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ORIGINAL ARTICLE

Tolerance to individual and joint effects of arsenic and *Bacillus thuringiensis* subsp. *israelensis* or *Lysinibacillus sphaericus* in *Culex* mosquitoes

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Abstract Arsenic contamination of global water supplies has come to the forefront in policy decisions in recent decades. However, the effects of arsenic on lower trophic levels of insects inhabiting contaminated ecosystems are not well understood. One approach to document both acute and sublethal effects of toxicants like arsenic is to assay them in combination with microbial pathogens to evaluate shifts in survival curves of the test organisms. Larvae of *Culex quinquefasciatus* and *Culex tarsalis* were reared in water containing 0 or 1 000 μg/L of arsenate or arsenite. Fourth instars were then exposed to a range of doses of *Bacillus thuringiensis* subsp. *israelensis* (Bti) or *Lysinibacillus sphaericus* (Ls), with shifts in lethal concentrations determined. Arsenic accumulation in 4th instars was also quantified, and a relative growth index (RGI) calculated for the treatments and compared to controls. Larvae of both species accumulated between 4 447 $\pm$ 169 ng As/g and 6 983 $\pm$ 367 ng As/g, though RGI values indicated accumulation did not affect growth and development. In all cases, the LC_{50}'s and LC_{90}'s of *Cx. quinquefasciatus* exposed jointly with arsenic and Bti/Ls were higher than *Cx. tarsalis*. *Cx. tarsalis* reared in arsenite showed a significant reduction in their Bti LC_{90} values compared to the control, indicating a sublethal effect of Bti. When exposed jointly with Ls, arsenite was more toxic than arsenate in *Cx. tarsalis*. Overall, these results indicate tolerance of these *Culex* species to arsenic exposures, and why this may occur is discussed.

Key words arsenate, arsenite, Bsph, Bti, *Culex tarsalis*, *Culex quinquefasciatus*

Introduction

In toxicological studies acute mortality is frequently used as the experimental endpoint (Mogren & Trumble, 2010); however, this negates the more subtle effects of a toxicant that may occur at sublethal concentrations and the ecological processes that may be disrupted (Stark & Banks, 2003; Boyd, 2010). While acute mortality provides valuable information, some insects may appear to be resistant to pollutants at ecologically relevant concentrations (e.g., perchlorate, Sorensen *et al.*, 2007; selenate, Jensen *et al.*, 2007) if only mortality is considered. Thus, the population level effects depend on the parameters being measured, and it may be possible for a toxicant's sublethal effects to be missed entirely. One way to efficiently evaluate the effects of toxicants in insects is to assay them in combination with microbial pathogens to evaluate shifts in survival curves. This approach has been applied as a means of evaluating whether joint exposure of a pollutant and microbial control agent can lower the rates of microbial control applications needed in contaminated wetland areas (Sorensen *et al.*, 2007). However, this joint

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exposure could also be applied as a means to test whether a particular toxicant is inducing a sublethal physiological stress.

This joint exposure technique is particularly suitable for aquatic systems that contain mosquitoes, such as *Culex quinquefasciatus* Say and *Culex tarsalis* Coquillett (Diptera: Culicidae), because biological control agents for these species, including the bacteria *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Lysinibacillus sphaericus* (Ls, formerly *Bacillus sphaericus*, Ahmed *et al.*, 2007) are commercially available. Both mosquito species are of medical concern and are known to vector the causative agents of important diseases such as St. Louis encephalitis, avian malaria, and West Nile fever (Reisen, 1993; Farajollahi *et al.*, 2011). However, these insects also play an important ecological function, serving as filter feeders of fine particulate organic matter and as food sources for higher trophic levels (Wallace & Walker, 2008). Given their propensity for survival in moderately to severely polluted environments, they are good organisms to use for assessing both lethal and sublethal physiological effects of the aquatic pollutant, arsenic.

Arsenic is a common pollutant of surface waters worldwide as a result of geothermal and weathering processes and anthropogenic inputs (Nriagu, 1994; Ravenscroft *et al.*, 2009; Rahman & Hasegawa, 2012), and is considered a priority toxic pollutant by the U.S. Environmental Protection Agency (EPA). Naturally occurring environmental concentrations may be as high as 10 000 μg/L (Smedley & Kinniburgh, 2002), though the U.S. EPA has set the maximum safe concentration for chronic exposure as 150 μg/L for freshwater life (U.S. EPA, 2006). In the environment, arsenic exists in numerous inorganic and organic forms as a result of complex redox chemistry, seasonal fluctuations, and prokaryotic transformations (Lloyd & Oremland, 2006; Rahman & Hasegawa, 2012). The arsenic forms most often encountered in aquatic environments are inorganic arsenate [As(V)] and arsenite [As(III)], with arsenate being the more thermodynamically favorable form in oxic waters and arsenite predominating in reducing environments (Tamaki & Frankenberger, 1992; Rahman & Hasegawa, 2012). Biologically, arsenite is considered to be the more toxic form (National Research Council, 1999).

In this study, we test whether chronic arsenic exposure and accumulation is detrimental to *Cx. quinquefasciatus* and *Cx. tarsalis*. We hypothesized that arsenic accumulation induces sublethal physiological stress in larvae that will lower the LC_{50}'s (concentrations that will kill 50% of a population) and LC_{90}'s of Bti and Ls compared to controls, with the associated null hypothesis being no effect of arsenic. Further, we wanted to evaluate whether arsenite is more toxic than arsenate, and hypothesized that we would observe lower LC_{50}'s and LC_{90}'s of Bti and Ls in larvae exposed to arsenite than arsenate, with the null hypothesis being no difference in toxicity.

Materials and methods

Mosquito rearing

Egg rafts of *Cx. tarsalis* and *Cx. quinquefasciatus* were obtained from colonies maintained at the University of California, Riverside. Eggs were hatched in shallow white enamel pans (39 × 23 × 10 cm or 39 × 23 × 6 cm) containing 3 L of tap water. For both mosquito species, arsenic treatments contained 1 000 μg/L of either sodium hydrogenarsenate heptahydrate, 99.998% (Sigma-Aldrich, St. Louis, MO, USA) or sodium arsenite (Fisher Scientific, Pittsburgh, PA, USA). This concentration was chosen because it represents a high, yet still ecologically relevant concentration of arsenic (As) encountered in aquatic systems (Smedley & Kinniburgh, 2002). Concentrations of As in the pans (including controls) were validated using Inductively Coupled Plasma Optical Emission Spectrometry (Perkin-Elmer Optima 7300 DV, Waltham, MA, USA) and were found to be within 0.675% of the target concentration for the 1 000 μg/L treatments for arsenate and arsenite, and below the limit of detection for controls.

Pans were maintained in an environmental rearing chamber under a 16 : 8 L : D cycle at 28.0 ± 0.25 °C. Larvae were fed daily a mixture of a 3 : 1 (w : w) ground mouse chow (mouse/rat diet, Harlan/Teklad, Madison, WI, USA) and brewer's yeast (MP Biochemicals, Aurora, OH, USA) as a 10% suspension in deionized water (after Van Dam & Walton, 2008).

Mosquito growth assays

Larvae were transferred using an eyedropper to 100 mL glass jars containing 100 mL of deionized water and either 0 or 1 000 μg/L arsenate or arsenite, and covered with plastic lids. In order to avoid mortality to the young mosquito larvae, individuals were not transferred until they reached the 2nd instar. For *Cx. tarsalis*, 15 individuals were assayed per arsenic treatment (control, arsenate, and arsenite) with 3 replicates per treatment in a static test system. Similarly, 20 individuals were assayed per arsenic treatment for *Cx. quinquefasciatus* with 3 replicates per treatment. Survival and instar were monitored daily until individuals became adults or expired. Larval instars were determined based on observations of head capsule size. Larvae were fed the mouse chow suspension

described above with rates adjusted daily to maintain a light cloudiness in the water.

In order to determine if physiological stress induced by chronic arsenate and arsenite exposure affected growth and survival of *Cx. tarsalis* and *Cx. quinquefasciatus*, ten 2nd instar larvae were transferred to a 100 mL jar containing either control or 1 000 μg/L of arsenate or arsenite water, and fed the mouse chow suspension daily. Mortality and molting were assessed daily in order to calculate a growth index (GI) and relative growth index (RGI), where:

$$\mathrm{GI} = \frac{\sum_{i=1}^{i_{\max}}[n_{(i)} \times i] + \sum_{i=1}^{i_{\max}}[n_{(i)} \times (i-1)]}{N \times i_{\max}},$$

where $i_{\max}$ is the highest attainable instar by the insect and n is the total number of insects tested (after Zhang *et al.*, 1993). The RGI was then calculated as:

$$\mathrm{RGI} = \frac{\text{GI of test group}}{\text{GI of control group}}.$$

The RGI values were calculated daily across treatments using the maximum control GI as the denominator in order to maintain continuity on the RGI plot (following Jensen *et al.*, 2007). Differences between treatments were analyzed using a log-likelihood ratio test (R Statistical Software, v.2.15.0) for each species, with day and treatment as the fixed variables, replicate as the random effect, and the RGI value as the response variable.

Arsenic accumulation

Fourth instars of both species were reared as described above and analyzed for arsenic accumulation. Five replicates of 10 individuals from each species for each treatment (control, arsenate, arsenite) were frozen and oven dried at 50 °C to constant mass prior to microwave digestion and analysis using Hydride Generation Atomic Absorption Spectroscopy (HGAAS), as previously described (Ringmann *et al.*, 2002; Mogren *et al.*, 2012). Briefly, samples underwent a 2-step microwave digestion process with sodium persulfate, sodium fluoride (Sigma-Aldrich, St. Louis, MO, USA), and nitric acid in HP-500 Teflon PFA (perfluoroalkoxy) digestion vessels (CEM Corporation, Matthews, NC, USA). Once cooled, the digestate was diluted and an aliquot was prereduced using concentrated HCl and a 5%/5% w/w KI (potassium iodide, Sigma-Aldrich, St. Louis, MO, USA)/L-ascorbic acid (Fisher Scientific, Pittsburgh, PA, USA) solution. Analysis was conducted using a Perkin-Elmer (Waltham, MA, USA) Analyst 800 Atomic Absorption Spectrophotometer, with a Perkin-Elmer FIMS 400 flow injection mercury system coupled with an As-90 autosampler. The minimum detection limit of the HGAAS was previously determined as 0.050 μg/L for arsenic (Mogren *et al.*, 2012). Data were analyzed using one-way ANOVA in SAS v.9.2 (SAS Institute, Cary, NC, USA).

Larvicidal activity

In order to evaluate the physiological effects of sublethal exposure to arsenic, both mosquito species reared in these toxicants were then exposed to either Bti or Ls. Stock solutions of the pathogens were mixed using technical powder for Bti (lot 122–267-W5–02, ABG 6164 biological larvicide, 100% w/w, Valent Biosciences, Libertyville, IL, USA) and VectoLex technical powder for Ls (lot 117–140-N5–01, 1483 ITU/mg, Valent Biosciences, Libertyville, IL, USA) in Milli-Q HPLC-grade water. *Cx. tarsalis* was tested at 0.01, 0.015, 0.02, and 0.05 mg/L for Bti and 0.001, 0.002, 0.005, and 0.01 mg/L for Ls. *Cx. quinquefasciatus* was tested at 0.05, 0.08, 0.10, and 0.20 mg/L for Bti and 0.005, 0.01, 0.02, 0.05, and 0.08 mg/L for Ls. All of these doses were based on the estimated LC_{50} for each species and pathogen combination as determined in preliminary trials.

Ten 4th instars of *Cx. quinquefasciatus* and *Cx. tarsalis* were transferred to 100 mL glass jars containing 100 mL of deionized water and the appropriate concentration of arsenate or arsenite in which the larvae were reared. The Bti and Ls stock solutions were further diluted such that a 1 mL addition to the test jars resulted in the desired test concentration. Controls received 1 mL of HPLC-grade water only. Mortality was assessed after 24 h for Bti assays and 48 h for Ls assays (Zahiri *et al.*, 2004; Sorensen *et al.*, 2007). Larvae were fed as described for the mosquito growth assays at the start of the Bti and Ls trials and again after 24 h for the Ls trials. The assays were conducted at 4 different times using different batches of eggs, with 2 replicates at a time, giving a total of 8 replicates for each dose for both pathogens that were tested with each toxicant and each species.

Control mortality never exceeded 10%; however, Abbott's formula was applied to correct for any control mortality that did occur (Abbott, 1925). In order to determine the Bti and Ls LC_{50}'s and LC_{90}'s for the treatments, corrected data were analyzed using Probit analysis (Finney, 1971) in SAS (Proc Probit). Differences between arsenic treatments for the LC_{50}'s and LC_{90}'s were considered significant if the 95% fiducial limits did not overlap. In order to detect whether arsenic treatment significantly altered the effects of Bti or Ls across their respective concentrations, the proportions of dead larvae

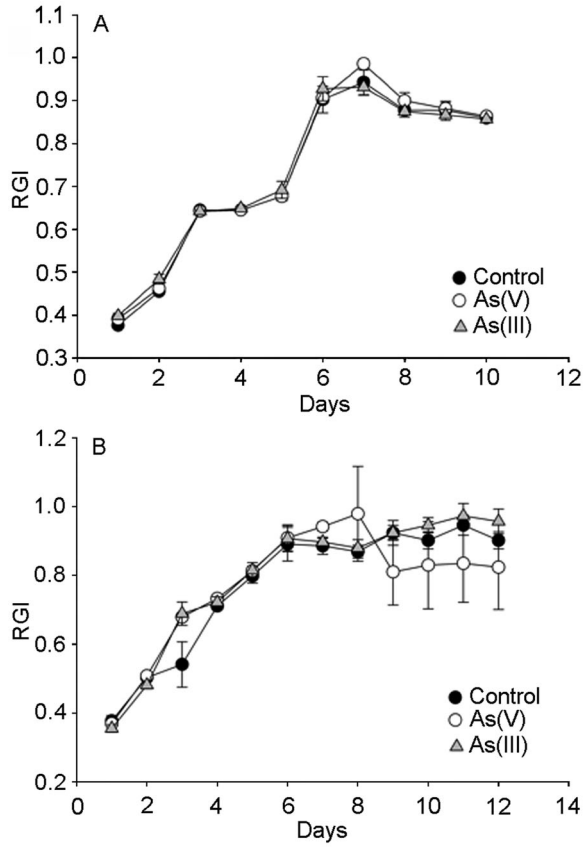

Fig. 1 The mean relative growth index (RGI) for *Cx. quinquefasciatus* (A) and *Cx. tarsalis* (B) calculated daily, starting from the 2nd instar. Error bars represent the SE calculated for each treatment on each day ($n = 3$ for each treatment).

for each replicate were arcsine [sqrt(y)] transformed to achieve normality and homogeneous variances and analyzed using general linear modeling (Proc GLM, SAS). *Post hoc* comparisons were made using Tukey's test.

Results

Mosquito growth assays

For *Cx. quinquefasciatus*, the RGI increased rapidly to day 6, after which point it leveled off until all individuals had either emerged or were dead by day 10 (Fig. 1A). A similar pattern was observed for *Cx. tarsalis*, where RGI increased until day 6 before leveling off until day 12 (Fig. 1B). There were no significant differences in RGI values between controls, As(V), and As(III) treatments for *Cx. quinquefasciatus* (Friedman's $\chi^2 = 0.1643$, df = 2, $P = 0.921$) or *Cx. tarsalis* (Friedman's $\chi^2 = 0.5530$, df = 2, $P = 0.758$). In this case, it did not appear that a simple measure of growth provided any indication of a sublethal effect of arsenic exposure in the mosquito larvae.

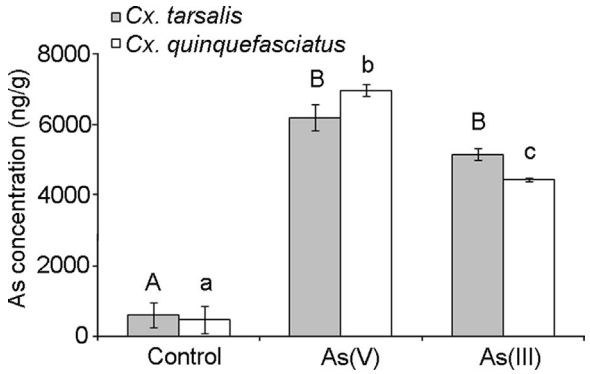

Fig. 2 Mean arsenic accumulation ± SE for *Cx. quinquefasciatus* and *Cx. tarsalis*. Capital letters indicate the significant differences between treatments for *Cx. tarsalis* and lower case letters indicate the significant differences between treatments for *Cx. quinquefasciatus* ($P < 0.05$).

Arsenic accumulation

The HGAAS analysis of digested mosquito larvae revealed significant differences between controls, As(V), and As(III) for both mosquito species (*Cx. quinquefasciatus*: $F = 224.09$, df = 2,10, $P < 0.001$; *Cx. tarsalis*: $F = 55.75$, df = 2,10, $P < 0.001$) (Fig. 2). For both species, accumulation in As(V) and As(III) treatments was significantly greater than controls ($P < 0.001$), though there was no difference in As accumulation for *Cx. tarsalis* between the As(III) and As(V) treatments ($P = 0.119$). There were no significant differences between the species in their arsenic accumulating abilities ($F = 0.01$, df = 1,20, $P = 0.912$).

Larvicidal activity

An examination of LC_{90} values revealed that for *Cx. tarsalis*, 39% less Bti was needed to achieve 90% mortality in the arsenite treatment as compared to the control. There was no difference between arsenate and arsenite treatments. For Ls exposure, mortality was greater in arsenite exposure than arsenate exposure, with only 33% of the Ls concentration from the arsenate treatment needed to cause 90% mortality in the arsenite treatment. Within both species and across arsenic treatments, there were no significant differences in LC_{50} values (Table 1).

Table 1 LC_{50}'s and LC_{90}'s for joint arsenic exposure with Bti and Ls.

Treatment	LC_{50} (μg/mL), FL[†]		LC_{90} (μg/mL), FL	
	Bti	Ls	Bti	Ls
Cx. quinquefasciatus				
Control	0.140[a‡]	0.019[a]	0.417[a]	0.088[a]
	0.123–0.168	0.016–0.022	0.305–0.710	0.067–0.127
1 000 μg As(V)/L	0.124[a]	0.023[a]	0.332[a]	0.125[a]
	0.111–0.0.143	0.019–0.027	0.256–0.505	0.091–0.198
1 000 μg As(III)/L	0.138[a]	0.021[a]	0.352[a]	0.254[a]
	0.090–0.899	0.016–0.031	0.187–441	0.122–1.06
Cx. tarsalis				
Control	0.021[a]	0.0043[a]	0.046[a]	0.012[ab]
	0.019–0.024	0.0038–0.0050	0.037–0.064	0.010–0.017
1 000 μg As(V)/L	0.019[a]	0.0046[a]	0.034[ab]	0.013[a]
	0.017–0.021	0.0040–0.0054	0.029–0.046	0.011–0.019
1 000 μg As(III)/L	0.018[a]	0.0043[a]	0.028[b]	0.0087[b]
	0.017–0.019	0.0037–0.0049	0.024–0.035	0.0076–0.0105

[†]FL, 95% fiducial limits.

[‡]Letters indicate significant differences between treatments for a mosquito species, based on overlapping fiducial limits.

There was also no significant difference in LC_{90}'s for *Cx. quinquefasciatus* for Bti or Ls exposure. In all cases, LC_{50}'s and LC_{90}'s for *Cx. quinquefasciatus* were significantly greater than those for *Cx. tarsalis*.

There was a significant difference in Bti efficacy between *Cx. quinquefasciatus* and *Cx. tarsalis* ($F = 70.84$, df = 1,190, $P < 0.001$) and between doses of Bti ($F = 51.65$, df = 2,190, $P < 0.001$), though the interaction between the two was also significant ($F = 77.66$, df = 1,190, $P < 0.001$). This indicates that the overall effect of Bti at a particular dose was dependent upon the mosquito species being tested, particularly at higher doses of Bti. However, the efficacy of Bti was not found to be significantly different between arsenic treatments ($F = 0.26$, df = 2,190, $P = 0.774$) (Fig. 3). Arsenic treatment was again not significant when tested with Ls ($F = 0.17$, df = 2,238, $P = 0.842$) (Fig. 4), though mosquito species and dose were (species: $F = 17.47$, df = 1,238, $P < 0.001$; dose: $F = 418.17$, df = 1,238, $P < 0.001$). There was also a significant species *x*-dose interaction ($F = 276.83$, df = 1,238, $P < 0.001$), indicating the overall effect of Ls at a particular dose was also dependent upon whether *Cx. quinquefasciatus* or *Cx. tarsalis* was being tested, particularly at higher doses.

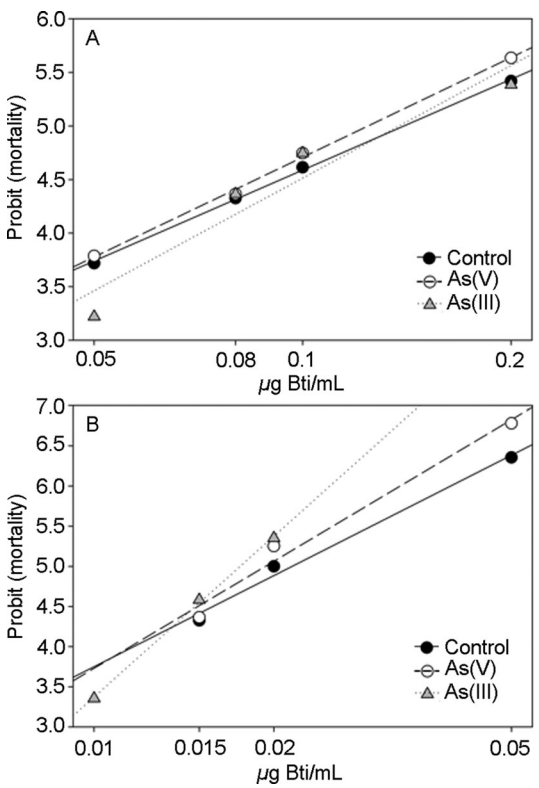

Fig. 3 The probit transformed mortality of *Cx. quinquefasciatus* (A) and *Cx. tarsalis* (B) exposed jointly to Bti and either control, 1 000 μg/L As(V), or 1 000 μg/L As(III). Bti dose has been log transformed on the *x*-axis.

Discussion

The presence of arsenic in surface and ground waters has been documented as a worldwide phenomenon

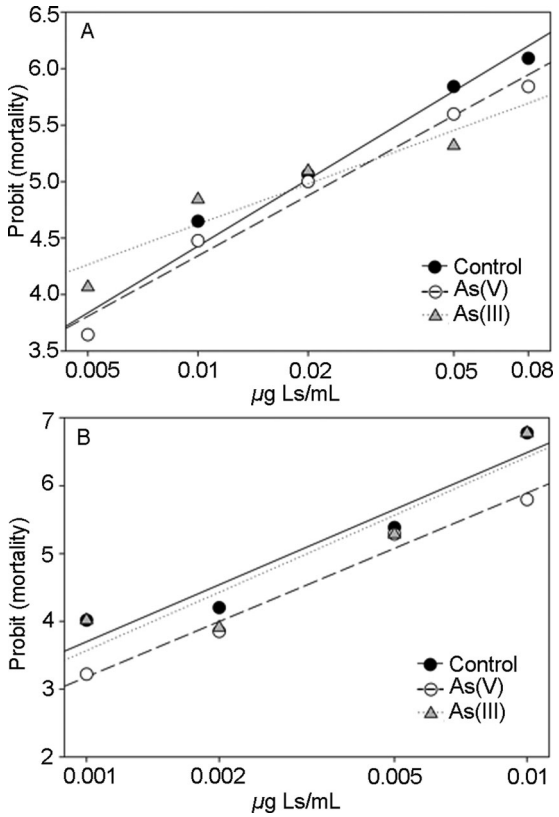

Fig. 4 The probit transformed mortality of *Cx. quinquefasciatus* (A) and *Cx. tarsalis* (B) exposed jointly to Ls and either control, 1 000 μg/L As(V), or 1 000 μg/L As(III). Ls dose has been log transformed on the *x*-axis.

(National Research Council, 1999; Smedley & Kinniburgh, 2002; Ravenscroft *et al.*, 2009), and recent discoveries of the presence of arsenic in rice and apples in the United States have further prompted national discussions about food safety (Melnick, 2011; Associated Press, 2012). Arsenic in rice may result from irrigation with contaminated water (Williams *et al.*, 2006), while arsenic in apples often results from lead-arsenate insecticide residues persisting in soils decades after the last applications (Creger & Peryea, 1994). In both cases, runoff into streams and lakes exposes aquatic life to concentrated and potentially harmful concentrations of arsenic, in addition to what they are already exposed to naturally (de Guzman *et al.*, 2012). In this study, we wanted to determine if arsenic that results naturally or from runoff events exerts a physiological effect on aquatic dipterans.

When larvae of *Cx. quinquefasciatus* and *Cx. tarsalis* were exposed to 1 000 μg As/L in the form of arsenate or arsenite, there was no significant effect of exposure on survival and growth in either species. However, there was a significant amount of arsenic accumulated in both mosquito species when exposed to either arsenate or arsenite. There was also significantly more arsenic accumulated in *Cx. quinquefasciatus* when exposed to arsenate than arsenite, which may be due to arsenate being taken up preferentially via phosphate transporters as a result of its chemical similarity to phosphate (Nriagu, 1994; Oremland & Stolz, 2003). Given that there was a significant amount of arsenic accumulated in the larvae of these mosquitoes, we wanted to test whether it could be exerting a sublethal physiological stress.

Exposure to metals has been previously shown to increase susceptibility to pathogens. In their study examining susceptibility to the fungus *Beauveria bassiana* in the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), Dubovskiy *et al.* (2011) found that sublethal nickel exposure reduced resistance to the fungus when compared to controls, despite heightened glutathione-S-transferase activity. In a different study using nickel accumulating insects, mortality of *Lygus hesperus* and the Ni accumulator *Melanotrichus boydi* (Hemiptera: Miridae) was also greater when simultaneously exposed to *B. bassiana* (Boyd, 2002).

The efficacy of pathogens against insects in aquatic systems when the insect is jointly exposed to an environmental toxicant has also been evaluated. Sorensen *et al.* (2007) showed that Bti and Ls were more effective against *Cx. quinquefasciatus* when there was joint exposure with hexavalent chromium; this exposure led to a significant increase in the efficacy of both pathogens, and decreased resistance of the mosquito. The Bti and Ls formulations used in this experiment were technical powders, and thus do not contain active bacterial cells, though they do contain active toxins and spores. It has been previously shown that insects launch an immune response against spore-crystal formulations (Ericsson *et al.*, 2009), with a significant response being measured as soon as 3 h after ingestion (Huang *et al.*, 2009). Therefore, the possibility existed that arsenic could alter susceptibility of the mosquitoes to Bti or Ls spore-crystals. In this study, we found that chronic exposure to arsenate and arsenite did not decrease the efficacy of Bti and Ls when exposed jointly with the pathogens, and the reported LC_{50} values for Bti and Ls are consistent with what have been reported elsewhere for *Cx. quinquefasciatus* (Wirth *et al.*, 2004, 2007), indicating arsenic tolerance in these species.

When determining the efficacy of Bti and Ls across a range of concentrations for the arsenic treatments, the efficacy of the pathogens was found to interact significantly with the mosquito species and the dose of the pathogen.

Cx. tarsalis reared in arsenite showed a significant reduction in their Bti LC_{90} compared to the control. Therefore, we can only conditionally accept our hypothesis that exposure to arsenic induces sublethal physiological stress, with the caveat that in our experiment we were only able to show significance at a single Bti concentration for 1 species, as opposed to across a range of concentrations for both species. To date, only a few other studies have evaluated the sublethal effects of arsenic on aquatic insects. Mogren *et al.* (2012) found significant reductions in the reproductive capacity of *Chironomus riparius* (Diptera: Chironomidae) females, as well as an increase in the time between male and female emergence. Martinez *et al.* (2006) reported mentum deformities in *Chironomus dilutus* that were exposed to arsenite spiked soils. The mayfly *Baetis tricaudatus* (Ephemeroptera: Baetidae) experienced reduced nymph growth and development when exposed to 1 000 μg/L of arsenate and arsenite (Irving *et al.*, 2008). The mechanisms by which arsenic induces toxicity are varied (Kumagai & Sumi, 2007) and are not well understood in insects, although research suggests glutathione synthetase may play a role in detoxification (Muñiz-Ortiz *et al.*, 2007; Andrahennadi & Pickering, 2008).

Our 2nd hypothesis tested whether arsenite is more toxic to *Culex* mosquitoes than arsenate. In this case, we can accept that this is true at the concentration tested (1 000 μg As/L) in *Cx. tarsalis* when exposed jointly with Ls. Interestingly, there was no significant difference between arsenate and arsenite in *Cx. tarsalis* when exposed to Bti, indicating that the different arsenic forms may interact with the toxins produced by the different pathogen species before a significant effect is observed. The proteinaceous Cry and Cyt toxins in Bti bind to the midgut epithelia and cause cell lysis, while BinA and BinB are the predominant toxins responsible for the same effect in Ls (reviewed in Lacey, 2007). Both Bti and Ls must be ingested and activated by midgut enzymes that cleave the protoxins into the toxic forms. This potentially puts them into direct contact with arsenic, which has been shown to accumulate and biotransform in the midgut of immature insects (Andrahennadi & Pickering, 2008, Mogren, unpublished data) and thus an interaction between arsenic form and Bti or Ls toxins is not unexpected, particularly as there is evidence for Ls entering target cells (Davidson *et al.*, 1987). Unfortunately, there is no detailed understanding of the mode of action of Ls at the molecular level (Berry, 2012). Just how the toxins of Bti and Ls interact with arsenic and other environmental pollutants at the molecular level specifically requires further investigation. Furthermore, because larvae are shown to accumulate As, future research on trophic transfer is warranted.

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Disclosure

The authors have no financial, relationship, or affiliation conflict of interest that would compromise the subject of the manuscript.

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