SALINITY TOLERANCE OF *LEPTOLEGNIA CHAPMANII* (OOMYCETES: SAPROLEGNIALES), A FUNGAL PATHOGEN OF MOSQUITO LARVAE

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Several oomycetous fungi have shown potential as microbial control agents for mosquito larvae. Unfortunately the most promising among them, *Lagenidium giganteum* Couch, cannot infect larvae in water with greater than 1 ppt (part per thousand) of NaCl (Merriam and Axtell 1982, Lord and Roberts 1985). This precludes its use in the salt marsh breeding sites of many important pest species.

Leptolegnia chapmanii Seymour is a water mold that is a virulent pathogen whose host range appears to be restricted to mosquitoes (McInnis et al. 1985). Recently the authors isolated this species from an unidentified *Culex* larva collected from a ground pool at the edge of a salt marsh in Levy Co., Florida. The saline conditions in the area of the pool provided impetus to evaluate the fungus for NaCl tolerance to assess its potential for control of salt marsh mosquitoes. Three facets of salt tolerance were measured. Vegetative growth was tested on agar medium, and infection rates were tested using submersed washed mycelium or previously infected larvae as sources of infective zoospores.

Capacity for vegetative growth in saline was evaluated on 100-mm petri dishes of Sabouraud dextrose agar (SDA). Sodium chloride concentrations in the SDA were 0, 2.5, 5.0, 7.5, 10.0 and 12.5 g/liter (ppt) in the first experiment. A concentration of 15 ppt was added to the second trial, and concentrations of 15 and 20 ppt were included in the third. The SDA was inoculated with 7 mm disks of 1-week-old cultures on agar containing sunflower seed extract (SFE) diluted to 1 g/liter of protein content. Five replicates were run for each treatment in each experiment. Radial growth was measured after 24 and 48 hr of incubation at 25–27°C.

The results of the above experiments indicate that NaCl at concentrations up to about 5 ppt enhances mycelial growth (Fig. 1). Higher salt concentrations are progressively inhibitory, with growth failing to occur at 20 ppt. This is similar to the growth rate pattern of *Lagenidium giganteum* on saline agar (Merriam and Axtell 1982). In practical terms, salinity would not affect parasitic development of the fungus within its host, and it would probably not be an impediment to potential saprophytic growth in the breeding sites of most salt marsh mosquitoes.

The ability to infect mosquito larvae in saline water was evaluated by two approaches using 3-day-old *Aedes taeniorhynchus* (Wiedemann) larvae as the target insects. In the first, mycelium cultured for one week in liquid SFE (0.3 g/liter protein) was washed, strained, and cut into blocks of ca. 10 mg wet weight. The mycelium was submersed in 100 ml of deionized water of the appropriate NaCl concentration in plastic cups and allowed to stand for 18 hr at room temperature (ca. 23°C). After this period, when sporulation was well under way, 20 test larvae were added to each cup. The second experiment was similar except that infected 3rd instar *Culex quinquefasciatus* Say larvae were used in place of washed mycelium. Three larvae that had been infected 18 hr earlier, but not yet bearing extruding hyphae or sporangia, were placed in each treatment cup. The potential zoospore production was ca. 6,700 per ml from cadavers and ca. 1,400 per ml from mycelium. Mortality data for both tests were taken 48 hr after exposure. Low control mortality and microscopic examination verified that nearly all of the mortality was due to fungal infection and that reduction of transmission rates was primarily due to failure to complete zoosporogenesis.

Zoosporogenesis was much more sensitive to salinity than mycelial growth. Regression lines for inhibition of infection are shown in Fig. 2. The inhibitory concentrations for 50% reduction of infection (IC_{50}) (95% confidence interval) were 4.0 (2.76–5.02) for zoospores produced in vivo and 4.7 (4.57–4.91) for zoospores produced in vitro, as calculated by log-probit regression analysis. The slopes and χ^2 values were −3.60 and 3.16 (6 d.f.) and −2.23 and 123.03 (8 d.f.) for in vitro and in vivo sporulation respectively. The variability of the data for zoospores produced in vivo probably reflects the variability of the condition of the fungus in its parasitic state. When infected *Cx. quinquefasciatus* were the zoospore source, some of the mosquito cadavers failed to produce zoospores even in salt-free water, probably due to bacterial invasion through the wounds caused by heavy fungal infection (Steinhaus and Tanada 1970). On the

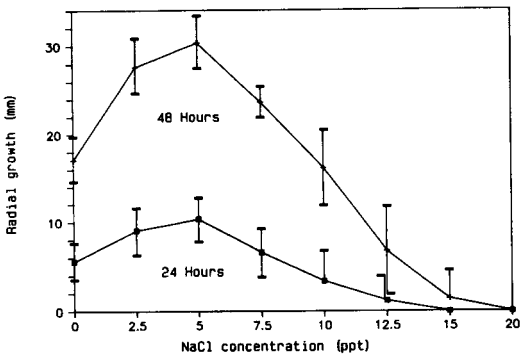

Fig. 1. Mean radial growth (95% confidence interval) of *Leptolegnia chapmanii* mycelium on Sabouraud dextrose agar containing various concentrations of NaCl.

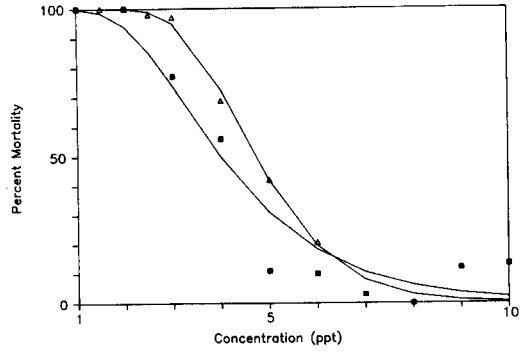

Fig. 2. Inhibition of infection of *Aedes taeniorhynchus* larvae by *Leptolegnia chapmanii* in NaCl solutions when zoospores are formed in *Culex quinquefasciatus* cadavers (■) or from washed mycelium cultured in vitro (△).

other hand, occasional test larvae became infected even in 10 ppt of NaCl, the highest concentration tested. Te Strake (1959) attributed a paucity of saprolegniaceous fungi in estuarine waters to a lack of nutrients. Transmission between mosquito larvae suggests a robust physiological state and rapid development associated with near optimal nutrition provided by the host.

Leptolegnia chapmanii infected mosquitoes in water with NaCl concentrations well above the tolerance limits of *Lagenidium giganteum,* but the difference between rates of infection associated with washed mycelium and infected cadavers was not so pronounced with *L. chapmanii.* Shielding of the fungus from a saline environment by the host's integument apparently enhances in vivo transmission of *Lagenidium giganteum* (Lord and Roberts 1985). In this case, sporangia are formed within the cadaver, and only the final stage of zoospore differentiation takes place externally. With *L. chapmanii,* the entire sporulation process is external to the host and is therefore subject to solute inhibition.

The salinity of many breeding sites for salt marsh mosquitoes ranges from less than 1 to occasionally above 20 ppt (Peterson and Chapman 1970). The results of this study indicate that salinity would be a limiting factor to achieving *L. chapmanii* infections in some but certainly not all salt marsh habitats.

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