

Nomenclature, Pathogenicity, and Conidial Germination of *Phyllostictina pyriformis*

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ABSTRACT

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Chlorotic and necrotic leaf spots of *Dendrobium* spp. were found to be caused by *Phyllostictina pyriformis*. Incubation periods were 6–12 wk for chlorotic and 6–8 mo for necrotic spots. Conidial germination of *P. pyriformis* on water agar was less than 1%, compared with approximately 90% on water agarose prepared with SeaKem Agarose.

During field examinations and samplings of *Dendrobium* blossom blights and flecks (5) caused by various Hyphomycetes, numerous chlorotic and necrotic leaf spots were observed (Fig. 1). The fungus most frequently isolated from both types of spots was *Phyllostictina pyriformis* Cash & Watson. In general, the leaf spots were not destructive—affected plants continued to produce flowers in abundance, although reduced photosynthetic capacity and yield are suspected. Some leaf drop was associated with the disease. Because of the developing market for blooming house plants, growers are concerned that many plants are unsalable or reduced in value because of the unsightly appearance of chlorotic and necrotic spots on leaves.

This disease is highly associated with *P. pyriformis* and is common on orchids in many genera. Based only on its association with the disease, *P. pyriformis* has been assumed to cause leaf spot of *Dendrobium* and other orchids (1,2,6). We report on the pathogenicity and conidial germination of the fungus.

MATERIALS AND METHODS

Isolations were made from chlorotic spots 1–3 mm in diameter and necrotic spots 1–12 mm in diameter obtained from leaves of *Dendrobium* hybrids. Seedlings of the crosses *D. macrophyllum* Richard

× *D. bigibbum* Lindley and *D. phalaenopsis* Fitzgerald × *D. grantii* White approximately 1 yr old were used in pathogenicity studies.

Phyllostictina isolate 689, obtained from a chlorotic spot, was our primary test organism. The fungus was grown on vegetable juice agar (10 ml of Campbell's V-8 juice, 0.2 g of CaCO₃, 1.8 g of agar, and 90 ml of deionized water) for 2–4 wk at 24–26 C, under 2,700 lux of continuous light provided by cool-white fluorescent lamps. Conidial slurries were made by

collecting freshly extruded spores (wet spore mass) with a glass needle and mixed with 1–2 ml of deionized water. The inoculum was then carefully spread with a glass rod over the wet, abaxial surface of half the leaf. Three leaves on each of three plants were inoculated. The uninoculated half of each leaf served as control. The plants were placed in plastic bags, moistened inside to maintain moisture saturation, for 24 or 48 hr at 25 ± 1 C, and then returned to greenhouse benches (22–30 C) to allow symptom development. Three inoculation tests were made.

Conidial suspensions for germination studies were prepared by placing in sterile, deionized water freshly extruded conidia aseptically taken from 16–22 day old cultures. The conidial suspension was then spread on substrate surfaces with a glass rod. The following media, each presumed low in nutrients, were the test substrates: 1) 1.8% water agar made with

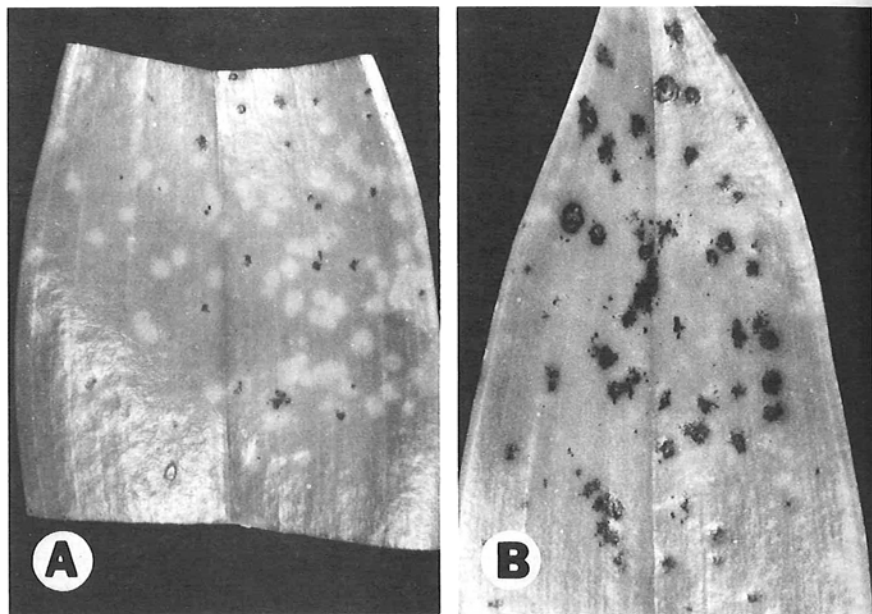


Fig. 1. *Dendrobium* leaves naturally infected by *Phyllostictina pyriformis*: (A) young infections, (B) older infections.

Difco Bacto Agar, 2) 1.8% water agar made with Difco Special (Noble) Agar, and 3) 0.8% water agarose made with SeaKem HGT-P Agarose (Marine Colloids, Rockland, ME). Each medium was prepared with deionized water. A deionized water drop control on a clean depression glass slide was also established.

Conidia of *P. pyriformis* germinated well on SeaKem Agarose but poorly or not at all on Difco Bacto Agar, suggesting that some substance(s) inhibited spore germination in the latter. To test this hypothesis, a 1.8% deionized water suspension of Difco Bacto Agar was allowed to stand overnight in a refrigerator. The supernatant was used to prepare 0.8% agarose with SeaKem Agarose. Agarose prepared with deionized water served as control, and these media were inoculated as described above.

The conidial germination studies were conducted at 24 C; observations were made and data collected after 24 and 48 hr. A conidium with germ tube longer than the conidial length was considered to have germinated. At least 200 spores were counted for each treatment. All germination tests were repeated.

Freshly extruded conidia from 7–20 day old cultures were suspended in 0.05% water agarose for measurements to confirm species identification. Conidial length, diameter, and appendage length were measured on 25 conidia from each of four isolates, including isolate 689. Spore length and diameter of 25 conidia from each of seven other isolates were also measured.

RESULTS

Faint, slightly depressed chlorotic spots approximately 1 mm in diameter were first observed 6 wk after inoculation and became distinct (Fig. 2) in another 4–6 wk. The change from chlorotic to necrotic spots was first noticed approximately 6 mo after inoculation, and within five more weeks most of the original chlorotic spots had become necrotic.

In other plants, chlorotic symptoms were not observed until 8–12 wk after inoculation, and the eventual development of necrotic spots took as long as 8 mo. Reisolations from chlorotic and necrotic spots yielded primarily *P. pyriformis*, although occasionally an unidentified fungus or bacterium was also recovered. Recovery rates of *P. pyriformis* from 60 chlorotic and 60 necrotic lesions were 87% and 92%, respectively. No disease developed on the uninoculated halves of leaves.

Conidial germination was less than 1% in water drops or on Difco Bacto Agar, approximately 15% on Difco Special (Noble) Agar, and about 90% on SeaKem Agarose. Germination on water agarose prepared with the supernatant from an aqueous suspension of Difco Bacto Agar was less than 1%, compared with 90% on

a control water agarose prepared with deionized water.

The conidial dimensions of the four isolates selected for measurement were so uniform that they were combined to give the following means and standard deviations: conidial length $12.0 \pm 1.4 \mu\text{m}$, diameter $7.5 \pm 0.4 \mu\text{m}$, length-to-diameter ratio 1.5 ± 0.2 , and filiform appendage length $21.4 \pm 7.9 \mu\text{m}$. Conidial dimensions of the other seven isolates were also pooled to give the following means and standard deviations: conidial length $11.9 \pm 1.0 \mu\text{m}$, diameter $7.3 \pm 0.3 \mu\text{m}$, and length-to-diameter ratio 1.6 ± 0.2 .

DISCUSSION

In a monograph of the genus, van der Aa (6) reestablished *Phyllosticta* Pers. ex Desm., recognizing two species, *P. capitalensis* P. Henn. and *P. aplectri* Ellis & Everh., as occurring on Orchidaceae, and placed *Phyllostictina pyriformis* in synonymy with *Phyllosticta capitalensis*. However, Punithalingam (4) argued that there is no evidence that anyone has examined the type selected by Persoon for *Phyllosticta convallariae* and that based on Persoon's notes it is impossible to distinguish *P. convallariae* generically from other leaf-inhabiting fungi. On these bases, Punithalingam (4) supported the separation of *Phyllostictina*, a small but well-defined genus, from *Phyllosticta*. Furthermore, van der Aa (6) acknowledges a problem with limits of *Phyllosticta* in his statement, "The large genus *Phyllosticta* as a whole has not yet been revised according to modern criteria."

In conidial dimensions, the present test fungus, represented by 11 isolates, fits the description of *Phyllostictina pyriformis* (= *Phyllosticta capitalensis*) given by Cash and Watson (2) and van der Aa (6).

However, the filiform conidial appendage, averaging approximately $21 \mu\text{m}$, is substantially longer than the 5–12 μm given by van der Aa and the "short appendage" of Cash and Watson. In our studies, many conidia without appendage or with appendage approximately equal to the conidial length were seen. The appendage length was highly variable and, unless developed and measured under defined conditions, has only limited value in species characterization. Except for appendage length, the fungus fits the conidial and pycnidial characteristics of *P. pyriformis* well, and the *Dendrobium* leaf-spotting organism is given this designation.

The 6–12 wk incubation period for chlorotic spots and 6–8 mo needed for development of necrotic spots were unexpectedly long, possibly accounting for the lack of records demonstrating the pathogenicity of *P. pyriformis* to orchids. The reproduction of typical leaf spot symptoms on *Dendrobium* and the reisolation of *P. pyriformis* fulfill requirements for demonstration of pathogenicity.

Conidial germination was usually less than 1% when tested on water agar prepared with Difco Bacto Agar and 10–20% on Difco Special (Noble) Agar. These agars have been shown to be sufficiently impure to support spore germination of nutritionally dependent organisms (3), whereas these same fungi do not germinate on water agarose prepared with SeaKem Agarose (3). The conidial germination of *P. pyriformis* on these substrates, which is completely the reverse of nutritionally dependent organisms, prompted the preliminary interpretation that there is a water-soluble, inhibitory substance among the

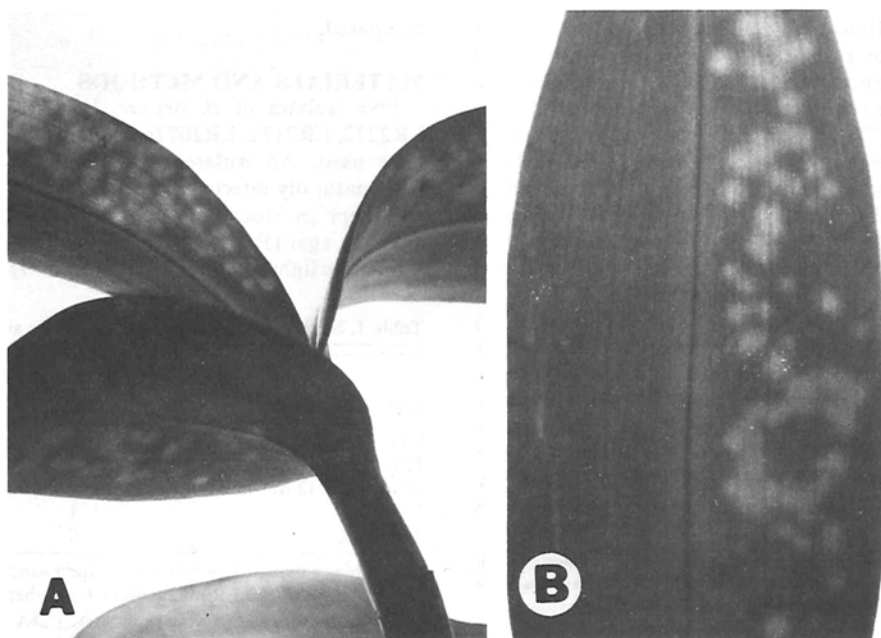


Fig. 2. Chlorotic spots on artificially inoculated *Dendrobium* leaves: (A) entire plant, (B) closeup of detached leaf.

impurities in Difco Bacto Agar and Difco Special Agar that is either absent or present at nontoxic levels in SeaKem Agarose. Furthermore, conidia in suspensions obtained by conventional flooding techniques from vegetable juice agar routinely made with Difco Bacto Agar do not germinate (*unpublished*), accounting for previous failures in inoculation tests.

The poor germination of *P. pyriformis* conidia in water drops was noted by van der Aa (6). This suggested that there may

be a substance in SeaKem Agarose that promotes the conidial germination of *P. pyriformis*. Alternatively, the conidial germination may proceed poorly in liquids. Studies are continuing to identify inhibitory substance(s) in agar and the nutrient requirement for germination of *P. pyriformis* conidia.

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