Technical Papers

A *Physoderma* Disease of Barnyard Grass

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Leaves of barnyard grass *Echinochloa crusgalli* (L.) Beauv. collected near Patna, Bihar, India, showed the presence of numerous brownish linear spots 1–2.5 mm long. The spots were distinct and aggregated, imparting a pale yellow color to infected leaves. Infections were localized and not systemic.

P. maydis are pustulate and erupt, releasing free sporangia, whereas in the *Physoderma* species on barnyard grass the sori are nonerumpent, and resting sporangia are probably released on decay of the host tissue.

Cross-inoculation experiments were carried out at College Station, Texas, using resting sporangia from corn, which were available in abundance. Seed of *E. crusgalli, E. walteri* (Pursh) Hellar, and *E. colonum* (L.) Link from five localities, and corn as a check, were planted in 6-in. clay pots in which the sterile soil had been inoculated by mixing with finely ground infected corn-leaf sheaths containing numerous rest-

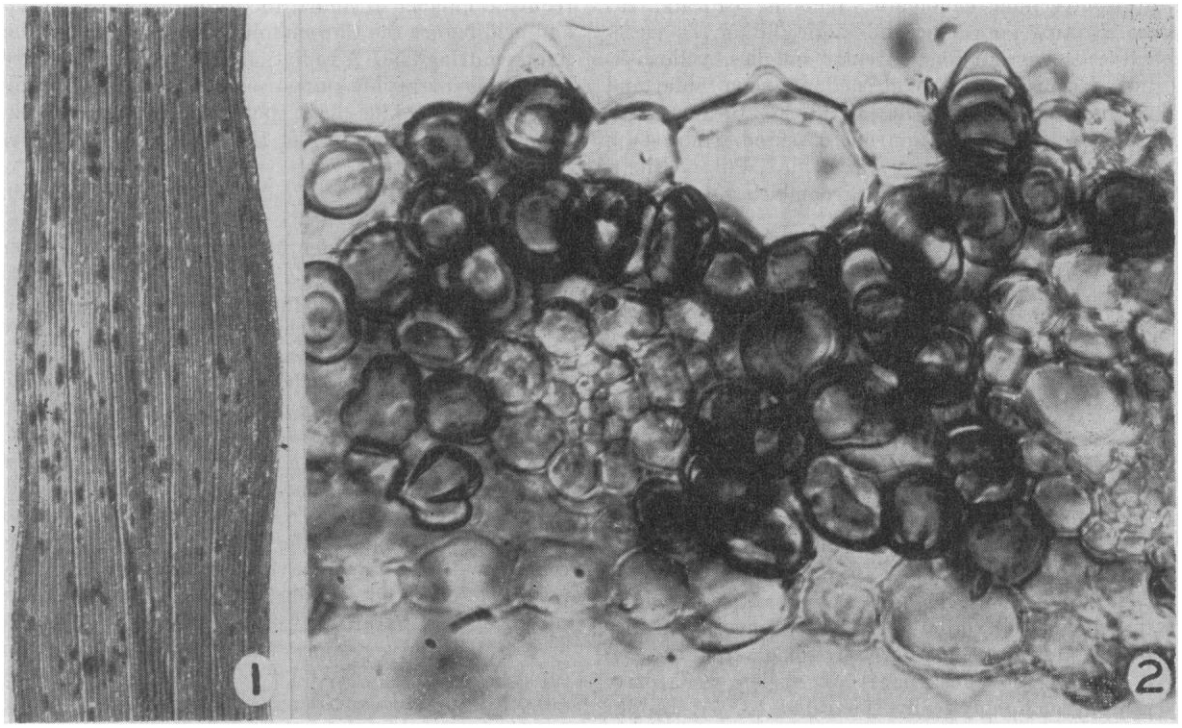

FIG. 1. Enlarged view of infection spots on leaves of *E. crusgalli* (× 7).

FIG. 2. Photomicrograph showing intracellular resting sporangia (× 320).

Microscopic examination of infected material stained with acid fuchsin in lactophenol revealed the presence of a *Physoderma* with tenuous rhizomycelium traversing the host cells, and numerous intracellular resting sporangia. Mature resting sporangia were yellowish-brown, ovate-elliptic, flattened on one side, and measured 18–26 × 10–16 μ. The epispore is smooth and no haustorial processes or appendages have been noticed.

The *Physoderma* species on barnyard grass was collected in corn fields where the corn was infected with *P. maydis* Miy. Comparative morphological studies indicated that the two are separate species. Apart from differences in the sizes of resting sporangia, the sori of

ing sporangia. At the fifth leaf stage, the plants of both corn and barnyard grass were inoculated hypodermically into the growing point with a suspension of resting sporangia of *P. maydis.* Infection readings were made 30 days after hypodermic inoculation. The inoculated corn plants showed severe infections with *P. maydis,* but no abnormalities occurred on barnyard grass. Companion experiments were carried out in India, where the sporangia from barnyard grass were used to inoculate corn plants, and barnyard grass was used as a check. No infections were obtained on corn, whereas the barnyard grass check plants became heavily infected. The fact that no barnyard grass has been

noted to be infected by *Physoderma* in Texas, although corn becomes severely infected with *P. maydis,* indicates that barnyard grass is not a collateral host for *P. maydis* and that the *Physoderma* observed in India is a separate species. None of the graminicolous species of *Physoderma* given by Karling (*1*) are morphologically similar to the one under study. The fungus is presented as a new species with the name *P. echinochloae.*

Physoderma echinochloae sp. nov.[1] Rhizomycelium endobioticum, tenue, delicatulum, ramosum; sporangia perdurantia endobiotica, cellulas mesophylli fasciculos vasculares circumdantes implentia, luteo-brunnea, hemisphaerica, in latere uno applanata et operculum circumscissilem retegentia, 18–26 × 10–16 μ; episporium leve; germinatio non visa. Hab. in foliis *Echinochloae crusgalli,* Patna, Bihar, India.

Rhizomycelium endobiotic, tenuous, delicate, ramose. Resting sporangia endobiotic, filling the mesophyll cells surrounding vascular bundles, yellowish-brown, hemispherical, flattened on one side and revealing the circumscissile lid, 18–26 × 10–16 μ. Epispore smooth. Germination not observed. Habitat on leaves of *Echinochloa crusgalli* Beauv., Patna, Bihar, India, 12-7-1952, leg. M. J. Thirumalachar.

Reference

1. KARLING, J. S. *Lloydia,* **13**, 29 (1950).

[1] The authors are indebted to Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, U.S.D.A., for the preparation of the Latin diagnosis.

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Separation of the Purines and Pyrimidines by Ionophoresis on Filter Paper

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The technique of ionophoresis on filter paper is being extensively employed in separations of various types of mixtures, as reviewed by some authors (*1–3*). Nucleotides, the constituents of nucleic acid, have also been ionophoretically separated (*4, 5*). In this communication successful separations by ionophoresis on paper of the purine and pyrimidine bases, adenine, guanine, cytosine, thymine, and uracil, the building units of nucleic acids, are described.

The equipment used for this purpose was essentially the same as described by Durrum (*6*) with slight modifications and simplifications. A jar (used as a dome for candles) is inverted over a wooden base coated with paraffin. The opening at the top of the jar is ordinarily kept closed by a cork. One or more paper strips (each 1 cm wide and 57 cm long) of Whatman 1 filter paper are supported over a horizontal glass rod within the jar, the ends of the papers dipping into the buffer or any other conducting solution, contained in two different containers. The level of the liquid in the two containers is made the same by temporarily connecting them through a siphon tube. Voltage is applied from the mains (220 v) through platinum electrodes. A milliammeter is connected in a series to record the current passing through the strips. After rinsing the paper strips with the conducting solution and adjusting the level of the liquid, the mixture to be separated is applied in state of solution from a micropipet at the apex of the supported strip (apex height about 28 cm) by temporarily opening the cork at the top. Usually 0.01 ml of the solution containing 5–15 μg of each of the purine and pyrimidine bases was found to be sufficient for the purpose. For advantages of detection, the constituents of the mixture usually were run individually side by side with the mixture on separate strips of paper. After passage of current for the requisite number of hours, the papers were taken out and held before the Chromatolite lamp (an ultraviolet lamp emitting 2537 A radiation and specially designed for chromatographic purposes). Purines and pyrimidines appear as blue-black spots on a fluorescent background due to quenching of the fluorescence of paper in those regions (*7*).

Since purines and pyrimidines are mainly basic in character, buffers of acidic pH were tried as conducting medium for ionophoretic separations. After trials, citric acid-phosphate buffer of pH 2 was found to be suitable for the purpose. An average current of 0.15 ma flowed through each strip when 220 v were applied. Guanine, adenine, and cytosine were found to move toward the cathode whereas thymine and uracil remained practically stationary at the original starting line. Thus, separation of thymine and uracil was not possible under such conditions; fortunately, however, they do not occur in the same nucleic acid. They can be separated from each other using other conducting medium. Of the remaining three, guanine was slowest and cytosine the fastest, with adenine rather close behind cytosine. Though 2 hr were quite sufficient for separation of either guanine and adenine or guanine and cytosine, 6–8 hr were required for separation of adenine and cytosine. An 8-hr-run of a mixture of uracil (or thymine), guanine, adenine, and cytosine was found to be quite sufficient for their neat separations. The distances traveled by the components are found to vary slightly from experiment to experiment. Average distances are: uracil and thymine, 0 cm; guanine, 11–12 cm; adenine, 15–16 cm; and cytosine, 17–18 cm.

Thymine and uracil were separated from each other by using alkali solution as the conducting medium. The bases were applied as sodium salts by dissolving them in alkali. Using 0.1 *N* alkali, the two separate in about 8 hr, uracil being a bit ahead of thymine (uracil, 10–11 cm; thymine 9–10 cm). Runs for longer hours did not improve the results, but instead created disadvantages due to considerable electrolysis. Using stronger alkali (0.2 *N*), separation can be effected in a shorter period (4 hr), uracil being a bit ahead of thymine. (uracil, 4–5 cm; thymine, 3–4 cm). In this

[1] My sincerest thanks are due to Dr. D. M. Bose for his kind interest and encouragement.