Induced Immune Response against *Bacillus thuringiensis* in the Developmental Stages of the Greater Wax Moth, *Galleria mellonella* (Lepidoptera: Pyralidae)

A Thesis
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Master of Science in Entomology

BY

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DEDICATION

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ABSTRACT

The present study provides direct tests of *Galleria mellonella* immune strength across different age groups following injection of *Bacillus thuringiensis kurstaki* (*B.t.*). Such current tests were quantified using standard immunocompetence assays. All of these assays were performed at different time intervals post injection with sub-lethal concentrations (LC_{20}) of *B.t.* Preliminary results concerning *B.t.* toxicity indicated that the pupae are more resistant and the larvae are more susceptible. Results concerning physiological immunity indicated that larvae had more body water content and haemolymph volume than pupae and adults. Bacterial injection decreased the body water content and, in the same time, increased the haemolymph volume in all developmental stages. Sex difference in the mass loss was detected; increasing in males over females.

Injection of *B.t.* produced several pathological events on the haemocytes, in addition to significant changes in both differential haemocyte counts (DHCs) and total haemocyte counts (THCs). Sexual differences in the haemocyte counts were detected, where females were found to have more haemocytes in the circulation.

Quantitative and qualitative estimations of haemolymph proteins revealed differences between the different developmental stages and between both adult sexes, in their responses to bacterial injection. It also approved the transfer of immune proteins of treated insects through metamorphosis and their persistence in the later stages.

Stage-specific differences were shown to phagocytosis and antibacterial immune reactions, as these immune responses were most effective in the pupal, next the larval and then the adult stage. There is no effect of sex on the mounting of immune response. In addition, a transfer of immunological memory through the metamorphosis was observed.

Key words:

Galleria mellonella, haemolymph volume, haemocytes, haemolymph proteins, phagocytosis, antibacterial activity and immunological memory.

CONTENTS

ABREVIATIONS

ABS	**:**	Absorbency
AMPs	**:**	Antimicrobial peptides
ADs	**:**	Adipohaemocytes
AGERIN	**:**	Agricultural Genetic Engineering Research Institute
Bis	**:**	N, N-methylenebisacrylamide
BSA	**:**	Bovine serum albumin.
B. t.	**:**	*Bacillus thuringinsis*
CBB	**:**	Coomassie brilliant blue
CFU	**:**	Colony formed unit
ºC	**:**	Celsius degree
cm	**:**	Centimeter(s)
COs	**:**	Coagulocytes
DHCs	**:**	Differential haemocyte count.
E-coli	:	*Escherichia coli*
G. mellonella	**:**	*Galleria mellonella*
GRs	**:**	Granulocytes.
GWM	**:**	Greater wax moth
h	**:**	Hour(s).
HV	**:**	Haemolymph volume
Ig	**:**	Immunoglobulin.
JH	**:**	Juvenile hormone
g	**:**	Gram
kDa	**:**	Kilo Dalton(s)
LC	**:**	Lethal concentration.
LPS	**:**	Lipopolysaccharides
µl	**:**	Microliter
mg	**:**	Milligram
min	**:**	minute(s)
ml	**:**	Milliliter
mm^3	**:**	Millimeter cubic
MW	**:**	Molecular weight
µm	**:**	Micrometer
nm	**:**	Nano meter
OEs	**:**	Oenocytoids
OD	**:**	Optical Dinesty
P	**:**	Plasma
PAMP	**:**	Pathogen-associated molecular pattern
pH	**:**	Power of hydrogen
PLs	**:**	Plasmatocytes

PPO	**:**	Prophenoloxidase
PO	**:**	Phenoloxidase
PRP	**:**	Pattern recognition proteins
PRs	**:**	Prohaemocytes
Rpm	**:**	Round per minute
SE	**:**	Standard error
SEM	**:**	Scanning electron microscope
SDS-PAGE	**:**	Sodium duodecyl sulphate-polyacrylamide gel electrophoresis
SP	:	Species
SPs	**:**	Spherulocytes
TEMED	**:**	N, N, N', N'-Tetramethylethylenediamine
THCs	**:**	Total haemocyte count
THP	**:**	Total haemolymph protein

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