A MOSAIC DISEASE OF HYBRID AMARYLLIS CAUSED BY CUCUMBER MOSAIC VIRUS

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INTRODUCTION

The mosaic disease of Amaryllis has long been thought to be caused by a virus. Recently the causal virus has been isolated from infected Amaryllis spp. and identified as strains of cucumber mosaic virus (CMV) by Kahn and Smith (10) and by the author. The virus isolated from Florida-grown plants does not appear to be identical to that isolated by Kahn and Smith. Cucumber mosaic virus has a wide host range and is not restricted to ornamental plants; it is one of the most commonly occurring plant viruses in Florida. Since Amaryllis spp. are widely grown in Florida, both commercially and in home gardens, information concerning the causal agent of the disease and its control should be of general interest. This report will summarize the results of current research and show how these results might be used to develop control measures for this disease.

REVIEW OF THE LITERATURE

In 1935, Townsend (13) observed the spread of mosaic in a planting of Amaryllis in Florida but was unable to determine the causal virus or the vector responsible for its spread. In a report published in 1948, dealing with diseases of the Amaryllidaceae, Brierley (3) described the symptoms of the mosaic disease in Amaryllis and reviewed the earlier literature on this topic. Hannibal (5) published a list of the various amaryllids known to be infected with the disease. Johnson (8) reported the occurrence of long, thread-like flexuous, rod-shaped particles in electron micrographs of leaf exudate from mosaic-infected Amaryllis. He apparently made no attempt, however, to determine the relationship of such particles to the disease. That such particles described by Johnson have a causal relationship to Amaryllis mosaic may be questioned in view of the fact that Scott (12) recently showed CMV to have spherical-shaped particles. Virus-like bodies were observed as early as 1928, when McKinney, Eckerson and Webb (11) were able to demonstrate the presence of intracellular bodies in mottled leaves of Hippeastrum johnsonii Bury. Holmes (6), working with Hippeastrum equestre Herb., was able to demonstrate similar intracellular bodies. The familiar symptoms of Amaryllis mosaic were described in later publications by Traub (14) and Kahn and Smith (9, 10) and the disease was noted in the 1960 Index of Plant Diseases (2) as being caused by an unidentified virus. Anderson (1) was perhaps the first to report an association of CMV with mottled Amaryllis, but he failed to obtain the additional evidence necessary to show that the virus he isolated was the primary causal agent of the disease. It remained for Kahn and Smith (10) in 1963, to present conclusive evidence that CMV can produce a mosaic disease in Amaryllis spp.

SYMPTOMATOLOGY

As the name implies, the symptoms most generally associated with the disease are mosaic-like patterns in the foliage which may vary from an almost symptomless mottling to a very conspicuous mosaic pattern (Fig. 1). Those most commonly seen in Florida appear as light-green to yellow mosaic patterns on a dark green background. The coloration tends to form a longitudinal pattern, giving the leaf a striped appearance. Ring-like or diamond-shaped patterns have been reported by Kahn and Smith (10), such patterns being obtained, however, only as a result of mechanical inoculation. Other foliage symptoms, including necrosis, are not generally associated with this disease (3) ; there is, however, at least one report (18) of infected plants showing progressive deterioration in foliage development, including a reduction in size and number of leaves.

Flower-breaking in some lines such as A. belladonna and A. striata may be associated with the leaf mottling symptoms (9), although there is no direct proof that CMV causes symptoms in floral parts of Amaryllis plants. Kahn (9) reports that Prof. Nelson has observed a general
loss of vigor in infected bulbs of *A. evansiae* at the Southwestern Louisiana Institute; quantitative data, however, are not available to support this observation. Seed production is apparently not affected by the presence of CMV in one or both of the parent plants.

**Transmission**

1. **Mechanical Transmission:**—The Amaryllis virus used in this study was transmitted by the gauze-pad method. Juice from infected leaves was rubbed over the surface of healthy leaves that were previously dusted with 500-mesh Carborundum. Difficulty was often encountered in the original transfer from Amaryllis, but subsequent transfers in tobacco presented no problems. Transmission was less difficult when young *Amaryllis* leaves were used as the source of inoculum; possibly old leaves contain either a low virus titer or inhibitors of virus infection.

2. **Insect Transmission:**—The pattern of field spread of Amaryllis mosaic suggested to previous investigators (9, 13, 14) that an insect vector was involved. No vector for the virus was actually demonstrated, however, until Kahn and Smith (10) demonstrated that the virus was transmitted by the green peach aphid, *Myzus persicae* Sulz. from amaryllid or tobacco source plants to healthy tobacco or Amaryllis seedlings. In the present work, it was found that the CMV strain isolated from Florida Amaryllis is also transmitted by the green peach aphid. From the results of research carried out with other strains of CMV, we know that the virus can be transmitted by the winged and wingless forms of the vector after very short acquisition periods (5 to 30 seconds) on infected plants. There is no evidence that the virus persists or multiplies in the insect vector; therefore the aphid loses its ability to transmit the virus a short time after it acquires the virus or after feeding on one or two healthy plants. It is necessary for the aphid to acquire additional virus from infected source plants in order to continue transmitting to other plants.

3. **Transmission Resulting from Propagation:**—There are no reports of the mosaic virus being carried through true seed of Amaryllis, even though both parent plants are severely infected. Amaryllis is also propagated vegetatively, either by off-sets, by fractional scale-stem methods, or by bulb-scale methods, and it is quite likely that the virus is perpetuated in the new plant derived by one or another of these vegetative methods. Indeed, the question remains for further research to determine whether or not Amaryllis can be freed from the virus by propagating plants by one or another of these methods.

**Identification of the Virus**

1. **Host Range:**—The virus was initially transferred from its natural host to a series of "indicator plants," including *Nicotiana tabacum* L., *N. glutinosa* L., *Cucumis sativus* L., *Vinca rosea* L., *Datura stramonium* L., and *Chenopodium amaranticolor* Coste and Reyn. (Fig. 2). The symptoms induced in these and other indicator plants were similar to those reported for CMV, except that the symptoms in *Nicotiana* spp. were more severe than those reported for other strains of CMV. Moreover, the Florida strain failed to
Fig. 2.—Symptoms produced in three indicator plants by the Florida isolate of CMV from hybrid Amaryllis. A) Symptoms in inoculated leaves of tobacco; B) Symptoms in leaves of tobacco systemically invaded by the virus; C) Inoculated leaf of Chenopodium amaranticolor, and D) Nicotiana glutinosa systemically invaded by the virus.
produce visible symptoms in *Vigna sinensis* Savi.

2. **Physical Properties:** Additional evidence for identification of the *Amaryllis* virus was obtained from its various physical properties. The Florida isolate retained biological activity after heating for 10 minutes at 65°C but not at 70°C, was infectious at a dilution of 1:316 but not 1:1000, and lost 90% of its biological activity after standing for 24 hours at 20°C. These data are similar to those reported by Kahn and Smith (10) for their isolate of CMV from *Amaryllis*.

3. **Cross-Protection Tests:** Cross-protection tests were carried out using the opposite half-leaf technique. Half leaves of tobacco plants were inoculated with the American Type Culture Collection CMV strain No. 12 (ATCC No. 12) as the protecting virus. To serve as controls, the opposite halves of these leaves were rubbed with juice from healthy tobacco. At 2-day intervals thereafter, both leaf halves were challenged with the *Amaryllis* isolate, which produces local lesions in leaves of tobacco. By the 8th day after the original inoculation, CMV-ATCC No. 12 gave almost complete protection against the formation of local lesions by the *Amaryllis* isolate.

4. **Serological Tests:** Serological tests (Ouchterlony agar double-diffusion technique) were carried out to compare the 2 virus isolates from *Amaryllis* (the Florida isolate and one obtained by Kahn) with 2 known strains of CMV (ATCC No. 10 and ATCC No. 127). The antiserum used for these tests was prepared by Dr. Howard Scott and contained specific antibodies for the ATCC No. 127 strain of CMV. Positive serological reactions were obtained between the antiserum used and each of the four virus antigens; no reaction however, was obtained between this antiserum and juice from healthy control plants.

The evidence obtained from host range studies, physical properties data, insect transmission studies, cross-protection tests and serological tests show that the virus responsible for mosaic symptoms in Florida-grown *Amaryllis* is a strain of CMV. The symptoms induced by the Florida isolate in certain indicator plants warrant its consideration as a distinct strain, different from ATCC No. 10, No. 12, No. 127 or the strain isolated from *Amaryllis* by Kahn and Smith (10).

**CONTROL**

At present, satisfactory chemical control measures for virus diseases are not available; thus increased emphasis must be placed on preventive measures. With the information which we have concerning the virus from *Amaryllis* plus that reported for other strains of CMV, the following control practices can be recommended.

1. **Insect Control:** Greenhouses used for growing *Amaryllis* should be completely screened with fine-mesh screening to prevent the entrance of viruliferous aphids. To insure against insect transmission within the greenhouse, a routine insecticide spray program should be followed. Such a program would also be beneficial in removing other insects which may be vectors of other viruses, such as tomato spotted wilt virus, to which *Amaryllis* plants are susceptible. Insecticides should be applied regularly to outdoor-grown *Amaryllis* and to other ornamental and weed plants growing in close proximity to *Amaryllis*. Such plants may serve as virus reservoirs and sources of virus which may be transmitted to *Amaryllis*.

2. **Isolation:** *Amaryllis* propagating stock or valuable specimen plants should not be grown adjacent to mosaic-infected *Amaryllis* or other plant species showing virus-like symptoms. All new acquisitions or outdoor-grown plants should be grown in isolation to determine their freedom from virus diseases before they are moved into the greenhouse. One should avoid the introduction of other vegetatively propagated plants into greenhouses used for *Amaryllis* and likewise should avoid planting *Amaryllis* adjacent to cucurbit and legume crops which are likely to be sources of CMV.

3. **Sanitation:** Care should be taken not to handle healthy plants after handling diseased plants without first washing one's hands thoroughly with an abrasive soap followed by rinsing with copious amounts of water. To avoid contamination during the propagation procedures, knives and other tools should be thoroughly disinfested after each bulb. This can be done in any one of the following ways:

   a. washing with soap or detergent, followed by thorough rinsing,
   b. immersing tools in boiling water for 10 minutes,
   c. soaking the tools in a saturated solution of trisodium phosphate, followed by thorough rinsing,
   d. immersing the knife blade in ethyl alcohol, removing from the alcohol and igniting; excess alcohol should be allowed to burn off and cool before using.
Resistant Varieties:—That resistance to the mosaic disease might be available is indicated by the work of Hannibal (5), who found that *Amaryllis rutila* (the type) is resistant whereas the variety *fulgida* and hybrids are not. It appears from the literature, however, that no concerted effort has been made to locate additional *Amaryllis* spp. that are resistant to this virus and to incorporate this resistance, along with other desirable characteristics, into new hybrids which are being developed.

Discussion

Although we now know that CMV can cause the mosaic disease in *Amaryllis*, much still remains to be learned about this disease. Is the *Amaryllis* virus a unique strain of CMV, restricted primarily to members of the *Amaryllidaceae* and other monocotyledonous plants, or are all strains of CMV able to incite mosaic symptoms in *Amaryllis*? If this is a unique strain of CMV, what plant is serving as the source of the infection? Is the virus spread among *Amaryllis* plants by the aphid vector; or is it introduced into *Amaryllis* from other ornamental or crop plants by the insect vector; or is it spread merely by contamination and vegetative propagation? Is it possible to heat cure an *Amaryllis* plant as has been reported by Holmes (6) for rose plants infected with mosaic? Perhaps the scale propagation technique reported by Brierley (4) for freeing Easter lilies from CMV might also be successful in freeing *Amaryllis* from CMV. Early detection of the virus, made possible by new serological techniques and sensitive indicator plants, coupled with stringent control measures and improved methods of vegetative propagation may, in the future, help eliminate mosaic as a major disease in Florida-grown *Amaryllis*.

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Literature Cited


The Influence of Harvest Seasons and Storage Temperatures on Florida Gladiolus Corms

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Numerous investigations have shown that low temperatures (32°F to 50°F) are essential in breaking dormancy of gladiolus corms uniformly and maintaining corms in a non-vegetative state during long storage periods (3, 5, 6, 7, 8, 9, 11). Also, exposure of corms to 70°-90°F for 1-2 weeks following cold storage stimulates early sprouting.