and slender roots (2–3 in number) appear, though much later than the shoots.

Cytokinin induced shoot buds, on being transferred to the basal medium, grew into healthy and vigorously-growing plants. Roots were formed extensively. The leaves also developed a characteristic dark-pink pigmentation on the abaxial surface.

Auxins, when tried alone, failed to evoke any response with regard to shoot bud formation, except with IBA (at $10^{-5}$ M), ca 18% cultures formed a few shoots on the cut end of the leaf. Root formation was profuse at the proximal cut end in all the three auxin-supplemented media (Fig. 1D). The anti-auxin tri-iodobenzoic acid, at 1 and 5 ppm, was without any effect in inducing shoot buds. Gibberellic acid supplemented to the medium did not favour shoot bud induction but, at a lower concentration, in a few cultures (ca 16%) rooting was noted.

According to earlier reports, isolated leaves of this species can regenerate roots but are unable to form buds. In leaves of a cultivar, 'Sidse1' of *Begonia semperflorens* cultivar, Heido reports 40% bud formation in controls, using 4 cm long leaves. In his treatment, leaves were submerged in test solutions for a minute, and planted in perlite-peat mixture; distilled water treatment served as control. In the present investigation no bud formation occurred on Nitsch basal medium, with excised laminae using aseptic culture techniques. Heide showed that, with increasing concentration up to 100 $\mu$M of BAP, shoot bud formation was increased to about 68%, but higher concentrations tended to suppress this promotion.

As regards bud formation, the present study undoubtedly stresses the necessity of a cytokinin. Auxins employed alone fail to achieve induction of shoot buds. Similar were the results of Ringe and Nitsch who found simultaneous application of a cytokinin as well as an auxin necessary for the shoot bud formation on leaf discs of *B. multiflora* and *B. evansiana*. But the slight promotive effect of auxins in low concentrations, noted in their studies without the addition of cytokinin, could not be confirmed with *B. semperflorens* (present study).

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Department of Botany, University of Delhi, Delhi-7, India, March 3, 1973.

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Not seen in original.

**A NEW ENTOMOGENOUS FUNGUS ON THE ROOT GRUB, HOLOTROCHICA SERRATA F. FROM MYSORE**

The root grub, *Holotricha serrata*, is widely distributed throughout the country. It is a serious pest on sugarcane, groundnut, pulses, vegetables, oilseeds, areca, coffee and millets. Recently, the root grub infected with the fungus was collected and isolated from an areca garden in N. Kanara District of Mysore State. The fungus has been identified as *Beauvaria bronchii* (Sacc.). Petch, and this has earlier been isolated from locusts in Algeria by Brongniart in 1891 and reported as *Botrytis sp.* In 1892, Saccardo referred it as *Botrytis bronchii*. Petch in 1926 transferred this species to *Beauvaria* and named it as *Beauvaria bronchii*. Ramaraje Urs et al. (1965) reported *Beauvaria bassiana* (Bals.) Vuill causing disease on cabbage semilooper from Mysore.

The earliest symptoms of the disease on the root grub, *Holotricha serrata*, include a change in colour and the appearance of white patches on the cuticle. The normal colour of the grub is dull white, but when diseased the colour changes to brown. The white patches may appear either posteriorly, anteriorly or more commonly around the spiracles on either side of the body and these patches usually spread later over the other parts. During the later stages a week before death, the grub loses its appetite, and does not feed nor makes any attempt to move. The death results usually within 7–10 days after the initial symptom of infection. Three weeks later, the diseased grub becomes white and almost completely enveloped by mycelium of the fungus (Fig. 1). The body of the insect gets shrunken, wrinkled and finally becomes stiff and mummified. Under high temperature and humid condition, a cottony growth envelops the grub and becomes a white pulpy mass. The fungus attacks all stages of the insect except egg. It can remain viable in soil for a long time.

The pure culture of the fungus of the infected insects was obtained in potato dextrose medium and it sporulated readily in about five days. The spores were oval in shape and measured, $4 \times 2.5 \mu$. 

**Letters to the Editor**
The fungus identified as *Beauvaria brongniartii* has been reported for the first time on root grub *Holotrichia serrata* from India. The pure culture of this fungus has also been deposited in the Commonwealth Mycological Institute, Kew, Surrey, England, and in the collections maintained in the Department of Plant Pathology, Agricultural College, Bangalore.

![Fig. 1. Root grub infected with Beauvaria brongniartii (Sacc.) Petch showing white pulpy mass of mycelium.](image)

Authors are grateful to the Director, Commonwealth Mycological Institute, Kew, Surrey, England, for identifying the fungus.

Dept. of Plant Pathology and Entomology, Agricultural College, Univ. of Agril. Sciences, Hebbal, Bangalore-560024, March 6, 1973.

**LIPASE PRODUCTION BY THE RICE BLAST PATHOGEN**

**Presence** of abundant phospholipid globules in the chlorophyll containing parenchyma cells of rice (*Oryza sativa* L.) leaf tissue and their ready discoloration in response to fungal, bacterial, and viral infection has been recently shown. The tissue composition to which the pathogen is adapted influences the enzymes produced by it. Although the production of lipase by many plant pathogenic fungi has long been known, the ability of the blast pathogen of rice, *Pyricularia oryzae* Cav. to produce lipase and the role of this enzyme in symptom expression is little understood.

Fifty milliliters of Tanaka's medium containing 20 g of glucose (control), 50 g of coconut oil (amendment A) or olive oil (amendment B) per liter as carbon source at pH 7 were placed in 250 ml Erlenmeyer flasks, and sterilized at 15 lb/sq inch pressure for 20 min. The required amounts of thiamine and biotin were then added to each flask under aseptic conditions, inoculated with a 4 mm culture disc of either P26, the least virulent or P150, the most virulent Philippine races of *P. oryzae*, selected on the basis of their pathogenicity on Philippine differential rice varieties and incubated at 28°C for 20 days, after which the exo- and endo-cellular lipase activities were assayed. The growth was determined by weighing the harvested mycelia after drying it for an hour in an oven at 100°C.

Enzyme in the culture filtrate was precipitated by the addition of alcohol to get a final concentration of 80% in a cold room at 4-5°C. The flocculent precipitate formed was separated by filtration after standing for an hour at 0-2°C and dissolved in 10 ml of McIlvaine's buffer at pH 7. The endo-cellular enzyme was prepared by suspending the mycelia in buffer solutions to get 10% extract, homogenized in a Sorvall omni-mixer for 10 min, centrifuged at 3,000 g for 30 min in cold and the supernatant liquid was used as the enzyme source. The protein in the enzyme solutions was determined by the method of Lowry et al.

Lipase activity was determined by the titrimetric estimation of the fatty acids liberated from the substrate. The reaction mixture contained 5 ml of buffer, 5 ml of 10% egg albumin and one milliliter of the enzyme extract. The blanks were incubated without the enzyme which was added immediately before titration. After incubation at 36°C for 5 hr with occasional shaking, 25 ml of 1:1 alcohol-acetone mixture were added and titrated against 0.05 N NaOH, using phenolphthalein as indicator. Activity is expressed as the number of lipase units.