LABORATORY BIOASSAY OF *BACILLUS THURINGIENSIS* (H-14) AGAINST *CULICOIDES* SPP. AND *LEPTOCONOPS* SPP. (CERATOPOGONIDAE)[1]

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The bacterium *Bacillus thuringiensis* (serotype H-14) de Barjac has been documented as a highly efficacious larvicide of mosquitoes (Goldberg and Margalit 1977), black flies (Gaugler and Finney 1982) and certain other nematocerous Diptera. Apart from preliminary field and laboratory tests of *B. thuringiensis* on *Culicoides* spp. conducted by Kelson et al. (1980) and Larget and de Barjac (1981), no detailed published accounts are available on the effects of *Bacillus* pathogens on *Culicoides* and *Leptoconops* species. It was the objective of this study to determine the larvicidal activity, if any, of *B. thuringiensis* (H-14) on several ceratopogonid species under laboratory conditions.

Field collected late-instars of *Culicoides mississippiensis* Hoffman from salt marsh (Yankeetown, FL), *C. guttipennis* Coquillett from tree holes (Gainesville, FL), *Leptoconops* spp. from sand dunes near Vero Beach, FL, and laboratory-reared *C. variipennis* (Coquillett) were exposed to 20 ppm of the primary powder of Bactimos (Biochem Products, Montchanin, DE), a commercially produced spore-crystal powder of *B. thuringiensis* (H-14). When numbers permitted, 20 larvae were used per replicate and 3 replicates conducted for each species. The larvae were exposed to *B. thuringiensis* (H-14) in 100 ml of well water (24°C) in wax-coated paper cups (120 ml). Due to the predaceous nature of *C. mississippiensis* and *C. guttipennis*, several hundred nematodes (*Panagrellus redivivus* (L.), Cephalobidae) were added to each cup at the beginning of each test to facilitate ingestion of the inoculum. *Leptoconops* and *C. variipennis* were provided with a small amount of the larval medium used for maintaining *C. variipennis* in the laboratory (Jones et al. 1969) 24 hr after exposing them to *B. thuringiensis* (H-14). The larvae were observed at 48 and 72 hr for mortality. Exposure of late-instar *C. mississippiensis* to 20 ppm of *B. thuringiensis* (H-14) was also conducted in filtered sea water under the aforementioned conditions.

In order to determine if the larvae of *C. variipennis* and *C. mississippiensis* were ingesting *B. thuringiensis* (H-14), 3 lots of 10 larvae each for both species were exposed to 10 ppm of Bactimos primary powder (with a manufacturer-determined toxicity of 7000 International *Aedes aegypti* Toxicity Units). One lot of *C. variipennis* was exposed to the inoculum for 24 hr in the medium in which they were reared; the remaining 2 lots were exposed in well water for <30 sec and for 24 hr. One lot of *C. mississippiensis* was exposed to the inoculum for 48 hr in 1% salt water in the presence of the nematode utilized for prey, and the remaining 2 lots were exposed in well water in the presence of prey for 48 hr. Ten additional unexposed larvae of each species served as controls. After exposure, each lot was surface sterilized in 95% alcohol for ca. 30 sec, rinsed in 500 ml of sterile deionized water, placed in 3 ml of sterile water and ground in a Pyrex No. 7727 (7 ml) tissue grinder. The triturated larvae and accompanying water were pasteurized for 12 min at 80°C in a water bath. From each lot, 0.1 ml of the triturated larval suspension and 0.1 ml of 10^{-1} and 10^{-2} dilutions were plated on tryptose blood agar base and incubated for 48 hr. After plate counts were made they were incubated for an additional 3 days to permit sporulation and facilitate identification. Identification of *B. thuringiensis* colonies was based on colony morphology and the presence of parasporal crystalline inclusions.

None of the above larvae tested were affected by *B. thuringiensis* (H-14). Under nearly identical conditions (24 hr exposure) in concurrent tests (Lacey and Singer 1982), the LC_{50} value of Bactimos tested against 4th instar *Aedes aegypti* (L.) was 0.038 ppm. The toxicity of *B. thuringiensis* (H-14) in susceptible species is exclusively attributed to the δ-endotoxin found in the parasporal crystalline inclusions. The count in larvae exposed for <30 sec was 192 colony-forming units (cfu) per larva indicating that the surface sterilization was not adequate for removal of all the spores adhering to the cuticle. Subtracting this figure from that recorded in the 24 hr exposure yields the number of spores ingested/larva exposed for 24 hr. A low colony count in the controls (96 cfu/larva) confirmed that the pasteurization reduced but did not eliminate background counts due to vegetative stages and/or spores of other bacteria in the rearing medium. Nevertheless, counts of 0.9–1.41 × 10^5 cfu/larva for *C. variipennis* larvae exposed to *B. thuringiensis* (H-14) in larval rearing medium and well water indicate that a large quantity of inoculum was ingested. The presence of spores in the larval gut is a strong

[1] This paper reflects the results of research only. Mention of a pesticide, commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

indication that parasporal inclusions were also ingested. Sporulating *B. thuringiensis* was observed in the vast majority of the colonies on five-day-old plates from the <30 sec and 24 hr exposures. Colony forming unit counts from *C. mississippiensis* exposed in well water were low to negligible and only 8.6×10^3 cfu/larva were recorded from the salt water exposures. Ostensibly, ingestion of prey and inoculum were strongly influenced by the type of water employed.

Both *C. variipennis* and *Leptoconops* sp. utilize bacteria in the larval diet (Laurence and Mathias 1972, Parker et al. 1977). It is probable that sufficient quantities of *B. thuringiensis* (H-14) were ingested to produce mortality if the species had been susceptible to the δ-endotoxin. Ingested parasporal inclusions must be activated in the larval midgut in the presence of a fairly high pH and the proper proteolytic enzymes to become toxic. It appears that one or both of these conditions are lacking in the ceratopogonids which were tested.

Garcia et al. (1980) reported 48–100% mortality in *Palpomyia* sp., a tree-hole ceratopogonid, after 4 days of exposure to very high concentrations (10^6–10^7 spores/ml) of *B. thuringiensis* (H-14). In our study, 20 ppm of Bactimos equates to ca. 1.59×10^6 spores/ml in the test cups. Based on the results from these tests, those of Kelson et al. (1980) and Larget and de Barjac (1981), it is apparent that *B. thuringiensis* (H-14) affords no promise in the practical control of *Culicoides* or *Leptoconops*.

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