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VIRUS AND VIRUS-LIKE DISEASES OF SHADE TREES[1]

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The number of known virus diseases affecting shade trees is limited. This may reflect limited observations rather than limited disease occurrence. The pressure for research on the more economically important food and fiber crops has stifled needed research on plants grown for esthetic purposes. Trees are continuously exposed to possible infection by viruses; therefore, it is surprising that so few have been found. Trees may be infected but the expression of the symptoms is so mild that they are not readily recognized. However, the presence of viruses in mature trees showing no obvious symptoms has been demonstrated in Australia. An entomologist collected aphids and leaf hoppers from mature Eucalyptus trees and caged these on young seedlings. He obtained a wide range of symptoms on these seedlings and was able by grafting to transmit the causal agent, indicating the presence of a virus in the mature, but symptomless, trees. Although most of these disorders are due to a virus, other parasites such as the mycoplasma and Rickettsiae can elicit similar symptoms on their host.

When a shade tree is infected with a virus the symptoms usually are easily detected. Such symptoms may be broadly classified into the following types: mottling or mosaic, yellows, ringspot, and witches' broom. Other factors, including mineral deficiencies and herbicides may elicit symptoms on the stressed host resembling those caused by a virus. The leaves of birch trees growing in the alkaline, boron-deficient soil of Eastern British Columbia are nearly white. These symptoms can not be reproduced on healthy plants by the routine methods of virus transmission and these symptoms disappear with applications of boron sprays. Pin oaks often have dwarfed, distorted leaves in mid-spring. Such foliar malformations are characteristically those associated with virus infection but may be due to 2,4-D injury. Leaves of pin oaks growing on shallow soils, generally with a high calcium content and an alkaline pH, frequently become chlorotic to completely yellow with necrotic margins. Since the agent causing this disorder can not be transmitted and the affected trees respond to applications of iron chelate, pin oak chlorosis has been shown to be due to lack of available iron. Witches' broom is so prevalent and species-specific that it is often used to identify hackberry. These symptoms are typical for virus-like disorders, but this disease is caused by a mite-mildew complex. Bunch on walnut is another witches' broom type of disease. This disorder affects black walnut (*Juglans nigra*), English or Persian walnut (*J. regia*), butternut (*J. cinerea*), and heartnut (*J. sieboldiana* var. *cordiformis*) with the severity of the disease increasing in an ascending order from symptomless to mild on black or Persian walnut to death of heartnut. Although the incriminating agent has not been isolated in pure culture, electron micrographs and remission studies with tetracycline place it in the diseases incited by the mycoplasma-like organisms (MLO).

Diseases Caused by Mycoplasma-Like Organisms (MLO)

Few transmissible diseases possess symptoms as spectacular as witches' broom or yellows. Previous to 1967, such diseases were considered to be of a virus etiology, since these could

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be transmitted by grafting, dodder, and/or leaf hoppers. None of these disorders could be transmitted mechanically nor were there virus particles detected by electron microscopy. In 1967, however, Doi *et al.* (5) found ploeomorphic bodies in the phloem elements of several of these witches' broom and yellows disorders. Simultaneously, Ishiie *et al.* (13) found that applications of tetracycline would suppress symptoms of these disorders. The morphological similarity of these ploeomorphic bodies to the mycoplasma-inciting animal diseases was striking. Further evidence of similarity was the suppression of symptoms by tetracycline. MLO's have been isolated in pure culture only in two cases; therefore, until the incriminating organisms can be cultured, those diseases with a mycoplasma-like etiology based on electron microscopy and remission by tetracyclines are considered as MLO-induced diseases.

Ash witches' broom was first reported in New York (11) where an occasional tree in a population of declining white ash showed symptoms of a yellows-type disease. These symptoms included small and chlorotic leaves, shortened internodes, and shoot growth from axillary and terminal buds which normally remain dormant until the following growing season. The transmissibility of this disorder (14) was demonstrated by attaching apical cuttings (5-10 cm) of dodder, *Cuscuta subinclusa,* grown from seed and maintained on tobacco, on witches' broom shoots. The shoots with attached dodder were enclosed within a plastic bag containing a screened ventilation to exclude insects. Eight weeks after establishment, apical cuttings of this dodder were removed and attached to healthy *Vinca rosea*. Symptoms of a yellows-type appeared on these Vinca four to five months later. Transmission to other healthy Vinca by cleft grafting resulted in a reproduction of symptoms while dodder attached to carrots resulted in the appearance of symptoms typical of those described for yellows diseases by Kunkel (15). Electron micrographs confirmed the presence of MLO in the mature sieve tubes of midveins and petioles of diseased ash, carrot, and Vinca.

Witches' broom of black locust was found in 1898 (24) when the typical brooming symptoms appeared on locust stump sprouts in Maryland. This was later transmitted by grafting. Symptoms included the proliferation of shoots and a reduced size of leaves with the broom always being erect or ascending. Occasionally a cushion 4-5 cm in diameter and about 1 cm thick formed on the trunk. This cushion bore groups of buds and tiny leaves on its surface and represented an extreme form of bunch. Brooming is frequently found on sprouts arising from the roots and stumps of trees which were symptomless prior to cutting. These generally show little resemblance to comparable healthy locust growth. Frequently, the broom develops terminally or in the axils of normal leaves near the top of the sprout. The presence of MLO in the sieve elements and companion cells of the phloem of stems, roots, and petioles of black locust with witches' broom symptoms places this disease in the MLO type of disorder. This disease is rather widespread in eastern USA and has been reported in Europe.

Bunch on walnut (Fig. 1) was reported by Waite in 1932 (23) and described in detail by Berry (3). Symptoms include a curling, cupping, chlorosis, and narrowing of the leaflets on small, wiry shoots. The bunch symptoms result from the development of shoots from buds on the primary stems in mid to late summer. Ordinarily these buds remain dormant until the following spring. These shoots are juvenile and succulent and do not become dormant in the late fall, thus, generally winter-kill. This annual late summer growth and subsequent dieback is so severe on some heartnut cultivars that they are killed within two years. Circumstantial evidence suggests that different strains of the bunch MLO exist. A single tree of the heartnut cultivar, Stranger, showed marked brooming but later completely recovered (R. Oaks, unpublished data). Bunch was considered to have a virus etiology until MLO's were found (16,20) in the sieve tubes of leaf, stem of root phloem cells.

Lethal yellows of palm is a destructive disease in Florida and the Caribbean region. It was described by Carter *et al.* (4) who indicated that yellowing was a general symptom which covered other disorders as well. Mechanical damange or a localized concentration of scale insects can cause tip yellowing, which is a specific symptom that can cause temporary confusion. Leaflets near the tips of fronds in the middle of the crown become bright yellow at, or just after, nutfall. The number of yellowed tips per frond varies from 1-11 and may involve a few leaflets or as much as half the length of the frond. In about four weeks these symptoms progress to complete death of the frond. Infrequently, patch yellows occurs, first appearing in 3-7 leaflets in the middle of the frond. On affected fronds the color spreads both ways and the leaflets dry up in about four weeks. A general yellowing is another symptom. This starts in one or more of the oldest fronds and progresses towards the center of the frond. These yellowed fronts (approximately ⅔ of the crown) will fall off in about two weeks. The remaining fronds of the crown may remain intact for about four months, then turn yellow changing to brown when the entire crown breaks off. Final diagnosis of lethal yellows is based on the appearance of a blackened inflorescence. The spathe may open normally with a fully developed inflorescence, then the tips of the male spikelets turn brown or black. The number of spikelets showing this dark color vary from one to all within the inflorescence and this discoloration continues downward. The necrosis progresses to include the female flowers and within four weeks the entire inflorescence blackens. This process accelerates and involves all of the emerging spathes. The basal portions rot, leading to death of the tree.

Fig. 1. Typical bunch symptoms on walnut caused by MLO (courtesy, C.E. Seliskar).

Cole (4) considers the brooming of branches and shoots as the most distinguishing symptom of pecan bunch. Bunch may appear on small and/or large branches and on sucker growth. Axillary buds of the current year's growth develop laterals which often equal the length of the primary shoot. Lateral buds on the lateral shoots also may be forced into growth. In advanced stages of bunch, the new shoots are short and slender but the buds are weak and may fail to develop. The color of the leaves on these witches' brooms varies from dark green to chlorotic. The width of the terminal leaflets are narrower. The leaflets usually are thinner and wider but abscise in late summer or early fall, starting with the lower leaves and progressing upwards. Leaflets on the broom appear earlier than those on the healthy trees. Production of nuts is reduced on the severely affected trees and the nuts may be small and poorly filled. The presence of MLO's in the phloem elements of the twigs and petioles adds pecan bunch to our growing list of diseases caused by MLO's.

Swingle (21) described phloem necrosis of elm in 1938 after an extensive dying of American elms in the central and lower Ohio rivershed. Symptoms of phloem necrosis first appear in the extreme tips of branches. Foliage becomes thin and the leaves droop due to the downward curvature of the petioles. The leaf blades curl upwards at the margin, producing a trough-like effect that makes the leaves appear narrow and greyish-green. Such leaves are often stiff and brittle. Later, the foliage becomes yellowish-green and finally yellow, followed by dropping. These symptoms occur throughout the entire crown. In advanced stages the roots die with the small, fibrous ones succumbing first. Typical discoloration, confined to the phloem and cambium, precedes death of the larger roots and frequently may be found extending into the trunk and branches. In large trees, this discoloration usually occurs in large roots at the base of the trunk, just before death of the tree. The cambium first becomes light yellow or golden followed by a yellowing of the phloem which turns butter-scotch brown with small, scattered black flecks. Soon thereafter the phloem becomes dark brown and necrotic and produces an odor resembling wintergreen.

Table 1. Transmissible diseases of trees with a MLO etiology

Common name	*Type*	*Host*	*Reference*
Ash witches' broom	*yellows*	*Fraxinus americana*	Hibben & Wolanski, 1970. Phytopathology 60:1295 (Abstr.)
Brooming of black locust	yellows	*Robinia pseudoacacia*	Seliskar *et al.* 1973. Phytopathology 63:30
Bunch of walnut	yellows	*Juglans* spp.	Hutchins & Wester. 1947. Phytopathology 37:11 (Abstr.)
Lethal yellows of palm	yellows	*Cocos nucifera*	Plavsic-Banja *et al.* 1972. Phytopathology 62:298.
Pecan bunch	yellows	*Carya illinoensis*	Seliskar *et al.* 1974. Phytopathology 64:1269.
Phloem necrosis of elm		*Ulmus americana*	Wilson *et al.* 1972. Phytopathology 62:140.

Economical implications. Lethal yellows of palm is rapidly killing the coconut palms in south Florida and the Caribbean. This disease responds to tetracycline therapy but this is practical only for lawn and avenue trees and then only to provide time for the planting and growth of resistant clones. Brooming of black locust appears to be widespread east of the Mississippi River, thus, the wisdom of planting this species in those areas where the disease is endemic seems questionable. The similarity of bunch symptoms on pecan and walnut suggest that the same, or strains of the same, MLO may be responsible. Both the heartnut and butternut are so susceptible to bunch that they should not be planted in areas where bunch is present. There is circumstantial evidence that bunch is responsible for a heavy mortality of butternuts in southern Minnesota, thus, more information on the spread and the vector responsible is critically needed. Neither ash witches' broom nor phloem necrosis of elm appear to be serious diseases at this time. The former has a rather restricted range but could become a real threat should environmental changes occur which would result in a rapid spread. With the devastating spread of Dutch elm disease (DED), phloem necrosis seems unimportant. The disease, however, is spreading in those areas which are still free from DED. This disease should be taken into consideration in current breeding programs for resistance to DED.

Diseases Caused by Viruses

The list of viral infections on shade trees is less imposing than that of the MLO's but this may be a function of visibility and persistence. Unlike the diseases caused by MLO's where brooming and yellowing are obvious and persist, the symptoms of virus infections often are mild and, in many cases, become latent after passing through the shock phase. Only when these diseases contribute to unmistakable loss of vigor or death and the symptoms are recurrent and clearly visible, do we recognize these disorders. Nonetheless, as our interests in landscape and environmental concerns increase, this list will grow.

Amelanchier mottle was reported in 1956 (18) when a bud from an apple tree showing symptoms of severe stem pitting (9) was placed into a shad bush, *Amelanchier* spp. Thirteen months later when the shoots of the shad bush were 10 cm long, a striking mottle (Fig. 2) appeared on the leaves of the shoot above the inserted apple bud. These leaves were dwarfed and rugose, in sharp contrast to the smooth leaves on the adjoining healthy clone. The identity of the virus causing this mottle was not established but the presence of chlorotic leaf spot (CLSV) in the inocula and the similarity of symptoms on other indicators (19) suggest that CLSV was responsible.

Ash dieback featuring a progressive dying back of the branches and ultimate death of the ash tree has been a serious problem in the northeast for several decades. Dieback is particularly serious on white ash, *Fraxinus americana,* but occasionally is found on green ash, *F. pennsylvanica* var. *lanceolata.* In 1963 and 1964, Hibben (11) found virus-like symptoms on the leaves of white ash in all stages of branch dieback. These symptoms include faint chlorotic spots and rings, irregular

Fig. 2. Mottle on *Amelanchier* spp. thirteen months after inoculation with a bud from infected apple. Left, mosaic pattern on leaves from inoculated tree. Right, leaves of healthy noninoculated control.

chlorotic sectors and line patterns along the main and secondary veins, green spots and rings in chlorotic or reddish tissue, and reddish spots and rings in green tissue. These chlorotic sectors often became necrotic and turned black. Occasionally premature reddening of the foliage preceded this dieback. In 1966 Hibben (11) transmitted this disorder to bean, cv. Scotia, and cowpea, cv. Early Ramshorn, but only from those ash leaves showing chlorotic or reddish spots and rings. Additional transmission from bean to bean and to tobacco, Datura, zinnia, cucumber, Crotalaria, and petunia, all of which showed typical symptoms of ringspot were evidence of the virus etiology of this disorder. The host range and similarity of symptoms to those of tobacco ringspot (TRSV) indicated that TRSV is associated with ash dieback.

Elm mosaic was first described by Swingle *et al.* (22) but it was apparently found as early as 1927 in the vicinity of Cleveland, Ohio. Prior to the rapid spread of DED, this disease had a widespread distribution (4). Foliage of affected trees show typical mosaic symptoms but some leaves remain normal in size and texture. Others are abnormally small, stiff, and frequently dwarfed with a yellow and green mottling accompanied by a rugosity. This mottling is more diffuse on some trees and there may be a mild brooming of the branches. Elm mosaic was shown to be caused (8) by a strain of the tomato ringspot virus (TomRSV) which may account for its widespread occurrence prior to the onset of DED.

Epinasty of flowering cherry is not a serious disease due to its tendency for latency and the lack on a known vector. Initially, this epinasty was thought to be due to the ringspot virus (17). After it was shown to be caused by the green ring mottle virus, the cultivar, Kwanzan, has been used as a virus indicator in state certification programs. This epinasty ranges from mild to severe, depending upon the virus strain. Portions of the midvein or lateral veins become necrotic, resulting in a twisting and curling of the leaves, similar to that induced by aphid feeding. Internodes of the elongating terminals are shortened and as the bark matures, it may be roughened by the development of fissures. Terminal growth becomes symptomless in early summer as the temperatures become elevated.

Birch decline is a serious disease in northeastern USA and eastern Canada, affecting both white and yellow birch (*Betula papyrifera* and *B. alleghaniensis*). Most of the merchandable trees in the severely affected areas were killed in the 1935-55 period. Hansbrough (10) in 1953 transmitted a gold ringspot of white birch to seedlings but did not associate this virus with the decline. Later Berbee (2) transmitted the line pattern of yellow birch to seedlings. The line pattern symptoms on both species consist of chlorotic lines forming oak-leaf designs, irregular rings or linear flecks, sometimes accompanied by a mild mosaic. Until fully expanded, emerging leaves on infected trees generally are symptomless and some infected trees have a few, or no, foliar symptoms. These symptoms may be restricted to a few leaves on a few branches. The leaves remain on the trees until the end of the growing season. Chlorotic tissue turns nearly white during midsummer. The virus has been mechanically transmitted to Chenopodium, cowpea, cucumber, squash, and bean. Serological and host range studies demonstrate that this virus-causing line pattern of birch is a strain of apple mosaic.

Palm mosaic, affecting Mexican fan palm, *Washingtonia robusta*, is a recently described disease of probable virus etiology. This palm is a hardy, relatively disease-resistant species used extensively in the warmer parts of California as an ornamental. This disease became a problem when a mosaic pattern was found on several small fan palms in a retail nursery. Symptoms consisted of a bright mosaic on leaves (Fig. 3A) that were reduced in size. On the older leaves, the obvious mosaic symptoms disappear and bright yellow and ring patterns develop. Limited observations indicate that infected trees have reduced growth rates. While mechanical transmission has not been successful and no insect vectors have been identified, electron micrographs revealed the presence (Fig. 3B) of long, flexuous rods (686 X 13 nm) in the diseased material. No rods were found in the healthy preparations. An examination of thin-sectioned leaf material confirmed the presence of pinwheel inclusion bodies, characteristic of viruses belonging to the potato Y group.

Zonate canker of elm is a virus disease of shade trees that should be relegated to history. This disease has been found in New Jersey, Ohio, and Missouri either alone, or in combination with elm mosaic. Although the foliar symptoms are similar to those of elm mosaic, the symptoms differ in that on inoculated trees leaves develop necrotic spots in the first flush of growth. These are later shed. Zonate canker also causes small, necrotic cankers to develop on the bark of the upper stems and branches of the inoculated trees. In old, established trees it may appear as a latent infection with little or no obvious symptoms.

Economic importance. Two of the viruses, epinasty of flowering cherry and mottle on *Amelanchier*, have little, if any, economic importance. Flowering cherry infected with the epinasty virus can serve as a reservoir for infection of peach or cherry but the lack of a known vector minimizes this danger. Mottle on *Amelanchier* has been found only on experimentally inoculated plants. Zonate canker of elm also is unimportant, since most of the susceptible elms have been replaced with other species. Elm mosaic also is no longer a serious disorder but its causitive entity, TomRSV, is a soil-borne virus with a wide host range. Certainly any replacement species should not be susceptible to TomRSV. The limited distribution of ash ringspot indicates that this disease presently is not serious. Ash ringspot,

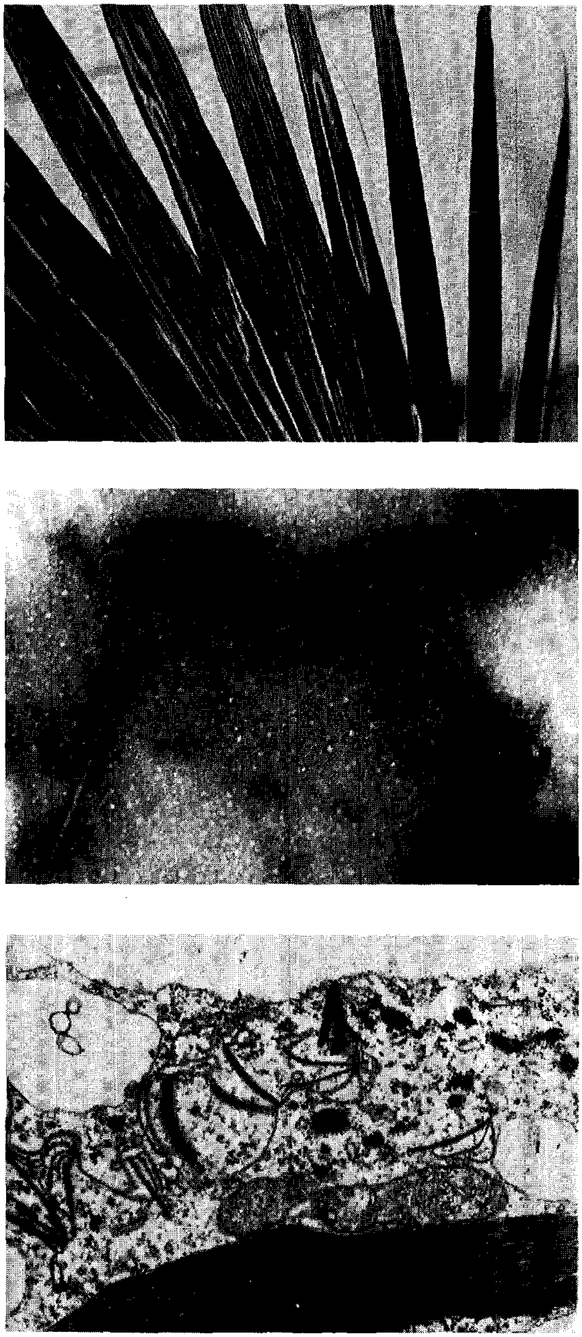

Fig. 3. Palm mosaic (courtesy, D.E. Mayhew). (upper) Mosaic patterns on leaves of infected Mexican palm, *Washingtonia robusta* Wendl. (middle) Electron micrograph showing long, flexuous rods (686 × 13 nm). (lower) Electron micrographs showing pinwheel inclusion bodies.

Table 2. Transmissible diseases of trees with a virus etiology[1]

Disease		*Identity*	*Reference*
Amelanchier mottle	*Amelanchier* spp.	CLSV	Millikan & Guengerich. 1956. Phytopathology 46:130
Ash ringspot	*Fraxinus americana*	TRSV	Hibben. 1966. Phytopathology 56:323
Elm mosaic	*Ulmus americana*	TomRSV	Swingle *et al.* 1943. Phytopathology 33:1196
Epinasty on flowering cherry	*Prunus serulata*	GRMV	Fridlund & Deiner. 1958. Plant Dis. Rptr. 42:830
Line pattern on birch	*Betula papyrifera; B. alleghaniensis*	ApMV	Gotlieb & Berbee. 1973. Phytopathology 63:1470
Palm mosaic	*Washingtonia robusta*	PVY	Mayhew & Tidwell. 1978. Plant Dis. Rptr. 62:803.
Zonate canker of elm	*Ulmus americana*		Swingle & Bretz. 1950. Phytopathology 40:1018.

[1] Abbreviations used in this table are as follows: ApMV = apple mosaic virus; CLSV = chlorotic leafspot virus; GRMV = green ring mottle virus; PVY = potato virus Y; TomRSV = tomato ringspot virus; TRSV = tobacco ringspot virus.

however, is caused by a strain of TRSV, a soil-borne virus with a wide host range, including maple (1) and several other woody species (24) which have been experimentally inoculated. Our information on palm mosaic is insufficient at the present time for any proper assessment but if it proves to be widespread, then other species among which there is a limited choice must be found for ornamental plantings.

Current Investigations

As indicated in Table 3, there are several disorders whose symptoms strongly indicate their infectious nature. One of these, a variegation of sugar maple, has been studied by Gotlieb in Vermont, an important producer of maple syrup. In Missouri we have found a bunch disease on sassafras and a mosaic on persimmon (Fig. 4). Both of these diseases are very debilitating to the host.

The economic importance of sugar maple in the confectionary business and landscape plantings emphasizes the need for further investigation of this disorder. Although sassafras is not one of our more desirable species, it grows on poor sites under unfavorable conditions. Limited observations suggest that sassafras bunch is a widespread and degenerative disease and merits further study. Persimmon mosaic appears to be limited in occurrence but was found on two beautiful landscape specimens. The unsightly dieback and unattractive mottling of the foliage accompanying infection with persimmon mosaic is evidence of the potential seriousness of this disease.

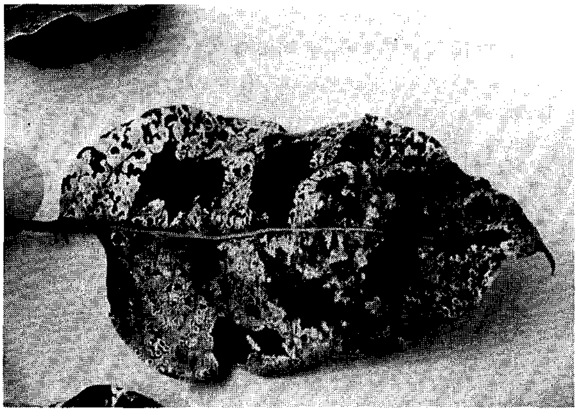

Fig. 4. Mosaic on persimmon.

Table 3. Tree diseases of unknown etiology under current investigation.

Disease	*Host*	*Reference*
Maple variagation	*Acer saccharum*	Gotlieb, unpublished data
Persimmon mosaic	*Diospyrus virginiana*	Millikan *et al.*, unpublished data
Sassafras bunch	*Sassafras albidum*	Millikan *et al.*, unpublished data

Summary

The number of known transmissible virus and virus-like disorders on shade trees is limited at the present time. Since three of the disorders were described on American elm, the list is even less impressive. Those with a MLO etiology outnumber those with a viral nature and one of these, lethal yellows of palm, is eliminating one species of coconut in South Florida and the Caribbean region. Bunch of walnut and pecan along with brooming of locust also appear to be widespread but their effects on the host vary from none, or little, to death, depending upon the clone. Among the diseases of a virus etiology, two occur on hosts inoculated with known fruit tree viruses. Line pattern of birch affects two species of *Betula* and is associated with a decline which is eliminating these species in their natural habitat of northeastern USA. Neither ash ringspot nor elm mosaic are considered as serious diseases at the present time. Since these diseases are caused by strains of TRSV and TomRSV, are nematode-transmitted and have a wide host range, they may cause disease to other hosts.

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