

# Alternative Inoculation Methods in *Manduca sexta*

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## Introduction

- *Manduca sexta*, the tobacco hornworm are a model organism because of their rapid development from eggs to adult, their large size, and their simplicity to care for.
- *Manduca sexta* are often used for studies of immunity with scientists injecting them with bacterial suspensions.
- Because injections cause stress, increased metabolic rates, and increased neuroendocrine interactions that may alter immune function, we want to find the best method of delivering a bacterial infection to the caterpillars.
- To find the best way to inoculate *Manduca sexta*, we performed 3 methods of delivering a bacterial infection to give them an immune challenge.
- There are still many unanswered questions about insect immunity, therefore it is critical that researchers have the right methods to use for conducting experiments to study the insect immune system.

## Hypothesis

Delivering bacteria via the spiracle will be the best method because this method may not cause harm and disrupt the immune system unlike injections.

## Methods

### Methods of Infection

#### 1. Spiracles

- PBS or *E. coli* mixed with dye
- 1 µl on each spiracle
- open spiracles with CO<sub>2</sub>

#### 2. Injections

- 10 µl PBS or *E. coli* injected
- In between 1<sup>st</sup> and 2<sup>nd</sup> proleg

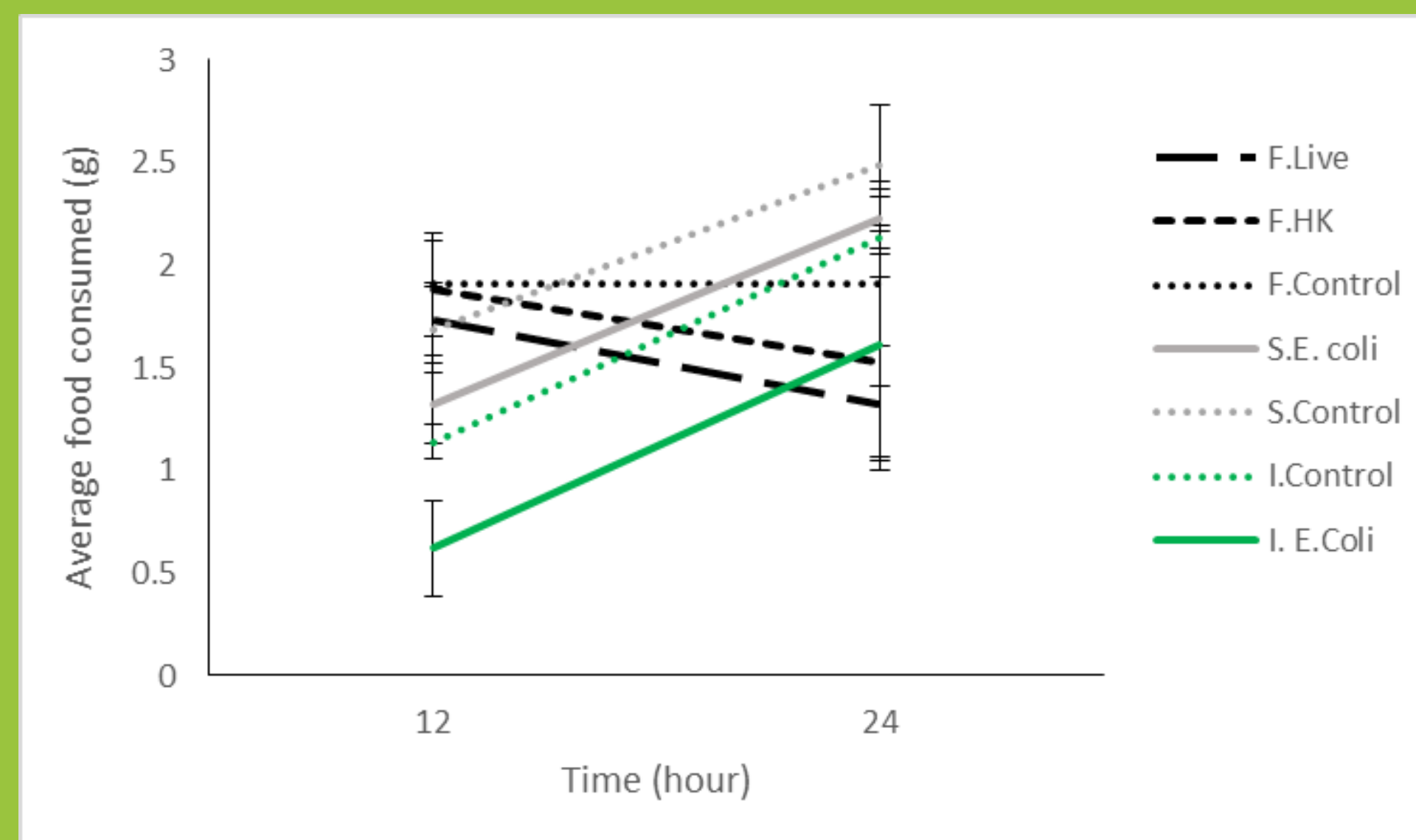
#### 3. Feeding

- 10 µl live or heat-killed *E. coli* added to each side of the food cube
- Counted bites for 3 min

Measurements were taken for the mass of the caterpillar and food, and survival rate at 0, 12 and 24 hour time points.

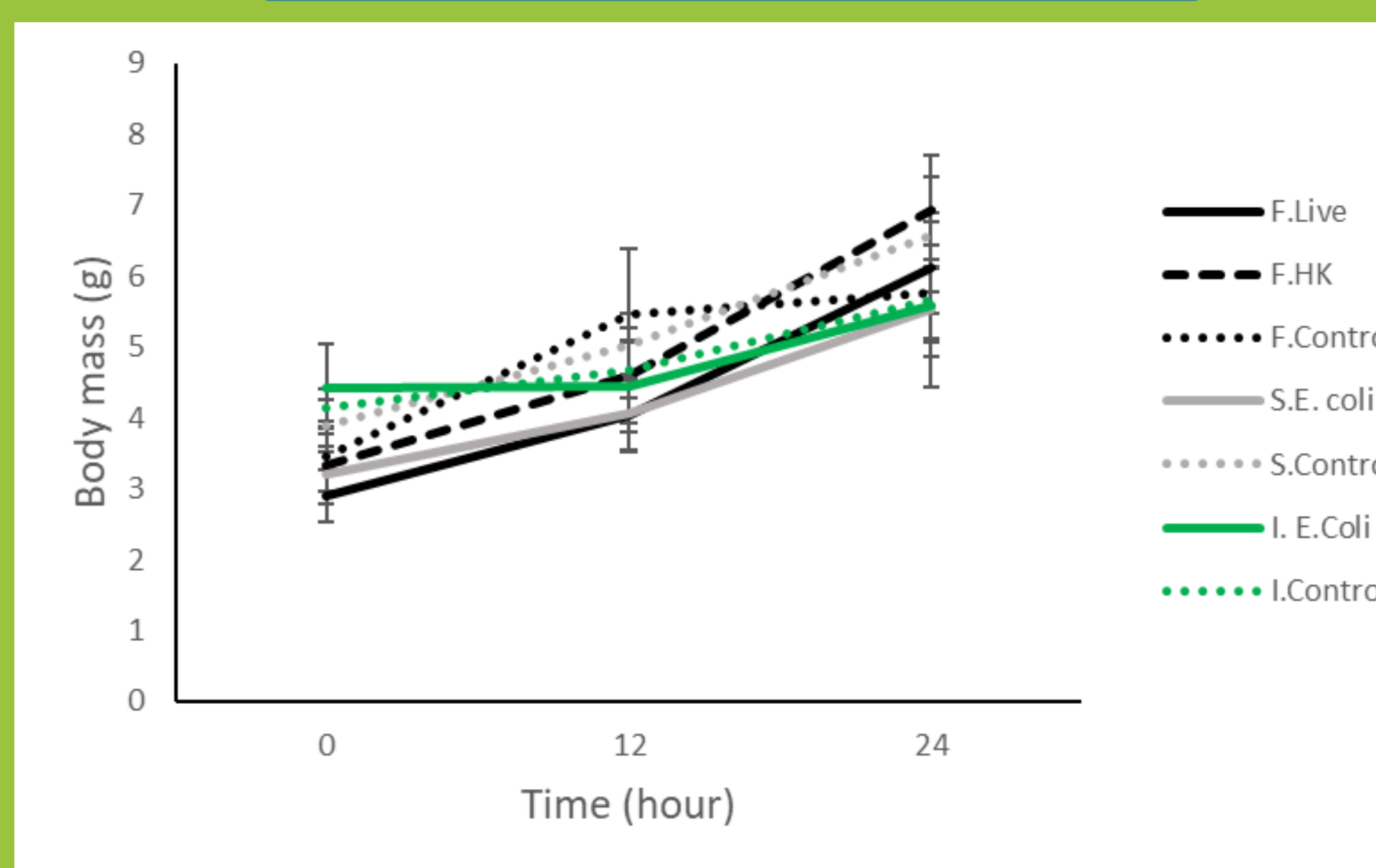
## Results

### Food consumption



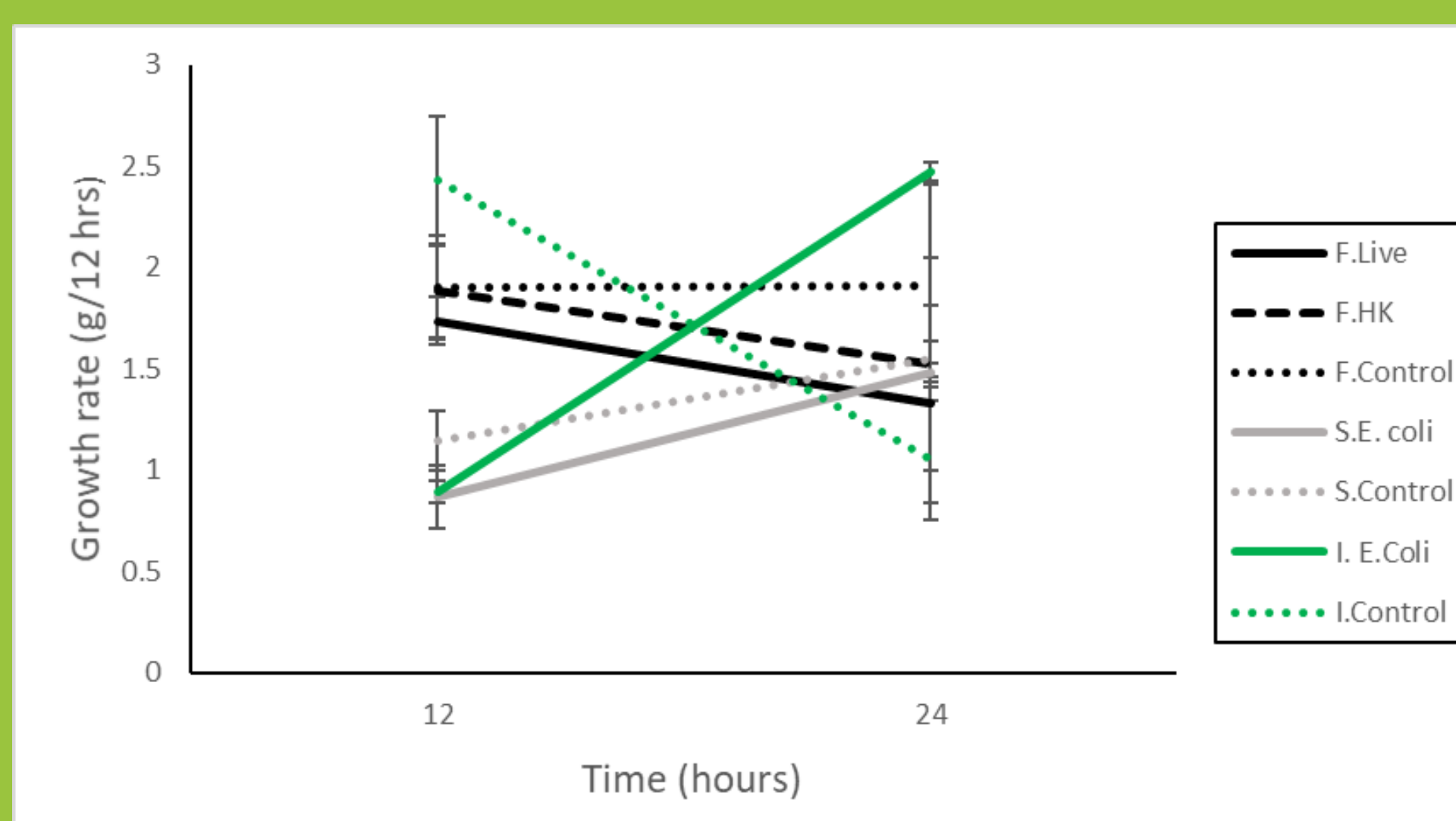
The average mass of food consumed from each method and treatment group over the course of 12 and 24 hours. There was a sign of sickness induced anorexia (SIA), because the caterpillars ate less food than the control group. Bacteria was also found on the bacteria plates. F.- Feeding S.- Spiracles I. Injections

### Average Mass of the Caterpillars



The average mass of the caterpillars for each method and treatment group over 12 and 24 hour time points. F.- Feeding S.- Spiracles I. Injections

### Growth Rate



The growth rate of the caterpillars for each method and treatment group over 12 and 24 hour time points. All caterpillars continued to grow throughout the 24 hour time period but the caterpillars fed with HK and live bacteria did not eat much of their regular food. F.- Feeding S.- Spiracles I. Injections

## Conclusion

### Feeding

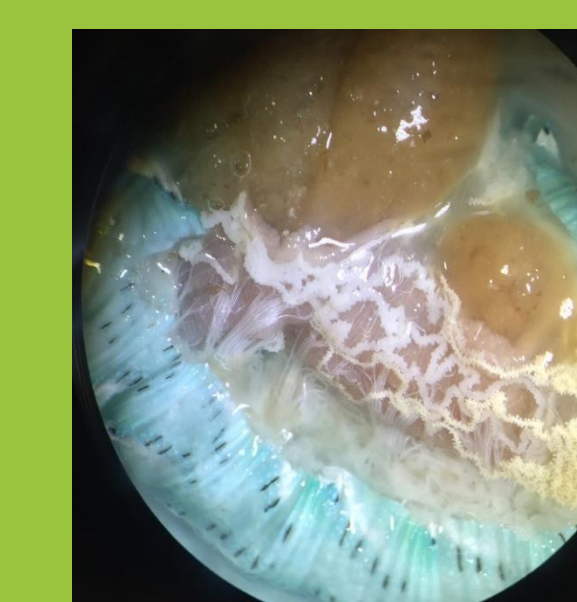
- There was a sign of sickness induced anorexia (SIA), because the caterpillars ate less food than the control group. Bacteria was also found in the bacteria load. This method would be best for infecting *M. sexta* because it does not cause any harm and they may not have different behavior unlike with injections.

### Spiracles

- Only a few of the caterpillars showed that *E. coli* made it into the airway, because we saw no dye in most of them, therefore, my hypothesis was refuted.
- This method may not be the best for infecting *M. sexta* because they also did not show SIA.



There is no sign of purple dye inside the caterpillar at the time of dissection.



There is a light purple color inside of the caterpillar at the time of dissection.

## Future

- Next time, there should be more bacteria to ensure a more impactful infection.
- The bacterial loads will be analyzed.



Bacteria load from a caterpillar injected with *E. coli*.

## References

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