Spore Persistence in the Environment Drives Infection Dynamics of a Butterfly Pathogen

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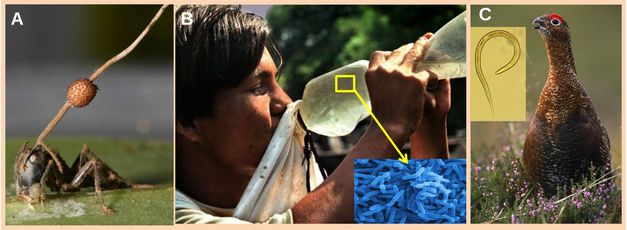
1. Conceptual Background

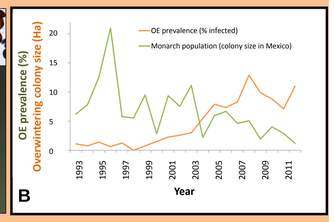
- Environmentally transmitted parasites commonly infect humans and wildlife.
- Environmental transmission is particularly important for insect pathogens; yet the factors affecting the persistence of environmental stages are poorly understood.
- Environmental transmission stages can facilitate pathogen persistence in fluctuating host populations, especially for vertically transmitted parasites that induce fitness costs to the host.



Examples of pathogens with environmental stages. **A.** An ant is parasitized by an entopathogenic fungus, *Ophiocordyceps unilateralis*. **B.** Humans are parasitized by the bacterium Cholera. **C.** Red grouse are parasitized by a strongstyle worm, *Trichostrongylus tenuis.*

2. Study System

- Monarch butterflies are commonly infected by the protozoan *Ophryocystis elektroschirrha* (OE).
- OE has two transmission modes: adults shed spores on to (1) eggs (vertical transmission) or to (2) milkweed leaves that are consumed by unrelated larvae (environmental transmission).
- Parasites develop internally and adult monarchs emerge with millions of dormant parasite spores.
- Rates of spore deposition and how long spores can persist in the environment are currently unknown.
- Monarchs have been steadily declining in eastern North America since 2000; at the same time parasite prevalence has increased.



A. An infected adult monarch can have millions of OE parasite spores (inset, shown at 100X) on its external abdomen. **B.** Adult monarch populations have decreased between 1993 and 2012, while OE prevalence has increased in this same period.

3. Research Questions

- How long do spores persist and does this depend on environmental conditions?
- How do spore deposition rates and spore longevity affect monarch population size and infection prevalence?

4a. Experiment

We conducted an experiment to investigate how the duration of environmental exposure to parasite spores affected infectivity to live monarchs.

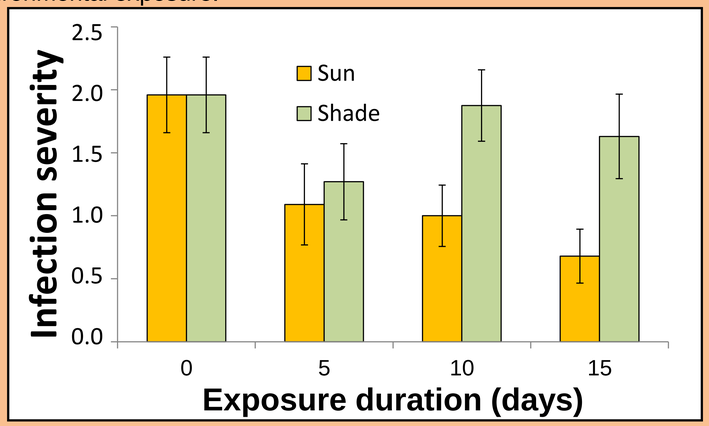
1. We added parasite spores to swamp milkweed plants (200 spores/leaf for 5 leaves/plant) to mimic spore deposition by infected monarchs. We used three parasite isolates.
2. Plants were placed outdoors in environmental and time treatment groups (6 plants/group).
3. After treatments were complete, we randomly assigned inoculated leaves to be fed to susceptible monarch larvae (~25 larvae per treatment group).
4. We raised larvae until pupation and adulthood, when monarchs were assessed for **infection status** and **infection severity**.

Treatment groups for milkweed leaves inoculated with parasites	
Sun (high UV light, rain exposure, high temperature)	Shade (less UV light, no rain exposure, low temperature)
Time: 0, 5, 10, 15 days left exposed in environment	Time: 0, 5, 10, 15 days left exposed in environment

A. Inoculation of milkweed leaves; spores were manually transferred to the underside of leaf surfaces. **B.** Larva inoculated with infected milkweed leaf. **C.** Milkweed plants in the environmental shade treatment.

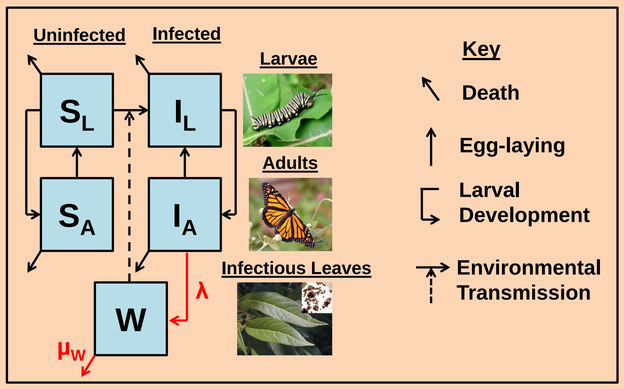
4b. Experimental Results

- We used generalized linear models to test for effects of time plants were left outdoors (0, 5, 10, 15), environment (sun, shade), and parasite lineage (3,10,13) on monarch infection status and severity.
- Time and parasite lineage, but not environment, were significant predictors of the infection status.
- Infection severity decreased with time, exposure to sun, and also depended on parasite lineage.
- Most inoculated leaves were still able to induce infections after 15 days of environmental exposure.



5a. Model

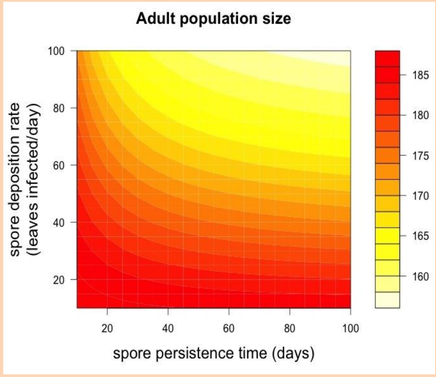
- We developed a differential equation model to describe monarch-OE dynamics
- Monarchs were divided into **S**usceptible or **I**nfected **L**arvae and **A**dults.
- The environmental pathogen stage is measured as the number of leaves receiving an infectious spore dose, **W**.
- We varied the **spore deposition rate (leaves infected per day, λ)** and **environmental pathogen persistence (time leaves remain infectious, 1/ μ_W)**.
- The model was solved in R and the number of adult monarchs and infection prevalence in adults was recorded at the end of the breeding season **(t=100 days)**.

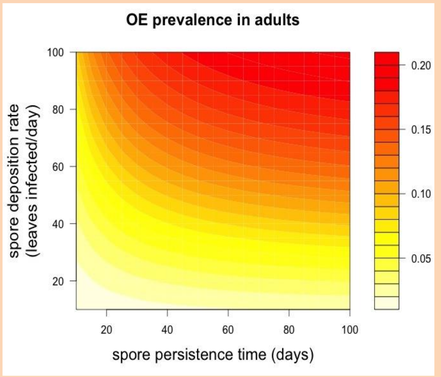


A. Student and mentor laugh in the face of differential equations.

5b. Model Results

- Adult population size decreases and prevalence increases as spore deposition rate and spore longevity increase.
- The range of prevalence predicted by the model agrees well with observed late-season infection prevalence in the midwestern U.S. (6-20%, 2006-2009).
- To obtain the minimum observed prevalence the model predicts that spores must persist >20 days and monarchs must infect >30 leaves per day.
- This is consistent with our experimental finding that most leaves remain infectious >15 days.





6. Conclusion

- The experiment showed that environmental stages of OE can persist >15 days in the environment, and increased time of exposure to sun resulted in less intense infections.
- Consistent with the experiment, the model showed that infected leaves had to remain infectious for >20 days to match minimum observed field prevalence.
- OE parasite spores have a long-lived environmental stage necessary for persistence in wild monarch populations.
- The model also revealed that infected monarchs must shed infectious spore doses onto >30 leaves per day to match field prevalence, motivating future studies of spore deposition rates in the field.

7. Acknowledgements

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