

CARNATION NECROTIC FLECK VIRUS

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Among the viruses of carnation, carnation latent virus and carnation vein-mottle virus have been known as elongated viruses. The authors isolated an elongated virus different from the known two viruses from carnations collected in Okayama and Shizuoka Prefectures that showed grayish white or reddish purple necrotic flecks (Plate I; A). The virus was newly named as carnation necrotic fleck virus (CNFV) on the basis of the characteristics of the symptoms on carnation. This paper mainly deals with the investigation on transmission, host range, symptoms, and electron microscopy of the virus.

MATERIALS AND METHODS

Isolation of the Virus An isolate of CNFV mainly used in this study was isolated by an aphid, *Myzus persicae*, from a diseased plant of carnation var. Coral collected in Kurashiki in 1969 showing reddish purple flecks on leaves. The isolate was maintained successively on carnation and *Dianthus barbatus* in glasshouse by aphid inoculation.

Inoculation Sap inoculation was conducted by carborundum rubbing method. Most of the inoculation test was carried out using an aphid, *Myzus persicae*. As the indicator plant for virus testing *D. barbatus* was used.

Electron microscopy Virus particles were observed under a Hitachi HS-6 and a Hitachi HU-12 electron microscope. Specimens for observation were prepared by dip method or by direct negative stain method using 2% PTA or 1-2% uranyl formate. Small pieces of infected leaf tissues of carnation and *D. barbatus* were fixed with Dalton's chrome-osmium solution. After fixation tissues were dehydrated by a graded series of ethanol, and embedded with epoxy resin. Thin sections were cut by Porter-Blum MT-1 microtome using glass knives, and stained with uranyl acetate and lead citrate.

RESULTS

1. Transmission

Sap Inoculation The virus was transmitted by plant sap with some difficulties. After about 10 days, local grayish white necrotic lesions developed on the inoculated leaves of the seedlings of carnation and *D. barbatus* (Plate I: H, I). Most of the inoculated seedlings were not infected systemically, but some of the seedlings of *D. barbatus* produced faint veinal chlorosis on the upper leaves.

Aphid Transmission CNFV was transmitted by *M. persicae*. Mode of transmission by this aphid was investigated using *D. barbatus* as the virus source and indicator plants. To know the time of acquisition feeding the aphids were fed on source plants for the period varying from 5 min to 24 hrs, and then transferred to indicator plants and allowed to feed for 24 hrs. Table 1 shows the results. The aphids transmitted the virus by acquisition feedings longer than 30 min, but not by shorter than 10 min. Efficiency of transmission was increased by feeding longer than 4 hrs.

TABLE 1.
Effect of varying acquisition feeding time on the transmission of
carnation necrotic fleck virus by *Myzus persicae*

| Experiment | Duration of acquisition feeding* | | | | | | Aphids fed on healthy plants |
|------------|----------------------------------|--------|--------|------|-------|--------|------------------------------------|
| | 5 min | 10 min | 30 min | 1 hr | 4 hrs | 24 hrs | |
| I | 0/3 | 0/3 | 0/3 | 0/3 | 1/3 | 3/3 | 0/3 |
| II | 0/3 | 0/3 | 1/3 | 1/3 | 2/3 | 1/3 | 0/3 |
| III | 0/6 | 0/6 | 1/6 | 0/6 | 1/6 | 4/6 | 0/6 |
| Total | 0/12 | 0/12 | 2/12 | 1/12 | 4/12 | 8/12 | 0/12 |

Denominator indicates the number of test plants colonized, the numerator the number that became infected.

*) After the acquisition feeding on infected *D. barbatus*, aphids were transferred in groups in fives to individual test plants (*D. barbatus*) for 24 hrs.

Table 2 shows the results of experiments about the time of inoculation feeding. The aphids which had received sufficient feeding for virus acquisition were transferred to indicator plants and allowed to feed for inoculation for the period varying from 5 min to 24 hrs. The virus was

TABLE 2.
Effect of varying inoculation feeding time on the transmission of
carnation necrotic fleck virus by *Myzus persicae*

| Experiment | Duration of inoculation feeding* | | | | | | Aphids fed on healthy plants |
|------------|----------------------------------|--------|--------|------|-------|--------|------------------------------------|
| | 5 min | 10 min | 30 min | 1 hr | 4 hrs | 24 hrs | |
| I | 0/1 | 0/1 | 0/1 | 0/1 | 1/1 | 1/1 | 0/1 |
| II | 0/3 | 0/3 | 1/3 | 0/3 | 2/3 | 1/3 | 0/3 |
| III | 0/3 | 0/3 | 0/3 | 1/3 | 1/3 | 2/3 | 0/3 |
| IV | 0/6 | 1/6 | 3/6 | 3/6 | 3/6 | 3/6 | 0/6 |
| Total | 0/13 | 1/13 | 4/13 | 4/13 | 7/13 | 7/13 | 0/13 |

Denominator indicates the number of test plants colonized, the numerator that became infected.

*) After being fed on source plants (*D. barbatus*) for a period sufficient for acquisition for 24 hrs, aphids were transferred in groups of fives to individual test plants (*D. barbatus*) and fed for given periods.

transmitted by inoculation feedings longer than 10 min, but not by a short time feeding for 5 min. Efficiency of transmission was increased by longer periods of feeding for 30 min or more.

Results of daily serial transfer tests in Table 3 showed that *M. persicae* retained their infectivities for 2 days after they left from the source plants.

TABLE 3.
Retention of carnation necrotic fleck virus by
Myzus persicae in daily serial transfer

| Experiment | Daily serial transfer [*]) | | | |
|------------|--------------------------------------|------|------|------|
| | 1 st | 2 nd | 3 rd | 4 th |
| I | 3/3 | 1/2 | 0/3 | |
| II | 5/6 | 0/6 | 0/6 | 0/3 |
| Total | 8/9 | 1/8 | 0/9 | 0/3 |

Denominator indicates the number of test plants colonized, the numerator the number that became infected.

^{*}) After being fed on source plants (*D. barbatus*) for a period sufficient for virus acquisition for 24 hrs, aphids were transferred in groups of fives to individual test plants (*D. barbatus*) daily and fed for 24 hrs.

2. Host Range and Symptoms

So far tested CNFV was infectious to 3 species in *Callyophyllaceae*, such as *Dianthus caryophyllus*, *D. barbatus*, and *D. chinensis*. The following plants were not infected with the virus by sap and/or aphids: *Nicotiana glutinosa*, *N. tabacum*, *Datura stramonium*, *Zinnia elegans*, *Arctium lappa*, *Beta vulgaris*, *Chenopodium amaranticolor*, *Spinacia oleracea*, *Cucumis sativus*, *Pisum sativum*, *Phaseolus vulgaris*, and *Rhaphanus sativus*.

Symptoms on susceptible plants caused by aphid inoculation were briefly described below.

On carnation, grayish white necrotic spots and flecks which were sometimes followed by reddish purple discoloration of leaves appeared 2-3 weeks after the inoculation (Plate I; B-D). Some of the seedlings severely affected were stunted and killed. However, most of the chronically infected plants became masked.

Symptoms on *D. barbatus* were characterized by chlorosis of veins and reddish discoloration of leaves. After 2-3 weeks from the inoculation, chlorosis of main veins appeared on several fully expanded young leaves (Plate I; E-G). Affected leaves were frequently showed yellow net symptoms (Plate I; E, F). The leaves, then, produced reddish discoloration followed by leaf blight from the tips (Plate I; J). On several young leaves grown to the next, the symptoms were observed only at the leaf tips. The upper leaves grown later were almost symptomless.

Symptoms observed on *D. chinensis* are almost similar to those on *D. barbatus*, but rather indistinct.

3. Electron Microscopy

Virus Particles. Particles of CNFV were long and very flexuous rods, 12–13 nm wide and 1.4–1.5 μ long (Fig. 1 and Plate II; A). In leaf-dip preparations treated with uranyl formate, cross banding of particles with an average pitch of 3.4 nm was clearly seen (Plate II; B).

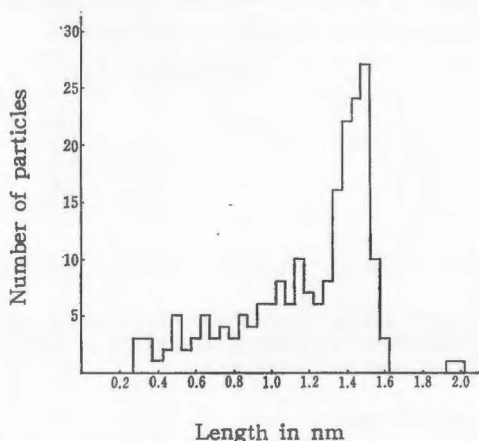


Fig. 1. Particle length of carnation necrotic fleck virus.

Ultrastructures of Infected Plant Cells. In thin sections of infected carnation and *D. barbatus*, virus particles were observed in phloem cells (Plate III). Characteristic vesicular structures were observed also in these cells (Plate III; C, D). Some of the phloem cells were almost filled with large masses of flexuous particles (Plate III; A, B), and/or particle aggregates and vesicular structures (Plate III; C, D). Necrosis of some phloem cells were frequently observed. Aggregates of particles and/or vesicular structures were seen in epidermal cells of infected *D. barbatus* as well, which may well correspond to the X-bodies in epidermal strip in light microscopy (Plate II; C). In mesophyll cells neither virus particles nor vesicular structures were so far found.

4. Distribution of CNFV in Carnation

Diseased samples of 18 varieties of carnation were collected from farmer's fields at Kurashiki and Kasaoka, Okayama Prefecture, Doi, Kawazu, and Minami Izu, Shizuoka Prefecture, and from the stock nursery of Okayama Agricultural Experiment Station. Electron microscopic observations on negatively stained dip preparations were conducted for detection of virus particles. In the samples of 15 out of 18 varieties isometric and/or rod-shaped particles were detected, which were presum-

ably carnation mottle virus or carnation ringspot virus for isometric particles and carnation latent virus or carnation vein mottle virus for rods. Particles of CNFV, long very flexuous and narrow rods, were detected in 13 out of 18 varieties, all of whose samples showing typical grayish white or reddish purple necrotic flecks were included. Double or triple infections with these particles were frequently observed.

DISCUSSION

CNFV causes grayish white and/or reddish purple necrotic flecks or streaks in carnation. Jones (1945) reported that causal agent of carnation streak of which symptoms were reddish spots and streaks was transmitted by *Myzus persicae*, but not by plant sap. Carnation streak virus reported by Brierley and Smith (1957) was transmitted by grafting, but probably not by insects. CNFV apparently differed from Brierley and Smith' carnation streak virus in aphid transmission. Symptoms and aphid transmission of CNFV appeared somewhat resembled to those of Jones' carnation streak. However, sap transmission of CNFV was not similar to Jones' virus. The nature of the pathogen of carnation streak has not been defined (4), and virus particles of it also have not been reported. Therefore, the authors preferred CNFV rather than streak for the name of the virus in the present study.

Several viruses of which particles were long very flexuous rod about 10 nm wide and over 1 μ long have been known, such as beet yellows virus (1, 3, 14, 17, 18), citrus tristeza virus (9, 12, 13, 16), Festuca necrosis virus (15), wheat yellow leaf virus (7), and a virus from burdock (Bd-F) (5). Very flexuous rods with a helix pitch of 3.8 nm, 12 nm wide, and 600-620 nm long were also known to be particles of apple chlorotic leaf-spot (10) and apple stem grooving (11) viruses. Characteristics of the CNFV in particle morphology and aphid transmission probably in a semi-persistent manner resembled to beet yellows and several other viruses mentioned above rather than the viruses of apple. Further, the characteristics observed in thin sections, the aggregation of virus particles in phloem cells and phloem necrosis, were very similar to those reported in beet yellows, citrus tristeza, and wheat yellow leaf viruses. Vesicular structures, usually associated with filamentous particles observed in epidermal and phloem cells infected with CNFV were similar to those reported in beet yellows virus (3).

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SUMMARY

A virus was isolated from carnation showing symptoms of grayish white or reddish purple necrotic flecks, and was newly named as carnation necrotic fleck virus (CNFV).

The virus was transmitted by plant sap with some difficulties, and easily by an aphid, *Myzus persicae*. The aphids, which had received acquisition feedings longer than 30 min, transmitted the virus, but not after feeding shorter than 10 min. After sufficient time of feeding for virus acquisition on source plants, the aphids transmitted the virus when fed on test plants for 10 min or more. Efficiency of transmission was increased by longer period than 4 hrs and 30 min for acquisition and inoculation feeding, respectively. In daily serial transfer tests, the aphids retained their infectivities for 2 days.

CNFV was infectious to 3 species in *Caryophyllaceae*, such as *Dianthus caryophyllus*, *D. barbatus*, and *D. chinensis*, but not to 12 species in the 6 families examined. In carnation the virus caused necrotic flecks, grayish white or reddish purple in colour, which usually became masked in chronically infected plants. *D. barbatus* produced yellowing of veins followed by reddish discoloration of leaves. Chronically infected plants usually became masked.

Particles of CNFV were long very flexuous rods with helical symmetry. Length of the particles was 1.4-1.5 μ , width was about 12-13 nm, and pitch of helix 3.4 nm. In thin sections of CNFV infected plants, necrosis of some phloem cells was observed. Masses of particles and/or vesicular structures with which some of the cells were almost filled were usually observed in epidermal and phloem cells of infected plants. Cellular inclusions (X-bodies) observed in epidermal strip of infected *D. barbatus* in light microscopy probably corresponded to the aggregates of particles and/or vesicular structures.

Electron microscopic observations for CNFV particles proved that the virus was rather commonly distributed in 13 out of 18 varieties of carnation collected in Okayama and Shizuoka Prefectures.

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EXPLANATION OF PLATE

Plate I. Symptoms caused by carnation necrotic fleck virus (CNFV) in carnation and *Dianthus barbatus*.

- A) Naturally infected carnation (var. Coral) with grayish white and/or reddish purple necrotic flecks and streaks.
- B-D) Necrotic flecks and streaks in carnation infected with CNFV by aphids.
- E) Veinal chlorosis on the lower leaf surface of *D. barbatus* infected with CNFV by aphids.
- F, G) Veinal chlorosis and necrosis on the upper leaf surface of *D. barbatus* infected by aphids with CNFV.
- H, I) Local lesions in an inoculated leaf of *D. barbatus* by plant sap after 25 days (H), and 40 days (I) from the inoculation.
- J) Veinal necrosis and reddish discoloration in *D. barbatus* infected with CNFV by aphids.

Plate II.

- A) Particles of CNFV in negatively stained dip-preparation mounted in PTA. Bar represents 1 μ .
- B) Particles of CNFV in uranyl formate. Bar represents 50 nm.
- C) Inclusion bodies in epidermal cells of *D. barbatus* infected with CNFV in light microscopy. X: Inclusion body n: Nucleus

Plate III. Ultrastructure of phloem cells of *D. barbatus* infected with CNFV

- A) Banded aggregates of CNFV particles in a phloem cell. Bar represents 1 μ .
- B) A phloem cell almost filled with masses of CNFV particles. Bar represents 1 μ .
- C) A phloem cell filled with masses of CNFV particles and vesicular structures. Bar represents 1 μ .
- D) Part of a phloem cell filled with masses of CNFV particles and vesicular structures. Bar represents 0.5 μ .

Abbreviations

F: Virus particle

V: Vesicular structure

W: Cell wall

Plate I

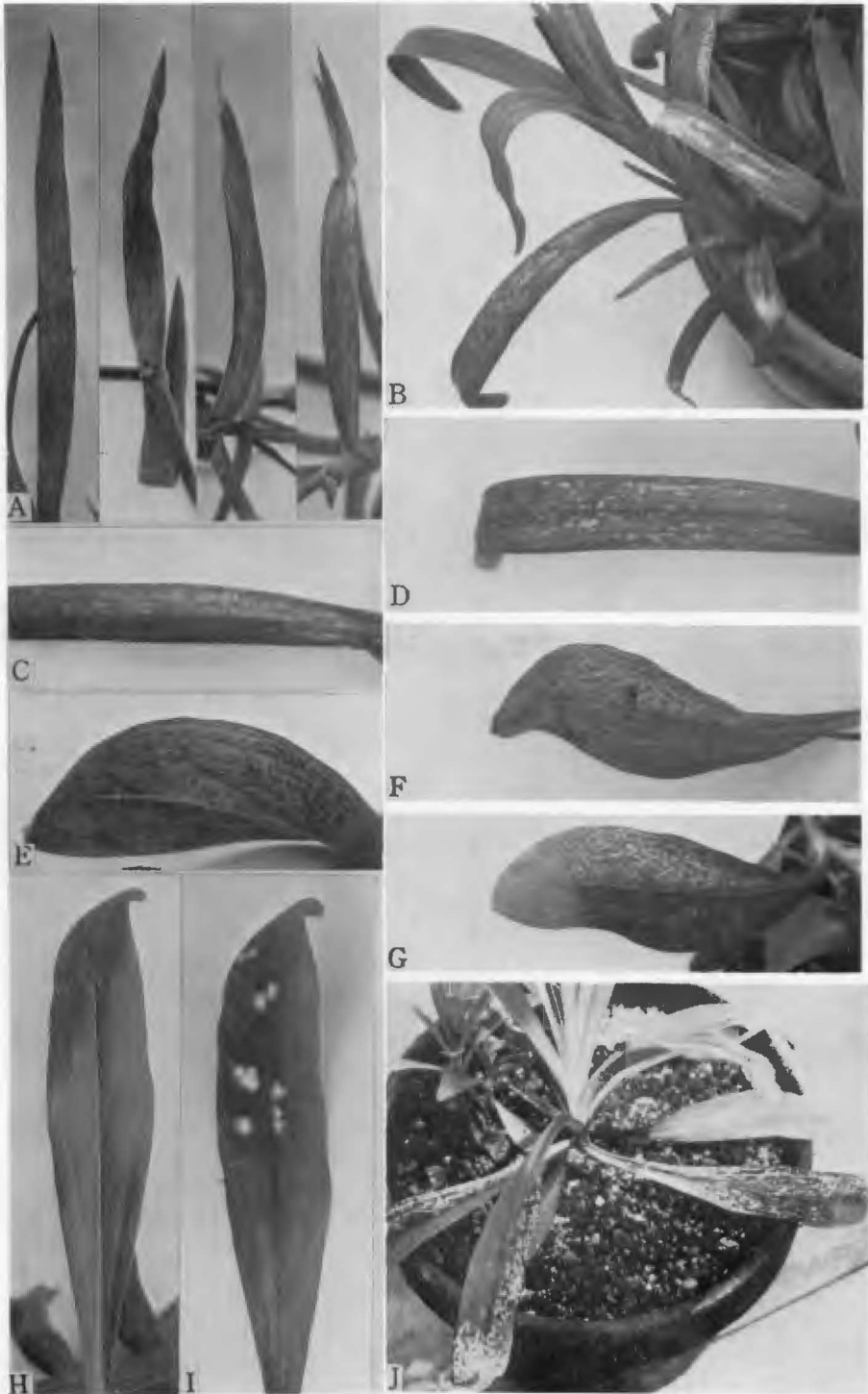


Plate II

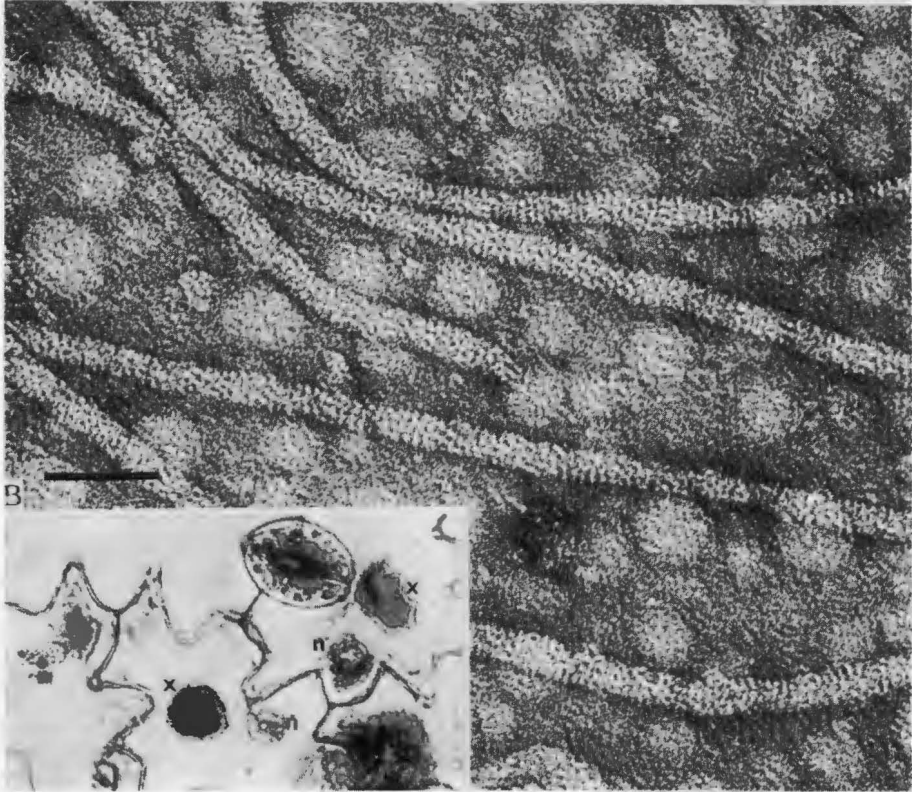
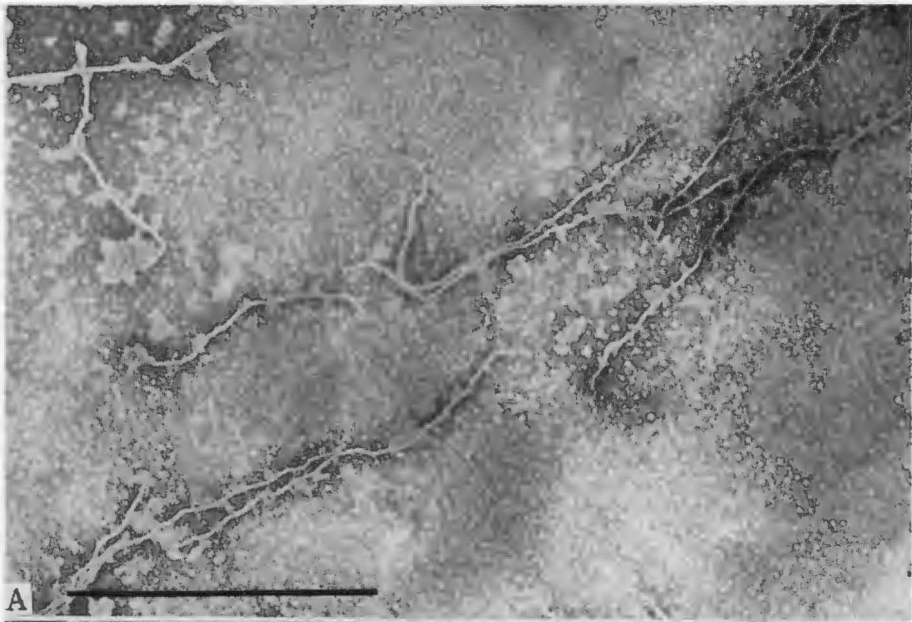


Plate III

