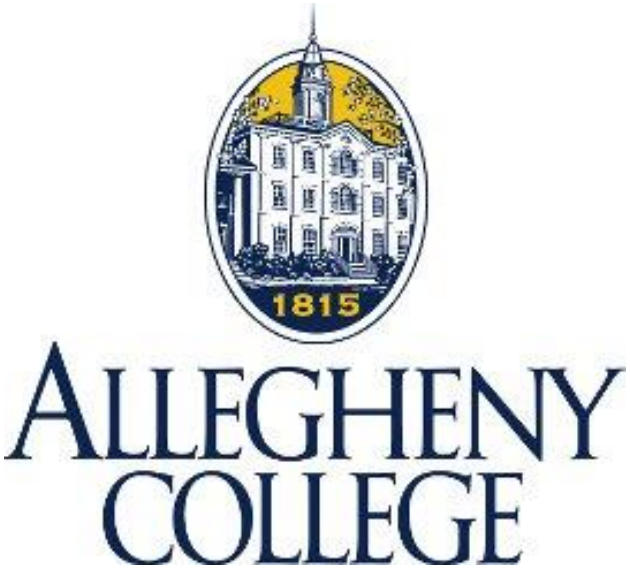
# Survival Rates of *Folsomia candida* When Subjected to Structured Concentrations of an Alcohol Ethoxylate Surfactant in a Controlled Environment

**By**

**Tyler Hunt**




**Allegheny College**

**Department of Environmental Science**

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**Tyler Hunt**

Department of Environmental Science

Allegheny College

Meadville, Pennsylvania

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Dr. Beth Choate 12/12/2017 Dr. Casey Bradshaw-Wilson 12/12/2017

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**Abstract**

Alcohol ethoxylates (AE) are used throughout agriculture and residential areas as adjuvant surfactants to increase the efficiency of herbicides. These surfactants leach into the soil and alter soil chemistry, water absorption, and nutrient exchange due to their chemical structures. The lethality of an AE commonly added to herbicides was tested on the soil invertebrate species, *Folsomia candida*. *Folsomia candida* are an ecotoxilogical soil invertebrate, that is found in numerous types of soils, and have been important lab test subjects for various chemical studies. Concentrations of surfactants were added to controlled environments containing *F. candida*, the resulting survival rates indicated the toxicity of AE on *F. candida*. AEs were found to completely eliminate *F. candida* populations at the highest surfactant concentration, 40%. Different population sizes, of 20 and 40 individuals within trials, did not significantly change survival rate when subjected to the same AE concentrations ($p=0.34$). AE concentrations significantly decreased the survival rates of *F. candida* when concentrations were at the two highest levels of AE concentrations of 4% and 40% ($p<0.05$). Concentrations of 0.4%, 0.04% and 0.01% were not statistically different than any other AE concentration or control replications (Tukey HSD). Therefore, general use of AE (concentrations of 0.4%) as an adjuvant surfactant should not significantly alter the population sizes of *F. candida* found in natural soil systems. This study's results are consistent with other studies that have been focused on soil ecotoxilogical invertebrates and AEs, and studies that researched the effects of other nonionic surfactants on *F. candida*. This is a novel study, the lethality of AE surfactants on the ecotoxilogical soil invertebrate, *F. candida*, has never been investigated.

**Introduction**

Worldwide use of herbicides has risen drastically over the years (Benbrook, 2016; Gianessi, 2013). The use of glyphosate, a common herbicide, increased worldwide from approximately 51.3 million kgs in 1995 to approximately 747 million kgs in 2014, due to the introduction and increasing availability of Roundup Ready® genetically modified crops (Benbrook, 2016). These genetically modified crops allow farmers to spray herbicides throughout their fields without any fear of damaging their cash crops. In the United States agriculture uses the vast majority of herbicides, consuming approximately 200 million kgs, while industries and governmental agencies used approximately 21 million kgs, and private home use was approximately 20 million kgs of herbicides (Grube et al., 2011). In total, the United States used approximately 241 million kgs of herbicides in 2007 (Grube et al., 2011).

To properly use an herbicide, the addition of a surfactant and water are needed. Water decreases the concentration of the herbicide to more effectively and efficiently kill unwanted plants. The surfactant allows the herbicide to enter the plants system, rather than just washing off and pooling in the soil. Surfactants account for approximately ¼ volume/volume of the total herbicide mixture (Curran et al., 2009). Surfactants are referred to as adjuvant surfactants in herbicide mixtures, since adjuvant chemicals aid in the herbicides' effectiveness once sprayed (Benbrook, 2016; Krogh et al., 2003). Surfactants specifically, are commonly added to herbicide applications due to their ability to decrease coagulation of the herbicide and increase the penetration of herbicides on plant material (Krogh et al., 2003). While surfactants aid in the binding of the herbicide to the unwanted plants, the herbicide mixture still makes its way into the soil by dripping off the plant, direct descent from the herbicide applicator through over spraying or poor aim, mixture leaks from holding containers, or cleaning of herbicide application equipment. Agriculture is the largest user of herbicide mixtures (Grube et al., 2011), and it accounts for 37% of worldwide land area usage (World Bank, 2011). Additionally, land is used for residential lawns, which are often cared for using herbicide mixtures (Grube et al., 2011). In both instances, surfactants are added to the mixtures and spread over the land right alongside the herbicides. Therefore, there are vast swaths of land and soil that are affected by the introduction of adjuvant surfactants.

Surfactants degrade the waxy coating of plants which allows the herbicide to attach and kill unwanted plants (Curran et al., 2009; Cserhati et al., 2002). Surfactants allow herbicides to adsorb or stick to various substances due to their hydrophobic and hydrophilic ends (Ishiguro & Koopal, 2016; Comber et al., 2008; Bester et al., 2001). Surfactants generally alter the permeability of plant cuticles, by creating links between the watery herbicide and the waxy cuticle with the hydrophilic and hydrophobic ends (Curran et al., 2009). This allows the herbicide to spread out on the foliage and penetrate the plant tissues. The chemistry behind surfactants is what makes them so useful as a cleaner and binder, but is also what may create problems in soil environments. Soil microorganisms and invertebrates in soils rely on the

transportation of nutrients and water through cell membranes for survival. Surfactants have the ability to inhibit or facilitate transportation of water and nutrients through cell membranes (Cserhati et al., 2002; Lee et al., 2013; Li, 2008). Krogh et al. (2003) suggest that surfactants have little direct effect on organisms within the soil. Instead, the presence of surfactants impacts the movement of nutrients and other pollutants found in soils (Krogh et al., 2003), which they suggest may have negative effects on soil invertebrates. There are several types of surfactants that differ in their molecular charges, this is indicated by their nomenclature: cationic, anionic, nonionic, and organosilicon (Curran et al., 2009; Ishiguro & Koopal, 2016). Most adjuvant surfactants are considered nonionic surfactants (Curran et al., 2009; Krogh et al., 2003). Nonionic surfactants do not contain any charge, allowing them to mix evenly for many different types of herbicides. Nonionic surfactants are most widely used due to this compatibility with most any herbicide (Curran et al., 2009).

Non-ionic surfactants consist of a wide range of products (detergents, soil wetting agents, etc.). Several studies have evaluated their environmental impacts when they are the primary chemical agent (Hoctor et al., 2013; Jensen, 1999; Lee et al., 2013). Some studies have found that surfactants can aid different soil invertebrates in water retention. Hoctor et al. (2013) attempted to aid nematodes in a relatively water-resistant soil by the addition of several soil surfactants. The surfactants allowed the nematodes to hold greater quantities of water next to their bodies, which increased their movement and predation abilities (Hoctor et al., 2003). In addition, some surfactants may be broken down by microorganisms, contributing to microorganism growth which could be a potential food source for other invertebrates (Lee et al., 2013).

Generally, surfactants have a short degradation period of several days to several weeks (Domene et al., 2009; Gejlsberg et al., 2003; Lara-Martin et al., 2008; Lee et al., 2013); however, many surfactants adsorb to soil particles (Bester et al., 2001; Comber et al., 2008; Cserhati et al., 2002; Ishiguro & Koopal, 2016; Kollmann et al., 2003; Lara-Martin et al., 2008) leading to a slower degradation period of one (Comber et al., 2008; Cserhati et al., 2002) to several months varying with time of year, aerobic conditions, and soil chemistry (Cserhati et al., 2002). Longer degradation periods mean there is more time for soil dwelling organisms to come into contact with these chemicals. Chronic exposure generally leads to greater toxicity to invertebrates (Cserhati et al., 2002) and disruption of bio-membranes and denaturation of proteins (Jensen, 1999). In addition, greater concentrations of the surfactant lead to greater toxicity (Cserhati et al., 2002; Domene et al., 2010; Domene et al., 2009; Jensen, 1999; Krogh et al., 2003; Singh et al., 2002).

Studies have also found that while nonionic surfactants are toxic in aquatic environments (Singh et al., 2002), their effects on similar organisms (planarians and nematodes) in a terrestrial setting were unnoticeable (Li, 2008; Comber et al., 2008). Nonionic surfactants have differing effects depending on

the organisms that come into contact with them. Nonylphenol, a common detergent (not an herbicide adjuvant), has been shown to reduce survivorship in populations of Collembolas, a soil-dwelling invertebrate (Domene et al., 2010). Contact with the surfactant had the greatest impact on Collembolas, *Folsomia candida* Willem, when at the reproductive stage (Domene et al., 2009). Several studies also report that populations of *F. candida* and a cousin species *Folsomia fimetaria* Linnaeus experience reduced reproduction at lower concentrations of nonylphenol surfactants than at concentrations that lead to death in juvenile stages (Domene et al., 2010; Domene et al., 2009). Adult stages are almost unaffected by the nonylphenol surfactants (Domene et al., 2010; Domene et al., 2009).

While various surfactants have been tested on soil invertebrates, surfactants comprised of Alcohol Ethoxylates (AE) have only been tested on a limited number of species. AEs are a type of non-ionic surfactant that are mainly used for detergents, but also have a large stake in agricultural use (HERA, 2009). AE surfactants are comprised of a hydrophobic alkyl chain and a hydrophilic ethylene oxide (Government of Canada, 2011). Studies on AEs have focused on their effects in aquatic settings (HERA, 2009; Gillepsie et al., 1996; Krogh et al., 2003; Lewis & Suprenant, 1983), since many AE products are detergents which would be flushed down the drain, however, studies have shown that when AEs are present in soil environments, they adsorb to soil particles, slowing down their degradation process (HERA, 2009; Krogh et al., 2003). The extent to which the AE adsorbs to soils depends on the length of side chains present and the specific properties of the soil (HERA, 2009). The greater the length of the alkyl chains, the greater the adsorption to soil particles, while reducing the amount of degradation (Krogh et al., 2003). In addition, the length of the alkyl chains has also been linked to the lethality of AE on invertebrates (Gillespie et al., 1996; Gillespie et al., 1997; Krogh et al., 2003). AE surfactants generally do not breakdown through natural abiotic processes when in aerobic conditions, such as photolysis, but require biotic degradation (HERA, 2009). Unfortunately, the AE that was used in this study did not specify the CAS registry number (Chemical Abstracts service number related to a specific chemical structure) for proprietary reasons. Therefore, exact chemical structure of the AE that was used is unknown. The CAS number would have allowed this AE to be related to previous studies that determined the number of alkyl chains, the length of those chains, and the adsorption rate of the AE to certain types of soils.

AE effects on soil invertebrates have been limited to earthworms (Krogh et al., 2003) and invertebrates that reside in sediment within aquatic settings (HERA, 2009). This means previous studies have only researched the effects of AEs in aquatic settings and in mesocosms where sewage sludge containing detergent AEs were applied to agricultural fields (HERA, 2009; Krogh et al., 2003). Thus, the data that has been collected, has focused on detergent AEs with minimal herbicide AE surfactants. The studies that have focused on herbicide surfactants, have only tested the effects of AEs on earthworms

(Krogh et al., 2003). To date, there has not been an expansion of herbicide AE testing for additional soil invertebrates.

For over 40 years *F. candida* have been used as a standard soil invertebrate for soil toxicity studies (Fountain & Hopkins, 2005). Collembola use in studies is due to their abundance in agriculture and forest soils (Hopkin, 1997), their general sensitivity to a broad range of chemicals (Frampton et al., 2006), as well as the ability to use them in laboratory settings (Fountain & Hopkins, 2005; San Miguel et al., 2008; Waalewijn-Kool et al., 2014). In 1999 a standardized laboratory process for maintaining *F. candida* populations and setting up their soil environments was published to create a base for all studies using this soil invertebrate (Fountain & Hopkins, 2005). Since there are many different types of environments and soils, a common soil invertebrate like *F. candida* could be used for toxicity testing. This soil species would be beneficial for such a study since they are so widespread and have a structured laboratory maintenance procedure (Fountain & Hopkin, 2005).

The widespread abundance of *F. candida* in various soil types (Hopkin, 1997) indicates their ability to survive in variable conditions. Therefore, when populations of *F. candida* are being affected by contaminants, other soil invertebrates will also show detrimental effects. In addition, *F. candida* play vital roles in soil community health. *Folsomia candida* generally feeds on soil fungi (Hopkin, 1997) that are often found around root systems. The feeding on fungi can actually benefit the plants in the area by removing bacteria that kill fruiting trees (Hildebrand et al., 2001). The health of the root systems maintains the health of the soil community by maintaining nutrient and oxygen flow. *F. candida* have also been found to be important in the spread of beneficial bacteria between root systems (Williams et al., 1998). As grazers, *F. candida* gain energy from fungi, and must pass that energy on to the next trophic level, representing an important connection between producers/decomposers and carnivorous soil invertebrates (Jesperson & Toft, 2003). The health of *F. candida* populations provides important information about the ecology of the of the soil community and therefore, represent not only an indicator species but a species closely connected to the health of the food web.

The objective of this study is to determine the survivorship of *F. candida* at different population densities, when exposed to 5 different concentrations of the AE surfactant, Drexel Hum-AC 820, which is one of many common surfactants used for agriculture (Curran et al., 2009). The survival rate of *F. candida* when exposed to an AE has yet to be evaluated, and as was seen in various other studies, the combination of surfactant, soil, and organism could create novel results and a better understanding of these specific variables. The AE was added to controlled environments containing juvenile *F. candida* in variable concentrations. The death rates for each concentration will be determined. It is expected that the survival rates of *F. candida* will decrease as the concentration of the AE surfactant increases.

**Methods**

Trials were constructed based on ISO protocols (Fountain & Hopkins, 2005). One hundred-fifteen grams of topsoil was placed within plastic containers (828 mL) to provide a substrate for the Collembola. First, various concentrations of AE were added to the soil. Then, *F. candida* were added to the containers. Each container received the same amount of liquid (30mL), with the control trials only receiving water. Each concentration (including the control) had two trials; with 20 Collembola and 40 Collembola in each container. The concentrations of adjuvant surfactants were; 0% (control), .01%, .04%, 0.4%, 4%, and 40% (0mL, $3x10^{-3}$mL, $1.2x10^{-2}$mL, $1.2x10^{-1}$mL, 1.2mL, 12mL of AE respectively). The corresponding volumes of AE were diluted with water so that only 30mL of liquid was added to each soil environment. During each trial, there were 3 replications of each concentration treatment. This large gradation in surfactant concentrations is due to the lack of knowledge about AE concentrations in the environment (HERA, 2009). ISO protocol states that if a toxin is not well documented, concentrations of 10%, 100%, and 1000% can be used to gather baseline data of toxicology (Fountain & Hopkins, 2005). The concentrations that were used in this study, are centered around 0.4% (the concentration of surfactant used in an herbicide application), with gradations of 10% and 100%. The lowest concentration only went down to 0.01% due to the inability to measure out 0.01% of 30mL.

The soil invertebrates, *F. candida*, were purchased as a stock population from New England Herpeticulture (http://www.neherpetoculture.com/springtailcare). The stock population most likely had Collembolan in every life stage, so to standardize the results of surfactant lethality and remove old age death and chemical avoidance bias (Gillet & Ponge, 2003; Santos et al., 2012), the smallest Collembola were selected. In order to easily transfer the small Collembola from the stock population to the trial containers, samples of the stock population were dumped into a container with enough water that the soil becomes submerged. The Collembola float on top of the water, so they can easily be transferred using a pipette (Fountain & Hopkin, 2005). The smallest Collembola are easy to see when they are in a large mass floating on top of the water, but are very difficult to accurately count as they are being transferred. Therefore, a large group would be taken up into the pipette and released into a petri dish underneath a microscope. Once the correct number of Collembola were in the petri dish (either 20 or 40 depending on the trial), the petri dish was emptied into the container containing the soil and surfactant concentration. Trials lasted one week so that new *F. candida* would not be added to the trial environments through reproduction.

The soil remained wet throughout the week, with only minimal evaporation that condensed on the top of the containers. The containers were placed in a 16:8-hour light:dark regime and 2mg of yeast was sprinkled on the surface of the soil at the start of each trial as food for the Collembola. Container lids

were not removed during the week of experimentation to allow additional air into the controlled environments, since the population of Collembola in the containers was so small; removing the need to add additional air to the environments. (Fountain & Hopkins, 2005)

After one week, Berlese funnels were used to extract all the living *F. candida* from the containers (Domene et al., 2010; Domene et al., 2009; Fountain & Hopkin, 2005; San Miguel et al., 2008; Sorensen & Holmstrup, 2005). The Collembola that were found in the ethanol were counted as surviving the experiment.

The trials with 40 Collembola had to be repeated due to inaccuracies with the setup of the Collembola concentrations. The original trial consisting of containers with 40 Collembola, resulted in several replications with over 40 Collembola, one replication even reached 80 individuals. This was an obvious mistake linked to the preparation of the replications since the Collembola that were chosen would have been juveniles, not yet ready to reproduce. Even if older Collembola had been included in those replications and they attempted to reproduce, the eggs of the Collembola take more than one week to hatch. Since the only temporal limitation of the experiment was that the populations remained for 1 week, a single trial of 40 Collembola was initiated again.

The Collembola that were found in the ethanol, were not distinguished between for size. While there were some slight size variations, it was assumed that juvenile Collembola were only added and that some gained mass over the week of experimentation.

**Data Analysis**

Since the trial of 40 Collembola was replicated, data was analyzed with both 40 Collembola trials averaged and with the initial trial of 40 Collembola excluded. Differences between population size, AE concentration, and the interaction of population size and AE concentration on the outcome of survival rate (percentage of Collembola that survived) were determined through a least squares fit model. In addition, a Tukey HSD test was conducted when statistical significance was found, in order to determine what factors resulted in the statistical difference. The least squares fit model and Tukey HSD were run through JMP 13.2.1 (SAS, 2016). Visual representations of data were created through Excel 2016 (Microsoft, 2016).

**Results**

The addition of the AE surfactant at the highest concentration (40%) eliminated all *F. candida* throughout all trials. In addition, all trials exhibited control groups with half of the initial populations at the end of the experiment (Fig. 1). The additional trial containing 40 Collembola required two separate analyses: the first averaged both 40 Collembola trial data, and the second removed the initial 40 Collembola data.

With the trials of 40 Collembola averaged, the variation in Collembola populations were found to not impact the survival ($F=0.92$, $df=1$, $p=0.34$). Survival rates of Collembola varied significantly with AE concentrations ($F=4.16$, $df=5$, $p<0.01$). There was no interaction between the AE concentration and population size ($F=0.44$, $df=5$, $p=0.82$). The control replicates showed significantly higher survival rates (Fig. 1) than replicates with 1.2mL and 12mL AE ($F=2.69$, $df=53$, $p=0.01$). The middle three concentrations of AE (0.01%, 0.04%, and 0.4%), exhibited differing survival rates, but were not significantly different than any other AE level or the control.

When the initial trial of 40 Collembola was excluded, there were no differences in survival rates between population sizes ($F=4.02$, $df=1$, $p=0.06$). However, trials containing 40 Collembola did exhibit lower survival rates than trials with 20 Collembola. Differences of the survival rates were found between AE concentrations ($F=8.39$, $df=5$, $p<0.01$). Control and 0.04% AE replicates showed higher survival rates of *F. candida* than the 4% and 40% AE replicates ($F=4.65$, $df=35$, $p<0.01$) (Fig. 2). In addition, the highest AE concentrations (4% and 40%) had lower survival rates than the lowest three AE concentrations (control, 0.01%, 0.04%). The 0.4% AE concentration replicates exhibited similar survival rates to all other AE concentrations. Survival rate of Collembolas was not affected by the interaction of population size and AE concentration ($F=1.05$, $df=5$, $p=0.41$).

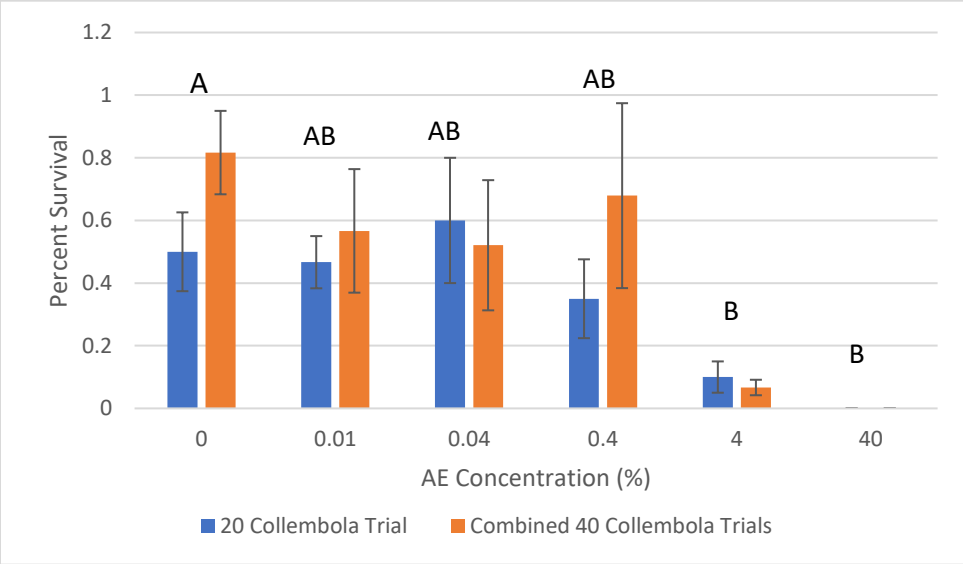



**Fig. 1** Percentage of *F. candida* that survived from the starting populations of 20 or 40, when subjected to the various AE concentrations. The two trials of 40 Collembola were averaged. Concentration levels that have the same letter above them (A or B) have survival rates that are statistically similar (Tukey HSD test).

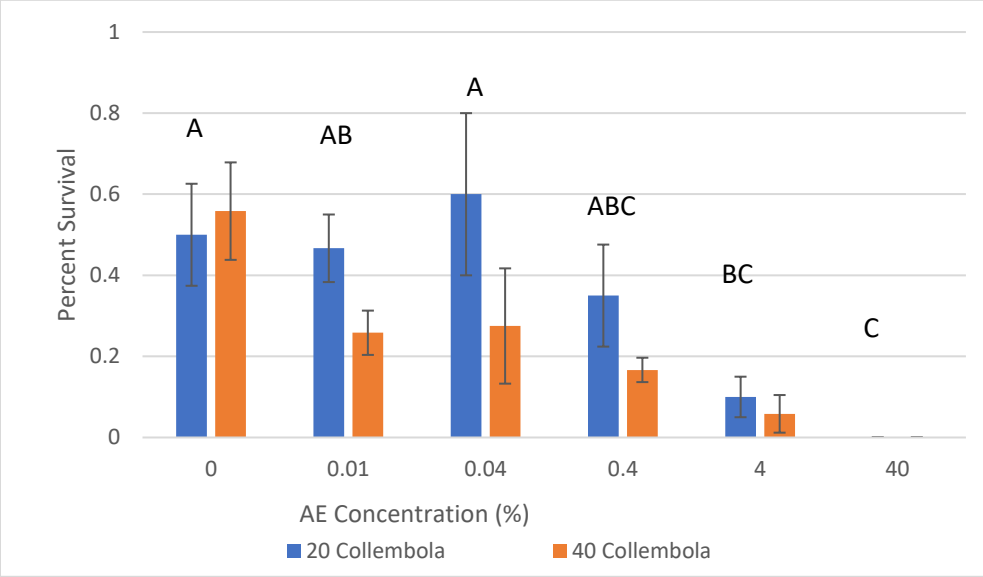



**Fig. 2** Percentage of *F. candida* that survived from initial populations of 20 or 40, when subjected to different concentrations of AE. The initial trial containing 40 Collembola was excluded. Bars with the same letter (A, B, or C) exhibited statistically similar Collembola survival rates.

**Discussion**

Using *F. candida* allows studies to compare results and findings when different chemicals are being tested (Fountain & Hopkins, 2005) and using ISO protocols, research can be compared when similar methodologies are used across the board. ISO protocols were followed as much as possible. Complications during the efforts to rear *F. candida* prohibited the use of *F. candida* juveniles from incubation chambers. Therefore, reproduction was allowed to occur in the original population containers, and the small juvenile Collembola were transferred over into the test containers. Since the project was only conducted for a week, results are comparable to data strictly on juvenile Collembola. The short time frame would prohibit an increase in numbers due to reproduction (Fountain & Hopkin, 2005).

The trials ran for a week, allowing ample time for *F. candida* to come into contact with the AE, while subjecting the Collembola to prolonged exposure. Keeping the exposure time short (1 week), also reduced the likelihood that the AE would aerobically degrade, since most AEs have a half-life of 20-40 days (Krogh et al., 2003). Alcohol Ethoxylates degrade through several different biotic processes (HERA, 2009; Madsen et al., 2001) thus resulting in various products that have not been tested on soil organisms (Madsen et al., 2001). The short testing duration allows the data that was collected to be correlated strictly to the AE surfactant, and not its subsequent products.

This study separated trials using different populations of *F. candida*. Generally, toxicology studies using *F. candida* incorporate the same number of Collembola throughout their trials (Domene et al., 2009; Domene, Chelinho & Sousa 2010; Hojer et al., 2001). Studies that have focused on Collembola consumption have variable populations of *F. candida* at the start of experimentation (Klironomos & Ursic, 1998). While this study was testing the toxicology of AE, the initial populations were differentiated to determine if population dynamics played into the survival of *F. candida* when subjected to the AE. There is no prior research on adjuvant surfactant AE and *F. candida*, so there has not been any data collected on the population dynamics of Collembola when subjected to these chemicals. This study differs from other toxicology research and mimics consumption research with respect to the different initial Collembola populations, but it also differs from most other toxicology research by not allowing the Collembola to reproduce. This study focused on the survival rate of juvenile Collembola. Increasing populations of Collembola have been seen to significantly alter the biotic environment within laboratory studies (Klironomos & Ursic, 1998), implying that greater densities of Collembola interact more with the other organisms in a controlled environment. This greater level of interaction, and the possibility of more contact with the AE was also being tested within this study. The greater interaction with the controlled environment could possibly explain the decrease in survival rate of the second 40 Collembola trial compared to the 20 Collembola trial. While the change was not significant, the decrease in survival rate was observed. Soils within agricultural fields have been found to contain very high populations of *F.*

*candida*, some reaching 120,000 individuals/$m^2$ (Axelsen & Kristensen, 2000). These highly concentrated areas of *F. candida* could mimic the results from this study, where greater populations increased contact with the AE, resulting in greater deaths. At a scale of 120,000 individuals/$m^2$, the loss of *F. candida* might be substantial enough to effect ecosystem processes.

*Folsomia candida* were seen to be severely affected by the highest concentration of AE (40%), as all populations of *F. candida* were killed within the week of experimentation. Previous studies have also found that concentrated levels of other toxins are severely detrimental to populations of *F. candida* (Amorim et al., 2012; Domene et al., 2009; Holmstrup & Krogh, 1996; Martikainen & Rantalainen, 1999). Similar results were also found for the Collembola species, *F. fimetaria*, where only the most concentrated levels of nonylphenol polyethoxylates (another common surfactant), and the surfactant byproducts, resulted in a significant population decrease (Domene et al., 2010; Domene et al., 2009). Krogh et al. (2003), notes that AEs are generally nontoxic in terrestrial and aquatic settings. Terrestrial results were found using several species of earthworms, all indicating a high concentration of AE to reach LC50 (lethal concentration resulting in death of 50% of test organisms) for all earthworm species (Krogh et al., 2003). Toxins that are created to kill invertebrates, such as dimethoate (an insecticide), have been found to severely affect populations of *F. candida* at high concentrations while less concentrated amounts, have minimal to no effect on the populations of *F. candida* (Amorim, 2012; Martikainen & Rantalainen, 1999). The lack of response by Collembola populations at less concentrated levels of toxins, was also exhibited within this study.

Toxicology studies generally orient toxin concentrations around the environmental concentrations that have been found through environmental studies (Fountain & Hopkin, 2005; HERA, 2009; Krogh et al., 2003). Environmental studies have not been conducted for the terrestrial concentrations of AE considering their rapid degradation in terrestrial settings (HERA, 2009; Krogh et al., 2003) and the overwhelming research focus for impacts of AE within aquatic environments (Gillespie et al., 1996; Gillespie et al., 1997; HERA, 2009; Krogh et al., 2003; Lara-Martín et al., 2008; Madsen et al., 2001; Singh et al., 2001). While studies have tested AE and their impacts in terrestrial settings, these studies have used sewage sludge, which mimic the introduction of AE to the environment through aquatic processes (these AEs are generally domestic or industrial cleaners that get poured down the drain) (HERA, 2009; Madsen et al., 2001) and not the direct process of spraying AE onto fields, as herbicide adjuvant AEs are introduced to the terrestrial system. This study examined the effects of AE as if it were directly applied to soils, therefore testing concentrations were not comparable to environmental concentrations (since there are none), but were comparable to concentrations that are generally used within herbicide applications.

Concentrations of AE below 4% resulted in similar survival rates as the control trials. The similar survival rates between control trials and low concentration AE trials (concentrations below 4%) could indicate the testing environments were optimal or at least reduced synergistic effects. Hojer et al. (2001) found that Nonylphenol (another nonionic surfactant) and drought, synergistically decreased *F. candida* populations. It has been found that Nonylphenol synergistically decreases *F. candida* populations with various environmental factors, with drought being the most prominent (Laskowski et al., 2010). The testing environments were noted to remain saturated throughout the testing week, meaning the environments never experienced conditions close to drought. Nonylphenol has been seen to severely affect Collembola populations even at low concentrations, when subjected to drought conditions either previous or post Nonylphenol subjection (Hojer et al., 2001; Laskowski et al., 2010). Drought conditions were not included in this study, possibly preventing detrimental synergistic effects on the *F. candida* populations when subjected to low AE concentrations. Additional research is needed to determine the full spectrum of synergistic affects between environmental factors and AE.

The slight decreases in *F. candida* populations are consistent with other studies that showed slight decreases in *F. candida* and *F. fimetaria* populations while subjected to varying concentrations of nonionic surfactants (Domene et al., 2010; Domene et al., 2009). Even though slight changes were observed, Domene et al. (2010) show that an extended period of time allows for populations of the relative species, *F. fimetaria*, to regain initial population amounts after exposure to a nonionic surfactant. The longer duration periods allow for the surfactants to degrade (HERA, 2009; Domene et al., 2010), while reproduction is also occurring. Surfactant levels that were lower than the highest concentration resulted in similar populations (Domene et al., 2010) indicating that with time, populations of Collembola can recover from toxins.

A survival rate of 60% was found in the 20 Collembola trial at an AE concentration of 0.04%, possibly indicating some type of beneficial effects for the Collembola at that concentration. Surfactants have been found to provide benefits for nematodes that reside in dry soils (Hoctor et al., 2003), however, unlike the study on nematodes, the soils that were used in this study remained wet throughout the duration of the tests. Surfactants aided nematodes by creating a wet region around them even when the soil was dry (Hoctor et al., 2003). Since the soil remained wet, the *F. candida* most likely did not benefit from a film of moisture held together by the AE. Low concentrations of surfactants have also been found to increase the Collembola population by initiating early egg laying (Domene et al., 2010). The lowest concentration of a nonionic surfactant was found to stress Collembolas into reproducing, resulting in a large increase in *F. candida* population after several weeks (Domene et al., 2010). This phenomenon would not have resulted in greater Collembola populations in this study, since it was specifically designed

to exclude the possibility of reproduction by only testing for one week, not enough time for eggs to hatch, even if they were immediately lain by Collembola (Fountain & Hopkin, 2005).

During the process of replicate setup, the AE/water mixtures were added to the test containers, and all were mixed together so all areas of soil were evenly covered with mixtures. However, areas of soil with higher organic or mineral content could have had higher AE adsorption (Government of Canada, 2011; HERA, 2009; Krogh et al., 2003; Ishiguro & Koopal, 2016), creating areas within the soil environment with higher concentrations of AE. In addition, *F. candida* exhibit toxin avoidance, often moving to areas where toxins are not present (Gillet & Ponge, 2003; Santos et al., 2012). Collembola have been found to avoid areas of heavy metal and insecticide pollution, instead either gathering food from other areas or remaining in areas with minimal pollution (Gillet & Ponge, 2003; Santos et al., 2012). *F. candida* would have most likely avoided those areas in the contained environments with elevated levels of AE. Replicates of lower AE concentrations could represent environments where *F. candida* were able to find areas with minimal AE. Higher concentrations, such as replicates with 4% and 40% might represent environments where the AE was unavoidable, and more direct contact was made by *F. candida*.

There is a possibility that this avoidance behavior is seen in the survival rates of the combined trials of 40 Collembola, where an increase in survival rate is found at an AE concentration of 0.4%. This concentration of AE represents the general concentration of AE when added to herbicide mixtures. Unfortunately, there was most likely a mistake when the populations of *F. candida* were being added to the initial trial of 40 Collembola. One replication, with 0.04% AE, contained 80 individual Collembolas at the end of the testing period. In addition, several other replications within the first 40 Collembola trials resulted in survival rates over 100%. *F. candida* take more than one week to lay eggs, and eggs take about 10 days to hatch (Fountain & Hopkin, 2005). Therefore, the increased numbers of *F. candida* at the end of experimentation is most likely due to accidental over counting when the Collembolas were being added to the testing containers. Even though the data was most likely skewed due to overcounting, the initial 40 Collembola data was averaged with the subsequent trial of 40 Collembola so that data could still be analyzed. The combination of both trials of 40 Collembola still ended with significant differences between survival rates and AE concentrations.

The minimal impact on *F. candida* by AEs is important considering they are an ecotoxilogical organism, and have been found to be one of the most toxin sensitive invertebrate species (Frampton et al., 2006). Within a controlled environment, *F. candida* was only severely affected by AE concentrations over 4%, which is ten times greater than the normal concentrations added to herbicide applications (Curran et al., 2009). While such high concentrations are unlikely to enter the environment (other than accidental spills), other studies have noted that lower concentrations affect Collembola when subjected to harsh environmental factors (Hojer et al., 2001; Laskowski et al., 2010), through interactions with

Collembola food sources (Kollmann et al., 2003) or by facilitating additional toxin movement through the soil (Krogh et al., 2003). Soil is affected by various environmental factors outside of controlled laboratory settings. There is a complexity of factors and their interactions between each other, such as how drought or extreme temperature changes act with surfactants against *F. candida*, to severely decrease populations (Hojer et al., 2001; Laskowski et al., 2010). Research must continue to test various environmental conditions with different toxins (Laskowski et al., 2010), including AE, for researchers to understand the full effects AE and other toxins will produce outside of the structured laboratory setting.

Agriculture fields containing nitrogen fixing cash crops or undergrowth can maintain *F. candida* populations of 120,000 individuals/$m^2$ (Axelsen & Kristensen, 2000). The sheer size of *F. candida* populations represents an important component of natural systems. The community interactions by *F. candida* in natural systems as a bacterial controller (Hildebrand et al. , 2001; Williams et al., 1998) and connection between producers and higher trophic levels (Jespersen & Toft, 2003) would most likely not be lost if normal levels of the AE are being applied to the environment. The main food source of *F. candida*, fungi (Fountain & Hopkin, 2005; Hopkin, 1997), have not been tested along with AE, but fungi growth, when subjected to another nonionic surfactant, was found to be unhindered, neither increasing nor hampering growth (Kollmann et al., 2003). If normal levels of AE were applied to fields, the survival of *F. candida* would be impacted slightly or not at all, but the remaining Collembola could repopulate the soil and continue existing in their niche. Only juveniles were used in this study, but it is important to note that Holmstrup & Krogh (1996) found that juveniles were more susceptible to a surfactant than the adult populations. In addition, reproduction was also affected by surfactants at low concentrations, actually inducing early egg laying in the cousin species *F. fimetaria* (Domene et al., 2010). Therefore, additional research must be conducted to fully understand the complex interactions that AEs have on *F. candida* populations and Collembola ages in a laboratory and natural environment.

**Conclusion**

The results from this study indicate the importance of using proper concentrations of adjuvant surfactants in herbicidal mixtures. Concentrations of AE exceeding 4% lead to severe decreases in *F. candida* populations in a controlled setting. Concentrations of 40% AE lead to total *F. candida* population death. Recommended levels of AE (0.4%) most likely will not impact *F. candida* populations when sprayed as an herbicide mixture. However, in environments with high densities of *F. candida*, there will most likely be some loss of population size, but the remaining Collembola will be able to repopulate the soil environment (Domene et al., 2010). The results indicate that the use of AE as an agricultural or private herbicide adjuvant is not an environmental risk for *F. candida*. The ecological role of *F. candida* also most likely would not be lost, considering surviving Collembola reproduce and repopulate so rapidly.

However, the possible spill or leak of concentrated AE should be taken seriously, considering the results of this study show complete mortality of *F. candida* at 40% AE concentrations.

Other environmental factors could increase the toxicity of AE at its recommended use concentrations, requiring more research to determine the extent to which toxicity of AE and its possible synergy impact populations of the indicator species, *F. candida*. In addition, studies must be conducted to ascertain the specific physiological effects high concentrations of AE have on *F. candida* individuals.

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