DIFFERENTIATION OF CERTAIN CRUCIFER VIRUSES BY THE USE OF TEMPERATURE AND HOST IMMUNITY REACTIONS

By GLENN S. POUND, formerly research assistant, and J. C. WALKER, professor of plant pathology, Wisconsin Agricultural Experiment Station

INTRODUCTION

During the several years that cabbage (Brassica oleracea var. capitata L.) mosaic has been under observation in southeastern Wisconsin, it has been noted that type and severity of symptoms vary considerably with the prevailing air temperature. Initial symptoms of the mosaic disease in the field in Wisconsin usually consist of pronounced vein clearing, vein banding, and a coarse distorting mottle. As the temperature at which the plants grow gradually increases, the vein clearing and vein banding become less intense, often being completely masked, and the mottle becomes more prevalent and pronounced. Toward the end of the season, however, as the plants mature in decreasing average temperatures, the mottle symptom gradually recedes and the vein clearing and vein banding again attain prominence. Walker, LeBeau, and Pound (16) have recently shown that these two types of symptoms are due to two distinct viruses operating together within the host plant, although evidently affected differently by high and low temperatures. The mottle symptom is due to a strain of turnip virus 1 Hoggan and Johnson (3) known as cabbage virus A, and the vein clearing symptom is due to a strain of cauliflower virus 1 Tompkins referred to herein as cabbage virus B (16). In nature these two viruses commonly occur together in host plants, at least in Wisconsin and in the Pacific Northwest, and there is no doubt that Larson and Walker (5) in earlier experiments were dealing with this virus combination.

In 1937 Tompkins (10) described a mosaic disease of cauliflower which he stated occurred in the cool coastal valleys of California. In 1938 Tompkins, Gardner, and Thomas (15) described a virus disease of cabbage which they named black ring. They made no study of the temperature relations of this disease but stated that it occurred chiefly in the cool coastal valleys of California during the winter months and that it was uncommon in the summer.

Walker et al. (16) have shown that a very close relationship exists between cabbage virus A and the black ring virus on the one hand, and between cabbage virus B and the cauliflower mosaic virus on the other hand. This paper is a report of studies directed toward the distinction of the two virus groups and of strains within each group by differential temperature reactions and by host immunity tests.

1 Received for publication March 27, 1944.
2 Numbers in parentheses refer to literature cited, p. 278.

Journal of Agricultural Research,
Washington, D. C.
MATERIALS AND METHODS

Cabbage viruses A and B used in this study were those employed in previous investigations in this laboratory (16). Cultures of the black ring and cauliflower mosaic viruses were supplied by Dr. C. M. Tompkins of the University of California. All four viruses were maintained in stock plants in aphidproof cages. Frequent inoculations were made to young cabbage plants in order to provide a constant supply of inoculum. All inoculations were made by rubbing leaves with absorbent cotton which had been dipped in juice extracted from diseased plants. Powdered carborundum was used regularly as an abrasive on all hosts. Greenhouses were fumigated weekly with vapors of nicotine and naphthalene to control insects.

When the reaction of the viruses on cabbage at various temperatures was being studied, young plants of Jersey Queen cabbage were inoculated with each virus or virus combination and kept in greenhouses with constant air temperatures of 16°, 20°, 24°, and 28° C. An equal number (usually 15) of healthy and inoculated plants was used at each temperature. Each experiment was repeated several times.

Reactions of all other hosts were determined by at least three separate tests with a minimum of five plants in each inoculation with each host, except in the reactions of the wild mustards to virus A and to the black ring virus, in which case only one trial each was made.

EXPERIMENTAL RESULTS

TEMPERATURE RELATIONS

REACTION OF CABBAGE TO THE INDIVIDUAL VIRUSES

It can be seen from table 1 that the incubation period of each of the four viruses increased in length as temperature decreased. At 16° and 20° C. the black ring virus characteristically produced symptoms 2 to 3 days earlier than virus A, but at higher temperatures both viruses produced symptoms concurrently. The incubation periods of virus B and cauliflower mosaic virus were practically identical. However, as will be pointed out later, virus B developed symptoms on certain cruciferous hosts 1 to 10 days later than the cauliflower mosaic virus.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Incubation period at—</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16° C.</td>
</tr>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td>Cabbage A</td>
<td>16-20</td>
</tr>
<tr>
<td>Black ring</td>
<td>14-18</td>
</tr>
<tr>
<td>Cabbage B</td>
<td>19-21</td>
</tr>
<tr>
<td>Cauliflower mosaic</td>
<td>18-21</td>
</tr>
</tbody>
</table>

A study of the symptoms produced at different temperatures revealed that the four viruses fell into two groups in regard to their reactions on cabbage at high and at low temperatures. In one group,
containing cabbage virus A and the black ring virus, mottling, stunt-
ing, distortion, and bloom reduction were very severe at 28° C. and
very mild at 16°. Necrosis with the black ring virus was much more
severe and pronounced at 16° and 20° than at 24° and 28°. Within
this group sharp differences were noted between the reactions of virus
A and the black ring virus. At 28° and 24° virus A was markedly
more severe than the black ring virus, producing a severe mottle
accompanied by extreme leaf distortion and severe chlorosis; the
black ring virus caused much less distortion and stunting and prac-
tically no diffuse chlorosis. At 16° and 20°, on the other hand, the
black ring virus was more severe than virus A, mottling and stunting
were more pronounced, and necrosis was very much more common
The difference in reactions of virus A and the black ring virus at high
and low temperatures is of special interest because the two viruses
occur in different regions. Virus A is prevalent in the midwestern
and northern States in which its economic hosts are primarily summer
crops. Its season of activity is therefore largely in the summer.
The black ring virus occurs in the cool coastal valleys of California
where economic hosts are cultivated throughout most of the year (10),
but the prevailing air temperatures are relatively low. Moreover,
Tompkins et al. (15) state that epidemics of the black ring disease
occur chiefly in the winter and are uncommon in the summer. Al-
though the two viruses are very similar, it may be that they have
become established in their respective regions because the prevailing
air temperatures in these regions are favorable for their activity.

In the other group, containing cabbage virus B and the cauliflower
mosaic virus, intensity of symptom development increased with
decrease in temperature. At all temperatures symptoms appeared as
a chlorotic vein clearing which was prominently expressed but which
rapidly became completely masked at 24° and 28° C. At 16° and
20° vein clearing and chlorotic vein banding were prominent and
persistent. At these temperatures enations occurred as warty, trans-
lucent excrescences along the veins on the undersurface of the leaf
more commonly than at higher temperatures. The enations, although
sporadic in their development, appeared to be associated with a pro-
nounced expression of vein clearing and vein banding. Hence they
were more common at low temperatures and occurred with equal
frequency with virus B and the cauliflower mosaic virus. Stunting
at low temperatures, in spite of pronounced symptoms, was no greater
than at higher temperatures and was less than that caused by virus A
and the black ring virus. The effect of temperature upon disease
development for the cauliflower mosaic virus was exactly parallel to
that for virus B. The two viruses differed in that symptoms of the
cauliflower mosaic virus were always milder than those of virus B.
The fact that B-infected plants were more chlorotic than those
infected with the cauliflower mosaic virus often served as a sharp
point of distinction between the two viruses.

Reaction of Cabbage to Various Combinations of the Viruses

When viruses A and B were inoculated simultaneously into the
same plants, the degree of interaction was somewhat clearly defined
by the temperature effect on symptom expression of the virus com-
bination. An examination of figure 1 will show that the combined
Figure 1.—Systemic symptoms on young leaves of Jersey Queen cabbage produced by cabbage virus B (Bv.), cabbage virus A (Av.), and cabbage viruses A and B (Av. + Bv.) together at various temperatures. Note that symptoms of virus B are masked at 28° and 24° C, but are prominently expressed at 20° and 16° C. Note also the progressive increase in severity of symptoms of virus A with increase in temperature and the pronounced increased severity at 28° and 24° when viruses A and B occur together.
effect of the two viruses was more severe than that of either virus alone. At higher temperatures the addition of the B component to virus A resulted in the appearance of vein clearing and rapidly changed the coarse chlorotic mottle into a fine mottle with much more chlorosis and increased severity, with the result that ultimate symptoms bore little resemblance to those of either virus alone. Stunting, leaf distortion, bloom reduction, and premature leaf abscission were very severe. Necrosis, quite uncommon to virus A, became very severe on some plants. Symptoms (other than stunting and chlorosis) of virus B alone at 24° and 28° rapidly became masked, but the presence of this virus in combination with virus A at these temperatures effected a striking change in the type and severity of symptoms produced. Whether the presence of this virus enhanced the activity of virus A, or vice versa, or whether this symptom change was the additive result of the effect of each individual virus on the metabolism of the host is a question prompting further investigation. At 16° and 20° the combined effect of viruses A and B resulted in more stunting and chlorosis than for either virus alone but there was no change in type of symptoms as at 28°. The effect of each virus was evident, and there was little apparent interaction between them. The activity of virus A was reduced such that little additive effect was evident, and the ultimate symptoms were largely those of virus B.

A few days after plants showing severe A+B symptoms were moved from a house at 28° to one at 16° C., subsequently developing leaves showed only a very mild mottle and a vein clearing which gradually increased in prominence. Symptoms on young leaves showed little resemblance to those on plants kept at 28°. Likewise, a few days after A+B plants showing typical low-temperature symptoms were transferred from 16° to 28°, the conspicuous vein clearing and vein banding disappeared, and young leaves showed a severe fine mottle accompanied by much stunting and distortion. When these two groups of plants were again removed to the houses of their original incubation, they once again developed symptoms characteristic of that specific temperature. These reversible temperature reactions of plants infected with both viruses fully confirm observations made of the disease in the field.

When plants showing systemic symptoms of virus A were reinoculated with virus B, or vice versa, there developed at each temperature symptoms typical of the A+B combination after the respective incubation period of the second virus introduced. Furthermore, the two viruses were easily recovered individually from such plants by the use of heat inactivation and differential hosts as described by Walker et al. (16). The fact that there was no immunization of the host by virus A toward virus B and vice versa would indicate no close relationship between the two viruses.

Symptoms produced by the mixture of cabbage A and cauliflower mosaic viruses (fig. 2) were practically identical with those described for the A+B combination. Just as symptoms of the cauliflower mosaic virus alone on cabbage were slightly milder than those of virus B, the symptoms produced by this virus in combination with virus A were slightly milder than those of the corresponding A+B combination.

Combinations of the black ring virus with virus B and with the cauliflower mosaic virus were very similar to the corresponding com-
Figure 2.—Symptoms produced on young leaves of Jersey Queen cabbage by cabbage A (Av.), cabbage B (Bv.), cabbage black ring (BRv.), and cauliflower mosaic (CAUlv.) viruses alone and in various combinations at 16° and 28° C. Note the similarity in symptoms of virus A and the black ring virus both when alone and in combination with virus B or the cauliflower mosaic virus. Note also the similarity of symptoms of virus B and the cauliflower mosaic virus.
binations of virus A except that the differences in the temperature reactions of virus A and the black ring virus were also manifested in the combinations of these viruses. At 24° and 28° the black ring virus combinations produced less chlorosis and less stunting than did the corresponding combinations of virus A. In addition, dark-green rings and ring spots typical of the black ring virus were manifested. At 16° and 20° all combinations of the black ring virus produced more necrosis and a slightly more prominent mottle than those of virus A.

Thus in the various combinations involving viruses of the two groups the reactions of the cauliflower mosaic virus were practically identical with those of virus B, and the reactions of the black ring virus were very similar to those of virus A (fig. 2). These similarities were expected since the same relations were manifested in the reactions of each of the viruses alone. Tompkins and Tompkins et al. (10) and Tompkins et al. (15) made no mention of the black ring and cauliflower mosaic viruses occurring together within the same plant in nature. Neither did they describe symptoms typical of the combined action of the two viruses.

REACTION OF SELECTED HOSTS AT VARIOUS TEMPERATURES TO CABBAGE VIRUS A AND THE BLACK RING VIRUS

When cabbage virus A and the black ring virus were studied over an extensive host range (16) certain hosts were noted on which the two viruses acted somewhat differently. These were studied further at 16°, 20°, 24°, and 28° C. to determine which could be used for cross-immunity tests at one or another temperature.

Nicotiana glutinosa L. The reactions of the two viruses on N. glutinosa differed only in minor respects, but the effect of temperature upon symptom expression was most striking. With both viruses the effect upon the host plant was most severe at 16° and 20° and least so at 28°, a temperature reaction quite the reverse of that noted for cabbage. Moreover, the type of symptom changed with increase in temperature. At 16° primary symptoms were necrotic flecks with chlorotic halos, while systemic symptoms were vein clearing, mottle, stunting, and necrosis. At 20° the symptoms were of the same general type but more severe. At 16° and 20° black ring symptoms were slightly more severe than those of virus A. At 24° there was a shift to more mottle, less necrosis and less stunting. At 28° primary symptoms were small necrotic flecks around which conspicuous zonate rings slowly developed. Similar ring spot symptoms occurred systemically (fig. 3.) The youngest leaves showed no symptoms (even after 90 days' incubation), and it was only when leaves were separated from the growing point by four to six internodes that symptoms appeared. Repeated attempts to recover the viruses from the symptomless young leaves were unsuccessful, even after 50 days' incubation. Recovery tests from leaves showing symptoms yielded the viruses in fair concentration. No necrosis, mottling, or distortion occurred at this temperature.

Thus on this host completely different types of symptoms were produced at different temperatures, the mildest symptoms occurring at 28° C. Since Nicotiana glutinosa L. was favored by relatively high
temperatures, and since each virus produced its greatest effects at temperatures unfavorable to the host and its least effects at temperatures favorable to the host, it would seem that the temperature effect upon the host-virus relationship was largely that of the temperature effect upon the host. This condition has its counterpart in the effect of temperature on the reaction of cabbage to the A and black ring viruses. Cabbage makes much better growth at 16° and 20° than at 28° C. It will be remembered that the symptom severity of the two viruses on cabbage decreased with decrease in temperature except for the increase in necrosis with the black ring virus at 20°. It is often stated that plants in a vigorous growing condition develop more severe symptoms than plants in a less active state. If this be true, then the reaction of *N. glutinosa* is exceptional, both in the mild symptoms produced and in the slowness with which the two viruses invaded actively growing young leaves at 28°.

It is evident that there must be also a temperature effect upon the activity of the viruses themselves. It was pointed out earlier that both viruses show incubation periods on cabbage, a low temperature plant, which increase in length with decrease in temperature. On tobacco, a high temperature plant, the same viruses also show incu-
bation periods which increase in length with decrease in temperature. However, as will later be shown, symptoms on Nicotiana rustica appear concurrently at low and high temperatures. Furthermore, on many hosts virus A and the black ring virus do not show the same temperature reaction in regard to the length of the incubation period. Walker et al. (16) pointed out that the black ring virus produces more necrosis than does virus A. This production of necrosis, especially on cruciferous plants, is restricted almost entirely to temperatures below 24° C. On many hosts this virus is more severe at 20° than at 28°, whereas virus A almost without exception is most severe at 28°. These facts clearly indicate that, as regards temperature effects, the reaction on any host is due to the temperature effect upon the specific host-virus combination and maybe due to the temperature effect upon the host, the virus, or both. The last of these three possibilities is probably the one which usually prevails.

Nicotiana rustica L.

When Nicotiana rustica was used the symptoms at all four temperatures appeared almost simultaneously. At 24° and 28° differences between the two viruses were slight. The black ring virus produced primary circular chlorotic lesions at 24° and 28°; virus A produced them indistinctly and only at 24°. Both produced zonate chlorotic rings and solid chlorotic spots systemically. At 16° and 20° the reactions of the two viruses were markedly different. The black ring virus produced numerous necrotic primary lesions and numerous chlorotic systemic lesions, 4 to 5 mm. in diameter and bordered by necrotic dots; stunting and leaf distortion were marked. The primary lesions caused by virus A were largely scattered chlorotic lesions while the systemic lesions were larger, fewer in number, and stunting was less pronounced. The systemic lesions of the two viruses are compared in figure 4.

Nicotiana multivalvis Pursh

On Nicotiana multivalvis the differences were most pronounced at 16° C., where virus A produced no primary symptoms while the black ring virus produced a conspicuous pattern of small white rings and ring spots. Systemically the symptoms were most severe at 16°, decreasing with increase in temperature as with N. glutinosa but without any marked change in symptom type. The black ring virus was the more severe at 16°, the difference becoming less pronounced with increase in temperature.

Wild Mustards

The effects of the two viruses on young plants of Brassica juncea (L.) Coss. (Indian mustard), B. nigra (L.) Koch (black mustard), B. arvensis (L.) Ktze. (charlock), and B. campestris L. (wild yellow mustard) at 20° were easily distinguishable (fig. 5). The black ring virus produced numerous, angular necrotic lesions on inoculated leaves. Necrosis spread, became systemic, and plants died within 3 weeks after inoculation. Except on charlock, virus A produced no primary necrotic lesions, and plants were not killed until several days
after death occurred to black-ring-infected plants. Symptoms were markedly milder than those produced by the black ring virus. At 28° disease development was so rapid with both viruses that plants were killed within 5 to 8 days after inoculation.

*Brassica oleracea* var. *gemmifera* DC. (Brussels sprouts)

At 16° and 20° C, symptoms of the two viruses on Brussels sprouts (var. Long Island Mammoth) were markedly different. The black ring virus produced a severe necrosis on inoculated leaves as circular lesions which expanded and coalesced, causing the entire leaf to die. Systemic symptoms appeared as circular chlorotic rings and spots which rapidly became necrotic to produce a conspicuous pattern of black rings and streaking of the veins. Virus A produced only chlorotic lesions, or no symptoms at all, on inoculated leaves and a systemic mottle which was very mild and never became necrotic until 4 to 5 weeks after inoculation. At 20° the effects of the two viruses were so different that they might easily have been considered as two distinct diseases (fig. 6). At 28° the symptoms of the two viruses were indistinguishable.
Differentiation of Certain Crucifer Viruses

FIGURE 5.—Symptoms produced on inoculated leaves of Indian mustard (B. juncea) at 20° C.: A, Virus A; B, black ring virus. Note the extreme difference in the effects produced by the two viruses.

FIGURE 6.—Systemic symptoms produced on Brussels sprouts at 20° C.: A, Mild mottle caused by virus A; B, Severe necrotic pattern produced by the black ring virus.
REACTION OF VARIOUS HOSTS TO CABBAGE VIRUS B AND CAULIFLOWER MOSAIC VIRUS

Brassica pekinensis (Lour.) Rupr. (Chinese cabbage)

On Chinese cabbage (var. Chihli) at 16° C. the cauliflower mosaic virus produced symptoms after 18 to 21 days as a conspicuous chlorotic vein clearing. This symptom became progressively more pronounced, and leaf midribs developed a marked curvature. Leaf laminae became severely ruffled and stunted, resulting in a conspicuous rosette in which a few old leaves showed no malformation, while severely stunted and distorted young leaves formed the central cluster. Virus B produced symptoms some 7 to 10 days later than the cauliflower mosaic virus. These appeared as vein clearing and leaf curvature and were not unlike the symptoms caused by the cauliflower mosaic virus except that severe stunting and rosetting did not occur. These much milder symptoms served as a sharp point of distinction between the two viruses. At 20° and 24° the symptoms of the two viruses became increasingly more alike and at 28° they were indistinguishable. In general, rosetting and vein clearing decreased with increase in temperature while chlorosis and stunting increased. As a means of differentiating the two viruses this host served best at 16°.

Wild Mustards and Rape

The reactions of the two viruses at 20° and 28° on young plants of Brassica arvensis (charlock), B. campestris (wild yellow mustard), B. juncea (Indian mustard), B. hirta Moench, (B. alba (L.) Rabenh. (white mustard)) and B. napus L. (rape var. Dwarf Essex) were studied. At 20° symptoms of the cauliflower mosaic virus became apparent in 12 to 15 days as a conspicuous chlorotic vein clearing and mild mottle. Young leaves exhibited a marked curvature of the midrib and a severe ruffling and stunting of the lamina. These severely stunted and inrolled young leaves produced a marked rosette on all hosts. Many plants of the wild mustard hosts were ultimately killed. Symptoms of virus B appeared 2 to 4 days later than those of the cauliflower mosaic virus as vein clearing followed by mild mottle with curving and wrinkling of the leaves. No marked rosette of young leaves developed on any of the hosts, and symptoms were much milder than those caused by the cauliflower mosaic virus.

At 28° the cauliflower mosaic virus again produced symptoms earlier and much more severe than did virus B. Stunting, distortion, mottling, and chlorosis were more severe, but rosetting was less pronounced than at 20°. Symptoms were very severe and most plants were finally killed. At both 28° and 20° the two viruses were easily separable by the degree of symptom severity rather than by the type of symptom.

HOST IMMUNITY REACTIONS IN CABBAGE

In the preceding section differential temperature reactions between virus A and the black ring virus and between virus B and the cauliflower mosaic virus on several hosts were described. As a result of these critical studies of the effects of temperature on symptom expression, certain host reactions were found which differentiated between the viruses in question so distinctly that they were selected
for use in cross-immunity tests. The efficacy of these selected hosts in differentiating between the viruses in question depended upon the use of a temperature which insured the expression of the differentiating symptoms. Although the differentiation was more distinct at one specific temperature, the hosts used were tested at all four temperatures to widen the scope of the test.

**Specific Immunity Against the Black Ring Virus Produced by Virus A**

Immunological tests involving virus A and the black ring virus were set up as follows: Each virus was inoculated to 10 cabbage plants. After all plants developed systemic symptoms the A-infected plants were reinoculated with the black ring virus, and the plants infected with the black ring virus were reinoculated with virus A, inoculations being made on systemically infected leaves. At the same time 10 healthy plants of the same age were inoculated (to serve as controls) with each of virus A, the black ring virus, and a mixture of the 2 viruses. Two weeks after systemic symptoms appeared in the 3 groups of control plants inoculations were made from all of the 5 groups of 10 plants each to *Nicotiana multivalvis*, *N. rustica*, and *Brassica oleracea* var. *gemmafera* at temperatures of 16°, 20°, 24°, and 28°. Extracts were taken from young leaves above inoculated leaves.

Symptoms produced on plants inoculated with extracts taken from the A-infected plants reinoculated with the black ring virus agreed in every respect with symptoms produced on these hosts by virus A alone. On all hosts at all temperatures symptoms showed no resemblance to those of the black ring virus but, on the contrary, were exactly identical with those of virus A as described in the preceding section. Correspondingly, extract from cabbage plants infected with the black ring virus and reinoculated with virus A produced symptoms exactly like those of the black ring virus alone. This, of course, was expected since the more severe symptoms of the black ring virus would cover up the mild symptoms of virus A if virus A were present. Figures 7 and 8 show the results of the inoculations to Brussells sprouts and *N. rustica*, respectively.

Inoculum taken from plants infected with the A-black-ring mixture produced symptoms similar to those of the black ring virus alone. This indicated that virus A does not inhibit the establishment of the black ring virus when the two are introduced into the plant simultaneously.

In another test 40 young Brussels sprouts plants were inoculated with virus A and incubated at 20°. After plants developed systemic symptoms, 20 of the infected plants were reinoculated with the black ring virus. At the same time 20 healthy plants of the same age were inoculated with the black ring virus. After 10 days the healthy plants inoculated with the black ring virus had developed a severe necrosis on inoculated leaves, and subsequent systemic necrosis was severe and typical of the black ring virus. Plants which were infected with virus A and reinoculated with the black ring virus developed no necrosis on inoculated leaves and no systemic necrosis until several days after systemic necrosis appeared in the black ring control plants.

In still another test 10 plants of *Nicotiana multivalvis*, infected with
FIGURE 7.—A, Systemic symptoms produced on Brussels sprouts at 16° C. when inoculated with extracts taken from cabbage plants used in the cross-immunity tests of virus A and the black ring virus. A, Leaves from plants inoculated with: a, Extract taken from cabbage infected with black ring virus alone; b, extract from cabbage infected with virus A alone; c, extract from cabbage infected with black ring virus and reinoculated with virus A; d, extract from cabbage infected with virus A and reinoculated with the black ring virus, respectively. B, primary symptoms produced on inoculated leaves (e, f, g, h) of same plants.

virus A and showing only a mild systemic mottle, were inoculated with the black ring virus. At the same time an equal number of healthy plants of the same age were inoculated with the black ring virus. After 5 days at 24° the healthy plants inoculated with the black ring virus developed numerous white rings and ring spots on inoculated leaves. Plants infected with virus A and reinoculated with the black ring virus failed to develop primary symptoms.

These tests would indicate a strain relationship between virus A and the black ring virus if the failure of the black ring virus to establish itself in plants infected with virus A were due to a specific acquired immunity.

Specific Immunity Against Virus A Produced by the Black Ring Virus

In an extensive host range study of virus A and the black ring virus (16) one host was found which appeared to be completely differential for the two viruses. This host, Solanum integrifolium Poir. (Chinese scarlet eggplant), was found to develop conspicuous, necrotic, primary lesions when inoculated with virus A at 28°, 24°, and 20°. This necrosis became systemic and appeared as numerous lesions 2 to 4 mm. in diameter and as streaking of the veins on young leaves. Leaf abscission resulting from the necrosis was marked. No mottle symptom developed. In four separate trials the black ring
FIGURE 8.—Symptoms produced on inoculated leaves of Nicotiana rustica at 16° C., with extracts taken from cabbage plants used in cross-immunity tests of virus A and the black ring virus. Leaves taken from plants inoculated with: A, Extract taken from cabbage infected with virus A alone; B, extract from cabbage infected with black ring virus alone; C, extract from cabbage infected with virus A and reinoculated with the black ring virus; D, extract from cabbage infected with black ring virus and reinoculated with virus A.
virus produced no symptoms, and attempts to recover the virus from the symptomless plants were unsuccessful.

No symptoms were produced on 27 plants of *Solanum integrifolium* with virus taken from cabbage plants infected with the black ring virus and inoculated with virus A. Neither did extracts from the plants infected with the black ring virus alone produce symptoms. Virus taken from cabbage plants infected with virus A and inoculated with the black ring virus produced symptoms similar to those of virus A alone (fig. 9). Inoculum taken from cabbage plants infected with the A-black-ring mixture produced symptoms similar to those produced by virus A, indicating that the black ring virus does not inhibit the establishment of virus A in plants when the two viruses are introduced into the plants simultaneously. Thus in this test (fig. 9, C) the black ring virus did not prevent the establishment of virus A in systemically infected cabbage plants. However, when cabbage plants were first inoculated with black ring virus and then inoculated with virus A the latter produced no symptoms (fig. 9, D).

**Specific Immunity Against Cauliflower Mosaic Virus Produced by Virus B**

To test the ability of virus B to protect cabbage plants against infection with the cauliflower mosaic virus a test similar to that just described for the A and black ring viruses was used. Ten young cabbage plants were inoculated with each of virus B and the cauliflower mosaic virus. After systemic symptoms appeared in all plants the B-infected plants were inoculated with the cauliflower mosaic virus, and the plants infected with the cauliflower mosaic virus were inoculated with virus B. At the same time virus B, cauliflower mosaic virus, and a mixture of the 2 viruses were each inoculated to 10 healthy plants of the same age. Two weeks after systemic symptoms appeared in the 3 groups of control plants, inoculations were made from each of the 5 groups of 10 plants to Chinese cabbage plants at 16°, 20°, 24°, and 28° C., a minimum of 5 test plants being used in each inoculation. The experiment was repeated 3 times and gave the same results each time.

Symptoms produced by an extract taken from B-infected plants which were inoculated with cauliflower mosaic virus were identical at each temperature with those produced by virus taken from plants infected with virus B alone. Symptoms produced by extracts taken from plants infected with the cauliflower mosaic virus alone and from plants infected with the cauliflower mosaic virus but later inoculated with virus B were identical. Inoculum taken from cabbage plants infected with a mixture of the 2 viruses produced symptoms characteristic of the cauliflower mosaic virus. In figure 10 are shown the results obtained in one of the experiments. One month later a new series of inoculations was made from the same groups of cabbage plants to young Chinese cabbage plants. Results similar to those in the first inoculations were obtained. These tests show that cabbage plants systemically infected with virus B are protected by the virus against infection by the cauliflower mosaic virus and indicate a strain relationship between virus B and the cauliflower mosaic virus.
FIGURE 9.—Symptoms produced on inoculated leaves of *Solanum integrifolium* with extracts taken from cabbage plants used in the cross-immunity tests of the A and black ring viruses. Leaves from plants inoculated with: A, Extract taken from cabbage infected with virus A alone; B, extract from cabbage infected with black ring virus alone; C, extract from cabbage infected with virus A and reinoculated with the black ring virus; D, extract from cabbage infected with black ring virus and reinoculated with virus A. Note that the black ring virus produced no symptoms.
FIGURE 10.—Symptoms produced on Chinese cabbage at 20° C. 30 days after inoculation with extracts taken from cabbage plants used in cross-immunity tests of virus B and the cauliflower mosaic virus. 

A, Extract taken from cabbage infected with virus B alone; 
B, extract from cabbage infected with the cauliflower mosaic virus alone; 
C, extract from cabbage infected with virus B and reinoculated with cauliflower mosaic virus; 
D, extract from cabbage infected with cauliflower mosaic virus and reinoculated with virus B; 
E, uninoculated control; 
F, extract from cabbage inoculated with a mixture of virus B and cauliflower mosaic virus. 
Note that extract taken from plants infected with virus B reinoculated with cauliflower mosaic virus produces symptoms indistinguishable from those of virus B alone.
DISCUSSION

The effect of temperature upon host-virus relationships has received disproportionately little attention in comparison with other phases of virus investigations. Many comparisons of viruses today are based upon symptoms and properties of the viruses. Evidence presented in this paper shows that type and intensity of symptoms may depend greatly upon the temperature to which the host plants are exposed. Other experiments under way indicate that temperature may radically alter the activity and concentration of the virus within the host. Since the determination of the physical properties of the viruses is influenced by the concentration of the virus in the expressed sap, it is suggested that the temperature under which they develop in the host may indirectly affect the physical properties of the virus as ordinarily determined. Thus in making comparison in symptomatology and properties of viruses prime consideration should be given to the temperatures of the environments in which the symptoms develop and the viruses multiply. High temperature is often reported to reduce the severity of a given virus. Observations reported herein indicate that the effect of temperature upon disease severity depends upon the specific host-virus complex and may be due to the temperature reaction of the host, virus, or both.

The effect of temperature upon host-virus reaction may have considerable bearing on virus classification and nomenclature. The inadequacies of a system of classification based primarily upon symptomatology are revealed by the reactions of cabbage viruses A and black ring on Nicotiana glutinosa and other hosts at different temperatures. For instance, the symptoms of these viruses on N. glutinosa at 28°C include those given by Holmes (4) for his family, Annulaceae, while the symptoms produced on this host at 16°C are typical of those given for the family Marmoraceae. The name black ring is very appropriate for the reactions of the black ring virus on crucifers at low temperatures but very inappropriate for symptoms at high temperatures.

Many investigators have tested numerous host plants with certain viruses in the hope of finding hosts which would give a differential reaction to related viruses or a local-lesion reaction which could be used in quantitative measurement of the viruses. Results given in this report show that by testing related viruses on selected hosts at various temperatures differential reactions may be obtained which may be more marked than those obtained by testing the viruses on a large number of hosts at only one temperature. Likewise, temperature studies may provide a local-lesion reaction which could not be found by trying several hosts at only one temperature. These values of temperature studies are well illustrated by the reaction of Nicotiana rustica to the cabbage A and the black ring viruses. At high temperatures the two viruses were not easily distinguishable on this host, but at low temperatures their reactions were very different. Furthermore, the reactions of the black ring virus at 16°C and 20°C were such that they could be easily applied to quantitative studies of the virus itself. Many hosts were found in this and other investigations (16) that gave a local-lesion reaction to this virus at low temperatures but not at high temperatures. Most of the differentiating symptoms used
in the immunity reactions reported in this paper resulted from finding
differential temperature reactions of the viruses in question.

The method of measuring specific acquired immunity employed in
this investigation proved just as effective as the ordinary local-
lesion-mottle test although it involved more work. Its effectiveness
shows that where two related viruses have a differential reaction on
any host their ability to immunize plants against each other can be
tested in any plant in which they develop systemically.

Significant features in the properties and host range of various
crucifer viruses are presented in table 2. These comparisons are made
in order to focus attention upon possible relationships in this hetero-
genous group of viruses. Although the writers are of the opinion
that these viruses fall into two distinct groups, it is realized that any
such relationships can be truly ascertained only by parallel studies in
the same laboratory. However, the viruses do show enough common
characteristics to justify a limited amount of speculation regarding
their relationships.

A study of table 2 reveals that in properties the first 14 viruses
listed fall more or less into 1 group, the properties of which are limited
by the following values: Aging in vitro, 48–384 hours; dilution,
1–600 to 1–100,000; thermal inactivation, 50°–68° C. The greatest
variation in these properties lies in the measure of dilution tolerance.
The fact that different test plants were used might account for some
of this variation. It is the writers’ experience that, with cabbage
virus A, infection on tobacco can be obtained at a lower concentra-
tion than on cabbage. This may be due to chance since much more
leaf area is covered in inoculating 10 tobacco plants than in inocu-
lating 10 cabbage plants. Furthermore, in only 1 trial was 1–100,000
the dilution inactivation point; and in each case in which inactiva-
tion was reached only at 1–50,000, the highest value at which infec-
tion occurred was 1–10,000. The property which varies the least is
thermal inactivation. Ten of the fourteen viruses have inactivation
points within 6° of each other. With 3 of the remaining 4 viruses
inactivation was obtained by heating at intervals of 3° to 5°. Had
closer measurements been made this variation would probably have
been reduced.

In regard to host range, the 14 viruses show many points of simi-
larity, and yet each differs from the others. All infect one or more
solanaceous and chenopodiaceous hosts. Local necrotic lesions are
produced on tobacco by all but the T₁ strain of turnip mosaic (7).
The virus of radish mosaic (13) is unique in that it becomes systemic
in this host. Within the Cruciferae it is significant that only virus
A, black ring virus, ring necrosis virus (6), the T₃ strain of turnip
mosaic (7), the turnip virus of Chamberlain (2), and the radish mo-
saic virus (13) are widely pathogenic on subspecies of Brassica oler-
acea, although cabbage was the only subspecies tested with turnip
virus 1 (3). Of the turnip mosaic viruses only the strain of Cham-
berlain (2) was pathogenic on wallflower; all were pathogenic on black
mustard and rape except Tompkins’ strain; only strain T₃ and T₉ (7)
affected kohlrabi. Neither of the stock viruses (12) was pathogenic on
members of B. oleracea. The mild stock mosaic virus affected wall-
flower.

In view of the points of similarity between these 14 viruses it seems
more logical to consider them as strains of one virus group rather than
<table>
<thead>
<tr>
<th>Virus</th>
<th>Properties</th>
<th>Reaction on selected hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage A (16)</td>
<td>1-10,000</td>
<td></td>
</tr>
<tr>
<td>Cabbage black ring (16)</td>
<td>1-1,000</td>
<td></td>
</tr>
<tr>
<td>Cabbage rine necrosis (6)</td>
<td>1-600</td>
<td></td>
</tr>
<tr>
<td>Turnip mosaic (8)</td>
<td>1-100,000</td>
<td></td>
</tr>
<tr>
<td>Turnip mosaic (11)</td>
<td>1-4,000</td>
<td></td>
</tr>
<tr>
<td>Turnip mosaic (2)</td>
<td>1-1,000</td>
<td></td>
</tr>
<tr>
<td>Turnip mosaic T&lt;sub&gt;1&lt;/sub&gt; (7)</td>
<td>1-50,000</td>
<td></td>
</tr>
<tr>
<td>Turnip mosaic T&lt;sub&gt;4&lt;/sub&gt; (7)</td>
<td>1-50,000</td>
<td></td>
</tr>
<tr>
<td>Turnip mosaic T&lt;sub&gt;6&lt;/sub&gt; (7)</td>
<td>1-5,000</td>
<td></td>
</tr>
<tr>
<td>Turnip mosaic T&lt;sub&gt;7&lt;/sub&gt; (7)</td>
<td>1-5,000</td>
<td></td>
</tr>
<tr>
<td>Severe stock mosaic (18)</td>
<td>1-1,000</td>
<td></td>
</tr>
<tr>
<td>Mild stock mosaic (10)</td>
<td>1-5,000</td>
<td></td>
</tr>
<tr>
<td>Rape mosaic (6)</td>
<td>1-7,000</td>
<td></td>
</tr>
<tr>
<td>Radish mosaic (18)</td>
<td>1-15,000</td>
<td></td>
</tr>
<tr>
<td>Cabbage B (16)</td>
<td>1-1,500</td>
<td></td>
</tr>
<tr>
<td>Cauliflower mosaic (10)</td>
<td>1-3,000</td>
<td></td>
</tr>
<tr>
<td>Broccoli mosaic (1)</td>
<td>1-3,000</td>
<td></td>
</tr>
<tr>
<td>Chinese cabbage mosaic (14)</td>
<td>1-6,000</td>
<td></td>
</tr>
</tbody>
</table>

1 Values given are points at which inactivation occurred.
2 S=Systemic infection; L=local reaction; O=no infection; no symbol=host not tested.
3 Inactivation was at 1-10,000 in 4 out of 5 trials.
as distinct viruses. The strain relationship of cabbage virus A and the black ring virus has been proved. These two viruses are considered as strains of turnip virus 1 (S) in view of the close similarities in symptoms, host range, and properties plus the fact that both virus A and turnip virus 1 were first obtained from the same area in southeastern Wisconsin. Turnip virus 1 is chosen as the type virus of the group because it was the first of the crucifer viruses for which critical property studies were made. Le Beau and Walker (7) have also considered turnip viruses T₁, T₆, T₈, and T₉ as strains of turnip virus 1. That the ring necrosis virus is a member of this group there is little doubt. It is the writers' opinion that the turnip viruses of Tompkins (11) and of Chamberlain (2), the two stock viruses, the rape mosaic virus (8), and the radish mosaic virus are also members of this group. The virus causing the ring spot disease of cabbage described by Smith (9) is very probably another strain of this group, but since no properties were given for this virus a close comparison with turnip virus 1 cannot be made.

Cabbage virus B, the cauliflower mosaic virus, the Chinese cabbage mosaic virus (14), and the broccoli mosaic virus (1) comprise a group that is quite distinct from the turnip virus 1 group in properties, symptoms, and host range. These viruses are confined to the Cruciferae except for the local reactions of the Chinese cabbage virus on tobacco and Nicotiana glutinosa. Their characteristic symptoms are vein clearing and vein banding. The strain relationship of virus B and the cauliflower mosaic virus has been proved, and virus B is classed as a strain of cauliflower virus 1. In parallel inoculations to a few selected hosts in this laboratory the Chinese cabbage virus appeared to be quite similar to virus B and the cauliflower mosaic virus both in symptoms and in its tendency to become masked in cabbage at 28°C.

There appear, then, to be two groups of crucifer viruses, one group being represented by turnip virus 1 and the other group by cauliflower virus 1. This and other studies (1, 3, 6, 7) indicate that members of the turnip virus 1 group are favored by relatively high temperatures, while those of the cauliflower virus 1 group are favored by relatively low temperatures. With each group, masking tends to occur at unfavorable temperatures. Strains within one group may show variation in their individual temperature reactions. For example, on some hosts virus A is distinctly more severe than the black ring virus at 28°C and 16°C. On other hosts the two are practically indistinguishable at high temperatures, but at low temperatures their reactions are widely different, owing chiefly to the increased severity of the black ring symptoms. It is apparent, then, that the black ring virus is favored by lower temperatures than some other members of this group. Little differential temperature reaction has been observed among members of the cauliflower virus 1 group.

**SUMMARY**

When the reactions of cabbage virus A, cabbage black ring virus, cabbage virus B, and cauliflower mosaic virus on cabbage were studied at various temperatures, it was found that they fell into two distinct groups.
In the turnip virus 1 group, containing cabbage virus A and the black ring virus, the progress and severity of disease development varied directly with the air temperature to which the plants were exposed, symptoms (except for necrosis with the black ring virus) being most severe at 28° C. and mildest at 16°. The characteristic symptom of both viruses was a coarse chlorotic mottle accompanied by leaf malformation. However, some marked differences between the reactions of the two viruses to temperature were observed. Symptoms of virus A were distinctly more severe than those of the black ring virus at 28° and 24°, but at 20° and 16° the exact reverse was true.

In the cauliflower virus 1 group containing cabbage virus B and the cauliflower mosaic virus, symptom intensity was also found to be directly proportional to the air temperature. However, in contradistinction to the turnip virus 1 group, symptom intensity increased with decrease in temperature and complete masking occurred at 28° and 24°. The change in reaction with change in temperature was exactly parallel with both viruses. The characteristic symptoms of these viruses were chlorotic vein clearing and vein banding.

When either virus A or the black ring virus occurred in cabbage together with either virus B or the cauliflower mosaic virus, the resulting disease reaction was more severe than that produced by either virus alone. The increased severity of symptoms was so pronounced at 28° and 24° that they appeared as those of an entirely different disease. At low temperatures the activity of virus A or the black ring virus was so reduced that combination symptoms agreed very closely with those of virus B or of the cauliflower mosaic virus. In such combinations the black ring virus reacted very similarly to virus A, and the cauliflower mosaic virus reacted similarly to virus B. When the temperature at which plants infected with a virus combination were growing was reversed from high to low, or vice versa, there resulted a corresponding reversal in symptom type.

At high temperatures symptoms produced by virus A on Brassica oleracea var. gemmifera, Nicotiana rustica, and N. multivalvis were practically indistinguishable from those produced by the black ring virus, but at low temperatures the reactions of the two viruses on these hosts were markedly different. A striking effect of temperature on the type of symptoms produced by these two viruses on N. glutinosa is described.

By use of the differential reaction between virus A and the black ring virus on the hosts mentioned above, virus A was shown to effectively immunize cabbage against infection by the black ring virus. Likewise, the black ring virus protected cabbage plants against infection from virus A, as measured on Solanum integrifolium, a host which was completely differential for virus A. Both virus A and the black ring virus are classed as strains of turnip virus 1.

At both high and low temperatures the reaction of the cauliflower mosaic virus was much more severe than that of virus B on such hosts as Brassica pekinensis, B. nigra, B. napus, B. arvensis, and B. campestris. By the use of the differential reaction on B. pekinensis, virus B was shown to immunize cabbage against infection by the cauliflower mosaic virus. Virus B is classed as a strain of cauliflower virus 1.
LITERATURE CITED


INFORMATION IN REGARD TO THE POLICY OF THE JOURNAL OF AGRICULTURAL RESEARCH AND SUGGESTIONS TO AUTHORS

1. The Journal accepts articles only from the United States Department of Agriculture and the State agricultural experiment stations.

2. Each article submitted must bear the formal approval of the chief of the department bureau or the director of the experiment station from which it emanates. The letter of transmittal must state that the manuscript has been read and approved by one or more persons (named) familiar with the subject, that the data as represented by the tables, graphs, summaries, and conclusions have been approved from the statistical viewpoint by someone (named) competent to judge, and that the computations have been verified.

3. Manuscripts originating at the State agricultural experiment stations should be forwarded to the chairman of the committee acting for the Association of Land-Grant Colleges and Universities, and those originating in the Department should be transmitted to the Division of Publications, which will forward them for approval to the committee, acting for the Department. Each manuscript is numbered and edited in the order received.


5. A recent copy of the Journal should be consulted and followed as to style, especially in regard to tables, illustrations, and literature citations.

6. Paper 8 x 10½ or 8½ x 11 inches, of good grade and medium weight, should be used.

7. All material except tables and quotations of more than three lines should be double-spaced. These may be single-spaced.

8. A table of contents properly indented to show the intended relationship between the different headings should accompany the manuscript.

9. Following the name of the author on the first page there should be given his official title and the name of the division, bureau, or station with which he is connected.

10. Each page of the manuscript should be numbered and should begin with a new paragraph; that is, no paragraph should carry over from one page to the next unless it is longer than one page.

11. Each footnote should be inserted in the text immediately after the line bearing the footnote reference.

12. Each table should be typed on a separate sheet, or on several if necessary. The page (or pages) carrying the table should immediately follow that containing the first reference to it. Each table should be referred to in the text and be numbered in the order of reference.

13. The illustrations in the Journal are usually shown as text figures, but to bring out fine detail plates may be used. Text figures and plates are each numbered in the order of reference. Each text-figure legend should be inserted in the text underneath the line carrying the first reference to it. Legends for plates should accompany the manuscript but should not be inserted in the text. All legends should be double-spaced and furnished in duplicate.

14. The major parts or units of illustrations are designated by capital italic letters; the subparts or subunits by lower-case italic letters. No final lettering on illustrations should be attempted, particularly on photographs. All lettering and necessary drafting will be done in the Section of Illustrations of the Division of Publications. Required letterings or markings should be indicated in the margins or lightly in pencil on the illustrations.

15. Graphs should be sent in final form, if possible, except for the lettering. If prepared in tentative form the curves and bars should be carefully indicated so that they may be accurately redrawn.

16. The plate or figure number and the title of the accompanying manuscript should be lightly written (not typed) on the back of each illustration. All photographs should be submitted unmounted, enclosed in an envelope.

17. Only references cited in the text should be listed in the literature citations. If there are seven or more they should be given at the end of the paper under the heading "Literature Cited." If fewer than seven they should be given as footnotes. All numbers referring to literature citations should be enclosed in parentheses in the text. The footnote reference to the first citation in the manuscript should be worded as follows: "Italic numbers in parentheses refer to Literature Cited, p. —." Material under Literature Cited should be double-spaced.

18. For further information consult Miscellaneous Publication No. 3 issued by the Joint Committee on Policy and Manuscripts. It may be obtained from the Division of Publications, United States Department of Agriculture.
One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of a special committee of the American Library Association headed by John E. Russell, the Librarian of the University of Rochester.

Because of the imminent paper shortage, attempts are being made to collect old periodicals for pulp. The Committee hopes to enlist the cooperation of subscribers to this Journal in preventing the sacrifice of this type of material to the pulp demand.

Questions concerning the project or concerning the value of particular periodicals should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.