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Nonmonotonic and Monotonic Effects of Pesticides on the Pathogenic Fungus *Batrachochytrium dendrobatidis* in Culture and on Tadpoles

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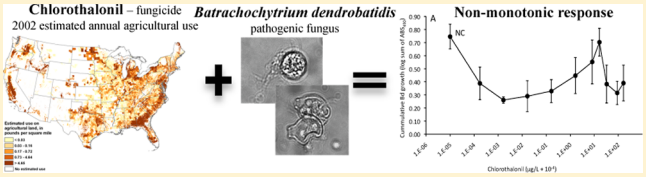
ABSTRACT: Pesticides and the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) co-occur and are implicated in the global decline of amphibians, a highly threatened taxon. Here, we investigated the effects of ecologically relevant concentrations of chlorothalonil and atrazine, two of the most commonly used, immunomodulatory pesticides in the United States, on tadpole (*Osteopilus septentrionalis*) survival and *Bd* growth. Tadpole survival was unaffected by the pesticides but was reduced by *Bd*. Atrazine monotonically (i.e., consistently increasing or decreasing) reduced *Bd* in culture and on tadpoles, and every concentration tested (0.0106−106 μg/L) significantly reduced *Bd* growth compared to controls. Chlorothalonil had a nonmonotonic (i.e., nonlinear) effect on *Bd* growth both in culture and on tadpoles, where low (0.0176−1.76 μg/L) and high (32−176 μg/L) concentrations inhibited *Bd* growth significantly more than did intermediate concentrations (8.2−17.6 μg/L) and controls. To our knowledge, this is one of only a handful of studies to document a nonmonotonic dose response of a nonvertebrate (*Bd*) to a pesticide. Although both pesticides reduced *Bd* growth on frogs, neither cleared the infection entirely, and because we know little about the long-term effects of the pesticides on hosts, we do not recommend using these chemicals to control *Bd*.



INTRODUCTION

Chemical contamination is one of the most diverse and widespread types of abiotic factors affecting aquatic organisms. Despite the high risk pollutants pose to the environment and threatened species, they are still understudied.[1] Some pesticides affect nontarget taxa nonmonotonically (i.e., nonlinearly; e.g., see refs 2 and 3); this makes it challenging to predict the effects of contamination on species interactions, such as host−parasite interactions. This is particularly important for amphibians because their global declines have been linked with both exposure to pesticides[4,5] and pathogens, such as *Batrachochytrium dendrobatidis* (*Bd*), a chytrid fungus that causes the disease chytridiomycosis and is implicated in the declines of hundreds amphibian species.[6]

Given that *Bd* is a fungus, fungicides, and perhaps other pesticides, might be directly toxic to it (e.g., see 7), affecting amphibian−*Bd* interactions. Moreover, several common pesticides, such as the most commonly used fungicide in the United States, chlorothalonil, and the second most commonly used herbicide in the U.S., atrazine,[8] are documented immunomodulators that have been shown to have nonmonotonic effects on nontargeted taxa (refs 2 and 3, respectively). Hence, these common pesticides might affect amphibian−*Bd* interactions in complex ways (as has been shown with other pesticides, see 9), such as by linearly or nonlinearly affecting *Bd* survival and/or affecting host resistance to *Bd*.

Here, we examine the effects of chlorothalonil and atrazine on *Bd* growth in culture and on tadpoles. Examining effects in culture allows us to isolate the direct effects of the chemicals on *Bd*, whereas examining effects of the chemicals on *Bd* growth on tadpoles allows us to capture the net effects of the chemicals on the hosts and parasites, or, in other words, how the chemicals affect the host−parasite interaction. In culture, we hypothesized that higher concentrations of both pesticides would decrease *Bd* growth because fungicides are designed to kill fungi and because atrazine has many documented effects on nontarget organisms[2,10] and other herbicides (e.g., glyphosate) have reduced *Bd* survival.[11] However, we predicted that the fungicide chlorothalonil would be more deadly to the fungus *Bd* than the herbicide atrazine. Finally, we hypothesized that *Bd* growth in vivo would be a function of both the direct effects of the pesticides on *Bd* and any immunomodulatory effects of these chemicals on hosts.[12] The directionality of this net effect is difficult to predict, and thus we predicted that the pesticides would affect *Bd* growth on the frogs without specifying directionality *a priori*.

Background on Pesticides. Atrazine and chlorothalonil are relatively mobile pesticides. They have been commonly found in groundwater, runoff, and precipitation, and they have

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even been found above the Arctic Circle, albeit at low concentrations.[13]

Atrazine is an herbicide that disrupts photosynthesis. Atrazine is a relatively persistent pesticide compared to other current-use pesticides, and though its half-life varies considerably based on environmental factors, it can be greater than three months.[14] Atrazine does not typically cause amphibian mortality at its expected environmental concentration (EEC) but its sublethal effects on amphibians are well documented.[2]

Chlorothalonil is a fungicide used to control blights, leaf spot, and mildews on crops, such as potatoes, peanuts, and tomatoes.[15] Chlorothalonil has a half-life that can be as long as 150 h but its breakdown products, which are often more toxic than the parent compound, can be much more persistent.[15] It is one of the most commonly used pesticides in Central America and has been detected at high levels in Latin American mountains[16] where many amphibian declines have occurred.[17] Chlorothalonil has been shown to cause significant mortality in tadpoles at concentrations 10,000 times below the EEC.[3]

MATERIALS AND METHODS

_Bd_ Culture and Inoculation. *Bd* inoculum was prepared by adding 1 mL of *Bd* stock (isolate SRS 812 isolated from *Rana catesbeiana* from Savanna River Site, SC) cultured in 1% tryptone broth, to a 1% tryptone agar plate, which was incubated for 8 d at 23 °C. Just prior to experimental inoculation, the plates were inspected to verify that zoospores were viable (motile). Each plate was then flooded with 3 mL of ultrapure water to suspend the zoospores. This zoospore suspension from all of the agar plates was homogenized, creating the *Bd* positive (*Bd*+) inoculum. Zoospore density was counted with a hemocytometer by averaging zoospore counts from four different fields of view from a 20-μL aliquot. The *Bd* negative (*Bd*−) inoculum was created at the same time using the same methods except that no *Bd* was added to the 1% tryptone agar plates. This process was repeated to create new inocula weekly.

Effect of Pesticides on _Bd_ Growth in Culture. This experiment measured the effects of chlorothalonil and atrazine on *Bd* growth in culture after one and two weeks. Four concentrations of chlorothalonil (0.0176, 0.176, 1.76, and 17.6 μg/L) and atrazine (1.06, 10.6, 58, and 106 μg/L; both pesticides were technical grade, purity >98%, Chemservice, West Chester, PA) and two controls for each pesticide (water and solvent: 500 ng/L acetone) were used. For both pesticides, a stock solution dissolved in acetone was serially diluted to obtain the desired concentrations. According to U.S. Environmental Protection Agency GENEEC v2 software, the approximate EECs for chlorothalonil and atrazine are 164 and 102 μg/L, respectively. The actual concentration of the stock solution of chlorothalonil was 176 μg/L (verified by the Mississippi State Chemical Laboratory, spiked recovery efficiencies: 95%) and of atrazine was 106 μg/L (verified with an ELISA kit; Abraxis LLC.). Concentrations reported are based on measurements of the stock concentrations. These measurements of the stock are used hereafter.

Inside a sterile, laminar-flow hood, 10-mL test tubes were filled with sterile 1% tryptone broth, *Bd*+ inoculum, and stock solution of the randomly assigned chemical (chlorothalonil, atrazine, or water or solvent controls). The tubes were incubated at 23 °C for either one or two weeks (n = 6 replicates/treatment/incubation time). Zoospore concentrations at one and two weeks were quantified by counting a 20-μL aliquot of each homogenized sample on a hemocytometer as described above.

Daily Effects of Pesticides on _Bd_ Growth in Culture. This experiment investigated the effect of pesticides on daily *Bd* growth for 8 d. Changes in *Bd* densities were quantified using a spectrophotometer (measured as change in optical density) following the validated methods of Rollins-Smith et al. (methods previously used by 18). There were 10 treatments of each pesticide created with a serial dilution from the same stocks used above (chlorothalonil: 0.000176, 0.00176, 0.0176, 0.176, 1.76, 8.8, 17.6, 35.2, 90.2, and 176 μg/L; and atrazine: 0.011, 0.106, 1.06, 10.6, 23, 58, 77, 106, 159, and 212 μg/L) and two controls (water and solvent: 500 ng/L acetone).

Bd+ and *Bd*− stocks were created for each concentration of pesticide and for each control (n = 6 *Bd*+ replicates and n = 2 *Bd*− replicates for each pesticide concentration and control). The *Bd*− treatment was used to control for the change in absorbance associated with the pesticide concentration in the absence of *Bd*. Each stock was composed of a pesticide (or control) treatment and a *Bd*+ or *Bd*− inoculum (*Bd*+ inoculum concentration: 5.6×10^5 zoospores/mL). In a sterile, laminar-flow hood, a 300-μL aliquot of each treatment (pesticide-by-*Bd*) was randomly placed in each of the 96 wells in the plate. Chlorothalonil and atrazine were loaded on separate plates with their own respective controls. The plates were covered with an optical adhesive cover (Applied Biosystems, Foster City, CA), sealed using a plastic applicator to prevent evaporation or contamination, and stored at 23 °C. The absorbance of each well was read in a 96-well plate reader (BioTek: EL808) at 490 nm at 0, 24, 48, 144, 168, and 192 h.

Effects of Pesticides on _Bd_ Growth on Tadpoles. *Osteopilus septentrionalis* (Cuban treefrog) tadpoles were collected from three clutches naturally oviposited in outdoor wading pools (1.5 m diameter, 30 cm deep) at the University of South Florida (USF) Botanical Gardens (N 28°03.537′ W 082°25.410′). Tadpole clutches were completely mixed before being distributed among treatments in the experiment. All tadpoles were Gosner stage 25[19] and the average tadpole weight at the start of the experiment was 0.086 ± 0.01 g (mean ± SEM from 10 randomly selected tadpoles). We employed a completely randomized design with 144 500-mL glass mason jars, each receiving 300 mL of artificial spring water (ASW[20]), five tadpoles, and one of 24 pesticides treatments (described below). The jars were maintained in a laboratory at USF at 23 °C and on a 14:10 light/dark cycle, and tadpoles were fed boiled organic spinach *ad libitum*.

Four concentrations of each technical grade pesticide (purity for both was >98%, Chemservice) and two controls for each pesticide (water and solvent: 500 ng/L acetone) were prepared. For both pesticides, a stock solution (the stock solutions described above were also used in this experiment; pesticide dissolved in acetone and diluted with water) was serially diluted to obtain the desired concentrations (chlorothalonil: 0.0176, 0.176, 1.76, and 17.6 μg/L; and atrazine: 1.06, 10.6, 58, and 106 μg/L). The highest concentration of chlorothalonil used was 17.6 μg/L because concentrations above 17.6 μg/L can be deadly to tadpoles.[3]

Half of the jars for each treatment received either 3 mL of *Bd* + (final *Bd* concentration in each *Bd*+ jar was 6.0×10^4 zoospores/mL) or 3 mL of *Bd*− inoculum (see above for *Bd* inoculum methods). Thus, there were six atrazine and six chlorothalonil treatments (including two controls for each

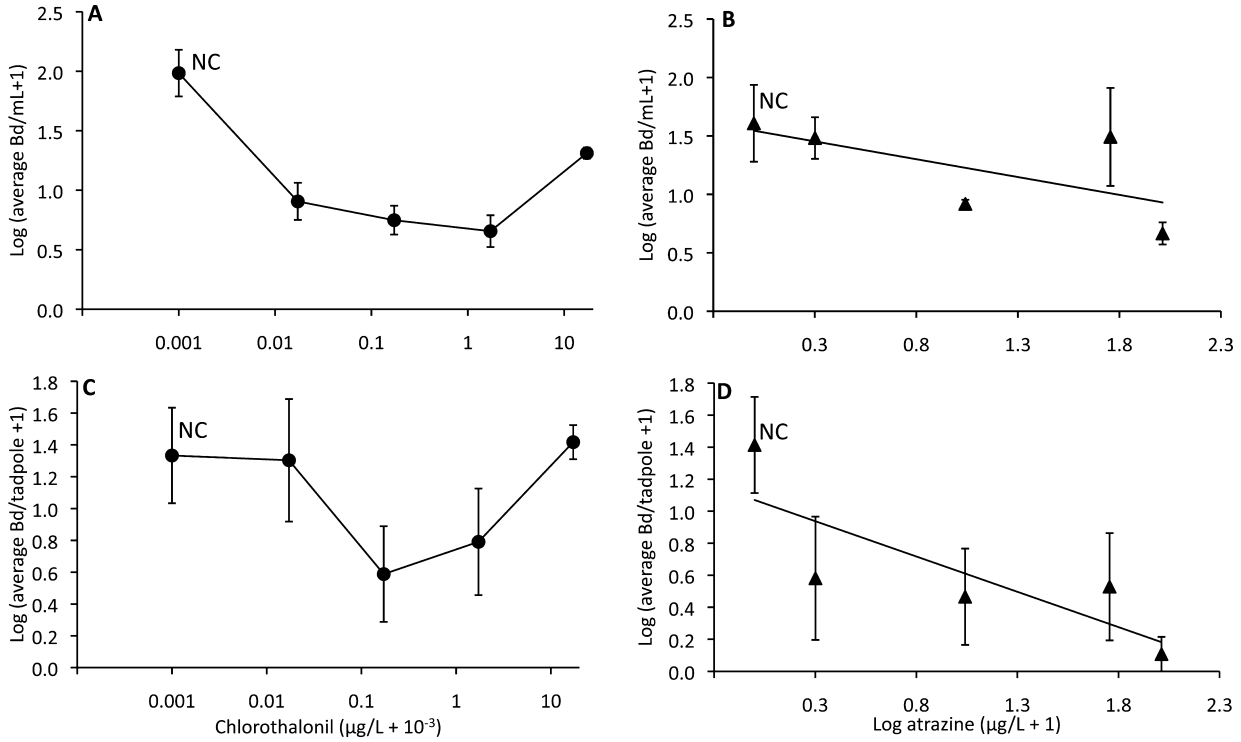

Figure 1. Densities of *Batrachochytrium dendrobatidis* (isolate SRS 812) when exposed to chlorothalonil (A) or atrazine (B) in culture, or when exposed to chlorothalonil (C) or atrazine (D) on tadpoles. Densities in culture were based on hemocytometer counts after 8 d of growth and we report the mean (±SE) of zoospores plus zoosporangia. Densities on tadpole mouthparts are based on mean (±SE) genome equivalents from quantitative PCR. NC means negative control.

pesticide) crossed with the presence or absence of *Bd*, resulting in 24 treatments (n = 6 replicates/treatment). The chemicals were added to the jars approximately 30 min before the tadpoles were added to give the chemical and *Bd* time to diffuse through the water column. Water was changed weekly to prevent fouling. To be sure the treatment exposures were constant, we re-exposed the tadpoles to the pesticide and *Bd* treatments after each water change. Dead tadpoles were preserved in 70% ethanol daily and the experiment lasted for 28 d. At the end of the experiment, all surviving tadpoles were euthanized in 1% benzocaine, weighed, and their mouthparts were removed and preserved in 70% ethanol for quantification of *Bd* abundance with qPCR.

We followed the procedure described by Hyatt et al.[21] to quantify *Bd* using quantitative PCR (qPCR; with a StepOne Real-Time PCR System; Applied Biosystems, Foster City, CA). DNA was extracted from the tadpole mouthparts with 40 μL of PrepMan Ultra (Applied Biosystems). The tissue was beat with 30 g of 0.5-mm zirconia/silica beads (BioSpec Products Inc.) using a bead beater (Disruptornie, Scientific Industries) for 45 s and then centrifuged at 13 000 rpm for 30 s (repeated two additional times). All samples were diluted 1:100 to reduce PCR inhibition. We added TaqMan Exogenous Internal Positive Control Reagents (Applied Biosystems) to every reaction well to assess inhibition of the PCR reaction[21] and any sample that was inhibited was diluted to 1:1000 and rerun using the procedure described above.

Statistical Analyses. There was never a difference between the water and solvent controls for any of the end points analyzed (P > 0.1), so they were pooled into one "control" treatment for all subsequent analyses.

Effects of Pesticides on *Bd* Growth in Culture. We conducted an analysis on each pesticide separately using a general linear model, where the response variable was the sum of zoospores and zoosporangia per field of view (mean of four fields of view), the continuous predictor was pesticide concentration, and the categorical predictor was time (one or two weeks). To determine which concentrations differed from one another, we treated concentration as a categorical variable and conducted a Fisher's least significant difference (LSD) multiple comparison test.

Daily Effects of Pesticides on *Bd* Growth in Culture. We analyzed each chemical separately. We conducted a regression analysis on *Bd* growth in culture where the area under the absorbance-time curve (AUC, sum of all daily absorbance data), independent of the effect of the pesticide on absorbance (see below for details), was the response variable, and log pesticide concentration was the continuous predictor. To control for the effect of pesticide concentration on absorbance, we calculated the mean absorbance for each pesticide concentration in the absence of *Bd* and subtracted that value from the absorbance for each replicate of the same pesticide concentration that received *Bd*. The log of this adjusted mean AUC value, which was independent of the effect of pesticide concentration on absorbance, was used as the response variable. To determine which concentrations differed from one another, we treated concentration as a categorical variable and conducted a Fisher's least significant difference (LSD) multiple comparison test.

Effects of Pesticides on *Bd* Growth on Tadpoles. Tadpole mortality was analyzed using a Cox proportional hazards regression model (function: coxph) censoring tadpoles that survived until the end of the experiment. We tested

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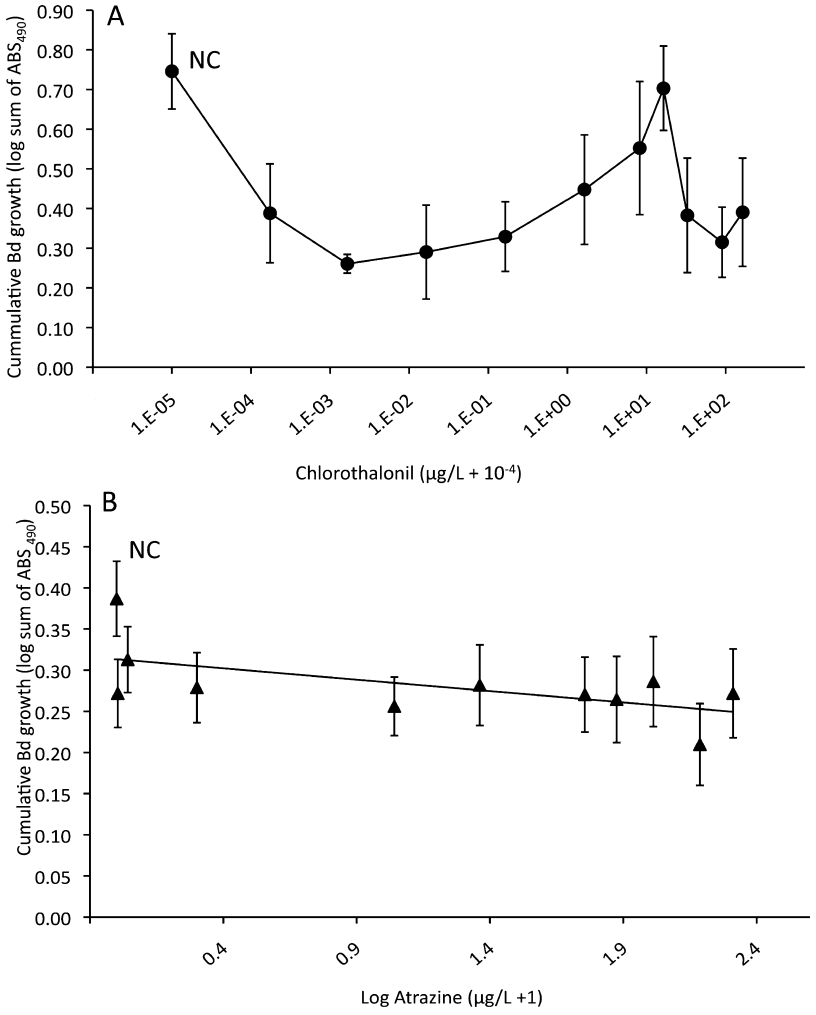

Figure 2. Growth of *Batrachochytrium dendrobatidis* (*Bd*; isolate SRS 812) in response to chlorothalonil (A) and atrazine (B). *Bd* was grown in a sealed, sterile 96-well plate for 8 days and the absorbance of each well was read at 490 nm at 0, 24, 48, 144, 168, and 192 h. There were *Bd*+ and *Bd*− wells for each treatment and the *Bd*− wells were used to control for the change in absorbance associated with chlorothalonil or atrazine concentration in the absence of *Bd*. Shown are log-transformed means (±SE) of the area under the absorbance−time curve. NC means negative control.

whether chemical type (atrazine, chlorothalonil), chemical concentration (continuous predictor), or *Bd* treatment (*Bd* +/*Bd*−) affected mortality compared to the controls (see 22 for information on survival analysis). These analyses were conducted in R statistical software[23] in the survival package.[24]

To test for effects on log hours alive or log average tadpole mass, we conducted an ANOVA fully crossing chemical type (atrazine, chlorothalonil), chemical concentration (continuous predictor), and *Bd* treatment (*Bd*+/*Bd*−). To test for effects of chemical type and concentration on *Bd* load, we conducted an ANCOVA fully crossing chemical type (atrazine, chlorothalonil) and chemical concentration (continuous predictor), with average tadpole mass as a covariate.

For both chemicals, if the dose−response appeared nonlinear after transformation, we conducted a polynomial regression to test for second- or third-order dose−response relationship. Significance was $P < 0.05$ and all statistical analyses, with the exception of the survival analyses, were conducted with Statistica v8.0 (Statsoft, Tulsa, OK).

RESULTS

Effects of Pesticides on *Bd* Growth in Culture. Chlorothalonil and atrazine appeared to exhibit different dose−*Bd* abundance relationships and thus their effects were analyzed separately. Log chlorothalonil concentration was quadratically associated with *Bd* abundance (log concentration^2: $F_{1,32} = 6.08$, $p = 0.02$, exposure duration: $F_{1,32} = 7.30$, $p = 0.01$; Figure 1A), with controls having significantly greater *Bd* abundance than every concentration of chlorothalonil tested except for the highest concentration of 17.6 μg/L (Fisher LSD: 0.0176 μg/L: $p = 0.0005$; 0.176 μg/L: $p = 0.005$; 1.76 μg/L: $p = 0.003$; and 17.6 μg/L: $p = 0.3$). Log atrazine concentration was negatively and monotonically associated with *Bd* abundance (log concentration: $F_{1,33} = 4.21$, $p = 0.048$), but only the highest concentration (106 μg/L) significantly reduced *Bd* abundance relative to controls (Fisher's LSD: $p = 0.03$; Figure 1B).

Daily Effects of Pesticides on *Bd* Growth in Culture. Log pesticide concentration in the absence of *Bd* increased absorbance (chlorothalonil: $F_{1,22} = 10.2$, $p = 0.004$; atrazine: $F_{1,8} = 10.2$, $p = 0.02$). Thus, we adjusted the AUCs to control for the effect of each pesticide concentration on absorbance as

described in Materials and Methods. The relationship between the adjusted log AUC, which estimates cumulative *Bd* growth in culture, and chlorothalonil concentration was significantly nonmonotonic (log concentration^3: $F_{1,68}$ = 9.47, p = 0.003; Figure 2A). Relative to controls, *Bd* growth was reduced by all chlorothalonil concentrations tested except 1.76, 8.8, and 17.6 μg/L (Fisher LSD: p > 0.05, for all; Figure 2A). All atrazine concentrations (except 0.1 μg/L) significantly reduced *Bd* growth compared to the controls (log concentration: $F_{1,8}$ = 7.38, p = 0.026; Figure 2B).

Effects of Pesticides on *Bd* Growth on Tadpoles. In this experiment, there were no significant interactions among chemical type, concentration, or *Bd* treatment for hours alive (all two-way and the three-way interactions: $F_{1,136}$ < 1.58, p > 0.2), and no effect of concentration on hours alive ($F_{1,136}$ < 1.58, p > 0.2). Tadpoles exposed to atrazine died sooner than control animals or those exposed to chlorothalonil (512.4 ± 11.18, 533.8 ± 10.06, 534.5 ± 10.38 h, respectively; $F_{1,136}$ = 4.98, p = 0.03). *Bd* was the only predictor that significantly decreased the proportion of tadpole surviving (*Bd*+: 0.34 ± 0.02, *Bd*−: 0.41 ± 0.02; X^2 = 5.78, d.f. = 1, p = 0.02).

Tadpoles exposed to atrazine had higher mass than tadpoles exposed to chlorothalonil treatments (mean ± SEM: 0.26 ± 0.02 g and 0.21 ± 0.01 g, respectively; $F_{1,129}$ = 8.21, p = 0.005), and, on average, longer-lived tadpoles grew to larger sizes ($F_{1,129}$ = 75.1, p < 0.001). None of the remaining predictors (pesticide concentration, *Bd*, and all interactions) significantly affected tadpole mass ($F_{1,129}$ < 1.15, p > 0.3).

The prevalence of *Bd*+ tadpoles was 73% in both controls. We evaluated whether the pesticides reduced *Bd* growth on tadpoles, which is a function of both the direct effects of the chemicals on *Bd* and the indirect effects of any pesticide-induced immunomodulation of host defenses.[12] There was a significant pesticide-by-concentration interaction for *Bd* load on tadpoles ($F_{1,62}$ = 7.38, p = 0.009), indicating that the dose−responses differed for the two chemicals. Hence, we analyzed the chemicals separately. Chlorothalonil had a quadratic effect on *Bd* abundance on the tadpoles (log concentration^2: $F_{1,30}$ = 4.70, p = 0.04; Figure 1C) that generally matched the dose responses in culture (Figures 1A and 2A). Log atrazine concentration was a significant negative predictor of *Bd* load on the tadpoles ($F_{1,30}$ = 14.54, p < 0.001; Figure 1D), with every concentration tested resulting in significantly less *Bd* than that found on control tadpoles (Fisher's LSD test: 1 μg/L: p = 0.02; 10 μg/L: p = 0.01; 56 μg/L: p = 0.004; and 102 μg/L: p = 0.001).

DISCUSSION

Atrazine and chlorothalonil both reduced *Bd* growth in culture and on tadpoles. The fact that chlorothalonil reduced *Bd* is not surprising given that it is a fungicide and *Bd* is a fungus. Although it might be surprising that atrazine, an herbicide, had adverse effects on a fungus, it was not completely unanticipated because atrazine has many documented effects on nontargeted organisms.[2,10] Additionally, another herbicide, glyphosate, has also been shown to reduce *Bd* growth or survival.[11] Further research is needed to identify the mechanism by which atrazine inhibits fungal growth. It is possible that atrazine disrupts important steroids in fungi, such as ergosterol,[25] because it is thought to disrupt steroidogenesis in amphibians, mammals, and reptiles.[26]

Chlorothalonil had a complex, nonmonotonic effect on *Bd* abundance, regardless of whether *Bd* was grown in culture or on tadpoles. Low and high concentrations inhibited *Bd* growth significantly more than did intermediate concentrations and controls. To our knowledge, this is one of only a handful of experiments to demonstrate a nonmonotonic dose response of a nonvertebrate (*Bd*) to a pesticide (e.g., see 27). Chlorothalonil had similar nonmonotonic patterns on the survival of several species of amphibians and on immune responses and hormone levels of *O. septentrionalis*.[3] The concentrations that did not reduce *Bd* growth in this experiment (0.017 and 17.6 μg/L) also generally caused the least mortality and changes to immune parameters and hormones in the amphibian species tested previously.[3] Given that chlorothalonil is designed to disrupt cellular respiration, a physiological process performed by virtually every organism on the planet, it is possible that a similar mechanism is responsible for the effects of chlorothalonil on amphibians and fungi.

Though the dose−response for *Bd* abundance differed for atrazine (monotonic) and chlorothalonil (nonmonotonic), the dose response for each chemical was consistent across the three experiments, where *Bd* was grown on the tadpoles (measured with qPCR), in test tubes (measured with a hemocytometer), and in a 96-well plate (measured with a spectrophotometer). The methods of quantification used do not determine if *Bd* is alive or dead, and therefore it was not possible for us to distinguish whether the pesticides inhibited growth by directly killing *Bd* or by inhibiting *Bd* reproduction. Ultimately, we need an effective way to determine the viability of *Bd* to truly understand the mechanism by which these pesticides are affecting *Bd* densities.

At the tested concentrations and exposure durations, neither chemical reduced the proportion of tadpoles surviving, although tadpoles exposed to atrazine died sooner than those exposed to chlorothalonil. These results are generally consistent with the amphibian literature for atrazine, which suggests that environmentally relevant concentrations of atrazine are often not directly toxic to amphibians (28−30, but see 31). For chlorothalonil, a previous study examined the same population of *O. septentrionalis* studied here and found similar effects of chlorothalonil on survival; in addition, this previous study revealed that chlorothalonil decreased survival of three other species of amphibians, killing some species at concentrations 4 orders of magnitude below the EEC.[3]

Although *Bd* is typically only lethal to postmetamorphic amphibians and not to tadpoles (32, 33, but see *Bufo boreas* in 34], we found that *Bd* reduced tadpole survival. Mortality may have been caused by pathogenic chemicals produced by *Bd*.[35] We may have detected significant *Bd*-induced mortality of tadpoles because of the 144 replicates and 720 total tadpoles used in this study, a high level of replication relative to many previous studies that often had <60 replicates.[34,36] On the other hand, we found no effect of *Bd* on tadpole growth, which has been demonstrated for *Bd* in other studies.[37] We did, however, find that tadpoles exposed to atrazine were larger than those exposed to chlorothalonil, which might simply be a product of differences in the toxicity of the two chemicals or other ecological factors.[38]

Although both chlorothalonil and atrazine reduced the intensity of *Bd* infections, we discourage their use as amphibian treatments for *Bd*. An ideal treatment for *Bd* would completely eliminate the infection with minimal documented short-term or long-term detrimental effects to the host and no unwanted effects on nontarget organisms. To our knowledge, no chemical has yet proven to be an ideal treatment for *Bd*. Both atrazine

and chlorothalonil have well-documented short- and long-term effects on amphibians.[2,3,39−41] Even the fungicide itraconazole, which is used commonly to treat amphibians infected with *Bd*,[42] can be immunosuppressive,[43] toxic to amphibians at low levels,[44] and occasionally ineffective at obtaining 100% clearance of *Bd* infections. Treating *Bd* infections with high heat is plausible[45−47] and there has been some success with this method, but we need more comprehensive multispecies experiments to determine effective and safe treatments for *Bd*.

In summary, this work emphasizes the need to more thoroughly understand the complex effects of pesticides on host−parasite interactions by simultaneously investigating host susceptibility to infections and parasite survival.[12,48] It is also important to identify more effective *Bd*-clearance treatments that do not have adverse effects on amphibian fitness.

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Author Contributions

T.A.M., J.M.R., and J.R.R. conceived and designed the experiments. T.A.M. and J.M.R. performed the experiments. T.A.M. analyzed the data and wrote the manuscript, and J.R.R. and J.M.R. provided editorial advice.

Notes

The authors declare no competing financial interest.

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