## Sphaeropsis Gall of Bottlebrush Tree, Callistemon viminalis, a New Host

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The assistance of Floyd J. McHenry and Eva Pryor is acknowledged.

Journal Series Paper No. 5098.

Accepted for publication 21 March 1974.

## ABSTRACT

During the past decade a gall disease of bottlebrush tree, Callistemon viminalis, has occurred in Florida. The fungus, Sphaeropsis tumefaciens, was isolated from the galls and found to be the causal organism. The fungus is a wound

invader requiring 21 to 105 days to induce symptoms. Galling can be sufficiently severe to kill branches.

Phytopathology 64:1001-1003.

Additional key words: canker, witches' broom.

Bottlebrush, Callistemon sp. is a popular ornamental tree in subtropical areas of the United States. During the past decade stem galls have occasionally been found on this tree in Florida. The disease is rarely found in nurseries, but it appears in mature plants.

Galls have a very irregular surface, with deep indentations between knobby swellings and often appear darker than the normal bark. Occasionally canker-like lesions developed instead of galls (Fig. 1-C). The central part of a canker was sunken to the stele and its bark flaked off easily. The margins were swollen and extended beyond the normal width of the branch upon which they were found. Small, black pycnidia occasionally occurred on the dead bark.

Isolations from stem galls and cankers consistently yielded, on potato-dextrose agar, Sphaeropsis tumefaciens Hedges (5, 7). This fungus also causes galls on citrus (1, 2, 3, 4, 9, 10, 11). This paper reports a new host, Callistemon viminalis G. Don. of S. tumefaciens.

MATERIALS AND METHODS.—Callistemon viminalis, Weeping Bottlebrush, was selected for inoculation tests. Small plants (1.0 - 1.5 m tall) of three cultivars, 'ESL', '75', and 'Jenifer Dunaway', were obtained from nurseries in Dade County, Florida. Branches on four trees of each cultivar were treated as follows: (i) a 0.5-cm square of a culture grown in a petri plate on (PDA) potato-dextrose agar was taped onto a shallow stem abrasion with grafting tape; (ii) the same as treatment number one except, in an effort to prolong the viability of the inoculum, a piece of moist gauze was placed over the inoculum before taping; (iii) the same as treatment number one, except no stem abrasion was made; and (iv) the same as treatment number one, except sterile PDA was used as control. All plants were maintained outdoors in 5-gal cans containing equal proportions of screened rock soil, peatmoss, and perlite.

Sphaeropsis tumefaciens was grown on PDA or steamsterilized bottlebrush stems (3-4 mm diam × 5.0 cm long) in petri plates with moistened filter paper. Pycnidia and conidia were produced on both media when cultures were kept under continuous fluorescent lights for ca. 3 wk. Thirty-eight pycnidia and 238 conidia were measured under a microscope from cultures on the bottlebrush stems. Mycelial growth rates of three isolates at five different temp, 20, 24, 28, 32, and 36 C, were determined on PDA in petri plates by measuring colony diam.

Isolations from gall tissue were made by surface sterilizing the gall surface with flaming 95% ethanol, and plating tissue from beneath the epidermis on PDA.

Gall development was estimated by rating each gall by odd integers (1 through 9). A rating of 1 indicated the diam of a swelling was over 1 mm thicker than the diam of the branch adjacent and distal to the wound. A rating of 9 indicated the gall had a diam at least 8 mm greater than the branch's normal diam. Height (along the branch axis), width, and thickness (measured at a right angle to the stem axis) of the galls were also measured.

RESULTS.—Gall development and sizes were recorded only for the 75 and Jenifer Dunaway cultivars. Data were not recorded for ESL cultivar since, with no inoculation, it naturally developed deep cracks in the stem. These cracks could not be easily differentiated from cankers due to infection.

Approximately 28 days after inoculation, callus tissue formed around the abrasions as wounds normally healed (8). After 35 days, three abrasions showed abnormally large amounts of swollen cortex which later proved to be early gall formation (Fig. 1-A). After 63 days, 11 incipient galls developed and a maximum of 16 galls were formed after 105 days on eight plants.

Gall size increased rapidly between 42 and 126 days after inoculation. Further incubation to 294 days, resulted in a decreasing rate of gall enlargement. Wounding of the stems prior to inoculation resulted in 15 galls, while only one gall formed without a wound at the inoculation site. The use of moist gauze did not enhance gall formation. Controls developed no galls.

After 105 days, the average distance along a branch axis (height) of an experimentally induced gall was 17.0 mm, with a width of 11.0 mm, and thickness of 11.2 mm. The thickness measurement can be compared to an average branch thickness near the gall of 7.4 mm. Galls on naturally infected trees have been observed to vary from 1-9 cm and older galls develop a witches' broom effect (Fig. 1-B, D). Such galls may occur in a series along a branch.

S. tumefaciens was isolated readily from gall tissue. Cultures on PDA or steamed bottlebrush stems formed pycnidia and conidia within 3 wk under continuous fluorescent lights. One-celled microconidia (mean  $4.6 \times$ 

2.3  $\mu$ m) were formed readily in the pycnidia. Macroconidia, formed later with the microconidia in the pycnidia (Fig. 1-E, F) consisted of single cells, were subhyaline, ellipsoid and rounded on one end; they

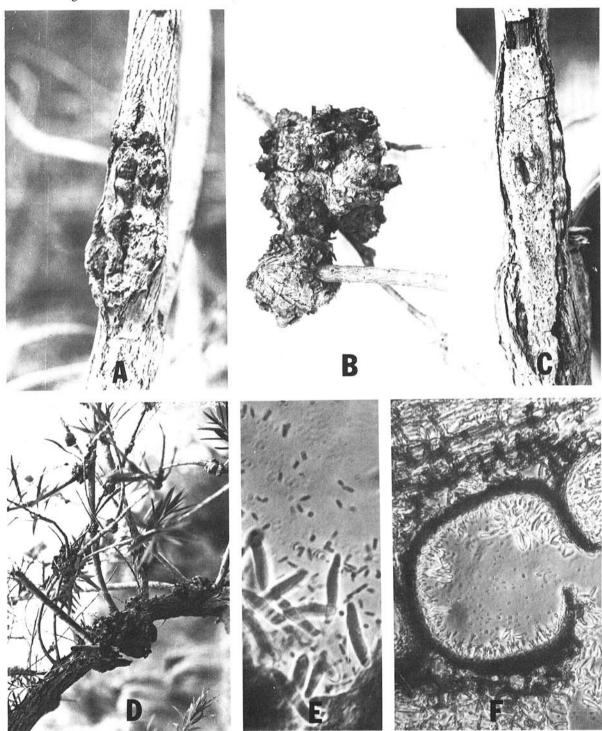


Fig. 1-(A to F). Galling of Callistemon viminalis caused by Sphaeropsis tumefaciens. A) Gall of C. viminalis following inoculation. B, D) Naturally occurring galls. C) Canker following inoculation. E) Macroconidia and microconidia of S. tumefaciens. F) Pycnidium and conidia of S. tumefaciens.

measured  $25.9 \times 5.75~\mu m$  (mean of 50 conidia) for one isolate and  $25.5 \times 4.47~\mu m$  for another isolate. Macroconidia germinated readily in distilled water within 24 h. Some macroconidia formed a single septum during germination. No germination of microconidia was observed.

Pycnidia were globose and 221.1  $\mu$ m in diam, and the pycnidial wall was approximately 10-20  $\mu$ m thick. Pycnidia were formed in a stroma on PDA and were scattered or in a stroma on steam sterilized bottlebrush stems. Pycnidia were found in relatively small numbers on galls of naturally infected branches. The conidia and pycnidia most clearly fit the description of *S. tumefaciens* Hedges (5, 6, 7).

Mycelial growth by three isolates of *S. tumefaciens* at temp of 20, 24, 28, 32, and 36 C showed isolates 658 and 1135 grew optimally at 32 C. Isolate 1147 showed increased mycelial growth at 36 C. The mycelium of young cultures was at first hyaline, but within 2-3 days, a dark pigment developed. The pigment developed most rapidly at 28 C.

## LITERATURE CITED

 ABBOT, E. V. 1929. Diseases of economic plants in Peru. Phytopathology 19:645-656.

- BLAZQUEZ, C. H., and P. HUNT. 1967. Sphaeropsis knot, a disease of rough lemon rootstock. Proc. Fla. State Hortic. Soc. 80:46-52.
- BLAZQUEZ, C. H., A. G. NAYLOR, and D. HASTINGS. 1966. Sphaeropsis knot of lime. Proc. Fla. State Hortic. Soc. 79:344-350.
- FAWCETT, H. S., and H. A. LEE. 1926. Citrus diseases and their control. McGraw-Hill, New York. 582 p.
- HEDGES, F. 1911. Sphaeropsis tumefaciens, nov sp., the cause of the lime and orange knot. Phytopathology 1:63-65.
- HEDGES, F., and L. S. TENNY. 1912. A knot of citrus trees caused by Sphaeropsis tumefaciens. U.S. Dep. Agric. Bull. 247. 74 p.
- HOLLIDAY, P., and E. PUNITHALINGAM. 1970. Sphaeropsis tumefaciens. Descriptions of Pathogenic Fungi and Bacteria No. 278. Commonw. Mycol. Inst., Kew, Surrey, England.
- KAUFERT, F. 1937. Factors influencing the formation of periderm in aspen. Am. J. Bot. 24:24-30.
- KNORR, L. C., R. F. SUIT, and E. P. DUCHARME. 1957. Handbook of citrus diseases in Florida. Fla. Agr. Exp. Sta. Bull. 587. 157 p.
- MOREAU, C. 1947. Two diseases of Citrus bigaradia Risso in the Cameroons. Rev. Mycologie Suppl. Colon xii (3):84-86
- STEVENS, H. E. 1918. Florida citrus diseases. Fla. Agr. Exp. Sta. Bull. 150:87-90.