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Is the foliar yellow vein of some ornamental plants caused by plant viruses?

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IS THE FOLIAR YELLOW VEIN OF SOME ORNAMENTAL PLANTS CAUSED BY PLANT VIRUSES?

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To my grandparents, Cesar and Sara

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ABSTRACT

Plant viruses have shown to be the cause of the yellow vein symptoms on the foliage of several ornamental plants. In this study five ornamental plants: oxalis (*Oxalis debilis* var. corymbosa) cv. Golden Veined Oxalis, geranium (*Pelargonium peltatum*) cv. Crocodile, pseuderanthemum (*Pseuderanthemum carruthersii*) cv. Golden Net Bush (narrow leaf and broad leaf variants), honeysuckle (*Lonicera japonica*) cv. Yellow Net Honeysuckle and coleus (*Solenostemon scutellarioides*) cv. Electric Lime, showing foliar yellow vein were used. Graft transmissions indicated that, except for coleus, the foliar yellow vein of all other plants was caused by infectious agents, likely plant viruses. With the exception of Golden Veined Oxalis, attempts to identify a virus as the causal agent of the foliar yellow vein in geranium, pseuderanthemum, and honeysuckle failed.

Successful whitefly (*Bemisia tabaci* biotype B) transmission of the foliar yellow vein of Golden Veined Oxalis suggested that a begomovirus was the causal agent. Therefore, PCR amplifications were conducted using degenerate primers for the genus *Begomovirus*. A 1.2 kb PCR product was obtained using the primer set PAL1v1978/ PAR1c496 and a 0.7 kb product with the primer set AV494/AC1048. These sets of primers have been used widely to detect and identify begomoviruses. Sequencing of the fragments confirmed the begomovirus nature of the amplified DNA from Golden Veined Oxalis. Sequence information and phylogenetic analyses revealed that the Golden Veined Oxalis begomovirus was closely related to *Tomato yellow spot virus* and *Sida mottle virus*, two begomoviruses reported to infect solanaceous and legume crops. The host range of the virus isolated from Golden Veined Oxalis begomovirus, designated Oxalis yellow vein virus, is not known. This finding illustrates the potential spread of plant viruses to different geographical areas through the commercialization of virus- infected ornamental plants.

CHAPTER I LITERATURE REVIEW

Viruses are submicroscopic agents composed of RNA or DNA and in most cases a coat protein. They contain their genetic information in the form of double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA) or single-stranded RNA (ssRNA), the last one being the most common in plant viruses (Matthews, 1992). The most common proteins coded by plant viral genes are the RNA or DNA polymerases which catalyze virus replication, the coat protein which protects the viral genome and the movement protein which is involved in cell-to-cell and systemic movement of the virus in the host plant (Hull, 2002). Viruses are known to cause diseases in economically important crops such as tomato [*Tomato yellow leaf curl virus* (TYLCV)], common bean [*Bean golden mosaic virus* (BGMV)], cucumber [*Cucumber mosaic virus* (CMV)] and cassava [*African cassava mosaic virus* (ACMV)], and often are associated with crop yield losses (Matthews, 1992).

1.1 Symptoms Caused by Plant Viruses

It is difficult to identify plant viruses based solely on the symptoms they cause because viruses seldom cause one type of symptom and usually several types of symptoms are present as viral diseases progress. It is very important to determine if the symptoms are local or systemic and they need to be differentiated from similar symptoms caused by insect pest, nutrient deficiency or herbicide injury (Matthews, 1992).

The most common external symptoms in plants caused by plant viruses are color deviations which result from chloroplast disorders. As the viral infection progresses, the tissue adjacent to the site of infection could become yellow. If the discoloration is distributed irregularly over the whole leaf lamina, the term variegation is often used and includes mottling, mosaics and line patterns. Line patterns can consist of vein mosaic, vein banding, oak-leaf pattern, concentric rings, and in flowers, color breaking (Gonsalves, 1989). Other symptoms caused by plant viruses are wilting, and withering which may be caused by water uptake imbalance; necrosis caused by death of cells and tissues; bronzing of the leaves when the epidermis died; malformation which is the uneven growth of leaf lamina; and enations which are outgrowths from the upper or lower surface of the leaf, forming ridges and dark-green leaf-like structures (Hamilton, 1980).

1.2 Mode of Transmission of Plant Viruses

In nature, plant viruses are spread from plant to plant by vectors, pollen, and through infected or infested true seed. Viruses are also known to spread through grafting on infected propagation materials and use of contaminated equipment (Hull, 2002).

1.2.1 Vector Transmission

Plant virus vectors include arthropods, nematodes, and fungi. Some arthropods include aphids(Family *Aphididae*), whiteflies (Family *Aleyroridae*), leafhoppers (Family *Cicadellidae*) and plant hoppers (Family *Delphacidae*), thrips (Family *Thripidae*), beetles (Family *Chrysomelidae*), scales and mealybugs (Families *Coccoidea* and *Pseudococcoidea*, respectively), and mites (Family *Eriophyidae*) (Ng and Falk, 2006).

Viruses can be divided into three groups according to the way they are transmitted by insect vectors: non-persistent, persistent, and semi-persistent. Non-persistent viruses can both be acquired and transmitted by a vector in a few seconds. Examples of virus genera transmitted in a non-persistent manner are the *Alfamovirus, Caulimovirus, Macluravirus* and *Potyvirus* (Hull, 2002). Persistently transmitted viruses require an acquisition time of 30 min to several hours or days. During that time the virus circulates in the body of the vector, reaches the salivary system and is secreted during feeding by the vector. One example in this group are luteoviruses such as *Potato leaf roll virus* (Hull, 2002). Semi-persistent viruses have intermediate characteristics between non-persistent and persistent viruses. The insect requires several hours to become viruliferous, but the virus does not circulate inside the vector. Examples of this type of transmission are *Caulimovirus* (*Cauliflower mosaic virus*) and *Closteroviruses* (*Beet yellow virus* and *Citrus tristeza virus*) (Ng and Falk, 2006).

Whiteflies, *Bemisia tabaci* (various biotypes), *Trialeurodes vaporariorum,* and *T. abutiloneus* are common vectors of virus members of the genera *Begomovirus, Closterovirus,* and *Crinivirus* (Bedford *et al*., 1994). The life cycle if this insect starts when eggs are deposited on the lower surface of the leaf. After a period of 3 to 10 days, eggs hatch, go through several instars (sedentary nymphs) and they reach the 3rd instar before they molt into a pupa. The pupal stage lasts for 3 to 6 days before the adults emerge. Acquisition periods of 8 to 48 h on the infected plant are needed for the whiteflies to become viruliferous. Similar time period is required for virus transmission. Some

characteristics such as previous starvation (1-3 h) and gender may affect their ability to transmit the virus (Dijkstra and Jager, 1998). Some examples of whitefly-transmitted begomoviruses, include, *Sweet potato leaf curl virus* (SPLCV) (Gutierrez *et al.,* 2003; Lotrakul *et al*., 2000) and TYLCV (Navot *et al*., 1991), both transmitted by *B. tabaci* biotype B. Whitefly-transmitted closteroviruses include *Beet pseudo-yellow virus* and *Cynodon chlorotic streak virus* (Rosell *et al*., 1997). The Crinivirus *Sweet potato chlorotic stunt virus* is transmitted by *B. tabaci* and *T. abutiloneus* (Karyeija *et al.*, 2000; Sim *et al*., 2000).

1.2.2 Graft Transmission

Plant viruses are known to spread through grafting. When an infected scion is grafted to a health root stock and vice versa, viruses are likely to move from infected to healthy tissue. Graft transmission is not common in nature (except in grafts between roots), but because grafting is widely used in commercial production of ornamentals, it has become a common mode of transmission and spreading plant viruses. An example of a graft transmissible virus is *Citrus tristeza virus*, a severe disease of citrus. Bar-Joseph *et al*. (1989) reported that severe tree decline occurred when virus-infected scions were grafted on sour orange rootstocks.

1.2.3 Mechanical Transmission

Many plant viruses are transmitted by mechanical means and *Tobacco mosaic virus* (TMV) is a classic example of this mode of transmission. Mechanically transmitted plant viruses are known to be very stable and they usually occur in high concentrations in plant tissues such as leaves, but there are some exceptions. One example is *Tobacco necrosis virus* which contains more virus in roots than in leaves (Smith, 1937). Another example of a virus that can be mechanically transmitted in nature is *Potato virus X* (PVX). This virus can be easily transmitted to adjacent plants by broken hairs (Hull, 2002).

1.2.4 Seed and Pollen Transmission

Some plant viruses are transmitted through seed and pollen. In seed transmission, the virus can be present on the seed coat or in the embryo (Lartey *et al.*, 1997). Other viruses such as *Alfalfa mosaic virus* are more efficiently transmitted by pollen than by seed (Frosheiser, 1974). Pollen transmitted viruses can cause two types of

infections,infection of the embryo or the direct infection of the mother plant. Cryptoviruses have been reported to be transmitted efficiently only by seed and pollen (Lisa *et al.*, 1996).

1.2.5 Vegetative Transmission

Vegetative transmission of plant viruses occurs when plants are propagated from virus-infected vegetative tissues such as tubers, bulbs, corms, stems, branches, or leaves. Vegetative propagation has been extensively used in plant production, but this practice has also become an efficient way to transmit plant viruses. Once infected, vegetative parts of the plant can remain infected throughout their entire life cycle. *Potato virus X*, *Sweet potato chlorotic stunt virus* (SPCSV), and *Sweet potato feathery mottle virus* are some of the most commonly plant viruses transmitted through vegetative propagation (Hull, 2002; Clark *et al.,* 2002).

1.3 Plant Viruses of Ornamental Plants

Some plants affected by plant viruses include ornamentals crops, which in the United States have a $165,839,000 sales (USDA, 2009). Dahlia stunt, ringspot of dahlias, nasturtium mosaic and yellow stripe of narcissus are well known diseases of ornamentals caused by plant viruses. Tospoviruses such as *Tomato spotted wilt virus* and *Impatiens necrotic spot virus* have been described in several ornamental plants around the world (Ghotbi *et al.*, 2005).

Although, viral infections of ornamentals may result in unmarketable plants. However, these infections can also cause desired variegation e.g. variegation of *Abutilon striatum* var. thompsonii caused by *Abutilon mosaic virus* (AbMV) (Pirone, 1978).

1.4 Variegations in Ornamental Plants

Plants with unusual foliage or exhibiting different colors are generally known as variegations. These variegations are often caused by genetic traits, physiological problems, or plant viruses. In some cases physiological disorders like nutrient deficiencies and plant viruses may cause similar symptoms (Valverde *et al*., 2012). Ornamental plants with variegations are of general interest due to their beauty and novelty and today, they are very common in many home and commercial landscapes. In most cases, these plants are commercialized as different cultivars (Valverde *et al.*, 2012).

Foliar and flower variegations caused by plant viruses have been described in the past. As earlier as in the 17th century, plant viruses were used to enhance the aesthetics of some ornamental plants (Cayley, 1928). Flower breaking or flower variegations is a common characteristic that can make one plant more commercially desirable over others. One of the first records of flower breaking caused by a virus was that of tulips grown by the Dutch tulip growers in the 17th century. This resulted in the characterization of a potyvirus (*Tulip breaking virus*) which was causing the tulip breaking (Cayley, 1928). Other well-known examples are the flowering maple (*Abutilon pictum*) infected with AbMV (Keur, 1934) and the variegated flowers of camellia (*Camellia japonica*) infected by Camellia yellow mottle virus (Plakidas, 1948). Although, many variegations in ornamental plants are caused by plant viruses, but some can result from genetic traits or physiological disorders.

Genetic variegations are classified into two groups. The first group includes pigment-related variegations which are caused as the result of chlorophyll imbalance [e.g. *Crocus vernus* and *Saururus chinensis* (Hara, 1957)] and pigment deficiency [e.g. *Coleus blumei* (Fisher, 1986) and *Tricyrtis* spp. and *Polygonum* spp. (Hara, 1957)]. The second group includes the structural variegation which is created by diffusion of light and a variation of epidermal thickness [e.g. *Oxalis martiana* (Hara, 1957)]. Most studies have been focused in variegation based on pigment-related and not as much on structural variegation group. The genus *Begonia* is a good example of genetic variegations; it contains more than 1500 species with genetic variegations (Sheue *et al*., 2012).

Many physiological disorders in ornamental crops including cut flowers, potted plants and bulbs have been reported. Some disorders can affect or reduce the shelf life, fresh weight, leaf and bud abscission, wilting, ethylene production, opening of younger flower buds and increased vase life (Watkins and Miller, 2004). Variegation in ornamentals due to physiological disorders can be caused by abiotic factors like ultraviolet radiation, low temperature, oxygen toxicity and nutrient deficiency. These factors can reduce the value of the ornamental plants (Li *et al.*, 2007).

1.5 Plant Viruses that Cause Foliar Yellow Vein

Foliar yellow vein in plants can be caused by several genera of plant viruses. The most common viruses reported to cause foliar yellow vein belong to the genera *Begomovirus* (family *Geminiviridae*) and *Rhabdovirus* (family *Rhabdoviridae*). One example of this is the begomovirus, *Argeratum yellow vein virus* (AYVV) which infects the weed *Ageratum conyzoides* causing yellow veins in the foliage (Saunders *et al*., 2000). Other plant viruses reported to cause yellow vein include the *Rhabdovirus* SYNV (Lampretch *et al*., 2009), the *Crinivirus Blackberry yellow vein associated virus* (Poudel *et al*., 2012) and the ipomovirus *Cucumber vein yellowing virus* (Gil-Salas *et al*., 2012). There are many ornamental plant viruses, particularly begomoviruses that cause yellow vein symptoms. Some of them include *Clerodendron golden mosaic China virus* (Valverde *et al*., 2012), AYVV (Saunders *et al*., 2003), *Honey suckle yellow vein virus* (Osaki *et al.*, 1979), and *Honeysuckle yellow vein mosaic virus* (Kitamura *et al*., 2004) among others.

1.5.1 Geminiviruses

Geminiviruses are made of circular single-stranded DNA genomes of approximately 2.5-3.0 kb (Stanley *et al*., 2005). The coat protein gene sequence is highly conserved and is involved in specificity for insect transmission (Briddon *et al*., 1990). Based on their type of insect vector, host range, and genome organization, geminiviruses are classified into four genera: *Mastrevirus*, *Begomovirus*, *Curtovirus* and *Topocuvirus* (Fauquet *et al*., 2008).

The Mastreviruses have monopartite genomes of approximately 2.6-2.8 kb circular ssDNA. They usually infect monocotyledonous plants and are transmitted by leafhoppers. The curtoviruses include viruses that only infect dicotyledonous plants and, have monopartite genomes of approximately 2.9-3.0 kb circular ssDNA and are transmitted byleafhoppers. The topocuviruses infect dicotyledonous plants, has a monopartite genome of 2.8 kb circular ssDNA and they are similar to the curtoviruses. However they are transmitted by treehoppers (Fauquet *et al*., 2008).

Begomoviruses contains the majority of the identified geminiviruses (Stanley *et al*., 2005). Begomoviruses have bipartite or monopartite genomes of approximately 2.5-2.8

kb circular ssDNA and they infect only dicotyledonous plants. This group of viruses are transmitted by several biotypes of the sweetpotato whitefly (*B. tabaci*) (Briddon, 2002). The B-biotype, has the broadest host range known among whiteflies (Brown *et al*., 1995). Symptoms of begomovirus infected plants consist of leaf curling, mosaic and more often foliar vein yellowing (Harrison and Robinson, 1999). Begomoviruses are divided into two groups. The old world begomoviruses and the new world begomoviruses (Padidam *et al*., 1995; Harrison and Robinson, 2002). Most of the new world begomoviruses have two genomic components which are denominated as DNA-A and DNA-B while most of the old world begomoviruses have only DNA-A (Fauquet *et al*., 2003). Begomovirus species are common and worldwide distributed. They represent an agricultural threat due to the damage they cause to their numerous plant host (Rojas *et al*., 2005). Some examples are BGMV, AbMV ACMV, *Ageratum enation virus, Ageratum leaf curl virus, Bean dwarf virus, Chayote yellow mosaic virus* and *Malvastrum leaf curl virus* among others (Fauquet *et al*., 2008).

1.5.2 Rhabdoviruses

Another group of viruses that cause yellow vein in several plant hosts are the rhabdoviruses. They can infect animals and plants. They have a bacilliform or bullet shape particle. They consist of linear, non-segmented, single stranded RNA in a negative sense orientation, reaching sizes of 15 kb (McGavin *et al*., 2011). Several proteins have been described in the genome like the N (nucleocapsid), P (phosphoprotein), M (matrix), G (glycoprotein), and L (polymerase) (Hull, 2002). Depending on the site of cell replication; they are divided into two groups: *Cytorhabdovirus* and *Nucleorhabdovirus*. Some viruses that cause foliar yellow vein included in this family are *Sonchus yellow net virus* (Jones and Jackson, 1990) and *Croton vein yellowing virus* (Jackson *et al*., 2005). Some rhabdoviruses have no known vector but the ones described are transmitted by aphids (*Aphididae*), leafhoppers (*Cicadellidae*), or planthoppers (*Delphacidae*). In the case of aphids, which are vectors of most known viruses, at least one day to acquire the virus have been documented (Jackson *et al*., 2005).

1.6 Ornamental Plants with Yellow Vein Symptoms

Currently throughout the world, there are many commercially available ornamental plants with yellow vein symptoms. As mentioned earlier, some of these symptoms are of genetic nature but others may be caused by plant viruses or by physiological disorders. In general the ornamental plants with unusual characteristics such as variegations and variations in leaf morphology are highly sought by horticulturists and ornamental plant enthusiasts (Valverde *et al.*, 2012).

1.6.1 Oxalis

Oxalis debilis var. corymbosa also known as wood-sorrel, is a species native to South America and belongs to the family *Oxalidaceae* (Lourteig, 1980). It was introduced into Taiwan as an ornamental plant (Wu *et al*., 2004). *Oxalis* is a morphologically diverse genus of at least 500 species, which have evolved a number of different root and stem morphologies like tubers, tuberous roots, caudices, rhizomes, and bulbs. *Oxalis debilis* is easily propagated from bulbs and, it is an aggressive weed that has achieved worldwide distribution, including Australia, Hawaii, Fiji, New Caledonia, the Galapagos Islands and China (Luo *et al.*, 2006). Research on oxalis evolutionary history is limited, but the major differences in morphology and growth habit suggest that the bulb-bearing groups in Africa and in the Americas arose independently (Tsai *et al*., 2010). In the United States several varieties are sold as ornamentals.

One important food crop within the genus *Oxalis* is Oca (*O*. *tuberosa*), which is consumed mostly on the Andean region of South America. The texture of the root varies from smooth to rough and there are a variety of colors (yellow, pink, black). This plant is usually cultivated in the highlands of South America and consumed because of its high value in carbohydrates, calcium, iron, fats and fiber (Flores *et al.*, 2003). Nevertheless, there is a study that reports that *Oxali*s species like oca, can produce oxalic acid (Ross *et al.*, 1999).

Oxalis tuberosa and other related plant species can be infected by *Potato virus T* (PVT) (Jones and Kenten, 1981). Also, a survey conducted on *Oxalis* sp. confirmed it to be a host of *Tomato spotted wilt virus* (Bitterlich and Macdonald, 1993). Coyier (1981) reported rod shaped virus like particles in *O. regnelli* which were associated with chlorotic ring spots and decay of the host plant. Several *Oxalis* species are known to be

troublesome weeds of greenhouses, nurseries, and turf in the northern hemisphere. Oxalis also has been found to be an alternate host for rusts of corn (*Zea mays*), sorghum, (*Sorghum bicolor*), and other grasses (Holt *et al*., 1988). *Oxalis tuberosa* has been reported infected by *Arracacha virus B*, *Papaya mosaic virus,* and *Ullucus mild mottle virus*. These viruses can be eliminated from plants by *in vitro* meristem culture and heat treatment (Fletcher and Fletcher, 2001).

The oxalis cultivar Golden Veined Oxalis (Fig. 1A) is currently available in several nurseries in the US and can be purchased online. Many customers prefer this cultivar due to its variegated leaves. The symptoms appear as yellow stripes, however some customers on web-based forums that share experiences on ornamental plants, mention that variegated oxalis can lose the variegations from season to season.

1.6.2 Geranium

Pelargonium (*Pelargonium peltatum*) also known as geranium (family *Geraniaceae*), includes annual and perennial species. Pelargoniums were first introduced into Europe in the 17th Century for medicinal purposes and their horticultural potential was recognized by the late 18th Century (James *et al.*, 2004). They are known to be resistant to drought and they are very popular for their ornamental properties reaching the denomination of ‘house geraniums’. The most popular commercial varieties come from South Africa.

There are more than 200 species in the family *Geraniaceae* divided in 13-17 sections, where sections *Ciconium*, *Dibrachya* and *Pelargonium* represent the most popular garden plants around the world and therefore, they are of considerable economic importance in the market of ornamental plants. The two most commonly cultivated pelargoniums in Europe and North America are the ‘zonal’ and ‘ivy-leaved’. Zonal cultivars (*Pelargonium x hortorum* Bailey; section *Ciconium*) are hybrids, derived from *P. zonale* and *P. inquinans* (Garcia-Sogo *et al*., 2012).

Pelargonium varieties are generated through selection, by crossing, and spontaneous mutations. The chosen plants are then propagated vegetatively. This propagation method reduces fertility in every generation (Becher *et al.,* 2000). Using this propagation method, horticulturists have developed several varieties that have some

desired characteristic traits and disease resistance. For example, geraniums are known for their resistance to many pathogens; however the scented geraniums (*Pelargonium* sp.) are more susceptible to *Ralstonia solanacearum* in comparison to their relative varieties like zonal (*Pelargonium x hortum*), regal (*Pelargonium x domesticum*) and ivy (*Pelargonium peltatum*) causing wilt symptoms (Norman *et al.,* 2009). But there are some pathogens like *Botrytis cinerea* which equally affect all *Pelargonium* species (Uchneat *et al*., 1999).

Geraniums have been reported as host for some viruses. For example CMV was isolated and reported in India causing mild mottling and stunting in *Pelargonium* sp. (Verma *et al*., 2006). A carmovirus, *Pelargonium flower break virus,* was described to cause flower break and chlorotic spots in *P. zonale* and *P*. *peltatum* (Franck *et al*., 1997).

The leaves of the ivy leaf geranium (*P. peltatum*) cv. Crocodile have an attractive yellow vein (Fig. 1B). The yellow-vein symptoms can be reproduced by grafting into related *Pelargonium* species and it has been suggested that a virus may be the cause of these symptoms (Cassells *et al*., 1982).

1.6.3 Pseuderanthemum

Pseuderanthemum (*Pseuderanthemum carruthersii)* belongs to the family *Acanthaceae* and it is a popular ornamental plant in Hawaii and Puerto Rico, particularly cultivars showing foliar yellow vein. Previous studies have shown that a begomovirus was the possible cause of a foliar yellow vein of a *Pseuderanthemum* sp. in Yemen. However, the virus was not transmitted by *B. tabaci* using several biotypes of whiteflies from different locations. Nevertheless, positive results were obtained with begomovirus-specific probe and the name, *Pseuderanthemum* yellow vein virus was proposed as the causal agent of the yellow vein of the *Pseuderanthemum* sp. (Bedford *et al*., 1994).

When rub-inoculated or sprayed, extracts of *P. bicolor* has been reported to reduce virus incidence in *Crotalaria juncea* and *Cucumis melo* var. momordica (Verma and Khan, 1984). Plants in this genus are also used for medicinal purposes. *Pseuderanthemum palatiferum* and *P. carruthersii* var. atropurpureum were used as a Panacea in Vietnam and some lignans (estrogen like compounds in plants which act as antioxidants) and

triterpenes (strong smelling compounds produced to protect plants against parasites) have been isolated (Vo *et al.,* 2012).

The cultivar Golden Net Bush (Figs. 1C and 1D) shows virus-like symptoms which consists of bright yellow coloration of the veins or complete yellow of the upper leaves. There are two varieties of this ornamental plant that show yellow vein; one with narrow leaves (Fig. 1C) and the other with broad leaves (Fig. 1D).

1.6.4 Honeysuckle

Honeysuckle (*Lonicera japonica)* is a common wild and cultivated perennial plant throughout the world. It belongs to the family *Caprifoliaceae*. Honeysuckle is propagated vegetatively through cuttings. It has been reported as a plant which grows in forest openings but can invade deeply shaded sites where it spreads slowly until the canopy is open (Randall, 1996). It is considered an aggressive invasive weed in the US due to its morphological plasticity in comparison to *L. sempervirens* (one of the native honeysuckle in United States) (Schweitzer and Larson, 1999). The allelopatic compounds produced in the root system have been demonstrated to compete with natural pine regeneration in forests (Skulman *et al*., 2004). Honeysuckle has been used in Chinese medicine to treat wind-heat, epidemic febrile diseases, sores, carbuncles and other infectious diseases. *Lonicera japonica* is used as food, cosmetics, and ornamental groundcover (Shang *et al.,* 2011). There are at least 12 cultivars available for sale through the internet. Some of them are Elegant Creeper, Hall's prolific, Cream Cascade, Mint crisp, and Interold Dart's World. The most popular and widely available cultivar, is Halliana also called Hall's honeysuckle (Schierenbeck, 2004).

Japanese honeysuckle was described to be an important larval host of the tobacco budworm (*Heliothis virescens*) and the corn earworm (*Helicoverpa zea*) due to the volatile chemical constituents in the flowers (Schlotzhauer *et al.,* 1996). Other pests reported are aphids and whiteflies. They are observed in the warmer regions of the US, but they do not appear to cause serious damage on the honeysuckle plants (Williams *et al.*, 2001). The whitefly *B. tabaci* B biotype, is able to produce phytotoxic disorders in honeysuckle in contrast to the non-"B" biotype (Bedford *et al.*, 1994).

Several viruses belonging to different families have been reported to infect this plant (Gulati *et al.*, 2011). Some of the cultivars such as Yellow Net Honeysuckle exhibit a yellow vein mosaic sometimes accompanied with enations along the veins abaxial surface (Fig. 1E). It has been shown that these symptoms can be caused by three whitefly-transmitted begomoviruses. Two of these viruses are: *Honeysuckle yellow vein mosaic virus* (HYVMV) (Kitamura *et al*., 2004) and *Tobacco leaf curl Japan virus* (TbLCJV). Ueda *et al*. (2008), Ogawa *et al*. (2008) and Park *et al*. (2011); have described HYVMV and TbLCJV as being able to infect tomato (*Solanum lycopersicum*). Wang *et al*. (2011) described a third virus called: *Honeysuckle yellow vein virus* which shared high nucleotide identity with HYVMV and TbLCJV and it can be found infecting tomato as well.

A rhabdovirus related to *Eggplant mottle dwarf virus* (EMDV) has been reported to cause yellow vein in honeysuckle in southern Italy (Martelli and Cherif, 1987). Other hosts for this virus include eggplant, tomato, tobacco, potato and cucumber. The rate of incidence of EMDV is higher in cooler regions and is one of the few rhabdoviruses which is mechanically transmissible. EMDV is transmitted by the leafhopper *Agallia vorobjevi* (Martelli and Cherif, 1987).

1.6.5 Coleus

Coleus (*Solenostemon scutellarioides)* is a widely cultivated plant for its bright colored foliage (Holcomb and Valverde, 1991). Coleus belongs to the family *Lamiaceae* and is native to south East Asia and Malaysia. Other plants that belong to this family include basil, mint, rosemary, sage, savory, marjoram, oregano, hyssop, thyme, lavender, and perilla (Cantino *et al*., 1992). An oak leaf pattern and yellow rings on the leaves of the coleus cultivar Alabama was reported to be caused by a strain of CMV (Holcomb and Valverde, 1991). A clonally propagated coleus cultivar Electric Lime (Fig. 1F), exhibiting yellow vein symptoms is commonly grown in home and commercial gardens in Baton Rouge, Louisiana. It is not known if the yellow vein is due to genetic origin or caused by a plant virus.

1.7 Detection and Identification of Plant Viruses

Virus characteristics such as biological, serological, and nucleic acid properties have been used for virus detection and identification. However, sometimes detection and

identification of plant viruses is not an easy task due to the low concentration and the uneven distribution of some viruses within the plant tissues (Esbenshade and Moyer, 1982). Some problems one could encounter while attempting to identify or detect a virus are the presence of phenolic compounds, latex or inhibitors (Abad and Moyer, 1992) and, as well as mixed viral infections (Valverde *et al*., 2008). Viruses can be detected by graft inoculation to susceptible indicator plants. Based on the presence of the coat protein of the virus, serological methods such as the enzyme-linked immunosorbent assay (ELISA) and western blots tests have been developed and used for virus detection (Cadena-Hinojosa and Campbell, 1981; Fuentes *et al*., 1996; Gutierrez *et al*., 2003). Other techniques commonly used for virus detection and identification are polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR) and real time PCR (qPCR) using specific and degenerate primers (Li and Mock, 2005; Szemes *et al.*, 2001). Other methods include molecular hybridization (Lotrakul, 2000), electron and light microscopy and nucleotide sequence analysis.

1.7.1 Mechanical Transmission

Many viruses, but not all of them can be transmitted mechanically. To achieve mechanical transmission, a wound is needed for virus particles (or nucleic acid) to penetrate the host plant. An effective way is to do this is by using abrasives like carborundum powder or celite dusted on the leaves before inoculation. The success of the mechanical inoculation depends on the virus characteristics such as stability, concentration, presence of other constituents in the inoculum and the host plant (Dijkstra and Jager, 1998).

One way to increase the efficiency of mechanical transmission is by choosing as a source of inoculum, a part of the plant in which the virus concentration is expected to be the highest. Young leaves and those showing clear virus symptoms usually contain high concentration of virus. Leaf material is ground in a sterilized mortar and pestle with phosphate buffer (0.01-0.05 M, pH 7.0) in a tissue-fluid ratio of 1:5 to 1:10 (w/v). The use of pure sap is not recommended because some plants have infectivity-inhibitor constituents which could decrease the success of transmission. Phosphate buffers are recommended, however, the buffer type depends on the virus. Other buffers include

borate, citrate or Tris. Low pH can inactivate most plant viruses by precipitation therefore an alkaline solution is recommended (Takebe and Orsuki, 1969).

As mentioned above, some compounds produced by plants like proteins, polyphenoloxidases (compounds which oxidize polyphenols to o-quininones), and tannins can interfere with the infection. This problem can be alleviated by diluting the sample, adding reducing agents, adding compounds which compete with tanins and the use of bentonite clay, respectively (Lawson *et al.,* 1990).

Test plants that are routinely used for mechanical inoculation are plants known to be susceptible to many viruses. Plant families commonly used are *Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae* and *Solanaceae*. The susceptibility of the test plant depends on several factors such as genotype, physiological condition, temperature, humidity, light intensity, nutrition and age. A rule of thumb is to use young plants or the first trifoliate leaves (Dijkstra and Jager, 1998).

1.7.2 Graft Transmission

Graft transmission is the universal method to transmit infectious agents. This technique is used when the virus vector is not known and mechanical inoculation has failed. For this technique to be successful, the tissues need to be compatible, which means plants should be from the same family, genera or species. However, in some cases, permanent tissue union is not needed and just the intimate contact could lead to successful virus transmission (Dijkstra and Jager, 1998). There are several graft techniques but the one to be used depends on the plant species. Some common graft techniques that are used to transmit plant viruses are the bud grafting (for woody perennials), wedge-grafting (herbaceous crops) and tuber grafting (for tuber or bulb forming species tulips, potatoes) (Hull, 2002).

1.7.3 Purification and Analysis of Viral dsRNA

The genome of about 90% of known plant viruses are made of RNA and dsRNA is generated during virus replication. A method has been developed for dsRNA purification from plants (Morris and Dodds, 1979) and has been used as a diagnostic tool for plant viral infections. DsRNA is resistant to enzymatic degradation in high salt

conditions. Several advantages over other diagnostic methods are: the high stability of the dsRNA, the host interference is reduced, the relatively low cost, and that results can be obtained in a relatively short period of time. Other advantages of this method are the detection of mixed infections and the fact that dsRNA can be used as reagent for other detection or identification techniques like RT-PCR and molecular cloning. However the limitations are that only RNA viruses can be detected, knowledge of molecular weight of different viruses is required, and that there are some virus families that yield low amounts of dsRNA (Valverde *et al*., 1990).

1.7.4 Polymerase Chain Reaction (PCR)

The PCR technique allows low amounts of viral DNA in the host to be amplified and detected (Matthews, 1992). To use this technique, the sequence (complete or partial) of the suspected virus must be known. The objective of PCR is to multiply DNA between two oligonucleotide sequences. Two primers are synthesized to be complementary to the desired DNA target, usually 150-500 base pairs apart (Sambrook *et al.*, 1989). Primer design is very important for detection. DsDNA is denatured to separate the strands in the presence of primers. Then temperature is reduced to allow primers to anneal. The next step involves annealing of primers under a certain temperature that the thermophilic DNA polymerase copies DNA strand. This process occurs several times and amplifies DNA in a logarithmic way. The problems that this technique has is that the primers need to be as specific as possible to bind its target, difficulties to reproduce similar results and non-target DNA could be amplified as well (Matthews, 1992). The reverse transcription PCR technique is used to detect RNA plant viruses and viroids (Hull, 2002). It is similar to PCR except that the RNA is previously converted in cDNA (copy DNA) to proceed with regular PCR amplification.

PCR has great sensitivity for virus detection. Numerous PCR procedures have been successfully used to detect some viruses such as *Tomato spotted wilt virus* (Jain *et al.,* 1998), *Wheat spindle streak mosaic virus* and *Wheat yellow mosaic virus* (Clover and Henry, 1999) and many whitefly transmitted geminiviruses (Rojas *et al.,* 1993; Wyatt and Brown, 1996; Briddon and Markham, 1994)

1.7.5 Virus Isolation and Purification

The objective of this technique is to separate virus particles from plant constituents. Purified virus particles can be used to determine the chemical and physical properties of the virus. Since viruses differ from each other (even at strain level) in physicochemical properties, there is no standard method for purification. However, some viruses belonging to the same taxonomic group share physicochemical properties, and therefore common techniques can be used. Several plant genera have been described to be “good hosts” for virus purification. Some of them are *Chenopodium*, *Cucumis*, *Nicotiana*, and *Vigna* (Dijkstra and Jager, 1998). One of the first steps in virus purification is clarification. This consists of the removal of plant material without losing significant amounts of virus. Clarification can be done by heating (50-60 ^{o}C for 10 min), freezing and thawing which coagulate proteins. However, some viruses cannot resist heat treatments. Other virus purification techniques include: precipitation with salts and polyethylene glycol (PEG) which is based on the dehydration of virus particles (Muniyapa *et al.*, 1991). Density gradient purification by utilizing sucrose gradients and high speed centrifugation separates plant contaminants from virus particles. High speed centrifugation which enables small particles in suspension to sediment and accumulate in the bottom of a tube can complement most of the approaches described above (Dijkstra and Jager, 1998).

1.8 Justification

In spite of the popularity of ornamental plants with foliar variegations, the cause of these variegations, particularly yellow vein of many ornamental plants remain unknown. These virus-like symptoms need special attention since these plants are very common in the US. Because of the potential transmission of viruses from infected ornamental plants to other economically important crops, it is important to determine if plant viruses are the cause of these variegations. Therefore, the objective of this investigation was to determine if the yellow symptoms of five commercially available ornamental plants: oxalis (*O. corymbosa*) cv. Golden Veined Oxalis, pseuderanthemum (*P. carruthersii*) cv. Golden Net Bush, ivy geranium (*P. peltatum)* cv. Crocodile, Japanese honeysuckle (*L. japonica*) cv. Yellow Net Honeysuckle, and coleus (*S. scutellaroides*) are caused by plant viruses or are of genetic or physiological nature.

The hypothesis of this proposal is that the yellow vein symptoms of the five ornamental plants described above are caused by plant viruses. Because of the yellow vein symptoms that these plants exhibit are often caused by members of the *Begomovirus* genus, the research focused on techniques to detect and identify begomoviruses.

CHAPTER II MATERIALS AND METHODS

2.1 Plant Materials and Sources

Two leaf variants (narrow and broad) of *P. carruthersii* cv. Golden Net Bush and *P. graciliflorum* cv. Twilight (Figs. 1C and 1D) were obtained from Kartuz Greenhouses, CA; *O. debilis* var. corymbosa cv. Golden Veined Oxalis (Fig. 1A) and *O. regnellii* cv. Atropurpurea (Fig. 2A) from Glasshouse Works, Stewart, OH; *P. peltatum* cv. Crocodile (Fig. 1B) and *L. japonica* cv. Yellow Net Honeysuckle (Fig. 1E) from Accents for Home and Garden, DE and *S. scutellarioides* cvs. Electric Lime (Fig. 1F) and Purple were provided by G. Holcomb, Louisiana State University. All the selected plants for this study showed various degrees of foliar yellow vein and they are currently available in several nurseries in US.

Oxalis debilis (pink woodsorrel) (Fig. 2B), *O. stricta* (common yellow woodsorrel) (Fig. 2C) and *L. japonica* (Fig. 2D) were obtained from local yards in Baton Rouge, LA. *Pelargonium zonale* cvs. Artic Frost (Fig. 2E) and Red and an unknown cv. of *Odontonema strictum* (firespike) (Fig. 2F), were obtained from local retail nurseries in Baton Rouge, LA.

The virus indicator plants *Nicotiana benthamiana*, *Chenopodium amaranticolor, Cucumis sativus*, *Phaseolus vulgaris,* and *Solanum esculentum* (tomato) cv. Sunny were provided by R. A. Valverde, Louisiana State University. These plants have been reported to be susceptible to several plant viruses.

All plants were propagated in a greenhouse located in the Greenhouse Service facilities of the Louisiana State University Agricultural Center, Baton Rouge Campus. The greenhouse temperature ranged from 20-30 ^{o}C. Plants were planted in a soil mix that consisted of one part of sand, two of river soil, and 3 of Miracle Grow potting mix. These proportion of soils showed to be efficient to grow all plants used for the experiments.

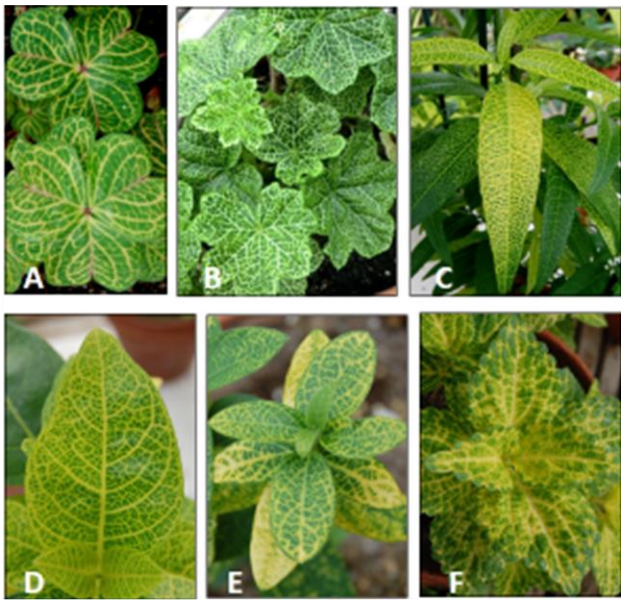

Figure 1. Ornamental plants with foliar yellow vein. A, Oxalis (*Oxalis debilis*) var. corymbosa cv. Golden Veined Oxalis; B, Geranium (*Pelargonium peltatum*) cv. Crocodile; C, Pseuderanthemum (*Pseuderanthemum carruthersii*) cv. Golden Net Bush (narrow leaf variant); D, Pseuderanthemum cv. Golden Net Bush (broad leaf variant); E, Honeysuckle (*Lonicera japonica*) cv. Yellow Net Honeysuckle; F, Coleus (*Solenostemon scutellarioides*) cv. Electric Lime.

Figure 2. Test plants used in mechanical and graft inoculations. A, Oxalis (*Oxalis regnellii*) cv. Atropurpurea; B, Pink woodsorrel (*Oxalis debilis*); C, Common yellow woodsorrel (*Oxalis stricta*); D, Wild honeysuckle (*Lonicera japonica*); E, Geranium (*Pelargonium zonale*) cv. Artic Frost; F, Fire spike (*Odontonema strictum*)

2.2 Attempts to Transmit Putative Viruses

2.2.1 Mechanical Inoculations

Foliar tissues from all five ornamental plants showing foliar yellow vein were used in mechanical inoculation experiments to selected virus-indicator hosts and when available, to the same or related virus-free plant species (Table 1). Inoculations were conducted by grinding tissues in a mortar using 0.01 M phosphate buffer, (pH 7.0) and sap extracts rub-inoculated to leaves dusted with carborundum. After the inoculation, inoculated areas of the leaves were rinsed with water. Plants were kept in the greenhouse and monitored for symptoms every week up to four weeks. At least five plants of each species were inoculated.

Table 1. Plants used in mechanical inoculations

Test plant	**Plants with foliar yellow vein symptoms**
Oxalis debilis (pink woodsorrel)	*O. debilis* var. corymbosa cv. Golden Veined Oxalis
Pelargonium zonale cvs. Artic Frost and Red	*P. peltatum* cv. Crocodile
Lonicera japonica (wild honeysuckle)	*L. japonica* cv. Yellow Net Honeysuckle
Odontonema strictum (firespike) *Pseuderanthemum graciliflorum* cv. Twilight	*P. carruthersii* cv. Golden Net Bush (broad and narrow leaf)
Solenostemon scutellarioides cv. Purple *Salvia coccinea*	*S. scutellarioides* cv. Electric Lime
Nicotiana benthamiana *Cucumis sativus* *Phaseolus vulgaris* *Chenopodium amaranticolor*	All plants

2.2.2 Graft Inoculations

Shoot samples from all five ornamental plants were grafted to the same or related plant species (Table 2) using the top wedge graft method (Fig. 3A). Because *Oxalis* species were not suitable for the top wedge graft method, a petiole graft method for the transmission of fern viruses reported by Valverde *et al*. (2009) was used. A symptomatic leaf (with 1-2 cm of the petiole) of Golden Veined Oxalis was grafted to the petioles of a healthy *O. debilis* using a razor blade. A v-cut was made to the petiole of the infected scion and tissues joined by pushing gently the scion into the cut petiole of the healthy plant (Fig. 3B). As with the wedge grafts, grafted tissues were wrapped with parafilm paper, watered, and covered with a plastic bag. The bag was removed a week after grafting. Symptoms and symptom progression was recorded weekly for six weeks. At least five grafts were conducted for each of the five ornamental plants. Negative controls consisted of scions of the same or related species as the rootstock. Only successful grafts (those where the scion survive six weeks) were evaluated.

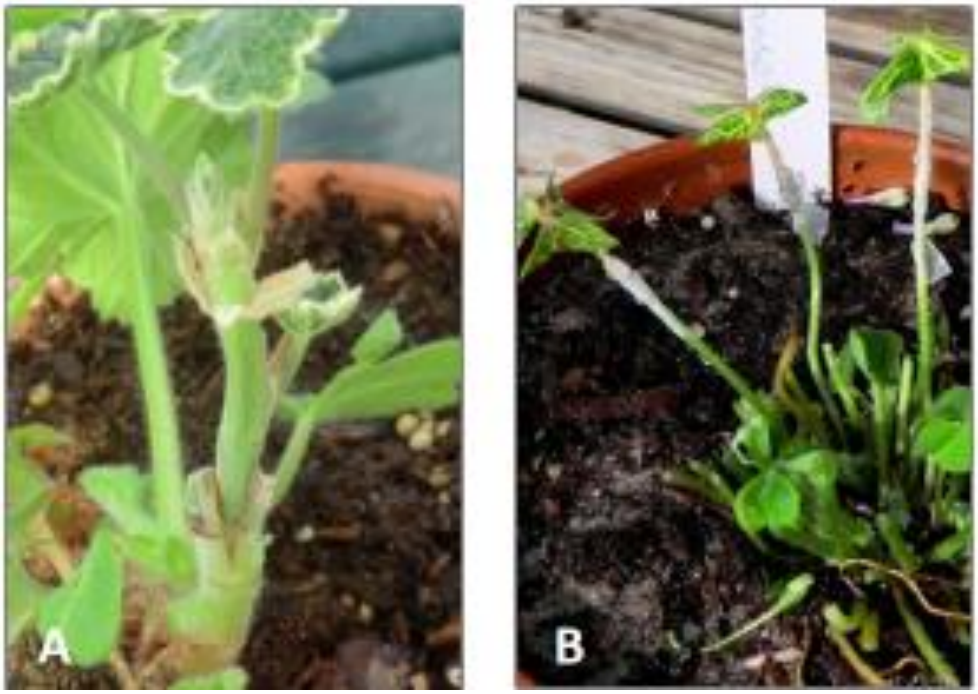

Figure 3. Grafting techniques used in attempts to transmit the foliar yellow vein symptom. A, top wedge graft; B, petiole graft.

Table 2. Plants used in graft inoculations

Indicator host	**Plant with foliar yellow vein symptoms**
Oxalis regnellii cv. Atropurpurea *O. debilis* (pink woodsorrel)	*O. debilis var.* corymbosa cv. Golden Veined Oxalis
Pelargonium zonale cv. Artic Frost *P. peltatum* cv. Red	*P. peltatum* cv. Crocodile
Pseuderanthemum graciflorum cv. Twilight *Odontonema strictum* (firespike)	*P. carruthersii* cv. Golden Net Bush (narrow leaf and broad leaf variants)
Lonicera japonica (wild honeysuckle)	*L. japonica* cv. Yellow Net Honeysuckle
Solenostemon scutellarioides cv. Purple *Salvia coccinea*	*S. scutellarioides* cv. Electric Lime

2.2.3 Whitefly Transmission

Since begomoviruses are often associated with foliar yellow vein symptoms, whiteflies were used in transmission experiments. All transmission experiments were conducted in the greenhouse. A whitefly colony, (*Bemisia tabaci* biotype B) (Figs. 4A-C) kept in plexiglass cages and feeding on cotton was used as a source for all transmission experiments. Groups of approximately 50 whiteflies were used in each transmission experiment. Whiteflies were allowed to feed for 48 h on the four plant species (individually) with foliar yellow vein symptoms listed in Table 3. Whiteflies were then transferred to cages containing the corresponding test hosts (single plants) listed in Table 3 and allowed a transmission period of 48 h (Fig. 4D). After that, plants were taken out and any remaining whiteflies killed. Test plants were monitored (in the greenhouse) for symptoms

weekly during a six week period. Plants showing symptoms were tested for begomoviruses by PCR. Randomly selected symptomless plants were also tested. In attempts to transmit the putative virus causing foliar yellow vein in oxalis to tomato, groups of 50 whiteflies were used as described above. In all cases, a minimum of five experiments were conducted. To ensure that the whiteflies were begomovirus vectors, transmissions of TYLCV, from tomato to tomato were used as positive control.

Figure 4. A, whiteflies (*Bemisia tabaci* biotype B) eggs and nymphs on a cotton leaf; B, *B. tabaci* adults on a cotton leaf; C, plexiglass cage used to rear whiteflies and conduct whitefly transmission experiments; D, whiteflies in the plexiglass cage feeding on a test plant (firespike).

Table 3. Plants used in whitefly transmissions

Test plant	**Plants with foliar yellow vein symptoms**
Oxalis debilis (pink woodsorrel) Tomato *Solanum lycopersicum* cv. Sunny	*O. debilis* var. corymbosa cv. Golden Veined Oxalis
Lonicera japonica (wild honeysuckle)	*L. japonica* cv. Yellow Net Honeysuckle
Odontonema strictum (firespike) *Pseuderanthemum graciliflorum* cv. Twilight	*P. carruthersii* cv. Golden Net Bush (broad and narrow leaf)
Solenostemon scutellarioides cv. Purple *Salvia coccinea*	*S. scutellarioides* cv. Electric Lime

2.3 Double Stranded RNA (dsRNA) Extraction

Since the genome of the majority of plant viruses is made of ssRNA and dsRNA is generated during replication, dsRNA extractions were conducted using desiccated foliar tissues from all plants showing foliar yellow vein symptoms listed in Table 1. A modification of the method for dsRNA extraction reported by Valverde *et al*. (1990) was used (Fig. 5). Foliar tissues (3.5 g) were desiccated overnight at 4 ^{o}C using silica gel (Fig. 6). The dried plant tissue was pulverized with a mortar and pestle and homogenized in two volumes (v/w) of STE buffer (0.1M NaCl, 0.05 M tris, 0.001 M ethylenediamine tetraacetic acid [EDTA] pH 7.0), 1% sodium dodecyl sulphate (SDS), 0.1% bentonite, and an equal volume of STE-saturated phenol. The mixture was shaken and then centrifuged for 15 min at 8,000 g. The upper aqueous phase was made to 16% ethanol and one cycle of fibrous cellulose (Sigma, St Louis, MO) column chromatography was performed using ethanol (16%) in STE as washing solution. The dsRNA was eluted from the columns with STE, precipitated with three volumes of ethanol and 1/20 volume of 3 M sodium acetate pH 5.5. Samples were stored overnight at -15 ^{o}C and centrifuged at 8,000 g for 30 min. Pelleted nucleic acid was resuspended in electrophoresis buffer (0.04 M tris, 0.02 M

sodium acetate, 0.001 M EDTA, pH. 6.8) containing 10 % glycerol and 0.01 % bromophenol blue.

dsRNAs extracts were electrophoresed in agarose (1.2%) gels using tris-acetate EDTA buffer (TAE). After electrophoresis, gels were stained in ethidium bromide and visualized over a UV light transilluminator. Positive controls consisted of common bean samples from plants infected with *Phaseolus vulgaris endornavirus* (PvEV) and soybean plants infected with CMV.

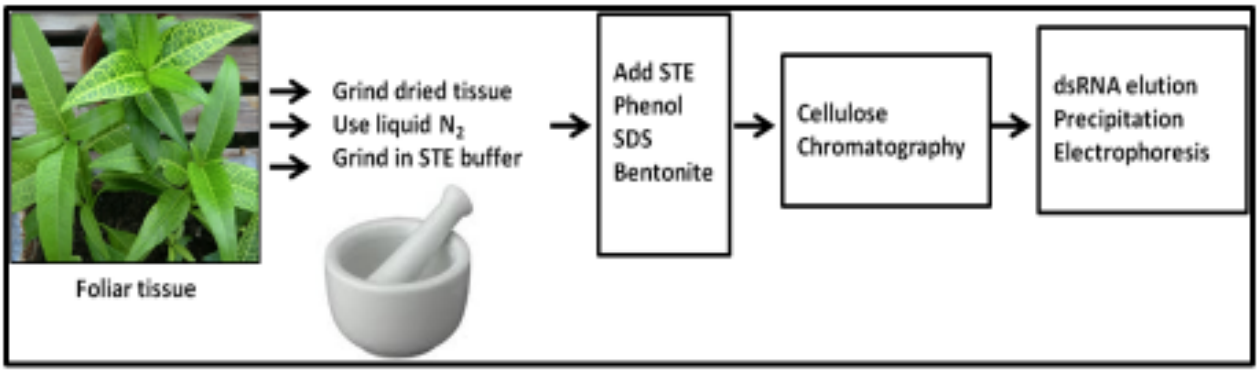

Figure 5. Diagrammatic representation of dsRNA extraction.

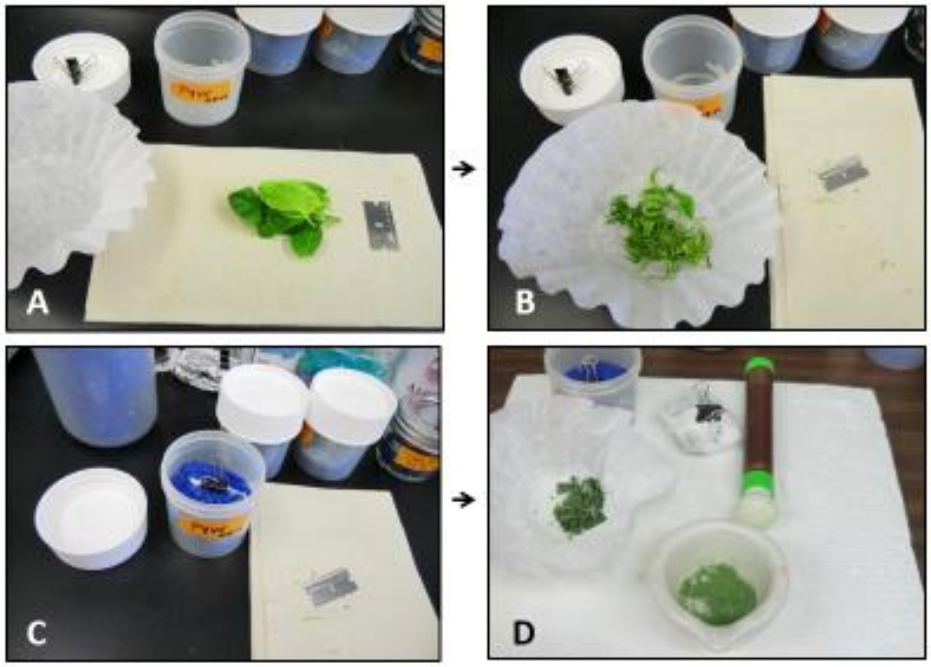

Figure 6. Steps involved in the silica gel drying of plant tissue samples and sample preparation for nucleic acid extraction. A, collected tissues; B, chopping tissues with razor blade and placing it in a coffee filter; C, placing coffee filter with tissue in a container with silica gel; D, grinding the dried tissue with a mortar and pestle.

2.4 Virus Purification

Attempts were made to purify virions from leaf tissue of plants with foliar yellow vein. Extractions were conducted with 0.1 M phosphate buffer (pH 7.2). Foliar tissue (50 g) was homogenized in a blender with 100 ml of phosphate buffer and 5 % (w/v) chloroform added to the mixture. The extraction/clarification step was followed by concentration using, 8% PEG 6000 precipitation. This was followed by two cycles of low (8,000 g for 15 min) and high (90,000 g for 2 h) speed centrifugations. Final pellets were resuspended in 0.01 M phosphate buffer, pH 7.2 and aliquots were sent to Dr. S. Sabanadzovic, Mississippi State University, for examination under the electron microscope.

2.5 DNA and ssRNA Extractions

Tissue samples of all five ornamental plants showing foliar yellow vein symptoms were collected for DNA and RNA extractions. DNA was extracted using a DNeasy Plant Mini Kit from Qiagen (Qiagen, Valencia, CA) from fresh (using liquid nitrogen) and silica gel dried tissues (as described above) following the manufacturer's guidelines. Other DNA extraction methods such as one described by Dellaporta *et al*. (1983), Plant DNAzol (Invitrogen, Carlsbad, CA) and CTAB (Desal *et al*., 1989) were also used. Quality and quantity of DNA was evaluated by gel electrophoresis. Total RNA was extracted using the RNeasy kit from Qiagen using fresh and dried tissues following the manufacturer's guidelines. DNA and RNA was also extracted from tomato infected with TYLCV and camellia infected with a putative rhabdovirus, Camellia yellow mottle virus (CYMV) respectively. When available, DNA and RNA tissues from healthy plants were extracted. To test the banding profile of the DNA extractions, extracted DNA was run in 1.2 % agarose gels in TAE buffer.

2.6 Polymerase Chain Reaction

Extracted DNA and RNA were used in PCR tests. Degenerate primers, commonly used to detect begomoviruses and rhabdoviruses were used to perform PCR and RT-PCR reactions (Rojas *et al*., 1993; Wyatt and Brown, 1996; Briddon and Markham, 1994; Lamprecht *et al.,* 2009) (Table 4). Some of the degenerate primers used to generate begomovirus fragments were generated from the alignment of nucleotide and amino acid

sequences that include the core region of the coat of many begomoviruses which is conserved in the *Begomovirus* genus (Rojas *et al.,* 1993; Wyatt and Brown, 1996).

Table 4. Degenerate primers used in polymerase chain reaction experiments

Reference	**Primer**	**Nucleotide sequence**
Rojas *et al*. (1993)	PAL1v1978	GCATCTGCACGCCCAGATYGTCTTY
Begomovirus DNA-A	PAR1c496	AATACTGCAGGGCTTYCTRTACATRGG
Rojas *et al*. (1993)	PCRc1	CTAGCTGCAGCATATTTACRARWATGCCA
Begomovirus DNA-B	PVL1v240	GCCTCTGCAGCARTGRTCKATCTTCATACA
Briddon and Markham (1994)	OWB-V	KSGGGTCGACGTCATCAATGACGTTRTAC
Begomovirus	OWB-C	CGGTCAGRRARACCCGGGGKTACTTAAGRAA
Wyatt and Brown (1996)	AV494	GCCYATRTAYZAGRAAGCCMAG
Begomovirus	AC1048	GGARTTDGARGCATGHGTACATG
Lamprecht *et al*. (2009)	RhabF	GGATMTGGGGBCATCC
Rhabdovirus	RhabR	GTCCABCCYTTTTGYC

Degenerate primers, PAL1v1978/PARc496 and PCRc1/PVL1v240 used to detect begomoviruses (Rojas *et al,.* 1993), were used to perform most PCR reactions. PCR mixtures consisted of 5 µl of 10X PCR buffer, 1 µl of 50 mM $MgCl_2$, 1 µl of 10 mM dNTP, 1 µl of 10 µM of each primer, 0.5 µl of 5 U of Taq DNA polymerase (GeneScript, Piscataway, NJ), 2 µl of DNA sample and 38.5 µl of nuclease-free water. PCR was performed in a Genius Thermocycler (Techne, Cambridge, UK) with 30 cycles of melting, annealing, and DNA extension of 94 ^{o}C for 1 min, 50 ^{o}C for 1 min, 72 ^{o}C for 3 min; and a final extension cycle of 72 ^{o}C for 10 min.

PCR conditions for other degenerate primers OWB-V/OWB-C (Briddon and Markham, 1994) for begomovirus detection consisted of 5 µl of 10X PCR buffer, 1 µl of

50 mM $MgCl_2$, 1 µl of 10 mM dNTP, 1 µl of 10 µM of each primer, 0.5 µl of 5 U of Taq DNA polymerase, 2 µl of DNA sample and 38.5 µl of nuclease-free water. PCR was performed in a Genius Thermocycler with 40 cycles of melting, annealing, and DNA extension of 94 ºC for 1 min, 50 ºC for 1 min, 72 ºC for 4 min; and a final extension cycle of 72 ºC for 10 min.

PCR conditions for a third set of primers AV494/AC1048 (Wyatt and Brown, 1996) for begomovirus detection consisted of 5 µl of 10X PCR buffer, 1 µl of 50mM $MgCl_2$, 1 µl of 10 mM dNTP, 1 µl of 10 µM of each primer, 0.5 µl of 5 U of Taq DNA polymerase, 2 µl of DNA sample and 38.5 µl of nuclease-free water. PCR was performed in a Genius Thermocycler with 35 cycles of melting, annealing, and DNA extension of 94 ºC for 1 min, 60 ºC for 20 sec, 72 ºC for 30 sec; and a final extension cycle of 72 ºC for 10 min.

Degenerate primers for the genus *Rhabdovirus* reported by Lamprecht *et al.* (2009) were used for RT-PCR experiments. Primers, RhabF and RhabR were used in a two-step RT-PCR method. The reaction consisted of 10 ul of the extracted RNA with 1 ul of RhabF primer (10 µM) and incubated for 70 ºC for 5 min. Then 2 ul of AMV reverse transcriptase (5 u/µl Promega, Madison, WI), 1 µl of Riboblock RNAse inhibitor (40u/µl, Fermentas), and 2 µl of 10 mM dNTP. The sample was incubated 25 ºC for 10 min, 42 ºC for 1 h and 99 ºC for 5 min to produce the cDNA. The second step consisted of a PCR mixture of 5 µl of the cDNA, 5 µl 5X buffer AMV/Tfl reaction buffer (Promega), Tfl DNA polymerase (5 u/µl, Promega), 50 mM $MgCl_2$, 1 µl of 10 µM of each primer, 1 ul of dNTP 0.5 µl of Taq polymerase and 36.5 µl of nuclease-free water. PCR was performed in a Genius Thermocycler with one cycle of 94 ºC for 2 min and 40 cycles of melting, annealing, and DNA extension of 94 ºC for 1 min, 37 ºC for 1 min, 72 ºC for 2 min; and a final extension cycle of 72 ºC for 7 min.

Positive controls for PCR reactions consisted of DNA from TYLCV-infected tomato and RNA from CYMV-infected camellia. PCR products were analyzed by electrophoresis in 1.2% agarose gels in TAE buffer, stained with ethidium bromide, and visualized under ultraviolet light. A 100 bp ladder (Promega) was used as marker.

2.7 Molecular Cloning of PCR Products and Sequencing

When obtained, PCR products were cleaned with the Quick Clean 5M PCR purification kit (GenScript) and cloned using pGEM-T Easy Vector system (Promega) following the manufacturer's guidelines. The products were ligated into pGEM-T vector (Promega) according to the manufacturer's instructions. Recombinant plasmids were transformed into competent cells (*Escherichia coli* JM 109) using the heat shock procedure (42 ^{o}C for 45 sec). Recombinant colonies were selected by cultivating them in 20 mg/ml of X-gal and 100 mg/ml of ampicillin on Luria-Bertani media (10 g bacto tryptone, 5 g bacto yeast, 10 g sodium chloride, pH 7.0 in 1L of water). The plasmids were purified using Wizard Plus SV miniprep kit (Promega) and inserts were excised using *Eco* RI (Promega).

Nucleotide sequences were determined by the automated sequence analysis performed with an ABI3730XL sequencer (MACROGEN, Rockville, MD).

2.8 Sequence Analyses

Nucleotide sequences of the PCR products and deduced aminoacid sequences were blasted using Blastn and Blasp National from the Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Dendrograms were generated using a Neighbor Joining method program developed by Saito and Nei (1987). Nucleotide sequence alignments were conducted using Zhang *et al.,* (2000) for DNA sequences available at NCBI. Relatedness to other sequences were used to determine the taxonomic status of the viruses.

CHAPTER III RESULTS

3.1 Mechanical Inoculations

All attempts to reproduce the foliar yellow vein symptoms to the same or related plant species or to induce virus-like symptoms on indicator hosts by mechanical inoculations failed. Symptoms were not observed four weeks after the inoculations.

3.2 Graft Inoculations

Graft transmission of the foliar yellow vein causing agent was successful when Golden Veined Oxalis was grafted onto *O. debilis* (pink woodsorrel) (Fig. 7A) and *O. stricta* (yellow woodsorrel) (Fig. 7B) but not in *O. regnellii* cv. Atropurpurea. The foliar yellow vein symptoms in yellow woodsorrel were mild. The foliar yellow vein causing agent was transmitted from geranium cv. Crocodile grafted into geranium cvs. Artic Frost and Red. All grafted geranium plants showed foliar yellow vein (Figs. 8A and 8B). Foliar yellow vein symptoms were observed in wild honeysuckle when grafted with Yellow Net Honeysuckle scions (Fig. 9). Golden Net Bush scions (narrow leaf) grafted into *P. graciflorum* cv. Twilight caused foliar yellow vein symptoms (Fig. 10A). However, grafting to *O. strictum* did not cause detectable symptoms (Fig. 10B). When Golden Net Bush (broad leaf) was grafted onto Twilight, foliar oak leaf and ringspot patterns were observed (Fig.10C) but when grafted to *O. strictum*, foliar yellow vein symptoms were obtained (Fig.10D).

In general, all the symptoms appeared 3-4 weeks after grafting. Symptoms were not observed when scions of Electric Lime coleus were grafted into Purple coleus or *S. coccinea.* (Figs. 11A and B). Golden Veined Oxalis petioles were grafted on tomato to test for possible transmissions of the putative begomovirus but symptoms were not observed.

Figure 7. A, Pink woodsorrel (*Oxalis debilis*) showing foliar yellow vein two weeks after grafting with a scion from Yellow Veined Oxalis; B, Mild foliar yellow vein symptoms on common yellow wood sorrel (*O. stricta*) after grafting with a scion from Yellow Veined Oxalis.

Figure 8. Foliar yellow vein symptoms on Pelargonium. A, foliar yellow vein on *Pelargonium zonale* cv. Artic Frost; B, foliar yellow vein symptoms on *P. zonale* cv. Red.

Figure 9. Foliar yellow vein symptoms on wild honeysuckle grafted with a scion from Yellow Net Honeysuckle.

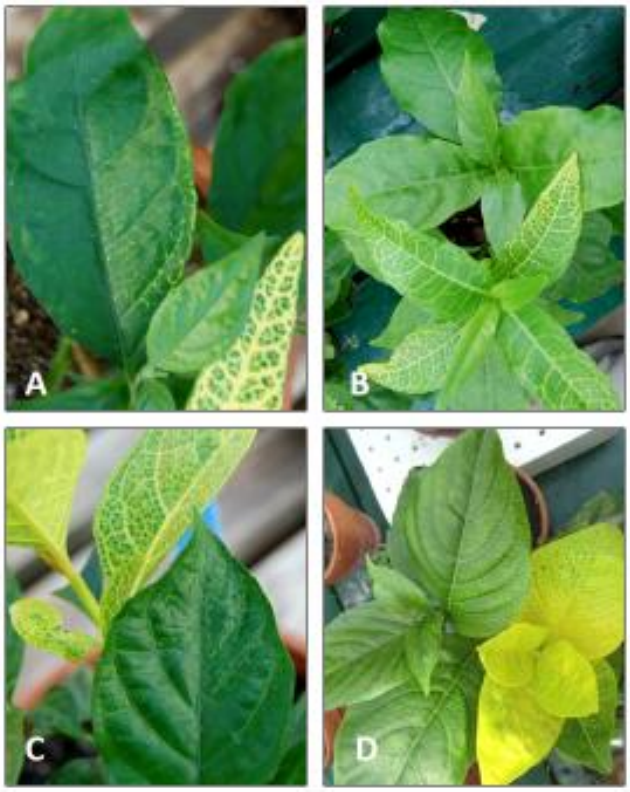

Figure 10. Symptoms on two plant species graft-inoculated with scions from two variants of Golden Net Bush (GNB). A, GNB narrow leaf grafted to *Pseuderanthemum graciliflorum* cv. Twilight; B, GNB narrow leaf grafted to *Odontonema strictum*; C, GNB broad leaf grafted to *P. graciliflorum*; D, GNB grafted to *O. stricta*.

Figure 11. Grafting of scions of coleus cv. Electric Lime to two plant species. A, scions of coleus Electric Lime grafted on coleus cv. Purple; B, scions of coleus Electric Lime grafted onto *Salvia coccinea*.

3.3 Whitefly Transmission

In two independent experiments, groups of 50 whiteflies transmitted the foliar yellow vein symptoms from Golden Veined Oxalis to pink wood sorrel. Eight other experiments did not result in transmissions. A plant used in one of the trials with successful whitefly transmission is shown in Fig. 12. PCR tests using begomovirus degenerate primers confirmed the success of the whitefly transmissions. Attempts to transmit the foliar yellow vein causing agents from pseuderanthemum, coleus and honeysuckle using whiteflies failed. Symptoms were not observed in any of the test plants. Transient mild foliar yellow vein symptoms were observed when wild honey suckle was exposed to whiteflies. However, this was associated with whitefly feeding. Attempts to transmit the foliar yellow vein putative virus from oxalis to tomato did not yield evidence of viral infections in tomato.

Figure 12. *Oxalis debilis* var. corymbosa plant showing foliar yellow vein three weeks after being exposed to whiteflies (*Bemisia tabaci*) which had fed on Yellow Veined Oxalis.

3.4 DsRNA Extractions

DsRNA extractions from all plants showing foliar yellow vein symptoms were negative while positive controls (PvEV and CMV) yielded expected dsRNAs. Increased dsRNA concentrations by pooling several dsRNA extracts of the same sample did not improve dsRNA results.

3.5 DNA and ssRNA Extractions

DNA was efficiently extracted in all tested plants. However, based on the banding profile after gel electrophoresis, DNeasy from Qiagen was the most reliable extraction method in comparison with Dellaporta *et al.* (1983) and CTAB (Desal *et al.,* 1989) (Fig.13). When the Qiagen kit was used to extract DNA from begomovirus-infected plants, followed by PCR, It consistently gave positive results.

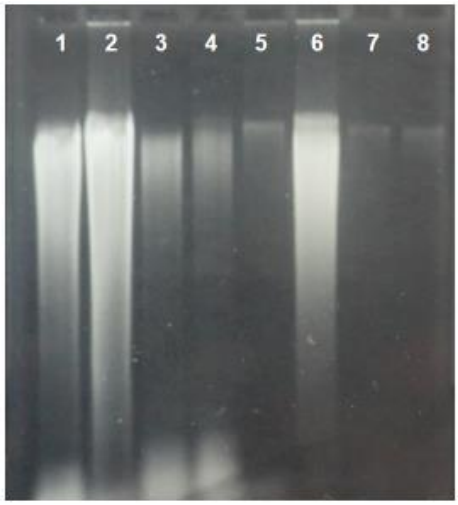

Figure 13. Agarose gel electrophoresis (1.2 %) of DNA extracted using Dellaporta *et al*. (1983), (1 to 4) and CTAB Desal *et al*. (1989) (5 to 8). 1, Tomato; 2, *Pseuderanthemum carruthersii*; 3, *Lonicera japonica*; 4, *Pelargonium peltatum*; 5, Tomato; 6, *P. carruthersii*; 7, *L. japonica*; 8, *P. peltatum*.

3.6 Polymerase Chain Reaction

PCR testing of Golden Veined Oxalis using degenerate primers for begomoviruses suggested that a begomovirus was the causal agent of the foliar yellow vein. Expected amplicons of 1.2 kb and 0.7 kb were obtained when PAL1v1978/PAR1c496 and AV494/AC1048 degenerate primers were used with DNA extracts (Figs. 14, 15, and 16). Positive results were also obtained when symptomatic plants from graft and whitefly

transmissions were tested. Attempts to obtain PCR products using primers PCRc1/PVL1v240 failed.

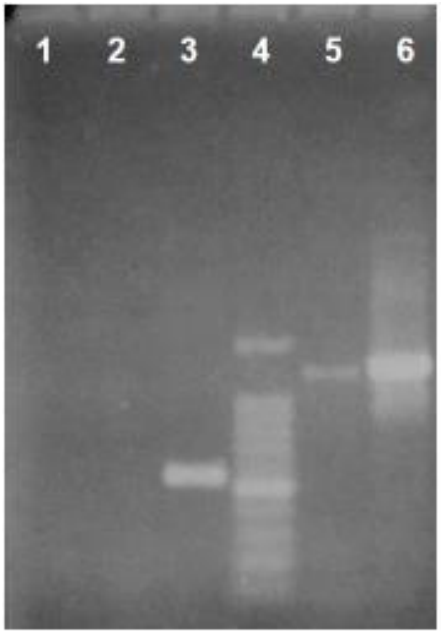

Figure 14. Agarose gel electrophoresis (1.2 %) of PCR products using DNA extracted from: 1, *Oxalis debilis* healthy; 2, Tomato healthy; 3, Golden Veined Oxalis; 4, 100 kb DNA Marker; 5, Golden Veined Oxalis and 6, Tomato infected with *Tomato yellow leaf curl virus*. Primers used were: AV494/AC1048 in lane 3 and PAL1v1978/PAR1c496) in lanes 5 and 6.

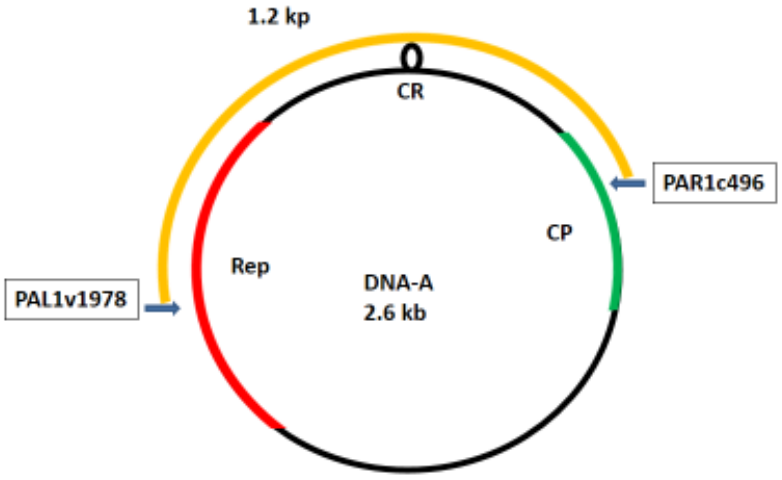

Figure 15. Schematic representation of amplified PCR product using Golden Veined Oxalis DNA as template and degenerate primers (PAL1v1978/PAR1c496).

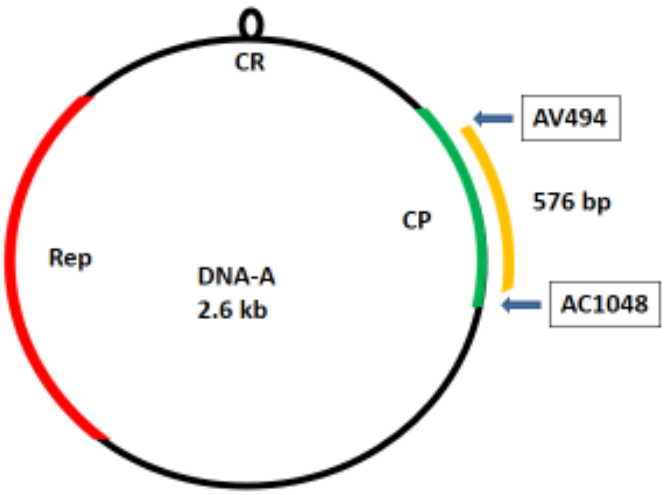

Figure 16. Schematic representation of amplified PCR product using Golden Veined Oxalis DNA as template and degenerate primers (AV494/AC1048).

All other plants showing foliar yellow vein tested negative by PCR using both primer sets. Attempts to obtain PCR products using rhabdovirus degenerate primers also failed. However, RNA extract of a putative rhabdovirus from camellia yielded a 350 bp fragment (Fig. 17).

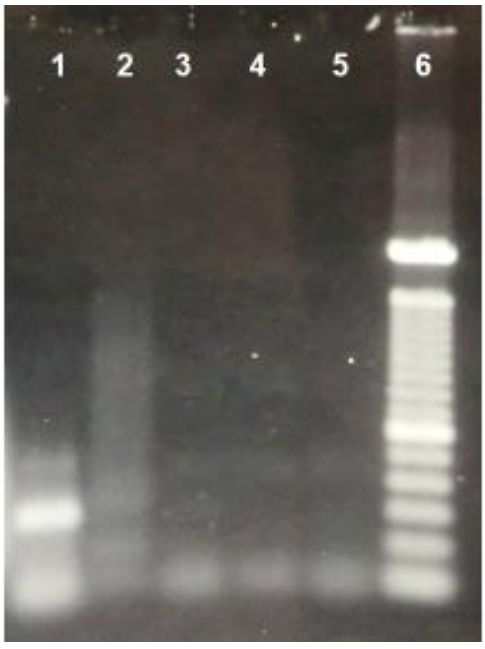

Figure 17. Agarose gel electrophoresis (1.2%) of RT-PCR products using RNA extracted with the RNeasy kit. 1, camellia infected with Camellia yellow mottle virus; 2, Electric Lime coleus; 3, Yellow Net Honeysuckle; 4, Crocodile geranium; 5, Golden Net Bush pseuderanthemum; 6, 100 bp DNA Marker.

3.7 Sequence Analyses

Sequences of both PCR products obtained from Golden Veined Oxalis are shown in Fig. 18A and 18B. Sequence analyses of the PCR products resulted in a nucleotide sequence identity of 87% with the begomoviruses *Leonurus mosaic virus, Sida mottle virus* (SiMoV), and *Tomato yellow spot virus* (ToYSV) (Table 5 and 6). The most similar virus was SiMoV. Sequence identities of 85-87% suggested that the virus causing foliar yellow vein in oxalis may be a new begomovirus species and the name of Oxalis yellow vein virus (OYVV) is proposed. Dendrograms using the obtained sequences from OYVLV were obtained after conducting a blastn analysis with nucleotide sequences in the GenBank (Figs. 19 and 20).

Figure 18. Nucleotide sequence of two PCR products obtained when DNA from Golden Veined Oxalis was used in PCR reaction with primers (AV494/AC1048) A, and primers (PAL1v1978/PAR1c496) B.

Table 5. Nucleotide percentage identity between the Golden veined oxalis begomovirus and selected begomoviruses using fragment amplified with primers PAL1v1978/PAR1c496

Begomovirus	% Identity
Sida mottle virus isolate BR:Vic10:10	87
Tomato yellow spot virus strain BR:MCR7:Le:09	87
Okra mottle virus – [Brazil:okra] isolate 6319	86
Sida Micrantha mosaic virus	85
Leonurus mosaic virus	83
Tomato yellow mosaic virus isolate Valle del Cauca	80
Tomato mild mosaic virus isolate BR:Vic7:10	77

Table 6. Nucleotide percentage identity between the Golden veined oxalis begomovirus and selected begomoviruses using fragment amplified with primers AV494/AC1048

Begomovirus	% Identity
Sida mottle virus-[Brazil] DNA-A, complete sequence	91
Tomato leaf distortion virus isolate BR:Pda4:05 segment DNA-A	90
Leonurus mosaic virus isolate PR49	90
Abutilon mosaic Brazil virus isolate BgV01A.1.C22 segment DNA-A	88
Passion flower little leaf mosaic virus isolate LNS-BZ coat protein gene	88

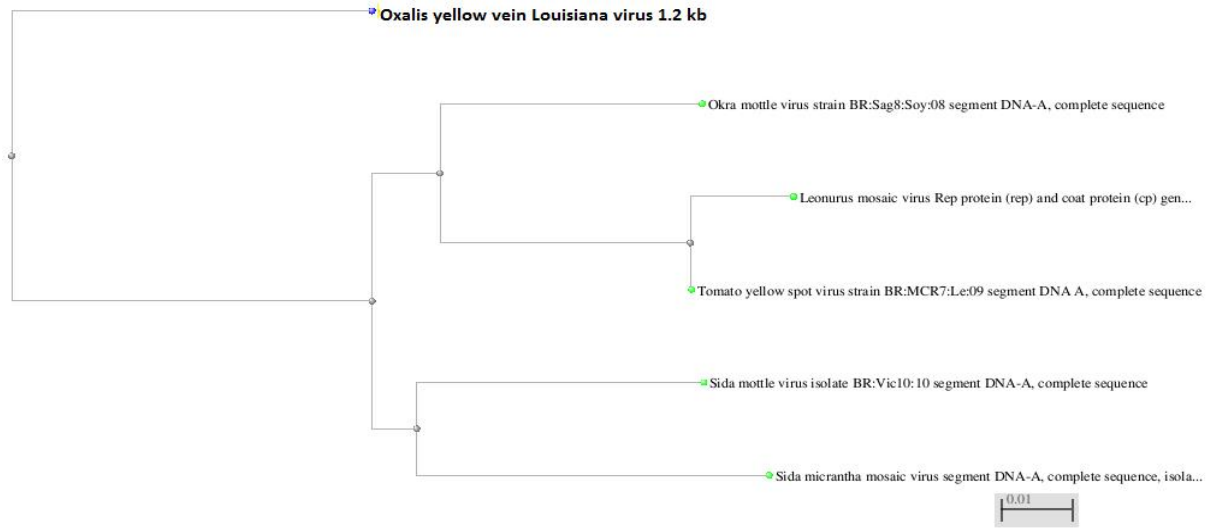

Figure 19. Tree generated after blasting the 1.2 kb sequence of the PCR product obtained with primers PAL1v1978/PAR1c496. The tree was constructed with the Neighbor Joining method.

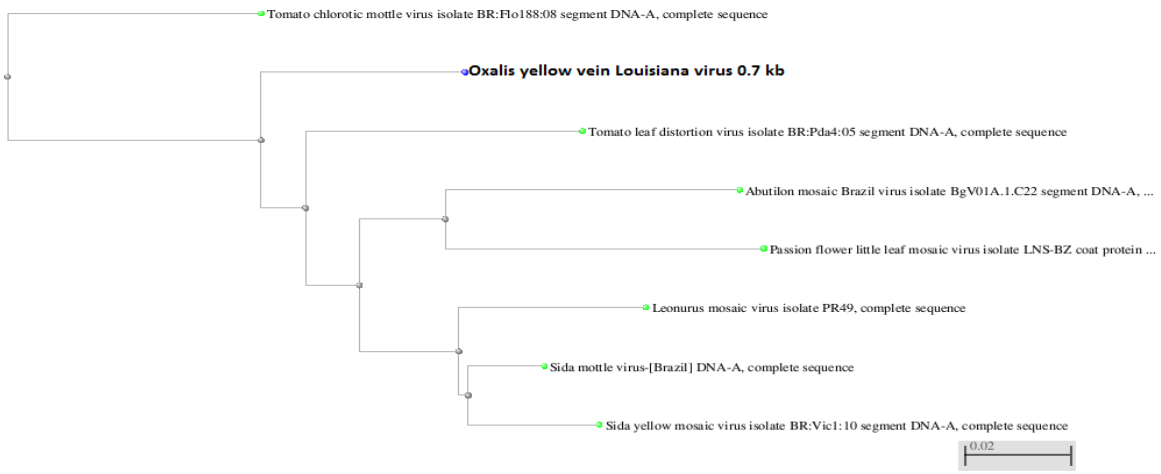

Figure 20. Tree generated after blasting the 0.7 kb sequence of the PCR product obtained with primers AV494/AC1048. The tree was constructed with the Neighbor Joining method.

CHAPTER IV DISCUSSION

Foliar and flower variegations in ornamental plants increase their aesthetics and market value. One type of foliar variegation is yellow vein. Plants with these symptoms are commercially available and desired among plant enthusiasts; but in most cases, the cause of the yellow vein symptom is not known. Cultivars of five ornamental plants with foliar yellow vein symptoms: oxalis, pseuderanthemum, geranium, honeysuckle, and coleus were chosen to investigate the nature of their yellow vein foliage. These plants are commercially available and currently grown in different parts of the US. Results of this investigation provide evidence, that at least in one case, the foliar yellow vein is caused by a virus, three others by graft-transmissible agents that are likely viruses, and one by a genetic factor. In attempts to identify the putative viruses, several virus-detection and virus identification techniques were used with all five ornamental plants and included biological and molecular tests.

The failure to mechanically transmit the putative viruses from four ornamental plants to various plant species was not surprising. Many plant viruses are not mechanically transmissible; particularly those restricted to the phloem tissues and some causing yellow vein or chlorosis symptoms such as begomoviruses (Fauquet *et al.,* 2008), closteroviruses (Jones, 2003), and rhabdoviruses (Jackson *et al.,* 2005). It is also possible that the host plants contain plant constituents that interfered with successful mechanical transmissions (Dijkstra and Jager, 1998).

In plants, grafting is the universal method of transmission of an infectious agent and often used to prove that a biological entity is involved or the cause of a symptom. Sometimes, in spite of the lack of a successful graft union, some viruses can be transmitted during the first contact of the tissues (R. A. Valverde, personal communication). With the exception of coleus, all graft inoculations resulted in the reproduction of the foliar yellow vein symptom. These results support the hypothesis that an infectious agent, possibly a virus is the cause of the foliar yellow vein of the four ornamental plants.

The plant virus genus *Begomovirus* contains many members that cause foliar yellow vein symptoms and are transmitted by whiteflies of the genus *Bemisia* (Hofer *et*

al., 1997). Therefore this investigation focused on the detection and identification of putative begomoviruses that may be causing the foliar yellow vein symptoms in the ornamental plant cultivars Golden Veined Oxalis, Golden Net Bush, Crocodile, and Yellow Net Honeysuckle.

The first approach to determine if begomoviruses were the viruses involved with the foliar yellow vein symptoms was to attempt whitefly (*B. tabaci* biotype B) transmissions. This approach yielded successful transmissions with the foliar yellow vein agent of Golden Veined Oxalis but failed with the foliar yellow vein agent of the other three ornamental plants. In spite of using groups of 50 whiteflies for each experiment, transmission rates of the putative begomovirus infecting Golden Veined Oxalis were extremely low. Nevertheless, low whitefly transmission rates have been reported for some begomoviruses such as SPLCV (Valverde *et al.* 2004; Gutierrez (2008). Furthermore, some begomoviruses are more efficiently transmitted by some *B. tabaci* biotypes than others (Bedford *et al*., 1994). It is important to point out that although the *B. tabaci* colony used caused silver leaf symptoms on squash (*Cucurbita pepo*) and therefore assumed to the B biotype, the homogeneity of the colony was not determined. It is also possible that the putative begomovirus infecting oxalis may have lost the ability to be efficiently whitefly-transmitted due to continued clonal propagation of the infected plant as reported for other begomoviruses (Lotrakul *et al.,* 1998; Wu *et al.,* 1996).

The second approach to investigate if begomoviruses were the viruses causing the foliar yellow vein symptoms was testing the plants by PCR using three sets of degenerate primers commonly used to amplify begomoviruses. With these primers, PCR products were obtained only with DNA from Golden Veined Oxalis. These primers to amplify begomoviruses have been used successfully by many plant virologists to detect and identify begomoviruses from many plant species (Rojas *et al.,* 1993; Wyatt and Brown, 1996; Briddon and Markham, 1996). When different PCR parameters and/or different methods for DNA extraction were used results were the same. Positive PCR amplicons were obtained consistently when TYLCV DNA was used as template. Based on the negative PCR results, one can conclude that the cause of the foliar yellow vein in those plants is not likely to be a begomovirus.

The lack of dsRNA yields from the extractions using tissues from four ornamental plants with foliar yellow vein together with negative PCR and mechanical inoculation results suggest that a crinivirus or a rhabdovirus may be the causal agent of the symptom. It is well known that criniviruses and rhabdoviruses do not yield dsRNA detectable by gel electrophoresis. Although, some rhabdoviruses are mechanically transmissible, criniviruses are not (Hull, 2002). PCR reactions using rhabdovirus degenerate primers and DNA extracted from the plants with foliar yellow vein did not yield expected amplicons.

Sequence information of the two PCR products obtained from Golden Veined Oxalis confirmed that a begomovirus was present and possibly the causal agent of the yellow vein in oxalis and the tentative name of Oxalis yellow vein virus (OYVV) was proposed. This finding has been reported in a poster presentation at the 2013 Annual Meeting of the American Phytopathological Society in Austin, TX (Herrera *et al*., 2013). Efforts to purify virus particles from OYVV-infected oxalis failed. This was not surprising because many begomoviruses, particularly those poorly transmitted by whiteflies are difficult to purify (Lotrakul *et al.,* 1998). This may be due to the low virus concentration and/or lack of efficient virion assembly (Dijkstra and Jager, 1998).

The sequence analyses of the PCR amplicons indicate that OYVV is closely related but not identical to the begomovirus *Sida mottle virus* (SiMoV). Both nucleotide-nucleotide blast (BLASTn) and protein-protein blast (BLASTp) yielded similar results. In Brazil, SiMoV is reported to infect non cultivated plant species but was recently found infecting tomato (Rocha *et al*., 2013). Other begomoviruses that were closely related included *Tomato yellow spot virus* (Andrade *et al*., 2006), *Okra mottle virus* and *Sida micranta mosaic virus*, the last two have been reported to infect plant members of the *Solanaceae, Malvaceae and Fabaceae* (Fernandes-Acioli *et al*., 2011). All of the begomoviruses that were found to be closely related to OYVV affect soybean and tomato crops in Brazil (Andrade *et al*., 2006; Fernandes-Acioli *et al*., 2011; Rocha *et al*., 2013). Furthermore, recombinant forms of these viruses are often found infecting tomato and soybean (Rocha *et al*., 2013; Faria *et al.,* 2009; Fernandes *et al.,* 1999).

There are two types of begomoviruses: those with a monopartite genome (a single genomic DNA of approximately 2.9 kb), and those with a bipartite genome (two DNA components, each approximately 2.6 kb, referred to as DNA-A and DNA-B). Begomoviruses of the new world have a bipartite genome. In contrast, begomoviruses of the old world have a monopartite genome (Briddon *et al*., 2010) Since the limited sequence of OYVV indicated that it is more similar to bipartite begomoviruses, attempts were made to amplify DNA-B using DNA-B degenerate primers for DNA-B (Rojas *et al*., 1993). However, this was not successful. Recently, for the first time, a new world begomovirus, Tomato leaf deformation virus (ToLDeV), was reported from South America (Melgarejo *et al*., 2013). The authors concluded that ToLDeV did not have a DNA-B. Since OYVV is related to this virus (90 % identity) and DNA-B could not be detected, it is possible that OYVV is another case of a new world monopartite begomovirus. These monopartite begomoviruses may have evolved from the DNA-A of new world bipartite begomovirus (Melgarejo *et al*., 2013).

Because of the close relationship of OYVV with tomato-infecting begomoviruses reported in South America, transmission experiments to tomato were conducted using whiteflies. However, attempts to transmit OYVV to tomato seedlings failed. Nevertheless, before ruling out that this virus can infect tomato, more transmission tests using other *B. tabaci* biotypes should be conducted. The closeness of this begomovirus isolated from oxalis to several viruses that infect tomato and soybean in Brazil, may be an aspect to consider in further research since it has been shown that begomoviruses can recombine and expand or change their host range (Moriones and Castillo, 2008).

The foliar yellow vein causing agent of pseuderanthemum (broad and narrow leaf variants) was transmitted to two related plant species. In the case of pseuderanthemum, it is interesting to notice that it appears that a strain of the putative virus infects the broad leaf variant and a different strain the narrow leaf variant. This is based on the different symptoms obtained when two hosts were grafted with the two variants.

The foliar yellow vein was transmitted from Yellow Net Honeysuckle to wild honeysuckle. In Korea and Japan, there have been several reports of honeysuckle being infected with begomoviruses and being the source of inoculum for transmissions to

tomato (Ogawa *et al.,* 2008; Wang *et al.,* 2011; Ueda *et al.,* 2008). Because of these reports, it is important to determine if the yellow vein causing agent of honeysuckle is a begomovirus. Limited data from this investigation including whitefly transmission and PCR experiments suggest that a begomovirus may not be the causal agent of the foliar yellow vein in honeysuckle. Many attempts to transmit the infectious agent in honeysuckle by *B*. *tabaci* failed. A mild vein yellowing on the leaves was inconsistently seen a week after the test plants was exposed to the whiteflies. However, these plants recovered from the symptom and it is likely that whitefly feeding (adults or nymphs) were the cause.

Geranium foliar yellow vein symptoms have been shown to be caused by a graft transmissible agent (Kemp, 1966; Cassells *et al.,* 1982). However, until now, the causal agent has not been characterized. Graft experiments conducted in this investigation confirmed these results. The foliar yellow vein agent from Crocodile geranium was transmitted to Artic Frost and Red geraniums. The limited data in the experiments conducted in this investigation suggests that the causal agent of the foliar yellow vein in Crocodile geranium is not a begomovirus. Meristem culture has been reported to eliminate the foliar yellow vein from geranium (Cassells *et al.,* 1982), but due to the plant enthusiasts interest on the unique foliar yellow vein, vegetative propagating of these yellow veined cultivars is practiced.

Based on the lack of mechanical and graft transmission, lack of dsRNA, PCR products, and whitefly transmission, it is likely that the foliar yellow vein of coleus is due to genetic factors and not an infectious agent.

The use of plant viruses to increase the aesthetics of plants is becoming more popular and infected ornamental plants are commercially distributed worldwide. The lack of knowledge of the identity and epidemiology of these viruses and their potential to infect other economically important crops. There is a need to characterize these viruses and determine their potential threat to crops. It is well know that these plants are often freely moved from country to country, the potential for epidemics of viral diseases in non-ornamental crops is real. Once these viruses are introduced to a new area where the vector is present, mutations, or recombinations could occur and they can expand their host range and infect susceptible crops.

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VITA

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