Morphogenesis of Cucumber Mosaic Virus-Induced Crystalline Inclusions in Peppers

G. W. Moorman and W. C. Woodbridge

Assistant professor and research assistant, respectively, Suburban Experiment Station, University of Massachusetts, Waltham 02254.
Present address of senior author: Department of Plant Pathology, Pennsylvania State University, University Park 16802.
Research supported by the Massachusetts Agricultural Experiment Station. Journal series paper 2562 of the Massachusetts Agricultural Experiment Station.
Accepted for publication 22 February 1983.

ABSTRACT

Moorman, G. W., and Woodbridge, W. C. 1983. Morphogenesis of cucumber mosaic virus-induced crystalline inclusions in peppers. Phytopathology 73:1106-1108.

Plants of *Capsicum annuum* 'Yolo Wonder' inoculated with cucumber mosaic virus were incubated at 21, 27, and 32 C with a 20-hr light period of fluorescent illumination (8,608 lux [800 ft-c]). Two types of crystalline inclusions were observed in the epidermis covering the abaxial surface of the veins of the inoculated leaves. Hexagonal crystals in the cytoplasm were smaller than the cell nucleus. Angular plates were often larger than the nucleus and formed asterisk-shaped aggregates as they increased in size. Angular plates initially appeared in the cells 3–5 days after inoculation in plants grown at 27 and 32 C, reached maximum size in 11 days, and disappeared in 25 days at 27 C and in 19 days at 32 C. At 21 C, crystals first appeared in 5–7 days, reached maximum size in 21 days, and disappeared in 28 days.

Cucumber mosaic virus (CMV) causes a severe disease of sweet bell peppers in the lower Merrimack River valley of northeastern Massachusetts and is also prevalent in the provinces of Ontario (4) and Quebec (5) in Canada. Fresh-market pepper production has been eliminated from some Massachusetts farms as a direct result of this disease. CMV is a deterrent to increased pepper production wherever it occurs. Leaves infected with CMV are chlorotic, have mild mottling, and sometimes exhibit necrotic line patterns. Affected fruits are also chlorotic, have numerous small, firm, sunken brown spots on their sides, and have a bitter taste.

Christie and Edwardson (3) reported that CMV particles form massive crystalline inclusions primarily in pepper leaf mesophyll cells. They also depict CMV-induced crystalline inclusions in epidermal cells of *Cucumis sativus* and *Nicotiana* × *edwardsonii*. These crystals may be of diagnostic value because similarly shaped crystals have not been reported in other virus groups. Moorman (6) consistently found the crystals in the epidermis covering the abaxial surface of pepper leaf veins. However, since the crystals were not always found, they could not be relied upon for determining whether CMV was present in pepper leaves collected during field surveys (Moorman, *unpublished*). Pasko (7) demonstrated that in peppers, if CMV-infected leaves containing observable crystalline

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

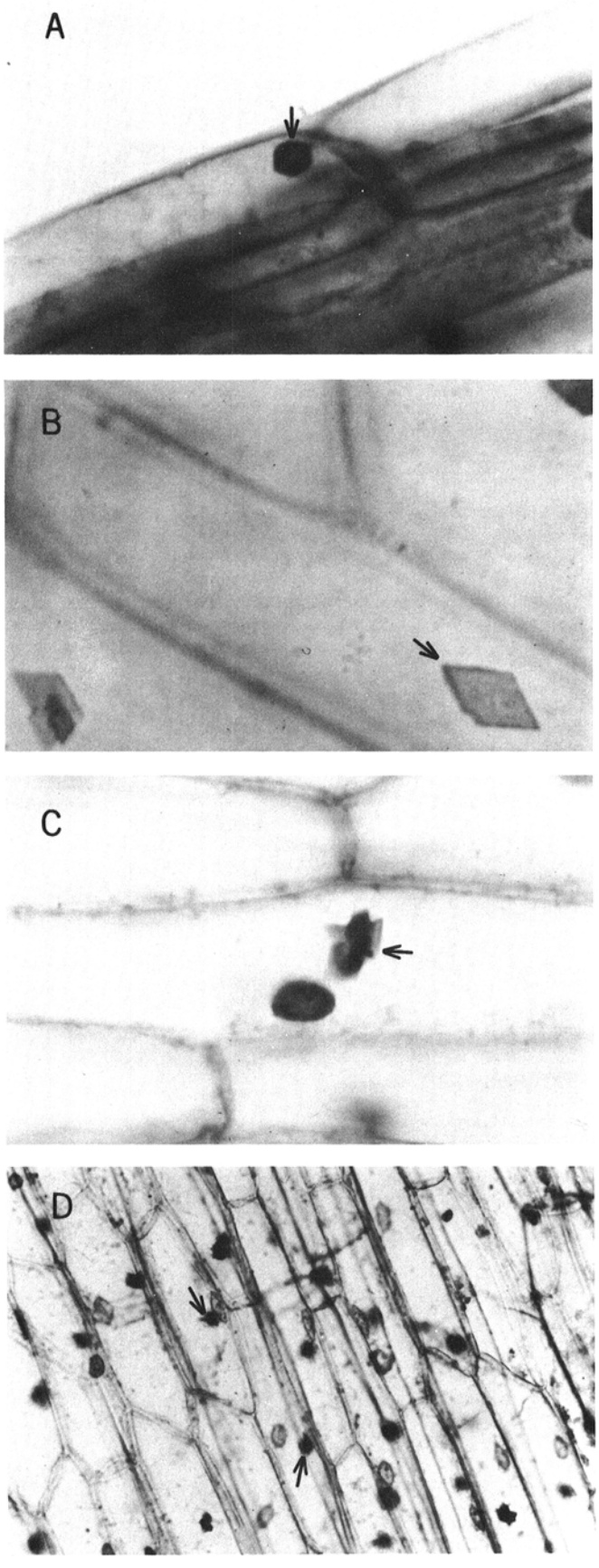

Fig. 1. Cucumber mosaic virus-induced crystalline inclusions in inoculated pepper leaf epidermal cells. **A,** Hexagonal crystal (arrow) with apparently hollow center (×1,000). **B,** Angular plate crystal (arrow) (×1,700). **C,** Angular plates in asterisk-shaped aggregate (arrow) larger than adjacent cell nucleus (×1,100). **D,** Epidermal cells containing aggregates (arrows) of maximum-size angular plates (×250).

inclusions of any size were used as inoculum, mechanical transmission was usually 100% successful. Leaves that exhibited typical CMV symptoms but that lacked observable crystals were extremely poor sources of inoculum.

The experiments reported in this article were initiated to determine when the crystals first appear after inoculation, the time required for crystals to reach maximum size, and the duration of their existence in pepper leaves under controlled conditions.

MATERIALS AND METHODS

Pepper leaves (*Capsicum annuum* L. 'Lady Bell') infected with cucumber mosaic virus (CMV) were collected from commercial fields in Methuen, MA, during the summer of 1979. CMV was maintained in cultivars Yolo Wonder and Midway peppers by mechanical transmission. One gram of leaf tissue was triturated in a sterile mortar with 9 ml of 0.05 M potassium phosphate buffer, pH 7–7.2. Leaves centrally located on the stems of pepper plants in the 4–8 leaf stage were dusted with Carborundum (320 grit) and mechanically inoculated with a cotton-tipped applicator stick that had been dipped in the triturated-leaf suspension. The inoculated leaf was immediately rinsed and patted dry with a paper towel.

In three experiments, at 21, 27, and 32 C, 10 inoculated and two uninoculated Yolo Wonder peppers were grown in a growth chamber (model E-30, Percival Refrigeration and Manufacturing Co., Inc., 1440 Walnut Street, Des Moines, IA 50307) at 8,608 lux (800 ft-c) fluorescent illumination with a 20-hr light period and a 4-hr dark period.

The leaf epidermis covering the abaxial surface of the veins of inoculated leaves contained large concentrations of crystalline inclusions as compared to other plant tissues (6). Small segments of tissue torn from inoculated leaves and from leaves of noninoculated plants were stained with 0.1% azure A using the procedure of Christie and Edwardson (3). Strips were scanned with a light microscope with bright-field optics. On each sampling date, five angular plates or aggregates of angular plates in an epidermal strip from each plant were selected at random and measured with an eyepiece micrometer. The length of the longest axis of the plate or plate aggregate multiplied by the width, measured perpendicularly to the longest axis, is the crystal size.

RESULTS

Two types of crystals were observed. Hexagonal crystals, apparently with hollow centers, were always smaller than the cell nucleus (Fig. 1A). These crystals were found only occasionally. Angular plates (Fig. 1B), present in all inoculated plants, were often larger than the cell nucleus, and were found either solitary or grouped into asterisk-shaped aggregates (Fig. 1C).

The angular plates initially appeared 3–5 days after inoculation at 27 and 32 C and in 5–7 days at 21 C. Their appearance coincided with the first leaf-mottling symptoms. Maximum crystal size was reached 11 days after inoculation at 27 and 32 C and in 21 days at 21 C (Fig. 2). When crystals were at maximum size, most of the abaxial vein epidermal cells contained a crystal aggregate (Fig. 1D). Crystals began to disappear after reaching maximum size. In each of the three experiments at 32 C between days 13 and 17, crystals again increased in size slightly but disappeared rapidly thereafter. The crystals became very granular in appearance and could no longer be found by day 28 at 21 C, day 25 at 27 C, and by day 19 at 32 C.

DISCUSSION

The length of time observable crystals were present in inoculated pepper leaves was correlated with the incubation temperature. At 32 C, observable crystals were present for 14–16 days, but they were present for approximately 18 days at 27 C and for 22 days at 21 C. The production of crystal-containing leaves can be hastened by incubating inoculated plants at 27–32 C and maintained for extended periods by incubating at 21 C. The fluctuating temperatures normally encountered in greenhouses may delay the

initial formation of crystals and prolong crystal existence in the plants.

The timing of the crystal formation and subsequent disappearance agree with the research of Simons (8) and Cheo and Pound (2), who used aphid or mechanical transmissibility of CMV in pepper and spinach as an indication of virus titre. CMV concentration and transmissibility increased over a period of 1–2 wk, but then decreased rapidly (8). Higher temperatures (24–28 C) shortened the period of high virus titre and good transmissibility, but lower temperatures delayed it (2). The high incidence of crystalline aggregates in the leaf vein epidermis is noteworthy. Bradley (1) reported that 84% of the aphids feeding on CMV-infected tobacco leaf veins were viruliferous while 73% feeding between veins carried the virus. Further work is required to confirm that crystal presence and size are a function of virus concentration in the epidermal cells.

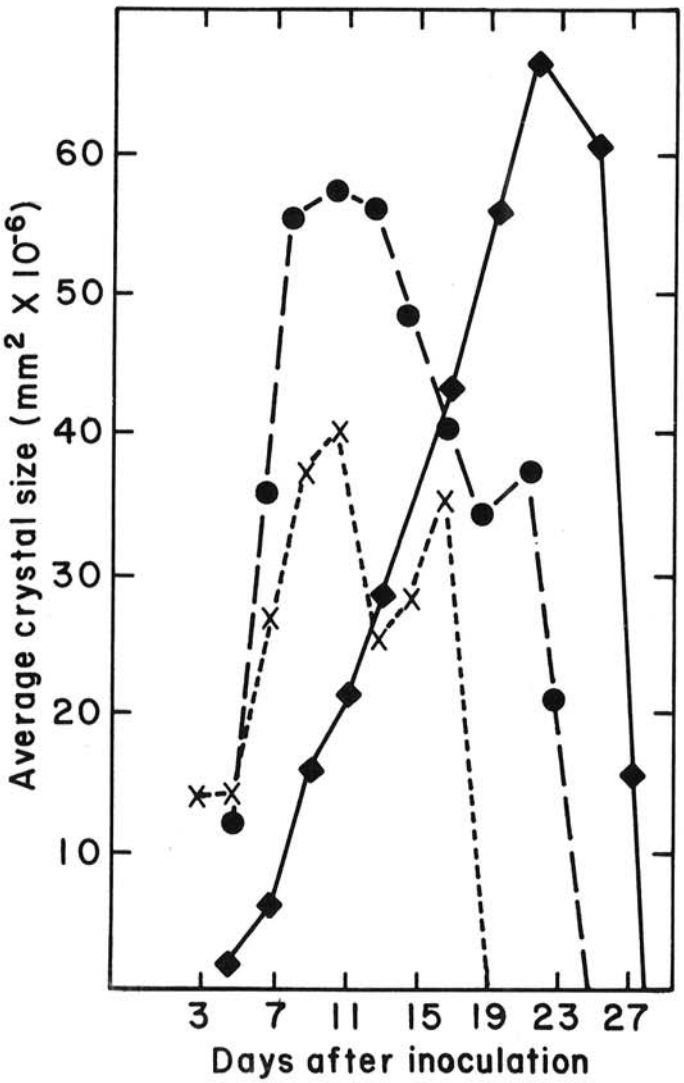

Fig. 2. Angular plate crystalline inclusion size in epidermal cells covering the abaxial surface of inoculated pepper leaf veins. Plants were inoculated with cucumber mosaic virus and incubated at 21 (▲——▲), 27 (•---•), and 32 C (x---x) with a 20-hr (8,608 lux [800 ft-c]) day length. Crystal size is the length of the longest axis multiplied by the width measured perpendicularly to the longest axis.

Several questions concerning CMV crystalline inclusion morphology and morphogenesis remain to be answered. Hexagonal crystals were not always found, but when they were, angular plates were also present in the epidermal strip. Hexagons were occasionally observed in experimental plants but also at random in both young and relatively old plants used as sources of virus and maintained either in growth chambers under controlled conditions or in greenhouses where temperature and light intensities varied widely. Conditions that influence formation of hexagonal crystals remain to be elucidated. Epidermal strips were taken from inoculated leaves to study crystal formation and disappearance. Experiments should be done in which young leaves above the inoculated leaf are sampled to determine whether crystals form in each flush of new growth.

The CMV crystals described in this article are readily observed by light microscopy following a simple stain procedure (3). A×100 oil immersion lens is not needed unless very small crystals are observed early in the infection or unless very granular dispersing crystals are observed late in infection. This rapid, inexpensive technique has been used to confirm the presence of CMV in samples collected from field plots that were observed on a weekly basis (Moorman, *unpublished*) and supplemented host range inoculation information. Since crystal appearance coincides with initial leaf mottling, mottled leaves should be sampled soon after symptoms develop. Mixed infections can be detected when crystal types other than those characteristic of CMV are observed.

LITERATURE CITED

1. Bradley, R. H. E. 1966. Tobacco leaf veins as a source of a cucumber mosaic virus for aphids. Virology 34:172-173.
2. Cheo, P. C., and Pound, G. S. 1952. Relation of air temperature, soil temperature, photoperiod, and light intensity to the concentration of cucumber virus 1 in spinach. Phytopathology 42:306-310.
3. Christie, R. G., and Edwardson, J. R. 1977. Light and electron microscopy of plant virus inclusions. Fla. Agric. Exp. Stn. 9. 155 pp.
4. Kemp, W. G., and Troup, P. A. 1978. A weather index to forecast potential incidence of aphid-transmitted virus diseases of peppers in the Niagara peninsula. Can. J. Plant Sci. 58:1025-1028.
5. Lana, A. F., and Peterson, J. F. 1980. Identification and prevalence of pepper viruses in southern Quebec. Phytoprotection 61:13-18.
6. Moorman, G. W. 1982. Cucumber mosaic virus disease-induced crystalline inclusion appearance, enlargement, and dispersal in sweet peppers. Phytopathology 72:939.
7. Pasko, P. J. 1982. Requirements necessary for consistent transmission of cucumber mosaic virus and screening peppers (*Capsicum* spp. L.) for CMV resistance. M.S. thesis. Plant and Soil Sciences Department. University of Massachusetts, Amherst. 37 pp.
8. Simons, J. N. 1958. Titres of three nonpersistent aphid-borne viruses affecting pepper in south Florida. Phytopathology 48:265-268.