present in scions grafted to a resistant stock. Some of the tubers from such plants developed severe necrosis (Fig. 1B). This necrosis was of a more severe type than the diffuse necrosis described in earlier trials. An extreme necrosis of the cortex and vascular ring was obtained in one case when virus A and virus X infected Green Mountain scions were approach grafted to the Saco variety. Successful isolations of virus X from Saco tubers were made (Table 4) by the following method.

Whole tubers were triturated in a mortar and pestle. A slight amount of water was added to the ground tissue to moisten the pulp and make it suitable for inoculum. Carborundum 400 mesh was dusted on the leaves of D. tatula and into the inoculum, and mechanical inoculations were made using a glass spatula. Inoculum was allowed to dry on the leaf surface after which the leaves were washed thoroughly with water. Temperatures during the experiment were 17° C.

FIGURE 1.

A. Typical internal necrosis in freshly harvested tubers of S.41956 plants grafted with virus X infected Irish Cobbler.

B. External and internal views of freshly harvested tubers of the Saco variety with advanced necrosis following graft inoculation with viruses X and A.

C. Typical small, spindly plants grown from necrotic tubers produced on "immune" stocks grafted either with virus X carrying scions, or with scions doubly infected with viruses X and A. Healthy plants were grown from non-necrotic tubers produced under conditions similar to the necrotic tubers.

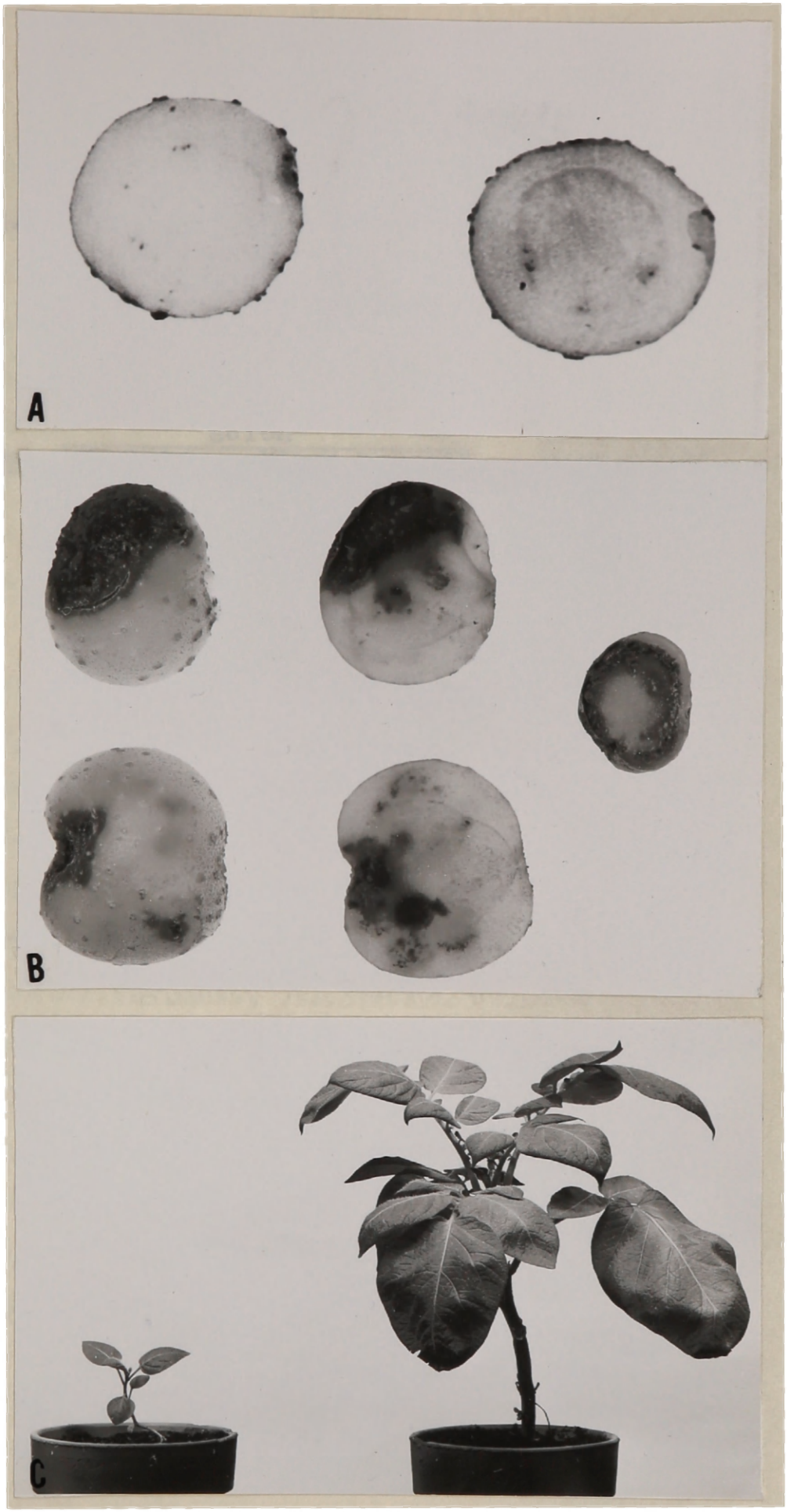
A
B
C

Table 4. Isolation of virus X from necrotic tubers produced on Saco stocks grafted with virus X infected scions, or grafted with scions doubly infected with viruses X and A.

Tuber	Scion		Isolation of virus X from necrotic tubers
	Variety	Virus present	1/
1	Irish Cobbler	X	+
2	" "	X	+
3	" "	X	+
4	" "	X	-
1	Green Mountain	X	+
2	" "	X	+
3	" "	X	-
4	" "	X + A	+
5	" "	X + A	+
6	" "	X + A	+
7	" "	X + A	+

1/ +, virus X isolated; -, virus X not isolated

When a virus mottle was produced in the inoculated D. tatula, the virus was successively transferred 4 times through that host to eliminate virus A after the method of MacLachlan et. al. (1953). Specific tests for the identification of virus X were applied to all unknown virus cultures following the fourth transfer. Virus X was identified in 9 of 12 isolation attempts (Table 4) indicating at least some survival of virus X in necrotic tubers of plants grafted with a virus X infected scion, or a scion doubly infected with viruses X and A.

Necrotic and non-necrotic tubers from S.41956 stocks grafted with Irish Cobbler were stored for a period of 3 months and planted in the greenhouse. Plants produced from necrotic tubers (Fig. 10) were always stunted and spindly while plants grown from non-necrotic tubers developed in a normal manner. Attempts to isolate virus X were made from 4 locations from healthy appearing, and spindly plants. Locations included: 1) leaves, 2) stems, 3) roots, and 4) necrotic and non-necrotic seed pieces. Virus X could not be isolated from 4 spindly plants. Furthermore, 4 healthy appearing plants or their seed pieces yielded no virus X. Successful isolations of virus X were made, however, from all 4 necrotic seed pieces which produced the spindly plants.

Necrotic tubers produced by Tawa stocks which had been grafted with virus A carrying Green Mountain were tested under similar circumstances. A parallel situation was found to exist. No successful isolations were made from healthy tubers or spindly and healthy plant parts. Again, successful isolations were made from 3 of 12 necrotic seed pieces.

In no instance was the virus re-isolated from tubers of mechanically inoculated plants of any immune variety.

Discussion

Three rather well defined types of resistance to virus X in potato have been reported from a number of sources. This literature has been recently reviewed by Hooker et. al. (1954). Immune types have been described as those varieties in which the virus fails to become established. Hypersensitive types are those in which top necrosis develops after virus inoculation. Certain tolerant varieties exhibit considerable resistance to field spread of the virus whereas many are completely susceptible. Investigations of the immune, and hypersensitive types of resistance have been carried out (Cadman, 1942; Cockerham, 1943; Hutton and Wark, 1952; Clinch, 1942, and 1944; Raleigh, 1936; Anonymous, 1952). Clinch (1944) using an intermediate scion of S.41956 grafted to an X-free susceptible stock with an X-infected scion found that the virus moved downward through the intermediate scion

in an unrestricted manner to infect the virus X-free stock but that upward movement was very slow. She failed to isolate the virus from the tissues of S.41956 and concluded that the inability of the virus to multiply in the cells of S.41956 was due to some substance or physical condition necessary for virus X synthesis. Hutton and Wark (1952) studied the immune reaction with a seedling similar to S.41956 in its resistance to virus X. Attempts were made to isolate virus X from mechanically inoculated leaves of plants with various types of resistance. They considered the "immunity" of S.41956 due to an inactivating system as evidenced by extremely restricted development of the virus in leaves of the X-immune type as compared to the susceptible types. Others (Anonymous, 1952) suggest that immunity and hypersensitivity are phases of the same reaction since necrotic spots developed on leaves of S.41956 following grafting to an X-infected stock.

It is suggested by the isolations herein reported that the virus survives, at least to a limited extent, in the tissues of "immune" varieties. It seems probable that it is either restricted in its development or short lived in these "immune" varieties. Therefore, under these conditions, the term "immune" should be used advisedly since the virus is present in the tissues. The mechanism for resistance has yet to be clearly defined. Furthermore, the possibility exists

that the necrotic condition of the tubers could even be a phase of the hypersensitive reaction and that the assumptions of certain investigators (Hutton and Wark, 1952; Anonymous, 1952) who believe that the immune reaction is similar to the hypersensitive reaction, could be correct.

The type of resistance found in S.41956, Saco, and Tawa, to virus X is very useful in the breeding program. For purposes of potato breeding, these varieties should still be considered "immune" but with some reservations since it has been demonstrated that the virus will not move from stored tubers into a growing plant. Spindly appearances of plants produced from necrotic tubers were probably due to the necrotic condition of the tubers rather than virus X itself. When more refined techniques are available for virus assay, however, it is possible that the virus may then be isolated from plants one generation or more away from graft inoculation with virus X.

Certain evidence suggests that virus X is confined to the vascular tissues and moves in the phloem of "immune" varieties. This evidence summarized is: (1) aerial tuber formation which suggests the impedance of translocation; (2) isolation of the virus from areas with abundant vascular tissues; (3) failure of the virus to move upward from necrotic tubers into growing portions of highly resistant varieties; and (4) the impedance of upward transmission of the virus

through a highly resistant intermediate scion; and (5) early necrosis of the inner phloem in varieties hypersensitive to virus X (Quanjer, 1931).

That the virus was never isolated from leaf tissues in earlier trials or later experiments may be of some significance. Possibly the succulence of tissues, and the comparative lack of vascular tissues as compared to stems and roots, lends itself to excess dilution of the virus, and a smaller quantity of virus containing tissues. It is also probable, however, that the virus is precluded in its upward movement into the tissues of the leaves.

In view of these assumptions, several reasons should be considered for success of virus isolation from tissues of highly resistant "immune" varieties. (1) Tissues with abundant phloem were used in isolation rather than succulent leaves, and the triturated tissue was left as nearly undiluted as possible. (2) Refined inoculation techniques have been developed in recent years. (3) A necrotic strain of virus X was used which was easily distinguished upon inoculation to D. tatula. (4) The virus was inoculated after the grafts had become established instead of using virus X infected scions at the time of grafting. (5) Since an extended period of time was sometimes necessary for symptom development in D. tatula test plants, earlier investigators may have discarded their test plants prematurely.

In general, length of incubation period in D. tatula was inconsistent. Incubation periods of up to 23 days were sometimes necessary before symptoms were observed. Delayed symptom development has been shown to be a function of low virus concentration (Hooker and Benson, 1958). With lower concentrations of virus X the incubation period was extended beyond the normal period required for expression of symptoms in D. tatula. It is probable that an incubation period of 5 days represented a considerably higher initial concentration of virus in the tissues than was the case with a 23 day incubation period.

There is also some evidence that certain "immune" varieties may be more resistant than others. This is indicated by increased frequency of successful isolations of virus X from the Tawa variety as compared to the number of successful isolations from other varieties. Generally, there was more uniformity of infection of the test plants, and greater numbers of successful isolations.

Summary

Varieties of potato considered to be immune from virus X were approach grafted with scions susceptible to the virus. These were either (1) free of virus X at the time of grafting and inoculated with the virus after the graft had become established, or (2) scions were infected with virus X at the time of grafting. All leaves were

trimmed from below the graft union on the highly resistant stocks. Isolations of virus X were attempted from such stocks at various time intervals following virus X inoculation. These isolations were attempted from: (1) the complete root system, (2) areas immediately below the graft union, (3) stem sections from below the soil line, and (4) necrotic tubers grown on stocks of grafted plants. Triturated tissues from the above mentioned plant portions were inoculated to Datura tatula (L.) Torr. which responded to infection with this strain of virus X by a strong systemic mottle. The presence of virus X in these varieties was confirmed by the following tests for the identification of virus X: (1) serological precipitin tests, (2) local lesions on Gomphrena globosa L., (3) cross protection with a mild strain of virus X on D. tatula, (4) synergistic reaction with virus Y on Nicotiana tabacum L.

Virus X was isolated at a very low frequency from all plant parts tested. Virus X was isolated successfully from 11 plants in 21 attempts from USDA seedling clone S.41956, 10 in 27 attempts from the variety Saco, and 10 in 19 attempts from the Tawa variety. The evidence suggests the possibility of differences in degrees of resistance between "immune" varieties. Virus X was isolated more frequently and consistently from Tawa than from either S.41956 or Saco.

Incubation periods of the virus in D. tatula were inconsistent, varying from 5 to 23 days (before a definite

virus mottle was distinguishable). It was later demonstrated that the length of incubation period was a function of low initial virus concentration.

In no instance was the virus isolated from leaves of highly resistant varieties grafted with virus X carrying scions.

The virus was isolated from freshly harvested necrotic tubers with some consistency. The frequency of isolation was reduced when attempts were made to isolate the virus from germinated seed pieces after a period of storage. Even though the virus was isolated from the seed pieces, isolations from plants produced from those seed pieces were not successful.

For all practical purposes, highly resistant varieties of this type may still be considered "immune" since isolations were not successful from plants one generation removed from the original inoculation.

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Time of symptom response in *Datura tatula* L. (Torr.) to virus X as a function of virus concentration

Introduction and review of literature

In the re-isolation of virus X from inoculated plants of potato varieties considered to be immune to virus X, the time between mechanical inoculation and symptom expression in plants of *Datura tatula* (L.) Torr. was considerably longer than that normally required with this virus. Virus X in mechanically inoculated *D. tatula* ordinarily requires approximately 5 to 9 days for symptom expression depending upon environmental conditions in the greenhouse. When isolations were made from graft inoculated immune varieties such as S.41956, Saco, and Tawa (Benson and Hooker, 1958) the incubation period ranged from 10 to 24 days for full expression of virus symptoms. In all other characteristics, the virus isolated under these conditions was identical to the virus used for original inoculation of the resistant potato varieties. It seemed probable that virus X was present at a very low concentration in the tissues of such inoculated plants, and that the delay in symptom expression was a function of initial virus concentration.

Delay in symptom expression as a function of virus concentration has been described with a number of animal viruses which include: Shope papilloma virus in rabbits (Bryan and Beard, 1939); encephalomyetitis virus of mice

(Gard, 1940); meningopneumonitis virus of mice (Gogolah, 1953; Crocker, 1954); and the avian erythromyeloblastic leukosis virus (Eckert, Beard and Beard, 1954). The research herein reported was undertaken to determine if a similar relationship exists in plant viruses.

Materials and methods

A virulent strain of virus X, isolate X5, previously described by Timian et.al. (1955), was cultured on Nicotiana glutinosa L.

The virus was clarified by extraction from frozen leaves, grinding in a mortar and pestle, filtering through 4 layers of cheesecloth, and centrifuging at 3000 x G. for 15 minutes. The supernatant was heated to 55° C for 10 minutes, cooled quickly and again centrifuged as before. This process of freezing and centrifuging was repeated and the virus preparation was transferred to 1 ml. ampoules which were sealed hermetically and frozen until used.

Vigorously growing Datura tatula (L.) Torr. plants of similar age were inoculated with a series of dilutions of the described virus preparation. The inoculation procedure finally decided upon was as follows. One series of inoculations was made using a glass spatula in the conventional manner. Prior to rubbing the virus suspension on the D. tatula leaf surfaces, the leaves were uniformly dusted with 400 mesh carborundum, and carborundum was also added to the inoculum. Approximately equal volumes of virus

suspension were used on each leaf, as the spatula was dipped into inoculum only once before rubbing each one of the several leaves in an inoculation series. Each of 2 leaves of a single plant was supported with a paper towel and rubbed lightly by stroking 14 times with a glass spatula. There was practically no macroscopically visible mechanical injury with the inoculation method. The time required for expression of systemic symptoms in the uninoculated leaves was recorded for each plant after daily observations.

In a second series of tests (Table 5), leaves were covered with the original virus suspension diluted 10^2 uniformly dusted with 400 mesh carborundum, and gently rubbed with a glass rod which had been drawn out to a flexible tip, and a small ball formed, by melting the end of the glass tip. An area approximately 1 mm. in diameter was gently rubbed at each inoculation site. Three leaves of each test plant were inoculated. In series 1, one site per leaf was inoculated, in series 2, three sites per leaf were inoculated and in a third series, 15 sites per leaf were inoculated. Care was taken to inoculate the interveinal areas by avoiding as much as possible the larger veins of the leaves. This test approximated the situation obtained at the low levels of virus concentration in a dilution series by controlling the area of infection. Noninoculated controls were maintained, as well as controls in which undiluted virus was used with the conventional glass spatula inoculation procedure.

In other trials relative sensitivity of Gomphrena globosa L. as a local lesion assay plant for virus concentration was compared with D. tatula.

In a final confirmatory test on D. tatula at 17° C (Table 6), a highly purified stock preparation of isolate X 5 containing 6 mg. of virus X per ml. was used for inoculation. Two drops of diluted virus preparation were placed on 3 leaves of each D. tatula plant by means of a thin glass tube of the type commonly used for melting point determinations. Average weight of drops from such tubes was 25.5 mg. Virus X was therefore applied at rates of 9.18×10^{-3} mg., 9.18×10^{-4} mg., 9.18×10^{-5} mg., 9.18×10^{-6} mg. and 9.18×10^{-7} mg. for each plant in the test. A parallel test was prepared using half leaves of G. globosa to determine the sensitivity of that host to virus X as compared to D. tatula.

Experimental results

A total of 5 dilution tests were made with clarified virus using the conventional mechanical inoculation technique with a glass spatula and carborundum. Initial tests were designed to determine the most reliable inoculation technique. Results were inconsistent and variable when limited amounts of carborundum were sprayed on leaf surfaces and into inoculum.

The least variation in the experimental results (Table 1) were obtained with liberal amounts of carborundum dusted on the leaf surfaces and in the inoculum. The second treatment appeared to be the most reliable. Plants developed

Table 1. Comparison of 2 inoculation techniques, and time required for expression of systemic symptoms of virus X infection in Datura tatula following inoculation with virus at different concentrations.

Treatment	Virus dilution	Plants inoculated no.	Datura tatula plants with symptoms after 1/							
			days no. 5	days no. 6	days no. 7	days no. 8	days no. 9	days no. 10	days no. 11	days no. 12
1 2/	$1/10^1$	9	6	9						
	$1/10^2$	9	6	9						
	$1/10^3$	9	1	5	9					
	$1/10^4$	9	-	1	6	7	8			
	$1/10^5$	9	-	-	2	2	2			
	$1/10^6$	9	-	-	-	-	-			
2 2/	$1/10^1$	9	9							
	$1/10^2$	9	8	9						
	$1/10^3$	9	1	7	9					
	$1/10^4$	9	1	1	8	8	8	9		
	$1/10^5$	9	-	-	2	2	3	3	4	5
	$1/10^6$	9	-	-	1	1	1	1	1	2

1/ Greenhouse temperature approximately 28° C. Figures represent cumulative numbers of plants showing symptoms at each observation interval.

2/ Treatment 1. Carborundum dusted into inoculum and on leaves, inoculum rubbed 4 times and allowed to dry partially, leaves rubbed 10 times.
Treatment 2. Similar to treatment 1, but, leaves washed after rubbing 10 times.

symptoms at a virus concentration of $1/10^6$ whereas the symptoms were expressed at a virus concentration of $1/10^5$ in the first treatment. Results were otherwise essentially similar.

The dilution end point of the clarified virus preparation was determined in 2 experiments with limited numbers of plants (Table 2). In the first experiment, symptoms in non-inoculated leaves of D. tatula were not apparent until 11 days after inoculation. This was probably due to the fact that rather old plants were used for inoculation. The dilution end point of the virus preparation was $1/10^6$ as shown in the second test. Results of these tests were similar to the following more critical experiments.

In the fourth test (Table 3) ten plants were inoculated at each level of virus dilution. Average greenhouse temperatures were approximately 25° C during the month of August. Where a high concentration of virus X was used, systemic symptoms were expressed in non-inoculated leaves of all plants within 8 days. At lower concentration levels the expression of symptoms was progressively retarded. There was no infection at concentrations of $1/10^6$ and above.

A fifth trial was prepared with average greenhouse temperatures approximating 20° C (Table 4). The average period for symptom development was slightly longer than that obtained at 25° C. Once again there was an increase in the

Table 2. Dilution end point of clarified virus X as determined by 2 tests, and time required for expression of systemic symptoms of virus X infection in Datura tatula following inoculation with virus at different concentrations

Test[1]	Virus dilution and greenhouse temperature		Plants inoculated no.	Datura tatula plants with symptoms after[1]											
				days no. 6	days no. 7	days no. 8	days no. 9	days no. 10	days no. 11	days no. 12	days no. 13	days no. 14	days no. 15	days no. 16	day no 17
1	$1/10^2$	19°C	4	-	-	-	-	-	1	2	4				
	$1/10^3$	19°C	4	-	-	-	-	-	-	-	2	3	4		
	$1/10^4$	19°C	4	-	-	-	-	-	-	-	1	3	3	3	4
	$1/10^5$	19°C	4	-	-	-	-	-	-	-	-	2	3	3	4
2	$1/10^4$	20°C	9	1	5	6	7	8	9						
	$1/10^5$	20°C	9	-	3	3	4	5	5	6	7				
	$1/10^6$	20°C	9	-	-	1	1	1	1	2	2	3	4		
	$1/10^7$	20°C	9	-	-	-	-	-	-	-	-	-	-		
	$1/10^8$	20°C	9	-	-	-	-	-	-	-	-	-	-		

1/ Represents cumulative numbers of plants showing symptoms at each observation interval.

Table 3. Number of local lesions on Gomphrena globosa and time required for expression of symptoms of virus X infection in Datura tatula following inoculation with virus at different concentrations.

Virus dilution	Local lesions on G. globosa[1/]	Plants inoculated	Datura tatula plants with symptoms after[2/]								
			5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days
	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.
$1/10^2$	27.2	10	2	2	6	10					
$1/10^3$	0.5	10	-	-	1	6	9	10			
$1/10^4$	0.0	10	-	-	-	2	6	6	6	10	
$1/10^5$	0.0	10	-	-	-	-	1	3	4	5	7
$1/10^6$	0.0	10	-	-	-	-	-	-	-	-	-
$1/10^7$	0.0	10	-	-	-	-	-	-	-	-	-
$1/10^8$	0.0	10	-	-	-	-	-	-	-	-	-

1/ Local lesions on 12 leaves of G. globosa after 6 days at 25° C.

2/ Greenhouse temperature approximately 25° C. Figures represent cumulative numbers of plants showing symptoms at each observation interval.

Table 4. Time required for expression of systemic symptoms of virus X infection in Datura tatula following inoculation with virus at different concentrations.

Virus dilution	inoculated no.	Datura tatula plants with symptoms after[1/] 9 days no.	10 days no.	11 days no.	12 days no.	13 days no.	14 days no.	15 days no.	16 days no.	25 days no.
1/10	27	1	27							
$1/10^4$	27	-	-	4	14	24	27			
$1/10^5$	27	-	-	1	16	19	22	22	22	22
$1/10^6$	27	-	-	3	12	17	18	18	19	20

1/ Greenhouse temperatures approximately 20° C. Figures represent cumulative numbers of plants showing symptoms at each observation interval.

virus incubation period at low concentrations of virus X. Infection at dilutions of $1/10^6$ was obtained.

In the test (Table 5) using known concentrations of purified virus, symptoms were produced in plants to the final concentration of 9.18×10^{-7}, this being an actual dilution of $1/10^8$ of the original virus preparation, and being close to the reported dilution end point for virus X on Nicotiana tabacum L. (Bawden, 1943). Local lesions on G. globosa were produced only to the 9.18×10^{-5} concentration of the virus. This was in general agreement with earlier trials although dilution end point in G. globosa using the clarified virus preparation (Table 3) was $1/10^3$.

In the second series of inoculation trials small interveinal areas were rubbed with a small glass ball on the end of a flexible glass rod. The virus concentration in this instance was held constant at a dilution of $1/10^2$ of the original inoculum (Table 6). This concentration produced symptoms in the minimum 5 days period when rubbed uniformly over the leaf. In this trial, all plants ultimately developed symptoms even though leaf rubbing was confined to a very limited area on each leaf. The influence of temperature on the incubation period was apparent in each treatment. Where the complete leaf was inoculated, the difference was not well defined. At 23° C one plant expressed systemic symptoms in 5 days and the remainder at 7 days. Symptoms were expressed in all plants after 7 days at 18° C. In the other treatments

Table 5. Number of local lesions on Gomphrena globosa and time required for expression of symptoms of virus X infection in Datura tatula following inoculation with virus at different concentrations.

Virus concentration	Local lesions[2] on G. globosa no.	Plants inoculated no.	Datura tatula plants with symptoms after [1] 7 days no.	8 days no.	9 days no.	10 days no.	11 days no.	12 days no.	13 days no.	14 days no.	15 days no.	16 days no.	17 days no.	18 days no.	19 days no.	20 days no.	21 days no.	22 days no.
9.18×10^{-3} mg.	31.54	15	4	15														
9.18×10^{-4} mg.	5.0	35	-	7	18	26	34	34	35									
9.18×10^{-5} mg.	0.38	35	-	6	15	24	30	30	34	35								
9.18×10^{-6} mg.	0.0	35	-	-	-	4	9	10	15	22	26	27	29	31	35			
9.18×10^{-7} mg.	0.0	35	-	-	-	-	-	7	9	14	16	20	21	21	23	24	24	25

1/ Local lesions on 11 half leaves of G. globosa after 5 days at 22°C.

2/ Controlled greenhouse temperature 17°C. Figures represent cumulative numbers of plants showing symptoms at each observation interval.

Table 6. Time required for expression of systemic symptoms of virus X infection in *Datura tatula* at different levels of inoculation severity.

No. of places each of 3 leaves rubbed and greenhouse temperature		Plants inoculated no.	*Datura tatula* plants with symptoms after 1/															
			5 days no.	6 days no.	7 days no.	8 days no.	9 days no.	10 days no.	11 days no.	12 days no.	13 days no.	14 days no.	15 days no.	16 days no.	17 days no.	18 days no.	19 days no.	20 days no.
Entire leaf	23°C	6	1	1	6													
15	23°C	5	-	1	3	5												
3	23°C	8	-	-	1	5	5	8										
1	23°C	13	-	-	6	8	8	10	12	13								
Entire leaf	18°C	6	-	-	6													
15	18°C	5	-	-	2	3	4	5										
3	18°C	8	-	-	-	-	5	5	5	6	6	7	8					
1	18°C	13	-	-	-	-	3	6	7	7	8	8	10	12	12	12	12	13

1/ Figures represent cumulative numbers of plants showing symptoms at each observation interval.

the incubation period was increased substantially and became more apparent with decreasing numbers of inoculation sites per leaf. The pattern of symptom development followed closely the trends exhibited in the first two dilution trials (Table 3 and 4). The delay of symptom expression was evident with each decrease in inoculation severity.

Discussion

Although there have been plant virus research papers suggesting that length of incubation periods were influenced by virus concentration there are no clear cut discussions of the subject. Maromorsch (1950) demonstrated that the incubation period of the aster-yellows virus varies in its insect vector according to initial concentration. He also found (Maromorsch, 1953) that occasionally there was an abnormally long incubation period in the plant before development of symptoms when inoculation was made by an insect on its first day of transmitting the virus. After the first day of transmission the incubation period was reduced in length. Jensen (1951) in 10 minute thermal inactivation tests, increased the incubation period of the mosaic virus of Cymbidium orchids with increases in temperature. Latent periods ranged from 43 to 72 days. This suggested reduced virus concentration following heat treatment.

Apparently incubation periods of virus X in D. tatula are a function of initial concentration of the virus since

the average number of days for symptom development increased correspondingly with a reduction in virus concentration.

Although there was overlapping of incubation periods in individual plants between treatments, the average number of days for symptom development of virus X in D. tatula was greater with lower virus concentrations. This phenomenon may have been caused in part by variations in the physiological conditions of the test plants in spite of efforts to select plants of uniform type. It is also reasonable to assume that differences in numbers of infection courts may have varied from plant to plant in spite of efforts to provide uniform inoculation.

Possibly there is a threshold infection phenomenon giving rise to differences in incubation period. If the infection courts were relatively large in number the apparent incubation period might be in the normal 5 to 9 day range of symptom development. As the virus concentration decreased, little noticeable change in incubation periods might be observed until the number of infection courts were limited. Virus multiplication above this point apparently requires a certain minimum time for development of systemic symptoms. Below the threshold point, an abnormally long incubation period is necessary before virus X symptoms become apparent in D. tatula due to time required for spread from individual inoculation sites until the bulk of susceptible cells have been invaded. In this light it is interesting to speculate on the

possibility that plant viruses might display asynchrony of infection as well as some animal viruses (Cairns, 1957), and that latent periods become constant when all initially susceptible cells become infected (Prince, 1958).

The incubation period of virus X is increased at lower temperatures. This presumably is due to a slower rate of multiplication than at higher temperatures. The possibility exists that in this lower temperature range, the virus infects, spreads, and is synthesized at a slower rate than at higher temperatures.

G. globosa is a commonly used local lesion test plant for indicating the presence of virus X, and for determining virus concentration. In these tests, D. tatula was approximately 100 times more sensitive than G. globosa in detecting the presence of the virus.

Summary

Serial dilutions of clarified virus X and a highly purified strain of the virus, X 5, were mechanically inoculated to Datura tatula (L.) Torr. Test plants which were inoculated with high concentrations of the virus developed symptoms within the expected minimum time limits. As the virus was decreased, time required for symptom development increased correspondingly and incubation periods in some cases were as long as 25 days.

Delayed symptoms were obtained when leaf area and inoculum volume were the limiting factors. In this respect results were similar to those obtained in the virus dilution studies.

In 2 tests where known amounts of virus X were inoculated to the Datura leaf surfaces and to Gomphrena globosa, the latter host was considerably less sensitive to virus X. D. tatula was approximately 100 times as sensitive as G. globosa.

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Certain physiological and morphological reactions of potato seedling clone S. 41956 (*Solanum tuberosum* L.)

Introduction and review of literature

Raleigh (1936) described the immunity of potato seedling clone S. 41956. He demonstrated that aerial tubers were produced on a scion infected with virus X when grafted to a highly resistant stock. It was later shown (Timian et al, 1955) that in certain instances such grafted scions failed to produce aerial tubers. Webb and Schultz (1957) later found that aerial tubers developed quite rapidly and with more uniformity under short day periods (8 hours) than under long days (12 or 16 hours). Their results were not completely uniform as one plant in 16 failed to produce aerial tubers, enlarged nodes or axillary buds under the most favorable conditions.

Rootstock deterioration of immune plants grafted with virus X carrying scions has been well known since Raleigh (1936) first described the immune reaction of S. 41956. It was speculated that root necrosis and lack of development of underground portions of the stock was caused by nutritional deficiency of the grafted stock.

This study was undertaken to determine: (1) graft-scion relationships of virus X - immune plants with various stock and scion graft types; and (2) whether the cause of necrosis in underground portions was either associated with

roots, or more directly due to the necrotic action of virus x.

Materials and methods

Reactions of grafted plants.

Graft experiments were made to determine the reaction of S. 41956 when grafted with a number of virus X-infected and virus X-free scion types. Scions were approach grafted to S. 41956 and cut 14 days after grafting leaving a virus X susceptible scion on an immune stock. In other instances the S. 41956 top was left intact producing a side branch scion susceptible to virus X.

In the first experiment the variety Irish Cobbler was grafted as the scion to S. 41956. The section of the scion below the graft union, and the stock above the graft union were cut leaving a single Irish Cobbler scion on a highly resistant stock. One series of plants was placed in a warm chamber at 25° C, and a second series was left on the greenhouse bench at 18° C under normal greenhouse winter conditions.

A second trial was prepared in which grafted plants were placed in constant temperature tanks at temperatures of 16° C, 22° C, and 28° C. S. 41956, used as the stock in all cases, was approach grafted (1) to (healthy) virus X infected Irish Cobbler, (2) to Irish Cobbler doubly infected with viruses X and Y, and (3) to tomato plants of the Bonnie Best variety. Tomato scions were also grafted

to a virus Y infected S. 41956 stock. Tomato plants were either inoculated 7 days after grafting with a severe strain of virus X, X 5 (Timian et al, 1955), or left as uninoculated checks. Grafts were cut 14 days following grafting leaving half the plants with single tops and half the plants with bifurcated tops at each temperature range. Observations of plant reactions were recorded periodically.

A third graft trial was conducted to determine patterns of starch accumulation in grafted plants using various stock and scion types (Table 1). All grafted plants were reduced to single scion tops. Grafts were harvested 12, 24 and 45 days after virus X inoculation of scions free from virus X. Upon harvest, grafts were cut longitudinally through both the scion and stock, and the presence of starch determined by iodine-potassium iodide applied to the exposed plant tissue. Similar trials were prepared using Datura tatula (L.) Torr. and tomato (Lycopersicum esculentum L.) as the virus X-infected and X-free scions.

Relative carbohydrate deficiency in the presence of virus X.

To determine if root necrosis may have been caused by carbohydrate deficiency, roots of grafted plants were analyzed semi-quantitatively for relative deficiency of carbohydrate. Plants were grown in sand and watered twice a week with a production type nutrient solution. S. 41956 stocks were grafted with scions of potato S. 41956, potato seedling

Table 1. Starch accumulation in stocks and scions of grafted plants involving hypersensitive Epicure and immune S. 41956 1/

Stock	Scion type											
	Immune S. 41956		Virus X infected Cobbler		Virus X free Erlaine selfed		Virus X inoculated Erlaine selfed		Virus X infected Epicure		Virus X free Epicure	
	Test for starch in		Test for starch in		Test for starch in		Test for starch in		Test for starch in		Test for starch in	
	Stock	scion	stock	scion	stock	scion	stock	scion	stock	scion	stock	scion
Immune S. 41956	0	0	0	+	0	0	+	+	0	+	0	0
Virus X infected Cobbler	0	0	0	0	*	0	-	-	-	-	0	0
Virus X free Erlaine selfed	0	0	0	0	0	0	0	0	-	-	0	0
Virus X inoculated Erlaine selfed	0	0	-	-	*	0	-	-	0	0	*	0
Epicure	-	-	0	+	0	0	*	+	0	0	0	0

1/ 0 negative starch text, + positive starch test, * occasional starch test, and - graft type not included in experiment.

Erlaine selfed, L. esculentum and D. tatula. Stocks of the Epicure variety, resistant to virus X by virtue of hypersensitivity, were grafted with L. esculentum, and potato seedling clone Erlaine. Some plants of the varieties Epicure and S. 41956 were left ungrafted to serve as controls. Approach grafted plants were cut 14 days following grafting, leaving plants with both single and bifurcated tops. At the time grafts were cut, those plants intended for virus X inoculation were rubbed with a suspension of the X 5 strain of the virus. Grafted check plants were rubbed with a suspension of carborundum and water. Plants were arranged in pairs according to size and general vigor.

Roots were harvested for analysis 7, 14, and 21 days after virus inoculation. Samples of roots were washed thoroughly in water to eliminate sand and debris, and dried to constant weight in a 60° C oven. Dried samples were ground in a Wiley Mill over a 40 mesh screen. Aliquots of paired samples were weighed to within 0.3 mg. of one another.

Somogyi's test for reducing sugars (Somogyi, 1952) was used. Sugars were extracted from samples in 80% ethanol, 2 ml. being added per 10 mg. of dried sample. Samples were placed at 4° C for 36 hours and filtered. The residue was washed with 0.5 ml. of 80% ethanol for each 10 mg. of sample and the washing portion collected with the original sample. 2 ml. of the prepared samples were placed in 25 ml. volumetric

flasks, and 2 ml. of Somogyi's reagent (Somogyi, 1952) were added. Checks consisted of 2 ml. of 80% ethanol and 2 ml. Somogyi's solution. Flasks were placed in boiling water for 15 minutes, removed and allowed to cool. 2 ml. of Nelson's reagent (1944) were added to bring out a stable color. The final solution was diluted to 25 ml. with distilled water and read on a Klett-Summerson colorimeter. Readings of all solutions were converted to mg. equivalents of glucose which would produce the same color under the conditions of the reaction minus the check values.

To substantiate the validity of the preceding test, a semi-quantitative glucose determination was run on certain samples. Commercial Glucostat, a specific glucose oxidase (Keilen and Hartree, 1948; Huggett and Nixon, 1958) was used for the determination. Because of the possibility that the alcohol might interfere with the action of the enzyme, 3 ml. of each sample extracted in 80% ethanol were evaporated in a water bath at 65° C. Residues of each were resuspended in 1 ml. of distilled water, and the complete sample was used in glucose determinations. 2.5 ml. of the reagent was dispensed into a tube and water was added to make the final volume 5.0 ml. The 1 ml. sample was added to the enzyme preparation and after a reaction time of 10 minutes, 1 drop of 4 N HCl was added to stop the reaction. The resultant colored solutions were read in a Klett-Summerson colorimeter. In all cases, glucose standards were run with each set of determinations.

Experimental results

Reactions of grafted plants.

In the first experiment, Irish Cobbler plants, which are known to carry virus X in a latent condition, were approach grafted to S. 41956. Grafts were cut leaving an Irish Cobbler scion on an immune stock and paired plants were placed at two temperatures, 18° C and 25° C.

S. 41956 stocks following grafting reacted generally in the same manner at both temperatures. Of 32 plants with single tops, 10 stocks became completely necrotic 18 to 32 days after grafting, and died before completion of the test. Aerial tubers were produced on the remaining 22 plants with Irish Cobbler scions and growth continued to a limited extent. After 75 days, two differences were apparent (Fig. 1a). In half of the remaining 22 plants, the scions at first wilted and later became completely necrotic. Necrosis proceeded down the stock 1 or 2 internodes below the graft union. At this point, apical buds proliferated and the stock began to grow vigorously. The balance of the plants in the test which did not become necrotic developed scions which were small and weak, with large aerial tubers in all cases. In these plants, the apical bud which was present above the graft union proliferated greatly. The plant then grew in a normal manner. Roots of all plants which were harvested at the last observation were only slightly, if at all, necrotic. Thus approximately

FIGURE 1

A. Scion-stock reaction in plants when virus X-carrying scions were grafted to immune stocks.

Upper left exhibits complete necrosis of scion and stock.

Upper center shows necrosis of the scion, and necrosis of the stock 2 internodes below the graft. An axillary bud has produced a normal plant.

Upper right shows a bifurcated graft in which scion and stock remain vigorous. A small aerial tuber may be seen at the graft.

B. Starch accumulation in potato scions grafted to S. 41956 stocks.

From left to right:

1. Virus X free scion on S. 41956.
2. Virus X-carrying Irish Cobbler scion on immune S. 41956 26 days after grafting.
3. Immune S. 41956 scion on an Erlaine selfed 12 days after the stock had been inoculated with virus X.
4. Erlaine selfed scion on S. 41956, 12 days after inoculation of the scion with virus X.

one third of the plants in the test became rapidly necrotic and died, another third became necrotic below the graft but the necrosis did not become general while a third of the group developed aerial tubers.

With the possibility that viruses X and Y could react synergistically, and break down the immunity of S. 41956, a second experiment was carried out at 16° C, 22° C and 28° C soil temperatures. Plant reactions were again independent of temperature. Variations in graft reactions from the above trend differed in only 2 respects. When S. 41956 was grafted with virus Y infected Irish Cobbler 16 of 24 plants developed complete necrosis. It is probable, if the remaining 8 plants had been left for a longer period, they also would have become necrotic. It is believed that this reaction was due in a large measure to the necrotic influence of virus Y. A similar situation developed when tomato scions were grafted to a virus Y carrying S. 14956 stock and later infected with virus X, strain X 5. Stocks of S. 41956 became almost completely necrotic before the tomato scions began to wilt and die. Virus X inoculated tomato scions on a healthy S. 41956 stock occasionally displayed the same phenomenon with slightly less rapidity.

Virus X was not isolated from any portions of the above grafted plants.

Grafted plants in which only virus X was involved were cut leaving bifurcated tops. Plants of this type

continued to grow vigorously throughout the experiment. When virus X only was involved in the grafted plants reactions were usually similar to the previously described experiment.

In previous trials it was determined that virus X-infected scions grafted to an immune stock developed a strong positive test for starch immediately above the graft union and several cm. above when treated with an iodine solution. Plants were grafted in a number of combinations to determine the starch reaction when virus X was, or was not present in the graft tissues.

A strong positive test for starch was apparent 12 days (Fig. 1B) following virus X inoculations of virus X-free scions, and 76 days after grafting with virus X-infected scions. Positive tests for starch have been observed as early as 10 days following virus X inoculation. Accumulation of starch was not apparent in scions where virus X was absent from grafted plants (Table 1). There was similarity in starch accumulation patterns in the hypersensitive (Epicure) and immune (S. 41956) types. When virus X-infected Irish Cobbler scions were grafted to either immune, or hypersensitive stocks, starch accumulated in the scions. The most striking similarity, however, was when virus X-free scions were grafted to both immune (S. 41956) and hypersensitive (Epicure) types and later inoculated with the virus. S. 41956 stocks reacted

consistently with iodine to give a positive test for starch 3 cm. or more below the graft. Epicure stocks reacted occasionally in the same manner. Apparently, delay in inoculation of virus X to the susceptible scions, shifted the site of starch accumulation from the scion to an area somewhere in the stock. When either D. tatula or tomato was used as the scion and later inoculated with virus X, the same pattern of starch accumulation in stocks of S. 41956 below and above the graft was apparent. Starch accumulated occasionally when virus free scions were grafted to virus X-carrying, or virus X inoculated stocks.

A phenomenon hitherto unreported was also noted in this graft test. When scions either carrying virus X or scions free of the virus and later inoculated were grafted to Epicure stocks, either enlarged nodes or enlarged axillary buds suggesting very small aerial tubers were sometimes present on the scions. All aerial tubers reacted strongly with iodine indicating the presence of starch.

Relative carbohydrate deficiency in the presence of virus X.

This phase of the study was undertaken to obtain some information concerning the cause of necrotic roots on immune stocks which had been grafted with virus X free scions and later inoculated with the virus after the graft had become established. Semi-quantitative estimations were made

of reducing carbohydrates in roots of such grafted plants using Somogyi's test (1952) for reducing sugars.

For the purpose of this discussion, the phrase relative carbohydrate deficiency in the presence of virus X, has been used to indicate relative amounts of carbohydrates present in paired grafted plants. The scion of one of the paired plants was free of virus X, while the scion of the other plant was inoculated with virus X after graft establishment. Since carbohydrate levels were usually low when virus X was present in the scion, the term relative carbohydrate deficiency is used to denote the difference.

No relative carbohydrate deficiency in the presence of virus X was observed in roots of approach grafted S. 41956 7 days after inoculation with the virus (Table 2). At this time there was some relative carbohydrate deficiency in bifurcated plants. Relative carbohydrate deficiencies were apparent 14 days after inoculation. When tomato and D. tatula were used as single scions, relative carbohydrate deficiency in the presence of virus X was more apparent than when Erlaine selfed potato clones were used as scions. Relative carbohydrate deficiency with virus X, in roots of plants with bifurcated tops, was essentially similar at the 14 day reading as at the 7 day reading.

When roots were harvested 21 days after virus X inoculations, relative carbohydrate deficiency was not as

Table 2. Carbohydrate reserves in roots of potato seedling clone S. 41956 grafted to scions either free of Virus X or inoculated with the virus 1/

Number of days after inoculation with virus X	Type of graft	Type of scion: Erlaine selfed X-free mg. equiv.	Erlaine selfed X-infected mg. equiv.	Erlaine selfed Difference %	Datura tatula X-free mg. equiv.	Datura tatula X-infected mg. equiv.	Datura tatula Difference %	Tomato X-free mg. equiv.	Tomato X-infected mg. equiv.	Tomato Difference %	S. 41956 grafted mg. equiv.	S. 41956 ungrafted mg. equiv.
7	approach	1.92	1.78	92.7	1.30	1.35	103.9	0.90	0.90	100.0	1.51	3.51
7	approach	1.50	1.53	102.0	-	-		0.89	0.95	106.7	1.56	3.60
7	bifurcated	3.43	3.98	116.0	2.82	2.47	87.6	5.66	3.83	67.7	-	-
14	approach	0.67	0.45	67.2	1.12	0.52	46.4	3.43	0.91	26.5	0.56	1.28
14	approach	2.10	1.70	81.0	1.45	0.75	51.7	1.98	0.76	38.4	2.44	5.12
14	bifurcated	2.15	2.12	98.0	2.74	2.31	84.3	3.00	2.07	68.1	-	-
21	approach	1.24	1.02	82.3	2.01	1.64	81.6	2.12	1.62	76.4	-	-
21	approach	1.22	1.25	102.5	2.09	1.68	80.4	2.18	1.66	76.2	-	-
21	bifurcated	3.03	2.55	84.2	4.83	2.73	56.5	6.24	2.68	43.0	-	-

1/ Reduction carbohydrates measured in mg. equivalents of glucose.

great in roots of plants with single tops as in roots of similar plants harvested a week earlier. Relative carbohydrate deficiencies in plants with bifurcated tops was greater at the 21 day period than at either of the earlier analyses.

Relative carbohydrate deficiencies with virus X in roots of S. 41956 stocks grafted with potato scions were not as great from week to week as were the deficiencies in any comparable treatment with tomato or with D. tatula scions.

Trends were similar when the hypersensitive variety Epicure was used as the stock (Table 3). Relative carbohydrate deficiencies with virus X were greater in roots of Epicure than in roots of S. 41956 on the same harvest dates (Table 2). Furthermore, relative carbohydrate deficiency remained relatively constant 7 to 21 days after inoculation with virus X (Table 3).

The relative carbohydrate deficiency with virus X in roots of bifurcated Epicure plants remained fairly constant throughout the test. In this respect the reaction was somewhat different from that of S. 41956 in the 21 day analysis.

Glucose analysis of certain samples from Tables 2 and 3 were made using a colorometric test with commercial glucose oxidase, Glucostat (Tables 4 and 5). Analyses were limited because of insufficient samples for analysis from

Table 3. Carbohydrate reserves in roots of the variety Epicure grafted to scions either free of virus X or inoculated with the virus 1/

Roots harvested after virus X inoculation days	Type of graft	Type of scion: Erlaine selfed X-free mg. equiv.	Erlaine selfed X-infected mg. equiv.	Erlaine selfed Difference %	Tomato X-free mg. equiv.	Tomato X-infected mg. equiv.	Tomato Difference %	Epicure X-free mg. equiv.	Epicure X-infected mg. equiv.	Epicure Difference %	Ungrafted mg. equiv.
7	approach	4.83	1.16	24.0	2.14	0.64	29.9	2.04	1.72	84.3	4.73
7	bifurcated	6.93	4.71	68.0				5.83	5.21	89.4	
14	approach	6.19	1.44	23.3	2.83	1.01	35.7	2.68	2.08	77.6	5.12
14	bifurcated	8.24	6.87	83.4				6.15	5.13	83.4	
21	approach	5.43	1.01	18.6	5.14	1.27	24.7	1.94	1.63	84.0	5.45
21	bifurcated	9.85	6.89	70.0				3.58	2.91	81.3	

1-/ Reduction carbohydrates in mg. equivalents of glucose.

Table 4. Glucose reserves in roots of S. 41956 grafted to scions either free of virus X or inoculated with the virus 1/

Number of days after inoculation with virus X	Type of graft on S. 41956	Tomato			S. 41956		
		X-free mg. equiv.	X-infected mg. equiv.	Difference %	grafted mg. equiv.	ungrafted mg. equiv.	Difference %
7	approach	N 2/	N	-	0.13	0.34	261.5
7	bifurcated	0.70	0.40	57.1	-	-	
14	approach	0.32	N	-	0.15	0.66	440.0
14	bifurcated	0.33	0.13	39.4	-	-	
21	approach	0.14	0.07	50.0	-	-	
21	Bifurcated	0.93	0.23	24.7	--	-	

1/ Glucose measured in mg. equivalents in 3 ml. of extracted samples.

2/ No measurable glucose in the sample.

Table 5. Glucose reserves in roots of Epicure grafted to scions either free of virus X or inoculated with the virus 1/

Number of days after inoculation with virus X	Type of graft on Epicure	Erlaine selfed			Epicure		
		X-free mg. equiv.	X-infected mg. equiv.	Difference %	grafted mg. equiv.	ungrafted mg. equiv.	Difference %.
7	approach	0.63	N 2/	-	0.14	0.65	464.3
14	approach	0.97	N	-	0.18	0.75	416.7
21	approach	0.73	0.08	4.1	0.19	0.75	394.7

1/ Glucose measured in mg. equivalents in 3 ml. of extracted samples.

2/ No measurable glucose in the sample.

the previous determinations. Virtually the same trends were observed as in the previous determinations. Relative glucose deficiencies with virus X were of greater magnitude than the relative carbohydrate deficiency reported in the previous experiment. Furthermore relative glucose deficiencies were apparent in roots of S. 41956 7 days after scion inoculation with virus X, whereas relative carbohydrate deficiencies at that harvest date in the previous experiment were not apparent.

In certain other trials prepared for root analysis, stocks of grafted plants were wrapped with aluminum foil after the graft had become established and the scion had been inoculated with virus X. This was done to preclude the possibility of photosynthesis occurring in the stocks and subsequent translocation to the root. In every case where the stocks were wrapped, the plants wilted and died prematurely and root analyses could not be made. Inoculated and uninoculated plants without covered stocks remained vigorous throughout the experiment.

Discussion

Reactions of grafted plants.

When virus X carrying scions were grafted to stocks of S. 41956, the stock usually became necrotic. Necrosis sometimes extended 1 or 2 internodes below the graft into tissues of the immune stock. It is possible that necrosis resulted from movement of virus X into the immune tissues.

In some respects this resembled a hypersensitive type of reaction. Axillary buds commonly developed on the immune stock producing a lateral branch and necrosis failed to develop in the stock.

Epicure (hypersensitive) and S. 41956 (immune) stocks reacted with some similarity in their patterns of starch accumulation when virus X was involved in the graft. Furthermore, suggestions of aerial tuber formation were observed on virus X carrying scions grafted to Epicure stocks. Starch accumulation in both types was possibly due to incompatibility of the graft union conditioned by the presence of virus X. Grafts between potato varieties when virus X was absent were within the limits generally accepted for compatibility.

Differences in locations in which starch accumulated were obtained when the virus was either introduced at the time of grafting, or inoculated after the graft had become established. When scions free of virus X were inoculated with the virus after the graft had become established, a positive test for starch was obtained up to 3 cm. below the graft union in the S. 41956 stocks. When immune stocks were grafted with Irish Cobbler scions already carrying virus X, starch accumulated in the scions above the graft union and did not accumulate in the S. 41956 stocks. A sharp distinction between stock and scion was observed when the starch-iodine test was applied to the graft.

Possibly the virus was able to move into the tissues of the immune stock when virus X inoculation of the susceptible scions was delayed until after the graft had become established. If a virus X carrying scion was grafted to an immune stock, the presence of the virus may have precluded establishment of a compatible graft.

Relative carbohydrate deficiency in the presence of virus X.

It seems probable that necrosis of roots (Raleigh, 1936) of immune stocks when grafted with virus X carrying scions, is associated with a depletion of nutritional substances in the roots. Relative carbohydrate deficiencies with virus X in roots of grafted plants was greatest 2 weeks after inoculation of the scion with the virus. There was a decrease in degree of carbohydrate deficiency in roots of grafted plants 21 days following inoculation.

Potato varieties considered to be immune to virus X are possibly hypersensitive to that virus. The evidence is summarized as follows: (1) Virus X was isolated from plant parts and necrotic tubers of "immune" varieties. (2) Aerial tuber formation and starch accumulation in both hypersensitive Epicure and "immune" S. 41956 suggests a similarity between the 2 types of resistance. (3) Portions of "immune" stocks grafted with virus X infected scions became necrotic suggesting that tissues were killed by the virus. (4) Tubers of both hypersensitive and "immune"

stocks became necrotic under certain conditions when grafted with virus X carrying scions. (5) Necrotic spots were developed on leaves of "immune" seedlings when grafted with a scion infected with virus X. (Anonymous, 1952)

Summary

USDA potato seedling clone S. 41956 was grafted with virus X carrying scions and scions free of the virus, but later inoculated. In general, 3 distinct plant reactions followed.

If both scion and/or stock became necrotic early the plant was killed. When scions and stocks remained vigorous for a relatively long period of time, scions later became necrotic, the stock was killed for a short distance below the graft union, and apical buds below the necrotic area developed lateral branches. A few plants which survived remained small and weak, and developed large aerial tubers on the scions. Plants with bifurcated scions, for the most part, remained healthy and vigorous until normal senesence.

When grafts of plants, similar to those mentioned above were cut longitudinally and tested for the presence of starch, a positive reaction was obtained only when virus X was present in the grafts. Virus X carrying scions on hypersensitive stocks reacted in a similar manner, with starch accumulation above the graft. Small aerial tubers were also present on the virus X carrying scions grafted

to hypersensitive stocks of the variety Epicure.

When virus X inoculation was delayed until after the susceptible scion had become established on an immune stock, starch accumulated up to 3 cm. below the graft union.

Tests for reducing carbohydrates and glucose were made on roots of S. 41956, and the variety Epicure grafted with virus X free, and virus X inoculated scions.

Varieties of potato immune to virus X may in reality possess an extreme degree of hypersensitivity to that virus.

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