A Marasmiellus Disease of Maize in Latin America

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ABSTRACT

Latterell, F. M., and Rossi, A. E. 1984. A Marasmiellus disease of maize in Latin America. Plant Disease 68:728-731.

An undescribed leaf spot and associated stalk rot of maize in Mexico and Central America are caused by a basidiomycete of the genus *Marasmiellus*. Symptoms include blanching of elongate marginal areas of leaves, with lesions often extending to the midrib. Lesions on stalks extend downward from sheath-blade junctures. Minute agaric basidiomes form amphigenously on leaf lesions. The pathogen grows vigorously in culture, producing cottony white colonies with feathery margins. Greenish black layers of leathery compact mycelium may develop in the midst of a white colony. The disease is given the common Spanish name "borde blanco" (=white border).

A basidiomycete of the order Agaricales (ATCC 56473), identified as a species of *Marasmiellus* by H. Bigelow and A. H. Smith, is the causal agent of a distinctive leaf and stalk disease of maize that we have observed in humid areas of Mexico and Central America (7).

The leaf blight aspect is characterized by blanched marginal lesions that may originate at any point along the leaf from the sheath-blade juncture to the apex but always beginning at the margin (Fig. 1). Similarly, stalk lesions (Fig. 2) originate at the margin of the sheath, but the fungus penetrates through several sheath layers into the stalk. Leaf lesions range from 5 to 25 cm long and from 1 to 3 cm wide. The fungus apparently is extensively established within the tissues before blanching occurs, hence no lesions smaller than 5 × 1 cm were observed. Lesions are characterized by concentric zonation of slightly different degrees of decolorization. The pathogen grows rapidly in artificial culture and likewise appears to grow rapidly within tissues after initial infection. Tiny stalked basidiomes (1–4 mm high) are formed amphigenously in advanced lesions (Fig. 3), especially in the center zone. Several black elliptical rings 2–6 mm in diameter are often apparent in these lesions. These rings are composed of tissue reminiscent of dark greenish black stromatic layers that often develop spontaneously in culture in the midst of a snow-white cottony colony. These structures will be discussed in a later section.

We propose the Spanish name "borde blanco" (=white border) for this disease because of its prevalence in Latin America and because of the characteristic white border lesions that glisten in the sunlight in severely infected fields. The disease is not limited to senescent tissues; lesions may be found on leaves of all ages on vigorous green plants at least as early as tassel emergence. Stalk lesions are numerous in fields showing a high percentage of infected leaves, and severe stalk rot has been observed in humid areas where foliage is dense. The potential for damage caused by stalk rot under such conditions has not been determined.

There are many reports on species of *Marasmiellus* and the related genus *Marasmius* pathogenic to maize and other Gramineae (5,8,11–13,15–18). We have examined these reports and have found none that fits the description of the borde blanco fungus, either in morphology or in symptoms induced. Basidiomes of the described species are appreciably larger, with broader pileus, sturdier or longer stipe, and more numerous lamellae, being readily visible to the naked eye, whereas those of the pathogen on which we are reporting are barely or not discernible without magnification. Symptoms attributed to the graminicolous species are primarily seedling blights, wilts, or root/foot rots, with fruiting structures developing on adventitious roots or stalks near ground level. The borde blanco fungus is primarily a pathogen of the leaf blade and sheath and not associated with roots or the basal regions of stalks. Fruiting structures are borne on leaf lesions.

MATERIALS AND METHODS

Observation of fungal structures. Characteristics of the pathogen were studied by incubation of naturally and artificially infected leaves in petri-dish moist chambers.

Isolation of cultures. Cultures were isolated by plucking young basidiomes from incubated lesions with sterile

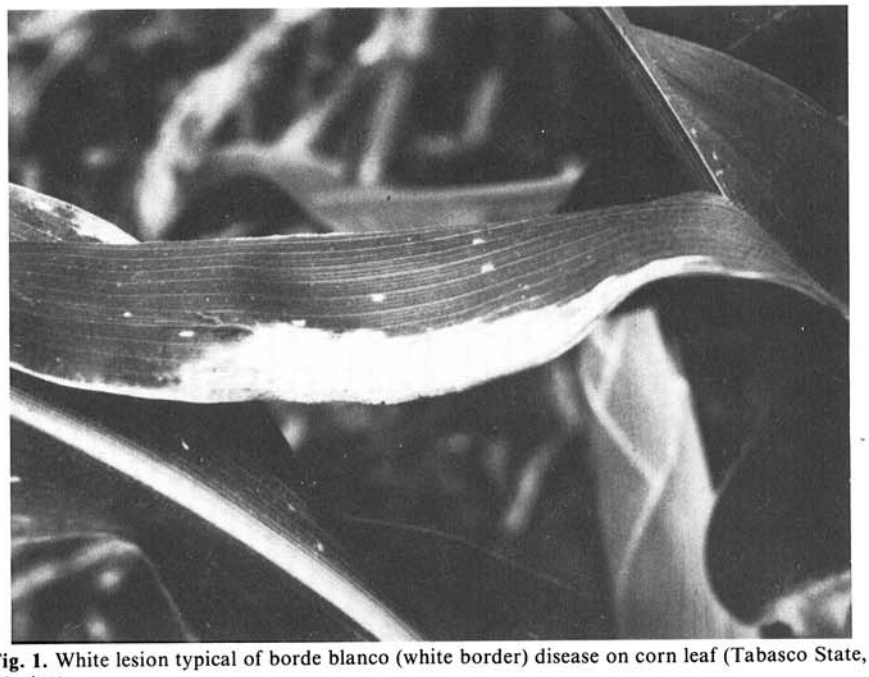

Fig. 1. White lesion typical of borde blanco (white border) disease on corn leaf (Tabasco State, Mexico).

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Accepted for publication 23 April 1984.

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forceps and placing them on sterile pieces of corn leaf on wet filter paper in 9-cm petri dishes or deep storage dishes. Wefts of mycelium growing from the basidiomes were transferred aseptically to several agar media.

Culture media. The fungus was cultured on various solid and liquid media. Agar media tested were potato-dextrose, V-8 juice, cornmeal, and rice polish (2% Gerber's baby food with 1.7% agar). Petri-dish moist chambers, each containing an autoclaved corn leaf piece on filter paper, were tested under various light regimes as substrates for support of basidiome development. Liquid media were yeast extract-dextrose and modified Fries' solution (4), the latter being used to support the possible production of an antifungal metabolite.

Pathogenicity tests. Cultures grown on sterile corn leaf pieces were used as inocula. Strips of infested tissue were placed on upper and lower leaf surfaces of corn leaves so that the inoculum was in contact with the leaf margin. Plants were incubated in a dew chamber (10) at 25 C for 24 hr.

Histological studies. For observing mycelial development in diseased tissue, whole mounts of naturally and artificially infected leaves were prepared by clearing 5-mm^2 leaf pieces in 40% aqueous chloral hydrate at 60 C for 16 hr followed by staining for 4 hr in 0.1% aniline blue in chloral hydrate. Transverse sections of infected leaves were prepared by slicing on the Lab Line/ Hooker Plant Microtome at 12–24 μm and mounting in 0.1% cotton blue in lacto-phenol. The basidiomes were prepared for mycological study in the following manner: Several apparently mature basidiomes were plucked with fine forceps from the leaf surface and dropped into a 5-ml vial of formalin-alcohol-acetic acid (FAA). Standard procedures (6) were used for dehydration, embedding in paraffin, and sectioning at 6 μm. The only special technique derived from the difficulty in transferring the minute basidiomes, each being only about 0.5 mm long after fixing in FAA; transfers were made using a Pasteur pipette to avoid losing the specimens by pouring. Surfaces of fresh basidiomes were photographed by scanning electron

Fig. 2. Borde blanco stalk lesions originating at sheath-blade juncture (Guápiles, Costa Rica).

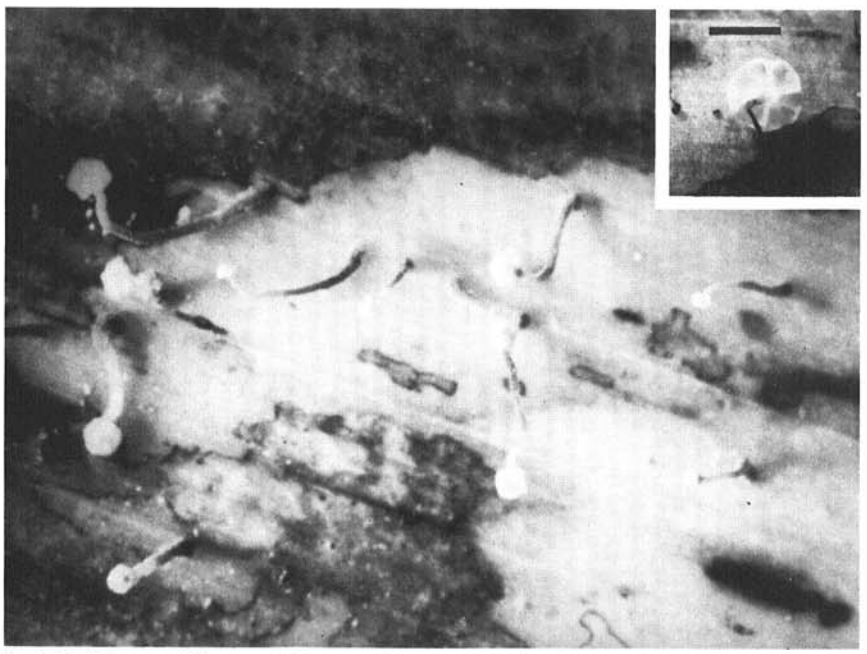

Fig. 3. Basidiomes of the borde blanco pathogen developing in a leaf lesion after incubation in moist chamber. **(Inset)** Basidiome showing venate lamellae. Scale bar = 2 mm.

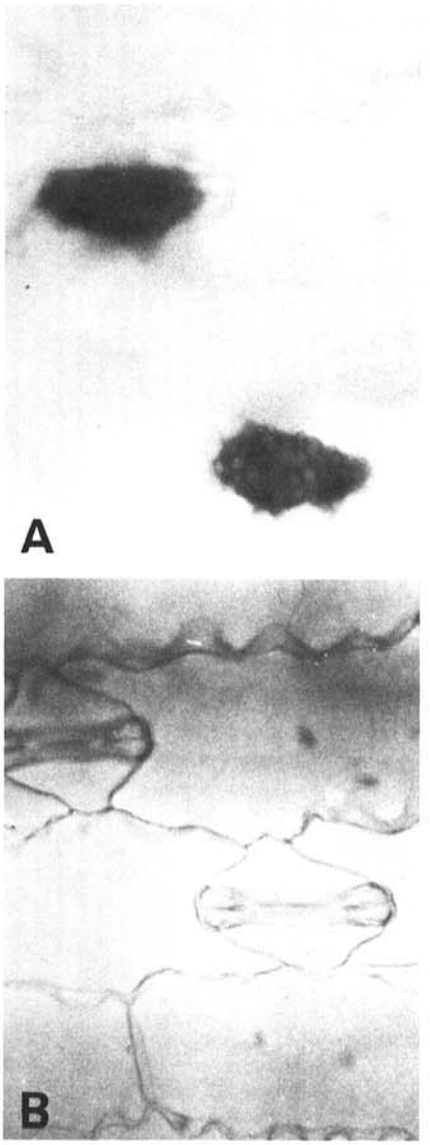

Fig. 4. Stomata on adaxial surface of corn leaf. **(A)** Optical section of leaf showing plugging below stomata by borde blanco fungus in leaf lesion. **(B)** Stomata on healthy leaf tissue adjacent to lesion.

microscopy in an Etec Autoscan by the methods of Crawford and Gonda (3).

RESULTS

Growth characteristics in vivo. Lesions incubated in a moist chamber for several weeks sometimes gave rise to young basidiomes (Fig. 3). The habit, consistency, and size of the basidiomes, the aculeate basidioles, clamped hyphae, inamyloid pileus and stipe, and diverticulate pileipellar hyphae all indicate that this fungus is a species of *Marasmiellus*. In addition to the black elliptical rings mentioned previously, fine black lines are often observed in lesions in the field. These lines continue to develop during incubation of lesions, ultimately extending to the edges of the leaf piece and often forming a complete border. Another unusual phenomenon related to these lines is the almost complete lack of growth of secondary fungi within the blanched area enclosed by the black lines, suggesting antibiosis. These structures are analogous to the black lines and plates formed by many Ascomycetes and Basidiomycetes (1,2), including genera closely related to *Marasmiellus* (9,14). S. A. Redhead (*personal communication*) suggested that these black plates may act as pseudosclerotia, forming impenetrable physical barriers that wall out other fungi and delimit the colonized substrate for the primary invader. Another consistent characteristic of the blanched zone of lesions was the plugging of stomata, so that they appeared black under low magnification. The nature of this plugging is described in the histological section.

Cultural characteristics. Cultures grew vigorously and produced dense white cottony mycelium with feathery edges on all solid substrates tested. In deep dishes, the growth was so luxuriant that within 2 wk in darkness, the mycelium entirely covered the inside walls and inner surface of the lid. The cultural counterpart of the black lines noted on infected tissue took the form of greenish black, rough, leathery tissue that appeared spontaneously in most cultures. Examined under high magnification, this tissue was composed of dark olivaceous green, densely woven hyphae and was almost impenetrable with dissecting needles. Viewed from the outside of a culture tube or from the bottom of a petri-dish culture, the boundaries of these structures appeared as black lines, in striking contrast to the pure white mycelium of the main body of the culture. Clamp

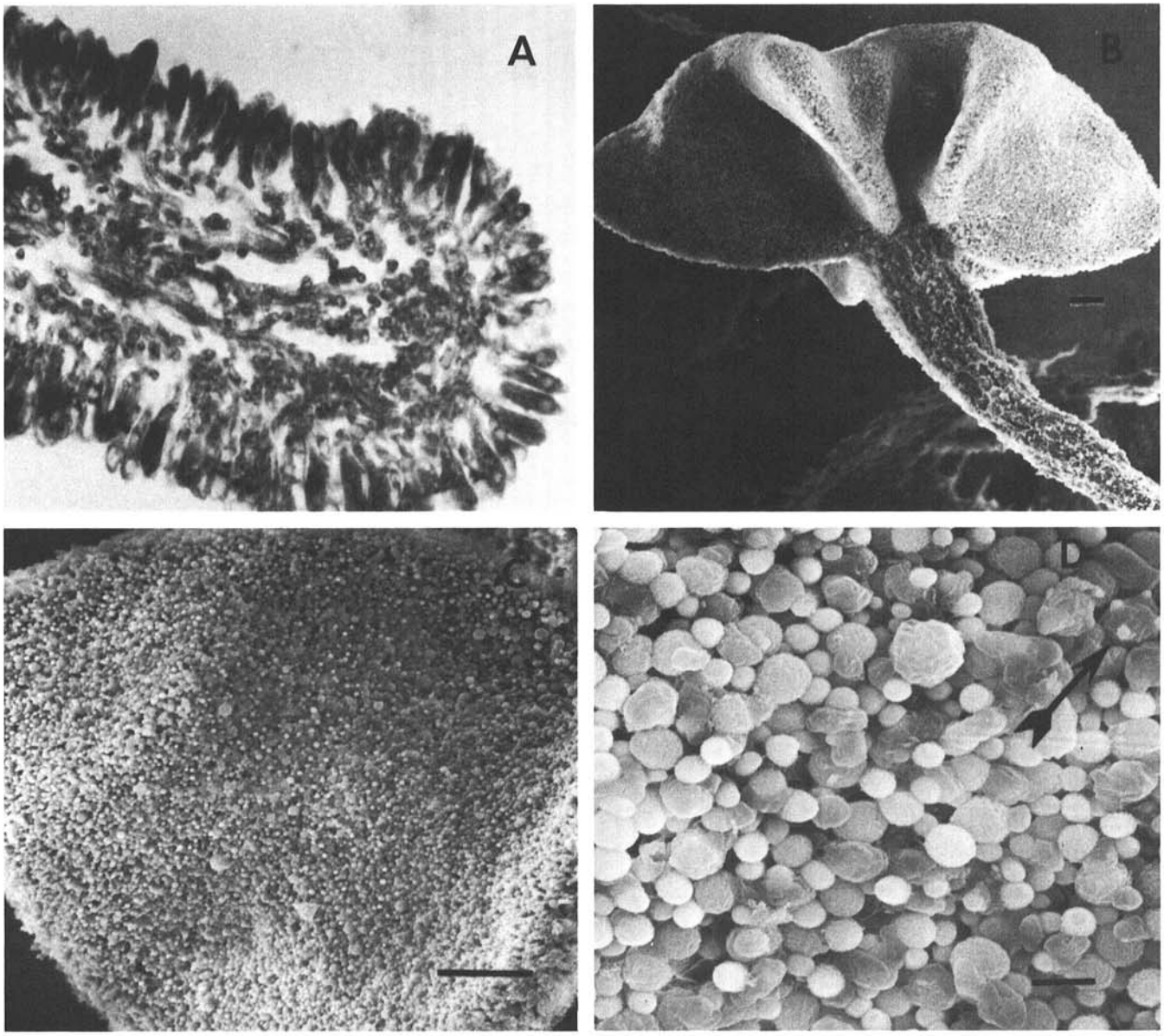

Fig. 5. Structures on lower surface of pileus. **(A)** Longitudinal section showing immature basidia and cystidioles (pointed ends). **(B)** Scanning electron micrograph (SEM) of underside of pileus. Scale bar = 100 μm. **(C)** SEM of a vein on lower surface of the pileus showing end view of immature basidia and cystidioles. Scale bar = 100 μm. **(D)** SEM of same surface at higher magnification; arrow points to sterigmata developing on tip of one basidium. Scale bar = 10 μm.

connections formed abundantly in culture and on hyphae within and on incubated lesions. Structures resembling dendrophysoid elements were also formed in agar culture. No basidiomes developed on any of the substrates. The fungus grew well in yeast extract-dextrose broth but not in Fries' solution. Results from efforts to explore the possible production of an antifungal metabolite in vitro were inconclusive.

Pathogenicity. Results of artificial inoculations were variable. Because we had no spores to use as inoculum, we relied on hyphal invasion of the tissues. This resulted in some of the characteristics of naturally infected leaves, but we were not able to promote "zoning," complete blanching, or development of black lines. The tissue exposed to mycelial growth from a leaf-piece culture became water-soaked within 24 hr, and intercellular hyphal growth was rapid but localized. Stomata became plugged with the same type of knobby ochraceous projections observed in naturally infected tissue, but lesions failed to enlarge appreciably beyond the initial area, even after further exposure in a dew chamber. The fungus was reisolated from such lesions, fulfilling Koch's postulates.

Histological studies. Cleared and stained whole mounts of both naturally and artificially infected tissue showed dense intercellular hyphal growth with abundant clamp connections. The stomata were plugged by the outgrowth of hyphae from the subepidermal tissues, but the growth terminated with knobby projections (Fig. 4A) that appeared to have a resinous character, both in color and in texture. Normal stomata are shown in Figure 4B.

Tangential sections of the pilei cut at 6 μm (Fig. 5A) showed the formation of basidia and probably cystidioles; diverticulate hyphae were found in squash mounts of revived tissue of the pileus. Scanning electron micrographs of fresh basidiomes showed immature basidia developing on the veinlike lamellae on the underside of the pileus (Fig. 5B–D). The formation of sterigmata (Fig. 5D) predicts the imminent development of basidiospores.

DISCUSSION

Borde blanco disease of maize is prevalent in the humid lowlands of Mexico and Central America and in the highlands where "hanging mists" are common during the rainy season. A basidiomycete identified as a species of *Marasmiellus* is associated consistently with the disease in Mexico, Nicaragua, and Costa Rica. Basidiospores are presumed to be propagules of the pathogen. Although the leaf-spotting aspect of the disease has not been observed to be of serious consequence, the associated stalk rot that occurs in densely planted maize in humid lowlands is a potential threat to grain production under these conditions, which are marginal for growth of the crop.

ACKNOWLEDGMENTS

We wish to thank Howard E. Bigelow, University of Massachusetts, and Alexander H. Smith, University of Michigan, for their invaluable assistance in identification of the fungus. We also thank Matthew A. Gonda, Program Resources Inc., NCI-FCRF, Frederick, MD, for the scanning electron micrographs and Scott A. Redhead, Agriculture Canada, Ottawa, for constructive criticism of the manuscript.

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