Infection of Rice Brown Planthopper, 
*Nilaparvata lugens* (Homoptera: Delphacidae), 
by Field Application of Entomopathogenic Hyphomycetes (Deuteromycotina)

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ABSTRACT Five entomopathogenic Hyphomycetes were tested under field conditions for 
biological control of brown planthopper, *Nilaparvata lugens* (Stål), in rice. Suspensions of 
conidia of *Metarhizium anisopliae* (Metsch.) Sorokin, *M. flavoviride* Gams & Roszpal, 
*Beauveria bassiana* (Bals.) Vuill., and *Hirsutella citriformis* Speare were applied at a rate 
of 4–5.107 conidia per ha. In addition, *M. anisopliae* and *Paecilomyces lilacinus* (Thom) 
Samson were applied as preparations of dry mycelium at a rate of 1.5–2 kg/ha. Mortality 
due to fungus infection ranged from 63 to 98% 3 weeks after application. There were no 
consistent differences between fungus species. The mycelium preparation sporulated on the 
plant and was as effective as the conidia suspension in infecting brown planthopper. Hy-
phomycetous fungi should be evaluated further for control of brown planthopper in rice.

KEY WORDS *Nilaparvata lugens*, rice, fungi, biological control, mycelium application

DURING RECENT decades, brown planthopper (BPH), *Nilaparvata lugens* (Stål), has risen from a 
sporadic occurrence to the status of a major pest of rice in many areas of tropical Asia. There are 
several reports of the effectiveness of natural bio-
logical control of BPH (e.g., Rao 1983, Greathead 1983, Chiu 1979). However, present control tactics 
focus mainly on the introduction of resistant rice 
varieties and on application of chemical pesticides 
(Heinrichs 1979). Unfortunately, in some areas new 
biotypes of BPH have developed that overcome 
resistance in the host plant (Pathak 1975). Also, 
serious resurgence of the pest resulting from treatments with various chemical pesticides continues to be a common phenomenon (Heinrichs & Mochida 1984, Kenmore et al. 1985). Therefore, to help ensure long-term control of BPH, more ecologically sound integrated pest management programs should be developed and implemented.

A complex of fungal pathogens, including various hyphomycetous and entomophthoralean fun-
ghi, has been identified from BPH populations (un-
published data). Previously published studies on the use of entomogenous fungi for BPH control are limited to experiments using small containers in the laboratory (Srivastava & Nayak 1975). In our study, tests with several hyphomycetous pathogens for the control of BPH were conducted in field plots. Treatments included applications of conidial suspensions of *Metarhizium anisopliae* (Metsch.) Sorokin, *M. flavoviride* Gams & Roszy-
pal, *Beauveria bassiana* (Bals.) Vuill., and *Hirsu-
tella citriformis* Speare. In addition, mass-pro-
duced preparations of dry mycelium of *M. anisopliae* and *Paecilomyces lilacinus* (Thom) 
Samson were tested.

**Materials and Methods**

**Field Plots.** A variety of a Korean (BPH-sus-
cceptible) glutinous rice was transplanted to a field near Victoria (Laguna Province, Luzon, Philip-
pines). The experiment was carried out during the rainy season with medium to heavy rains mostly in the afternoon. About 3 weeks before the start of the experiment, the pyrethroid insecticide Cy-
permethrin was applied at half the recommended rate to eliminate natural enemies and induce re-
surgence of the pest (Heinrichs & Mochida 1984). The field was divided into 25 plots, each 4 m2 and separated by 2-m intervals. A few days after insecticide application, potted rice plants infested with large numbers of BPH were introduced at the center of each plot; this ensured development of BPH populations. BPH from the potted plants were allowed to develop to the next generation; mixed BPH populations of different instars and adults were present at the start of the experiment. Each of seven fungal treatments was applied in three plots; four control plots were treated with water. Treatments were arranged in a randomized complete block design. A gauze cage enclosing 12
hills of rice (ca. 1 m²) was erected in the center of each plot after application of the pathogens.

Preparation of Fungal Materials. *M. anisopliae* (Ma), *M. flavoviride* (Mf), two isolates of *B. bassiana* (Bb and Bb252), and *H. citriformis* (Hc) were applied as suspensions of conidia. *M. anisopliae* (Mam) and *P. lilacinus* (Plm) were also applied as dry mycelium. Origins of the fungal isolates are given in Table 1.

Conidia of the fungi were produced on standard mycological media: Ma and Mf on Emerson’s YPS agar and Bb and Hc on Sabouraud dextrose agar enriched with 1% yeast extract. Conidia were washed from the surface of the plates by 75–100 ml of a solution of 0.02% Tween80. Concentrations of the conidia were determined with standard hemocytometer techniques. Over 98% of the conidia germinated in Sabouraud dextrose broth after 24 h incubation on a rotary shaker in viability tests just before field application.

Conidia of *H. citriformis* are difficult to produce due to low sporulation rates, slime production of the mycelium, and irregular growth patterns. Therefore, a mixture of conidia and mycelium particles of *H. citriformis* was applied in this experiment.

Conidia of strain Bb252 were produced on wheat bran by a small mass-production unit using a diaphasic fermentation process. The material used in these experiments was produced in 1982 and stored at 4°C for >3 years. Over 95% of the conidia germinated in viability tests in a liquid medium.

Mycelium (Mam and Plm) was produced in 12-1 bubbler-type fermentors in a molasses (1.5%) and yeast extract (1.5%) broth. The mycelium was dried, milled, and stored at −20°C for >1 month. This production method is similar to the marcescent process described in detail by McCabe & Soper (1985) for entomophthoralean fungi. Before application, samples of the mycelium were rehydrated and incubated on moist filter paper in a petri dish for 4 days; >95% of the mycelial particles sporulated.

Application of Fungal Materials. Before application in the field, the conidial suspensions were diluted to the appropriate concentrations with water. Two liters of conidial suspension (treatments Mf, Ma, Bb, Bb252) were applied to each plot by knapsack sprayer at a rate equivalent to 4–5×10⁶ conidia per ha. A similar rate of combined conidia and mycelium particles of Hc was applied. The dry mycelium preparations were soaked in water for several hours before application. The moist cake was crumbled by hand onto the plants in the center of the hills within each plot at a rate of 0.5–1 g per plot. Because of the inefficient application method, this rate was estimated to be equivalent to ca. 1.5–2.0 kg of dry mycelium per ha.

Evaluation of Effects. Counts of live and infected BPH were made from 12 hills within each cage and from 12 hills outside each cage from each plot. Counts were taken 1 day before the application of the pathogens and at 7, 14, and 21 days thereafter. The last count was done a few days before harvest of the rice. Infections of BPH by entomogenous Hyphomycetes are typical and can be recognized easily in the field; the fungi affix the dead insects to the plant. Field samples, which were regularly brought back to the laboratory, confirmed these identifications. At week 0 there were differences in starting numbers of BPH be-

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ARSEF numbers refer to the Collection of Entomopathogenic Fungi, USDA-ARS Plant Protection Unit, Boyce Thompson Institute for Plant Research at Cornell, Ithaca, New York.
between the replicates. For this reason, comparison of numbers of live insects in the different treatments was not possible. Comparison of infection levels avoided these differences in insect densities because percentages are compared.

Differences in mortality levels between the treatments were analyzed on a weekly basis. Analysis of variance (ANOVA) was applied to the mean percentage mortality (arcsine transformed) data from the three fungus treatments and four control replicate cages. Significance was accepted at $P = 0.05$.

### Results and Discussion

The ranges of the percentage mortality due to fungus infection are given in Table 2, the results of the ANOVA in Table 3. Outside the cages, significant differences between fungal treatments and the control were present 7 days after treatment; however, general infection rates were low, and these differences are not of practical value. Inside the cages, no differences were observed at that time. Fourteen and 21 days after treatment, the infection rates of BPH inside and outside the cages were obviously higher in all fungus-treated plots compared with the control.

There were no consistent differences in infection rates between the fungal treatments at 14 or 21 days after treatment. This included the treatments with fungi isolated from hosts other than BPH, Mam, Plm, and Bb252, and treatments with dry mycelium, Mam and Plm. By 21 days, 100% mortality had occurred in many replicates. There was a slightly larger variation between the fungal treatments inside compared with outside the cages. The reason for this is not known. A low (<10%) mortality of BPH in the control plots was caused by *M. flavoviride* from a natural inoculum.

Cameron (1969) proposed application of mycelial particles as field inoculum. Using this method, the fungus *H. thompsonii* Fisher was successfully applied in the field by McCoy et al. (1971, 1975) against the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead). In general, mycelia of entomogenous fungi do not infect the host, but under suitable conditions of temperature and relative humidity, infective conidia are produced by the mycelium in the field. This use of mycelium greatly simplifies the mass-production process, because the solid phase (the production of conidia by mycelium on a solid substrate) is eliminated. Therefore, the present results might provide a basis for future mass production and application. In our experiments, sporulating mycelium fragments were observed sticking to the rice plants over periods <1 week after application. The effect of this continuous supply of infective conidia on the development of mycosis in the BPH populations should be further investigated.

The dosage equivalent to $4\times10^3$ conidia per ha applied in the treatments Ma, Mf, and Bb was sufficient to cause infection. It may be that lower dosages than used in this experiment are also effective. The rate of 1.5–2.0 kg of dry mycelium was probably an overdose. Significant control levels can be achieved using rates as low as 700 g/ha (unpublished data). With better formulations and more efficient application methods, much lower effective dosages are expected.

It is not certain whether all fungi and preparations will be similarly effective in controlling the BPH population under other conditions of relative humidity, temperature, insect density, and insect stress since these factors are known to be important in infection and subsequent development of epidemics in insect populations (see various authors in Burges [1981]). The possible role of these organisms in the biological control of BPH should be further investigated.

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### References Cited


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