Non-destructive Methods to Determine Microsporidian Infection Levels in Cinnabar Moth Larva

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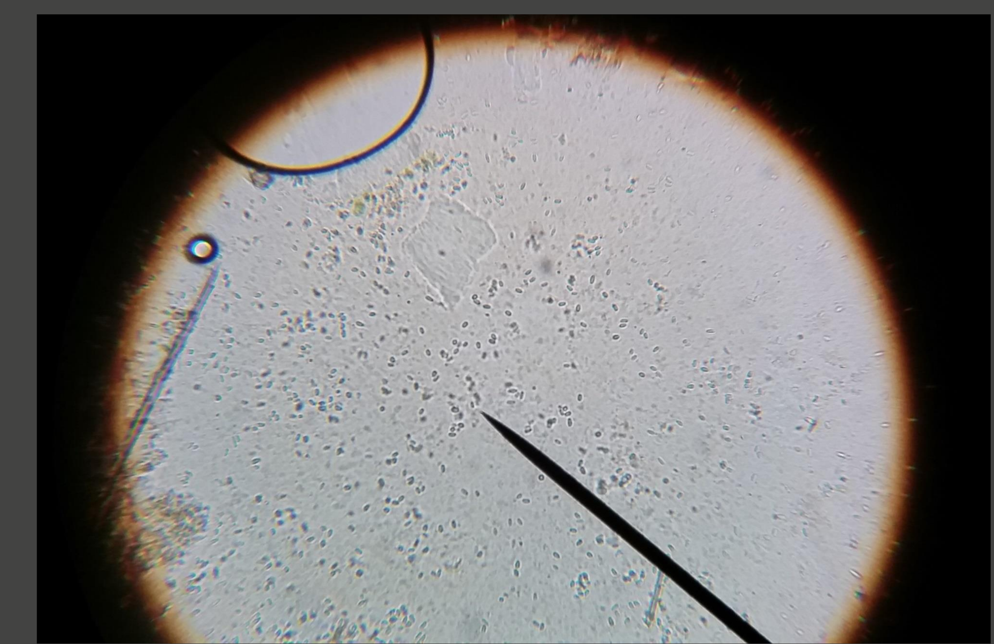

Introduction

The Cinnabar moth, *Tyria jacobaeae*, is native to Europe. This moth has been introduced to North America in an effort to control the invasive plant *Jacobea vulgaris*. *Nosema tyriae*, a microsporidian classified as a fungus, was accidently co-introduced with the moth. The Cinnabar moth is the main host of this parasite. When infection levels are high, *N. tyriae* can reduce growth, slow development, and decrease survival and reproduction of the Cinnabar moths (Karacetin 2007). *N. tyriae* mainly infects the gut wall, silk glands, and fat body (Canning, et al. 1999).

The objective of this study was to find alternative methods to determine *N. tyriae* infection levels in cinnabar moth larvae to overcome the limitations of destructive sampling for disease monitoring. We tested the following two approaches:

1) Using sibling larvae as proxies: We determined if spore concentrations in larvae from the same egg mass were similar

2) Using infection levels in frass: We determined if spore concentrations in frass reflected spore concentration in larvae

Methods

Sibling Larvae Infection Levels

- 8 egg masses, 10 larvae each
- Each larva homogenized with mortar and pestle in 2 drops of water
- Hemocytometer was used to determine spore concentration.
- Analysis of Variance used to test whether variance within families was smaller than variance between families

Frass to Larval Infection Level

- 30 egg masses with variable infection levels were used, 10 larvae were taken from each egg mass
- Individual larva numbered and put in cups with ragwort.
- When reached 3rd, 4th, and 5th instars, larva put in clean cup for 24 hrs. After, larva weighed and crushed in 3 drops of water from a disposable pipet. All equipment rinsed with hot water between larva.
- Equal amounts of frass from previous 24 hours collected and weighed, homogenized in 2 drops of water for 3rd instars, 6 drops 4th instars, 12 drops 5th instars
- A hemocytometer was used to count spore concentrations in frass and larval samples.

Results

Sibling Larvae Infection Levels:

The variance of infection levels between sibling larvae was not significantly smaller than the variance between families ($F_{1,77}$ = 1.27, P = 0.26). **Therefore, sibling larvae should not be used as proxies to determine infection levels in larvae of interest.**

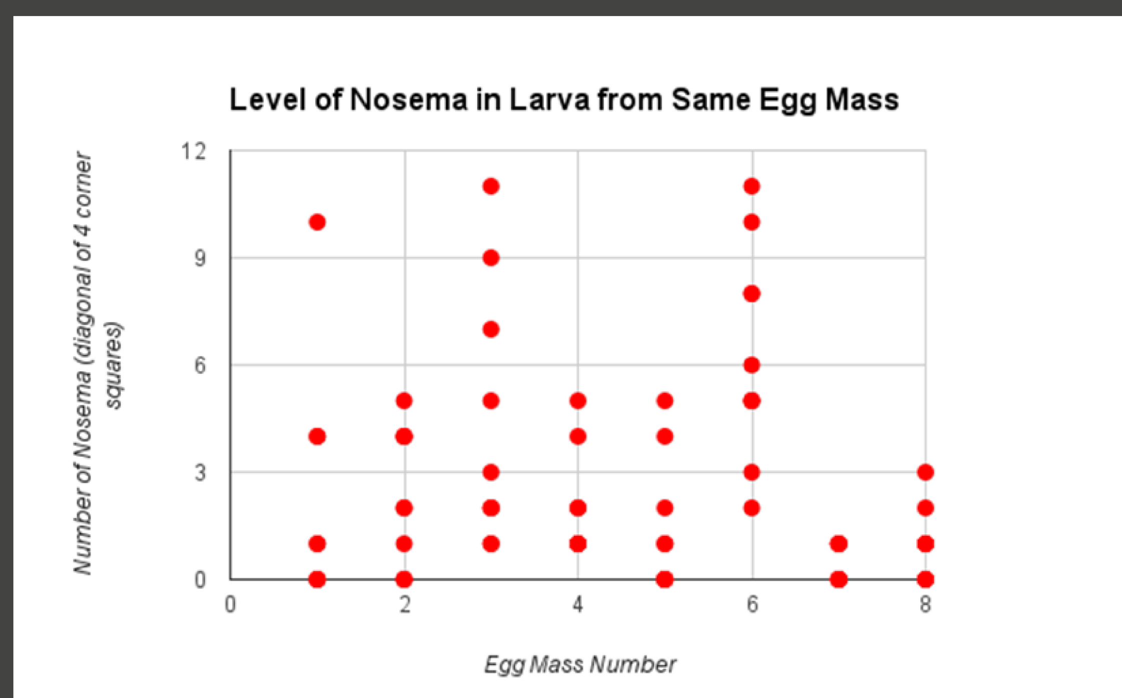

Figure 1. Variation of N. tyriae spore concentration within and between families of T. jacobaeae larvae. Variance within family was not significantly smaller than between families ($F_{1,77}$ = 1.27, P = 0.26) , thus sibling larvae cannot be used as proxies to non-destructively determine spore concentration in a larva.

Frass to Larval Infection Levels:

There was a positive correlation between spore concentration in frass and spore concentration in larvae that **was significant for 4th instar** ($F_{1,22}$=7.99, P=0.01), **marginally significant for 5th instar** ($F_{1,22}$=3.52, P=0.07) and **non-significant for 3rd instar** ($F_{1,22}$=2.00, P=0.17). However, low R^2 values of 0.27, 0.14 and 0.08 for 4th, 5th and 3rd instar, respectively, indicate that **this correlation cannot be used to accurately predict spore concentrations in larvae**.

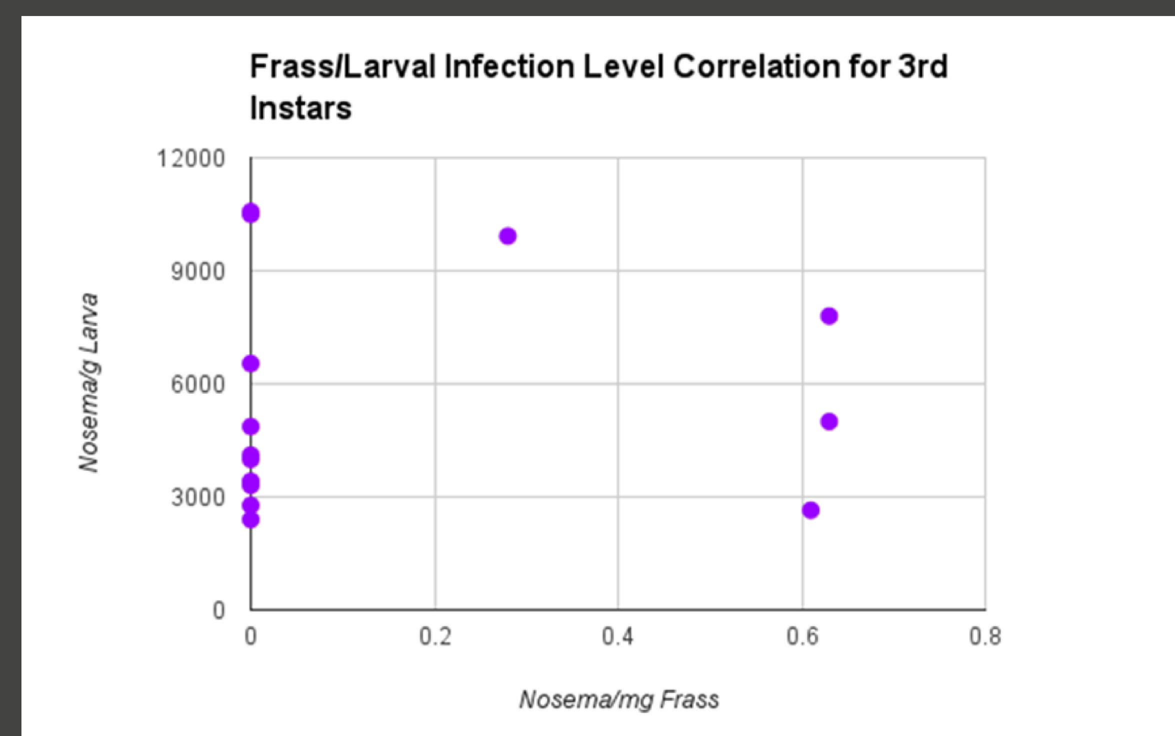

Figure 2. Correlation between N. tyriae spore concentration in frass and larvae for 3rd instars of T. jacobaeae. No significant correlation was found ($F_{1,22}$=2.00, P=0.17, R^2=0.08) and low R^2 values indicate that spore concentration in frass cannot be used as a predictor for spore concentration in larvae.

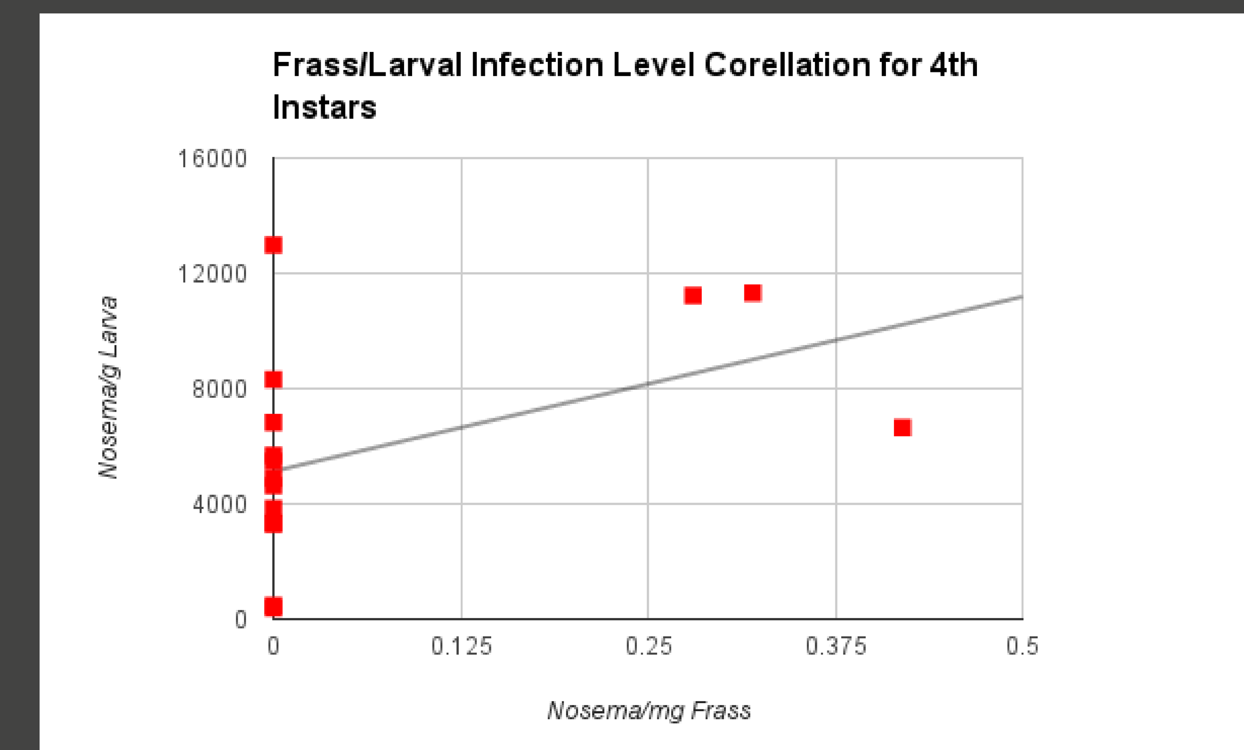

Figure 3. Correlation between N. tyriae spore concentration in frass and larvae for 4th instars of T. jacobaeae. A significant correlation was found ($F_{1,22}$=7.99, P=0.01, R^2= 0.27), but low R^2 values indicate that spore concentration in frass cannot be used as a predictor for spore concentration in larvae

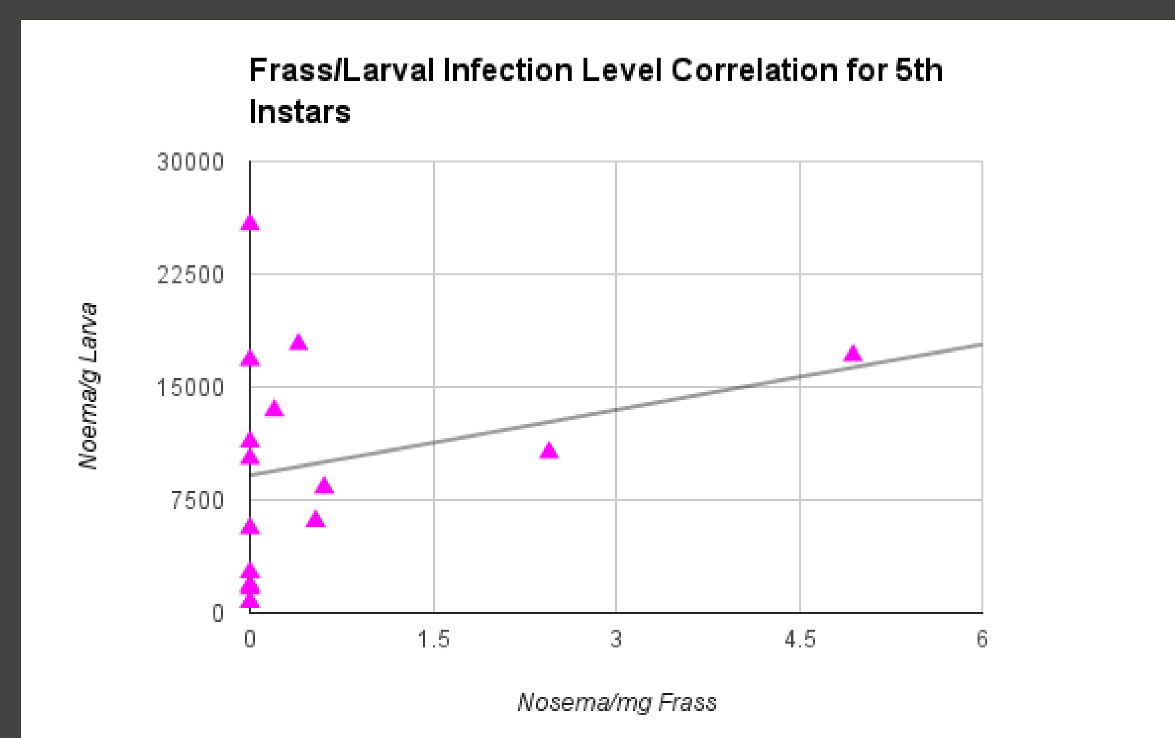

Figure 4. Correlation between N. tyriae spore concentration in frass and larvae for 5th instars of T. jacobaeae. A marginally significant significant correlation was found ($F_{1,22}$=3.52, P=0.07, R^2=0.14), but low R^2 values indicate that spore concentration in frass cannot be used as a predictor for spore concentration in larvae.

Discussion

The results of this study show that sibling larvae do not emerge with the same infection level of *N. tyriae* and that the correlation between spore concentration in frass and spore concentration in larvae contains too much variation to be reliably used for prediction. Based on these results, we thus conclude that frass sampling cannot be an alternative to destructive sampling for *N. tyriae* detection in Cinnabar moths.

Another experiment done with *Choristoneura occidentalis* and *Nosema fumiferanae* found that the start of spore egestion varied from 11 to 15 days post inoculation, depending on temperature (Campbell 2007). Possibly, low levels of *N. tyriae* were found in the Cinnabar larva frass of our study due to low levels of initial infection. Naturally occurring, vertically transmitted spore concentrations were used versus in other experiments, larvae were artificially inoculated with high spore doses.

Future studies could address the detection of *N. tyriae* in Cinnabar moths by artificially inoculating larvae to control levels of infection and investigate how the two transmission pathways might result in different levels of spore concentrations in frass. Similarly, future studies could extend the time period of samples to gain a better understanding of the time-lags between infection and appearance of spores in the frass.

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