Four Sugarcane Seedling Diseases in Hawaii: Causal Agents, Control, and a Selective Medium for Isolation

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ABSTRACT

Cochliobolus lunata, Drechslera rostrata, Drechslera hawaiiensis, and *Curvularia senegalensis* were isolated from 1- to 10-week-old sugarcane seedlings. The latter three fungi have not been previously associated with sugarcane seedling blight. *Cochliobolus lunata* and *D. rostrata* were the more virulent of the four pathogens. A selective medium containing benomyl, nonionic surfactant (NPX), and streptomycin was developed to aid in isolations. Evidence would indicate that inoculum is present on the seed when it is planted. Fungicide applications at sowing, emergence, and at weekly intervals thereafter controlled the disease adequately.

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Additional key words: Saccharum sp., panogen.

Propagation of sugarcane (*Saccharum* sp.) seedlings from true seed (fuzz) is an essential step in the development of new commercial clones. Approximately 2 million seedlings are raised each year at the Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii. At this early stage of development, losses due to diseases are important and should be controlled.

Limited information is available concerning sugarcane seedling diseases and their control. *Alternaria tenuis, Helminthosporium halodes* (13), *H. sacchari* (8), *H. tetramera, Curvularia lunata,* and *Pythium* sp. (2, 18) caused seedling blights in various areas of the world. In Hawaii, Wismer (18) controlled *Pythium* damping-off by using Dexon. Ziram was effective in controlling *H. halodes* and *A. tenuis* in India (13). Martin (9) reported control of a *Helminthosporium* sp. "possibly *sacchari*" in Hawaii using mercurous and mercuric chloride.

In 1969, seedling losses from greenhouse flats in Hawaii ranged from 8 to 31% and averaged 16% (6) despite the routine use of Dexon [Sodium-*p*-(dimethylamino)benzenediazosulfonate], suggesting a nonpythiaceous problem. This paper describes four causal agents associated with seedling diseases, recommends a control, and describes a selective medium for their isolation.

MATERIALS AND METHODS.—*Isolations.*—Diseased seedlings were surface-sterilized with a 1% sodium hypochlorite solution for 4 min and plated on cane leaf agar (CLA) (19) containing 50 μg/ml streptomycin. Pathogen cultures were maintained on CLA.

A selective medium (BNSCLA) was developed to enable the isolation of the pathogens from the fuzz. The seed remains enclosed in the dried flower parts, which are fluffy and are thus referred to as "fuzz". Cane leaf agar was amended with 20 μg/ml benomyl, 1,000 μg/ml NPX (nonyl phenyl polyethylene glycol ether plus 10.5 moles ethylene oxide, Union Carbide Corp., New York, N. Y.) and 80 μg/ml streptomycin. Isolations were made by placing florets directly on BNSCLA, or from aqueous washes of the fuzz. Fuzz washes were accomplished by agitating 5 g fuzz in 200 ml water containing 50 μg/ml Tween 20 (polyoxyethylene sorbitan monolaurate). The suspension was filtered through glass wool and concentrated 20 times by centrifugation. Tenfold serial dilutions of the supernatant liquid were made. A 0.1-ml aliquot was suspended in 10 ml of warm (45 C) BNSCLA in a petri dish, and incubated in the dark at 25 C. Colonies of the pathogens were distinguishable in 4 to 7 days.

Greenhouse testing.—The fuzz used in greenhouse testing was a mixture derived from several polycrosses. It was propagated by the standard practice of surface planting in wooden flats containing methyl bromide-treated soil. The flats were covered with wax paper until germination occurred (4-7 days). Inoculum was sprayed onto the surface of the flats at sowing. Fungicides were applied by spraying a sufficient quantity to drench the surface (0.04 ml/cm^2). A standard fertilizer and Dexon application (18) was made 2 weeks after planting.

RESULTS. *Isolation and pathogenicity.*—Four fungi were consistently isolated from diseased seedlings. They were identified by M. B. Ellis of the British Commonwealth Mycological Institute as: *Drechslera rostrata* (Drechsler) Richardson & Fraser; *Drechslera hawaiiensis* (Bugn.) Subram. & Jain; *Curvularia senegalensis* (Speg.) Subram.; and *Cochliobolus lunata* Nelson & Haasis (*Curvularia* state) (Fig. 1). Spores of these isolates were sprayed onto fuzz at sowing time. *Drechslera rostrata* and *C. lunata* were more virulent than were *D. hawaiiensis* and *C. senegalensis.* Disease incidence in the inoculated flats ranged from 53 to 81%. The isolation of these fungi from the seedlings was indicative of their pathogenicity.

Symptoms.—Various symptoms occurred on naturally and artificially inoculated seedlings, and no single pathogen was associated with one particular type. Symptoms were generally similar to those caused by *H. sacchari* (8) and *H. halodes* (13).

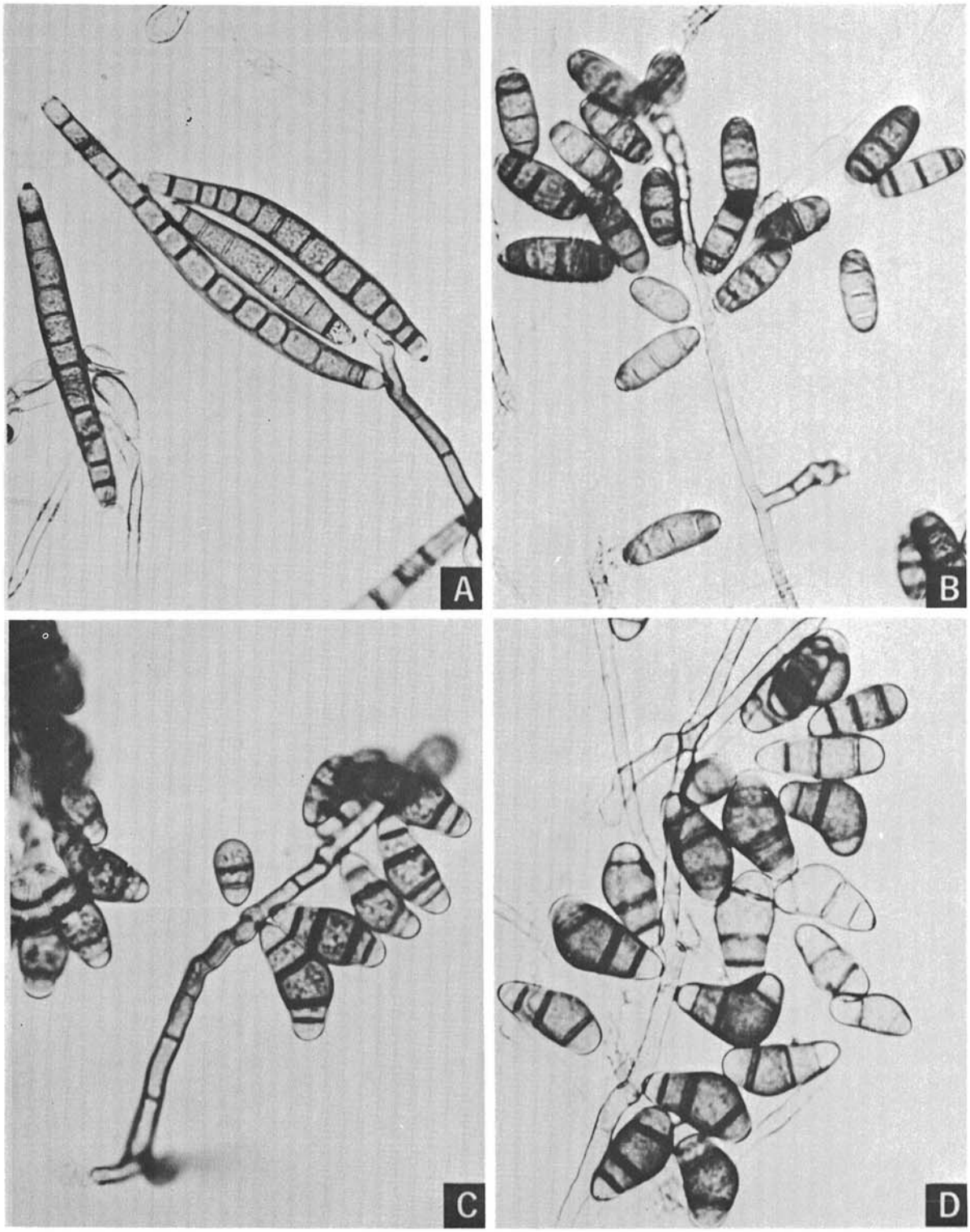

Fig. 1. Conidia of the seedling blight pathogens. **A)** *Drechslera rostrata*; **B)** *Drechslera hawaiiensis*; **C)** *Curvularia senegalensis*; **D)** *Cochliobolus lunata.*

Fig. 2. Severe seedling blight of 2-month-old transplanted sugarcane seedlings.

Lesions were located on coleoptiles, stems, leaves, and roots. Symptoms varied in color from black to brick red, and in shape from small elliptical lesions to complete girdling. Often the lesions were located at the ground line. Primary and then secondary leaves often became chlorotic; bronzing of these leaves followed by necrosis was initiated at the apex and moved downward. Susceptible plants died within 3 or 4 days after germination. Resistance increased as the seedlings aged, and generally, infections were not fatal after the plants became 2 to 3 weeks old. Occasionally, older plants were predisposed to infection due to poor growing conditions (Fig. 2). All four pathogens have been isolated from 1- to 3-month-old plants apparently predisposed to infection by nutrient stress, deep transplanting, or stress imposed by transporting plants to test sites on other islands (2 to 3 days in transit). Young seedlings derived from year-old fuzz (stored at 0 C) had a high disease incidence, possibly due to loss of seedling vigor.

Selective media.—It was suspected that the pathogens were being introduced with the fuzz at sowing time. Introduction in the methyl bromide-treated soil seemed unlikely. Because of high microfloral populations in the fuzz, a selective medium was used to detect the presence of the pathogens in fuzz. A 4,000- and 1,000-fold reduction in fungal and bacterial colonies, respectively, was achieved by the additions of benomyl, NPX, and streptomycin. The effectiveness of the selective medium was tested by adding a known quantity of spores to aqueous fuzz washes and determining the number of colonies recovered. Recovery ranged from 43 to 68% (Fig. 3). None was recovered from the nonamended CLA. Benomyl had little effect in eliminating several rapidly growing *Mucor* spp., but addition of NPX reduced their growth sufficiently to allow observation of the pathogenic fungi. At the concentrations used in the medium, NPX reduced linear growth of the pathogenic fungi ca. 3.3-fold; benomyl and streptomycin had no effect.

Using this selective medium, the pathogens were isolated both from naturally inoculated florets and fuzz washes. In addition, spores of *D. rostrata* and *Curvularia* sp. were observed directly in the fuzz washes.

Control.—Low concentrations of panogen inhibited spore germination of the four fungi in 1% potato-dextrose broth. ED_{50} values of 0.015, 0.022, 0.04, and 0.06 μg/ml were obtained for *D. hawaiiensis, C. senegalensis, C. lunata,* and *D. rostrata*, respectively. Bordeaux mixture, reported as a control of *C. lunata* (16), was not effective in these tests. In greenhouse tests, disease incidence 2 weeks after planting was 18, 13, and 6% in flats spray-drenched at sowing, and 1 week after sowing with 0.1, 1.0, and 10 μg/ml panogen, respectively. Twenty-four per cent of the plants in the flats having no treatment were diseased. Thus, 10 μg/ml panogen was used in further testing. No phytotoxicity was associated with fungicide applications.

Adequate control was obtained in greenhouse flats inoculated with the four pathogens by spray drench applications of 10 μg/ml panogen at planting, emergence, and 1-week intervals until transplanting (usually 4 to 5 weeks after planting) (Table 1). Applications only at sowing or at sowing and emergence gave initial control, but later failed to stop the spread of disease. Likewise, a single application at emergence failed to give control.

DISCUSSION.—*Curvularia senegalensis* (Syn. *Brachysporium senegalense*), pathogenic on at least five gramineae (12), and *Drechslera hawaiiensis* (Syn. *Helminthosporium hawaiiense*), pathogenic on rice (7), have not been previously reported on sugarcane. Both fungi were less pathogenic than *D. rostrata* or *C. lunata. Dreschlera rostrata* (Syn. *Helminthosporium rostratum, Dipolaris rostrata*) causes a leaf spot on sugarcane (4, 11) and on other gramineae (12, 17). It is also the causal agent of a severe blight of *Cynodon dactylon* (14), but has not been associated with seedling blights on sugarcane. *Cochliobolus lunata* (Syn. *Curvularia*) has a wide host range (12), and is responsible for a rice (1) and a sugarcane (2) seedling blight. This is a first report of *D. rostrata* and *C. lunata* on sugarcane in Hawaii.

Losses from these diseases apparently vary according to the physiological status of the host. Unfavorable growth conditions predisposed sugarcane seedlings to *H. sacchari* (8). We observed greater losses in seedlings that had been subjected to apparent nutrient stress or deep transplanting, and on plants germinating from 1-year-old fuzz.

Evidence would indicate that the pathogens are carried in the fuzz and introduced into the flats at

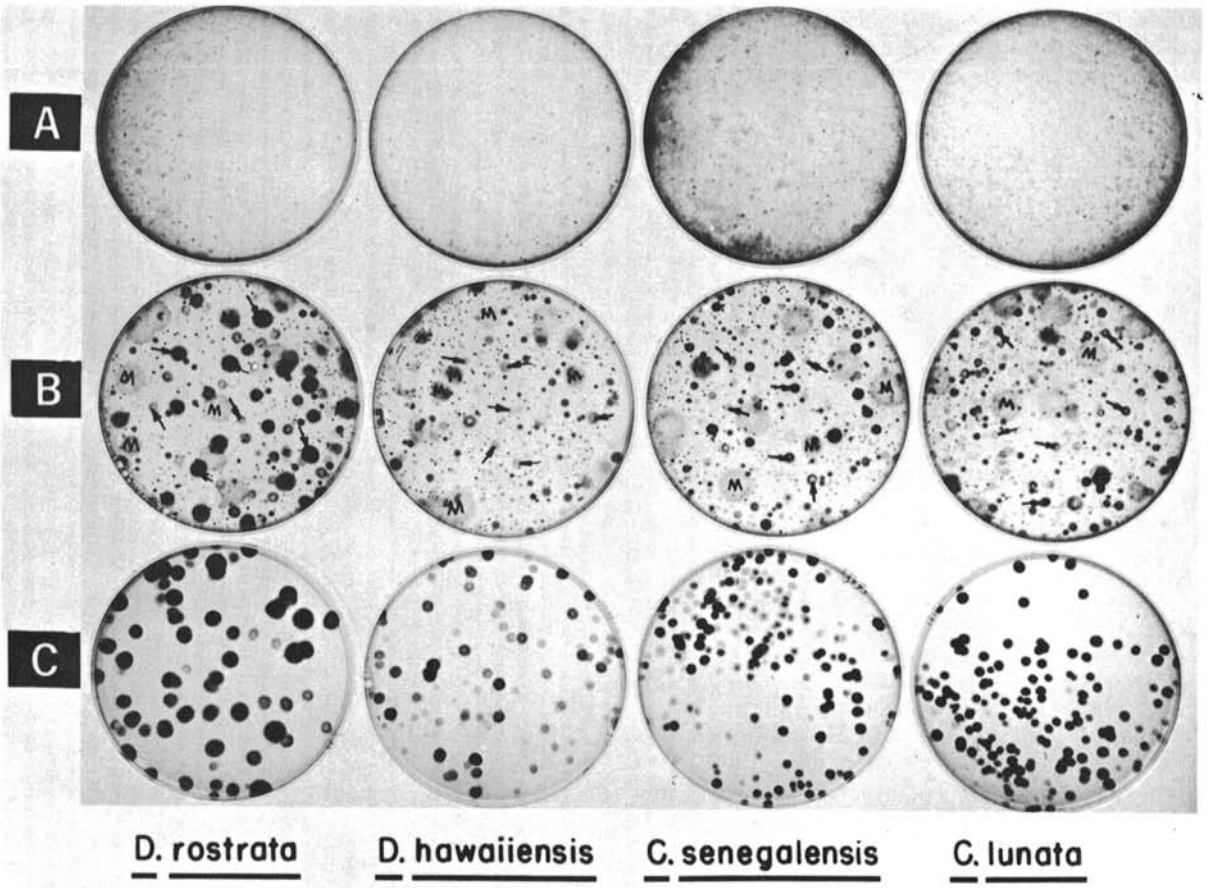

Fig. 3. Comparison of selective medium (BNSCLA) to cane leaf agar (CLA) for the recovery of seedling blight pathogens from fuzz washes. **A)** CLA with additions of 0.1 ml fuzz wash and 0.1 ml pathogen spore suspension (an average of 2.1×10^5 bacteria and 5.9×10^4 nonpathogenic fungi/plate); **B)** BNSCLA with additions of 0.1 ml fuzz wash and 0.1 ml pathogen spore suspension (an average of 240 bacteria and 13 nonpathogenic fungi/plate); **C)** BNSCLA with addition of 0.1 ml pathogen spore suspension. Arrows identify several pathogen colonies and (M) indicates several *Mucor* spp.

TABLE 1. Incidence of seedling blight of sugarcane affected by times of spray drench applications of 10 μg/ml panogen in artificially inoculated flats

Application time of of panogen	Disease incidence following planting [c]		
	9 days	21 days	28 days
Days	%	%	%
None	47	55	49
0[a]	11	38	49
0, 4[b]	5	18	48
0, 4, 11	3	9	23
0, 4, 11, 18	3	8	12

[a] 0 day indicates application at sowing.
[b] Plants emerged 4 days after sowing.
[c] Average of four replications, including 200 seedlings in each.

sowing. Alternate hosts of these pathogens, maize, *Cynodon dactylon*, papaya, rice, banana, citrus, mango, and *Eleusine indica*, grow in the vicinity of the breeding station, in addition to the 4,200 clones of sugarcane representing five species. It seems likely that the moist conditions normally present during the flowering season would favor sporulation and spread of these fungi. Wind-borne spores are probably trapped in the inflorescence, and remain in the fuzz until planting. Spores of *D. rostrata* and *Curvularia* sp. were observed in washes of the fuzz, and all four pathogens were isolated from fuzz. It is also possible that the spores may germinate and the fungi establish themselves either parasitically or saprophytically in the tissues of the old flower parts, to be introduced in a mycelial state. Loveless & Smith (8) suggested that dormant mycelium rather than spores were normally responsible for initial infection of seedlings by *H. sacchari*.

Once introduced into the flats, the pathogens may be disseminated readily. The methyl bromide-treated soil probably aids in their saprophytic activity, as many of the soil competitors have been eliminated. Abundant sporulation of *D. hawaiiensis* was observed on the surface of seedling flats. *Drechslera rostrata* has the ability to grow over straws placed on natural soil (10), and would be expected to spread rapidly in the flats. Splashing water from surface irrigation would also contribute to dissemination.

The possibility exists that the selective media used to isolate these pathogens from fuzz could be modified to isolate *Helminthosporium* and *Curvularia* spp. from soil. Benomyl was effective in controlling most of the fungi associated with the fuzz. Bollen & Fuchs (3) recently compiled a summary of the reaction of numerous fungi to benomyl, and suggest its use in selective media for benomyl-tolerant fungi. They indicated, however, that it would have limited use in selective media for soil organisms, because Zygomycetes are tolerant to benomyl and are universally present in soil. However, we found that additions of NPX, as previously reported (15), would sufficiently reduce the growth of *Mucor* sp. so that pathogenic fungi could be isolated and identified. We have also used benomyl successfully in a selective medium for *Pythium* (5). The control of these seedling blights as outlined in this report is now being successfully used as routine practice at this experiment station.

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