

# Chlorotic Mottle: A Newly Recognized Disease of Chrysanthemum

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## ABSTRACT

A chlorotic mottle of chrysanthemum (*Chrysanthemum morifolium* Ramat.) first noted in the cultivar Yellow Delaware proved to be graft-transmissible to a large number of chrysanthemum cultivars. Scions of some cultivars, although infected, remained symptomless, whereas others initially exhibited marked yellow-green mottling, but eventually developed pronounced general chlorosis and were somewhat dwarfed. Scions of some cultivars exhibited marked initial reaction, but later became symptomless though still carrying active incitant. The relative brevity of the early phase of striking variegation was an outstanding characteristic of the

syndrome. No pathogenic bacterium or fungus was found associated with the diseased plants. The probability of virus etiology was indicated by successful inoculation of healthy chrysanthemums by standard sap inoculation techniques. Negative results of sap inoculations of standard indicator plants suggested that it was not caused by the viruses of chrysanthemum stunt, chrysanthemum mosaic, chrysanthemum aspermy, chrysanthemum dwarf-mottle, Noordam's chrysanthemum virus B, tobacco mosaic virus, or cucumber mosaic virus. We propose that the disease be designated by the term "chlorotic mottle". *Phytopathology* 61:415-419.

During the summer of 1967, our attention was called to a malady affecting chrysanthemums in a commercial glasshouse range in upstate New York (3). We were advised that the problem was limited mostly to the cultivar Yellow Delaware, and to a minor extent to the "parent" cultivar Delaware. The malady had been observed in a number of glasshouses in southern USA, but only occasionally in the north. It appeared that most, if not all, diseased material originated from clonal stock plants grown in Florida.

Symptoms of the disease included (i) mild mottling or variegation of the young leaves and occasional distinct chlorotic spotting; (ii) general chlorosis of new leaves, often following mottling of early-formed leaves; (iii) dwarfing of leaves, flowers, and the entire plant; and (iv) delay in development of blossoms.

Expression of symptoms within a group of plants varied at any given time and from time to time. Plants of some cultivars might at one time be very chlorotic, 1 or 2 weeks later appear almost wholly recovered, then again exhibit severe symptoms. Often the parent plant and the rooted cuttings would appear perfectly healthy, severe symptoms developing only after the cuttings were planted by the unsuspecting customer. Frequently, only two or three of the five plants in a pot developed symptoms, but the pot was thus rendered valueless (Fig. 1).

No described disease of chrysanthemum incited by a fungus, bacterium, or nematode (10) induces symptoms resembling in any way those associated with the chlorotic mottle disease. A yellow strapleaf disease, apparently caused by accumulation of certain amino acids in the root zone, described in Florida in 1959 (14), induces general chlorosis, distortion, and extreme narrowing of newly formed leaves. It seemed possible that the symptoms observed in Yellow Delaware might be a varietal response to amino acid accumulation or to some nutritional factor. Several described virus diseases of chrysanthemums (2, 12) produce various degrees of mottling, variegation, and chlorosis but none,

in our opinion, induces symptoms matching those of the present disorder.

If the malady were an inherent genetical aberration or deficiency limited to identified clones of Yellow Delaware and Delaware, it could be eliminated quickly by destroying these clones. If, on the other hand, it were caused by a systemic pathogen and the pathogen were transmissible to other clones and varieties, it could be an extremely important threat. Because of the urgency of determining whether the malady was nutritional, genetical, or a transmissible disease, the studies reported here were undertaken.

*Attempts to isolate fungi and bacteria.*—To determine whether a systemic fungus or bacterium might be involved, stem and leaf tissues from diseased plants were plated out on potato-dextrose agar. No fungus or bacterium was isolated consistently. Negative results of inoculations with those organisms that were isolated indicated that the disease was not caused by any bacterium or fungus readily recoverable by common isolation techniques.

*Sap inoculation.*—Attempts were made in February and again in April 1968 to transmit the incitant from diseased to healthy chrysanthemums by rubbing sap extracted without buffer from diseased Yellow Delaware leaves on Carborundum-dusted leaves of 10 healthy Yellow Delaware plants. In both tests, symptoms failed to develop after 3 months and the plants were discarded. In December 1969 and again in January 1970, additional sap inoculation tests were conducted in which inoculum was obtained from the highly reactive variety, Deep Ridge. Both extracted sap and cut stacked leaves (15) were employed with and without buffer, using healthy plants of Deep Ridge and Yellow Delaware as test plants. Infection and conspicuous symptom development resulted in a high percentage of the test plants of both cultivars, whether buffer was used or not. These results indicated the probability of virus etiology of the disease.

Sap inoculations also were attempted in April 1968



**Fig. 1.** Yellow Delaware chrysanthemum plants from a commercial glasshouse. (Left) healthy; (right) chlorotic mottle-infected. These plants were removed from a single pot and repotted. Note normal appearance of lowest leaf, mild mottle of next two, and general chlorosis of upper leaves of infected plant. All five plants in the original pot were uniform and appeared normal when planted.

to 12 plants of *Chenopodium amaranticolor* Coste & Reyn., and in September and October to 6 plants of *Petunia hybrida* Vilm. 'White Cascade', 17 plants of *Nicotiana glutinosa* L., 12 plants of *N. clevelandii* Gray, 8 plants of *N. tabacum* L. 'Turkish', and 16 plants of *Tetragonia expansa* Murr. The inoculated plants were retained for at least 4 weeks, but in no case did any local or systemic symptoms indicative of virus transmission develop. Although negative evidence is equivocal, these tests suggest that the diseased chrysanthemums did not carry tobacco mosaic virus, cucumber mosaic virus, chrysanthemum aspermy virus, Noordam's chrysanthemum virus B, or chrysanthemum dwarf-mottle virus—or, alternatively, that the chrysanthemum sap from diseased plants carried virus inhibitors or that the technique was faulty. Previous studies in our laboratory and elsewhere (5) have not indicated the presence of a general virus inhibitor in chrysanthemums that would do more than reduce the number of infection sites. Symptom expression of the above-named virus diseases on these hosts following sap inoculation usually develops within 4 weeks (1, 5, 6):

*Graft transmission.*—Rooted cuttings of Yellow Delaware in which no abnormalities had ever been noted were obtained from a chrysanthemum propagator in

California and established as healthy stock plants. Twenty-eight rooted cuttings from this healthy stock and 29 rooted cuttings from diseased Yellow Delaware plants were selected for uniformity and planted in 5-inch pots. Terminal shoots from the healthy Yellow Delaware stock plants were then cleft-grafted to the healthy and the diseased Yellow Delaware cuttings, which were "topped" about 2 inches above the soil line. The grafted plants were covered with plastic bags for 8 to 10 days to promote union.

Within a month, 29 of 29 "healthy" scions on diseased Yellow Delaware understock were distinctly mottled, whereas 28 of 28 healthy scions on healthy understock were completely symptomless, with solid, dark-green color. After 3 months, the latter remained perfectly normal in appearance, with good color and vigor. The scions on diseased Yellow Delaware had developed a variety of symptoms, five becoming generally yellow and dwarfed, three or four reverting to almost healthy-appearing foliage, the rest showing mild mottling or flecking, mild chlorosis, and some dwarfing. It was evident that a disease incitant had been transmitted through 100% of the grafts from diseased understock to healthy scion. The later variation in symptoms was consistent with that observed in commercial plantings.

Two additional types of grafting studies were undertaken: (i) grafts to determine whether two of the known chrysanthemum viruses, stunt and mosaic virus Q (11), might be involved; and (ii) grafts to determine the reaction of other chrysanthemum cultivars to the Yellow Delaware disease incitant.

In the first category, scions of the chrysanthemum stunt virus indicator cultivars, healthy Mistletoe and mosaic Q-infected Blanche, were grafted onto diseased and healthy plants of Yellow Delaware and, for symptom reference, onto stunt-infected plants of the cultivar Fanfare. Also, healthy Yellow Delaware scions were grafted onto stunt-infected Fanfare and mosaic-infected Blanche. After 1 and 2 months, the healthy Mistletoe scions on diseased Yellow Delaware showed mild chlorosis, marked inward-curling of the young leaves, and some intensification of the "normal" yellow flecking, but not the typical large yellow spotting of stunt, as witnessed by the Mistletoe scions on stunt-infected Fanfare.

The mosaic-infected Blanche scions on diseased Yellow Delaware did not exhibit leaf distortion typical of the mosaic/stunt reaction on Blanche (11), although this was extreme in the mosaic-infected Blanche scions on stunt-infected Fanfare. The healthy Yellow Delaware scions on stunt-infected Fanfare, although showing some chlorosis and vein-clearing, did not show symptoms typical of chlorotic mottle. The healthy Yellow Delaware scions on mosaic-infected Blanche developed very mild vein-clearing, but not the mottling and chlorosis characteristic of Yellow Delaware disease.

Although it was evident that a disease incitant was transmitted to healthy Mistletoe from diseased Yellow Delaware, consideration of all graft combinations indicated that it was not a typical strain of chrysanthemum stunt virus. The grafts likewise indicated that chrysanthemum mosaic virus Q was not primarily involved.

In the second category of graft studies, presumably healthy cuttings of a large number of popular greenhouse and garden chrysanthemum cultivars were obtained, and scions taken from them were grafted onto plants of diseased and healthy Yellow Delaware. All were observed for at least 9 weeks. The results indicated that many cultivars are susceptible and will develop distinctive visible symptoms. Scions of many varieties, however, remained symptomless (Table 1). When such symptomless scions were cut off, rooted, and allowed to develop on their own roots, scions of healthy Yellow Delaware and the highly reactive cultivar Blue Ridge grafted onto them developed pronounced typical symptoms of chlorotic mottle, indicating that these seemingly resistant cultivars are potential symptomless carriers. In some instances one or more branches of a single plant appeared healthy, but here again indicator scions grafted onto them showed pronounced typical symptom reaction. Scions of all cultivars grafted onto healthy Yellow Delaware remained symptomless.

*Transmission by tissue implantation.*—Transmission was also effected by tissue implantation (4). In early tests, diseased leaf tissue was inserted in longitudinal

TABLE 1. Symptom response of scions of selected chrysanthemum cultivars following grafting to stocks of Yellow Delaware showing chlorotic mottle

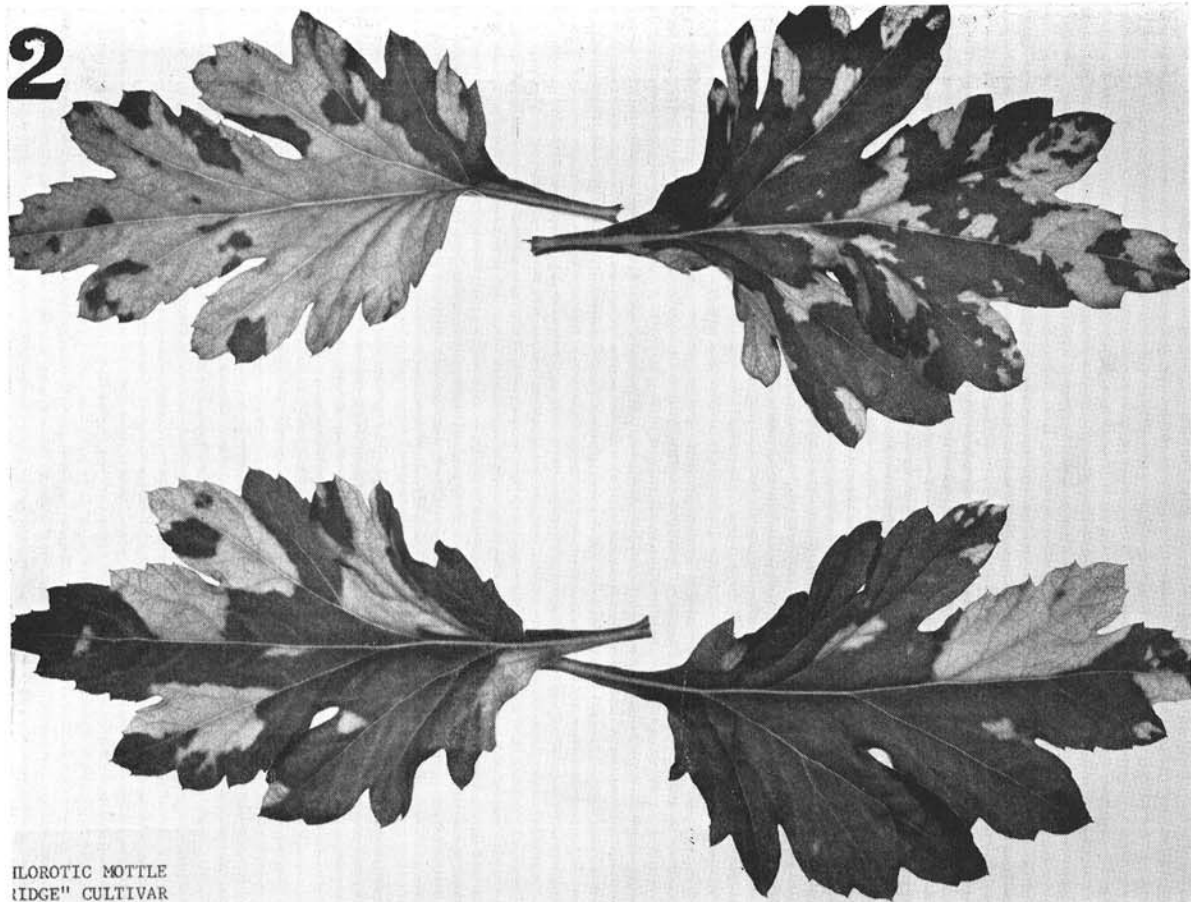
Symptoms	Cultivars
Marked mottling, followed by general chlorosis	Yellow Delaware, Delaware, Blue Ridge, Deep Ridge, Tinker Bell
Marked mottling and chlorosis, later growth reverting to normal or near-normal vigor and color	Dark Red Star, Giant Betsy Ross, Hurricane, Icecap, Mandalav, Torch, Ruby Mound, Rosey Nook
Chlorotic spotting, vein clearing, very mild chlorosis	Knob Hill, Matador, Mermaid, Red Cap, Winter Carnival, Zonta
No visible symptoms	Albatross, Blue Chin, Bright Golden Anne, Fanfare, Fred Shoemith, Good News, Indianapolis No. 4, plus 18 garden varieties.

slits in stems of healthy plants. The injured stem areas were then wrapped with paraffin film. Later, very small plugs or cores of leaf or stem tissue from infected plants were inserted in holes made by removing similar cores from stems of healthy plants. In all cases, the degree of transmission was high, being 100% when donor tissue was taken from near the stem apex and implanted in a similar position in the acceptor stem.

*Temperature and symptom expression.*—Tissue implantation proved particularly useful in determining the effect of temperature on symptom expression. Forty small Deep Ridge plants were selected for uniformity, each was implanted with chlorotic mottle tissue near the apex, and 10 plants were placed immediately in each of four growth chambers held at the following day (14 hr) and night (10 hr) temperatures (C), respectively: 15.5:10.0; 21.1:15.5; 26.6:21.0; 32.2:26.6. No symptoms developed even after 4 months at 15.5:10.0; moderate symptoms developed only after 30 days at 21.1:15.5; very pronounced symptoms developed within 12 to 15 days at 26.6:21.1; pronounced symptoms developed within 17 to 20 days at 32.2:26.6. There was surprising uniformity within each group both as to time and intensity of symptom development. This clear-cut picture, which we had not been able to obtain by any other approach, was crucial to the development of indexing procedures. Reliable indexing obviously requires growing temperatures well above 21.1 C. Studies currently in progress, however, indicate that disease development may be suppressed by relatively brief daily exposures of infected plants to 35 C or higher. These results provide a consistent explanation of seasonal variation in symptom expression as observed in glasshouse and field.

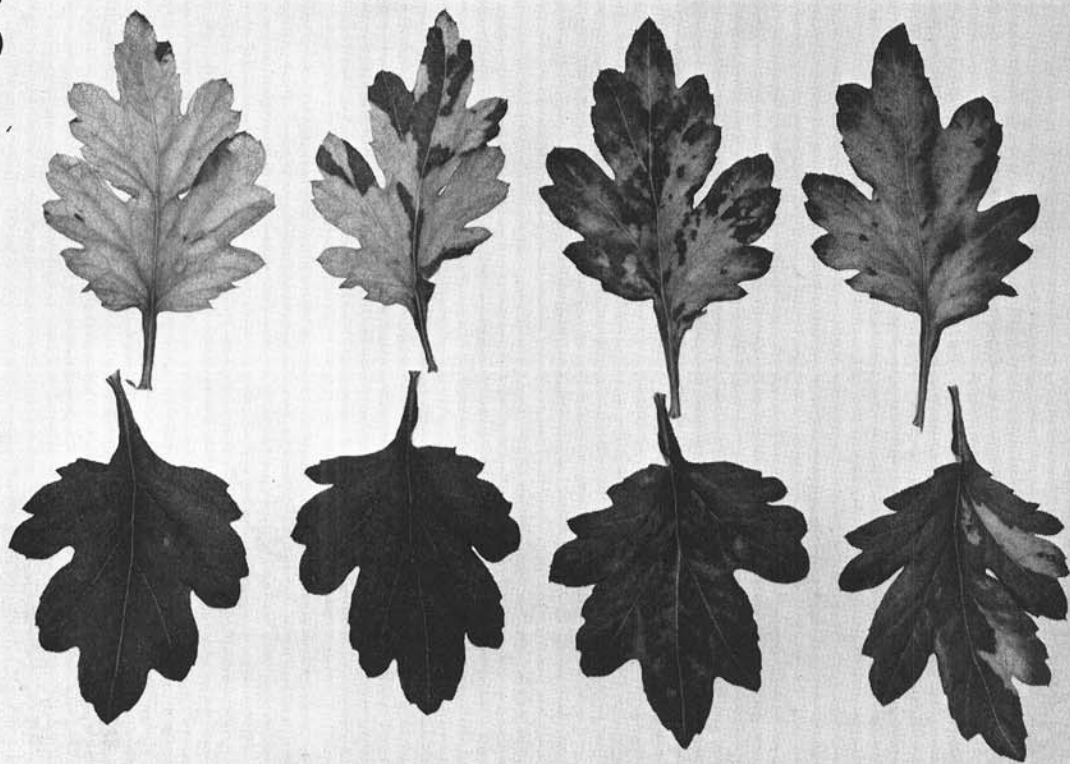
The sequence of symptoms developing in scions of the more reactive cultivars (e.g., Ridge, Delaware, and Tinker Bell) grafted onto diseased understock or in new growth above tissue implants has been quite regular and predictable. The first two or three leaves to expand following grafting or tissue implantation appear normal and uniformly dark green; then one or two

2



CHLOROTIC MOTTLE  
"RIDGE" CULTIVAR

3



CHLOROTIC MOTTLE  
"RIDGE" CULTIVAR  
27 DAYS FROM INPLANT  
10/3/69

**Fig. 2-3.** 2) Striking mottling of leaves of Blue Ridge chrysanthemum. These were taken from Blue Ridge plants infected with chlorotic mottle by tissue implantation. 3) Progression of symptoms in chrysanthemum following implantation of chlorotic mottle tissue approximately 2 cm below the apical meristem of healthy Blue Ridge plant. First new leaf to expand is at lower left, with subsequently expanding leaves in counter-clockwise order.

leaves may show vein-clearing and mild mottling (first evident in about 2 weeks); the next one or two leaves develop marked mottling (Fig. 2), with sharply defined yellow and dark-green areas (evident only 1 or 2 days after the preceding stage); finally, the subsequently developing leaves are uniformly yellow with only a trace of green pigmentation (Fig. 3). With less reactive varieties, symptom expression may progress to the stage of distinct mottling, with subsequent reversion of new growth to normal green color. Occasionally even the more reactive varieties will show symptom reversion, but tests mentioned earlier indicate that this is masking of symptoms, not loss of the systemic incitant. It should be noted that, in general, once leaves on artificially infected plants have developed distinctive mottling they do not change; the yellow areas remain yellow, the dark green remain dark green.

Electron microscope studies of thin-sectioned mesophyll tissues and "dip" preparations have failed thus far to demonstrate viruslike particles or other structures of possible etiological significance.

Because of the prominence of the distinctive mottling and subsequent general chlorosis following artificial inoculation of reactive varieties, we propose the term "chlorotic mottle" to distinguish this disease. The terms "vein mottle", "necrotic mottle", "dwarf mottle", "mild mottle", "stunt mottle", "slight mottle", "dull yellowish mottling", and "leaf mottling" have previously been employed in discussing chrysanthemum diseases (2, 6, 7, 8, 9, 13), but we have not found the term "chlorotic mottle" so employed.

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