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Allelopathy of Invasive Brazilian Pepper (Schinus terebinthifolius) on Mobile Invertebrates from the Indian River Lagoon

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ABSTRACT: Brazilian pepper *Schinus terebinthifolius* is a highly invasive plant species that can be found in many different ecosystems, including the edges of estuaries in south and central Florida. Brazilian pepper fruits contain chemicals that are toxic to native salt marsh plants. Previous researchers found that high densities of crushed Brazilian pepper fruits negatively impacted growth and final biomass of the black mangrove *Avicennia germinans* and the red mangrove Rhizophora mangle. Here, we investigated the impact of *S. terebinthifolius* on the viability of mobile estuarine invertebrates commonly found in the Indian River Lagoon, as well as brine shrimp, a test organism commonly used in aquatic ecotoxicology bioassays. Our null hypothesis was that *S. terebinthifolius* would have no effect on the survival of all tested invertebrates. Our alternative hypothesis was that *S. terebinthifolius* would have a significant negative impact on the survival of the test invertebrates. Specifically, percent survival would decrease as the density of fruits increased, and crushed fruits would result in a lower percent survival than intact fruits. Bioassays containing 0 fruits (control), 5 crushed fruits, 5 intact fruits, 10 crushed fruits, or 10 intact fruits were tested on a variety of mobile invertebrates. We found that contact with any fruits of *S. terebinthifolius* significantly reduced survival in some trials (*Sphaeroma quadridentata*, *Artemia salina* Trial 1), significantly reduced survival of some trials only at the highest density of crushed fruits (*Ilyanassa obsoleta, Artemia salina* Trial 3), and had no effect on some trials (*Petrolisthes armatus, Artemia salina* Trial 2).

INTRODUCTION AND HYPOTHESES

Human-initiated movements of flora and fauna all over the globe have had drastic impacts on natural ecosystems when the non-native organisms were released into the wild (Sharma, Raghubanshi, and Singh, 2005). The exotic species may out-compete and eventually eliminate native species in newly recruited areas. These invasive species usually have characteristics that facilitate the species's establishment in the new environment. Such characteristics include abundant seed or gamete production, rapid growth rates, no natural predators, and tolerances to abiotic factors found in the new environment (Sharma et al., 2005). Another factor that may influence the success of invasive species is called the "novel weapons hypothesis" (Callaway and Aschehoug, 2000). This hypothesis proposes that exotic species have an advantage over native species due to possession of "new weapons," such as allelopathic chemicals, against which native species have no current defense (Orr, Rudgers, and Clay, 2005).

Many exotic plant species have been brought to Florida with the intention of beautifying the landscape or removing excess water from the land (Jones and Doren, 1997). Some of these species have become extremely problematic, causing Florida to invest large amounts of funding in removal and control (Cuda, Ferriter, Manrique, and Medal, 2006). One species that now presents a daunting problem in Florida is *Schinus terebinthifolius* (Brazilian pepper). This plant is native to South America and was introduced to Florida for ornamental use in the 1800s (Williams, Overholt, Cuda, and Hughes, 2005). *Schinus terebinthifolius* is found as scattered individuals in its native habitat and co-exists with other plants in native areas, but when *S. terebinthifolius* invades areas in Florida, it typically forms monocultures (Cuda et al., 2006). This species has been so successful, in part, because it possesses all of the characteristics of successful invasive species, including rapid recovery following physical damage, tolerance for a wide range of environmental conditions, fast growth rates, and profuse seed production (Jones and Doren, 1997; Cuda et al., 2006).

Schinus terebinthifolius is an evergreen, woody perennial with a multiple-stemmed trunk that is capable of reaching heights of seven meters (Jones and Doren, 1997). This dioecious plant generates small flowers on branched inflorescences, usually between August and October in Florida (Cuda et al., 2006). *Schinus terebinthifolius* flowers are pollinated by insects, and the seeds are dispersed by birds and small mammals or by water movement in estuaries (e.g., Rejmanek and Richardson, 1996; Jones and Doren, 1997; Mielke, Furtado de Almeida, Gomes, Mangabeira, and Da Costa Silva, 2005; Morgan and Overholt, 2005; Donnelly, Green, and Walters, 2008). It produces clusters of small red fruits on female trees annually between November and February, and these fruits can stay on plants for up to eight months (Cuda et al., 2006). Observations have shown that these seed clusters have more than 100 individual fruits per stalk.

As a close relative to poisonwood, poison oak, and poison ivy, *Schinus terebinthifolius* can negatively affect its surrounding environment by producing noxious secondary compounds in its fruits (Inderjit and Callaway, 2003; Morgan and Overholt, 2005). When secondary chemicals produced by an organism negatively affect other species, this chemical defense is called allelopathy (Orr et al., 2005). Other well known species that evince allelopathic properties include the black walnut tree (*Juglans nigra*), tobacco (*Nicotiana rustica*), and rice (*Oryza sativa*) (Rivenshield, 2005). Previous studies have shown high densities of crushed *S. terebinthifolius* seeds at high salinities reduced survival and growth rates of the black mangrove *Avicennia germinans* and the red mangrove Rhizophora mangle (Donnelly et al., 2008). Based on the results of Donnelly et al. (2008), we decided to test fruit density variation on survival of estuarine invertebrates.

Because *S. terebinthifolius* has become a problem along the Indian River Lagoon, specifically Mosquito Lagoon (Donnelly et al., 2008), we chose this as our collection site for our field-collected organisms. The only species used in the research that was not collected in Mosquito Lagoon was the brine shrimp *Artemia salina*, which is commonly used as a model organism in aquatic biological assays for toxicity because it is inexpensive and easy to obtain in large quantities. Hence, we ran our trials three times with *A. salina* to look for potential variation among trials. To minimize impact on Mosquito Lagoon, all wild-collected organisms were only used in one trial.

In our research, we investigated the impact of *S. terebinthifolius* on the viability of mobile invertebrate species. Specifically, we asked if *S. terebinthifolius* fruits were lethal to a variety of mobile invertebrates that are commonly found in Mosquito Lagoon. Our null hypothesis was that *S. terebinthifolius* would not have an effect on the survival of the test organisms. Our alternative hypotheses were that *S. terebinthifolius* would have a significant negative impact on organism survival, that percent survivals would decrease as the quantity of fruits increased, and that crushed fruits would result in lower percent survivals than whole fruits.

METHODS

Mosquito Lagoon, which is the northernmost part of the Indian River Lagoon system, extends from Ponce Inlet to the north end of Merritt Island and covers 288.5 km2 of surface area (Dybas, 2002). Mosquito Lagoon is part of one of the most diverse estuaries in North America and serves as a spawning and nursery site for organisms from the lagoon and nearby ocean (Dybas, 2002). For our trials, approximately 90 green porcelain crabs (*Petrolisthes armatus*), 90 isopods (*Sphaeroma quadridentata*), and 90 eastern mud snails (*Ilyanassa obsoleta*) were collected from Mosquito Lagoon in February 2008. Once collected, all organisms from the lagoon were transported to Orlando in air-conditioned vehicles in five-gallon buckets filled with saltwater from collection sites. Trials started within two hours of collection. Over 1000 brine shrimp (*A. salina*) were purchased from two local aquarium stores. The first two trials, each from a different store, began on 9 February 2008. The third trial commenced a week later (16 February 2008), after the aquarium shop received a new shipment of brine shrimp.

Green porcelain crabs, *Petrolisthes armatus*, are 1-1.5 cm in carapace width in the Atlantic and are somewhat larger in the Pacific. These crabs are filter feeders and live in rocky rubble, oyster reefs, and shallow inter-tidal and sub-tidal zones (King and Knott, 2008). The Eastern mud snail *Ilyanassa obsoleta* is commonly found in soft-sediment habitats. It is mainly a deposit feeder that focuses on diatoms, blue-green algae, and bacteria, but it will also actively feed on macroalgae when present (Kelaher, Levington, and Hoch, 2003). The isopod *Sphaeroma quadridentata* is an intertidal or shallow subtidal organism, and is a wood borer that frequently inhabits pilings and mangrove roots (Kensley, Nelson, and Schotte, 1995). Brine shrimp (Arthropoda) are approximately 10 mm in length as adults and feed on phytoplankton in the water column as well as benthic algae (USGS, 2005).

To test the toxicity of *S. terebinthifolius* fruits on these invertebrates, 48-hour bioassays were performed. Materials needed for each assay included either Corning Inc. Costar 2.3 x 1.6 cm sterile 12-well, cell culture cluster, tissue culture treated, non-pyrogenic polystyrene well-plates or disposable 60 x 15 mm, sterile, polystyrene petri dishes (Falcon 1007). Each well or petri dish contained one organism. Each well in the *Artemia salina* trials included 2 mL of saltwater. *S. quadridentata* and *I. obsoleta* trials used 5 mL of saltwater in each well and *P. armatus* trials in petri dishes included 12 mL of saltwater per dish. The saltwater used in each trial matched the salinity of the collection site. The variation in water quantities was due to the size of the organisms; our goal was to minimize the amount of water needed per organism while still enabling the organisms to swim freely. Salinities were 35 ppt for all *A. salina* and *I. obsoleta* trials, 36 ppt for the *S. quadridentata* trial, and 40 ppt for the *P. armatus* trial. All of the assays were carried out at room temperature (22o C).

Immediately before commencing each trial, *S. terebinthifolius* treatments were randomly added to each of the petri dishes or wells. These treatments consisted of 0 fruits (control), 5 intact fruits, 5 crushed fruits, 10 intact fruits, or 10 crushed fruits. Fruits were crushed by placing them in the well or petri dish and crushing them with the bulbous side of a pipette. All pieces of the fruits were left in the container and then water and organism were added. Trials lasted 48 hours and observations of animal mortality were made at 0.5, 1, 2, 3, 6, 12, 24, and 48 hours. Organisms were recorded as dead if they were not moving and did not move upon agitation. Results were examined separately for each trial. The data were not normally distributed; therefore, non-parametric Kruskal-Wallis statistics followed by a posteriori comparisons of the ranks were used to determine if there were significant differences in survival among treatments at the end of 48 hours.

RESULTS

Different test species were impacted in various ways by *S. terebinthifolius.* All treatments with *S. terebinthifolius* had a significant negative impact on the survival of the isopod *S. quadridentata*, relative to the control, but there were no significant differences among the *S. terebinthifolius* treatments (Figure 1A). The green porcelain crab *Petrolisthes armatus* was not significantly affected either by density or the condition of the *S. terebinthifolius* fruits (Figure 1B). For the eastern mud snail *I. obsoleta*, only the treatment with 10 crushed fruits significantly reduced survival relative to the control and all other treatments (Figure 1C).

The first trial with the brine shrimp, *A. salina*, found no significant differences among treatments with 5 intact fruits, 5 crushed fruits, and 10 intact fruits (Figure 2). However, survival in these treatments was all significantly reduced relative to the control. The treatment with 10 crushed fruits significantly reduced survival relative to the control. The treatment with 10 crushed fruits significantly reduced survival relative to all other treatments. The second brine shrimp trial found no significant differences among treatments (Figure 2). Finally, in the third brine shrimp trial, survival was only significantly reduced by the treatment of 10 crushed fruits (Figure 2).

DISCUSSION

Schinus terebinthifolius can have a significant negative impact on the survival of a range of unique mobile invertebrate taxa, depending on *S. terebinthifolius* fruit density and the condition of the fruits (crushed or intact). Although the patterns were often the same, with the highest density of crushed fruits having the greatest impact on survival, each animal reacted differently to the treatments. Likewise, multiple trials with the brine shrimp documented similar variability. From this it can be inferred that the presence of *S. terebinthifolius* can affect a wide range of estuarine invertebrates.

Schinus terebinthifolius fruits proved to be lethal for *S. quadridentata, I. obsoleta,* and *A. salina*, while having no significant effect on the survival of *P. armatus*. Furthermore, for *I. obsoleta* and *A. salina*, only exposure to the highest tested density of crushed *S. terebinthifolius* fruits caused a significant negative effect on their viability. This negative effect could be due to the fact that the mud snail *I. obsoleta* has an external shell, in which it can close itself off from the outside environment for short periods of time, potentially reducing the surface area of the organism that is exposed to the toxin for prolonged periods of time.

Although the second trial of *A. salina* showed no significant impact of *S. terebinthifolius* on survival, it is still believed that *S. terebinthifolius* had a negative effect on *A. salina* given the results from the other two trials. The low p values for trials 1 and 3 both showed *S. terebinthifolius* significantly impacted *A. salina's* survival. This skew in the data from trial 2 could have been a result of differences in age, size, or condition of the purchased brine shrimp. These measurements were not collected at the time of the trials, but it is common to purchase brine shrimp that range in age from four days to two weeks old and vary in size from 2 – 10 mm. All specimens are simply sold as brine shrimp and any variation depends on the distributors.

Interestingly, *P. armatus* was the only organism tested that showed no significant impact due to the presence of *S. terebinthifolius* fruits. One possible explanation for this difference is the variation in water volumes among the organisms. While the treatments were kept the same, the volume of water used for the *P. armatus* trials was greater than the trials with other organisms. This greater water volume could have been significant in that the toxic chemicals from the *S. terebinthifolius* would be more diluted in comparison. It was also postulated that the exoskeleton of *P. armatus* helped to slow the accumulation of toxins within the tissues of the organisms, thus giving it an advantage during the bioassays. Previous research has demonstrated that the absorption of certain metals, such as zinc and lead, is slower through the exoskeleton than in the soft tissues (Dallinger and Rainbow, 1993). All of the organisms in this study possessed exoskeletons, but the exoskeleton of a brine shrimp, an isopod, or a snail is different from a crab exoskeleton. A crab exoskeleton is a fused carapace, which creates less exposed soft tissue surface area. Also, the exoskeleton of a crab is both tanned and calcified, which renders the cuticle impermeable (Langston and Bebianno, 1998). While a snail also possesses a calcified shell, the exposure of the soft tissue in this organism is much higher once the snail begins crawling and feeding. It is thought that *P. armatus* could become more vulnerable after molting to the toxins from the fruits and show an increased mortality rate. This hypothesis is based on other studies demonstrating increased absorption of polycyclic aromatic hydrocarbons during the molt cycle of blue crabs (Neff, 2002). This enlarged absorption is due to increased permeability of the soft shell, and additional water uptake during time of the molt. All of our crabs had hard, calcified shells. Further research would need to be conducted to see if the lack of a calcified exoskeleton could significantly reduce the survival of *P. armatus* by additional intake of the toxin through the organism's outer membranes.

One question that deserves attention is whether the inhibitory chemicals in *Schinus terebinthifolius* are soluble in water. Although not directly tested, we assume we are dealing with a water soluble chemical, as the crushed fruits did change the water color from clear to slightly red. If not soluble in water, then the organisms might only be impacted if coming in direct contact with the fruits. Such impact was likely in the small containers used in our trials. In the field, it is possible the allelopathic chemicals are indirectly important to the survival of our invertebrates, even if the chemicals do not directly kill them. If these chemicals primarily influence the surrounding species, especially prey species, then it interacts through indirect effects, not direct interference (Inderjit and Weiner, 2001).

Inderjit and Weiner (2000) suggested that inhibitory chemicals alter the abiotic components of the environment, such as availability of nutrients, which ultimately can affect the survival of the species that come into contact with the allelopathic plant. However, this theory would need further testing to show whether it holds true with respect to our plant-animal interactions, since the test subjects are able to travel in their environment.

Our study documents the negative effect of fruits of the exotic Brazilian pepper on marine invertebrates, which are important prey sources for higher taxa and serve as an example of how an exotic plant species can cause cascading effects through an ecosystem. As was observed with multiple salt marsh plant species (Donnelly et al., 2008), the overall impact of *S. terebinthifolius* fruits varied with the species tested. In order to predict more accurately which other Indian River Lagoon species could be negatively impacted by the fruits of *S. terebinthifolius*, further research should be done to determine the chemical composition of the toxin and determine how each taxa responds to specific fractions of it. Once this is known, researchers should be able to predict which species the toxin will be able to enter and harm, due to varying membrane permeability, which will increase our understanding of the effect that the *S. terebinthifolius* invasion has on estuarine systems.

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REFERENCES

Callaway, R.M., Aschehoug, E.T. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. ***Science, 290,*** **521-523.**

Cuda, J.P., Ferriter, A.P., Manrique, V., Medal, J.C. (2006). Florida's Brazilian; peppertree management plan: Recommendations from the Brazilian peppertree task force Florida Exotic Pest Plant Council. Retrieved October 1, 2008, from http://www.fleppc.org/Manage_Plans/2006BPmanagePlan5.pdf

Dallinger, R., Rainbow P.S. (1993). *Ecotoxicology of Metals in Invertebrates.*London: CRC Press.

Donnelly, M.J., Green, D.M., Walters, L.J. (2008). Allelopathic effects of fruits of the Brazilian pepper Schinus terebinthifolius on growth, leaf production and biomass of seedlings of the red mangrove Rhizophora mangle and the black mangrove Avicennia germinans. *Journal of Experimental Marine Biology and Ecology, 357*, 149-156.

Dybas, C.L. (2002). Florida's Indian River Lagoon: an estuary in transition. *Bioscience, 52,* 554-559.

Inderjit, Callaway, R.M., (2003). Experimental designs for the study of allelopathy. *Plant Soil, 256,* 1-11.

Inderjit, Weiner, J. (2001). Plant allelochemical interference or soil chemical ecology? *Perspectives in Plant Ecology, Evolution and Systematics, 4,* 3-12.

Jones, D.T., Doren, R.F. (1997). The distribution, biology and control of Schinus terebinthifolius in Southern Florida, with special reference to Everglades National Park. In: Brock, J.H., Wade, M., Pysek, P., Green, D. (Eds.), *Plant Invasions: Studies from North America and Europe. Leiden: Backhuys Publishers, 594,* 81-93.

Kelaher, B.P., Levington, J.S., Hoch, J.M. (2003). Foraging by the mud snail, Ilyanassa obsoleta (Say), modulates spatial variation in benthic community structure. *Journal of Experimental Marine Biology and Ecology, 292,* 139-157.

Kensley, B., Nelson, W.G., Schotte, M. (1995). Marine isopod biodiversity of the Indian River Lagoon. *Florida. Bulletin of Marine Science, 57, 136-142.*

King, R.A. and Knott, D.M. (2008). Petrolisthes armatus - an introduced species in the South Atlantic Bight? Retrieved February 13, 2008, from http://www.dnr.sc.gov/marine/sertc/P%20armatus%20SOM.pdf

Langston, W., Bebianno, M. (1998). *Metal Metabolism in Aquatic Environments.* New York: Springer.

Mielke, M.S., Furtado de Almeida, A.A., Gomes, F.P., Mangabeira, P.A.O., Da Costa Silva, D. (2005). Effects of soil flooding on leaf gas exchange and growth of two neotropical pioneer tree species. *New Forests, 29*, 161-168.

Morgan, E.C., Overholt, W.A. (2005). Potential allelopathic effects of Brazilian pepper (Schinus terebinthifolius Raddi, Anacardiaceae) aqueous extract on germination and growth of selected Florida native plants. *Journal of Torrey Botanical Society, 132.*1, 11-15.

Neff, J. (2002). *Bioaccumulation in Marine Organisms: Effect of Contaminants from Oil Well Produced Water.* Amsterdam: Elsevier.

Orr, S.P., Rudgers, J.A., Clay, K. (2005). Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. *Plant Ecology, 181,* 153-165.

Rejmanek, M., Richardson, D.M. (1996). What attributes make some plant species more invasive? *Ecology, 77,* 1655-1661.

Rivenshield, A. (2005). Cornell Science Inquiry Partnerships. Allelopathy. Retrieved January 16, 2009, from http://csip.cornell.edu/Projects/CEIRP/AR/Allelopathy.htm

Sharma, G.P., Raghubanshi, A.S., Singh, J.S.(2005). Lantana invasion: an overview. *Weed Biology and Management, 5*, 157-165.

USGS. (2005). Brine Shrimp and Ecology of Great Salt Lake. Retrieved October 1, 2008, from USGS: Science for a Changing World Web site: http://ut.water.usgs.gov/shrimp/index.html

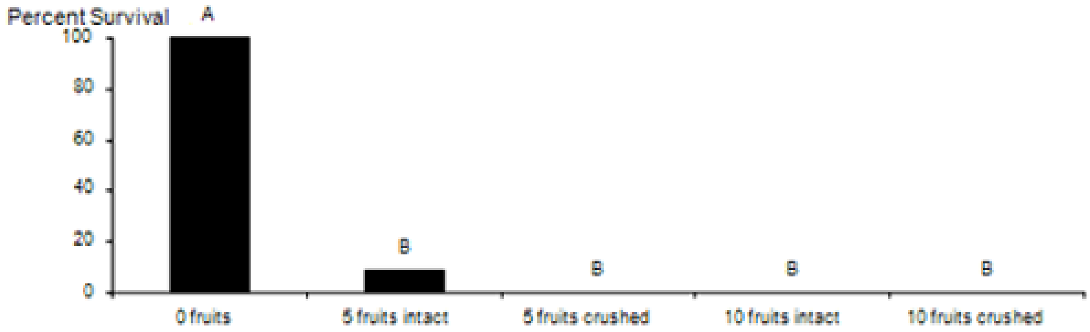
Williams, D.A., Overholt, W.A., Cuda, J.P., Hughes, C.R. (2005). Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian pepper (Schinus terebinthifolius) in Florida. *Molecular Ecology, 14*, 3643-3656.

Table 1: *Results of Kruskal-Wallis tests and comparisons of ranks to examine significant differences in survival among treatments. C = control (0 fruits), I5 = 5 intact fruits, I10 = 10 intact fruits, C5 = 5 crushed fruits, and C10 = 10 crushed fruits. If $p < 0.05$, then there were significant differences overall. These differences are shown in ranks as ">" signs.*

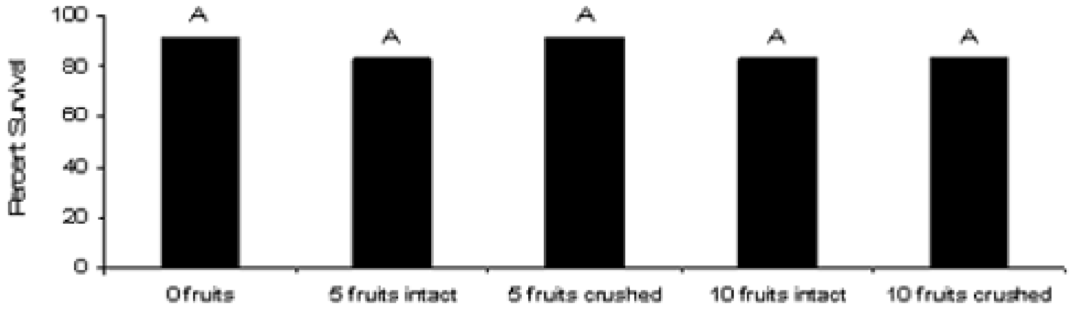
Organism	K-W Statistic	p value	Ranks
Sphaeroma quadridentata	53.6218	$p < 0.0001$	C > I5 = C5 = I10 = C10
Petrolisthes armatus	0.8510	$p = 0.9315$	C = I5 = C5 = I10 = C10
Ilyanassa obsoleta	21.4545	$p = 0.0003$	C = I5 = C5 = I10 > C10
Artemia salina 1	21.2869	$p = 0.0003$	C > I5 = C5 = I10 > C10
Artemia salina 2	2.1455	$p = 0.7090$	C = I5 = C5 = I10 = C10
Artemia salina 3	24.7201	$p = 0.0001$	C = I5 = C5 = I10 > C10

Figure 1. Percent survival by treatment for each test species.

A. Percent Survival of *Sphaeroma quadridentata*



B. Percent Survival of *Petrolisthes armatus*



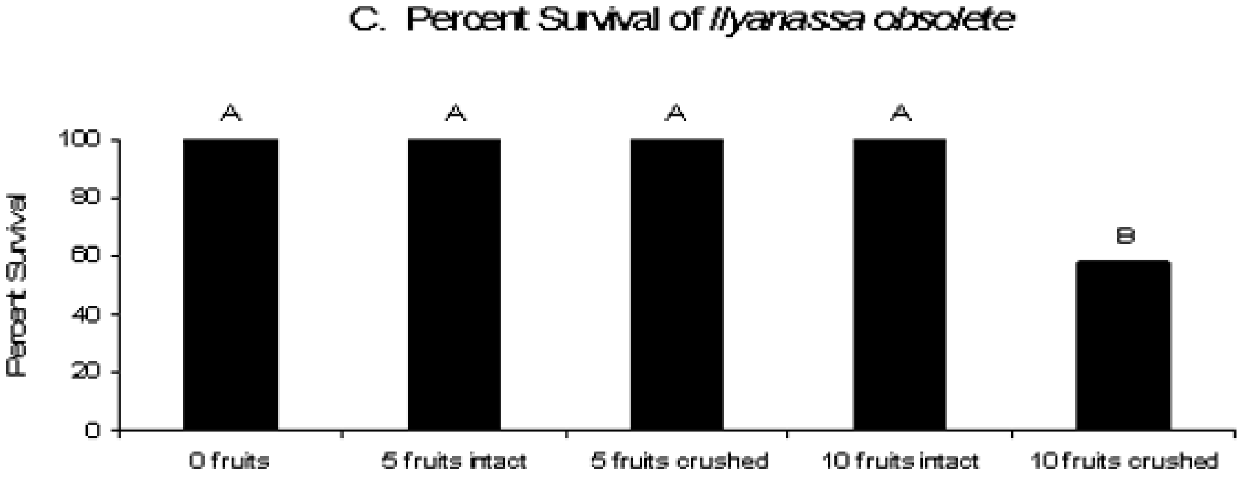


Figure 2. Percent survival by treatment for three trials with *Artemia salina*. Each trial was run independently of the other trials. For significant differences see Table 1.