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Vegetative Propagation and the Identification of Five Viruses Infecting *Verbena x hybrida* in Florida[1]

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INTRODUCTION: Vegetative propagation is a method of asexual reproduction in plants. It occurs naturally in some plants and it can be done artificially by human beings. Cuttings can be taken from so called "mother plants" and rooted to produce genetically identical plants. Smaller pieces of the mother plant can be removed under sterile laboratory conditions and grown into mature plants using a process called tissue culture. The ornamental industry has been using these techniques for years for many popular plants because it reduces the amount of time to raise mature plants to sell, it increases the amount of plants that can be grown to sell, and it maintains the genetic makeup of the desired plant.

While vegetative propagation increases the amount of material that can be sold commercially, it also heightens the risk of viral infection of plants. Kraus *et al.* 2008 reported that, "Vegetatively propagated crops are particularly prone to virus infections..." This is true even for those plants propagated using plant tissue culture techniques. Although tissue culture methods are used specifically to eliminate plant pathogens, they are not 100% effective at eliminating virus infections from new plants (Matthews 2010). A case in point was brought home recently in Florida when a *Verbena* plant sent to the Division of Plant Industry in 2013 for pathogen diagnosis was found to harbor five different viruses.

VERBENA AND ITS VIRUSES: *Verbena*, particularly *Verbena x hybrida* or garden verbena, is a popular ornamental landscape plant because of its ease of growth, its lack of pest problems, and its beautiful long lasting blooms. *Verbena* hybrids are the result of careful cross breeding and the selection of the best varieties (House Plants Guru, 2015). Given this and their popularity, *Verbena* species are often vegetatively propagated by cuttings or tissue culture.

Verbena plants are known hosts for many different viral pathogens. They include:

- *Alfalfa mosaic alfamovirus* (Mumford *et al.* 2005)
- *Angelonia flower break carmovirus* (Assos Filho *et al.* 2006)
- *Bean yellow mosaic potyvirus* (pea strain) (Guaragna *et al.* 2004)
- *Bidens mottle potyvirus* (Florida Department of Agriculture and Consumer Services)
- *Broad bean wilt fabavirus* (Breman *et al.* 1995)
- *Carnation ringspot dianthovirus* (Brunt *et al.*)
- *Clover yellow mosaic potyvirus* (Baker *et al.* 2004)
- *Coleus vein necrosis carlavirus* (Kraus *et al.* 2008)
- *Cucumber mosaic cucumovirus* (Mumford *et al.* 2005)
- *Impatiens necrotic spot tospovirus* (Mumford *et al.* 2005)
- *Melilotus mosaic potyvirus* (Brunt *et al.*)
- *Nemesia ring necrosis tymovirus* (Mathews and Dodds 2006)
- *Tobacco mosaic tobamovirus* (Mumford *et al.* 2005)
- *Tomato spotted wilt tospovirus* (Mumford *et al.* 2005)
- *Tobacco etch potyvirus* (Florida Department of Agriculture and Consumer Services)
- *Tobacco ringspot nepovirus* (Florida Department of Agriculture and Consumer Services)
- *Verbena latent carlavirus* (Cohen *et al.* 2003).

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- *Verbena virus Y* (*potyvirus*) (Kraus *et. al.* 2010).

In addition, several publications have indicated the presence of two or more virus species in a single infected plant of *Verbena* spp. (Mathews and Dodds 2006; Mumford *et al.* 2005; Kraus *et al.* 2006, and 2010). A research project being done at The Department of Plant Pathology and Microbiology, University of California, Riverside, to improve virus detection in tissue cultured plants, used *Verbena* as the host plant for their research because it is commonly infected with multiple viruses. Many times there are 3-4 different viruses in the same plant (Matthews 2010).

SYMPTOMS: Given that *Verbena* plants are often diagnosed with more than a single virus, the symptoms can vary widely. Plants with various abnormal discolorations (mottle, mosaic, chlorosis, ringspots, etc.), necrosis, reduced vigor or stunting (Fig. 1 a-e) are often submitted for plant diagnosis. However, the real problem with plants that are vegetatively propagated either by cuttings or tissue culture is well stated by Matthews (2010): "new viruses may escape detection since most routine screening relies on specific antibody or nucleic acid based tests for known viruses, and because symptoms may be absent or quite subtle, especially in young plantlets."

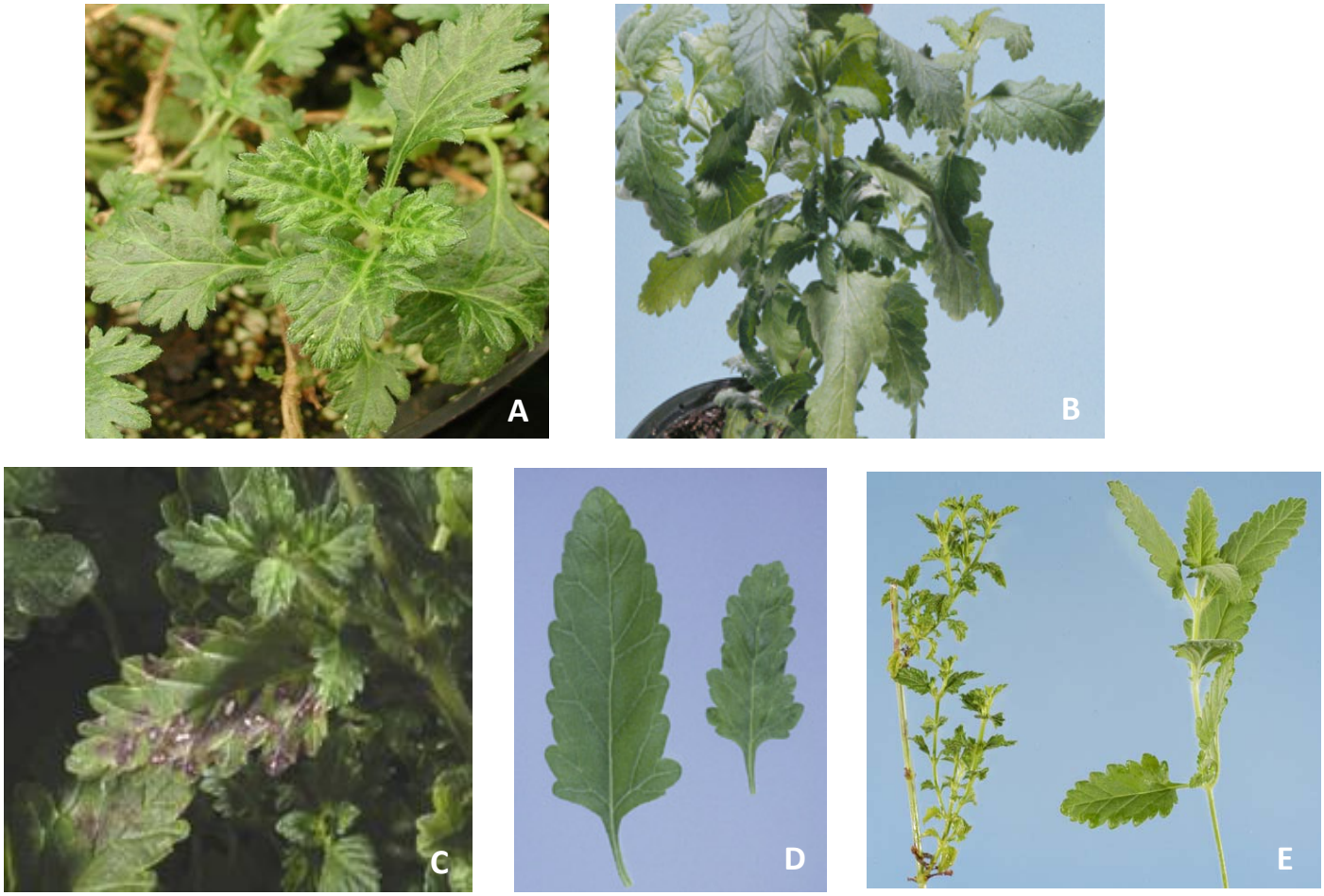

Fig. 1. Various symptoms found on *Verbena hybrida*: **A.** Chlorosis (Hammond *et al.*), Photography credit: USDA. **B.** Deformed leaves, **C.** Necrotic spots, and **D-E.** Stunting. Photography credit: Mariana Beckman and Jeffrey Lotz, FDACS-DPI.

Those small plants are then sold and as they grow they develop symptoms that make them no longer saleable. In addition, if the infected plants are retained and used for propagation, other viruses can accumulate in some plants over time leading to the circumstances found in 2013.

DETECTION AND DIAGNOSIS: To test for plant viruses in *Verbena*, primers were made for eight different viruses known to infect *Verbena* spp. They included *Bean yellow mosaic potyvirus* (pea strain), *Broad bean wilt fabavirus, Clover yellow mosaic potyvirus, Coleus vein necrosis carlavirus, Impatiens necrotic spot tospovirus,*

Nemesia ring necrosis tymovirus, Verbena latent carlavirus, and *Verbena virus Y (potyvirus)*. Nucleotide sequences from the GenBank were used to design specific primers for each virus using the CLUSTAL-X alignment program, and then the primers were used in a RT-PCR test. Total RNA was extracted from 100 mg leaf tissues of the Verbena specimen using a QIAGEN RNeasy kit. The test produced the expected DNA bands for *Bean yellow mosaic potyvirus* (pea strain), *Clover yellow mosaic potyvirus, Impatiens necrotic spot tospovirus, Nemesia ring necrosis tymovirus, and Verbena latent carlavirus.*

CONTROL: Given the importance of *Verbena* to the ornamental industry and the difficulty of growing it by seed (Syngenta Flowers), vegetative propagation will continue to be the major means of growing new plants for the marketplace. The same is true for many other commercially grown plants (*i.e.*, *Phlox* and *Passiflora*). Thus, the only means of virus control in these plants is constant vigilance. The plants used for cuttings or tissue culture should be tested for virus infections and those that test negatively for known viruses should be maintained in an insect-free environment. No new plants should be added to the mother plants until they have been tested and found free of viruses known to infect them. Even under these circumstances, vigilance should be maintained. Given how many viruses are known to infect *Verbena*, it is always possible for yet another unknown virus to manifest itself in the propagated material.

SUMMARY: Unlike plant diseases caused by fungi or bacteria, there are no chemicals that can cure a virus-infected plant or prevent its infection. The only thing that can be done is to reduce the occurrence and transmission of viruses by using the practices listed below:

- Purchase only virus-free plants.
- Maintain strict insect and mite control.
- Remove all weeds since these may harbor both viruses and potential insect vectors.
- Remove all crop debris from benches and the greenhouse structure.
- Immediately isolate plants with suggestive viral symptoms and obtain a diagnosis from your plant disease clinic.
- Discard virus-infected plants.
- Disinfest tools used for vegetative propagation frequently by placing them in a chlorine solution, hydrogen peroxide, or a quaternary ammonium disinfectant for at least 10 minutes. Rinse thoroughly with tap water.
- Propagate plants via seed rather than vegetatively when possible. Note that some viruses of certain crops can be carried in or on the seed. In these cases, purchase only virus-free seed (Moorman 2014).

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