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Author(s):
Van den Wyngaert, Silke; Vanholsbeeck, Olivier; Spaak, Piet; Ibelings, Bas W.

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HOST MICROBE INTERACTIONS

Parasite Fitness Traits Under Environmental Variation: Disentangling the Roles of a Chytrid's Immediate Host and External Environment

Silke Van den Wyngaert · Olivier Vanholsbeeck · Piet Spaak · Bas W. Ibelings

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Abstract Parasite environments are heterogeneous at different levels. The first level of variability is the host itself. The second level represents the external environment for the hosts, to which parasites may be exposed during part of their life cycle. Both levels are expected to affect parasite fitness traits. We disentangle the main and interaction effects of variation in the immediate host environment, here the diatom *Asterionella formosa* (variables host cell volume and host condition through herbicide pre-exposure) and variation in the external environment (variables host density and acute herbicide exposure) on three fitness traits (infection success, development time and reproductive output) of a chytrid parasite. Herbicide exposure only decreased infection success in a low host density environment. This result reinforces the hypothesis that chytrid zoospores use photosynthesis-dependent chemical cues to locate its host. At high host densities, chemotaxis becomes less relevant due to increasing chance contact rates between host and parasite, thereby following the mass-action principle in epidemiology. Theoretical support for this finding is provided by an agent-based simulation model. The immediate host environment (cell volume) substantially affected parasite reproductive output and also interacted with the external herbicide exposed environment. On the contrary, changes in the immediate host environment through herbicide pre-exposure did not increase infection success, though it had subtle effects on zoospore development time and reproductive output. This study shows that both immediate host and external environment as well as their interaction have significant effects on parasite fitness. Disentangling these effects improves our understanding of the processes underlying parasite spread and disease dynamics.

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S. Van den Wyngaert · P. Spaak · B. W. Ibelings
Aquatic Ecology, Eawag, Ueberlandstrasse 133, P.O. Box 611, 8600 Duebendorf, Switzerland

P. Spaak · B. W. Ibelings
Institute of Integrative Biology, ETH Zürich, CH-8092, Zürich, Switzerland
e-mail: silke@uglylab.eu

O. Vanholsbeeck
Institute of Evolutionary Biology and Environmental Studies, University Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

B. W. Ibelings
Institut F.-A. Forel, Université de Genève, 10 Route de Suisse, Versoix, Switzerland

B. W. Ibelings
Netherlands Institute of Ecology, Department of Aquatic Ecology, Droevendaalsesteeg 10, 6708, PB Wageningen, The Netherlands

Present Address:
S. Van den Wyngaert (✉)
Laboratory of Aquatic Biology, Department of Biology, K.U. Leuven Kulak, E. Sabbelaan 53, 8500 Kortrijk, Belgium
e-mail: silke@uglylab.eu

Introduction

The way an infectious disease spreads through a host population is driven by the complex interplay between host, parasite and environment (disease triangle; [37]). From a parasite's perspective, the environment can be viewed in two dimensions: (i) the host itself (immediate environment), nested in (ii) the surrounding environment (host population density and habitat of the host), with both dimensions being highly interdependent [24, 39]. The immediate (i.e. the host) environment consists of both genetic and ecological components. A substantial part of research on host parasite interactions has focused on the genetic mechanisms underlying parasite infectivity/host susceptibility (reviewed in [47]). However, ecological mechanisms, either

directly through phenotypic traits of individual hosts or indirectly through environmental-induced changes in host condition may also affect parasite fitness and consequently play a large role in parasite population dynamics [13, 21, 33, 14].

Parasite fitness is an integrative measure of several parasite life history traits such as infection success, development time, per capita reproductive output and survival. Each of these traits can be differentially affected by the immediate host and/or external environment. Vale and Little [40] for example disentangled parasite fitness traits in a *Daphnia*-bacteria host parasite system and demonstrated that infectivity depended mainly on host genetics (immediate host environment) whereas parasite spore production was determined by temperature (external environment). Another study [11] found that pre-exposure of leopard frogs to a pesticide mixture did not influence host recognition or penetration by a parasitic lungworm. However, the parasite matured and reproduced earlier in hosts that were exposed to pesticides in their environment.

Disentangling the responses of single parasite fitness traits in heterogeneous environments improves our knowledge on the mechanisms underlying parasite population growth and enhances our understanding and prediction of disease dynamics.

In this study, we aim at experimentally disentangling the main and potentially interactive effects of immediate host and external environmental variation on single parasite fitness traits such as infection success, zoospore development time and reproductive output. We designed our experiments based on the results of a previous epidemiological experiment that we conducted with the microbial host parasite model system *Asterionella formosa* (as diatom host) and *Zygorhizidium planktonicum* (as chytrid parasite) exposed to an environmental pollutant, the herbicide diuron. In that study, we observed that exposure to diuron modified the parasite population dynamics, and that prevalence of infection positively correlated to mean host population cell volume [42]. Compared to the control treatment, herbicide exposure prompted an initial inhibition of parasite transmission, though over longer time—with a gradual increase in host density—an enhanced spread of disease was observed with diuron exposure. We formulated the following hypotheses to disentangle the mechanisms that caused the modified parasite infection dynamics upon herbicide exposure.

1. Hypothesis no. 1 revolves around zoospore infection success. We hypothesize that acute herbicide exposure (external environment) constrains parasite infection success by interfering with host finding mechanisms based on chemotactic cues. Moreover, we hypothesize that at higher host density chance contact rates between host and parasite increase; therefore, at high host density, chemotaxis is less relevant for infection success [22].

Specific to our algal–chytrid model system, it was found that infection success of chytrid zoospores decreased with decreasing light intensity and becomes zero under dark conditions. [2, 4]. Observational studies on zoospore swimming behaviour demonstrated that under light conditions, zoospores were strongly attracted by host cells (swarm behaviour) swimming in tighter circles and remain "spinning" around the host colony. In the dark, they swam at random and exhibited a basic pattern of smooth gliding motion along straight or curvilinear paths before becoming immobile [5, 4] These observations led to the hypothesis that zoospores use photosynthesis exudates of the host as chemotactic cues to localize host cells. Similar to light limitation, the herbicide diuron inhibits photosynthesis [9] and could thereby interfere with chemotaxis.

2. Hypothesis no. 2 revolves around the negative effects of environmental (herbicide) stress on host condition and disease resistance (immediate host environment). We hypothesize that increased host exposure time to herbicides increases host susceptibility. Diuron affects photosynthetic electron transport and thereby causes depletion of ATP and NADPH supplied by the light reaction. Inhibition of photosystem II electron transport also generates reactive oxygen species (ROS) that have the potential to cause membrane protein damage [7]. Longer exposure to the herbicide may therefore disrupt physiological processes which could increase host susceptibility to parasites.
3. Hypothesis no. 3 revolves around the effects of host quality on parasite fitness (immediate host environment). Here, we hypothesize that the increased spread of infection in host populations with larger cell volume (as demonstrated by [42]) is mainly driven by a higher parasite reproductive output on larger host cells due to a richer host environment (increased resource availability) [34]. However, we also hypothesize that diuron pre-exposure negatively affects host nutrient quality by reducing the incorporation of photosynthetic products, thereby reducing zoospore production and/or development time.

By testing these three hypotheses, we aim to determine to what extent the immediate environment, which the host *Asterionella* constitutes for the chytrid parasite (variables host cell volume and host condition/quality through herbicide *pre*-exposure) and the external environment (variables host density and acute herbicide exposure) and their possible interaction affect parasite fitness traits.

Material and Methods

Host–Parasite Study System

The host *A. formosa* is a cosmopolitan freshwater diatom which forms stellate colonies. Reproduction in this species is predominantly asexual and characterized by a progressive

reduction in cell size. The parasite *Z. planktonicum* is an aquatic fungus belonging to the Chytridiomycetes [17]. It is an obligate, host-specific parasite of *A. formosa* [43]. Hence, the parasite cannot survive and/or reproduce in absence of its host. *Z. planktonicum* is an extremely virulent parasite, i.e. every infection inhibits host reproduction and quickly leads to host cell death. The life cycle of this chytrid parasite begins with free swimming motile stages called zoospores that actively find their host and attach to the surface of the host cell. After zoospore encystment, a germ tube is formed which enters the host cell through the girdle zone. Via the germ tube, nutrients are extracted from the host cell and used for the development of the sporangium. New zoospores are formed either asexually or sexually and are released from the sporangium by dehiscence [8, 15].

General Experimental Set-up

Both the host strains and the parasite strain were isolated during the spring bloom of 2008 from Lake Maarsseveen, (52.142828 N, 5.085711 E, The Netherlands). They were cultured in CHU-10 medium [36] as uniclonal but nonaxenic batch cultures.

To test our three hypotheses, we conducted two experiments. With a short-term infection experiment, we test hypotheses 1 and 2 by disentangling the effects of the external environment (hypothesis 1; host density and acute diuron exposure) and the immediate host environment (hypothesis 2; host condition by diuron pre-exposure) on parasite infection success. Experiment 2 is a test of hypothesis 3 because here, we test the effect of host quality upon diuron exposure and host cell size (immediate environment) on parasite reproductive output and development time.

Prior to the experiments, we kept the host strains and the parasite strain (on its host) in exponential growth in respectively 60 and 120 mL batch cultures under the experimental conditions (18 °C, 100 μE m^{-2} s^{-1} irradiance). Six days before the start of the experiments, we transferred one subpart of the host strains to control conditions, and another subpart we incubated with a sublethal concentration of 8 μg L^{-1} diuron (CAS 330 54 1, PESTANAL® analytical standard, Sigma-Aldrich Chemie GmbH, Germany). This concentration provokes ca. 50 % inhibition of the maximum photosynthetic yield and was determined from a preliminary toxicity test performed with three different *A. formosa* strains from Lake Maarsseveen [42]. Before the experiment was started, we measured the maximal photosystem II quantum yield after dark adaptation of both control and diuron incubated host strains on a PhytoPam (Walz GmbH, Germany); $(F_m - F_0)/F_m$, where F_m is the maximal fluorescence yield and F_0 is the minimal fluorescence yield [27]. Thereby, we ensured that the diuron exposure had effectively reduced photosynthesis. We obtained the parasite zoospore suspension by filtering the parasite culture through a 7-μm mesh-sized plankton net. We verified whether filtration and exposure to the experimental diuron concentration did not have any negative effect on zoospore survival by microscopic inspection of the swimming activity of the zoospores.

Experiment 1: Parasite Infection Success

In a factorial experiment, we examined the effect of the external environment (i) host population density (low, 10,000 cells mL^{-1} versus high, 80,000 cells mL^{-1}) and (ii) acute diuron exposure, and the immediate host environment (iii) host strain and (iv) host condition through diuron pre-exposure on parasite infection success. We used one parasite strain and five monoclonal host populations which we grouped in two different size classes (small: mean cell volume=253 μm^{-3} (±6.04) and large: mean cell volume= 301 μm^{-3} (±3.10), see Fig. 1) to test for the effect of host size on infection success. We divided the experiment in two replicated temporal blocks (1 week apart) which each contained three replicates of all treatments. The experiment involved 5 host populations×2 acute diuron treatments (yes/no)×2 host densities (low, high)×2 diuron pre-exposure treatments (yes/no)×2 blocks×3 replicates=240 experimental units. The infection experiment was conducted in 24-well plates with each well having a total volume of 2.5 mL. First, the host cell concentration in each of the experimental batch cultures (different host strains and treatments; control and diuron exposed) was determined with the Utermöhl method [43]. Then, the inoculation numbers to obtain the low host density (10,000 cells mL^{-1}) and high host density (80,000 cells mL^{-1}) treatments were calculated and added to the wells which were filled up to 2 mL with medium. Subsequently, diuron was added to the respective treatments to reach a final concentration of 8 μg L^{-1}. Thereby, we considered the amount of diuron already contained in the pre-exposed host strains. Since we did not want to manipulate the host differently compared to the other treatments (by filtering and washing), we have to notify that in case of the high host density conditions the amount of diuron was still relatively high in the pre-exposed, but non-diuron added treatment (approximately 6 μg L^{-1}). In the "Discussion", we will explain why this does not interfere with the interpretation of the results. In the low host density treatment, however, diuron that was transferred from the pre-exposed host strain batches was diluted to a concentration in the range of nanograms per litre. This concentration did not have an effect on photosynthetic ability (as measured with a PhytoPam (Walz GmbH, Germany)). After a 30-min incubation time, we added 0.5 mL of zoospore suspension to each treatment. To determine the zoospore concentration, we added Lugol's solution to a 1-mL subsample of the filtered zoospore suspension in order to let the zoospores settle. We counted a minimum of 30 fields of view using an inverted microscope

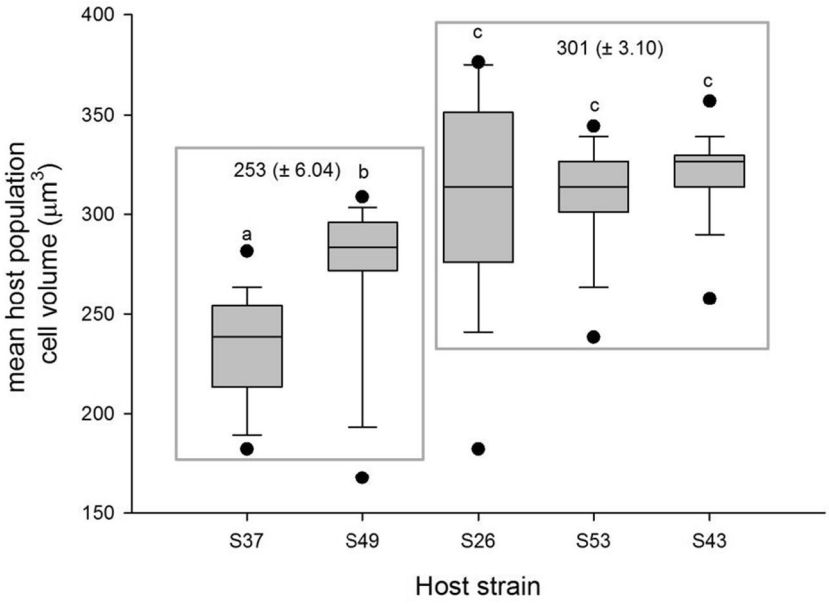

Fig. 1 *Box plots* of the cell volume for the five hosts populations (for each population $n=50$). The *horizontal line* represents the median and *whiskers* indicate the fifth and 95th percentiles. *Letters* indicate statistically different groups in pairwise post-hoc tests (Tukey's HSD; $p<0.001$). *Grey boxes* depict the host size classes (small and large) with their mean cell volume (±SE)

(Leitz Fluovert FS, Wetzlar, Germany). For block 1 and block 2, zoospore concentrations were 6,728 and 4,407 mL^{-1}, respectively. After 3 h of parasite exposure, we fixed the samples in the well plates with a mixture of paraformaldehyde (0.01 % final concentration) and glutaraldehyde (0.1 % final concentration). Samples were subsequently transferred into sampling tubes and stored cool and dark until further processing. We counted for each treatment ca. 350 host cells using an inverted microscope (Leitz Fluovert FS, Wetzlar, Germany) according to the Utermöhl method [43] and determined the number of infected cells and number of attached zoospores. We calculated parasite infection success as the proportion of attached zoospores from the initially added zoospore numbers.

Experiment 2: Zoospore Development Time and Production

In a factorial experiment, we tested for the effects of the external environment, (i) acute diuron exposure and the immediate host environment, (ii) host strain (smallest and largest of the strains used in experiment 1) and (iii) host quality through diuron pre-exposure of host cells on zoospore development time (time span between zoospore exposure and the release of new zoospores) and zoospore production. The experiment involved 2 host populations (small and large)×2 diuron treatments (yes/no)×2 diuron pre-exposure treatments (yes/no)×3 replicates=24 experimental units. We conducted the experiment in 6-well plates with each well having a total volume of 10 mL, containing 5 mL of filtered zoospore solution. The host density was 25,000 cells mL^{-1} and parasite zoospore concentration was 9,409 mL^{-1}. Based on an earlier experiment with the chytrid *Rhizophydium planktonicum*, the sporangia development time (defined as the period between zoospore attachment and sporulation) was estimated to be approximately 2 days under the experimental temperature conditions (18 °C) used in our experiment [1]. For each treatment, we had an additional replicate where we took subsamples at 4 or 5 h time intervals from day 2 onwards to verify the start of sporulation. After 62 h, we saw the first empty sporangia, indicating that zoospores had been released from the sporangia. From this time onwards, we took 1 mL subsamples from the experimental units in 2.5 or 3 h time intervals. We counted for each treatment and time interval at least 45 sporangia and calculated the percentage of empty sporangia. The counting error for counting 45 sporangia corresponded to 30 % and was estimated by the following formula: counting error (±%)=$2\times(100/\sqrt{n})$ [25]. We determined a factor we call $S_{25,}$ the time where 25 % of sporangia had sporulated. From the same samples, we measured the smallest diameter of 35 empty sporangia per treatment and calculated the sporangia volume assuming a spherical shape [2]. From the measurements of sporangium volume, we deduced the number of zoospore produced per sporangium by applying a conversion factor (sporangium volume×0.166) that was previously determined by [2]. In addition we measured the host cell volume associated with the empty sporangium assuming the shape of a rectangular box ($V=\text{length}\times(\text{width})^2$). We recorded phase contrast images (×800 magnification) with a digital camera (Leica DFC290 HD, Leica Microsystems GmbH, Wetzlar, Germany), using an inverted microscope (DMI 4000B, Leica Microsystems GmbH, Wetzlar, Germany) and used Leica image analysis software (LAS Image Analysis, Microsystems GmbH, Wetzlar, Germany) for the measurements.

Statistical Analysis

We performed a factorial ANOVA to analyse the effect of host strain, acute diuron exposure, host condition (diuron pre-exposure) and host density on parasite infection success. We included blocks as a fixed factor in the model since zoospore abundance differentiated between the two blocks. We used a logit transformation of the response variables as this is preferred over arcsine square root transformation of proportional data [46]. Afterwards, we used planned contrasts to test whether infection success was host size dependent. To analyse the effect of host strain, acute diuron and diuron pre-exposure on zoospore development time, we used the 25 % sporulation time, determined from the three replicates, as the dependent variable and host strain, diuron pre-exposure and acute diuron exposure as fixed independent variables. A similar analysis was performed for sporangia volume, including the associated host cell volume as a covariate to control for cell size effects between treatments. In both analyses, zoospore development time and sporangia volume (log transformed) complied with the assumptions of normality and homogenous variance, judged by visual inspection of residuals. We also conducted linear regressions to investigate the relationship between sporangia volume and host cell volume. All statistical analyses were performed using R, version 2.15.1 (R Development Core Team 2008).

Agent-Based Model (ABM)

The model is implemented using Uglylab, an agent-based modelling tool we developed in Java. The source code is freely downloadable from http://www.uglylab.eu. The complete model description can be found in Online Resource 1. The purpose of the model is to test the hypothesis of density dependence of chemotaxis (active host-finding mechanism) for parasite infection success and to compare the predicted patterns with our experimental results. We therefore defined four scenarios that simulate the experimental treatments: (1–2) low and high host density with chemotaxis (control) and (3–4) low and high host density without chemotaxis (diuron exposure).

The initial model contains two agent classes: hosts and zoospores (free-living life stage of the parasite) which are randomly placed (following a uniform distribution) in a three-dimensional space representing a volume of 0.125 mL. We considered a host being a colony of eight cells because these are commonly observed in nature [26]. We set the initial number of host colonies and zoospores according to the experimental host and zoospore densities. We introduced five host pools normally distributed around the mean host cell size of 40 μm (represents the larger host size class) to introduce host cell size variation.

The model contains three rules:

1. Zoospore movement: When chemotaxis is not active, zoospores show a random walking behaviour. When chemotaxis is activated, zoospores show a random walking behaviour until hosts are encountered in the defined sphere of chemotaxis influence. Once inside the sphere of chemotaxis, the algorithm computes a vector representing the chemotaxis strength. The chemotaxis strength is the sum of the individual strength of each host present in the sphere of chemotaxis influence. A host exerts a force inversely proportional to the distance host-zoospore and tends to zero as the distance tends to the radius of the sphere of influence.
2. Host movement: The host movement rule simulates Brownian motion (small-scale random motions) just to avoid that zoospores are trapped into a basin of attraction when chemotaxis is active. For example, when a zoospore finds itself at exact the same distance from two hosts, the chemotaxis strength will be equal for both hosts. The zoospore would then continue moving back and forth between them without being attracted to a host. This situation is avoided by simulating Brownian motion because hosts will always change their position to some small extend so that the distance to a zoospore will never remain completely equal between hosts.
3. Infection: If a zoospore comes into contact with a host, it will infect the host with a probability PI.

For all simulation scenarios the infection probability was set at 1 %, i.e. when a zoospore comes in contact with a host it has a probability of 1 % to infect the host. This low probability of infection was used with the purpose to simulate that not every contact with a host leads immediately to an infection but that the process needs a certain time. This is also observed under real conditions and is probably due to the fact that additional cell recognition steps (which may be regulated by different cues implying host genetics) are involved to complete infection. Cell recognition is not directly modelled in our simulations, but zoospores with chemotactic swimming behaviour increase their chances to infect because they stay attracted near the host cell leading to more frequent host contacts. Since we are interested in rapid infection processes, we used very short discrete time steps of 1 s. Simulations were run for 10,800 s (3 h) which corresponds to the duration of the infection experiment.

Results

Experiment 1: Parasite Infection Success

There was a significant block effect, with the first block, having higher initial zoospore concentration, resulting in a higher

overall infection success. Although there was a significant block×host density interaction (Table 1), the direction of the slope did not change and reaction norms did not cross between blocks (host density; block 1, slope=1.05, p=<0.001; block 2, slope=2.11, p=<0.001). Acute diuron exposure had a negative effect on parasite infection success, however only in the low host density treatment (Fig. 2a). Parasite infection success increased with an increase in host density, in both the control and diuron treatments. This increase in infection, however, was higher in the presence then the absence of diuron which resulted in a significant interaction between host density×diuron (Table 1, Fig. 2a). Host condition through diuron pre-exposure decreased parasite infection success in the non-acute diuron-exposed treatment, whereas additional acute diuron exposure did not further reduce infectivity. This resulted in a significant pre-exposure× acute exposure to diuron interaction (Table 1, Fig. 2b). Host strain (mean host population cell volume) had a significant effect on parasite infection success (Table 1). Lower infection success in host populations with smaller mean cell volume compared to populations with larger mean cell volume was shown by a significant contrast effect (T_{228}=2.51, p=0.013).

Experiment 2: Sporangia Development Time and Zoospore Production

There were significant main effects of host strain, host condition/quality through diuron pre-exposure and acute herbicide stress on zoospore development time (Table 2). Zoospore development was significantly faster on the larger host strain (Table 2a, Fig. 3). Acute diuron exposure significantly increased the zoospore development time whereas

Table 1 ANOVA results of the effect of host strain (HS), block (B), host density (HD), diuron pre-exposure (EXP) and diuron (D) on the proportion of *Z. planktonicum* zoospores successfully infecting *A. formosa* cells

Source	*df*	MS	*F*	*p*
Host strain (HS)	4	2.93	3.10	*0.017*
Block (B)	1	15.46	16.40	*<0.001*
Host density (HD)	1	142.99	151.68	*<0.001*
Diuron pre-exposure (EXP)	1	2.86	3.04	0.083
Diuron (D)	1	0.01	0.01	0.935
B×HD	1	17.40	18.46	*<0.001*
B×EXP	1	1.93	2.04	0.155
B×D	1	0.01	0.01	0.906
HD×D	1	20.49	21.74	*<0.001*
HD×EXP	1	0.11	0.11	0.738
EXP×D	1	6.26	6.64	*0.011*
HS×B	4	0.94	1.00	0.411
HS×HD	4	1.71	1.81	0.129
HS×EXP	4	0.31	0.33	0.857
HS×D	4	1.12	1.19	0.319
Error	154	0.943		

All three-way and higher-order interactions were not significant and therefore are not included in this table. Italicized numbers indicate significance

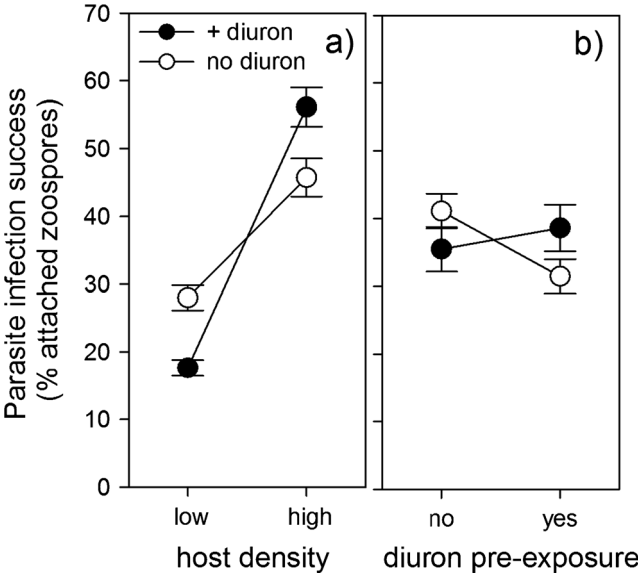

Fig. 2 Interaction effects of acute diuron and **a** host density and **b** diuron pre-exposure on parasite infection success (±SE). *White circles* designate the absence of diuron and *black circles* the presence of diuron

Table 2 Three-way ANOVA for zoospore development time and sporangia volume (zoospore production) by host strain (HS), diuron pre-exposure (EXP) and diuron (D) In case of sporangia size we included the covariate host cell volume (CV)

Source	*df*	MS	*F*	*p*
Zoospore development time				
Host strain (HS)	1	69.87	54.76	*<0.001*
Diuron pre-exposure (EXP)	1	25.94	20.33	*<0.001*
Diuron (D)	1	33.73	26.43	*<0.001*
HS×EXP	1	0.18	0.14	0.716
HS×D	1	2.01	1.58	0.227
EXP×D	1	4.38	3.43	0.083
HS×EXP×D	1	0.00	0.00	0.993
Error	16	1.28		
Sporangia volume				
Host cell volume (CV)	1	2.911	231.62	*<0.001*
Host strain (HS)	1	0.689	54.81	*<0.001*
Diuron pre-exposure (EXP)	1	0.108		*0.004*
Diuron (D)	1	0.055	4.39	*0.037*
HS×EXP	1	0.001	0.05	0.832
HS×D	1	0.094	7.51	**0.007**
EXP×D	1	0.111	8.83	**0.003**
HS×EXP×D	1	0.047	3.74	0.054
Error		275	0.013	

Italicized numbers indicate significance

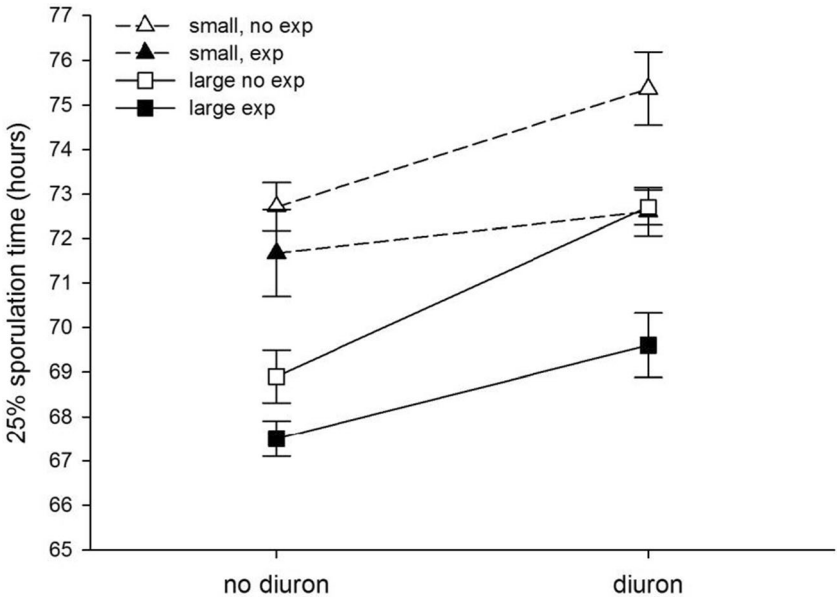

Fig. 3 Zoospore development time is expressed as the time where 25 % of the sporangia have released their zoopsores (mean and SD is plotted). *Triangles* and *squares* represent the development time on the smaller and larger host strain, respectively, in the presence and absence of acute diuron and upon diuron pre-exposure (*black symbols*) or no pre-exposure (*white symbols*)

diuron pre-exposure reduced it (Table 2, Fig. 3). For the dependent variable sporangia size, there were also significant main effects of host strain, acute diuron exposure and diuron pre-exposure. Parasites produced smaller sporangia on the smaller host strain compared to the larger host strain (Table 2, Fig. 4a). Applying the conversion factor for estimating number of zoospores, as determined by Bruning [2] (sporangia volume×0.166), the larger host strain produced on average 1.5 times more zoospores than the smaller (25 and 16 zoospores per sporangium, respectively). Pre-exposure to diuron led to a smaller sporangia volume. The difference was subtle, resulting in a 9 % reduction of zoospores per sporangium when pre-exposed to diuron. Similar as to the results on infectivity, additional diuron exposure did not enhance the negative effect of diuron pre-exposure on zoospore production which explains the pre-exposure×diuron interaction (Table 2, Fig. 4b). In addition, the negative effect of acute diuron exposure was dependent on the host strain. Only the smaller-sized host strain experienced a reduction in zoospore production (9 % less zoospores per sporangium compared to the control) upon acute diuron exposure (Table 2, Fig. 4a). The covariate host cell volume was positively related to sporangia volume, independent of host strain and treatment (linear regression; r^2=0.38, p<0.001, n=284, Fig. 5).

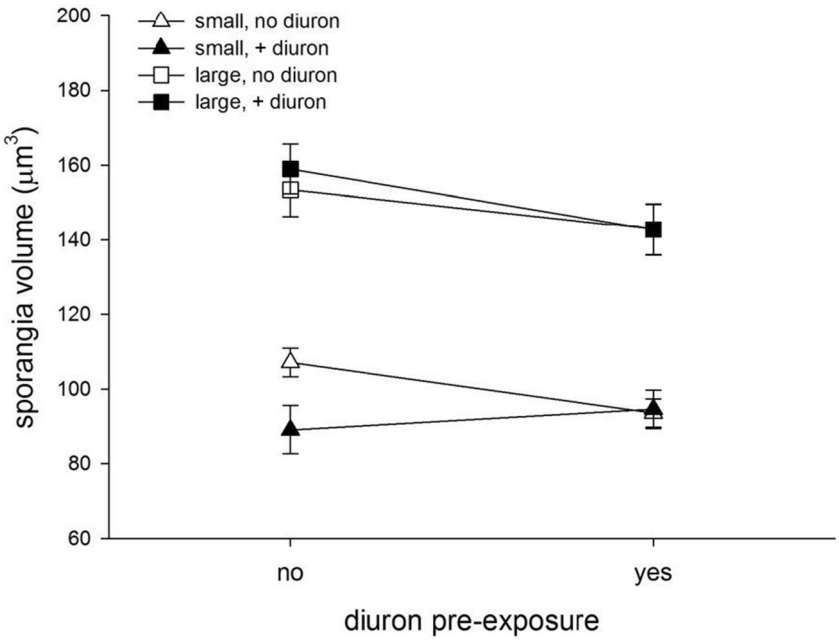

Fig. 4 Main and interaction effects of acute diuron exposure, diuron pre-exposure and host strain on sporangia volume (±SE). *Triangles* and *squares* represent the sporangia volume on the smaller and larger host strain, respectively, in the presence and absence of diuron pre-exposure and upon acute diuron exposure (*black symbols*) or no acute diuron exposure (*white symbols*)

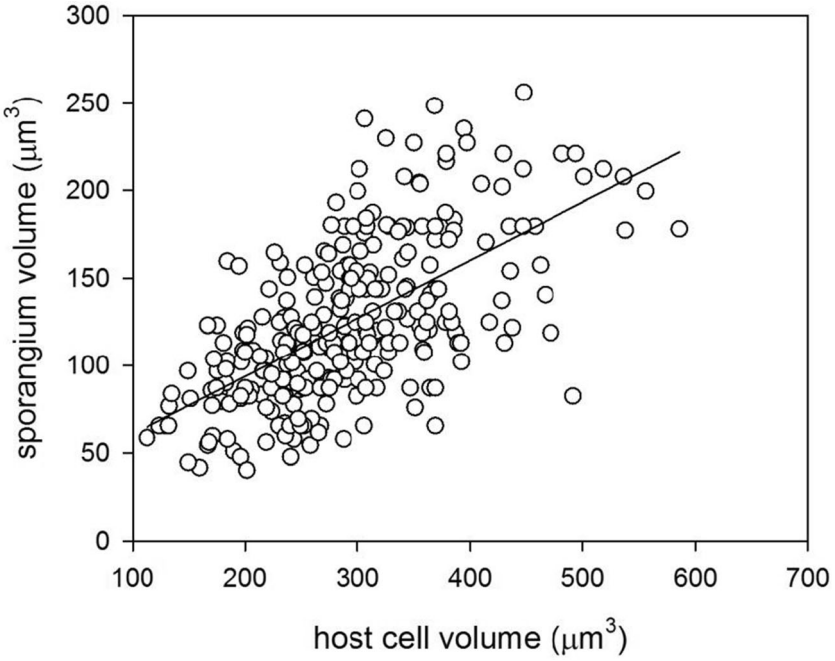

Fig. 5 Relationship between host cell volume and sporangia volume

Model Simulation Results

The simulations were run over a 3-h time span, equal to the experimental infection time. Since the objective of the model is to compare the simulation results with the outcome of the experimental results, we only report about the end result of the simulations.

Just by ex- or including chemotaxis and keeping all other parameter values constant, the model reproduced patterns similar to the outcome of the experiment. For the low host density scenarios, there was a clear difference between number of attached zoospores in the presence and absence of chemotaxis, although this difference was stronger in the model than observed in the experiment (57 and 36 % respectively; Fig. 6a, b). Under high host density conditions, the simulation results produced an overall higher parasite infection success compared to the experimental observations (Fig. 6a, b). However, the general pattern of the simulation was in agreement with the experimental results, e.g. the absence of chemotaxis did not have a negative effect on zoospore infectivity, even slightly increasing it (Fig. 6a, b).

Discussion

In this study, we evaluated to what extent the immediate environment, which the host constitutes for the parasite (variables host cell volume and host condition/quality through herbicide pre-exposure) and the external environment (variables host density and acute herbicide exposure) affect parasite fitness traits. We show that parasite infection success was mainly affected by the external environment while parasite reproductive output was mainly driven by the immediate host environment (quantity and quality of resources). However, we also demonstrate that both external environmental factors and the immediate (i.e. host) environment can interact to influence parasite fitness.

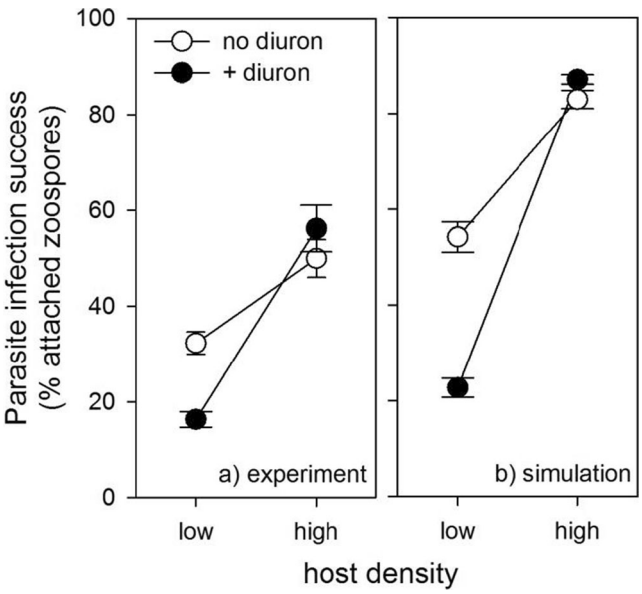

Fig. 6 Endpoint results on infection success from **a** experiment and **b** agent-based model simulations for the four scenarios after 20 simulation runs (mean and SD is plotted). Simulation results are plotted in a similar way as the experimental results for comparison. *White circles* (no diuron) represent the chemotaxis-present scenarios and *black circles* (+diuron) represent the chemotaxis-absent scenarios for each host density (low and high)

The presence of the herbicide diuron in the external environment negatively affected parasite infection success at low host densities. However, as hypothesized, in a high host density environment, this negative effect of diuron disappeared and infection success was even enhanced (Fig. 2). Although we cannot completely exclude the fact that diuron has a direct negative effect on zoospores, we did not observe any differences in zoospore swimming behaviour in the presence of diuron. Further, if diuron would act immediately on zoospores, we would also expect lower infection success at high host density. Therefore, we argue that the underlying mechanism explaining these results is the interference of the herbicide with host finding mechanisms (chemotaxis) below a threshold host density. We do not deliver direct proof for this, but the simulation scenarios of our ABM model were able to produce patterns similar to those observed in our experiment simply by in- or excluding chemotactic swimming behaviour of zoospores. Hence, both the experimental and simulation results support our first hypothesis that chemotaxis is important for locating hosts; however, it becomes less relevant with increasing host density.

Several studies indicate that motile organisms display chemotactic behaviour towards suitable hosts or nutrient sources. For example, the malaria mosquito uses human sweat components as host finding cues [19, 44], cercariae show chemoorientation towards micromolecules excreted by snails [12, 20] or nematodes and pathogenic bacteria are attracted to substances released by their hosts [45, 48]. There are also a few studies on chemotaxis of chytrid zoospores. The infamous chytrid pathogen of amphibians, *Batrachochytrium dendrobatidis*, for example, showed positive movement towards nutritional cues of host origin [30], and Muehlstein et al. [31] found evidence of chemotactic activity of a marine chytrid towards concentrated food sources. Although chemotaxis seems to be a widespread mechanism for parasites with free-living stages to increase their transmission potential, not many studies experimentally or theoretically incorporate host-finding efficiency (chemotaxis) as a parasite fitness trait explicitly in an epidemiological context.

Contrary to our expectations, lowering host condition through diuron pre-exposure did not increase parasite infection success and even showed a tendency to decrease it (Fig. 2). This suggests that hosts did not become more prone to infection upon prolonged stress exposure. Hence, we reject hypothesis no. 2. One could argue that diuron pre-exposed hosts transferred to clean medium had only a limited recovery time (ca. 30 min before parasite addition). For the photosynthesis-inhibiting herbicides atrazine and isoproturon, it was found that photosynthesis of the green algae *Scenedesmus vacuolatus* was for 75 % recovered after 45 min and 1 h, respectively [41]. Based on the results of Vallotton et al. [41], we cannot claim that 30 min is enough for total recovery of photosynthesis although it probably reduced photosynthesis inhibition already to a certain extent. The effect of host condition through pre-exposure could therefore have possibly been masked by the remaining acute negative effect of photosynthesis reduction by diuron on zoospore host finding. However, from the previous discussed results of host density and acute diuron exposure, one would expect that at high host density, host-finding mechanisms become much less relevant for the infection process and infection success then depends solely on the host susceptibility. When analysing the high host density treatment separately, there was no evidence that pre-exposure increases host susceptibility ($F=0.62$, $p=0.43$). Because acute diuron exposure alone had no negative effect on parasite infection success in a high host density environment, we can exclude the potential confounding effect of the remaining relatively high diuron concentration in the pre-exposed high host density treatment (see remark in Materials and Methods). A possible explanation for why infection was not enhanced upon increased diuron exposure time is that diuron may not interfere with potential defence mechanisms of the host. Or, another explanation is that hosts that are in poorer condition and/or damaged may be less "attractive" for zoospores. Chytrids are very dependent on the resources of their host, i.e. they consume almost their entire host. Selecting healthy, "good quality" hosts may therefore be an advantageous strategy that insures a high growth and transmission potential for the parasite.

However, a diuron main effect ($F=6.25$, $p=0.014$) indicated that in the presence of diuron parasite infectivity is even slightly higher. The positive effect of diuron on parasite infectivity in a high host density environment is not easy to explain, but was confirmed in the simulations. Gradients of exudates are likely to overlap when host density is very high, and this could deter zoospores from sensing spatial gradients essential for locating individual hosts (see [22]). Under such conditions, a reduction in the release of chemical cues—like caused by a photosynthesis inhibitor as diuron—may actually be beneficial for zoospores, thereby increasing the opportunities for successful location of hosts. However, cell densities as high as used in our experimental "high host density" treatment are unlikely to occur in natural populations (maximum ever observed in Lake Maarsseveen in order of several thousand *Asterionella* per millilitre [16]). Therefore, it is doubtful that such an "overdosis" effect of chemical cues may really occur under natural conditions.

In plants, it has been shown that various biotic and abiotic stress response pathways share common nodes [28, 35] so that pre-exposure to one stressor may positively influence subsequent resistance to another stressor [29, 32]. Such mechanism could lead to the observed interaction between diuron pre-exposure and acute diuron exposure. However, as already mentioned above, diuron pre-exposed hosts had only a limited recovery time (ca. 30 min before parasite addition) thereby probably still experiencing some inhibition of photosynthesis

activity upon parasite exposure. The weak interaction effect between pre-exposure and additional diuron exposure could then be explained by the remaining negative effect of photosynthesis reduction by diuron on zoospore host finding which is not further enhanced by additional diuron exposure.

Our third hypothesis was that increased spread of infection observed in host populations with larger mean cell volume is mainly driven by a higher parasite reproductive output and that herbicide pre exposure reduces parasite reproductive output because of lower host nutrient quality. As expected, we did find that larger hosts sustained significantly larger sporangia, resulting in ca. 1.5 times more zoospores produced on the host population with larger mean cell volume. In vertebrates or higher plants, size differences between hosts and parasites are generally large and parasites only consume a relatively small part of their host resources. In contrast, the biovolume of mature sporangia of phytoplankton parasites may constitute around half of the host's biovolume and parasites consume practically all of their host resources. This may explain why quantitative nutrient availability, as determined by host cell size, is such an important aspect of parasite fitness in this system. In addition, we also found that infection success was significantly higher and zoospore development time faster on host populations with larger mean cell volume. The delayed zoospore development on the smaller host could be due to a negative effect on zoospore searching time (as seen in the previous results on infectivity). Because in our experimental design, zoospore development time is the sum of zoospore searching time and sporangia development upon host attachment, no conclusions can be made upon which factor contributed to what extent to the final outcome. In any case, these results show that parasite fitness is enhanced in host populations with larger cell size via various mechanisms.

We also found support for the hypothesis that host quality through herbicide pre-exposure negatively affects zoospore production. Since diuron affects photosynthetic electron transport, it causes inhibition of sugar production in the Calvin cycle through the depletion of ATP and NADPH supplied from the light reaction. Pre-exposure to the herbicide therefore reduces the incorporation of photosynthetic products [6]. Kagami et al. [18] measured elemental ratios and content (carbon, nitrogen, phosphorous) of both host and zoospores and found that they have similar C:P ratios but zoospores have higher C:N ratios. Based on these results, carbon is expected to be more limiting than phosphorous for chytrid growth. In addition, large amounts of cholesterol (a carbon-rich molecule) were measured in zoospores [18] which are used as internal lipid storage reserves for zoospore dispersal during the chytrid's free-living stage. The high carbon demand of the chytrid combined with the negative effect of diuron on carbon assimilation by the host could explain the reduction in zoospore production. In addition, diuron exposure may not only reduce the number of zoospores but could also reduce their lipid content. Lower internal lipid reserves may further reduce the transmission potential of zoospores by reducing their dispersal (reduced swimming activity and/or longevity) and infection capacity (production of the germ tube during the initial part of infection). This, however, remains a hypothesis to be tested for in a further study. Studies on two different *Daphnia*-parasite systems showed that low quality nutrition of *Daphnia* reduced the reproductive output of its parasite [10, 13]. Though, in both cases poor nutrition of *Daphnia* also led too smaller-sized animals. In our case, diuron pre-exposure led to a smaller sporangia volume in both host strains (small and large). The differences in sporangia volume were subtle, resulting in a 9 % reduction of zoospores per sporangium when pre-exposed to diuron. Although at the individual level such a reduction may seem unimportant, it may still be relevant for disease spread when scaling up to the population level.

At the same time, we also found that host quality through diuron pre-exposure significantly reduced zoospore development time, though only by 3 %. We are aware that with a counting error of 30 % interpretations of such a minor reduction have to be treated with caution. The shorter development time can be explained by the smaller number of zoospores within sporangia growing on diuron pre-exposed host cells. The zoospore doubling time (calculated as $D \times \ln 2 / \ln N$, where D is average sporangia development time and N is average number of zoospores per sporangium, [3]) was, however, the same for both treatments (approximately 16 h). This means that chytrid parasites produce less zoospores at a similar rate on hosts that are pre-exposed to diuron compared to control hosts. The reduced development time does therefore not outweigh the negative effect of a diuron-exposed host environment on parasite fitness. In addition, the negative effect of acute diuron exposure was dependent on the immediate host environment (i.e. host size). Only the smaller host strain experienced a reduction in zoospore production (9 % compared to the control) upon acute diuron exposure. This may be due to the stronger negative effect of diuron on smaller-sized host strains. Acute diuron exposure may already induce physiological changes in the smaller host; whereas in the larger host strain, they occur only after a prolonged diuron exposure time (6 days of pre-exposure). Several studies found a negative relationship between cell volume and toxicant sensitivity in phytoplankton [23, 38]. In a previous study, we also observed that *Asterionella* populations with smaller mean cell size were more sensitive to the herbicide diuron, reflected in a stronger inhibition of population growth [42] Photosynthesis inhibition upon diuron exposure did not differ between the experimental strains (data not shown) which may explain why we do not see an interaction between host strain and diuron exposure for infection success. However, differences between larger and smaller host cells in the amount of storage compounds could be a mechanism explaining the stronger negative effect of acute diuron

exposure on smaller host cells. When diuron inhibits assimilation of carbon (by photosynthesis inhibition), larger cells may keep their cell functions and growth longer intact because they may contain more reserves that can be used as energy source. Therefore, smaller hosts not only provide fewer resources but may also constitute a lower qualitative nutritional environment for their parasites when acutely exposed to environmental stressors such as the herbicide diuron.

Concluding Remarks

Through our infection experiments, and supported by an agent based model, we demonstrated that both the immediate (i.e. host) and external environment and their interaction influence parasite fitness traits and consequently disease dynamics. Successful infection of free-living parasite stages was mainly affected by the external environment (host density and acute herbicide exposure), and parasite reproduction was mainly driven by the immediate host environment (quantity and quality).

This study also highlights that for parasites with free-living motile stages, efficient host-finding mechanisms are a relevant fitness trait for successful transmission in particular when hosts are not abundant. In many infection experiments, this is not accounted for when unrealistically high host densities are used and this may give unrealistic estimates of parasite transmission potential.

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