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A RHABDOVIRUS ASSOCIATED WITH A NEW DISEASE OF FLORIDA PAPAYAS[1, 2]

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Abstract. **Diseased papaya plants with distinctive symptoms were observed in groves in Dade County, Florida, during the autumn of 1980 and 1981. These symptoms were readily distinguished from those induced by papaya ringspot virus, which was much more prevalent. Examination of diseased tissues sectioned with a Cryostat and stained with calcomine orange and Luxol brilliant green revealed nuclear inclusions in phloem cells which were not found in either healthy papaya leaves or in leaves of plants singly infected with papaya ringspot virus. Ultrathin sections of phloem cells stained in uranyl acetate and lead citrate revealed rhabdovirus-like particles (87-98 x 180-254 nm) in the nuclei of infected tissues. These particles were similar to those previously described from papaya in Venezuela (Lastra, R. and E. Quintero. 1981. Papaya apical necrosis, a new disease associated with a rhabdovirus. Plant Disease 65:439-440). This is the first report of such a viral agent of papaya in Florida.**

The virus and virus-like diseases of papaya have been reviewed by Cook (7). In addition, Lastra and Quintero (13) have recently described a rhabdovirus infecting papaya from Venezuela.

The first report of a papaya virus disease in Florida was in 1940 (22). By 1960, viral diseases were considered to be major limiting factors in Florida's papaya production (10). Conover (4, 5, 6) described three distinct papaya viruses from Florida: papaya distortion ringspot (5), faint mottle ringspot, and mild mosaic (6). Faint mottle ringspot virus was considered to be a strain of papaya distortion ringspot virus (6). These viruses were further characterized by DeBokx (8) and Zettler et al. (23). Whereas papaya distortion ringspot virus is a potyvirus and is now referred to as papaya ringspot virus (PRV) (8, 17, 23), papaya mild mosaic virus is a potexvirus and is now referred to as papaya mosaic virus (8, 16, 23).

This paper describes an association of a rhabdovirus with a new disease of papaya occurring in Florida and discusses its possible relationship to the rhabdovirus disease from Venezuela (13). We propose the name "droopy necrosis" which is descriptive of the initial and terminal phases of the disease.

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Materials and Methods

Light Microscopy

Methods developed by Christie and Edwardson (2) were used for the study for virus induced inclusions. Leaf samples were fixed and stored in 2-methoxyethanol (ethylene glycol monomethyl ether). The stain was prepared by mixing water, calcomine orange, and Luxol brilliant green (v/v/v = 1:1:10). Sections cut with a Cryostat were placed on microscope slides and flooded in the stain for 15 min. The sections were then rinsed briefly in absolute ethanol, transferred to 2-methoxyethyl acetate for 1 min and blotted dry. The sections were then mounted in Euparal and examined with a compound light microscope with an oil immersion objective lens for the presence of virus induced inclusions. Similarly treated sections from PRV-infected and healthy papaya leaves were used as controls.

Electron Microscopy

Infected and healthy papaya leaf tissues were prepared for ultrathin sectioning. Small pieces of tissue (1 mm^2) were vacuum infiltrated in Karnovsky's solution (11) for 2 min and stored for 1.5 hr for fixation. The tissues were then washed with 0.1 M sodium cacodylate buffer, pH 7.2, and postfixed in 1% osmium tetroxide for 1.5 hr. Tissues were then sequentially washed with 0.05 M sodium cacodylate buffer (pH 7.2), dehydrated through an alcohol series, placed in 100% acetone, and embedded in Spurr's embedding medium (20). Ultrathin sections were made using a LKB Ultrotome III with glass knives. Sections were picked up on carbon-coated Formvar grids and poststained in 1% uranyl acetate for 15 min and in lead citrate (18) for 5 min. The grids were examined using a Hitachi 11C or a Hitachi H-600 electron microscope.

Mechanical Transmission

Inocula were prepared by triturating diseased papaya leaf tissue in 0.5% sodium sulfite with a mortar and pestle. Either celite or 600 mesh carborandum were used as abrasives. *Carica papaya, Nicotiana* x *edwardsonii,* and *Sonchus* sp. were used as test plants. Mechanical transmission was done by dipping a piece of cheesecloth into inoculum and by rubbing it onto the leaves of test plants. Three plants of each species were inoculated with 0.5% sodium sulfite as controls. Test plants susceptible to sonchus yellow net virus (SYNV), a mechanically transmissable rhabdovirus infecting sowthistle and *Bidens pilosa* in Florida (3), was also included in these trials as a measure of the adequacy of the transmission technique used.

Results

Symptomatology

Initial symptoms of droopy necrosis are drooping and recurvature of blades in the upper 1/3 to 1/2 of the crown. Petioles are arched downward. Blades of youngest leaves in the bud are pale yellow, do not expand normally and are sharply recurved (Fig. 1). Petioles are shortened and stiff. As the disease progresses during the summer, recurvature of blades becomes more pronounced, leaves become thickened and progressively smaller. Petioles become

Fig. 1. Early symptoms of papaya droopy necrosis. (left) Pale yellow, sharply recurved, and unexpanded young leaves, and recurved petioles. (right) Marginal necrosis of leaves in winter.

shortened and often develop greenish parallel lines that are somewhat ribbed. The crown becomes rounded and has a decided bunchy appearance. Internodes are very short. Staminate inflorescences are shortened and stiff. Flowers on pistillate plants abort, fruit set ceases but fruit do not abscise or develop symptoms. In contrast to papaya bunchy top, latex flows readily following wounding.

Droopy necrosis is much more severe during the winter than in summer with the main difference being in the way the bud and upper stem and attached leaves are affected. In initial stages in winter, blades on petioles 6″ to 10″ long often develop marginal necrosis (Fig. 1). Later the leaves near the stem tip begin abscising. Abscission continues until nearly all leaves are gone except for 1 to 3 tiny leaves near the stem tip (Fig. 2). Shortly after this the stem tip develops a necrosis which progresses down the stem until the plant is dead.

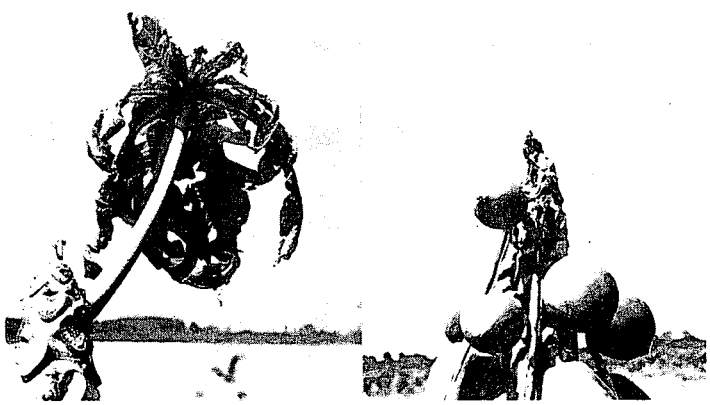

Fig. 2. Later symptoms of papaya droopy necrosis. (left) abscission of leaves, and (right) necrosis of the stem tip.

Droopy necrosis usually spreads through plantings that are previously infected with the papaya ringspot virus, and when this occurs further expression of ringspot symptoms is suppressed. In one instance, droopy necrosis appeared in a small planting of papaya which showed no symptoms of ringspot. Symptoms and development of the disease was the same as on ringspot-affected plants.

Light Microscopy

Nuclear inclusions which stained dark green were observed in the phloem parenchyma cells of infected papaya leaves (Fig. 3-A). Such nuclear inclusions were not found in epidermal or mesophyll cells of infected plants or in the cells of healthy papaya leaves.

Electron Microscopy

In ultrathin sections of diseased papaya leaves, aggregates of bacilliform virus particles were observed in the nucleoplasm of the phloem parenchyma calls (Fig. 3-B, 3-C). Infected nuclei were readily distinguishable from healthy ones by the presence of aggregates of virus particles. Virus particles were not enclosed in a membrane structure. Virus particles measuring 87-98 x 180-254 nm were seen not only in nuclei but also occasionally in the cytoplasm. These particles closely resembled those described for other rhabdoviruses (9, 12). In some cases, together with bacilliform particles, scrolls and pinwheels were observed in the cytoplasm. These structures are presumed to be induced by papaya ringspot virus (23). Neither pinwheels nor bacilliform particles were observed in ultrathin sections of healthy papaya leaves, however.

Transmission Study

No symptoms were observed in plants of *N. edwardsonii* or *Sonchus* sp. mechanically inoculated with diseased papaya extracts. Inoculated papaya plants, however, showed mottle and distortion symptoms on leaves, typical of those induced by papaya ringspot (5). Symptoms of papaya droopy necrosis were not observed in these plants, and thus these papaya seedlings are assumed to have been singly infected with papaya ringspot virus, which is readily transmitted mechanically (5). When SYNV was used as inoculum, however, *N. edwardsonii* and *Sonchus* sp., but not papaya, developed symptoms as previously described for this virus (3).

Discussion

This is the first published report of a rhabdovirus-like

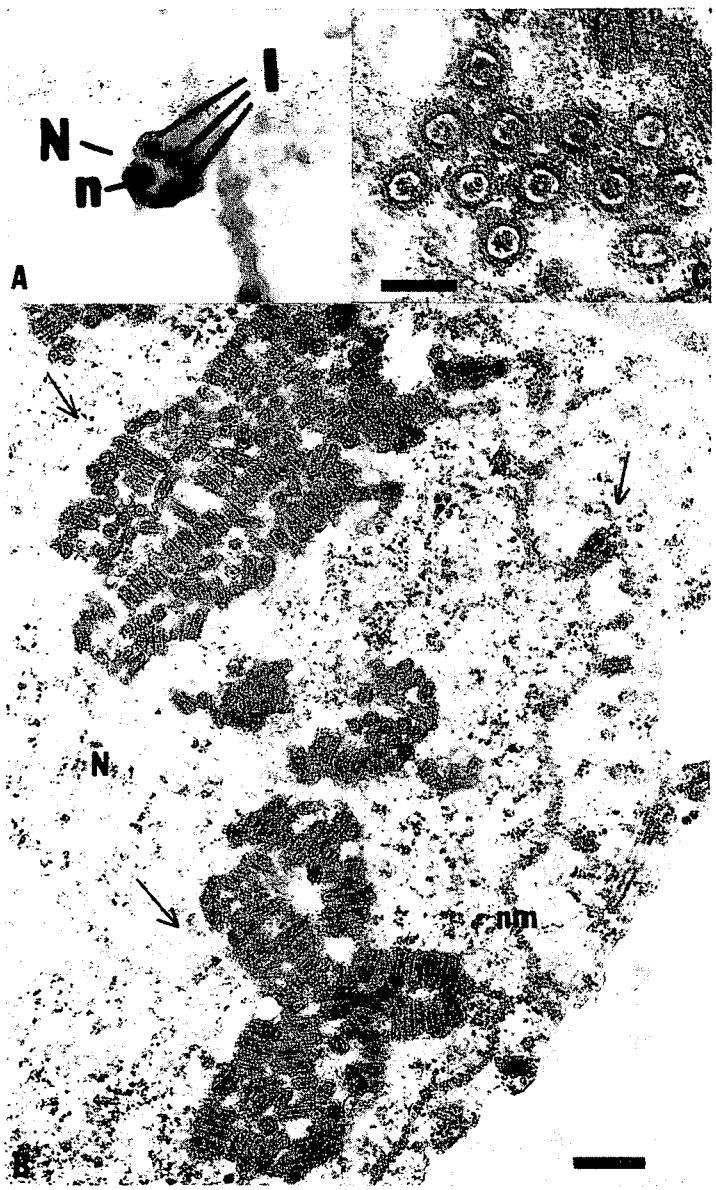

Fig. 3. Light and electron micrographs of rhabdovirus particles associated with droopy necrosis. A. Light micrograph of thin section of rhabdovirus-infected papaya leaf stained with calcomine orange and Luxol brilliant green. x 1,800. B. Electron micrograph of ultrathin section. Aggregates of bacilliform virus particles (see arrows) in nucleus. Scale bar = 360 nm. C. Electron micrograph of ultrathin section showing virus particles in cross section. Scale bar = 100 nm. N = nucleus, n = nucleolus, nm = nuclear membrane, I = virus inclusion bodies.

agent infecting papaya in Florida. In 1979, J. R. Edwardson (unpublished data) also found bacilliform virus particles in ultrathin sections of leaves of papaya affected by droopy necrosis. A rhabdovirus has been reported to be the cause of the apical necrosis disease of papayas in Venezuela (13). Whether or not the Venezuelan and Florida rhabdoviruses are the same has not been resolved. Particle sizes of the two are similar. Symptomatology of the two diseases is similar in some respects but differs in others. Neither virus was transmitted mechanically.

Sonchus yellow net virus is the only rhabdovirus previously reported from Florida (3). However, this virus, which may be transmitted mechanically, failed to infect papaya in experiments where it was transmitted to *Sonchus* sp. and *N. edwardsonii*. In parallel experiments the papaya droopy necrosis virus was not transmitted to any test plant. Thus it is concluded that these two rhabdoviruses are distinct.

The papaya leafhopper, *Empoasca papayae* Oman, was reported to be the vector of the Venezuelan rhabdovirus (13). No bacilliform virus particles, however, were observed in the papaya test plants inoculated with this vector although the test plants did develop typical symptoms of apical necrosis.

Empoasca papayae, which also transmits papaya bunchy top (1, 19, 21) is not known to occur in Florida (14, 15). However, the vector of the papaya droopy necrosis virus remains unknown. Based on the relative high numbers of bacilliform particles in the phloem of diseased papaya, it is likely that a phloem-feeding vector is involved, but whether the vector is an aphid (Aphididae), leafhopper (Cicadellidae) or planthopper (Fulgoridae) remains unresolved. Representatives of each of these insect taxa are reported as vectors of specific rhabdoviruses, and transmit them in a circulative manner (9, 12).

Surveys indicate a very high incidence of ringspot in Florida papayas. In contrast incidence of droopy necrosis is quite low although a few infected trees are seen in most year-old plantings in the Homestead area. Droopy necrosis is a lethal disease during winter, thus if its incidence increases significantly in future years, it could seriously affect papaya production in Florida. Since neither an alternative host nor a vector of the virus has been identified, the best control solution would be a program of eradication whenever diseased trees are found. At AREC Homestead droopy necrosis has increased to damaging levels when there was an overlap of old and new plantings. Providing for a distinct break between successive crops coupled with rogueing all suspicious plants has kept the disease at a low level for the past 3 years.

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POPULATION FLUCTUATIONS OF PLANT-PARASITIC NEMATODES ON BANANAS IN FLORIDA[1]

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Abstract. **Population levels of plant-parasitic nematodes in soil and roots were monitored for one year in a commercial banana (Musa x 'Burro') planting in south Florida during 1979-80. Common plant parasites included** Helicotylenchus dihystera **(Cobb) Sher,** H. multicinctus **(Cobb) Golden, and** Meloidogyne incognita **(Kofoid & White) Chitwood. Soil populations of** Helicotylenchus **spp. and** M. incognita **showed peaks in Oct.-Dec., which corresponded to the end of the rainy season. Treatment of individual mats with ethoprop at either 6.0 g ai/mat every 6 months or 3.0 g ai/mat every 3 months failed to significantly reduce populations of either nematode genus in soil or roots compared to untreated controls.**

Many different species of plant-parasitic nematodes are associated with bananas and plantains throughout the world, and several of these may adversely affect crop growth (2, 13, 17). As commercial production of bananas and plantains increases in Florida, knowledge of the nematode fauna and its control is essential. The burrowing nematode, *Radopholus similis* (Cobb) Thorne, has been reported from bananas in Florida (4, 8, 11), but in south Florida, the presence of high numbers of other nematode species, particularly *Helicotylenchus multicinctus* (Cobb) Golden, may constitute a more serious threat to commercial production (11). *H. multicinctus* has also been found to be the predominant pest in Cuba (15), Israel (12), Argentina (7), and South Africa (10). For these reasons, it is necessary that the biology and control of these nematodes be better understood.

The present study examines the efficacy of nematode control by ethoprop (Mocap® 10G), the only nematicide currently registered for postplant nematode control on bananas and plantains in Florida. In addition, the seasonal population fluctuations of the more common nematodes associated with bananas in south Florida are investigated.

Materials and Methods

This experiment was conducted in a planting of the banana cultivar *Musa* x 'Burro' (ABB Group), also known as *Musa* x 'Bluggoe' (14). The site was located several miles west of Florida City, Florida, on a Rockdale fine sandy loam soil with pH = 7.6. The bananas had been planted in March 1978, and the first treatments were applied on February 1, 1979. The 6 treatments were as follows:

1) ethoprop at 6.0 g ai/mat
2) ethoprop at 3.0 g ai/mat
3) iron chelate at 85 g/mat
4) ethoprop at 6.0 g ai/mat + iron chelate at 85 g/mat
5) ethoprop at 3.0 g ai/mat + iron chelate at 85 g/mat
6) untreated control

Ethoprop, formulated as Mocap® 10G, was applied within an 0.5 m radius around the base of each mat and lightly incorporated into the top 2-4 cm of soil. The 6.0 g ai treatment of ethoprop was reapplied every 6 months, while the 3.0 g ai treatment was reapplied every 3 months. The iron chelate consisted of Sequestrene 138 Fe Iron Chelate® applied in 13.2 liters of water to the surface of the soil around each mat. This treatment was reapplied in 6 month intervals. The iron chelate was included as a treatment to determine if plant yields would be further enhanced by adding a critical micronutrient in combination with the nematicide. The 6 treatments were applied to individual mats arranged in a randomized complete block design with 6 replications.

Soil and root samples for nematode analysis were collected on February 1, 1979, and at two-month intervals thereafter until April 7, 1980. An individual sample consisted of approximately 1000 cm^3 of soil and 10-20 g of roots taken from a single mat. Each composite sample was obtained from 3 locations around the mat with a hand trowel to a depth of 15 cm. Soil samples were passed through a 4 mm sieve to remove rock, and a 100 cm^3 subsample was then processed by decanting and sieving followed by suspension of the residues in modified Baermann funnels (1, 6). Roots from each sample were washed and cut into 2-cm segments and mixed. A 5 g subsample of fresh roots was placed in 200 ml of water in a 250 ml beaker after counting the root knot galls. A gentle stream of air was passed through the water, and after 7 days at 24°C, the nematodes that had emerged from the roots were counted.

Yield data were collected between September 6, 1979, and March 13, 1980, and the cumulative yield per mat over that time period was determined. Nematode and yield data were analyzed by analysis of variance and Duncan's new multiple range test.

Results and Discussion

The most common plant-parasitic nematodes found in soil samples at the study site were *Meloidogyne incognita* (Kofoid & White) Chitwood, *Helicotylenchus multicinctus,* and *H. dihystera* (Cobb) Sher, and the first two were also isolated regularly from root samples. Roots exhibited galling from *M. incognita* and the cortical lesions attributed

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