Occurrence of Sphaeropsis knot on citron (*Citrus medica* L.) in Puerto Rico[1]

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ABSTRACT

Citron (*Citrus medica* L.) stems and branches showing numerous round to elongated knots were brought to the laboratory for diagnosis. Microscopic studies of dried small knots revealed the presence of picnidia bearing conidia typical of the fungus *Sphaeropsis tumefaciens* Hedges. Original disease symptoms were reproduced through pathogenicity trials conducted in the greenhouse.

INTRODUCTION

Citron (*Citrus medica* L.) is cultivated in the humid mountainous region of the island, specifically at the municipality of Adjuntas. Almost all the farmers in that area are engaged in the commercial production of this fruit. In Puerto Rico, there are two processors, who supply U.S.A. and European markets with processed fruit. In 1977, a new disease, which represents a serious threat to the local citron industry was detected in one of the commercial orchards in that area. This malady is characterized by the presence of round to elongated deformations along the stems and branches of the affected trees, ranging from 2.5 to 13.0 cm in length and 1.9 to 21.3 cm in circumference (fig. 1). The knots, when young are smooth surfaced, but as they grow older their external tissues become necrotic and split off, debilitating affected areas and ultimately causing ruptures and breakage of the branches. Some knots become dark in appearance because of the presence of mycelium (fig. 1). Loss of apical dominance due to infection usually brings about a witches broom condition and in severe cases die-back of affected limbs is commonplace (fig. 1). Occasionally canker lesions occur in association with the knots (fig. 1).

Since this disease constitutes a new record for Puerto Rico, a detailed study of the malady and its etiology was undertaken. Results of these initial studies are reported herein.

MATERIALS AND METHODS

Field infected material was brought to the laboratory for isolation of the causal agent. Knots were thoroughly washed with a mixture of Alconox and Lysol, rinsed in tap water and then immersed for 5 min. in

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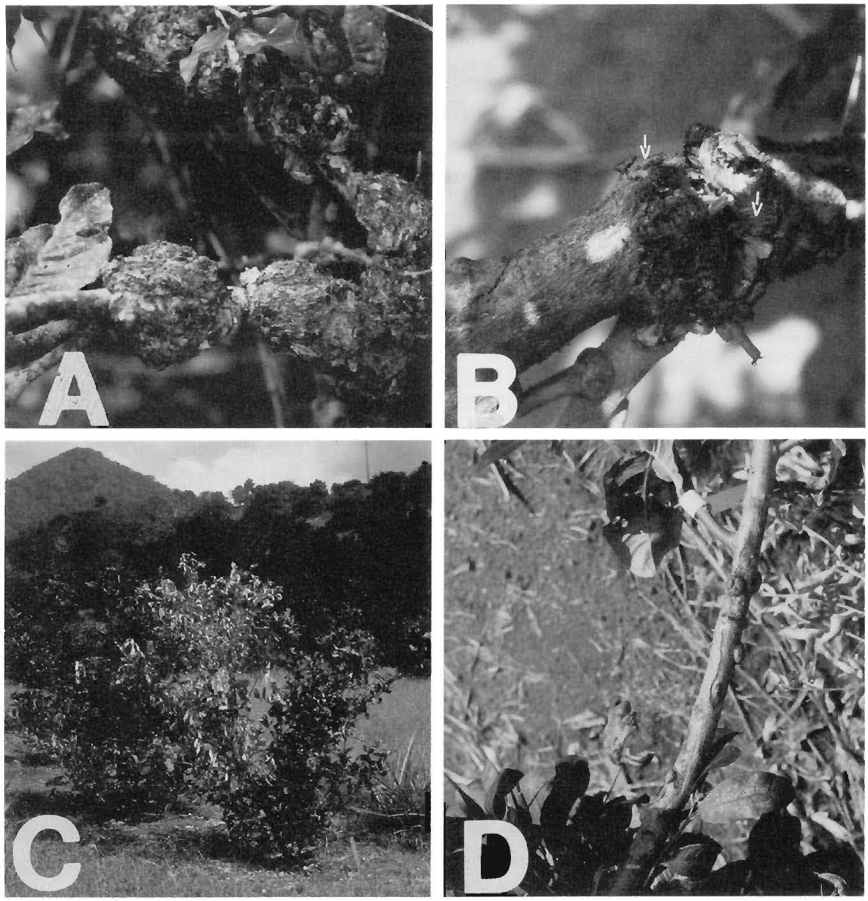

FIG. 1.—Symptoms of the disease caused by *S. tumefaciens*. A) Knots; B) Breakage of the branch at the knot area (darkening of the woody interior is evident); C) Die-back; D) Cankers associated with the knots.

70% ethyl alcohol. Superficial tissues were excised and discarded, leaving the woody interior for the intended isolations. Several pieces were cut, one-half of them were disinfested in 10% Clorox for 2 min. and the other half remained untreated. Material thus prepared was plated on PDA and incubated at 28° C for five days. The fungal colonies recovered were purified and tested on small citron trees.

Plants (presumably disease-free) obtained from a nursery at Adjuntas were brought to the greenhouse and kept under observation for 5 months to make sure that they were free of infection. After this quarantine

period, they were propagated by cuttings to maintain an adequate healthy population to be used throughout the trials.

Cultures of the fungus grown on PDA for 8 days at 28° C were mixed with Triton B-1956 (0.06% at the rate of 100 cm^3/plate), and homogenized in a blender for 5 min. Stems and branches of five test plants were sprayed with the inoculum thus prepared and covered with plastic bags for 48 h, at the end of which time the bags were removed and the potted plants kept on greenhouse benches under sprinkler irrigation. Checks were treated similarly except that they were sprayed with sterile PDA. Plants thus treated were observed daily for the appearance of symptoms.

RESULTS AND DISCUSSION

Pure colonies of a fungus with dark mycelium were obtained from diseased tissue when plated on PDA. These colonies were whitish when young, but turned dark with age. The formation of structures resembling fruiting bodies was observed but they appeared devoid of conidia. Due to failure of the fungus to produce fertile picnidia in vitro, pure cultures of the organism were forwarded to the Commonwealth Mycological Institute (CMI) for identification. Since the fungus always appeared in strict association with the diseased tissues, pathogenicity trials were conducted with several of these pure isolates. In all cases formation of the first knots were observed 1 month after inoculation. Pure colonies of a fungus very similar to that inoculated were recovered from pieces of the knots after plating in PDA. Some of the cultures were preserved in mineral oil.

Meanwhile microscopic examinations of small dried knots obtained from field infected material showed a large number of picnidia scattered throughout their surfaces. The picnidia are aggregate, subglobose, ostiolate and dark (fig. 2). Detailed observations of these fruiting bodies revealed the presence of macroconidia rounded at the apex and truncate at the base (22.0 to 31.9μ long × 5.5 to 6.6μ wide). The presence of microconidia associated with the macroconidia was also detected (fig. 2). These characteristics corresponded to those of the fungus *Sphaeropsis tumefaciens* Hedges, as described by Holliday and Punithalingam (5). Subsequently Dr. E. Punithalingam from CMI identified the fungal cultures as *Sphaeropsis tumefaciens* Hedges, thus corroborating our identification.

After approximately 1 year, the cultures preserved in mineral oil were transferred to PDA and active sporulation took place. Inoculations with these fertile cultures have proved almost 100% effective in all trials conducted. The capacity of our isolates to sporulate and maintain their virulence apparently is not affected by subculturing. When first isolated from fresh material, this fungus failed to sporulate; seemingly it requires

an aging treatment to complete its asexual reproduction. This is in agreement with Hedges' (4) findings, who reported that the orange strain of *S. tumefaciens* was sporulating after several months of growth in cornmeal flasks.

Sphaeropsis tumefaciens causes a severe disease on citron in Puerto Rico. This fungus was first described by Hedges from limes and orange orchards in Jamaica (4), where the disease is a limiting factor to the successful production of limes. Although in Puerto Rico, we have observed this disease only on citron, in Jamaica it has been detected frequently in other *Citrus* spp. such as rough lemon and sporadically in sweet oranges, tangerines and orthaniques (10). Blazquez et al. (2) reported that in five

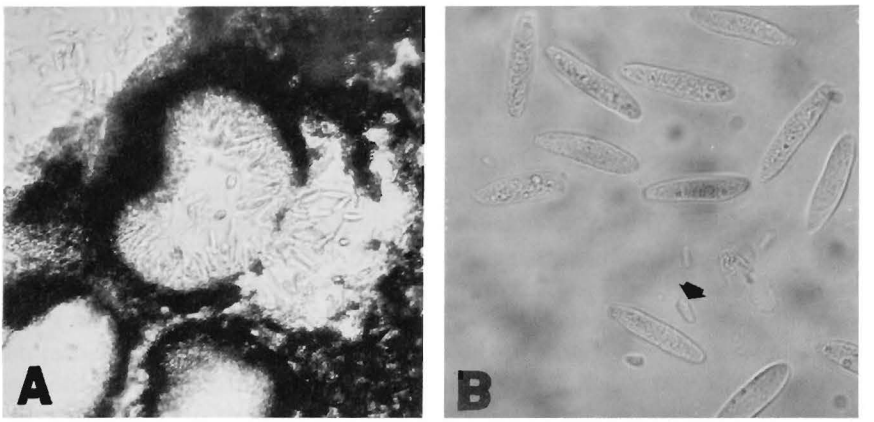

FIG. 2.—Section through the cortex of small dried knots. A) Picnidia; B) Macro and microconidia.

localities in Jamaica, the disease was very serious on 4-year old Valencia trees budded on rough lemon. They estimated it had killed approximately 40% of the trees because of severe rootstock infection. In addition, this pathogen has been recorded on limes from southern Perú (1) and India (11). In Florida it is not considered an important disease because of the low number of the cases reported (3). Curiously, in the Cameroons, where the disease was detected in Seville orange trees, no such knots occurred in the specimens observed but only numerous fruiting bodies bearing conidia (9).

The host range of *S. tumefaciens* is not limited to *Citrus* spp.; it has been reported as the causal organism of galls in *Callistemon viminalis* (6) and *Schinus terebinthifolius* (7) and of witches' broom of *Ilex* spp. (8) and *Nerium oleander* (12).

RESUMEN

En troncos y ramas del cidro (*Citrus medica* L.) aparecieron numerosos nudos entre redondos y alargados. Pedazos secos de dichos nudos vistos al microscopio revelaron la presencia de picnidios con conidios típicos del hongo *Sphaeropsis tumefaciens* Hedges. En ensayos patogénicos realizados en invernaderos se reprodujeron síntomas iguales a la afección original.

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