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Infectivity of the parasite *Metschnikowia bicuspidata* is decreased by time spent as a transmission spore, but exposure to phycotoxins in the water column has no effect

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Summary

1. Transmission from one host to another is a crucial component of parasite fitness. For some aquatic parasites, transmission occurs via a free-living stage that spends time in the water, awaiting an encounter with a new host. These parasite transmission stages can be impacted by biotic and abiotic factors that influence the parasite's ability to successfully infect or grow in a new host.

2. Here we tested whether time spent in the water column and/or exposure to common cyanobacterial toxins impacted parasite transmission stages. More specifically, we tested whether the infectivity, within host growth, and virulence of the fungal parasite *Metschnikowia bicuspidata* changed as a result of time spent in the water or from exposure to cyanotoxins in the water column. We exposed parasite transmission spores to different levels of one of two ecologically important cyanotoxins, microcystin-LR and anatoxin-a, and factorially manipulated the amount of time spores were incubated in water. We removed the toxins and used those same spores to infect one genotype of the common lake zooplankton *Daphnia dentifera.*

3. We found that cyanotoxins did not impact parasite fitness (infection prevalence and spore yield per infected host) or virulence (host lifetime reproduction and survivorship) at the tested concentrations (10µg/L & 30µg/L). However, we found that spending longer as a transmission spore decreased a spore's chances for successful infection: spores that were only incubated for 24 hours infected approximately 75% of exposed hosts, whereas spores incubated for 10 days infected less than 50% of exposed hosts.

4. We also found a negative relationship between the final spore yield from infected hosts and the proportion of hosts that became infected. In treatments where spores spent longer in the water column prior to encountering a host, infection prevalence was lower (indicating lower per spore infectivity), but each infected host yielded more spores at the end of infection. We hypothesize that this pattern may result from intraspecific parasite competition within the host.

5. Overall, these results suggest that transmission spores of this parasite are not strongly influenced by cyanotoxins in the water column, but that other aspects of spending time in the water strongly influence parasite fitness.

Introduction

A crucial component of parasite fitness is successfully transmitting from one host to another. For many parasites, transmission occurs via a free-living stage that spends time in the environment until it encounters a suitable host. In aquatic ecosystems, these parasite transmission spores spend time in the water column (before sinking out or moving on to their next life history stage), where they are exposed to abiotic and biotic factors that can ultimately affect the timing, size, and duration of an epidemic. While we know these transmission stages are very common for aquatic parasites and that many biotic and abiotic factors impact their transmission to hosts, we know less about how prolonged exposure to environmental factors such as cyanotoxins influences their ability to infect and propagate in new hosts.

One factor that likely influences the fitness of parasite transmission stages is time spent in the environment. In addition to the risks of spore predation (Thieltges *et al.*, 2013) and settling out of the water column (Brookes *et al.*, 2004), spores may lose viability over time, perhaps due to depleting the resources they acquired from their host (Amigó *et al.*, 1996; Malone, Gatehouse & Tregidga, 2001; Duffy & Hunsberger, 2019). Longer time in the environment also increases spore exposure to stressors such as light, temperature, secondary metabolites produced by other organisms, and anthropogenic pollutants (Heagle, 1973; Amigó *et al.*, 1996; Hallmann & Sikora, 1996; Vasemagi, Visse & Kisand, 2017; Shaw *et al.*, 2020). Together, this suggests that increased time in the water column should decrease parasite fitness.

When they are in the water column, parasite spores are exposed to secondary metabolites. These are often released from phytoplankton cells, becoming dissolved in water (Huisman *et al.*, 2018); some are known to have antibacterial and antifungal properties (Borowitzka, 1995; Ostensvik *et al.*, 2002; Volk & Furkert, 2006; Leflaive & Ten-Hage, 2007). While there is no clear consensus on why cyanobacteria produce these phycotoxins, one possibility is that these antimicrobial compounds are released in the water as part of an allelopathic attack by phytoplankton to deter enemies (Leflaive & Ten-Hage, 2007);

these same compounds may have a negative impact on the transmission spores of parasites. If so, this could impact parasite epidemics in aquatic ecosystems (Lafferty & Holt, 2003). Thus, we were interested in understanding how cyanotoxins might impact parasite transmission stages in the water.

Understanding the impact of cyanotoxins on parasite transmission stages is particularly important given that cyanobacterial harmful algal blooms (CHABs) are expected to increase in frequency and intensity in the coming years with climate change (Huisman *et al.*, 2018). CHABs and their toxins can increase infection rates (by making hosts more vulnerable to infection) and increase the negative effects of parasites on their hosts (Landsberg, 2002; Harvell *et al.*, 2004) including important zooplankton in freshwater ecosystems (Tellenbach *et al.*, 2016); however, positive effects on aquatic hosts of consuming chemically defended cyanobacteria have also been observed (Coopman *et al.*, 2014; Manzi *et al.*, 2019; Sánchez *et al.*, 2019). When considering the potential impact of CHABs on parasitism, to date the focus has been on one main mechanism: that toxins may alter host susceptibility or tolerance (Landsberg, 2002; Harvell *et al.*, 2004; Coopman *et al.*, 2014; Penczykowski *et al.*, 2014; Andersen *et al.*, 2016; Tellenbach *et al.*, 2016; Duperron *et al.*, 2019; Lassudrie *et al.*, 2020). Here, because some cyanotoxins are known to have antimicrobial properties, we consider the possibility that cyanotoxins have direct negative effects on parasite transmission spores in the water column.

Using the *Daphnia-Metschnikowia* host-parasite system, we tested whether time spent in the water column and cyanotoxins have negative consequences on the parasite's ability to infect and grow within a host, both of which are key components of parasite fitness. A variety of biotic and abiotic factors are known to impact *Metschnikowia* epidemics in lakes (Cáceres *et al.*, 2006; Hall *et al.*, 2010). In this study, we were concerned with how the duration of exposure to the environment impacts the free-living stage of the parasite with an emphasis on the duration of exposure to certain cyanotoxins. We also looked for impacts on parasite virulence, specifically looking at host reproduction and lifespan, both of which are influenced by infections.

Methods

In this study, we used the zooplankton *Daphnia dentifera*, which is common in stratified lakes in temperate North America (Tessier & Woodruff, 2002). For our experiments, we used the Midland 37 (MID37) genotype, which was isolated from Midland Lake in Greene County, Indiana and has been used in several prior experiments (e.g., Auld, Hall & Duffy, 2012; Auld *et al.*, 2014). We also used the common fungal parasite *Metschnikowia bicuspidata* ("Standard" isolate, originally isolated from Baker Lake in Barry County, Michigan). *Daphnia* become infected by inadvertently consuming transmission spores they encounter in the water column when feeding. By "transmission spore", we refer to the mature, needle-shaped ascus that contains the ascospore (Metschnikoff, 1884; Codreanu & Codreanu-Balcescu, 1981). After consumption by the host, infection can begin if the needle-shape spore crosses the gut barrier and is not fought off by a host hemocyte response (Metschnikoff, 1884; Stewart Merrill & Cáceres, 2018). Once infection has taken hold, the fungus replicates within the hemolymph of the host (Stewart Merrill & Cáceres, 2018). The parasite reduces the fecundity and lifespan of infected hosts (Auld *et al.*, 2012). *Metschnikowia* is an obligate killer, meaning it must kill its host in order to transmit to a new host (Ebert, 2005); transmission spores are released into the environment after host death, after which they can be consumed by a new host, completing the parasite's life cycle.

We incubated fungal transmission spores for different lengths of time in filtered lake water (Pall AE filters, 1 µm pore size). The incubation times were 24 hours, 3 days, 5 days, 7 days, and 10 days. We also added two common cyanobacterial toxins, microcystin-LR and anatoxin-a, to the water during the incubations. We chose these cyanotoxins because they are commonly produced during blooms (Huisman *et al.*, 2018) and because prior research suggested that they reduced infection prevalence (in the case of microcystin-LR) or that production of them can increase in the presence of *Metschnikowia*

(in the case of anatoxin; Sánchez *et al.*, 2019). Microcystin-LR is produced by some members of the genus *Microcystis,* which has been extensively studied due to concerns over its geographical expansion and capability of producing CHABs in both marine and freshwater ecosystems (Huisman *et al.*, 2018). Anatoxin-a is produced by some members of the genus *Anabaena.* Microcystin-LR is a hepatotoxin while anatoxin-a is considered a potent neurotoxin in vertebrate models (Christensen & Khan, 2020). Both toxins are also produced by other genera of cyanobacteria such as *Plantktothrix*, *Oscillatoria*, *Aphanizomenon*, *Cylindrospermum*, and *Dolichospermum*, all capable of producing CHABs (Huisman *et al.*, 2018). We also included two types of negative controls: a solvent control of 0.01% acetic acid (see toxin preparation for explanation) and a negative control with no toxin or solvent added. The incubation times were crossed factorially with the toxin treatments, as described below. After the appropriate incubation time, we carried out infection assays in which we exposed *Daphnia* hosts to these spores and measured infection, spore production, host reproduction, and host mortality over time.

Toxin preparation

Pure microcystin-LR standard was purchased from Cayman Chemical (Ann Arbor, MI) and suspended in 1mL of nano-pure water for a concentration of 1 mg/mL. Anatoxin-a standard was acquired from Abraxis (Warminster, PA). The anatoxin-a comes in a solution of 3:1 water and methanol and 0.01% acetic acid. We placed 1.5 mL of the anatoxin solution in a 2 mL Eppendorf tube and evaporated the methanol using an Eppendorf Vacufuge (Eppendorf, Hamburg, Germany) at 23 °C. Once evaporation had occurred, we added nanopure water to restore to the original volume/concentration. Toxins were frozen in between uses during the exposure period.

Spore preparation

Spores for the experiment were grown in vivo by infection of *Daphnia dentifera* ("Standard" genotype) in the laboratory. Infected animals with well-developed late-stage infections were collected from laboratory cultures and placed in 2mL Eppendorf tubes with 100-500 uL of milliQ water, then stored in the refrigerator at 4 °C. For this experiment, we used spores from animals that had been stored in the refrigerator for 2 weeks. To generate the spore slurry for experimental infections, we crushed infected animals to release spores, and then determined the density of mature ascospores using a hemocytometer (Hausser Scientific 3100, Horsham, PA, USA) and a compound microscope (Olympus BX53, Center Valley, PA, USA) at 400X magnification. Each incubation treatment was initiated with a new spore slurry (made from infected animals that were harvested live from the laboratory cultures, then stored in the refrigerator for 2 weeks) because we know that *Metschnikowia* spores lose infectivity over time, even in the refrigerator (Duffy & Hunsberger, 2019).

Transmission spores exposure to toxins

On the first day of the experiment ("day 1"), we initiated the longest incubation treatment (10 days) by placing 5000 mature transmission spores of *Metschnikowia bicuspidata* in 15 mL Falcon tubes filled with 10 mL of lake water that had been filtered through a Pall AE filter (Pall Corporation, Port Washington, NY). We then added microcystin-LR, anatoxin-a, acetic acid (negative control), or no toxin/chemical; for both microcystin-LR and anatoxin-a, we had two toxin levels: 10 and 30 µg/L. The concentrations we chose for this study are below and/or well within the range observed during natural CHABs blooms (Park *et al.*, 1998; Pawlik-Skowrońska *et al.*, 2004; Ibelings *et al.*, 2005; Ha, Hidaka & Tsuno, 2009), and therefore ecologically relevant. These treatment doses are below the LC50s reported in previous *Daphnia* toxicology experiments for microcystins and anatoxin-a (DeMott, Zhang & Carmichael, 1991; Pawlik-Skowrońska, Toporowska & Mazur-Marzec, 2019). Even though we did not expose hosts to these

toxins, we chose these concentrations because they should not cause high levels of stress and mortality in *Daphnia*, so any impact to pathogens could have a substantial impact on parasite-host interactions. While these concentrations are likely on the high side of what spores are likely to encounter in nature, if there is no impact of the cyanotoxins at these levels, it suggests that they are unlikely to significantly impact the free-living stages of this parasite in the water column. There were 10 replicates of each treatment (including the negative control treatment of acetic acid), with the exception of the no toxin controls (0 µg/L), which had 20 replicates; this yielded a total of 70 experimental units per incubation time treatment. The tubes with spores were left uncapped for 10 days inside a large plastic tote covered with a lid at 20 °C with a 16:8 L:D photoperiod. On day 4, the same procedure from day 1 was repeated. In this treatment, spores were incubated for 7 days at 20 °C with a 16:8 L:D photoperiod, yielding the 7-day incubation treatment. On day 6, the same procedure was used to initiate the 5-day incubation treatment. Additionally, on this day, adult *Daphnia* were set-up so that we could use their offspring in the infection assays. Adult *Daphnia* of the MID37 genotype were placed in 150 mL beakers (5 adults per beaker) filled with 100 mL of filtered lake water. Beakers were placed in incubators at 20 °C with a 16:8 L:D photoperiod for 24 hours. On day 7, we collected neonates (0-24 hours old) produced from mothers that had been set-up the previous day. We placed 10 neonates per beaker in 250 mL beakers with 150 mL filtered lake water for a total of 400 animals. Each beaker received 2 mg C per L of *Ankistrodesmus falcatus* food and was placed in incubators at 20 °C with a 16:8 L:D photoperiod. After that, 2 mg C per mL of *Ankistrodesmus* was added to each beaker each day until the day the experimental animals were exposed to parasites. On days 8 and 10, we used the same procedure as described above to initiate the 3- and 1-day incubation treatments, respectively.

Infection assays

On day 11, we carried out infection assays, in which we exposed *Daphnia* to transmission spores that had been incubated for different time lengths and with varying levels of exposure to toxins/chemicals. All Falcon tubes, containing spores with different exposure times and toxin levels, were collected and placed in a centrifuge (Sorvall ST 16, Thermo Scientific, Waltham, MA, USA) to spin down the spores. The tubes were spun at 3000 rpm for 10 min. We decanted the tubes using a 10mL pipette without disrupting the spore pellet at the bottom. In a pilot experiment, we confirmed that the original concentration of spores was recovered from vials after a 24-hour incubation followed by centrifugation. After the water was removed (which also removed the toxin), we resuspended the spores by adding 10 mL of filtered lake water and disturbing the pellet by vigorously pipetting the water in the tube. Then, we placed one 6-7-day old *Daphnia* in each Falcon tube and allowed the tubes to incubate again at 20 °C with 16:8 L:D photoperiod. Because we had originally placed 5000 mature transmission spores in each tube, this yielded an exposure dose of 500 spores/mL. Hosts were fed 1 mg C per mL of *Ankistrodesmus* on this day; using this lower level of food on the day of exposure is common in infection assays because it promotes infection. After 24 hours (that is, on day 12), each *Daphnia* individual was removed from the tubes with spores and placed in a 50 mL beaker filled with 30 mL filtered lake water that did not contain spores (one animal per beaker). Animals were fed 2 mg/L C of *Ankistrodesmus falcatus ad libidum* for the rest of the experiment (20 days post-infection, 32 days from day 1 of entire experiment).

During those 20 days, we tracked mortality in each of the beakers five days per week (Mondays through Fridays). Water changes were done twice a week; during these, we counted offspring in each beaker; offspring were removed from beakers and discarded. Any animals that died during the trial were placed in 2 mL Eppendorf tubes with 100 µL of nanopure water and stored at 4 °C for later spore counts. At the end of the experiment (20 days post-infection, 32 days from day 1 of entire experiment), any remaining live animals (130 total) were placed in a 2mL Eppendorf tube with 100 µL of nanopure water.

Animals were then ground to release spores, and spores in the ascus stage were counted using a hemocytometer under a compound microscope at 400X magnification.

Data analysis

For all our models, we analyzed data from the microcystin-LR and anatoxin-a treatments separately. This means that for each analysis described below, there was one performed for the microcystin-LR relevant data and another for the anatoxin-a data. The same no-toxin control data (0 µg/L; 20 replicates per incubation time) were used for the two sets of data (microcystin-LR and anatoxin-a). When analyzing the anatoxin-a data, the acetic acid treatments were included in the analyses (as an additional negative control) and were treated in our analyses as a low concentration (0.01%); statistical analysis did not find differences between the no toxin controls and the acetic acid controls (data not shown). Prior to analyses, the data was checked for normality using the Shapiro-Wilk test. Data that did not meet normality were analyzed using appropriate family error distribution link functions and checking for overdispersion. All analyses were carried out in R Studio Version 1.2.1335 using the stats v3.6.1 package.

We assessed environmental effects on the parasite's ability to infect by comparing differences in the number of animals that developed terminal infections (that is, infections that produced asci (Stewart Merrill & Cáceres, 2018)). In this analysis, our response variable had two outcomes (terminal infection or not). We performed a generalized linear model (GLM) with incubation time and toxin concentration as explanatory variables, using a binomial family error distribution.

We also evaluated if toxin concentration and incubation time (that is, the time spent as a transmission spore in the water) affected the number of mature transmission spores produced per infected host individual. For this analysis, we ran a GLM; here the number of spores was our response

variable and, similarly to the analysis of infections, toxin concentration and incubation time were used as the explanatory variables. In this model, we used a Gaussian error distribution.

To evaluate the effects of incubation time and toxin exposure on the parasite's virulence, we measured host offspring production, host survival over the 20-day experiment, and proportion of hosts who died within 20 days of parasite exposure. For these analyses, we ran GLMs with toxin concentration and time of exposure as explanatory variables; for the two former response variables—the number of offspring each host individual produced and the number of days each host survived (up to 20 days post infection)—we used a Poisson family error distribution. For the proportion of dead hosts at the end of the experiment, we used a binomial family distribution.

We ran two linear models analyzing the relationship between the proportion of infected individuals and the mean number of spores produced at the end of infection. In the first, we averaged across the different toxin exposure treatments, yielding one value for each incubation time (n = 5). In the second, we averaged within the toxin treatments, yielding five values per toxin treatment (one per incubation time treatment, total n = 20).

We also ran a second experiment to evaluate whether plastic absorbed considerable amounts of microcystin in the water and therefore negated the toxic effects on the parasites transmission spores. Studies have reported adsorption of microcystins by plastics (Hyenstrand *et al.*, 2001; Moura *et al.*, 2022), which raised the possibility that the results of our first experiment may have been due to toxin concentrations that were lower than we intended. Therefore, in the second experiment, we evaluated whether the plastic vessels we used in the first block adsorbed microcystin-LR from the water and whether using glass vs. plastic vessels for the incubations impacted infections. Additional methods and results from that experiment can be found in the supplementary materials. Briefly, we did not find any

effect of vessel (that is, glass vs. plastic tubes) on toxin concentration, infection prevalence, or spore production.

Results

Time spent in the water decreased infectivity, but toxin exposure did not

Transmission spores that spent longer in the water were less infectious, but toxin exposure did not significantly influence infectivity (Figure 1, Table 1 "Infection prevalence"). The number of hosts that became infected decreased with increased incubation time: spores that were only incubated for 24 hours infected around 75% of exposed hosts, whereas spores incubated for 10 days infected less than 50% of exposed hosts. The reductions in infectivity with increasing incubation time were consistent across different toxin concentrations (as indicated by a non-significant concentration x incubation interaction term in the GLM: microcystins, $Z = 1.160$, $p = 0.246$; anatoxin, $Z = 0.816$, $p = 0.415$).

Spore yield was not affected by toxin exposure

Neither microcystin nor anatoxin dose significantly influenced the number of spores produced per infected host (Figure 2; Table 1). However, in the anatoxin treatments (but not the microcystin treatments), infected hosts exposed to spores that were incubated for longer periods of time yielded more spores at death (or, for those that did not die within 20 days of exposure, at 20 days post-exposure; Figure 2; Table 1).

Virulence was not affected by toxin concentration or incubation time

Neither toxin concentration nor incubation time impacted host offspring production, lifespan, or survivorship measured up to 20 days post-infection. Host lifetime offspring production was consistent across the microcystin-LR treatments (Figure 3, left panel) and across the anatoxin-a treatments (Figure 3, right panel), and across incubation times (Figure 3, Table 1). Similarly, neither toxin concentration nor incubation time significantly influenced the number of days each host survived (up to 20 days post-infection) or the proportion of dead individuals at day 20 (Table 1). Thus, there is no evidence that exposure of transmission spores to toxins in the water, or the duration of time spent in the water column, influenced the virulence of this fungal parasite.

Greater parasites infectiousness is associated with lower spore yield

Treatments that had higher infection prevalence yielded fewer spores per infected host (Figure 4). When averaged across the different toxin treatments within incubation times, longer incubation times had fewer infections that yielded more spores per infected host ($R^2 = 0.80$, $p = 0.02$). If we average within toxin treatments by incubation times, we observe a similar pattern but with a lower amount of variance explained ($R^2 = 0.40$, $p = 0.001$).

Discussion

In this study, we found that the amount of time transmission spores spend in the water impacted their fitness. If spores encountered a new host within a few days of being released from their dead host, the number of hosts that became infected was high, suggesting high per-spore infectivity. However, the high prevalence did not translate into high spore yield; instead, hosts from treatments with high infectivity produced low numbers of mature transmission spores. On the other hand, spores

that spent longer in the water column after being released from a dead host infected relatively few hosts, but each infected host yielded more mature transmission spores as compared to a host infected with spores that spent less time in the water column.

A notable finding from our study is the absence of toxic effects of two common cyanotoxins on parasite transmission spores while in the water column. We predicted that long exposures to high concentrations of cyanotoxin would decrease parasite fitness and virulence. However, our analyses did not detect any effect of toxins. Previous studies have found that consuming toxin-producing cyanobacteria can make hosts more susceptible to parasites (Tellenbach *et al.*, 2016), can decrease the number of spores produced within the host (Manzi *et al.*, 2019), and in certain cases protects hosts from infection (Sánchez *et al.*, 2019). However, the conditions of parasite toxin exposure in the previous experiments were different from our current study. In the previous studies, parasites were exposed to these cyanotoxins when hosts consumed toxic cyanobacteria as part of their diet, so interactions between the toxins and parasites would occur in the host gut. The key difference is that, in our study, the toxin exposure only occurred in the water column, and hosts were not exposed to these compounds. This suggests that the location of exposure of spores to cyanotoxins is important. One possible reason why we did not observe effects of toxins on parasite fitness might be due to the structure of the transmission spore. *Metschnikowia* transmission spores are composed of an ascus, which is an outer structure surrounding the ascospore; the ascospore is the actual transmission spore that pierces the host gut and therefore causes the infection. One possibility is that the ascus, which protects the spore from chemical and physical damage (Lachance *et al.*, 1976), also shields against secondary metabolites in the water, which would explain the lack of effects from the cyanotoxins in this study. Taken together, the results of our study combined with other studies that have found effects of toxin and non-toxin producing cyanobacteria on parasite fitness (Coopman *et al.*, 2014; Tellenbach *et al.*, 2016; Manzi *et al.*, 2019; Sánchez *et al.*, 2019) suggest that the interaction between parasites and

secondary metabolites may be particularly important within the gut of the host. If true, this would suggest that, for parasites with a protective structure that envelops transmission spores, the interaction of toxins with parasites might primarily be important in parasite life stages that lack the protective coating.

There was an apparent tradeoff between per-spore infectivity and the number of spores yielded per infected host. One mechanism that could explain this result is within-host spore competition (Ebert, Zschokke-Rohringer & Hans, 2000). Spores that spent little time in the water column had a high per-spore infectivity, which likely led to multiple spores infecting an individual host; individual *Daphnia* can be infected by multiple *Metschnikowia* spores (Stewart Merrill & Cáceres, 2018). Infection by multiple spores would result in resource competition among spores inside the host, decreasing parasite spore production. In contrast, spores that spent longer in the water column were less viable; this would mean fewer spores were able to infect an individual host, decreasing resource competition and increasing within host reproduction. Prior work has shown that not all spores that make it to the gut pierce the gut—a critical first step of infection that must occur prior to growing in the host hemolymph (Stewart Merrill & Cáceres, 2018). Our results and this hypothesized mechanism are consistent with an earlier study using the same parasite but a different species of *Daphnia*; that study found parasite reproductive success decreased with increased spore dosage at the time of infection (Ebert *et al.*, 2000). It is also possible that spores that survived longer were, on average, 'stronger' than the spores that died during the incubation, meaning a non-random sample of spores generated the infections in the longer incubation periods.

It remains to be determined why spores lost infectivity so rapidly in the water column. One possibility is that light played a role. Our parasite incubations for the toxin exposures were done in the light (using a 16:8 light:dark photoperiod), and other studies have found that light, including UV and photosynthetically active radiation (PAR), decreases infectivity of *Metschnikowia* spores (Overholt *et al.*,

2012; Shaw *et al.*, 2020). Therefore, spores may have retained high infectivity for longer if our experiment had been done in the dark. However, in a previous experiment, even spores that were maintained in the refrigerator in the dark rapidly lost infectivity (Duffy & Hunsberger, 2019); thus, it is possible that multiple factors explain the rapid loss in infectivity of *Metschnikowia* spores in the water column.

One limitation of our study is that we only used a single host genotype and a single parasite genotype. There is substantial diversity in the susceptibility of *Daphnia dentifera* to *Metschnikowia* (e.g., Duffy & Sivars-Becker, 2007; Duffy *et al.*, 2012; Auld, Searle & Duffy, 2017). While there is much less diversity in *Metschnikowia*, there is some (Shaw, 2019). Future work exploring whether *Metschnikowia* genotypes vary in their sensitivity to phycotoxins and/or time spent in the water column would be valuable, as would studies assessing whether the patterns we found in this study are consistent when other host genotypes (and species) are exposed. Interestingly, a new study that incubated *Metschnikowia* spores with cyanobacterial extracts or a control solution, then exposed them to two genotypes of *Daphnia galeata* x *longispina* (Manzi *et al.*, 2022) suggests that these results might hold broadly. Consistent with our study, they did not find reduced infectivity of spores that had been incubated with the cyanobacterial extract; in fact, for one of the two host genotypes, infectivity of spores that had been incubated with the cyanobacterial extract was actually higher.

Here, we found that parasite spores rapidly lose infectivity in the water, but that two common cyanotoxins had no detectable effect on spores. We propose that *Metschnikowia* spores may be protected from toxins in the environment by their ascus structure. We also found that, while spores that had spent more time in the water were less infectious, they yielded more spores per infected host, results that might be driven by resource competition. This raises the intriguing possibility that there might be an intermediate spore age (or dose) that is most likely to fuel large epidemics. Given that

spores spend substantial time in the sediment between outbreaks (Decaestecker *et al.*, 2004), the joint impacts of these mechanisms are likely to be important drivers of *Metschnikowia* epidemics in lakes.

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Author contribution statement

Conceptualization: KFS, MAD. Developing methods: KFS, MAD. Conducting the research: KFS, BZ, JAA. Data analysis: KFS, BZ, JAA, MAD. Data interpretation: KFS, BZ, JAA, MAD. Preparation figures & tables: KFS, BZ, JAA. Writing: KFS, BZ, JAA, MAD.

Data availability statement

Data is accessible through DRYAD digital repository https://doi.org/10.5061/dryad.612jm6420

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Tables

Table 1. Statistical results of generalized linear models on the effects of toxin concentration and incubation time on the parasites fitness and virulence. Note that host reproduction and host survivorship are used here as proxies of virulence. "Conc*Incub" indicates the toxin concentration * incubation time interaction term.

Variable	*Estimate*	*Std. Error*	*Z-value*	*P-value*
		Infection prevalence		
Microcystin-LR				
Toxin concentration	-0.012	0.025	-0.497	0.619
Incubation time	-0.382	0.083	-4.630	<0.0001*
Conc*Incub	0.005	0.005	1.160	0.246
Anatoxin-a				
Toxin concentration	-0.003	0.026	-0.107	0.915
Incubation time	-0.527	0.087	-6.071	<0.0001*
Conc*Incub	0.004	0.005	0.816	0.415
		Spore reproduction		
Microcystin-LR				
Toxin concentration	466.76	386.33	1.208	0.231
Incubation time	2230.73	1528.03	1.460	0.149
Conc*Incub	-87.23	85.90	-1.016	0.313
Anatoxin-a				
Toxin concentration	326.10	355.22	0.918	0.361
Incubation time	4337.31	1508.30	2.876	<0.01*
Conc*Incub	-21.27	83.29	-0.255	0.799
		Host lifetime reproduction		
Microcystin-LR				
Toxin concentration	-0.0007	0.005	-0.140	0.889
Incubation time	0.012	0.019	0.643	0.520
Conc*Incub	0.0008	0.001	0.790	0.430

Anatoxin-a				
Toxin concentration	-0.003	0.005	-0.753	0.452
Incubation time	0.007	0.019	0.353	0.724
Conc*Incub	0.0005	0.001	0.506	0.613
		Host survivorship		
Microcystin-LR				
Toxin concentration	0.0008	0.004	0.227	0.820
Incubation time	0.013	0.015	0.854	0.393
Conc*Incub	-0.0002	0.0008	-0.327	0.744
Anatoxin-a				
Toxin concentration	7.074e-04	3.485e-03	0.203	0.839
Incubation time	1.451e-02	1.447e-02	1.003	0.316
Conc*Incub	-9.278e-05	7.997e-04	-0.116	0.908

Figures

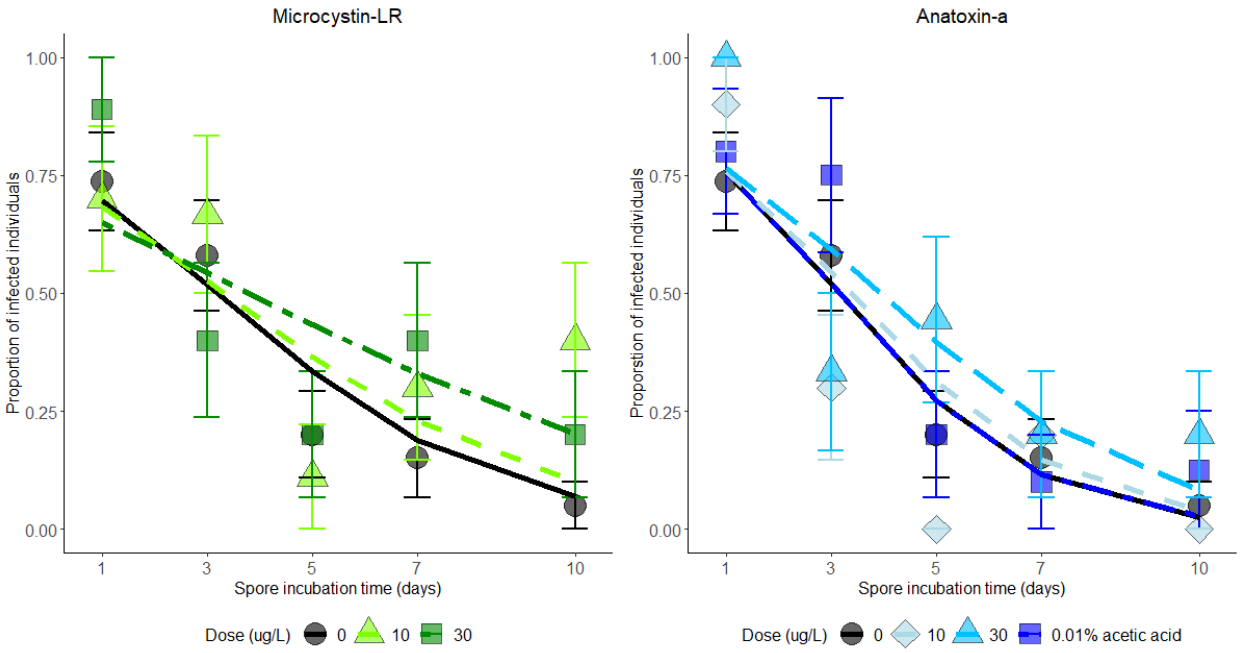


Figure 1. Infection prevalence of hosts exposed to transmission spores treated with different concentrations of pure cyanotoxins and incubated for different amounts of time. This figure shows the proportion of individuals that became infected in each treatment group and standard errors. For both toxins and in all concentration treatments, the number of individual hosts that became infected decreased with the amount of time the transmission spores spent in the water before being used for host exposure. Note that the 0 µg/L concentration treatment is the same set of data for both panels.

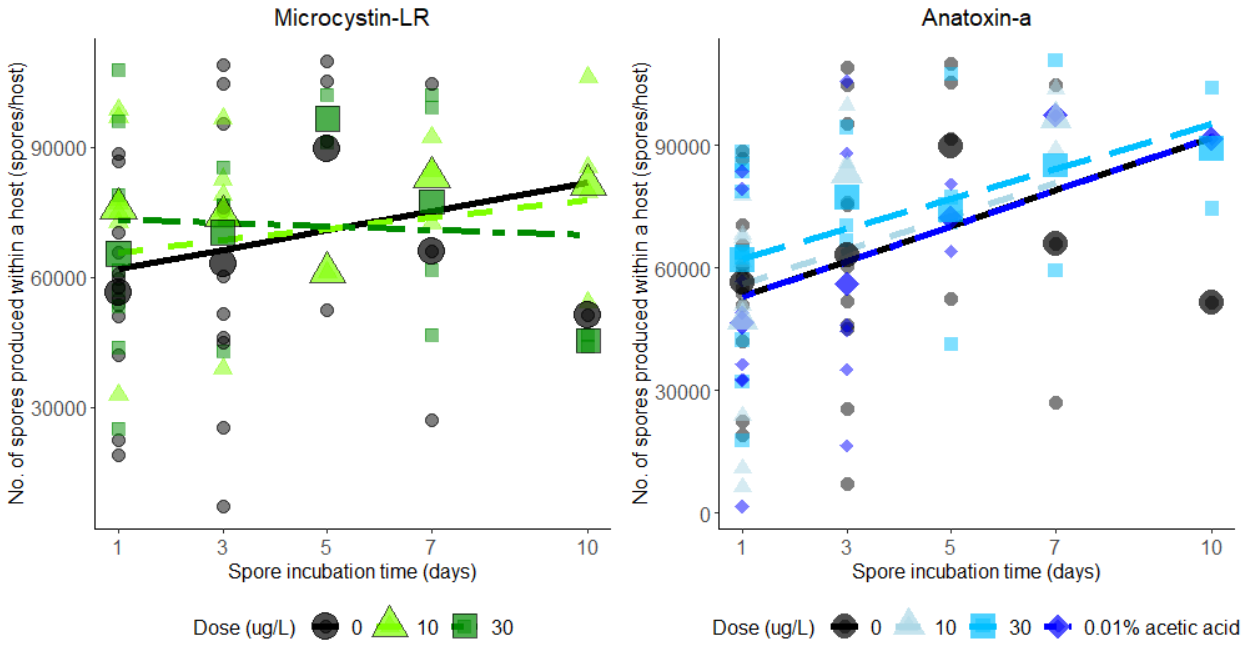


Figure 2. Parasite reproduction within infected hosts exposed to transmission spores treated with different concentrations of pure cyanotoxins that were incubated for different lengths of time. *(Left panel)* Spores that were treated with microcystin-LR did not appear to be affected by the concentration of toxin nor the amount of time these spores spend in the water. *(Right panel)* The number of mature transmission spores produced in all concentration treatments increased with longer periods of incubation time for transmission spores. Note that the 0 µg/L concentration treatment is the same set of data for both panels. Small symbols represent individual data points while large symbols represent means for a given treatment group.

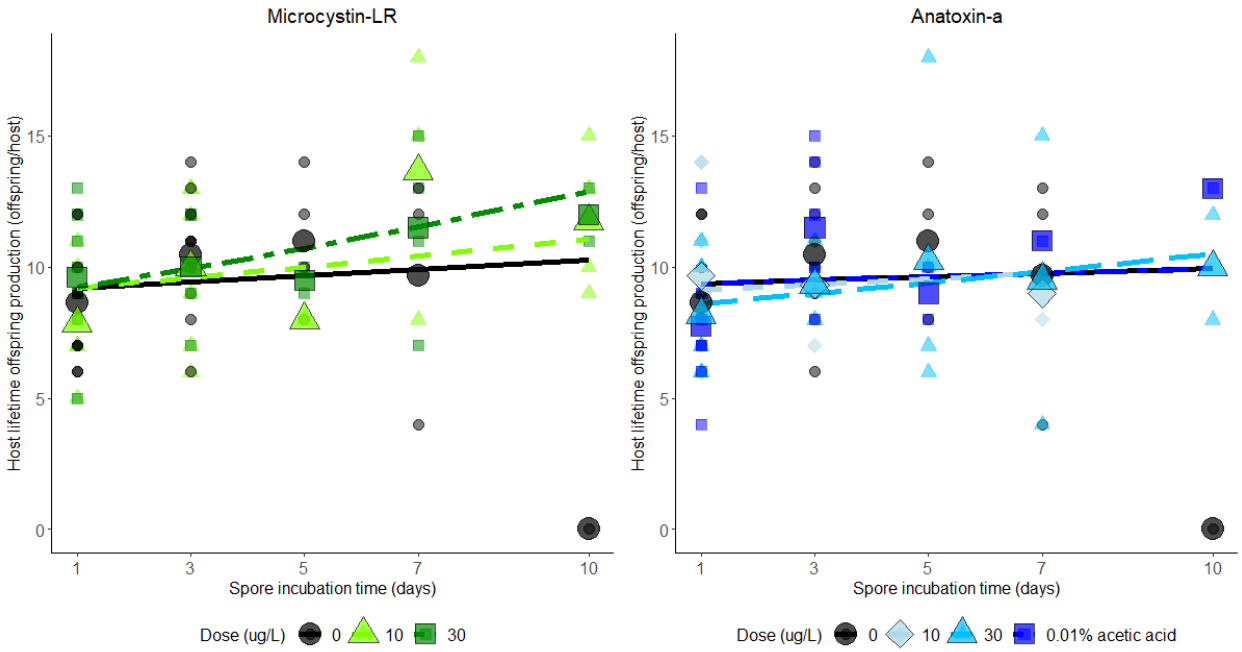


Figure 3. Lifetime reproduction of hosts that were infected with transmission spores treated with different concentrations of pure cyanotoxins and incubated for different lengths of time. Virulence of the parasite did not change based on the transmission spore treatments. Note that the 0 µg/L concentration treatment is the same set of data for both panels. Small symbols represent individual data points while large symbols represent means for a given treatment group.

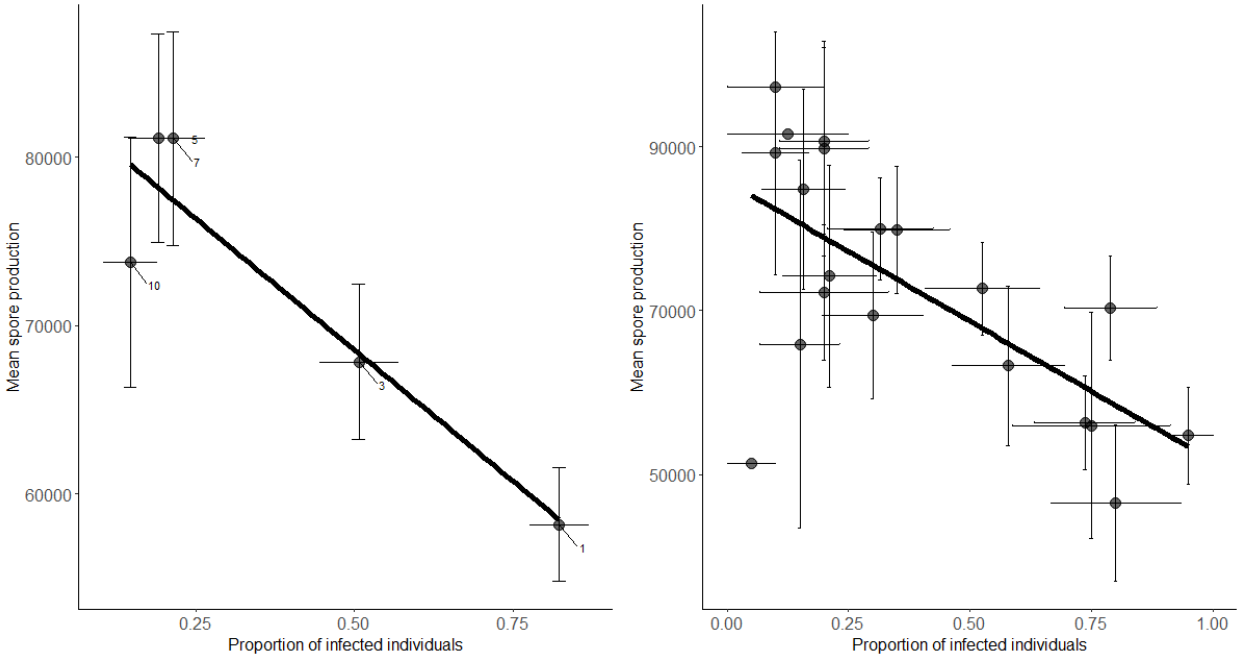
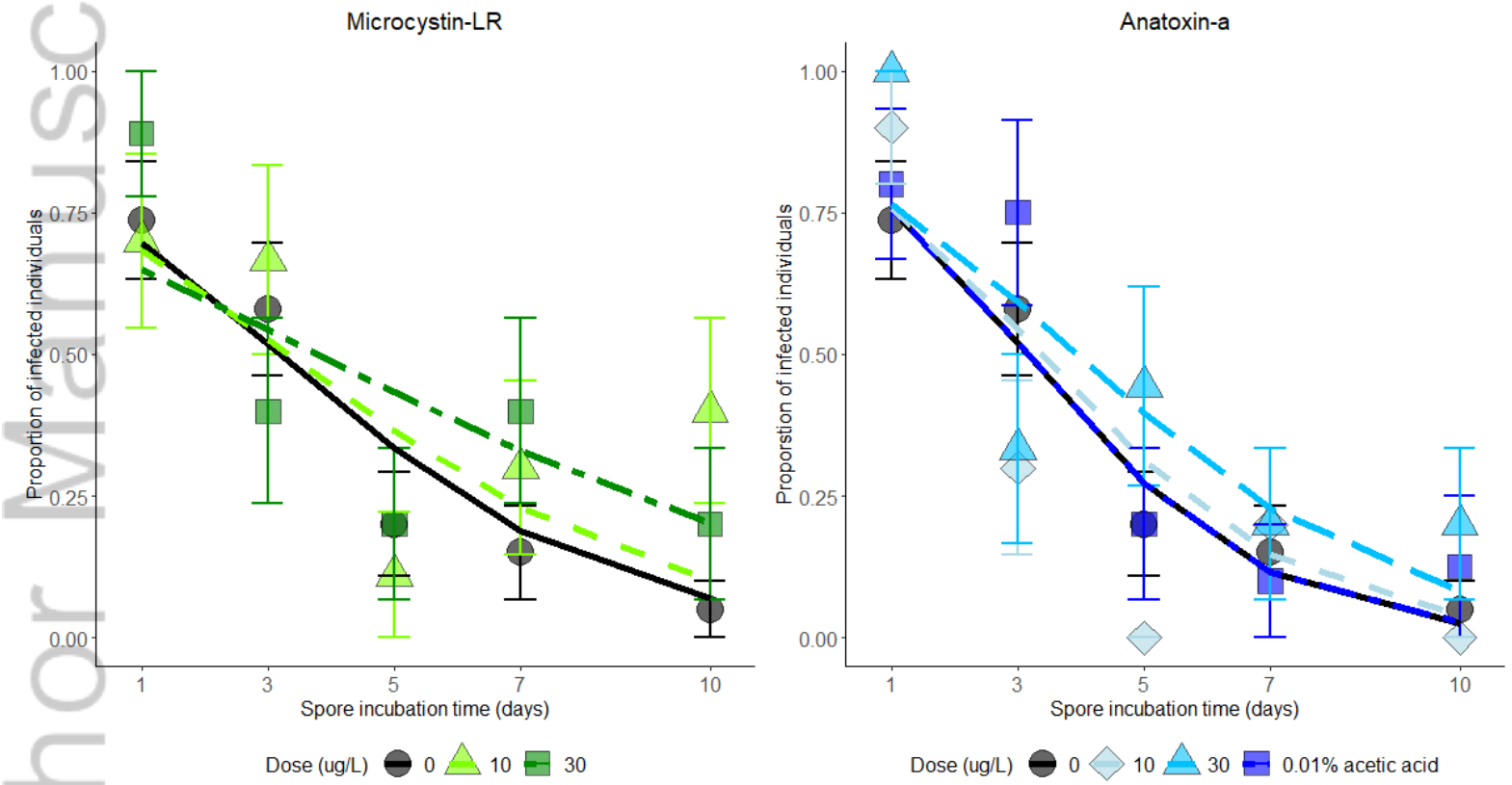


Figure 4. Linear regressions of the proportion of individuals that became infected versus the mean number of mature transmission spores produced in each treatment group. *(Left panel)* The data points in this regression are averaged by incubation time of transmission spores in all toxin treatments. The number in the label for each point represents the number of days those spores were incubated, R^2 = 0.80, p = 0.02. *(Right panel)* This correlation was done by averaging the data by toxin treatment and incubation period, R^2 = 0.40, p = 0.001. For both correlations we observed a decreasing number of spores produced within a host as the number of individuals that become infected in a treatment increased. Error bars in both panels represent standard error for infection prevalence and spore reproduction for the horizontal and vertical bars respectively.



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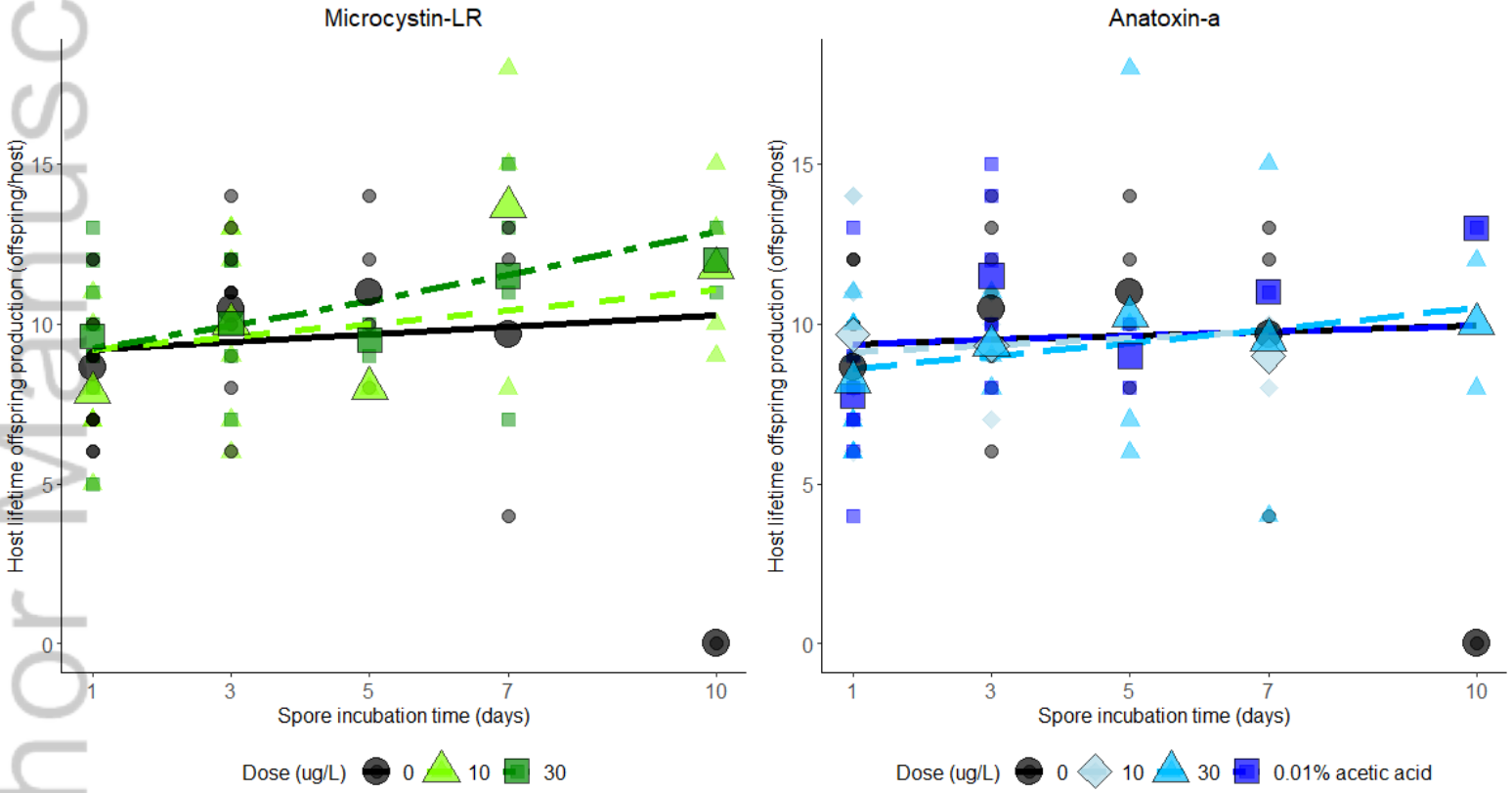

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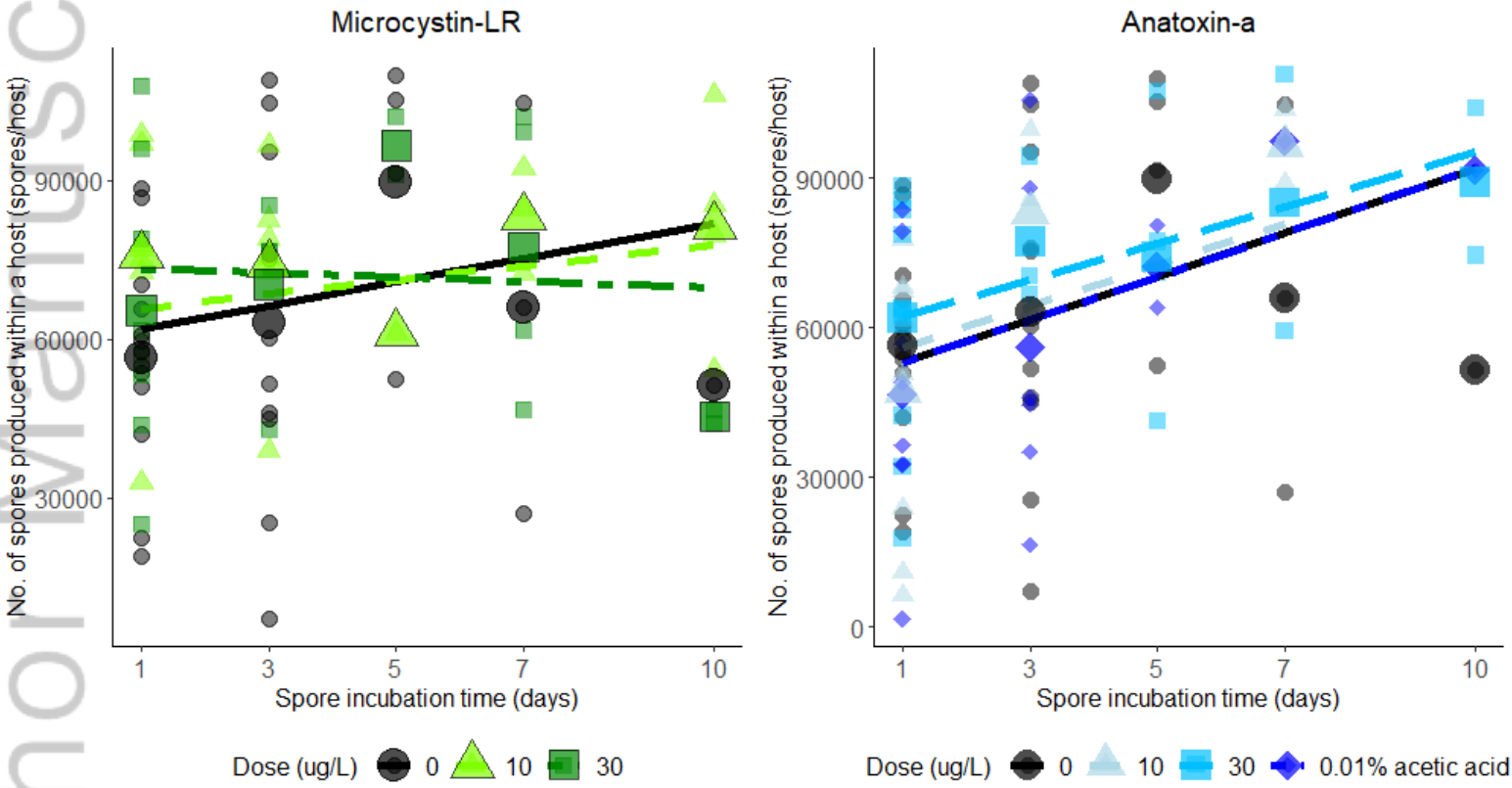

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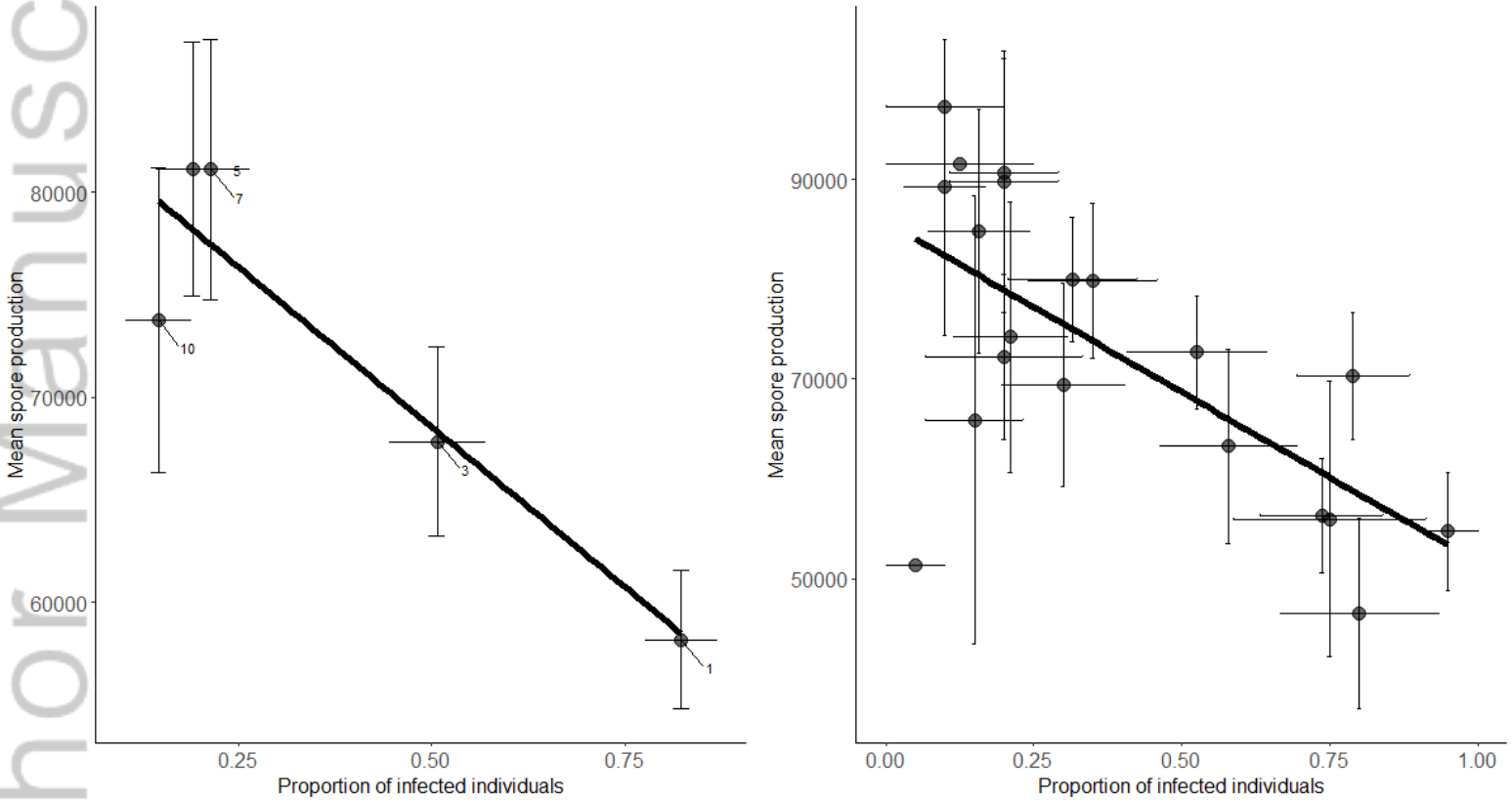

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