FACTORS INFLUENCING SYMPTOM EXPRESSION AND MULTIPLICATION OF POTATO VIRUS X IN A POTATO VARIETY HYPERSENSITIVE TO THE VIRUS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Heng-ji Su
1963

This is to certify that the

thesis entitled
FACTORS INFLUENCING SYMPTOM EXPRESSION AND MULTIPLICATION OF POTATO VIRUS X IN A POTATO VARIETY HYPERSENSITIVE TO THE VIRUS

presented by

Hong-ji Su

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Botany and Plant Pathology

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Date 8/6/63

FACTORS INFLUENCING SYMPTOM EXPRESSION AND MULTIPLICATION OF POTATO VIRUS X IN A POTATO VARIETY HYPERSENSITIVE TO THE VIRUS

by

Hong-ji Su

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
the Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major subject: Plant Pathology

Approved:

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Michigan State University
1963

ABSTRACT

FACTORS INFLUENCING SYMPTOM EXPRESSION AND MULTIPLICATION OF POTATO VIRUS X IN A POTATO VARIETY HYPERSENSITIVE TO THE VIRUS

by Hong-ji Su

This is a study of the environmental and physiological factors influencing pathogenesis and multiplication of potato virus (PVX) in a hypersensitive potato variety, Epicure.

The optimum post-inoculation temperature for disease development was 16°-20°C, with considerable reduction in severity of systemic symptoms at 24°C. At 28°C local symptoms developed very slowly and systemic symptoms were precluded. Disease severity of plants grown in continuous light at 16°-24°C was generally higher than that of plants in 8 hour day. At 28°C, disease severity was slightly greater in 8 hour day.

Pre-inoculation environment of 2 weeks at 28°C enhanced susceptibility of plants grown at post-inoculation temperatures ranging from 16° to 28°C. A relatively short (2 days) pre-inoculation treatment at 36°C was not effective in increasing susceptibility. Susceptibility was decreased in inoculated leaves treated within 30 minutes before or after inoculation by a 60 second immersion in 50 C water.

Symptom severity and virus multiplication in inoculated leaves were enhanced by growing plants in short photoperiods especially at 20° or 28°C, and were retarded by

pre-inoculation incubation in continuous light especially at 16°C for 2 weeks.

Symptom development and virus multiplication were very rapid in old leaves. Infectivity of PVX in stem epidermal tissue was higher in comparison with that in leaves and maintained high levels of infectivity over a relatively long period.

Virus infectivity in inoculated leaves was positively correlated to symptom severity. Two types of virus infectivity curves were obtained, i.e., type A was an upward curve flattened on top without or with a small downward drop of infectivity, and type B curve was characterized by a rapid rise and prompt decline in infectivity.

Type A curves were usually obtained in inoculated leaves of plants kept at 16°C, or in young leaves, or stem epidermal tissues. Type B curves were obtained in plants kept at 20°C, in long days after inoculation, in leaves predisposed at high temperature (28°C), or predisposed by short days before inoculation, and in old leaves.

The X_8 isolate of the virus caused slightly more rapid disease development in Epicure plants in comparison with X_5 isolate, although X_8 isolate was a mild isolate on some other potato varieties susceptible to PVX.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1963

ACKNOWLEDGMENT

The writer wishes to express his sincere gratitude to Dr. W. J. Hooker for his kind and careful direction and encouragement during the course of this investigation and his aid in preparation of this manuscript.

Thanks are also due to Dr. D. J. deZeeuw, Dr. H. H. Murakishi, Dr. N. R. Thompson, and Dr. E. H. Barnes for their kind guidance throughout the program.

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I. INTRODUCTION AND LITERATURE REVIEW

Degrees of resistance to potato virus X differ widely among the different potato varieties. The literature on the subject has been reviewed (Hooker et al. 1954). Three rather well-defined types of resistance have been identified as susceptible or tolerant, hypersensitive or field immune, and immune. Susceptibility or tolerance is characterized by the plants being infected as more or less symptomless carriers, and yet appearing to be healthy. Most commercial varieties presently grown in the United States are tolerant to PVX. These include Cobbler, Sebago, Katahdin, Green Mountain, and Triumph.

Potato varieties of hypersensitive type respond to inoculation by necrosis of the inoculated leaves and usually infected plants develop top necrosis. In this type, resistance is associated with extreme susceptibility or hypersensitivity to the virus. This reaction is manifest either as localized, necrotic lesions on inoculated leaves in which case the virus may not become systemic, or as systemic top necrosis following inoculation either through leaves or with X-infected scions. Only occasionally are diseased plants observed among the plants of this type in the field. Certain varieties necrotic to PVX are Epicure, Arran Crest, King Edward, Edgecote Purple, Ninetyfold, and Southesk.

The virus fails to multiply and survive in potato plants of immune type. Immune types in this country have been derived from S. 41956 which has not yet been typically infected with any isolate of PVX. Following graft inoculation of S. 41956, PVX has been obtained apparently in very low concentration from stems, roots (Benson and Hooker, 1960) and leaves (Bagnall, 1961). Certain collections of an uncultivated potato species, Solanum acaule Blossfeld, La Pena blanca, and Bukasor, contain individuals immune to PVX.

Little work has been done on the nature of the hypersensitivity or localized reaction of potato plant to PVX. The top necrosis of potato due to viruses was first described by Quanjer and Botjes (1929). Bawden (1936) made extensive studies on top necrosis (acronecrosis) of potato plants caused by viruses and stated that PVX and some other viruses induced top necrosis in the Epicure variety of potato. Cadman (1942) showed that hypersensitivity for PVX in potato varieties was inherited as a single dominant gene (N_x). Cocherham (1943b) investigated the reaction of potato varieties to virus X, A, B, and C and considered hypersensitivity as "field immunity" because under field conditions of infection with virus X, potato varieties and seedlings with a localized reaction remained free of this virus. He (1943a) also reported that the hypersensitive reaction of potato plants to strains of PVX, designated as either X^x or X^b was governed by a single pair of dominant genes (N_x and N_b), and emphasized the

particular value of variety stocks responding to PVX with top necrosis in seeking control of PVX. More recently, Bagnall (1961) described the specific genetic nature of the hypersensitive type of resistance to viruses A and X in Canadian and American potato varieties. His data agree with those of Cocherham that the reaction of varieties to virus A and the 2 groups of PVX isolates (X^x and X^b) was governed in each case by single allelic pairs of genes (N_a, N_x, N_b).

Hutton and Wark (1952) discussed the inheritance of resistance to PVX by the pattern of PVX development in inoculated leaves of immune, localized reaction, and susceptible phenotypes in potato. They indicated that the phenotypic reaction of Epicure to PVX, giving a localized reaction, could not be regarded as hypersensitive, the term resistance should take its place. They further proposed that a common virus-inactivation system determined resistance of both the hypersensitive and the immune types. Köhler (1958) studied the slope of the inactivation curve of PVX in inoculated potato leaves of tolerant, hypersensitive, and immune types. He used the data of Hutton and Wark (1952) for the interpretation of the hypersensitive response. He believed that a common virus-inactivating system determined the immunity of S. 41956 and the localized resistance of certain other potato varieties, such as Epicure.

The importance of air temperature in relation to development of symptoms of PVX in potato was first recognized

by Johnson (1922). He found optimum temperature for the disease development to be between 14° and 18°C. The response was further investigated by Tompkins (1926) and by Timian et al. (1955). Pound and Helms (1955) showed that the variation in symptom expression of PVX in Nicotiana species at different temperatures was correlated with changes in virus concentration. Multiplication of virus X was influenced by host varieties as well as by temperature and season variation.

The extent of the stimulatory effect PVY infection on PVX multiplication varied with temperature (Stouffer and Ross, 1961a). Concentration in both singly (PVX) and doubly infected leaves (PVX and PVY) was higher at 19° than at 28°, and higher at 28° than at 32°C. Ford and Ross (1962a) obtained 4.7 to 6.6 times as much infective PVX at 30°C, and 2.2 to 3.4 times at 20°C as much infective PVX in doubly (PVX and PVY) infected leaves of Samsun NN tobacco as in the leaves infected with PVX alone. Other factors also influenced the concentration of PVX in doubly infected plants (Stouffer and Ross, 1961b).

Ford and Ross (1962b) found that age of tobacco leaf had no marked effect on the level of PVX concentration. In leaves inoculated with both PVX and PVY, however, PVX concentration was higher in leaves 5 cm wide than in those that were either 2 or 8 cm wide.

Mature plant resistance to PVX was demonstrated in certain potato varieties such as Flava and Copella by Berks (1951), and Bintje and Voran by Beemster (1957). It is possible that additional other physiological conditions influence the PVX-Epicure interaction.

Except for the work of Hutton and Wark (1952), the course of infection and subsequent localization of the virus and death in hypersensitive potato varieties has received little attention. It becomes important to determine the degree of compatibility with the host-virus interaction under different environmental conditions before a common virus inactivating system as proposed by Hutton and Wark (1952) can be accepted. Information concerning the reaction of tolerant potato plants to PVX under different environmental conditions is available (Hooker and Kim, unpublished data). Numerous attempts to infect immune S. 41956 and to recover PVX from inoculated plants have failed to demonstrate virus multiplication within the tissues (Köhler, 1958, Benson and Hooker, 1960, Bagnall, 1961).

Köhler (1958) presented 3 different virus multiplication curves in potato plants of the 3 resistance types, i.e. the virus in immune potato plants was unable to multiply and was inactivated very quickly; in susceptible varieties the virus multiplied rapidly and virus titre was maintained at a relatively high level over the period of observation, while in hypersensitive varieties, the virus multiplied but declined rapidly.

In preliminary trials with the Epicure variety, virus infectivity curves were obtained which were quite distinct from those of Hutton and Wark (1952). Because of this discrepancy the present investigation was made in order to clarify the factors influencing pathogensis and multiplication of the virus in plants with the hypersensitive type of resistance.

II. MATERIALS AND METHODS

The potato variety, Epicure, hypersensitive to the virus X^x types of PVX (Cockerham, 1943), but tolerant to isolates of the X^b group, was used as a test plant in these trials. Potato plants from tuber units were generally grown in a green house at 22°C, although the green house temperature in summer could not be controlled. Plants were inoculated on the lower 3-5 fully expanded leaves when the potato plants were about 25 cm in height, 3-4 weeks old. Plants were grown at pre-inoculation temperatures in predisposition studies 10-14 days before inoculation.

Stock isolates of PVX, X_5 and X_8 (Timian et al. 1955a), tested for freedom of X^b by grafting to Katahdin, were passed serially through the local lesion host Gomphrena globosa L., and used as inocula. Stock cultures of the virus X isolates were maintained in plants of Datura tatula L. Both isolates were equally virulent on the Epicure variety of potato but on D. tatula isolate X_5 produced severe symptoms of necrosis and mottle while isolate X_8 was symptomless. For the inoculum, systemically infected leaves of D. tatula were ground and the juice diluted with water was used.

Potato leaves were dusted with 400-mesh carborundum and inoculated with juice using a glass spatula. Inoculated leaves were thoroughly rinsed with water following inoculation.

The inoculated plants were kept in separate houses thermostatically controlled at 17°, 22°, and 28°C, under normal winter illumination of 3,000 to 5,000 F.C. in bright

days. Plants were also grown in thermostatically controlled temperature boxes set at 16°, 20°, 24°, and 28°C, with continuous fluorescent artificial light totalling approximately 800 F.C. Short photoperiods (8 hour day) were accomplished by placing a metal box over the plants. Temperatures inside the boxes were the same as those of the incubator.

Disease records on the test plants kept under various conditions were determined at 2 day intervals and the disease index was calculated by the following method:

local symptoms

1 necrotic spots on inoculated leaves

2 severe necrotic spots or/and vein necrosis

3 leaf yellowing

4 death of inoculated leaves

systemic symptoms

5 necrotic spots on top young leaves

6 moderate top necrosis

7 severe top necrosis

8 death of top

Later separate disease indices were prepared, one for local and one for systemic symptoms.

For determining relative virus infectivity in the inoculated leaves at each harvest date, leaf discs were removed from each inoculated leaf with a 4 mm cork borer.

The collected leaf discs were placed in small vials containing 0.3 ml of phosphate buffer solution (0.1 M KH_2PO_4 adjusted to pH 7 with 0.1 M NaOH) and stored in a deep freeze. Sample weights were obtained by weighing these vials before and after addition of leaf samples.

For assay, samples were then ground in a mortar and diluted 1:5 with 0.1 M pH 7.0 phosphate buffer solution and the homogenate was refrozen. This was thawed, and clarified by: 1) centrifuging at 12,800 g for 15 minutes; 2) heating the supernatant at 55°C in a water bath for 10 minutes; and 3) centrifuging as above and saving the supernatant. These samples were stored in the refrigerator and tested on G. globosa later the same day. One sample was used at the 1-5 dilution and a second sample tested after 1-50 dilution by the 2-dilution method of Spencer and Price (1943).

The uppermost pair of fully expanded leaves of Gomphrena globosa L. plants grown under uniform conditions and selected for uniformity were used for assaying virus infectivity. Thus on a given harvest date, all samples were compared on a 2 dilution basis using the randomization plan of Youden for half leaves (1937). Local lesions were counted 5 days after inoculation. Relative infectivity in discs of inoculated leaves collected at several intervals

of time were expressed by plotting logarithms (n+1) of average lesion number (n) per half leaf, against time after inoculation.

III. EXPERIMENTAL RESULTS

Environmental Factors

1. Influence of Post-inoculation Environment on Symptom Expression and Infective Virus Content

Epicure plants inoculated in one test with X_5 and in another test with X_8 were maintained at different temperatures 16°, 20°, 24°, and 28°C, and grown under short day (8 hours light and 16 hours dark) or in continuous light. Six potted plants were used in each treatment, thus 48 plants were used in each test. Plants were observed periodically and the disease index was calculated as previously described.

Temperature had a greater influence on symptom expression than did differences in length of exposure to light (Fig. 1, A-D). Most rapid disease development occurred in the plants kept at 20°C and symptoms appeared a little earlier than in plants kept at the other temperatures. Plants at 16° showed almost as severe symptoms as plants at 20°C. At 24°C, there was considerable reduction in severity of systemic symptoms while local symptoms developed somewhat slower than on plants at 16°C. At 28°C, no systemic symptoms developed and disease developed in severity on inoculated leaves slower than at lower temperatures.

Disease indices of plants grown at 16°C, under continuous light were higher than those of the plants under short photoperiods. These differences were similar but less

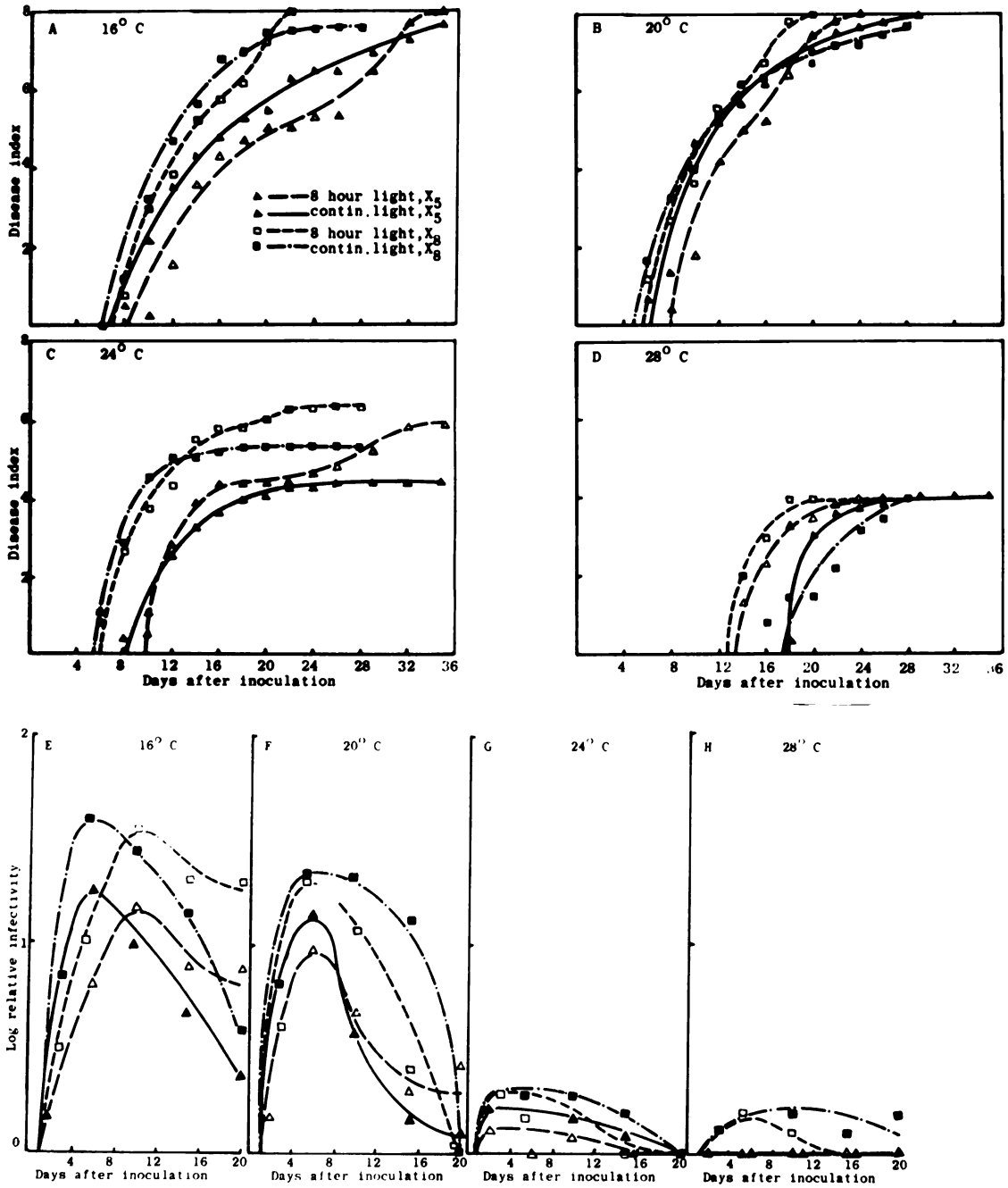


Fig. 1. A-D, Disease indices of inoculated Epicure plants grown in an 8 hour day and in continuous light at 4 different temperatures. E-H, Relative infectivity on half leaves of G. globosa in extracts from inoculated leaves of Epicure plants grown under various photoperiods and temperatures.

pronounced at 20°C. At 24°C, early disease readings were slightly more severe in continuous light than in the 8 hours day but after approximately 12 days, disease was more severe in the plants grown in 8 hour days. At 28°C, higher disease indices were obtained in the 8 hour days than in continuous light.

Generally, disease development on the plants inoculated with X_8 isolate of PVX was slightly more rapid than that of plants inoculated with X_5 isolate. Differences between these two isolates were greatest especially at temperatures not optimum for symptom development. At the optimum temperature for symptom expression, disease severity with two virus isolates were essentially similar (Fig. 1, A-D).

Increase in virus infectivity in inoculated leaves of potato plants grown under different temperatures and photo-periods was determined quantitatively as previously described. Six potted plants were used in each treatment and so 48 plants were used in each test. The leaf discs were collected periodically. On each harvest date about 200 leaf discs were removed from the plants in each treatment group. Relative infectivity was expressed in logarithms (n+1) of the average number of local lesions (n) on 7 half-leaves of G. globosa (Fig. 1, E-H). Infectivity was determined by the 2-dilution method (Spencer and Price, 1943) at 1-5 and 1-50 dilutions. Since graphs were essentially similar at either dilution but infectivity was low at the 1-50 dilution, only data derived from 1-5 dilution are presented.

There was no evidence that at the 1-5 dilution, infectivity had exceeded the level of sensitivity of the indicator plant leaves.

No infectivity was obtained from leaves one day after inoculation, but in a few instances infective virus was recovered two days after inoculation.

Essentially similar high levels of virus infectivity developed in inoculated leaves of plants at 16°, and 20°C, and the initial rise of the curves were quite similar at both temperatures. Low levels of infectivity were obtained in inoculated leaves at 24°, and 28°C. Infectivity at 24°C reached a low peak at about the same time, 4-10 days, as that at 16° or 20°C. At 28°C, X_5 failed to become infective and infectivity of X_8 slowly declined from a low level of infectivity.

After the peak had been reached, infectivity dropped rapidly at 20°C under both continuous light and short day, although the loss of infectivity with isolate X_5 was more rapid under long days and the loss of infectivity with X_8 was more rapid under short days. At 24°C, loss of infectivity was relatively slow from the initial low peak, and the infectivity was lower in short days than in long days. Relatively little loss of infectivity was obtained in inoculated leaves at 16°C in short days and the level of infectivity remained quite constant throughout the 20 days period of observation. This contrasts with the drop in infectivity in continuous light at 16°C.

Curves of both virus isolates were essentially similar although infectivity of X_8 was consistently higher than that of X_5. Higher infectivity was usually demonstrated during the initial rise in inoculated leaves of plants kept under long photoperiods than in plants in short photoperiods. Loss of infectivity was more rapid at 16°C in continuous light than in 8 hours day. At higher temperature, inactivation was somewhat more rapid in continuous light.

Severity of symptom development was positively correlated with virus infectivity in inoculated leaves. Both were highest at 16° and 20°C, intermediate at 24° and lowest 28°C. At 16°C, both disease severity and the initial rise in virus infectivity were slightly higher under continuous light than in the 8 hour day. At 20°C, differences in disease index and virus infectivity at the two photoperiods were less pronounced than at 16°C. In the early portion of the observation period at 24°C, both infectivity and disease index were higher in continuous light.

2. Influence of Pre-inoculation Environment on Symptom Expression and Infective Virus Content.

The influence of pre-inoculation temperatures on predisposition of Epicure plants to PVX and virus multiplication within such plants was determined at different post-inoculation temperatures. In three preliminary trials, two groups of uniform and healthy plants were grown in 17° and

28°C glass houses respectively for 2 weeks. Then all of the plants were inoculated with PVX_5 on the fourth and the fifth leaves from the top of every plant. One-half of the 8 plants in each group were kept at 28°C and the other half of the plants were kept at 17°C after inoculation. Disease development was observed periodically. Data from these 3 preliminary trials were in agreement and were similar to results from an additional more extensive trial (Fig. 2).

Approximately 3-week-old plants were uniformly divided into 3 groups and were grown in 17°, 22°, and 28°C glass houses respectively for 2 weeks. Each of 5 well-developed leaves of these plants was inoculated with PVX_8. Then the plants of each group were separated into 3 groups of similar plants and were kept at the 3 different temperatures respectively. The disease development was observed periodically and leaf discs were collected from inoculated leaves at time intervals of 4, 8, 12, 16, and 20 days after inoculation. Relative virus concentration in these leaf samples was determined by assay on 5 half leaves of G. globosa as previously described.

Similar results were obtained in the 4 preliminary trials. Symptom expression and virus multiplication in inoculated leaves were influenced by post-inoculation temperatures similarly to those shown in Fig. 1. The optimum temperature for local and systemic symptom development was 22°C. Both symptom types developed slightly later at 17°C. At 28°C,

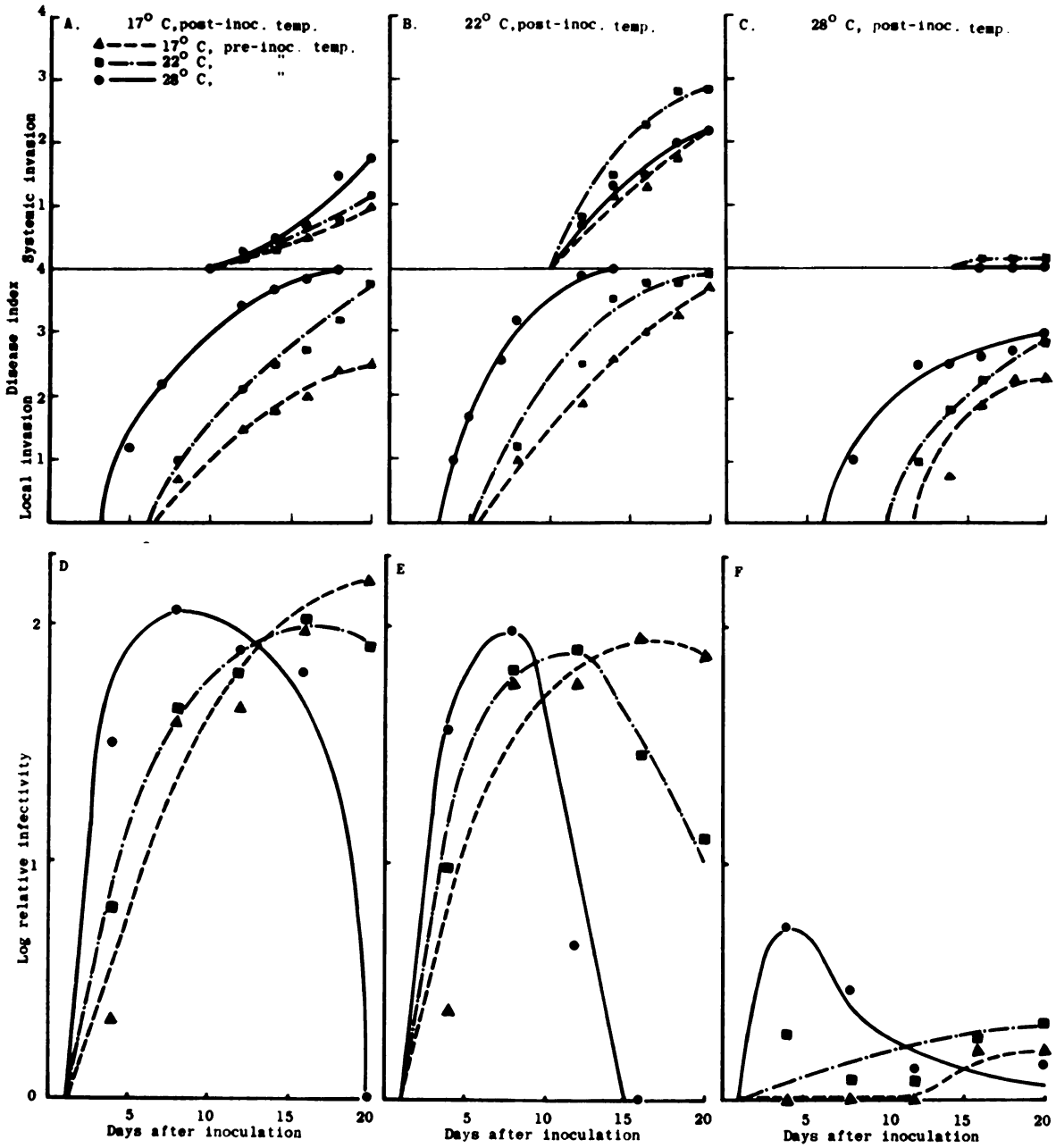


Fig. 2. A-C, Disease indices on plants predisposed under different pre-inoculation temperatures. D-F, Relative virus infectivity in inoculated leaves of plants mentioned above.

local symptoms were retarded and systemic symptoms almost precluded.

Pre-inoculation temperatures modified symptom expression in inoculated leaves at any post-inoculation temperature (Fig. 2, A-C). Local symptom development was increased at each post-inoculation temperature by high temperature (28°C) incubation before inoculation, and was decreased by pre-inoculation incubation at 17°C at each of the 3 post-inoculation temperatures. Plants grown at 22°C were intermediate and those grown at 17°C were most retarded in symptom expression. Systemic invasion was not consistently influenced by pre-inoculation temperatures. This may have been due in part to recovery from the influence of the predisposition environment during the 12 days required for development of systemic symptoms following inoculation and transfer to the post-inoculation environment.

Infectivity of virus from the treated plants (Fig. 2, D-F) kept at these 3 post-inoculation temperatures were in essential agreement with those previously presented. The influence of pre-inoculation temperatures on virus infectivity on potato plants was marked. The virus multiplied more rapidly, reached a peak more quickly and declined more rapidly in the leaves of the plants grown at 28°C, than within groups of plants kept at any temperature after inoculation. This was the only treatment in which inactivation of the virus in inoculated leaves was rapid and pronounced.

In contrast, virus multiplied slower in the leaves of plants grown at 17°C before inoculation, and virus concentration continued to increase at all post-inoculation temperatures up to the end of observation period. Virus multiplication in inoculated leaves of plants predisposed at 22°C was intermediate between that of plants predisposed at temperatures of 16° and 28°C.

To determine the predisposing influence of length of time of illumination, 9 uniform Epicure plants were grown under 8 hour illumination or continuous light at 16°, 20°, or 28°C. After 2 weeks, these treated plants were inoculated on 5 well-expanded leaves with PVX_8, and then all of the plants were kept in a 22°C glass house, about 10 hours day. Disease indices were determined periodically by symptom severity in inoculated leaves and by systemic symptoms. Leaf discs were collected for virus infectivity measurement from inoculated leaves at intervals of 3, 6, 10, and 20 days after inoculation and the relative infectivity of virus was determined as previously described (Fig. 3).

The influence of pre-inoculation temperatures on symptom expression (Fig. 3, A-C) at post-inoculation temperature of 22°C, and virus concentration was similar to that observed earlier. Symptom severity was highest in inoculated leaves of plants grown in an 8 hours day at 28°C, and 20°C were essentially similar, and was retarded at 16°C pre-inoculation temperature. Continuous light at all temperatures

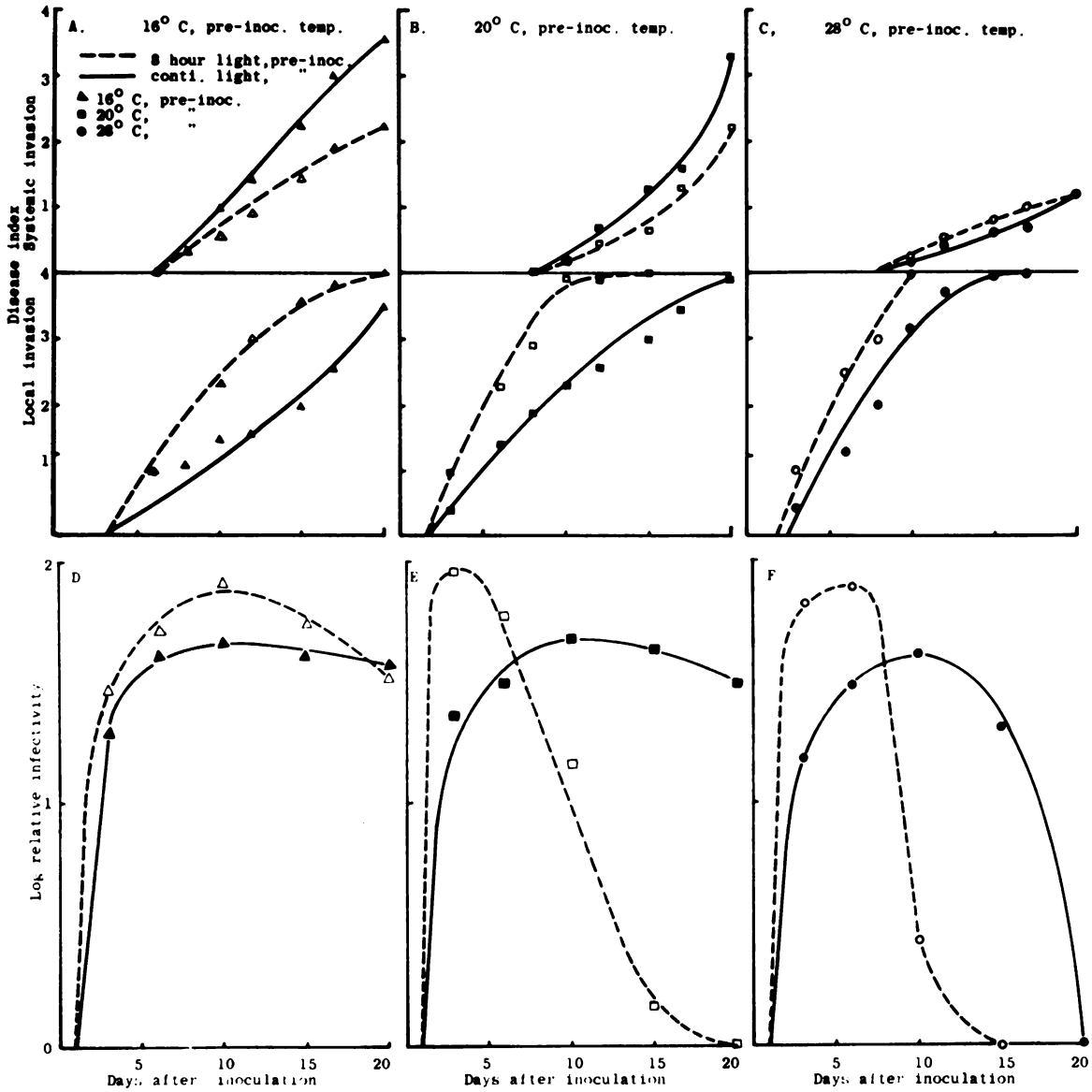


Fig. 3. A-C, Disease indices of Epicure plants predisposed under various pre-inoculation temperatures and photoperiods and grown at 22° C in a glass house: D-F, relative virus infectivity in inoculated leaves of plants mentioned above.

before inoculation retarded symptoms in inoculated leaves and slightly enhanced symptom severity in systemically invaded leaves at 16° and 20°C pre-treatment. Plants predisposed at 28°C in either day length developed less severe systemic symptoms than plants predisposed at 16° or 20°C (Fig. 3, A-C).

Infectivity of leaf disc homogenates (Fig. 3, D-F) from these plants was similar to those in previous trials. The level of infectivity remained relatively constant in plants predisposed at 16° and 20°C in continuous light and at 16°C in an 8 hours day. Loss of infectivity was most rapid when plants were predisposed in short days at either 20° or 28°C, and was delayed but pronounced with long day 28°C pre-treatment.

Six 4 week-old uniformly selected plants grown in a 22°C glass house were pre-treated at either 20° or 36°C in continuous light for 2 days before inoculation, and inoculated with isolate X_5 (Fig. 4). Then the plants were transferred to a 22°C glass house. Disease indices were calculated and leaf discs were collected for infectivity assay at time intervals of 2, 3, 5, 8, 12, 16 and 20 days after inoculation. Local symptoms developed more quickly on inoculated leaves of plants pre-inoculation treated at 36°C than at 20°C, while severity of systemic symptoms was retarded by the high temperature pre-treatment. Virus infectivity increased somewhat more rapidly in the inoculated leaves of plants predisposed at 36°C than in the control plants. Differences in this

trial were relatively small due possibly to the short pre-treatment period. Since potato plants tolerate high temperature poorly, a longer pre-treatment period did not seem advisable.

3. Influence of Hot-water Treatment on Symptom Expression.

The influence of hot-water treatment of inoculated leaves on the pathogenesis of PVX was determined in 2 trials. In one preliminary test, uniform Epicure plants were separated into 2 groups. The 6 plants of one group were inoculated on the leaves soon after heating in 50°C water for 60 seconds, and the other 6 plants inoculated on the non-heated leaves with PVX_5 were served as control. All of these plants were kept in a 22°C glass house under sun light in winter. The disease index of the plants inoculated soon after heating was lower than that of the control plants. The systemic symptoms occurred on the plants inoculated on non-heated leaves as early as 12 days after inoculation, while plants with inoculated heated leaves developed systemic spots on the upper young leaves at least 2 days later.

In an additional test (Fig. 5) uniform plants were divided into 7 groups of 6 plants each and PVX_5 was used as inoculum. The fifth and sixth leaves from the top of each plant were heated similarly either before or after inoculation at the following time intervals: 1) heated and inoculated as soon as possible after heating, 2) heated and then inoculated 30 minutes later, 3) heated and inoculated

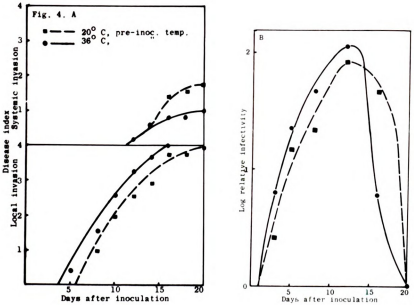


Fig. 4. A. Disease indices and B, relative virus infectivity in Epicure plants predisposed at 36° and 20° C and grown in 22° C glass house.

15 hours later, 4) inoculated and heated at once, 5) inoculated and heated 30 minutes later, 6) inoculated and heated 15 hours later, and 7) inoculated control, no heat treatment. All of the inoculated plants were kept in 22°C glass room in early spring.

Plants inoculated on leaves heated before or after inoculation developed relatively low disease indices in comparison with the control plants inoculated without heating or when heating preceded or followed inoculation by 15 hours (Fig. 5). Pre-inoculation heat treatment was slightly more effective in reducing disease indices than was post-inoculation treatment. Slightly lower indices were obtained on the plants inoculated on the leaves 15 hours after heating in comparison with those unheated plants.

4. Relation of Leaf Maturity on Disease Severity and Infective Virus Content.

In earlier trials there was some evidence that PVX induced severe and rapid disease development and multiplied more rapidly in the old leaves than in the young leaves. Ten Epicure plants of equal maturity were used in each of 2 trials. In one trial, 7-week-old plants were inoculated on 4 young leaves (approximately 2-week-old) and 4 old leaves (approximately 6-week-old) with X_5 and in another trial, 10-week-old plants were inoculated on 5 young leaves, approximately 2 weeks old, and 5 old leaves, approximately 8 weeks

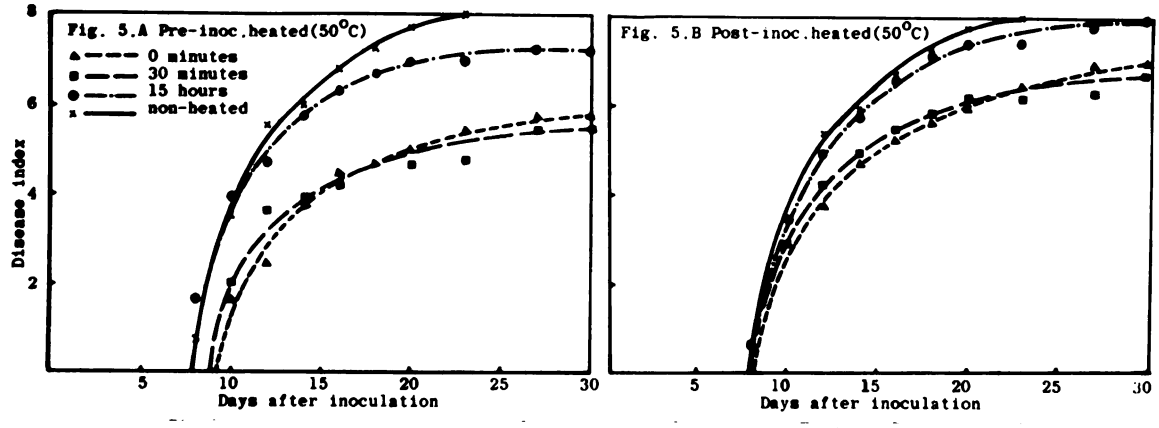


Fig. 5. A. Disease indices on plants inoculated on leaves heated in 50° C water for 60 seconds at various time intervals either before, or B, after inoculation.

old, with X_8. All of the inoculated plants were kept in a 22°C glass house. Disease development on both old and young inoculated leaves was observed and leaf discs were also collected periodically.

Similar results were obtained in each test involving the 2 virus isolates, X_5 and X_8 (Fig. 6, A). Old leaves inoculated with X_5 became yellow on the fifth day and died 7 days after inoculation. Infectivity of the virus in these leaves reached a peak on the third day, declined very quickly and was inactive by the eighth day after inoculation. The symptoms on inoculated young leaves developed more gradually, and leaves were still alive by the end of observation period. The virus in young leaves (Fig. 6, B) multiplied more slowly than in old leaves, and reached highest infectivity on the eighth day. Infectivity remained high throughout the observation period. In the trial with X_8, essentially similar results were obtained. In another trial with X_5, curves of virus infectivity in inoculated leaves grown at 22°C in the glass house were similar to those of Fig. 6, B. However, the drop in infectivity in the old leaves was not as rapid because in the previous test (Fig. 6, B) 6-8 week-old mature leaves had been used and, in the present test, 3-4 week-old leaves were used as mature leaves.

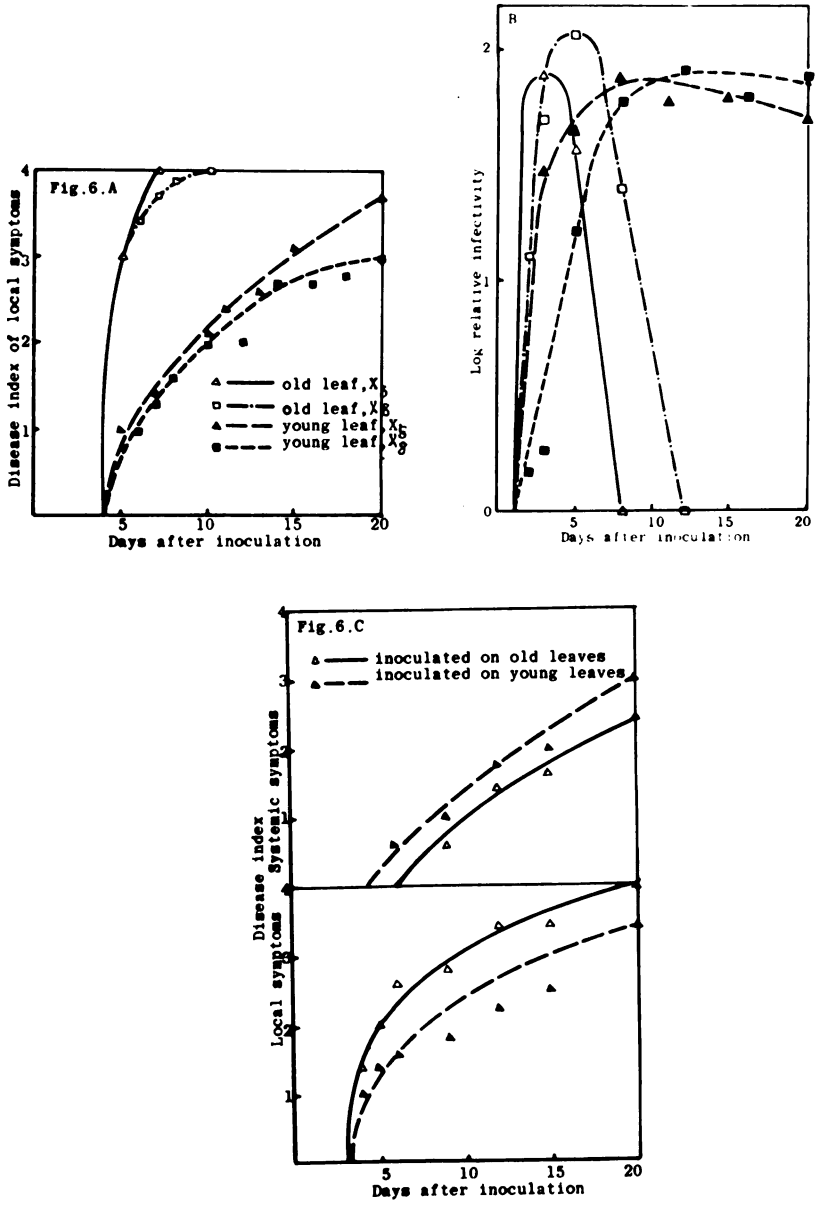


Fig. 6. Susceptibility of leaves of different maturity as measured by A, local symptom development and B, by virus infectivity, and C, by local and systemic symptoms.

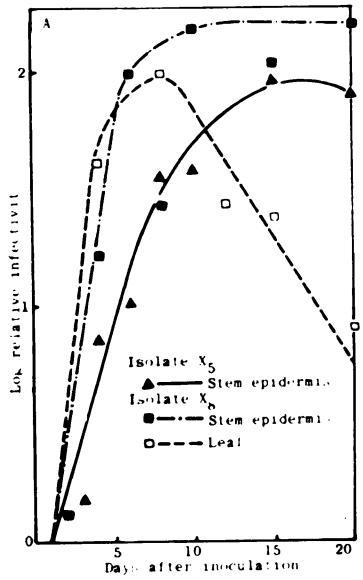
An additional test was made in order to confirm whether systemic infection was affected by infection through leaves of different maturities (Fig. 6, C). Uniform 5-week-old plants were inoculated with X_5 on 2 uppermost well-expanded young leaves, approximately 2 weeks old, and other 5 plants were inoculated on 2 lower leaves approximately 4 weeks old. Local symptoms developed more rapidly on the inoculated old leaves than on young leaves, while systemic symptoms developed more slowly on plants inoculated on old leaves.

5. Multiplication of PVX in Stem Epidermis of Epicure Plants.

Assay of stem epidermis was attempted because this tissue strips with relative ease and a relatively high percentage of inoculated cells were thus available for assay. About 40 plants uniformly selected were used in each of 5 trials. The mature portion of the stem was inoculated by rubbing carborundum dusted on the surface of the stem with a cotton ball saturated with virus suspension. Stems were thoroughly rinsed with water after inoculation. All of the plants were kept in a 22°C glass house under sun light condition in fall and winter. The stem epidermis of inoculated portions of

each 3 plants was stripped off periodically, weighed and frozen in a small vial. Such strips separated from the stem between the collenchyma layer and parenchyma layer, and thus they included epidermis, a layer of chlorenchyma (green parenchyma), and 2-3 layers of collenchyma tissue. Because virus infectivity in epidermal strips appeared to be too high for assay by the 2-dilution method, a 3-dilution system 1-5, 1-50, and 1-500 was used. However, the curves of the 1-50 dilution was satisfactory for the assay (Fig. 7, A).

All of the results in 5 similar trials involving either X_5 or X_8 were essentially similar. For comparing the virus infectivity in epidermal tissue with that in leaf tissue, data at 1-5 dilution derived from a test with X_8 in leaves in a 22°C glass house under sun light condition in fall are presented. Infection of PVX in stem epidermal tissue was established and the virus was recovered on the second day after inoculation. Infectivity rose considerably higher on the fourth day and reached a peak about 10 days after inoculation. Infectivity of virus in stem epidermal tissues remained high for relatively long periods, although the infectivity of virus in leaves was lost rapidly under the same environmental conditions. Virus infectivity was still detected in the strips collected on the thirtieth day. The infectivity of virus in stem strips at 1-50 dilution showed the curves similar to those shown by the virus infectivity in leaves at 1-5 dilution. It seemed that the virus in stem



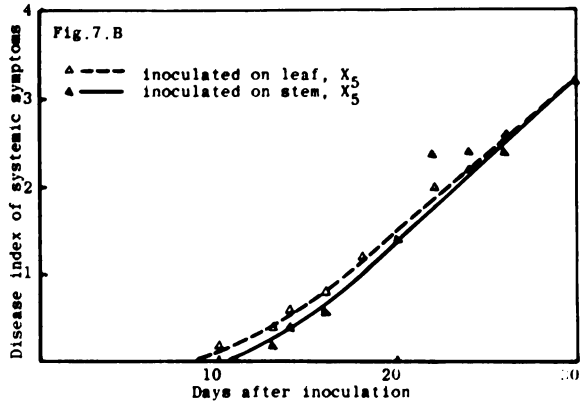


Fig. 7. A. Relative infectivity of virus in stem epidermal strips (1-50 dilution) or in leaves (1-5 dilution), B, disease indices of plants inoculated either on leaf or on stem surface.

epidermal tissues reached a concentration at least 10 times greater than that in the leaves and also that loss of infectivity was not as rapid.

Ten uniformly selected plants were divided into 2 groups and plants of one group were inoculated on stems and the other plants were inoculated on 2 well-expanded leaves with X_5. Disease development of systemic symptoms was shown in Fig. 7, B. No apparent difference in systemic disease development was observed between the 2 groups of plants, although the systemic symptoms developed slightly earlier on the plants inoculated on leaves.

IV. DISCUSSION

Virus multiplication curves in Epicure plants were influenced considerably by different environmental conditions. Two types of curves were obtained in the present investigation, i.e., type A was an upward curve flattened on top without or with a small downward drop of infectivity, and the type B curve was characterized by a rapid rise and prompt decline in infectivity.

Type A curves are typical of virus titre in tolerant potato plants grown at 16°-24°C (Hooker and Kim, unpublished data) and in Epicure plants as listed below. Type B curves were obtained in Epicure plants kept at 20°C, in long days after inoculation, in leaves predisposed at high temperature (28°C) or predisposed by short days before inoculation, in old leaves, and with certain other growing conditions. Type B curves were obtained when environmental or physiological factors were favorable for very early, rapid virus multiplication. This type of curve was demonstrated by Hutton and Wark (1952), with Epicure plants. Since the Epicure plants used by Hutton and Wark were inoculated under summer conditions, the type of curve they obtained is logical and results in their trial and mine are in agreement. Hutton and Wark did not test virus titre under varied environmental conditions. Hutton (1948) stated that such a hypersensitive reaction as localization could be complete under midsummer condition but was incomplete in mid winter.

Virus multiplication type A curves were usually obtained in inoculated leaves of Epicure plants kept at 16°C under short days after inoculation, in plants predisposed at 16°C, or in young leaves or stem epidermal tissues. These curves (type A) were distinct from those of Hutton and Wark and more nearly resemble virus titre curves of tolerant plants (Hooker and Kim, unpublished data).

Köhler (1958) accepted the theory of Hutton and Wark (1952) in that a common virus-inactivation system determined the immunity of S. 41956 and the localized resistance of Epicure. Both types of curves were obtained with Epicure in the present test. It is difficult to accept without reservation the theory that a common virus inactivating system is present in both hypersensitive and in immune types of potato since virus titres from inoculated leaves of Epicure more nearly resemble those of tolerant varieties than of immune types. Data obtained to date from inoculation of immune types such as S. 41956 or its derivatives (Hutton and Wark, 1952; Köhler, 1958; Benson and Hooker, 1960; Bagnall, 1961) suggest neither the A nor the B type of curve.

Mature plant resistance in tolerant potato plants to PVX was demonstrated by Bercks (1951) and by Beemster (1957) in tolerant potato varieties. They found that the older the potato plants were at the time of inoculation with PVX, the fewer were the tubers that became infected. At present, the cause of the mature plant resistance is unknown. In the

present trials, rapid virus multiplication followed by rapid decline in infectivity took place in the old leaves of Epicure plants. In young leaves, the tissue maintained high levels of infectivity for relatively long periods.

Bercks (1954, 1956) found that PVX concentration as determined by serological and electronmicroscopic methods was very low in the oldest leaves of Samsun tobacco plants and relatively high in the middle leaves. The older foliage contained mostly fractions with particles at most half the length of the whole virus particles and so he supposed that PVX tended to disintegrate in the old leaves. Possibly a slightly different situation is present in old Epicure leaves since virus multiplied very quickly, reached a high titre, and declined rapidly. Inoculated old leaves were killed quickly. Few of the Epicure plants inoculated on old leaves became systemically infected. These plants furthermore did not generally show top necrosis and were usually free of PVX as determined by the Gomphrena test. The fact that PVX multiplied more rapidly in old leaves followed by early defoliation probably played a role in mature plant resistance in this hypersensitive potato variety. The rapid disease development and quick death of the inoculated old leaves might be attributed to rapid increase of virus content in old leaves associated with the early attainment of an infectivity level which was high enough to cause death of old leaf tissue due to hypersensitivity. Probably some of these

plants escaped further systemic invasion because the inoculated leaves were quickly killed and dropped. The tissue of young leaves was more tolerant to high virus concentration, and virus infectivity remained high in such leaves even though relatively non-necrotic. At least some evidence is presented to suggest that within tissues of a hypersensitive potato plant, degrees of susceptibility exist suggesting tolerance (young tissue) as opposed to hypersensitivity (old tissue).

PVX in stem epidermal tissue maintained high levels of infectivity over a relatively long period. It seems probable that epidermal stem tissues were more tolerant to PVX infection or were not as hypersensitive as the leaves. Thus, different degrees of sensitivity to PVX may exist within different tissues of the host.

Timian et al (1955) found that local symptoms in potato plants of susceptible progenies developed equally well at 18° and at 24°C, and that systemic symptoms were more pronounced at 24° than at 18°C. At 28°C, no systemic symptoms developed and local disease developed slower on inoculated leaves. In the present tests with Epicure plants, the optimum temperature for disease development was within more narrow limits and more definitely inhibited by high temperature.

Disease developed more rapidly in hypersensitive plants in continuous light at temperatures below 24°C while at 28°C higher disease indices were obtained in the 8 hour

day than in continuous light. In contrast, increased symptom severity in susceptible plants subjected to reduced light has been reported (Timian et al, 1955b).

Yarwood (1956, 1958) reported that susceptibility of bean leaves to certain viruses was increased by pre- and post-inoculation hot water treatment. In my tests, no increased susceptibility was obtained in inoculated leaves heated to 50°C in hot water for 60 seconds before inoculation. In contrast, heating leaves within 30 minutes before or after inoculation reduced infection. Infection was decreased to approximately the same extent by either pre- or post-inoculation treatment. Conceivably susceptible sites may have been destroyed either before or after infection or some process in virus synthesis within the cell may have been impaired. Whatever the underlying reason, the treatment temperature was well below the thermal inactivation point of PVX, and within 15 hours of inoculation treated tissue had resumed a level of susceptibility similar to that of nontreated tissue.

Other types of pre-inoculation treatment such as variation in growing temperature and photoperiods were factors in susceptibility of Epicure plants to PVX. Predisposition by growing plants for 2 weeks at 28°C increased susceptibility, whereas at 16°C susceptibility was decreased. These results are difficult to interpret since 28°C was generally a very poor temperature for disease development and 16°C was very favorable. A relatively short exposure to 36°C

(2 days) was not as effective as 2 weeks at 28°C in enhancing susceptibility. Kassanis (1952) increased susceptibility of local lesion hosts to 5 mechanically transmitted viruses by keeping healthy plants at 36°C for 1-2 days before inoculation.

It is recognized that age of plants and conditions under which they have been grown affect virus susceptibility (Kassanis, 1957; Yarwood, 1959). In my trials, symptom severity and virus multiplication in inoculated leaves were enhanced by growing plants in short photoperiods especially at 20° or 28°C, and was retarded by pre-inoculation incubation in continuous light especially at 16°C for 2 weeks. Many viruses produce more severe lesions both local and systemic in plants grown under low than under high light intensities (Bawden and Roberts, 1947; Ross, 1953). The effect of environment on virus disease development was demonstrated by subjecting plants to various periods of darkness and reducing illumination before and after inoculation (Bawden and Roberts, 1948). Shaded leaves are much more fragile than those grown in bright light. Thus they may be more readily injured during inoculation and provide a greater number of entry points for virus. Leaves of plants grown in continuous light were tougher than those grown in short days. Mechanical resistance may at least play a part in this mechanism. However, other more subtle changes in physiology may be of greater importance in this response. Takahashi (1947) reported that TMV multiplied more rapidly in leaves in the

light than in the dark. According to Pound and Bancroft (1956), TMV concentration in tobacco plants kept under long photoperiods (12-16 hour day) was higher 4 days after inoculation than that of plants kept in short day (4-8 hour days) although these differences were reversed after 18-32 days.

Direct correlation between virus multiplication and symptom severity has been demonstrated in Nicotiana spp. with PVX (Pound and Helms, 1955) and other host-virus combinations (Harrison, 1956; Bancroft and Pound, 1956). In my trials, infectivity of virus in inoculated leaves was positively related to local symptom severity as mentioned above. High levels of virus infectivity were demonstrated in the inoculated leaves of plants which showed high disease indices.

Generally disease development in the plants inoculated with isolate X_8 of PVX was slightly more rapid than that in plants inoculated with isolate X_5, although isolate X_5 produced severe symptoms and isolate X_8 either no symptoms or mild symptoms on susceptible potato plants (Timian et al, 1955a). Cockerham (verbal communication) has indicated that for infection of necrotically reacting potato varieties, non-necrotic strains of PVX are relatively more effective than necrotic isolates.

V. SUMMARY

Effects of certain environmental factors and certain factors within host plant on disease development and multiplication of PVX in Epicure plants hypersensitive to PVX were studied. Most rapid disease development occurred in the plants at post-inoculation temperature of approximately 20°C, and plants at 16°C showed almost as severe symptoms as plants at 20°C. At 24°C, there was considerable reduction in severity of systemic symptoms, while local symptoms developed somewhat slower than on plants at 16°C. No systemic symptoms developed on plants at 28°C, and local symptom development was slower than that at lower temperatures.

Disease indices of plants kept in continuous light at 16°C were higher than those of plants exposed to 8 hour illumination in 24 hours. Difference between photoperiods were small at 20°C. At 24°C, early disease symptoms were more severe in plants with continuous light, whereas 12 to 14 days after inoculation symptoms were more severe in plants grown in an 8 hour day. At 28°C, disease developed in 8 hour day plants earlier than in plants grown in continuous light.

Virus infectivity in inoculated leaves was positively correlated to symptom severity. Higher relative infectivity of virus was demonstrated in inoculated leaves at optimum temperatures for disease development, and little infective

virus content was present in leaves at 24° and 28°C. Higher infectivity was usually demonstrated during the initial rise in long photoperiod than that in short photoperiod. Loss of virus infectivity occurred after 8 days in leaves under all conditions tested except at 16°C in short day. Differences in virus concentration were small between photoperiods at other temperatures.

At each post-inoculation temperature, local symptom development was increased by a pre-inoculation high temperature of 28°C and decreased by pre-inoculation incubation at 17° for 2 weeks. Plants grown at 22°C were predisposed intermediately. The virus multiplied more rapidly in the leaves of plants predisposed at 28°C, and the virus infectivity lost more rapidly. While the virus multiplied slower in leaves of plants grown at 17°C, and virus infectivity continued to increase up to the end of observation period. Virus multiplication in leaves predisposed at 22°C was intermediate.

Continuous light at all temperatures before inoculation retarded symptom development and virus multiplication in inoculated leaves, while symptom development and early virus multiplication were enhanced by predisposition in short day before inoculation. Loss of infectivity was most rapid when plants were predisposed in short day at 20° or 28°C. and was delayed by 16°C predisposition at either day length as well as by long day at 20°C.

Increased susceptibility to PVX was slightly increased by pre-inoculation temperatures of 36°C 2 days before inoculation.

Disease indices of inoculated plants were reduced by hot water leaf treatment (50°C), within 30 minutes before or after inoculation. After 15 hours tissues had recovered and disease indices were essentially similar to plants with untreated leaves.

Symptoms on inoculated old leaves developed very quickly. The virus multiplied very rapidly and infectivity was lost suddenly after reaching a high peak. Disease developed slower and virus multiplication was slightly retarded but infectivity remained high over a 20 day period in inoculated young leaves.

Infection of the virus in stem epidermal tissues was established. The virus titer in stem strips remained high for relatively long period, although in leaves it dropped rapidly under the same conditions. Infective virus content in stem epidermal tissues was at least 10 times greater than that in leaves. About the same degree of systemic infection was noticed among plants inoculated on leaf or stem tissue.

In general relatively low systemic invasion developed on the plants which showed higher local symptoms under the conditions favorable for virus multiplication. The X_8 isolate of the virus caused slightly more rapid disease development in Epicure plants in comparison with X_5 isolate, although X_8 was a mild isolate on some other potato varieties susceptible to PVX.

VI. LITERATURE CITED

Bagnall, R. H. 1961. Hypersensitivity to virus A and X in Canadian and American potato varieties. Amer. Potato Jour. 38: 192-202.

Bancroft, J. B., and G. S. Pound. 1956. Cumulative concentration of TMV in tobacco and tomato at different temperatures. Virology 2:29-43.

Bawden, F. C. 1936. The viruses causing top necrosis (acronecrosis) of the potato. Ann. Appl. Bio. 23: 487-497.

Bawden, F. C. and F. M. Roberts. 1947. The influence of light intensity on the susceptibility of plants to certain viruses. Ann. Appl. Biol. 34:286-296.

Bawden, F. C., and F. M. Roberts. 1948. Photosynthesis and predisposition of plants to infection with certain viruses. Ann. Appl. Biol. 35:418-428.

Beemster, A. B. R. 1957. Some aspects of mature plant resistance to viruses in the potato. Proceedings of the Third Conference on potato virus Diseases. 212-217.

Benson, A. P., and W. J. Hooker. 1960. Isolation of virus X from "immune" varieties of potato, Solanum tuberosum. Phytopathology 50:231-234.

Bercks, R. 1951. Weiter Untersuchungen zur Frage der Altersresistanz der Kartoffelpflanzen gegen das X-virus. Phytopath. Z. 18:249-269.

Bercks, R. 1954. Untersuchungen über Änderungen des Virusgehaltes in Tabakpflanzen während der Vegetationsperiode. Phytopath. Z., 22:215-226.

Bercks, R. 1956. Über Kozentration und Verhalten des X-virus in alten Blättern. Phytopath. Z. 26:35-40.

Cadman, C. H. 1942. Autotetraploid inheritance in the potato: Some new evidence. Jour. Genetics 44:33-52.

Cocherham, G. 1943a. Potato breeding for virus resistance. Ann. Appl. Biol. 30:105-108.

Cocherham, G. 1943b. The reactions of potato varieties to viruses X, A, B and C. Ann. Appl. Biol. 30:338-344.

Ford, R. E., and A. F. Ross. 1962a. Effect of temperature on the interaction of potato viruses X and Y in inoculated tobacco leaves. Phytopathology 52:71-77.

Ford, R. E., and A. F. Ross. 1962b. The interaction of potato virus X and Y in tobacco leaf tissue differing in age. Phytopathology 52:226-233.

Harrison, B. D. 1956. Studies on the effect of temperature on virus multiplication in inoculated leaves. Ann. Appl. Biol. 44:215-226.

Hooker, W. J., C. E. Peterson, and R. G. Timian. 1954. Virus X resistance in potato. Amer. Potato Jour. 31: 199-212.

Hutton, E. M. 1948. The separation of strains from a virus X complex by passage through potato seedlings. Aust. J. Sci. Res. Ser. B 1:439-450.

Hutton, E. M., and D. C. Wark. 1952. A relationship between immunity and localized reaction to virus X in the potato, Austr. J. Sci. Res. Ser. B 5:237-243.

Johnson, J. 1922. The relation of air temperature to the mosaic disease of potatoes and other plants. Phytopathology 12:438-440.

Kassanis, B. 1952. Some effects of high temperature on the susceptibility of plants to infection by viruses. Ann. Appl. Biol. 39:358-369.

Kassanis, B. 1957. Effects of changing temperature on plant virus disease. Advances in Virus Research 4: 221-241.

Köhler, E. 1958. Das Verhlten des X-virus in Damit Geimpften Blätten Unterschiedlichen Resistenztyps. Proceedings of the Third Conference on Potato Virus Diseases. 189-198.

Pound, G. S., and J. B. Bancroft. 1956. Cumulative concentrations TMV in tobacco at different photoperiods and light intensities. Virology 2:44-56.

Pound, G. S., and K. Helms. 1955. Effects of temperature on multiplication of potato virus X in Nicotiana species. Phytopathology 45:493-499.

Quanjer, H. M., and J. G. O. Botjes. 1929. Potato diseases of the streak and top necrosis type and the problem of latency and physiological specialization. Meded. Landb-Hoogesch., Wageningen, 33.(R.A.M. 9:481-482).

Resconich, E. 1961. Heat-induced susceptibility to TMV and thermal injury in bean. Virology 13:338-347.

Ross, A. F. 1953. Physalis floridana as a local lesion test plant for potato virus Y. Phytopathology 43: 1-8.

Spencer, E. L., and W. C. Price. 1943. Accurace of the local-lesion method for measuring virus activity. I. Tobacco mosaic virus. Amer. Jour. Bot. 30:280-290.

Stouffer, R. F., and A. F. Ross. 1961a. Effect of temperature on the multiplication of potato virus X in presence and absence of potato virus Y. Phytopathology 51:5-9.

Stouffer, R. F., and A. F. Ross. 1961b. Effect of infection by potato virus Y on the concentration of potato virus X in tobacco plants. Phytopathology 51:740-744.

Takahashi, W. N. 1947. Respiration of virus-infected plant tissue and effect of light on virus multiplication. Amer. J. Botany 34:496-500.

Timian, R. G., W. J. Hooker, and C. E. Peterson. 1955a. Immunity to virus X in potato: Studies of clonal lines. Phytopathology 45:313-319.

Timian, R. G., W. J. Hooker, and C. E. Peterson. 1955b. Immunity to virus X in potato: Studies of segregating seedling populations. Phytopathology 45:445-450.

Tompkins, C. M. 1926. Influence of the environment on potato mosaic symptoms. Phytopathology 16:581-610.

Youden, W. J. 1937. The use of incomplete block replications in estimating tobacco mosaic virus. Contrib. Boyce Thompson Inst. 9:41-48.

Yarwood, C. E. 1956. Heat-induced susceptibility of beans to some viruses and fungi. Phytopathology 46:523-525.

Yarwood, C. E. 1958. Heat activation of virus infection. Phytopathology 48:39-46.

Yarwood, C. E. 1959. Predisposition. Plant Pathology Vol. 1:521-562.