

Note

## Control of *Herpomyces* spp. (Ascomycetes: Laboulbeniales) infection in the wood cockroach, *Parcoblatta lata* (Dictyoptera: Blattodea: Blattellidae), with benomyl

The Laboulbeniales are a group of obligate parasitic ascomycete fungi that grow on the cuticle of arthropods. Most of the over 2000 species have been described from adult Coleoptera and Diptera, but the genus *Herpomyces* is found exclusively on cockroaches (Guthrie and Tindall, 1968; Richards and Smith, 1956; Roth and Willis, 1960). The haustoria of *Herpomyces* penetrate the cuticle of the cockroach, entering the underlying cells and causing a dermatitis with characteristic histopathology; however, there is no evidence of pathogenicity (Richards and Smith, 1956).

Nymphs and adults of wood cockroaches, *Parcoblatta lata* (Brunner), developed a severe *Herpomyces* spp. infection in our laboratory colony. The thalli were visible with the naked eye and grew in large numbers on the antennae, palpi, and cerci and in lower numbers on the legs, head, pronotum, thorax, and abdomen. The fungus did not cause high mortality, but many individuals had broken antennae, palpi, and cerci and some exhibited unusual color patches on their cuticle. Heavily infected individuals moved slowly and became moribund indicating, in contrast to the study of Richards and Smith (1956), a pathogenic effect of *Herpomyces* spp. on the cockroach colony. The aim of this study was to test the efficacy of the fungicide benomyl [methyl 1-(butylcarbamoyle)benzimidazol-2-yl-carbamate] as a treatment against *Herpomyces* spp. infecting *P. lata*. We chose this chemical because it exhibits relatively low toxicity to mammals (EPA class IV—practically nontoxic), it is in widespread use in agriculture, and it has been reported to be effective against microbial infections (microsporidia) in insects (Brooks et al., 1978). We tested the antifungal properties of several concentrations of benomyl in different formulations, as well as the toxicity of benomyl to healthy cockroaches.

Nymphs were reared in clear-plastic containers with an egg carton shelter. Drinking water was supplied in glass tubes with a cotton stopper; diet consisted of dog chow (Purina Dog Chow Nutritional Excellence Formula, Purina Mills, St. Louis, Missouri) provided ad libitum. The rearing room was maintained at  $27 \pm 1^\circ\text{C}$

under a 16L:8D photoregime. Benomyl (Sigma–Aldrich, St. Louis, Missouri) was given to the cockroaches mixed with agar diet, dry powder diet, or water. To prepare the agar diet 133 g of dog chow and 10.66 g of agar were added to 1 L water, mixed in a blender and autoclaved. Benomyl was dissolved in 10 ml of 10% ethanol in water and mixed with the diet after it cooled down to  $60^\circ\text{C}$ . The diet was poured into Petri dishes, and stored at  $4^\circ\text{C}$  until use. Three concentrations of benomyl were used: 250 ppm (250 mg/L, labeled B1), 500 ppm (500 mg/L, labeled B2) and 1000 ppm (1000 mg/L, labeled B3). Control agar diet was prepared in the same manner but without benomyl. To prepare the dry food diet the dog chow was sieved, autoclaved and mixed thoroughly with benomyl (374 mg/100 g dog chow, similar concentration to B2). Control dry food was prepared the same way but no benomyl was added. The benomyl-water treatment was prepared by dissolving benomyl in 10% ethanol and adding water to make 500 mg benomyl/L (equivalent to B2). Insects treated with benomyl water were fed the control dry powder food. Benomyl water was provided in glass vials with cotton plugs. All experiments were carried out in clear plastic containers with fine mesh metal screen on the lids for ventilation. The floor of the cages was lined with recycled paper towels for shelter; water was provided in glass vials with a cotton plug. The agar diet was cut into cubes ( $\sim 1\text{ cm}^3$ ) and placed on small aluminum foil sheets. Both the diet and the foil were replaced every 1 or 2 days. The dry food powder was placed directly on the paper towels and supplied ad libitum.

Late instar nymphs (4th instar and older) were used in the experiments. Twenty-five cockroaches were placed into a plastic container (20 cockroaches for the benomyl water treatment). Each treatment was replicated in three different containers. The colonies were maintained in the rearing room and their position on the shelf was randomized.

Insects were scored for presence or absence of fungus at the beginning of the experiment (pre-treatment group) and 50 days after the start of the experiment.

The initial estimate of the percentage of infected individuals in the lab colony was made from 50 randomly selected individuals. The presence of fungus was scored on CO<sub>2</sub>-anesthetized insects with the aid of a compound microscope. To determine if benomyl causes mortality in healthy nymphs, two benomyl concentrations in dry food, equivalent to B1 and B2 (187 and 370 mg/100 g dog chow, respectively), were prepared. As a control diet we used dog chow alone. Twenty healthy cockroaches per cage, three cages per treatment, were used and kept in an environmental chamber maintained at 27 ± 1 °C and a 16:8 L:D photoregime. The position of the cages on the shelves was randomized and mortality was recorded three months after the beginning of the experiment. All the statistical comparisons in this study were analyzed with the Ryan's multiple comparison test of proportions (significance level = 0.05, Ryan, 1960). Separate analyses of percentages of infection were performed on the agar and the dry food treatments.

The fungus growing on the cockroaches was identified as *Herpomycetes* spp. (Alex Weir, SUNY College

of Environmental Sciences, New York). Cockroaches treated with benomyl showed a marked decrease in the levels of infection whereas the untreated control insects had equal or greater percentages of infection than the pre-treatment group (Figs. 1 and 2). At the beginning of the experiment the percentage of infected individuals ranged from 66 to 72%. After the 50-day treatment period the levels of infection of insects treated with benomyl in agar diet decreased significantly to ≤ 10%, with the exception of antennae, where percentages of infection ranged from 14 to 49%. The antenna was the only body part that showed a linear response to changes in the concentration of benomyl in diet (Fig. 1).

Cockroaches fed dry food control diet had similar percentages of infection to those of the agar food control and to the pre-treatment sample (Fig. 2). The antennae of insects treated with benomyl in drinking water exhibited similar percentages of infection as the controls (62 and 82%, respectively), whereas in the rest of the body benomyl in water reduced infections below 27%. Benomyl mixed with dry food (500 ppm) provided the

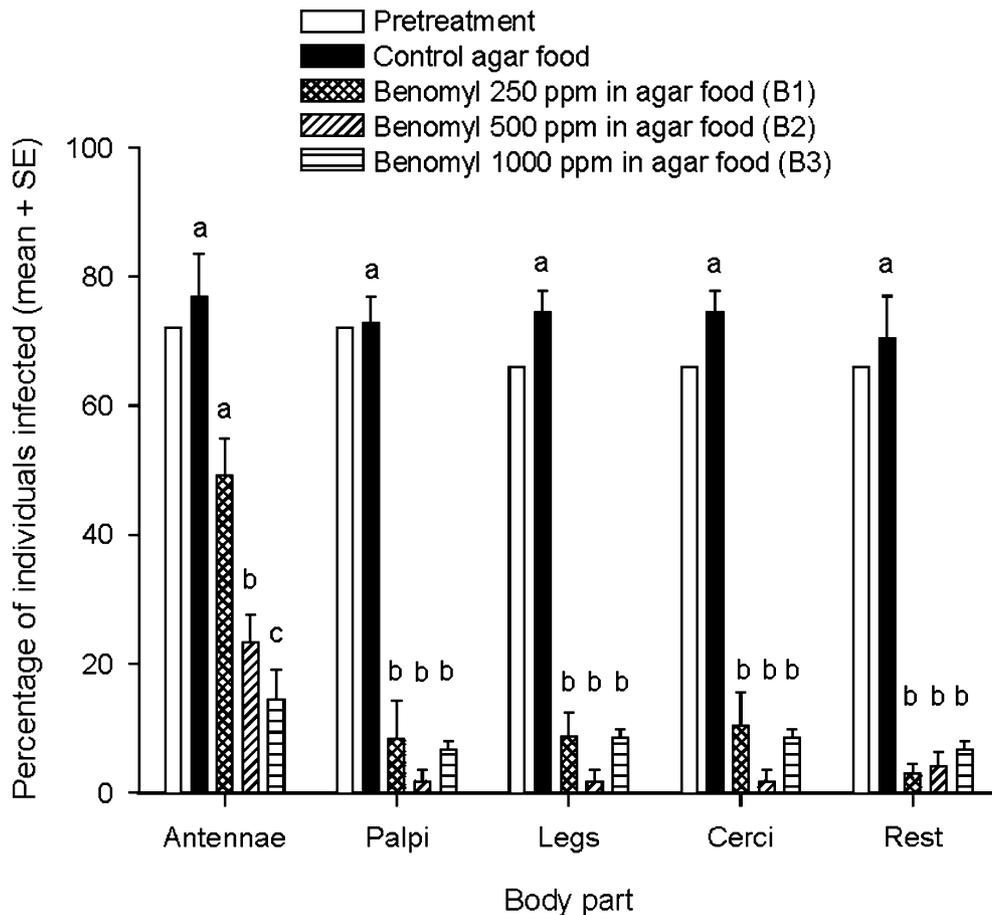


Fig. 1. Percentage of *P. lata* nymphs infected with *Herpomycetes* fungus before and after treatment with several dosages (B1, B2, and B3) of benomyl mixed in an agar diet. Different letters above the bars indicate significant differences ( $P < 0.05$ ) among the treatments, within each body part (Ryan's multiple comparison of proportions test).

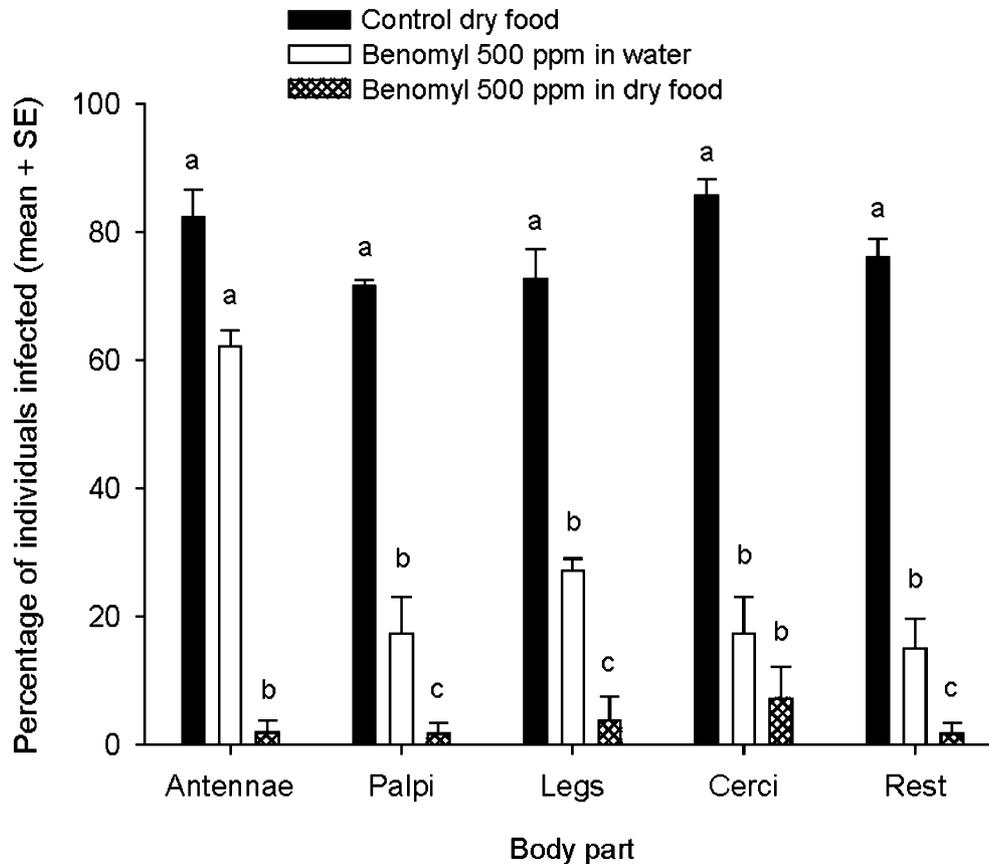


Fig. 2. Percentage of *P. lata* nymphs infected with *Herpomyces* fungus after treatment with benomyl mixed in a dry diet or in water. Different letters above the bars indicate significant differences ( $P < 0.05$ ) among the treatments, within each body part (Ryan's multiple comparison of proportions test). Pre-treatment percentages of infection same as in Fig. 1.

best treatment of *Herpomyces* spp. fungus in *P. lata*. Insects in this treatment exhibited the lowest percentages of infection in the antennae in all treatments (2%). The highest percentage of infection was only 7% in the cerci.

When we scored the fungus prevalence in the previous experiment, we also recorded mortality. This ranged from 18 to 37.5%, and it was as high for cockroaches treated with benomyl and as for the untreated controls ( $P > 0.05$ ). In contrast with the high mortality of the infected insects, the uninfected healthy cockroaches treated with benomyl showed minimal mortality. Three months after starting this experiment more than 90% of the healthy nymphs in the B1, B2, and control treatments were alive (mean  $\pm$  SE,  $90 \pm 2.36$ ,  $93.33 \pm 2.72$ ,  $91.66 \pm 1.36$ , respectively), and there was no significant difference among treatments ( $P > 0.05$ ).

Based upon these results, we recommend benomyl as an effective fungicide (374 mg/100 g of dry dog food) to control *Herpomyces* spp. infections in laboratory colonies of *P. lata*. At this concentration benomyl does not cause mortality. The use of benomyl in diet may be

suitable to control *Herpomyces* in beneficial insects, such as lady beetles, where it sometimes causes problems (Welch et al., 2001). Decreasing humidity and insect density, cleaning the cages frequently, removing the exuviae, and eliminating sites where the insects can aggregate, can probably further prevent the spread of *Herpomyces* infection.

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#### References

- Brooks, W.M., Cranford, J.D., Pearce, L.W., 1978. Benomyl: Effectiveness against the microsporidian *Nosema heliothidis* in the corn earworm, *Heliothis zea*. *J. Invert. Pathol.* 31, 239–245.

- Guthrie, D.M., Tindall, A.R., 1968. *The Biology of the Cockroach*. St. Martin's Press, New York.
- Richards, A.G., Smith, M.N., 1956. Infection of cockroaches with *Hepomyces* (Laboulbeniales). II. Histology and histopathology. *Ann. Entomol. Soc. Am.* 49, 85–93.
- Roth, L.M., Willis, E.R., 1960. The biotic associations of cockroaches. *Smithsonian Miscellaneous Collections* 141, 1–470.
- Ryan, T.A., 1960. Significance test for multiple comparison of proportions, variances, and other statistics. *Psychol. Bull.* 57, 318–325.
- Welch, V.L., Sloggett, J.J., Webberley, K.M., Hurst, G.D.D., 2001. Short-range clinal variation in the prevalence of a sexually transmitted fungus associated with urbanisation. *Ecol. Entomol.* 26, 547–550.

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