Acremonium Leaf Spot of *Syngonium*: Nomenclature of the Causal Organism and Chemical Control

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ABSTRACT

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Acremonium crotocinigenum was determined to be the cause of an important foliar disease of *Syngonium podophyllum* characterized by small, reddish-brown leaf spots that sometimes coalesced to a leaf blight. Characterization, identification, pathogenicity, and nomenclature of the pathogen are discussed. Of four fungicides, anilazine was the most effective in controlling the pathogen.

Additional key words: *Cephalosporium cinnamomeum*

During our studies of Ceratocystis blight of *Syngonium podophyllum* Schott (6), we frequently encountered a severe foliar disease characterized by bright reddish-brown leaf spots. Many of the spots were small (1–2 mm diameter), discrete, red lesions surrounded by a chlorotic halo. On severely diseased leaves, spots expanded and coalesced, resulting in extensive leaf blighting and yellowing around the necrotic area.

The most frequent and consistent organism isolated was a *Cephalosporium*-like fungus. A similar leaf-spotting disease caused by *Cephalosporium cinnamomeum* Linn was first reported from New York in 1940 (3). The disease was later reported to be a common, persistent, and troublesome problem in Florida (1) and was recently found to occur in California (4).

Preliminary microscopic examination of isolates from leaves of *S. podophyllum* revealed that the shape and dimensions of conidia and development of chlamydospores conformed to the description for *C. cinnamomeum*. However, the cinnamon-buff color that *C. cinnamomeum* develops on potato-dextrose agar (PDA) was not observed with our isolates.

In his treatment of *Cephalosporium*-like fungi, Gams (2) placed *C. cinnamomeum* among the excluded or insufficiently known species, stating that it was without a Latin description. Because a legitimate name for the leaf-spotting fungus of *Syngonium* was apparently unavailable, we undertook to identify the fungus, verify its pathogenicity to *S. podophyllum*, and develop control measures for this important disease.

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MATERIALS AND METHODS

Infected leaves of *S. podophyllum* 'Green Gold' with characteristic reddish brown, irregular to circular, flecks to small necrotic spots (0.5–2 mm diameter) surrounded by chlorotic zones were obtained from a commercial nursery. Thirty-eight tiny lesions were dissected out, dipped into 0.25% sodium hypochlorite for ca. 5 sec, then plated on water agar. Eighty-one percent of the specimen pieces were associated with a small-spored *Cephalosporium*-like fungus. The remaining pieces produced no organisms. Five isolates were randomly selected and compared. Little variation was observed, and two single-conidial isolates 902 and 903 were used in further studies.

For pathogenicity studies, conidia of isolate 902 were grown in petri dishes on V-8 juice agar (100 ml of Campbell's V-8 juice, 2 g of calcium carbonate, 15 g of agar, and 900 ml of deionized water) at 25 C under continuous, cool-white fluorescent light (2,200 lux) for 6 days. Inoculum was prepared by washing the conidia from the surface of the plates with Tween 20 (1:2,000) in deionized water and adjusting the concentration to 10^6 conidia per milliliter.

Rooted cuttings of popular cultivars Green Gold and Noack White were used in pathogenicity as well as fungicidal control studies. Inoculum was applied by spraying the leaves until runoff, followed by incubation of the plants in a moisture-saturated atmosphere for 24 hr. The plants were then returned to the greenhouse bench for symptom development. Data on disease development were collected 9 days after inoculation. Reisolation from resulting lesions was also undertaken at this time.

Characterization of test isolates 902 and 903 from *S. podophyllum* closely followed Gams' procedures (2). Cultures were grown on a) malt agar (1 g of malt extract, 0.25 g of peptone, 1.5 g of agar, and 100 ml of deionized water); b) oatmeal agar (7.2 g of oatmeal agar, 0.5 ml of glycerine, 0.05 ml of 85% lactic acid, 0.3 g of agar, and 100 ml of deionized water); and c) Sabouraud glucose agar (4 g of glucose, 1 g of peptone, 1.5 g of agar, and 100 ml of deionized water). The cultures were also grown on PDA. Growth rates and dimensions of conidia and conidiophores were measured after 10 days at 20 C in continuous darkness. Observations for chlamydospore production were made after two more days of incubation at 20 C but with continuous, cool-white fluorescent light at ca. 2,200 lux.

Mancozeb (Dithane M-45), anilazine (Dyrene), triadimefon (Bayleton), and glycophene (Rovral) were selected for chemical control studies. For in vitro testing, these fungicides were incorporated in a 2% V-8 juice agar (20 ml of Campbell's V-8 juice, 15 g of agar, and 980 ml of deionized water) at final concentrations for active ingredients of 1, 10, and 100 μg/ml for each fungicide. Fungicides were added to autoclaved, 2% V-8 juice agar cooled to 50 C and thoroughly mixed with the fungicides before dispensing into petri dishes. Test isolate 902 was grown as previously described for 5 days on V-8 juice agar to produce conidial inoculum. Plates containing test fungicides were inoculated by spreading masses of conidia to cover a 2- to 3-mm circle in the center of the test plates using a sterile wire loop. Inoculated plates were then incubated at 24 C in continuous darkness for 5 days before radial growth measurements were made.

Anilazine and triadimefon, which effectively reduced radial growth in plate tests, were used for disease control studies. Anilazine was applied at 60, 120, and 240 g a.i./100 L, and triadimefon was applied at 7.5, 15, and 30 g a.i./100 L to cultivars Green Gold and Noack White. One week after application of fungicide to leaves, test plants were inoculated as described above, and data on disease

control were collected 9 days later. All tests were repeated twice.

RESULTS

Numerous reddish brown lesions, frequently along the midrib and irregular to circular in outline, were observed on Green Gold and Noack White 3 days after inoculation. Chlorosis around lesions was not noticeable at this time. Seven days after inoculation, lesions expanded from approximately 0.5 to 3 mm in diameter (Fig. 1A). A few of the larger spots had raised, blisterlike areas. Wide chlorotic zones were conspicuous around the larger lesions and coalescing spots 7 days after inoculation. Broad translucent areas around clusters of lesions, especially in older leaves, were also a characteristic of this disease (Fig. 1B). Leaves with numerous lesions turned completely yellow after this period. Reisolations from the resulting lesions yielded only *Acremonium* sp.

Colonies grew rapidly on all experimental media, attaining diameters of 42–44 mm on Sabouraud glucose agar, 48–50 mm on oatmeal agar, and 50 mm on malt agar after 10 days at 20 C in continuous darkness. No cinnamon color developed in colonies on PDA, and the colony reverse color was white to pale orange yellow or buff on Sabouraud glucose agar. Hyphae were mostly 2.5–3.0 μm; some were up to 5 μm in diameter. Conidiophores were up to 180 μm in length, multiseptate, occasionally branched, with some of these branches longer than the primary stalk. The basal cells of some of the conidiophores were thick walled. The phialides were often wavy, with tapered to narrow apexes and short collarettes.

Conidia were mostly single celled, but two-celled spores were not uncommon.

Dimensions of conidia from isolate 902 were $8.1 \pm 2.2 \times 2.5 \pm 0.6$ μm, with a length:diameter ratio of 3.3 ± 0.6; dimensions of conidia from isolate 903 were $8.8 \pm 2.4 \times 2.4 \pm 0.6$ μm, with length:diameter ratio of 3.6 ± 0.6. Numerous chlamydospores were formed on oatmeal agar, either singly or in short chains of up to nine in number, and a few clusters were also observed. Chlamydospores were hyaline to light yellow, mostly intercalary, nearly spherical to ellipsoidal, 6–7 μm in diameter, and smooth walled. Only a few chlamydospores were formed on malt agar. These characteristics fit the description for *A. crotocinigenum* (Schol-Schwarz) W. Gams.

Evaluations of mancozeb, glycophene, triadimefon, and anilazine in vitro revealed anilazine and triadimefon to be promising, with ED_{50} values for restricting vegetative growth at less than 10 μg/ml a.i.; the ED_{50} values of mancozeb and glycophene were greater than 10 μg/ml and 100 μg/ml, respectively.

Results of both trials on Green Gold and a single test with Noack White were similar. Control of the disease with anilazine was highly significant ($P = 0.01$) at 60, 120, and 240 g a.i./100 L. For triadimefon, significant control ($P = 0.05$) was obtained only with the highest rate of 30 g a.i./100 L. No phytotoxic effects were observed at the rates tested.

DISCUSSION

A. crotocinigenum is distinguished from other species of *Acremonium* by its rapid growth, extremely long (over 125 μm), multiseptate conidiophores, and production of chlamydospores (2). Isolates in the present studies grew faster than the rate given by Gams (2) but appeared to be slower than growth measured by Schol-Schwarz (5); however, information on incubation temperatures were not provided. Long (up to 180 μm), multiseptate conidiophores with several lateral phialides extending beyond the central phialide, and the ready development of hyaline chlamydospores in our isolates, fit the description of *A. crotocinigenum* given by Gams (2) and Schol-Schwarz (5). Therefore, we concluded that the fungus causing reddish brown lesions on leaves of *S. podophyllum* was *A. crotocinigenum*.

With the exception of one characteristic, Linn's (3) original isolate from *S. podophyllum*, described as *C. cinnamomeum*, resembled that of *A. crotocinigenum*, as described by Gams

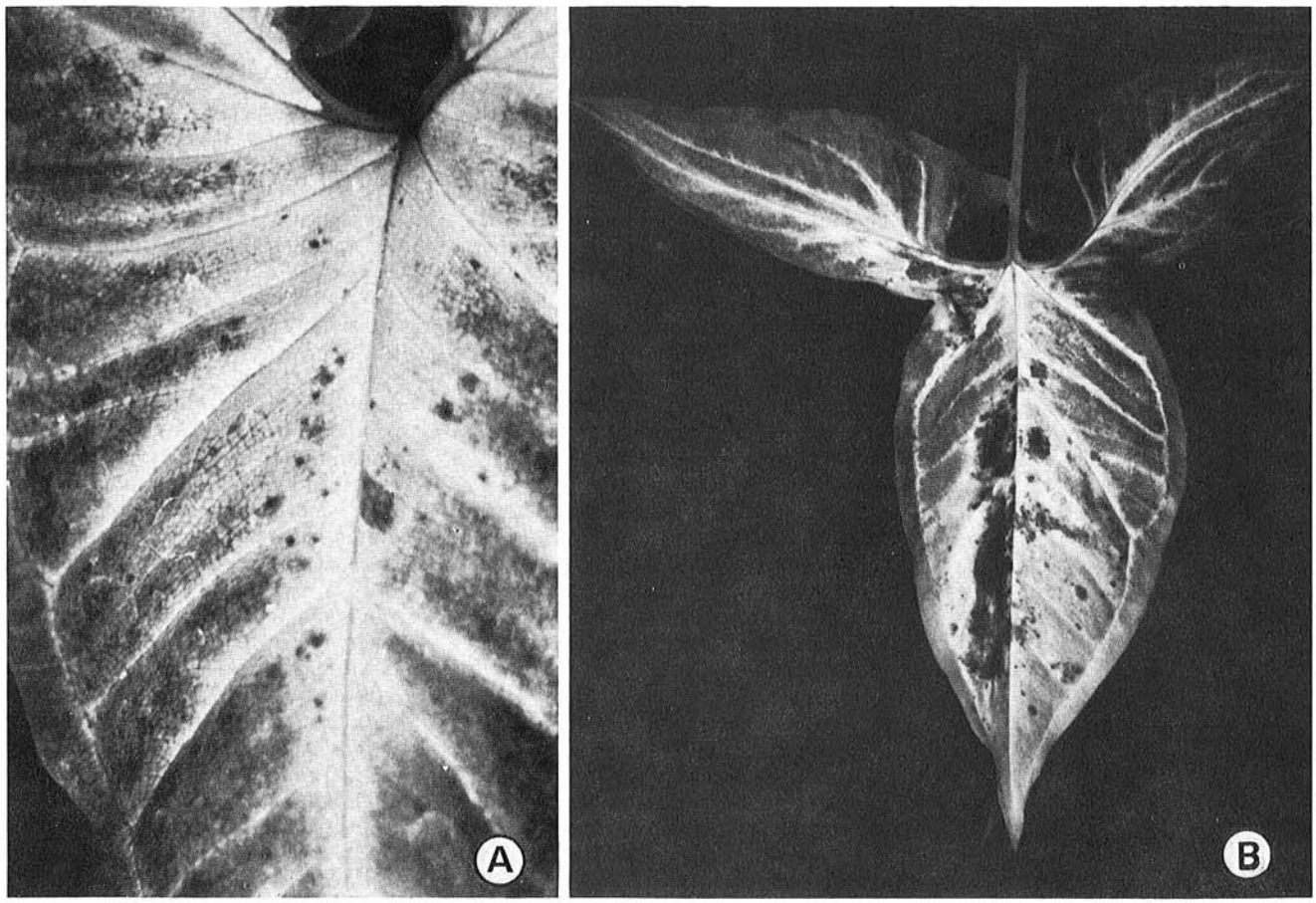

Fig. 1. Acremonium leaf spot of *Syngonium podophyllum*: **(A)** Close-up of flecks and spots. **(B)** Blight caused by *Acremonium crotocinigenum*.

(2) and Schol-Schwarz (5). This one characteristic was the observation of one to four septate, cylindric conidia on decumbent conidiophores. Gams (2) also noted septate conidia in the type specimen for *C. cinnamomeum*, but attributed them to conidia of *Cylindrocarpon* Wollenweber 1913. Conceivably, Linn's (3) multiseptate, cylindric conidia were similar to those that Gams (2) attributed to *Cylindrocarpon*. We have thus concluded that the *C. cinnamomeum* described by Linn (3) is probably conspecific with *A. crotocinigenum* as described in our study.

Colony reverse colors on PDA of pale pinkish cinnamon to pale ochraceous buff to light buff reported by Schol-Schwarz (5) are suggestive of Linn's (3) cinnamon buff colony color on the same medium. Isolates used in this study were cream colored on PDA. This may be a variable characteristic. On Sabouraud glucose agar, the pale to orange yellow colony reverse color of our isolates was in accord with that reported by Gams (2) and Schol-Schwarz (5).

The ability of *A. crotocinigenum* (= *C. crotocinigenum*) to produce antifungal crotocin is mentioned in the Latin description (5), but Gams (2) did not include this characteristic. The two isolates (902 and 903) from *S. podophyllum* in these studies gave no evidence for the production of any antifungal substance against several fungi, including *Colletotrichum, Gliocladium, Calonectria* (*Cylindrocladium*), *Stemphylium, Alternaria, Bipolaris,* and *Phytophthora* species (Uchida and Aragaki, *unpublished data*). When a broader spectrum of isolates of *A. crotocinigenum* from *S. podophyllum* is examined, isolates producing antifungal substances may be discovered.

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