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IDENTITY OF A CHLOROTIC RING-SPOT VIRUS FROM KALE[1]

(*Brassica oleracea* var. *acephala*)

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INTRODUCTION

A mosaic disease of kale (*Brassica oleracea* var. *acephala*) was found in vicinity of Ithaca, N. Y., by Dr. R. E. Wilkinson of the Cornell Plant Pathology Department, late in the fall of 1948. This mosaic is characterized by systemic mottling and chlorotic ring-spots on the leaves.

The occurrence of the disease in kale and perhaps in other crucifers in upper New York may be considered sporadic in nature and of no economic significance. The viral entity apparently is endemic in the State and has escaped recognition in cruciferous suscepts grown when the environment is not favorable for the multiplication of the virus, nor for its dissemination. There is the possibility, also, of its being a strain of low virulence, causing only mild symptoms on most crucifers.

Crucifer diseases due to viruses are considered, so far, of little economic importance in New York State. The first observation of mosaic in a crucifer was made by Stewart (15)[3] on cabbage (*B. oleracea* var. *capitata* L.), in 1910. The disease was characterized by necrotic flecks of the outer and inner head leaves. No mention was made of the nature of the disease.

Cauliflower (*B. oleraceae botrytis* L.) mosaic was first reported by Thatcher in 1927 (17). Clayton (2) described in 1930 a mosaic of rutabagas (*B. campestris* (L) *napobrassica* DC) which occurred commonly in the Port Jefferson section on Long Island. This mosaic has never attained economic importance on Long Island, nor elsewhere in the State. The highest proportion of diseased plants in the field never went beyond 10 percent, and this figure represented late infections in the growing season. Clayton indicated that rutabaga mosaic will never be of economic importance on Long Island, because the crucifers are grown when the temperature is relatively low and unsuited for development of the disease. He further said that, because of the usually mild winters on Long Island and of the fact that brussel

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[3] Numbers in parentheses refer to Literature Cited, p. 125.

sprouts (*B. oleracea* L. *gemmifera* DC) and cabbage are both relatively winter-hardy, they are the cruciferous suscepts that serve as primary sources of inocula for the next growing season.

The purpose of this paper is to present the data collected in regard to symptoms, suscept range, incubation period, and physical properties of a virus responsible for the mosaic of kale, and to express an opinion on the nature of the viral entity in agreement with recorded observations.

REVIEW OF LITERATURE

Cruciferous viruses tend to fall into two marked categories (11); one represented by the turnip virus 1 Hoggan and Johnson, including the cabbage virus A (22), the cabbage black ring virus (4), the cabbage ring necrosis virus (8), turnip virus described by Tompkins (19), Hoggan and Johnson's turnip virus (7), turnip virus described by LeBeau and Walker (9), and perhaps many others; the second represented by the cauliflower virus 1 Tompkins, which includes cabbage virus B (22), the cauliflower mosaic virus (18), Tompkin's Chinese cabbage virus (21), and the broccoli virus described by Caldwell and Prentice (1).

The first publication on a transmissible virus of cruciferous suscepts was made in 1921 when Gardner and Kendrick (3) obtained mechanical transmission of a virus from a mosaic-affected turnip to other crucifers, but not to radish (*Raphanus sativus* L). Schultz (13) using mechanical means and the vector *Myzuz persicae* (Sulz.) successfully transmitted from suscept to suscept a virus causing mosaic in turnip (*B. rapa* L.), Chinese cabbage (*B. pekinensis* (Lour) (Gagn), and mustard (*B. japonica* (Thumb) Sieb). Chinese cabbage mosaic has been reported from many parts of the world. Tompkins and Thomas (21), reported the disease from California and did extensive work on it. Turnip and rutabaga mosaic also is widespread and has been the object of studies by Tompkins (19). Reports of mosaic on *Raphanus sativus* caudatus (L) Bailey and on charlock (*B. arvenis*) have been made from India and Denmark (5). Noble (10) reported a cauliflower mosaic from New South Wales. He stated that it differs from the cauliflower mosaic reported by Tompkins from California. Hino (6) from Japan listed cruciferous suscepts of mosaic and mosaiclike diseases. Smith (14) reported a ringspot of cabbage from Cambridge, England, suggesting a similarity of this virus and the California virus.

In 1934 Tompkins transferred a cauliflower mosaic virus to cabbage. Similar results were obtained by Hoggan and Johnson (7) producing infections on cabbage with virus extracts from either turnips or horseradish and were the first to determine the properties of these viruses.

Tompkins (18) in 1937 reported a mosaic disease occurring on cauliflower in the cool, coastal valleys of California. In 1938 Tompkins, Gardner and

Thomas (20) described the black ring disease of cabbage, and indicated its common occurrence during the summer. Larson and Walker (8) described a ring necrosis of cabbage in Wisconsin. The presence of more than one virus entity was shown; virus A and B were found systemically in infected cabbage plants.

McWhorter (1936) reported a mosaic disease of crucifers, especially cabbage and cauliflower, in Oregon. This seems to be the same one that occurs in California.

MATERIALS AND METHODS

Plants used in the course of the work were grown in 4-inch clay pots or in flats, and kept at all times in an insect-free greenhouse at a temperature of approximately 22°C. The soil was sterilized with steam in all occasions. All material for inoculation such as cheesecloth, mortars and pestles, carborundum, test tubes, etc., were made aseptic by sterilization in the autoclave or in the Arnold sterilizer. All precautions to avoid contaminations were exercised.

Studies in the host range, the symptomatology and physical properties of the virus under consideration were conducted in the same greenhouse where the plants had been grown. Different trials were made in various compartments in the greenhouse at different air temperatures. To stimulate sap movement and new leaf formation in the plants to be inoculated, all excess leaves were removed and in some plants the process was to take away alternate leaves, or pinching off the terminal bud to promote suckering.

Mechanical inoculations were made by rubbing the inoculum with cheesecloth and with carborundum. The latter was sprayed over the leaves previous to inoculation. All plants that had not reacted to inoculation within a reasonable time were discarded. Cruciferous and noncruciferous plants with two or three pairs of leaves were inoculated and observed for virus-suscept reactions. In the case of noncrucifers, if any symptoms were detected, testing for infection was corroborated by taking juice from such plants and inoculating known cruciferous suscepts.

The physical properties of the virus were determined by treating viral extract from kale in the following manner: The inoculum was allowed to stand for a few minutes at room temperature (24°C), until the gross particles in suspension were precipitated. The supernatant liquid was removed with pipettes and immediately used to determine tolerance to dilutions and the thermal inactivation point. Part of the extract was kept in test tubes for the ageing trials. For tolerance to dilution, one cubic centimeter of the crude, decanted liquid was diluted with 9 cubic centimeters of sterile, distilled water; further dilutions being made to obtain various ratios.

In determining the thermal inactivation point, the clarified crude extract was distributed in serological, sterile tubes to about two-thirds of their capacity. Duplicates of these tubes were then treated at the desired temperatures in a thermostatically controlled water bath. For ageing, the crude, clarified extract was kept at 22°C. One tube was used each time for every required temperature.

EXPERIMENTAL RESULTS IN THE GREENHOUSE

Young crucifers and noncrucifers, with at least one pair of well developed leaves, were inoculated mechanically with infectious extracts from diseased kale. The technique described under the topic on methods was used. All crucifers were found susceptible to infection with the virus, the symptoms appearing within 10 to 20 days after inoculation. The lapse of time depended, apparently, upon the air temperature of the greenhouse and the type of plant or species utilized. The mosaic symptoms characteristic of the kale mosaic were reproduced in many of the crucifers; in others only some of the symptoms were evident. In some suscepts like rutabagas and kale, systemic mottling and chlorotic ring spots were reproduced while in cabbage and others vein-clearing, vein-banding, or mottling developed.

The data recorded strongly point toward the importance of air temperature, suscept species, and symptom expression, as well as to other events in connection with the incubation period. The crucifers reacted to inoculation within 10 to 14 days, if kept under an air temperature around 20° and took up to 20 days in a range from 14° to 17°C. No symptoms were noticed at temperatures either under 14° or above 30°C. Plants showing mosaic symptoms were taken to an environment with a temperature of less than 14°C., and the result was masking of the symptoms. When brought back to higher temperatures, the original symptoms reappeared.

In a study concerning the differentiation of crucifer viruses, Pound and Walker (11) determined that the cauliflower mosaic virus produced infection on the Jersey Queen cabbage seedlings in 18 to 21 days at a temperature of 16°C., in 14 to 17 days at 20°C., in 10 to 12 days at 24°C., and in 9 to 10 days at 28°C. These results indicate that the cauliflower mosaic virus from California and the kale mosaic virus from New York multiply more rapidly at the relatively higher temperatures. These workers have pointed out that cabbage virus B, the cauliflower mosaic virus, the Chinese cabbage mosaic virus, and the broccoli mosaic virus comprise a group confined to the Cruciferae, except for the Chinese mosaic virus which infects *Nicotiana glutinosa*. According to their work, virus B and the cauliflower mosaic viruses are related strains. It should be remembered, however, that virus B is cauliflower mosaic virus 1 category.

It has been shown by several workers (11, 12) that the severity of symptoms in infected plants varies with the species of the suscept and with the air temperature. Pound and Walker in Wisconsin noticed that mosaic-infected cabbage plants reacted with initial vein-clearing, vein-banding, and coarse mottling, and that as the temperature increased, the first two symptoms gradually disappeared, only mottling being present. When the air temperature decreased the first two symptoms again developed. They found that the Jersey Queen cabbage, when inoculated with cabbage virus A or B, reacted with severe symptoms at a temperature range from 16 to 20°C but the plants stayed symptomless at temperatures from 24 to 28°C. Indian mustard (*B. juncea*) showed mild spotting when inoculated with cabbage virus A at 20°C. Inoculation with virus B caused severe spot necrosis. *Nicotiana rustica* infected with virus A at 16°C. showed mild necrosis; with virus B severe necrosis.

In studying the mosaic disease of cauliflower Tompkins (18) found that pronounced symptoms were produced in this plant from 10 to 19° C. and that the symptoms disappeared in a temperature range from 20° to 30°C.

In determining the effect of air temperature and virus concentration Pound and Walker (11) found that in cabbage, virus A and black ring-spot virus multiplied rapidly at 28°, but very little at 16°C. Plants kept at 5°C. for 60 days showed no virus multiplication, but the titer became normal after the plants were brought back to 28°C.

The negative response to inoculation of noncrucifers, shows that the kale virus under consideration does not belong to turnip virus 1 category.

In table 1 it can be seen that *Beta vulgaris* reacted with pronounced anthocyanescence and small spot necrosis. However, the controls reacted in a similar manner. In order to clear this point, extract from beets was inoculated to cruciferous suscepts but no infection was obtained. Perhaps it is difficult to obtain the virus from beets in this way. If we examine the symptomatology and the extent of the suscept range of the viruses in the second category, it will be seen that the kale virus compares very closely with the cauliflower mosaic virus from California described by Tompkins in 1937. This investigator found that repeated inoculations with the cauliflower mosaic virus failed to infect *Nicotiana glutinosa*, *N. tabacum*, and *N. langsdorffii*. All crucifers were found susceptible and, so far, there are no suscepts from other families than the *Cruciferae*.

The possibility that the turnip virus from New York and the rutabaga virus described by Clayton (2) are identical, has been suggested (18). Tompkins found that the turnip virus causes infection on *Nicotiana tabacum*, *N. glutinosa*, rutabaga, and Pet-sai, and annual stock, but no infection was obtained on sprouting broccoli, kohlrabi, radish and *N. langdorsffii*, showing that the turnip virus and the cauliflower virus are

TABLE 1.—*Reaction of different species of cruciferous and noncruciferous plants to artificial inoculation with infectious extract obtained from kale mosaic infected plants*[1]

Family species and common name	Symptoms—	
	From 14 to 18°C.	From 20 to 27°C.
Brassica oleracea capitata L. (cabbage var Cape)	Symptoms similar to those above or marked	Light mottling, almost inconspicuous, no distortion of leaves, no necrosis
B. oleracea L. *gemmifera* DC. (brussel sprouts v. Long Island Improved)	No leaf malformation, no necrosis	Vein-clearing, later mottle
B. oleracea botrytis L. (cauliflower v. Snow Ball	Similar symptoms as for preceding species	Vein-clearing, faint mottle, no necrosis
B. oleracea gongylodes (kohl-rabi v. Early White Vienna)	Similar to above, but symptoms appeared somewhat later	Vein-clearing and systemic mottling with no leaf distortion and no necrosis
B. oleracea L. var *acephala* (kale v.)	Similar symptoms, but lighter mottling	Vein-clearing and systemic mottling, chlorotic rings, no necrosis, no distortion of leaves
B. campestris L. *napobrassica* DC. (rutabaga v. American Purple Top)	Similar symptoms as for above species	Vein-clearing and systemic mottling, chlorotic rings, no malformation, no necrosis, rings disappearing in older leaves
Raphanus sativus L. (radish v.)	Similar results as for above species	Light mottling as small chlorotic spots, no necrosis
B. rapa (L) Bois (turnip v. Flat Purple Top)	Similar results to the ones obtained with preceding species	Light mottlings as small chlorotic spot, no necrosis
B. pe-tsai Bailey (Chinese cabbage v.)	Light mottle	Vein-clearing, followed by mottling, distortion, and curvature of the midrib, stunting of plant, at high temperatures chlorotic rings and no necrosis

[1] Inoculations were made on March 7, 1949—not less than 5 plants of each species tested.

different entities and belong to different categories. Very little work has been done with the rutabaga virus and what was done did not include the study of the physical nature of the virus nor the immunological and serological reactions to compare with other similar viruses reported on crucifers.

So far the work done by Clayton (2) gives the only available description of this virus on rutabagas.

Noncruciferous, Resistant Species

The following species were tested and found not to become infected by the virus:

Nicotiana tabacum L. v. Turkish
N. glutinosa L.
N. langsdorffii Weinm.
N. rustica L.
Solanum melongena L. (American Black Beauty)
Lycopersicum esculentum Mill. (John Baer)
Datura stramonium L. (jimsonweed)
Solanum nigrum
S. integrifolium
Capsicum frutescens
Apium graveolens L. (celery)
Spinacia oleracea L.
Beta vulgaris L. (beets)
B. vulgaris var. cicla Moq. (swiss chard)
Lactuca sativa L. (lettuce v. Simpson)
Gomphrena globosa L. (bachelor's button)
Phaeseolus vulgaris L. (common bean)
Pisum stivum L. (garden pea)
Medicago sativa L. (alfalfa) (clover)
Vigna sinensis (black cowpeas)
Cucumis sativus L. (cucumber)
Zinnia elegans (zinnias)
Physalis sp.
Portullaca sp.

PROPERTIES OF THE VIRUS

In testing the physical properties of the Kale virus, infectious extract from diseased kale was applied with the help of carborundum to inoculate cruciferous suscepts. The results obtained showed that the virus has an approximately longevity in vitro of 13 to 14 days, at temperatures around 20°C. to 22°C. The virus was not inactivated at a temperature around 80°C. for 10 minutes. The tolerance to dilution was somewhere from 1:1800 to 1:2000. (See table 2.)

Tompkins (18) found that the cauliflower mosaic virus has an age in vitro of 14 to 15 days at 22°C., thermal inactivation was around 75°C, and its dilution tolerance is 1:2000. Others have reported a dilution tolerance up to 1:3000.

In accordance with the data obtained in the course of this exploratory work, it can be said that the behavior of the kale mosaic virus is very similar to that described by Tompkins (18) for the cauliflower mosaic virus from California. There is, however, an apparent disparity in the symptomatological reactions at different temperatures, the virus from kale reacting more definitely at temperatures around 20 to 22°C., while that of Tompkins ran from 10°C. to 19°C. This could perhaps be explained by the possibility that the entity from kale is a different virus, or a strain of the cauliflower mosaic virus.

TABLE 2.—*Properties of the kale virus as determined by mottling in a cruciferous suscept (Kohlrabi)*[1]

Longevity in vitro		Thermal death point		Tolerance to dilution	
Exposures at 22°C.	Reaction to inoculation	Temperature of the 10-minute exposures	Reaction to inoculation	Dilution rate	Reaction to inoculation
Days		°C.			
1	x	45	x	1:10	x
2	x	50	x	1:100	x
3	x	55	x	1:200	x
4	x	60	x	1:300	x
5	x	65	x	1:400	x
6	x	70	x	1:600	x
7	x	75	x	1:800	x
8	x	80	x	1:2000	x
9	x			1:1400	x
10	x			1:1600	x
11	x			1:1800	x
12	x			1:2000	—
13	x				
14	—				

[1] Not less than 3 plants per test.

In view of the limited amount of work done in determining the nature of the virus from kale, it cannot be stated definitely that the kale virus is the cauliflower mosaic virus. Further and more extensive work should be done in which the virus from the kale and the cauliflower are compared simultaneously under identical environment, the same species of suscept plants being used in both cases.

SUMMARY AND CONCLUSION

The results obtained from several pathogenicity trials with leaf extracts from a kale (*B. oleraceae* var. *acephala*) plant showing mottle and chlorotic

ring-spot symptoms, have disclosed that the physiological disturbance is produced by a virus.

The virus can readily be obtained from kale and transmitted mechanically with carborundum to several crucifers, but not to noncrucifers. The reaction of crucifers to inoculation is characterized by initial vein-clearing, vein-banding, systemic mottling, and chlorotic ring-spots on the leaves.

Among the crucifers tested, kohlrabi, cauliflower, Chinese cabbage, kale, and rutabaga showed the most conspicuous symptoms of mosaic. Kale, rutabagas, and Chinese cabbage developed chlorotic ring-spot symptoms and mottle; and Chinese cabbage reacted usually with faint mottle and leaf curling. The symptoms were seen clearly at temperatures around 20°C. and gradually became inconspicuous at temperatures below 14° or above 27°C.

Brussells sprouts and broccoli were slow in showing mottle symptoms and cabbage appeared to be the least to react to infections, very faint symptoms being produced at the optimum temperatures.

In comparing the cauliflower virus from California and the turnip virus from New York, Tompkins (18) found that the turnip virus produced symptoms in Colma cabbage and February cauliflower very similar to those described by Clayton (2) on brussels sprouts and cauliflower, but Tompkins demonstrated that turnip virus from New York caused symptoms on *Nicotiana tabacum* and *N. glutinosa*, brussels sprouts, rutabagas, and Chinese cabbage, and no infection on sprouting broccoli, kohlrabi, raddish, and *N. langdorsffii*, thus showing that the cauliflower mosaic virus is different from the turnip virus and the rutabaga virus described by Clayton from New York.

The work done in Cornell with the kale mosaic virus indicates that the kale mosaic virus and the rutabaga mosaic virus described by Clayton are related to the cauliflower mosaic virus described by Tompkins (18) from California. Clayton did not determine the physical properties of the rutabaga virus.

The virus from kale does not cause infection in noncrucifers; its physical properties are: Thermal inactivation point around 80°C., dilution tolerance of 1:2000, and aging in vitro approximately 14 days. All these strongly point to the conclusion expressed above.

An apparent discrepancy between the kale mosaic virus and that of the cauliflower rests on the temperature range for maximum symptom expression, though not for infection. Tompkins (18) has shown that the cauliflower mosaic virus produces the most marked symptoms in cauliflower at temperatures from 10°C., and that at temperatures from 20–30°C., the symptoms are masked. However, there is the possibility that, even

though there is masking of the symptoms, the rate of multiplication of the virus is not suppressed by higher temperatures, if we consider the results obtained by Pound and Walker (11) in which they showed that, at temperatures of 16°C., the cauliflower mosaic virus studied produced mottle symptoms in Jersey Queen cabbage in 18 to 21 days, while at higher temperatures the incubation period was gradually decreased, and at 28°C. the time for symptom expression was within 9 to 10 days. The titer of the virus at the different temperatures was not determined, therefore there is no evidence of the relation of time-of-incubation period and virus multiplication at various temperatures.

One explanation of our apparent discrepancy as to the epiphytology of this disease could be based on daily fluctuations of the temperatures in the greenhouse, which veils the true relations of the temperature and the incubation and symptom expression of the disease. One thing is obvious, and that is that the temperature ranges used in our work were very wide, and there were opportunities for symptoms to develop either when the temperature was at the lower or at the higher limit of the range, or at any point therein.

In order to have a clear picture of the situation it would be necessary to conduct further trials under constant air temperatures, running parallel tests with the cauliflower mosaic virus for comparative results.

In his work with the true cruciferous virus, Clayton found that rutabagas, white and black mustard, Chinese cabbage, turnip, and rape were susceptible; brussels sprouts and cauliflower not easily infected; and cabbage was either resistant or immune. These last results do not seem to be conclusive since Clayton did not use carborundum in his work. At 21 to 27°C. he found that Chinese cabbage and mustard developed streaks. At 12 to 18°C., brussels sprouts and cauliflower recovered completely and the symptoms disappeared either below 13°C. or above 27°C.

The difference between the true cauliflower and the cauliflower mosaic viruses is based on the virus-suscept reaction at different temperatures.

Tompkins' virus works best at temperatures from 10° to 19°C., and Clayton's at temperatures from 20° to 27°C.

The trials conducted here in connection with the kale mosaic virus are too limited to warrant the exact classification of the virus. They do, however, show that the kale mosaic virus belongs to the cauliflower virus category, and is very closely related to the cauliflower mosaic virus described by Tompkins from California. The kale mosaic virus is either the cauliflower mosaic virus or a very close virus entity. Further work on cross-immunity reactions and possibly serological tests could clarify the situation.

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