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Viruses Infecting Passiflora Species in Florida[1]

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INTRODUCTION: Two papers were published in the early 1990s concerning viruses found in *Passiflora* species in Florida (Elliot *et al.* 1991 and St. Hill *et al*. 1992). At that time, surveys found only two viruses. *Maracuja mosaic virus* (a *Tobamovirus*), was found in a germplasm collection in Miami-Dade County, Florida. The second, *Passiflora latent virus* (a *Carlavirus*), was found in a botanical collection in Alachua County, Florida. The surveys were conducted because the commercial production of passion fruit in Florida had increased, but there was no information about viruses of *Passiflora* spp. in Florida. Both papers warned of the possible spread of these two viruses and the introduction of other viruses of *Passiflora* spp. into South Florida due to the "free exchange of vegetative material between growers, collectors, hobbyists, homeowners and botanical gardens" (Elliot *et al.* 1991). Starting in 2004, this prediction became a reality as several samples of *Passiflora* sent to the Division of Plant Industry (DPI) were diagnosed with at least four, possibly six, additional viruses, all aphid-transmitted potyviruses.

THE HOST: The genus *Passiflora* (common name passionflower or passion vine) contains over 500 different species, nine of which are native to the United States. Others are native to South America, Eastern and Southern Asia, New Guinea, Australia, and New Zealand. Many species are cultivated outside their native habitats because of the beauty of their flowers, as food for butterfly larva, as a source of herbal medicine for the relief of anxiety, or for their fruit. *Passiflora edulis*, for example, is cultivated for its fruit in Africa, Australia, the Caribbean, Central America, Hawaii, South America, Southern Asia and the United States. The fruit is either eaten or juiced.

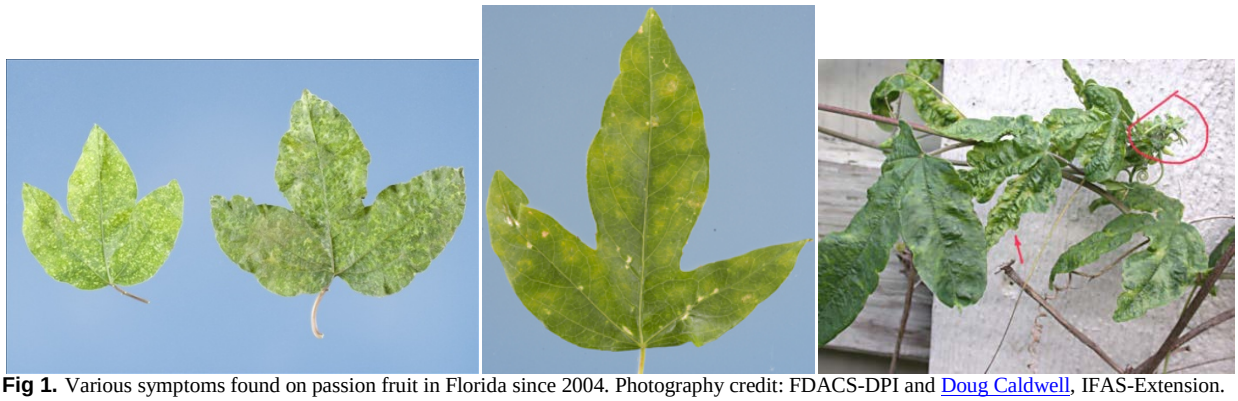

Fig 1. Various symptoms found on passion fruit in Florida since 2004. Photography credit: FDACS-DPI and Doug Caldwell, IFAS-Extension.

VIRUS DETECTION AND DIAGNOSIS: There are a number of different viruses reported in *Passiflora* species from around the world (Fischer and Rezende 2008, Koizumi 1995, Liberato and Zerbini 2014). Many of them are potyviruses. Potyviruses can be diagnosed by distinctive viral inclusions. There are three potyviruses of passion fruit, *Passionfruit woodiness virus* (Taylor and Greber 1973), *Passionfruit mottle* virus (Chang 1992) and *Passionfruit crinkle virus* (Chang *et. al*. 1996), that have been differentiated based on differences in their inclusion morphologies (Fig. 2a-c). Antiserum was made to each of these three viruses in the late 1980s and early 1990s and is available at the Division of Plant Industry in Gainesville, Florida. These antisera were used in sodium dodecyl sulfate (SDS)-immunodiffusion tests (Purcifull and Batchelor 1977) to confirm the presence of *Passionfruit woodiness virus*, *Passionfruit mottle virus and Passionfruit crinkle virus*. A fourth potyvirus, named *Passiflora chlorosis virus*, discovered in 2004 (Baker and Jones 2007), was diagnosed by its viral inclusions in

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leaf strips (Fig. 2d) and its lack of reaction with the antisera to the other three passion fruit potyviruses in SDS immunodiffusion tests.

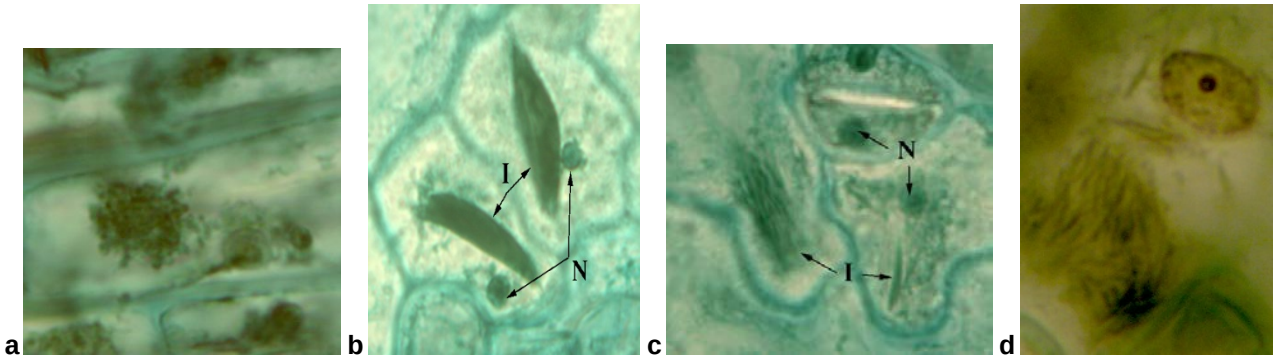

Fig 2. Various potyvirus inclusion bodies found in leaf strips of *Passiflora* in Florida since 2004. **a)** Inclusions of *Passionfruit woodiness virus*, **b**) Inclusions of *Passionfruit mottle virus*, **c**) Inclusions *of Passionfruit crinkle virus* (Chang *et al*. 1996*)*, and **d)** Inclusions of *Passiflora chlorosis virus*. N=nucleus I=Cylindrical inclusions. Photography credit: Dr. Carlye A. Baker, FDACS-DPI.

Passiflora chlorosis virus was also sequenced and compared to other potyvirus sequences. It did not match any of the virus sequences deposited in GenBank at the time. Unfortunately, not all of the potyviruses diagnosed in *Passiflora* spp. using inclusion morphology have been sequenced.

One that has been sequenced is *Passionfruit woodiness virus.* In an attempt to confirm this virus in Florida-grown plants, RT-PCR using degenerate potyvirus primers was used. To our surprise, sequence analysis of the RT-PCR product was identified as *Cowpea aphid-borne mosaic virus*. This virus was previously identified in *Passiflora edulis f. flavicarpa* in Brazil (Nascimento *et al*. 2006).

The next step to confirm the identity of *Passionfruit woodiness virus* was to design specific RT-PCR primers. Specific primers were designed for *Passionfruit woodiness virus*, *Cowpea aphid-borne mosaic virus*, *Passiflora chlorosis virus*, *Passiflora virus Y* and *Passiflora latent virus*. Again to our surprise, all five primers gave positive results in the two samples tested. These positive tests not only confirmed the presence of *Passionfruit woodiness virus* and the *Cowpea aphid-borne mosaic virus*, they also reconfirmed the *Passiflora chlorosis virus* and the *Passiflora latent virus* diagnosed earlier, and found another potyvirus, *Passiflora virus Y*, originally reported in Australia in *Passiflora foetida.* (Parry *et al*. 2004).

VIRUS SPREAD AND CONTROL: Potyviruses and carlaviruses are vectored by aphids. However, insects are probably not the major source of virus spread for these viruses into Florida. The major means of virus spread is the vegetative propagation of these plants, a fact that also contributes to multiple virus infections.

Vegetative propagation is the major means of plant reproduction in the ornamental industry. Increasing plants for sale is easier, faster and less expensive by rooting cuttings of popular plants and the progeny are true to type. Unfortunately, if any of the mother plants are virus infected, it is also easier and faster to increase the number of virus-infected plants. If there is more than one virus in the mother plants or insects bring in other viruses over time, the number of viruses in the plants can increase with continued vegetative propagation if clean techniques are not used. Plants found to be infected should be destroyed to avoid further spread of these viruses whether by vegetative propagation or by aphid infestations.

SUMMARY: *Passiflora* viruses have a long history of being assigned different names that are later found to be the same virus after sequencing. For example, *Passionfruit woodiness virus* was believed to be the same as what were called *Passiflora chlorotic spot virus*, *Passiflora mosaic virus* and *Passion fruit mosaic virus* (Liberato and Zerbini 2014). In 2011, work by Coutts *et al.* 2001 in Australia found Passiflora mosaic to be a distinct virus from *Passionfruit woodiness virus.* A virus once called *South African passiflora virus* has been found to be *Cowpea aphid-borne mosaic virus* (McKern *et al*. 1994). Until *Passionfruit crinkle virus* and *Passionfruit mottle virus* are isolated, sequenced and compared to the sequences in the GenBank, it is impossible to know if there are 4, 5 or 6 potyviruses that have been identified in Florida *Passiflora* spp. Most of the samples tested at DPI have either been diagnosed as *Passionfruit woodiness virus* by viral inclusions or have been mixed infections with *Passionfruit woodiness virus* making it difficult to isolate individual viruses. In addition, the sample that originally tested

positive for *Cowpea aphid-borne mosaic virus* also tested positive *for Passionfruit woodiness virus* and *Passionfruit crinkle virus* in SDS-immunodiffusion.

For now, we know that *Passionfruit woodiness virus* and *Cowpea aphid-borne mosaic virus*, which are "considered the most important diseases of *Passiflora* crops" (Fisher and Rezende 2008), have entered Florida, as has *Passiflora virus Y*. We know that *Passiflora latent virus* has been found outside of Alachua County, Florida. *Passiflora chlorosis virus*, first sequenced in Florida, has now been found in Europe (Cardin *et al*. 2009) and Africa (Ochwo-Ssemakula, *et al*. 2012). We also know that the concern of virus spread voiced in 1991 has been realized and significant virus spread has occurred.

To date, the viruses have only been found in collections or nurseries. We would like to reiterate the warnings of 1991… "the free exchange of vegetative material among growers, collectors, hobbyists, home owners and botanical gardens poses a great risk" – an even greater one now that several aphid-transmitted potyviruses that infect *Passiflora* spp. have been identified in Florida for the first time.

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