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Lysozyme in eggs of the cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae)

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Summary. Lysozyme exhibiting bacteriolytic activity was found in boll weevil eggs at a concentration of 2.22 ng of lysozyme per boll weevil egg.

Fleming first described in 1922 the bacteriolytic activity of lysozyme in hen egg white[1], and lysozyme was subsequently found to be widely distributed in various cells, tissues and secretions of many species[2,3]. Insect lysozyme was recently purified from the eggs of the Dipterous *Ceratitis capitata* and found to have a mol.wt of 23,200[4].

The enzyme lysozyme functions in host defense by its ability to lyse the cell walls of many bacterial species with gram-positive bacteria being more sensitive to lysozyme than gram-negative bacteria[5]. Lysozyme hydrolyzes glycosidic bonds between amino sugars of the bacterial cell wall murein layer leading to dissolution or lysis of the rigid cell-wall structure[6].

The boll weevil eggs were collected from 2-day-old wax-coated food pellets. After removing the wax coat along with any frass, the food pellets containing the eggs were expelled through a sterile 10-ml syringe having no needle into a beaker containing a 100% saturated NaCl solution. The food debris was allowed to settle and then was aspirated from the bottom of the beaker. The eggs, which floated on top of the saturated NaCl solution, were washed 5 times with the saturated NaCl solution and then 3 times with sterile distilled water. The eggs were counted after they had settled to the bottom of the beaker of distilled water. A total of 185 eggs (0.1 ml volume) were homogenized in 0.2 ml of 0.85% sterile saline. No extraneous particulate material was present. Lysozyme and protein determinations were then done on the egg homogenate.

Lysozyme concentration in the boll weevil eggs was determined according to the enzyme assay described by Wardlaw[7] using a lysozyme test kit (Kallestad Lab, Chaska, Mn.). In this test, triplicate wells cut in an agarose culture of *Micrococcus lysodeikticus* bacteria were filled with the boll weevil egg homogenate. After 18 h of incubation at room temperature, the cleared ring diameters around the wells due to the lytic action of lysozyme were measured in mm with a calibrating viewer (Kallestad Lab, Chaska, Mn.). The concentration of boll weevil egg lysozyme was then determined from a graph on 2 cycle semi-logarithmic paper against purified primary human urine lysozyme reference standards of known concentrations done at the same time (Kallestad Lab, Chaska, Mn.).

The total boll weevil egg lysozyme concentration for 185 eggs was found to be 0.411 µg. This would be 2.22 ng of lysozyme per boll weevil egg. The total boll weevil egg protein concentration for 185 eggs using the Lowry method[8] and egg albumin as the standard was found to be 4.73 mg. This would be 25.6 µg of protein per boll weevil egg. 1 boll weevil egg would thus contain 2.22 ng lysozyme/25,600 ng protein, meaning that 0.009% of the total protein of a boll weevil egg is lysozyme. The presence of lytic activity due lysozyme would attest to its importance in protection of the boll weevil egg against bacterial infection.

Lysozyme activity has also been found in boll weevil hemolymph and frass[9]. A mean lysozyme activity peak of 7.0 µg/ml was found in boll weevil hemolymph at 48 h following inoculation with heat-killed *Serratia marcescens*. Mean lysozyme activity of 3 µg/ml was also demonstrated in the hemolymph of uninoculated boll weevils.

The cotton boll weevil is a common insect pest to cotton production in the USA. Thus, the presence of lysozyme in boll weevil eggs and also in hemolymph and frass would serve a protective function against infection by the various bacteria found in the boll weevils' plant and soil environments.

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