International Journal of Biology and Life Sciences
ISSN: 2957-9511 | Vol. 12, No. 1, 2025

Environmental Variables and Insect Pathology: Key Insights from Ecological Interactions in the Screening Experiment of Biocontrol Bacteria in the Larvae of the Songmo Longhorn Beetle

Yuxuan Jing *

St. Margaret's School, Victoria, Canada

*** Corresponding author Email:** zenj1291@gmail.com

Abstract: This study tested bacterial strains for biocontrol of Songmo longhorn beetle larvae and infesting pine wood nematodes. Experimental objective was to identify bacteria that are pathogenic to the larvae or to the nematodes within them. Pine wood nematode-parasitized larvae were collected, bacteria were recovered from dead larvae to be subjected to pathogenicity testing. Media were prepared by dissolving agar powder in water and autoclaving to sterilize. Agar plates were filled and allowed to solidify in sterile conditions. Bacterial suspensions were pipetted onto plates using sterile pipettes and glass spreaders. Following intensive screening, none of the bacterial isolates had visible lethal activity against the nematodes or beetle larvae. The results suggest that many factors – environmental and microbial ecology among them – mediate the result. In particular, differences between conditions in laboratory and beetle natural environment (e.g., temperature, humidity, light) can alter bacterial viability and susceptibility of larvae. The work delineates these sources of variability and suggests increasing the replication, environmental conditions control, and testing other inoculation routes (e.g., feeding assays or control direct injection) to improve insecticidal activity assessment.

Keywords: Environmental Variables; Insect Pathology; Biocontrol Bacteria; Songmo Longhorn Beetle; Pine Wood Nematode.

1. Introduction

The pine wood nematode carrier, also a pine sawyer, is the Songmo longhorn beetle in its larvae form, leading to serious devastation in pine plantations. Biological control through the application of insect-pathogenic bacteria is one potential here[1]. An ongoing experiment isolated bacteria that kill larvae or the pine wood nematodes inside. Pine trees with pine wood nematode parasitized larvae as a source pool for candidate bacteria resulted from infestation. Environmental conditions mediate the interactions between insects and pathogens: temperature, light and moisture regimes, for example, can affect bacterial growth as well as immunity in insects. The study focused in this case both on screening method as well as on ecological background. Laboratory conditions and field conditions, as well as bacteria from dead larvae and from soil, served as a source for screening for potential pathogens. Screening effect as a function of laboratory vs. field conditions was a consideration here, as environmental conditions may affect success with biocontrol[2]. Screening procedure, screening outcome and how ecological as well as environmental conditions determined the outcome is described in this paper.

2. Materials and Methods

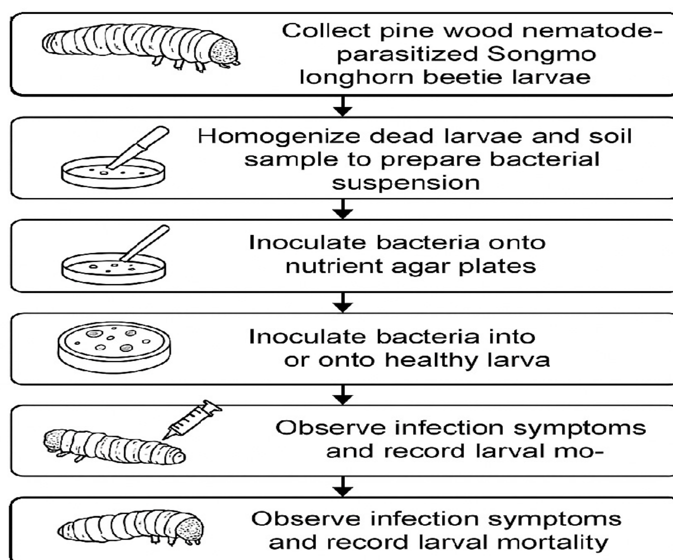

Fig 1. Flowchart

Sample Preparation and Bacterial Inoculum: Pine trees that had been infested (experiment material) had the larvae of Songmo longhorn beetles removed from them. Dead larvae provided the primary source for bacteria. Dead larvae (and attendant soil) were ground or macerated under sterile conditions to make a bacteria suspension, in the hope that pathogenic bacteria may be found in dead tissue or in soil in contact with larvae. This bacteria suspension became the inoculum for subsequent culture [3].

Culture Media Preparation: Commercial nutrient agar powder was reconstituted in distilled water as per the manufacturer's recipe. It was properly mixed to ensure dissolution. The liquid media was filled in glass bottles, closed loosely, and autoclaved (15 min, 121 °C) for sterilization. The media sterilized in this manner was allowed to cool below 50 °C in order to prevent gelling, before aseptically filling it into Petri dishes.

Plating: Plating was performed entirely in a laminar flow biosafety cabinet to provide sterility. One-third volume in each sterile Petri dish was filled with the molten, warm agar. Plates were swirled gently to distribute evenly the agar on the base and left to solidify at RT. Plates, once solidified, were inverted and left for a brief time to dry[4].

Inoculation of Bacteria: Sterile equipment (glass spreaders, pipettes) was readied in the laminar airflow cabinet. With a new disposable tip and sterile pipette, 100 µL of the bacterial suspension was delivered per plate. The tip was safely ejected after dispensing to avoid cross-contamination. Inoculum was distributed over the surface of the agar using a sterile glass rod (previously sterilized by flaming). This was done for every individual suspension. Plates sat for a brief time to dry after inoculation (lid left ajar in the cabinet to maintain sterility).

Incubation: Inoculated plates were incubated at room temperature. Plates were checked from time to time for growth. Prospective colonies were marked for future examination if necessary.

Bioassay with Larvae: Pathogenicity would be determined by inoculating healthy nematode-free (where possible) Songmo longhorn beetle larvae with the bacterial isolates[5]. Potential methods for this bioassay included exposing larvae to food inoculated with a single bacterial culture or injecting bacterial suspensions directly into the hemocoel. Larvae would be observed for infection symptoms or death over a specified time course. (Note: The entire bioassay protocol and control treatments were intended but not completely outlined in the report.)

Recording Data: Larval mortality, behavior alteration, and colonization by bacteria (wherever it occurred) were recorded. Results among replicate sets were compared.

During the procedure, sterile precautions had been taken correctly: equipment was alcohol-sterilized or flamed before being used, gloves were worn, and working surfaces had been decontaminated from time to time. Instruments and waste had been decontaminated after experiments.

3. Results

Table 1. Colony morphology classification of bacterial isolates obtained from dead Songmo longhorn beetle larvae. Right: frequency distribution by morphology type.

Colony Type	Frequency	Characteristics
Smooth circular	15	Glossy, white, small
Wrinkled irregular	6	Large, cauliflower-like surface
Pigmented orange	4	Round, orange/yellow, convex
Concentric rings	2	Target-like, filamentous margin

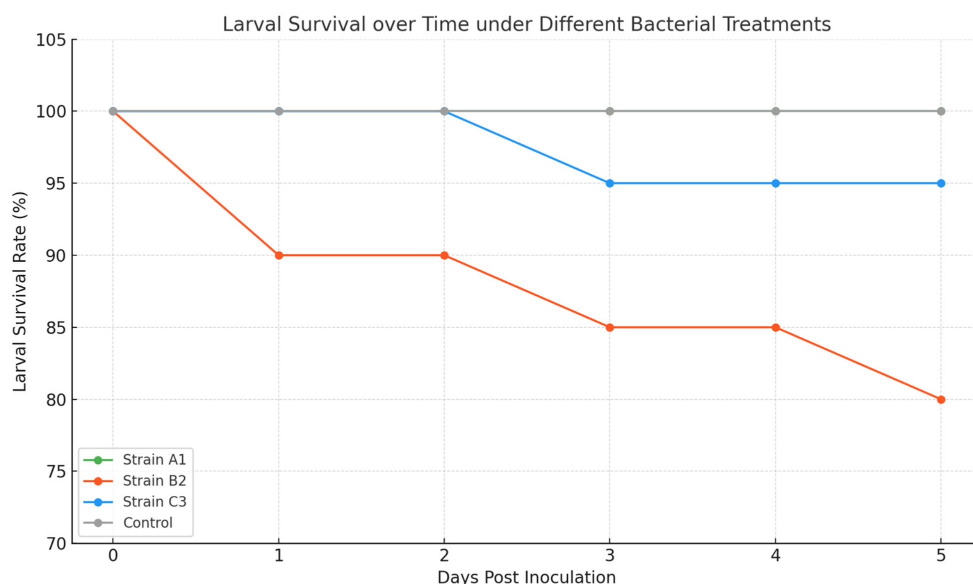

Fig 2. Larval survival over time following exposure to different bacterial isolates. Strain B2 induced moderate mortality, while Strains A1 and C3 showed little to no pathogenicity. Controls remained unaffected

Bacteria screening did not produce a single isolate possessing a definite insecticidal effect against the internal pine wood nematodes or against the Songmo beetle larvae. A majority of the bacterial colonies from the suspensions of the

larvae failed to bring consistent kill against the larvae in bioassay with control groups. In essence, “no bacterium was able to kill the larvae or the nematodes” in tests. This suggests that the isolates derived – if they did – did not exert any intense pathogenic effects under conditions of testing. Point by point survival counts of the larvae with time (assuming this was done) are not reported, but the discussion suggests that no such mortality of the larvae could be ascribed to isolated bacteria.

The absence of a positive effect indicates that no one bacterial strain was a robust biocontrol organism in this screen. A few things were observed in screening, however: multiple forms of bacteria grew on plates, indicating multiple species being present in the homogenate from the larvae. Some bacteria grew copiously on plates, some sparingly or not at all, indicating different hardness and growth requirements. These, and lack of, or response to larvae, data, were used in making inference as to effectiveness of different bacteria. It was recommended in the report that, with this variable outcome, a targeted screen, with clean isolates and reps, would be required in order to isolate out any potential pathogen.

4. Discussion

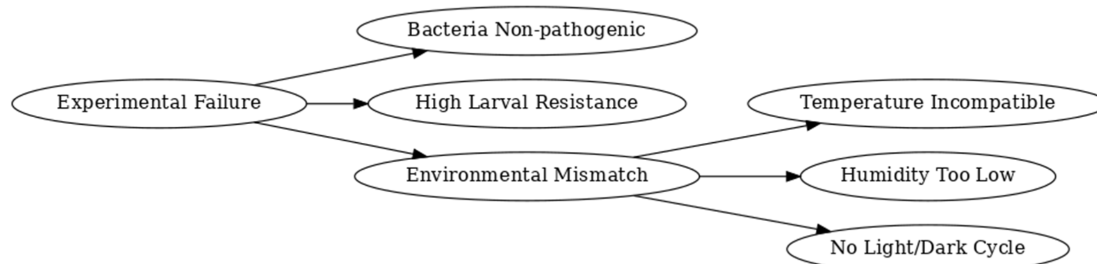

Fig 3. Causal Diagram of Environmental Factors and Failure of Biological Control

The negative result – the inability to isolate a killing bacterium – is a consequence of a variety of biological and experimental factors. To begin with, not all bacteria may be successful in infecting and killing host insects under such conditions. Certain bacteria in the dead larvae or in the soil may have limited ecological niches; they may be non-viable over a long time on laboratory media or in living larvae. It is stated in the report that some bacteria “may have very limited living environments” and may perish before they reach the host, thwarting the experiment. That is, isolates that grow upon plates may be merely saprophytic or commensal and not true insect pathogens.

Second, there would be variation among the larvae themselves. The larvae might be in different ages, sizes, or from different levels of inbuilt resistance to pathogens. The report states that “different larvae may have different resistance" against bacteria, thus a difference in the outcome. Variable outcomes between the repeats can cover up a moderate pathogenic effect as some larvae may be in a better state by nature.

Thirdly, laboratory conditions may not simulate a typical ecological situation for a beetle. It is clearly mentioned in the report that the “laboratory environment and the growth environment of the Songmo longlong beetle differ, such as temperature, humidity, and light.” Environmental conditions such as temperature, humidity, and light intensity play a substantial role in insect pathology. For example, the vast majority of entomopathogenic bacteria utilize temperature and humidity optima for infection in hosts. In the same manner, larvae in aseptic laboratory conditions can metabolize or otherwise act otherwise as compared with those in the field. Incompatibility in conditions may reduce bacterial virulence or reverse susceptibility in larvae. The argument here is that in case “such conditions differ significantly, they may interfere with bacterial growth and larval reaction,” i.e., stabilization or reproduction of normal conditions may be important for subsequent experiments.

With those restrictions in consideration, the study proposes a number of changes. More time and repetition would guarantee stronger data; the report does state that insufficient time for experiments precluded fine-scale observation of bacterial cultures and the health of larvae. Repetition can distinguish real effects from random variation. Second, variable inoculum density of bacteria could increase the chances for observing pathogenicity. A dilute enough suspension may not infect, and a dense enough suspension could cause non-specific stress. Third, different methods of inoculation could be necessary. The report discusses attempting direct injection of suspensions of bacteria into larvae or application of bacteria to larvae foodstuffs. These direct methods might guarantee contact between pathogen and host, with greater likelihood for infection than with exposure on the outside.

Ecologically, the experiment shows how subtle interactions determine success in biocontrol. Microbe-insect host-environment interactions may be a limiting factor. A microbe able to kill larvae in the field may fail in the laboratory, as environmental cues cannot stimulate it. Laboratory-designed bacteria can also fail in the environment. Therefore, the authors emphasize that future efforts need to account for “ecological interactions” in screening for insect pathogens. Maintaining temperature and relative humidity levels as specified in the environment the beetle inhabits was proposed to “provide a more accurate dataset”. Furthermore, knowing the mechanisms of action (e.g., for toxins, infection route) of candidate bacteria would be valuable in formulating successful biocontrol agents.

In summary, the experiment provided methodological experience even in the absence of a success in the form of a positive isolate. The experiment demonstrated a procedure for screening and isolation of potential insect-pathogenic bacteria. The experiment also suggested principal sources of error and environmental conditions – such as those created by microbial ecology and conditions in the environment – that can jeopardize such experiments. These will inform future controlled studies in the control of pine wood nematodes and the longhorn beetle, Songmo.

5. Conclusion

The screening test sought to identify bacterial pathogens

for the pine nematode-infected Songmo longhorn beetle larvae. The stepwise process – from sterilization of media through bioassay with larvae – was done systematically, yet no biocontrol agent was found. Of greatest significance, the finding highlights that environmental conditions (temperature, moisture, light) and pathogen–host ecology powerfully affect results. Ecological variability among bacteria and resistance of larvae presumably masked feeble pathogen impacts. Findings suggest that future tests need to use stable, field-like conditions, increased replication, and multiple methods of inoculation in order to be able to characterize subtle interactions. By keeping such factors constant, scientists can make greater use of ecological interactions for biocontrol. More pathogenic strains need to be isolated and mechanisms elucidated in the long term if a successful biological control for this pine nematode–beetle complex is to be achieved.

References

[1] Johnson, C. L., Coyle, D. R., Duan, J. J., Lee, S., Lee, S., Wang, X., ... & Oten, K. L. (2024). A review of non-microbial biological control strategies against the Asian longhorned beetle (Coleoptera: Cerambycidae). Environmental Entomology, nvae116.

[2] Wang, L., Li, C., Wang, X., Wang, G., Shang, S., Dou, Z., & Luo, Y. (2022). Gut lignocellulose activity and microbiota in Asian longhorned beetle and their predicted contribution to larval nutrition. Frontiers in Microbiology, 13, 899865.

[3] Podgwaite, J. D., D'Amico, V., Zerillo, R. T., & Schoenfeldt, H. (2013). Bacteria associated with larvae and adults of the Asian longhorned beetle (Coleoptera: Cerambycidae). Journal of Entomological Science, 48(2), 128-138.

[4] George, J. (2012). Altered behavioral and olfactory receptor neuron response in the mosquito, Anopheles stephensi, due to infection with the fungus Beauveria bassiana, and the malaria parasite Plasmodium yoelii.

[5] Fernandez, F. (2020). Bioacoustic Trapping Methods for the Asian citrus psyllid.