A Previously Undescribed Selenophoma Leaf Spot of Maize in Colombia

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ABSTRACT

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A species of *Selenophoma* is the pathogen of a severe leaf spot of maize growing at an altitude of 2,700 m in southwestern Colombia. Other species of this fungus have been described throughout the world primarily as pathogens of cereals and grasses. This is the first report of a *Selenophoma* species on maize and only the second on any host from South America.

Additional key words: corn, halo blight, halo spot, *Zea mays*

At least 10 species of the pycnidial fungus *Selenophoma* Maire (6) have been described as pathogens of cereals, grasses, and other hosts representing six plant families from 31 countries on all continents (1,5,14,15,17). This is the first report of a species of *Selenophoma* pathogenic to maize (*Zea mays* L.). No species of *Selenophoma* has been reported from South America, but Sprague and Johnson (15) considered a species described in 1906 as *Pseudoseptoria donacicola* Spegazzini from *Arundo donax* L. to be a synonym of *Selenophoma donacis* (Pass.) Sprague & A. G. Johnson. The species described from barley (*Hordeum vulgare* L.), wheat (*Triticum* spp.), and other small grains and grasses are distinctive in their unusually broad tolerance of temperature for spore germination, infection, and disease development. Spores are reported to germinate at temperatures from 1 to 35 C, with optimum occurring between 18 and 25 C, depending on the species (2,10). Park and Sprague (10) found that 72 hr of incubation of inoculated plants at 18–20 C was favorable for infection with four gramineous species of *Selenophoma*. Allison (2) inoculated field plots most successfully during cool, cloudy weather when the temperature did not exceed 20.5 C.

The disease on cereals and grasses caused by several species of *Selenophoma* is commonly known as "halo spot," descriptive of the pale border surrounding the tan elliptical lesions during early stages. Lesions on the Colombian maize specimens (Fig. 1) are typical "eyespots" with concentric zonations, as are those resulting from artificial inoculations (Fig. 2), but which are bordered by a yellow "halo" during the first several weeks of development. Lesions extend to a length of 2 cm under humid conditions (>70% relative humidity [RH]), and coalescing lesions can cause severe leaf necrosis (Fig. 1).

MATERIALS AND METHODS

Isolation of pathogen. Clippings of leaf lesions collected in Colombia (Vereda del Motilón, Lago de La Cocha, Pasto, Nariño, 27 November 1984) were incubated in petri-dish moist chambers at 26 C with 12 hr of fluorescent light/12 hr of darkness for 2 days. Pycnidia were excised with a sterile insect-mounting pin in a wooden handle and streaked with a microloop across the surface of 3% water agar in a petri dish for as long as the release of pycnidiospores could be observed under a ×96 stereoscopic binocular microscope. After a 24-hr incubation at 26 C, single germinating spores were cut out in agar cubes and transferred to various culture media.

Nutrition and incubation of cultures. Cultures were grown on various agar media: V-8-juice (16), fresh potato-dextrose (12), oatmeal (12), lima bean-V-8 (11), Difco malt, rice polish (16), and Czapek-Dox-V-8 (3). Cultures were grown also on corn-leaf-piece substrate (7-cm sections of corn leaves cut from greenhouse plants at the five- to eight-leaf stage, each placed on three pieces of wet filter paper in a 9-cm petri dish and autoclaved 15 min). Liquid media included yeast extract-dextrose broth (15 g of yeast extract and 3 g of dextrose per liter of water), V-8 juice broth, and fresh potato-dextrose broth. Various combinations of light and temperature were employed: 12 hr of 20W daylight fluorescent (18 and 26 C), 12 hr of 20W daylight fluorescent plus 15W Sylvania blacklight/blue (10 C), and natural diurnal light/dark with 1 hr/day under a Hanovia UV lamp/254 nm at 0.5 m (26 C).

Spore germination tests. Suspensions of pycnidiospores (about 3.5×10^5/ml) were atomized onto petri dishes (35 × 10 mm) containing 3% water agar. Dishes were placed on a gradient temperature plate and covered with aluminum foil. Temperatures tested were 9, 14.5, 19, 23.5, and 30 C, with four replicates at each temperature. Percent germination was determined after 48 hr, counting germinated spores as those with germ tubes one to four times the spore length.

Pathogenicity tests. Maize plants of cultivars Pioneer 3320 and 3334A were greenhouse-grown in 1-gal plastic pots, one plant per pot. Two pots of each cultivar were used for each test. Plants were sprayed at the five- to eight-leaf stage with spore suspensions (about 1×10^4/ml) from a DeVilbiss surgeon's atomizer, lightly covering both surfaces of leaves. Plants were incubated in a dew chamber (8) first set at 26 C, and in later tests, at 23 C, for 24, 48, and 72 hr. They were then moved to a Percival growth chamber model MB-60B, programmed for a 12-hr day at 24 C and a 12-hr night at 21 C. To increase humidity, the chamber was modified by inserting an inner Lucite cabinet (61 × 64 × 91 cm), which provided the programmed temperatures desired for day/night but eliminated the turbulence necessary to maintain those temperatures in the outer chamber. Temperatures within the cabinet were maintained at 24 C for a 12-hr day and at 21 C for a 12-hr night and monitored three times a day with probes (400 series) of a YSI Telethermometer. An orifice accommodated periodic misting inside the Lucite cabinet (mist for 1 min at 4-hr intervals during daylight hours for 1 wk after inoculation).

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RESULTS

Isolation of pathogen. The methods described were successful in obtaining single-spore isolates from the original diseased leaf specimens 1 wk after their collection in Colombia, 6 mo after their storage at 4 C, and from lesions resulting from artificial inoculations, thereby completing Koch's postulates.

Fungal and cultural characteristics. Pycnidia borne in lesions from field collections or from artificial inoculations were subepidermal, hyaline with membranous walls, extruding spores under moist conditions in mucoid globules, which upon drying, curled like rams' horns, presenting a pale peach color. Two types of pycnidiospores were formed in culture: lunate unicellular spores (α-spores) (Fig. 3) that germinated and infected the host and threadlike scolecospores (β-spores) (Fig. 3) that did not germinate or infect the host. Their apparent lack of a role in infection is comparable with that of β-spores of *Phomopsis* and *Stenocarpella* spp. Pycnidia on V-8-juice, V-8-Czapek-Dox, oatmeal, fresh potato-dextrose, or lima bean-V-8 agars and on corn-leaf-piece substrate extruded α-spores in large globules at 10 or 18 C but only β-spores at 26 C. The lunate pycnidiospores (α-spores) in culture were unicellular with rounded ends, 24–50 × 2.8–4.7 μm, mean = 40.7 × 3.3 μm (Fig. 3), similar in shape and size to those from lesions. Scolecospores (β-spores), produced in great abundance at 26 C, have not been reported for other species of *Selenophoma*. On the other hand, freeborne conidia reported in other species (4) were not observed in the maize pathogen. Cultures developed slowly into flesh to rosy pink colonies on all media except malt agar, on which they were gray with white tufts. Erumpent pycnidia formed a rough or finely tufted surface on most media. Colonies typically covered a 9-cm petri dish within 3–4 wk at all temperatures tested. The fungus grew vigorously in all liquid media at 10 and 18 C but not at 26 C.

The light/temperature combinations available for testing did not permit a controlled evaluation of the importance of light in formation of α-spores. There was such a striking response of the fungus to temperature in this respect that the effect of light, though lacking comparison with total darkness, appeared relatively noncritical.

Spore germination tests. Spores germinated at both ends and laterally, producing as many as five germ tubes from a single spore. The mean percentages for spore germination of four replicates for the five temperatures tested were as follows: 9 C/18.3%, 14.5 C/33.3%, 19 C/74.5%, 23.5 C/92.0%, and 29.8 C/0.5%.

Pathogenicity tests. Inoculated plants incubated 24–48 hr in a dew chamber at 26 C, then at 24 C day/21 C night in the unmodified Percival growth chamber,

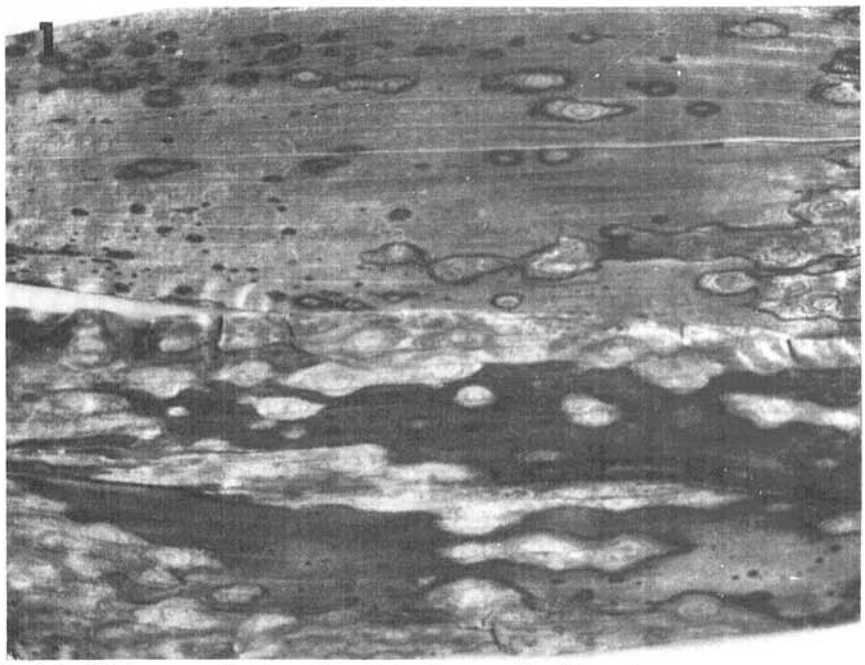

Fig. 1. Leaf lesions on maize leaves from Colombia caused by *Selenophoma* sp.

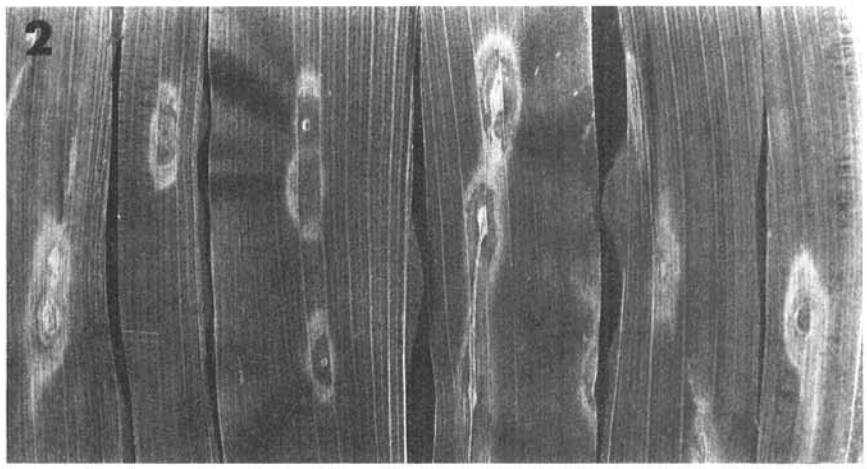

Fig. 2. Leaf lesions resulting 8 days after artificial inoculation with a suspension of *Selenophoma* pycnidiospores (about 1×10^4 per milliliter).

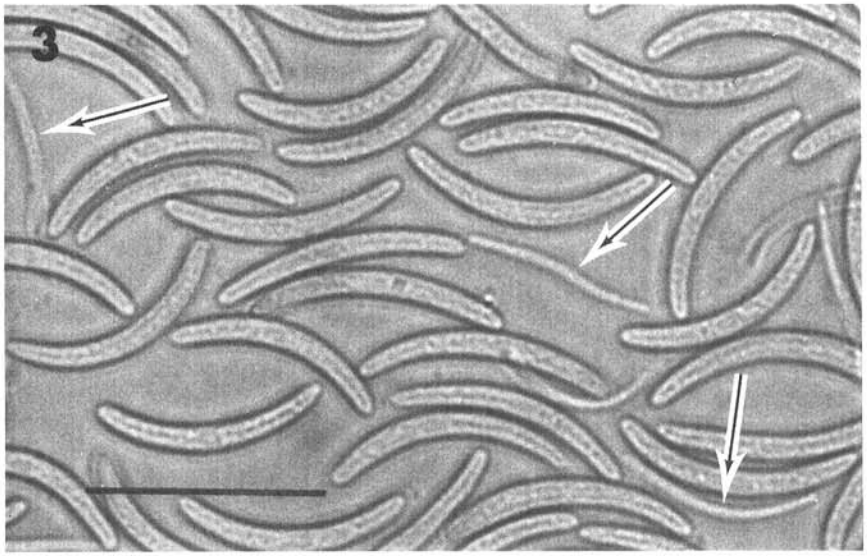

Fig. 3. Pycnidiospores (mostly α-type) from V-8 agar culture of *Selenophoma* sp. Several scolecospores (β-spores) are indicated by arrows. Scale bar = 40 μm.

did not become infected. Insertion of the inner Lucite misting cabinet within the Percival chamber at 24 C, however, combined with a prior 24- to 48-hr dew-chamber incubation at 22 C, provided conditions conducive to infection and lesion development. Lesions typical of those on leaves collected in Colombia were formed (Fig. 2) but not in numbers proportionate to the number of spores applied. We still lack information on optimal conditions for infection. Lesions on the two cultivars tested were similar in shape and size.

DISCUSSION

Selenophoma diseases of gramineous and other hosts characteristically cause greatest damage during cool, moist seasons and are most common in geographical areas or altitudes where such conditions prevail. *Selenophoma* diseases of cereals and grasses in the United States are known primarily in the Pacific Northwest and the north central states, but *S. bromigena* (Sacc.) Sprague & A. G. Johnson has been found attacking *Bromus* spp. in arid western regions from Idaho and Utah south to the Grand Canyon in Arizona and Tioga Pass in California (14). Their prevalence as far north as the Canadian Maritime Provinces (13), Greenland, Iceland, Finland (7), and northern USSR (9) indicates the tolerance (or preference) of these pathogens for low temperatures. Our experiments have shown a similar adaptation to lower temperatures by the maize *Selenophoma* as indicated by abundant production of infective propagules at 10 and 18 C but not at 26 C and infection of plants at 22 C but not at 26 C. We have not yet determined the critical RH for disease development with this species of *Selenophoma*; we report only on the satisfactory level within our experimental conditions. Although this fungus thrives at lower temperatures for spore germination and for pathogenesis (in nature as well as under artificial test conditions) than most other fungal pathogens of maize from Latin America, it resembles these pathogens, e.g., species of *Cercospora, Hyalothyridium, Phyllachora, Marasmiellus,* and *Stenocarpella*, in requiring free moisture and high (>70%) RH for maximal disease development.

Other species of *Selenophoma* have shown a high degree of host specificity (10). We have not yet tested the maize pathogen against other gramineous hosts. On the basis of morphological and cultural characteristics, we believe it is a previously undescribed species.

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