Using a Terrestrial Model Ecosystem to Examine the Effects on Planktonic Shrimp of Leachates and Runoff from Terrestrial Sites Impacted by Pesticide Use in Moorea, French Polynesia

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Abstract. Pesticides are used worldwide in agriculture, and are often detected in nearby aquatic habitats. Most have been shown to have negative impacts on non- target organisms. This study examined the effects on a marine planktonic shrimp of the soil runoff from lettuce fields and from two different methods of growing pineapples. Soil was collected from various sites impacted by agriculturally applied pesticides and fresh water was poured over it and the runoff and leachates were collected. This terrestrial model ecosystem crudely approximated what happens when rainwater washes agricultural pesticides into nearby streams, or leaches into groundwater. Planktonic shrimp were then exposed to the contaminated water for 48 hours and survivorship was measured. By measuring the mortality of planktonic shrimp exposed to leachates and runoff, this study identified some effects of the suite of chemicals that theoretically make it at least as far as groundwater or streams. Being primary producers that support entire food chains, plankton are essential components of marine ecosystems. Therefore this project can be extrapolated to show potential impact on the entire marine ecosystem. This study found that shrimp exposed to leachates and runoff from soil collected from a lettuce field have significantly lowered survivability.

Key words: plankton; shrimp; toxicity; pesticides; survivorship; Moorea, French Polynesia

INTRODUCTION

The use of pesticides (herbicides, insecticides and/or fungicides) for agricultural purposes is prevalent worldwide, and is considered by many to be necessary to agriculture and the prevention of vector born diseases. Pesticides often are not selectively toxic and many have adverse effects on non-target species (Klasasen 2008). This is a problem for human, animal and environmental health. On top of being generally toxic, many of these chemicals have the ability to leach into groundwater and/or enter streams and rivers via runoff. Once in these water systems agricultural pesticides may enter a variety of important and often sensitive ecosystems such as ponds, estuaries and lagoons. Chemical factors such as soil mobility (Koc), half life in soil and water, adsorption ability, solubility in water and environmental factors such as temperature, rainfall and soil makeup dictate the environmental fate of pesticides and their metabolites (Klassen 2008).

In the coastal tropics, unique environmental factors and agricultural needs, such as high numbers of pests and high rainfall, mean that large amounts of pesticides are used and high levels of these chemicals enter water systems and run into lagoons. Relatively high levels of organophosphates, an acutely toxic class of insecticide, and other pesticides have been detected in lagoons in tropical Mexico due to "surface runoff, river discharges and field drainage" (Carvalho et al. 2002). It is reasonable to expect that in tropical French Polynesia, which also has many rivers, streams, estuaries and lagoons the fate of agricultural chemicals would be similar.

Organic farming is almost nonexistent on Moorea, French Polynesia, and many farmers do not follow pesticide use regulations, resulting in overuse of agricultural chemicals. However, the School of Agriculture is engaged in testing the efficacy of less chemical intensive methods of agriculture, such as growing pineapples through biodegradable plastic tarps, where herbicides used are less toxic and/or less persistent and only sprayed in between rows. The goal of this study is to examine the effects on a marine planktonic shrimp of the soil runoff from lettuce fields and from two different pineapple treatments at the School of Agriculture. By examining the effects of leachates and runoff, I am examining the effects of the suite of chemicals that are transported as far as groundwater or streams.

Being primary producers that support entire food chains, plankton are essential components of marine ecosystems. In fact, "ecotoxicological risk to zooplankton [...] can be used as an early warning signal of risk to the health of marine ecosystems"(Fossi et al. 2001). Therefore this project not only aims to examine the toxicity of agricultural runoff to planktonic shrimp, but the results can also be extrapolated to show potential impact on the entire marine ecosystem.

MATERIALS AND METHODS

TME model

I performed a static, lethal toxicity test, and used a crude type of terrestrial model ecosystem (TME). A TME is "an appropriate tool to investigate potential impacts of a chemical stressor on terrestrial compartments, at the biological organization level of ecosystems" (Knacker et. al.). TMEs are "defined as controlled, reproducible systems that attempt to simulate the processes and interactions of components in a portion of the terrestrial environment" (Knacker et. al.). I ran fresh water through soil to simulate the effects of rain washing pesticides from agricultural lands into streams and out to the ocean. As personally observed, pineapple and lettuce farms are typically located in stream valleys. Therefore, it is possible that pesticides are transported by rainwater from soils at pineapple and lettuce fields to streams, and are then transported into estuaries and the ocean.

Sample collection

I used a trowel and plastic bags to gather soil samples from pineapple and lettuce plantations at the School of Agriculture in the Opunohu Valley (Fig. 1), on the Island of Moorea, French Polynesia (Fig. 1). Site A was a field of lettuce, sites B and C were "alternative" pineapple fields, where herbicides are only applied in between the rows of pineapples and biodegradable plastic is used to protect the actual plants. Site B was from in between the pineapple rows, where herbicides are applied, and site C was from underneath the plastic tarps, where no herbicides are applied. Site D was from a conventional pineapple field, and site E was a control, with soil collected across the road from and above the pineapple fields. This way the soil properties, except those changed by agriculture, will be the same but no agricultural chemicals will be present. Because pesticides are sprayed on the surface of the soil, sampling was biased away from deep soils. No dirt was collected from deeper than 15cm.

Pesticide information

Table 1. Summary of information relevant to toxicity and environmental fate of pesticides known to be used on sample fields.

	TAMARON	GRAMOXONE	ROUNDUP (10)	2,4 D (1)	DIURON	GESAPAX	ATRAZINE
Type	Insecticide	Herbicide	Herbicide	herbicide	herbicide	herbicide	herbicide
Class	Organophosphate	quartenary nitrogen compound	organophosphorus compound	chlorinated phenoxy compound	substituted urea compound	triazine	triazine
Chemical	Methamidophos	Paraquat	Glyphosate	2,4-Dichloro phenoxy acetic acid	Diuron	Ametryn	Atrazine
Toxicity	Class I (2)	Class I	Class II	Class III	Class III	Class III (12)	Class III
Soil Mobility		None in soils containing clay; Koc of 15,473-51,856 (3)	Low.	Low to moderate. Koc of 19.6 to 109.1	Low. Koc of 485 (11)		High to medium in most soils; average Koc of 122. (4)
Solubility in water (at 20*C)	High. 90g/L (8)	High. 700g/L (9)	12g/L	Slight. 0.5 g/L	Moderate. .42g/L (11)	High. .185g/L (14)	Moderate. .03 g/L (4)
Degradability (half life) in water	pH 5.0: 309 days pH 7.0: 27 days pH 9.0: 3 days Presence of sunlight expedites degradation. (8)	13.1 hours (6)	12 days to 10 weeks	10 to 50 days	Stable in water(16)		60 to 100+ days (5)
Other Relevant Information	Expected to be present in marine ecosystems due to runoff from agriculture (15); is highly toxic to aquatic organisms including larval crustaceans. (7)		Adheres to soil, especially clay; won't leach into groundwater. However, may enter aquatic ecosystems via surface runoff.		Persists in ground and surface water. High potential for polluting aquatic ecosystems (11) Has been found in groundwater. (16)	Highly toxic to aquatic crustaceans (13)	Found in drinking water wells and groundwater in the US; potential to leach into aquatic ecosystems. (4)

Notes:

1. All information for 2,4- D is from USEPA 2006 (2,4 D) unless otherwise noted.

2. Class I compounds are highly hazardous, class II compounds are moderately hazardous, class III compounds are slightly hazardous and class IV+ compounds are unlikely to present hazard in normal use (IPCS, 2005).
3. Juo and Oginni 1978
4. USEPA 2006 (Atrazine)
5. USDA 1990
6. Kosinski and Merkle 1984
7. Juarez and Sanchez 1989
8. USEPA 1989
9. Kidd and James 1991
10. All information for Glyphosate is from USEPA 2006 (Glyphosate) unless otherwise noted.
11. Green and Young 2006
12. Meister 1992
13. Briggs 1992
14. Thomson 1982
15. Méndez 2008
16. Howard 1991

Zooplankton collection

To collect zooplankton I used a light trap (Fig. 2) placed in the lagoon by the dock at Gump Station (Fig. 1) for approximately twenty minutes. I used shrimp because I could consistently collect them in large amounts.

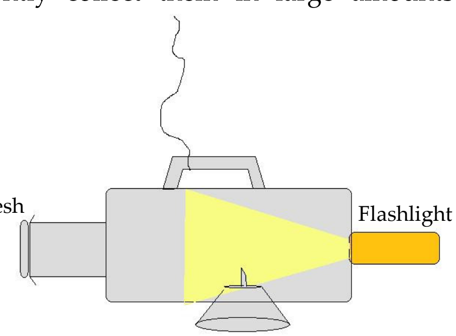

Figure 2. Diagram of a light trap. A flashlight is shone into the trap, which is submerged in the lagoon. The light attracts plankton, which swim through the funnel. When the trap is removed from the water the plankton are strained through the mesh.

Experimental methods

I placed a standardized amount of soil, between 96.4 and 97.9 grams, with an average of 97.4 grams and a standard deviation of .42, in a coffee filter and poured 100 ml of fresh water on it. The fresh water was collected from a stream at Afareaitu at a site above any agricultural land (Fig. 1). I collected the water in glass jars after it had run through the soil, and measured the pH of the water using Whatman pH strips before and after passing through the soil. I was very careful not to contaminate samples. I used separate jars, dishes, cups, scoopers and coffee strainers for each sample. Within half an hour of collecting the plankton I used a transfer pipette to place 7 to 13 shrimp, with an average of 9.57 shrimp, in each of six 5 inch diameter Petri dishes. 4 ml of filtered seawater was pipetted into each dish, and 4 ml of sample water was added, with one sample per dish. Sample F was a control using fresh water not run through soil. The Petri dishes were labeled and used with the same sample for each trial. I left the shrimp in the dishes for 48 hours, and counted the number of living shrimp at 10, 20, 24, 34, 39 and 48 hours after placement in the dishes.

RESULTS

Pesticide use at the School of Agriculture

Through personal communication with the chairman of the farm at the School of Agriculture, M. Serge Touzanne, I gathered as much information as possible on pesticide use at the school (Table 2).

Table 2. Basic information about pesticide use on soil samples.

Note: The information about the pesticides was gathered from a meeting with M. Serge Touzanne, through a translator, and may not be entirely complete.

SITE	Crop	Pesticides used (known)	Last sprayed/spraying frequency	Other relevant information
A	Lettuce	- Gramoxone - Roundup - Tamaron	Roundup and Gramoxone was sprayed ~ 6 months before soil collection. Tamaron is sprayed every 1-2 weeks.	Insecticides and fungicides are rotated and sprayed ~ every week, however I do not know exactly what is sprayed, or when.
B	"Alternative" Pineapple (sampled in between rows)	- Roundup - 2,4 D	Sprayed ~6 months before soil collection.	1 year ago this field was used to grow pineapples conventionally.
C	"Alternative" Pineapple (sampled under plastic)	None		1 year ago this field was used to grow pineapples conventionally.
D	Conventional Pineapple	- Diuron - Gesapax - Atrazine	Sprayed 1-3 months before soil collection.	
E	No Agriculture	None		

Change in pH of stream water after passing through soil

Though it was impossible to tell absolute pH with much accuracy, running the water through soil samples B, C, and D lowered the pH of the water. The stream water had a pH of ~7.0 and the ocean water had a pH of ~8.0. Stream water passed through soil samples A and E remained at pH ~7.0 and stream water passed through soil samples B, C and D dropped to pH ~6.0. Water sample pH was tested three times, and results did not vary.

Effects on survivorship

Exposure to water contaminated by running through soil collected from a lettuce field (treatment A) appeared to lower survivorship of the plankton (Figure 3). The difference in survivorship between treatment A and both both of the controls, which were treatments E and F, are significant (Tables 3 and 4).

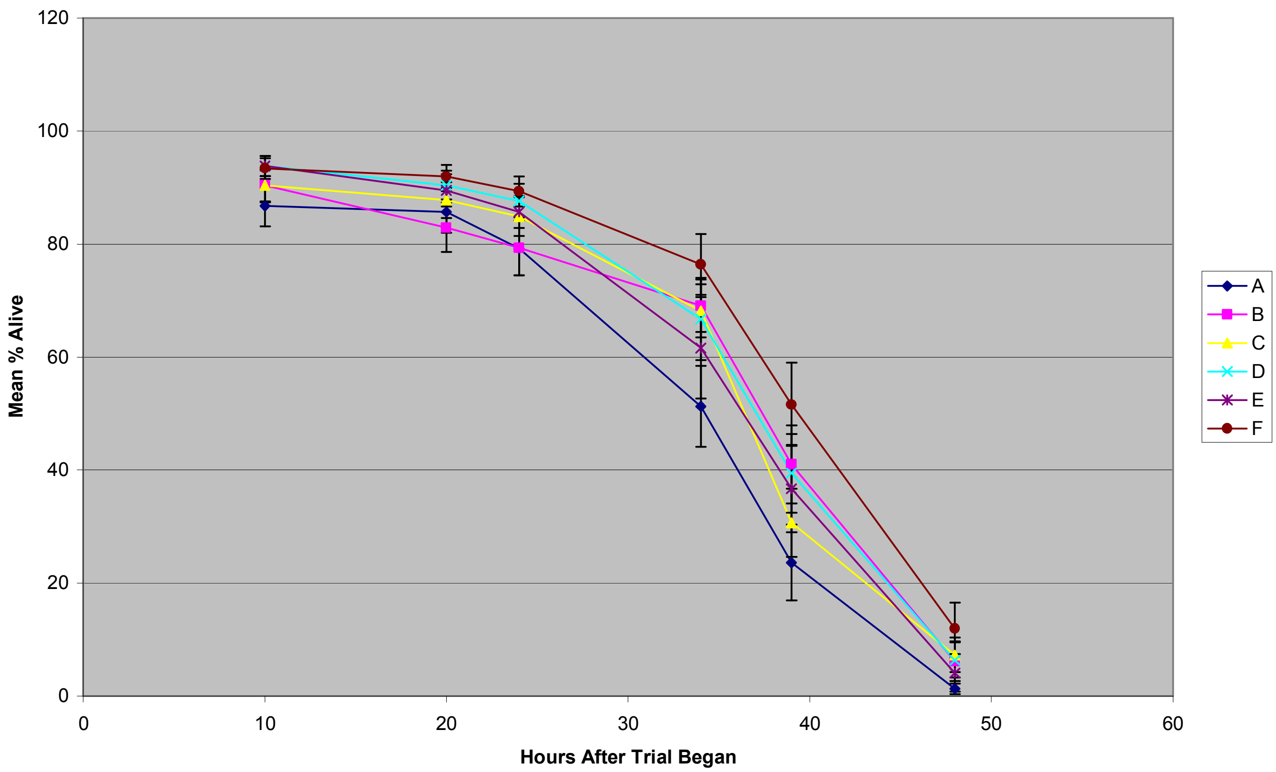

Figure 3. Total survivorship of planktonic shrimp for each treatment over time. A is lettuce field, B is "alternative" pineapple, sampled between rows, C is "alternative" pineapple, sampled under plastic, D is conventional pineapple, E and F are controls. Water for E is run though non-agricultural soil and F is unadulterated stream water. Error bars show standard error

Table 3. Chi square values and probability > chi square values of survivorship between treatments and control F.

TREATMENT	A	B	C	D	E
Chi square	30.1859	4.1372	6.2033	4.7567	11.5729
Probability > chi square	<.0001*	0.0419*	0.0128*	0.0292*	0.0007*

Note: Calculated using the Log-Rank test by JMP software, Kaplan-Meier survival platform. * denotes significance of difference.

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Table 4. Chi square values and probability>chi square values of survivorship between treatments and control E.

TREATMENT	A	B	C	D
Chi square	4.6007	1.6233	0.8259	1.6952
Probability > chi square	.0320*	0.2026	0.3635	0.1929

Note: Calculated using the Log-Rank test by JMP software, Kaplan-Meier survival platform. * denotes significance of difference

All treatments, including control E, significantly reduced survivorship compared to control F (Table 3) however only treatment A had a significant effect on survivorship compared to control E (Table 4). The differences in survivorship between each treatment and control F are shown in figures 4-7. That survivorship of A is reduced further than any other treatment is clearly shown.

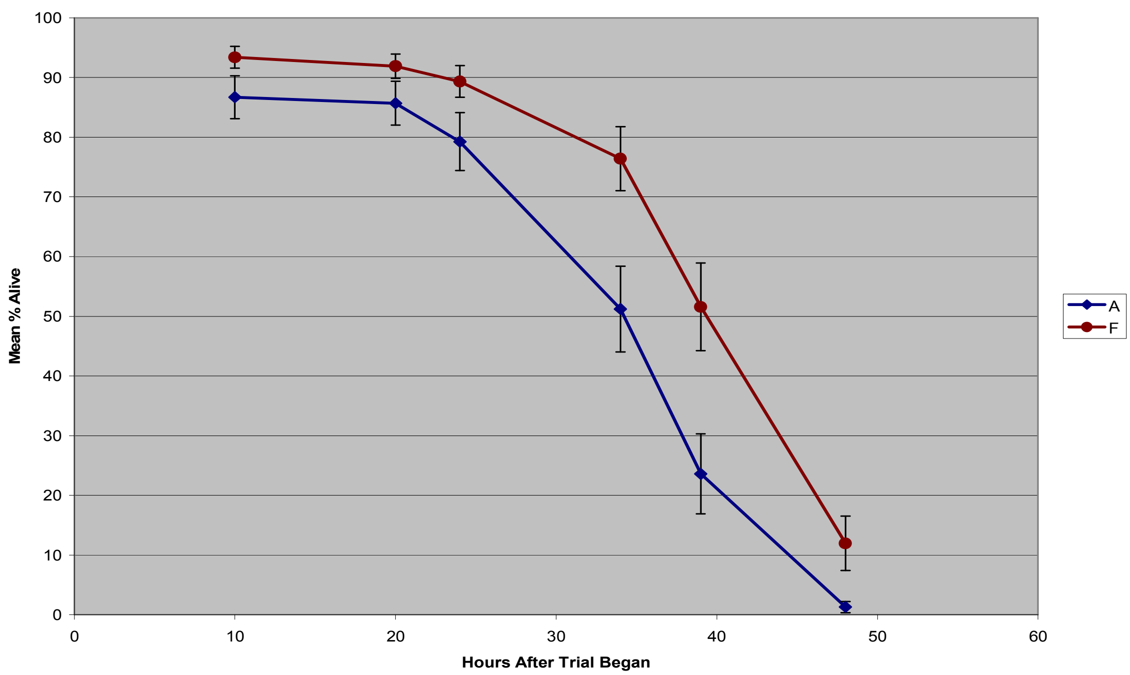

Figure 4. Total survivorship of planktonic shrimp for treatment A and control F over time. Error bars show standard error.

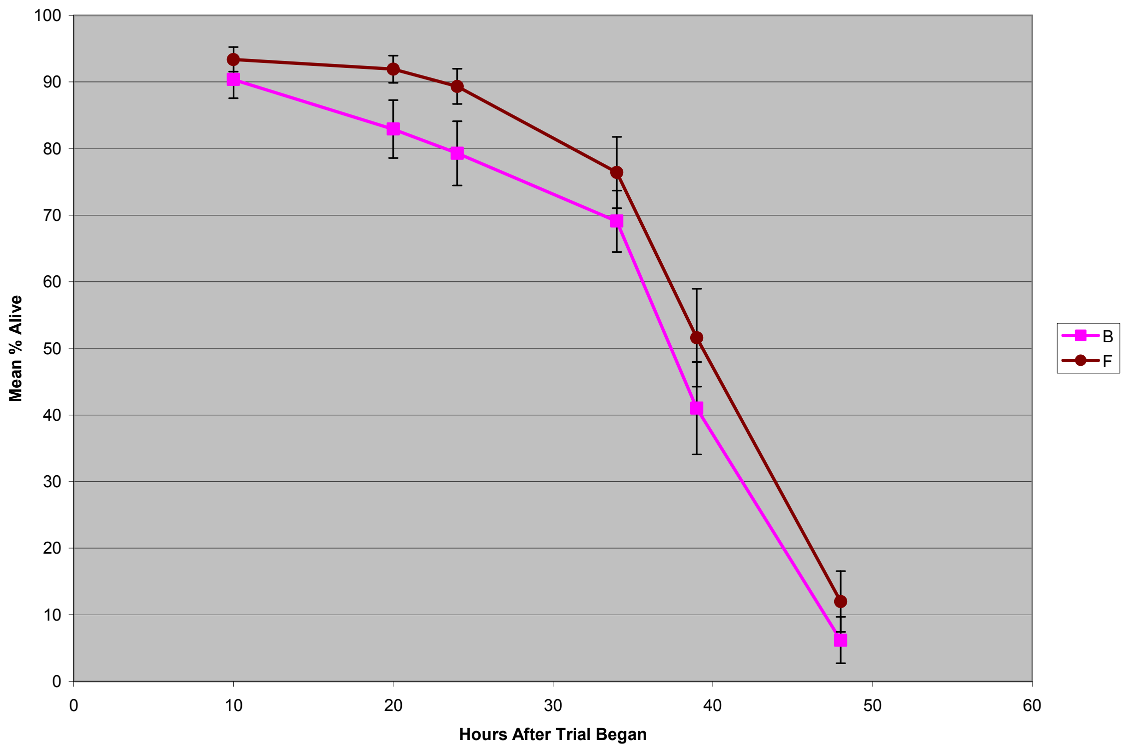

Figure 5. Total survivorship of planktonic shrimp for treatment B and control F over time. Error bars show standard error.

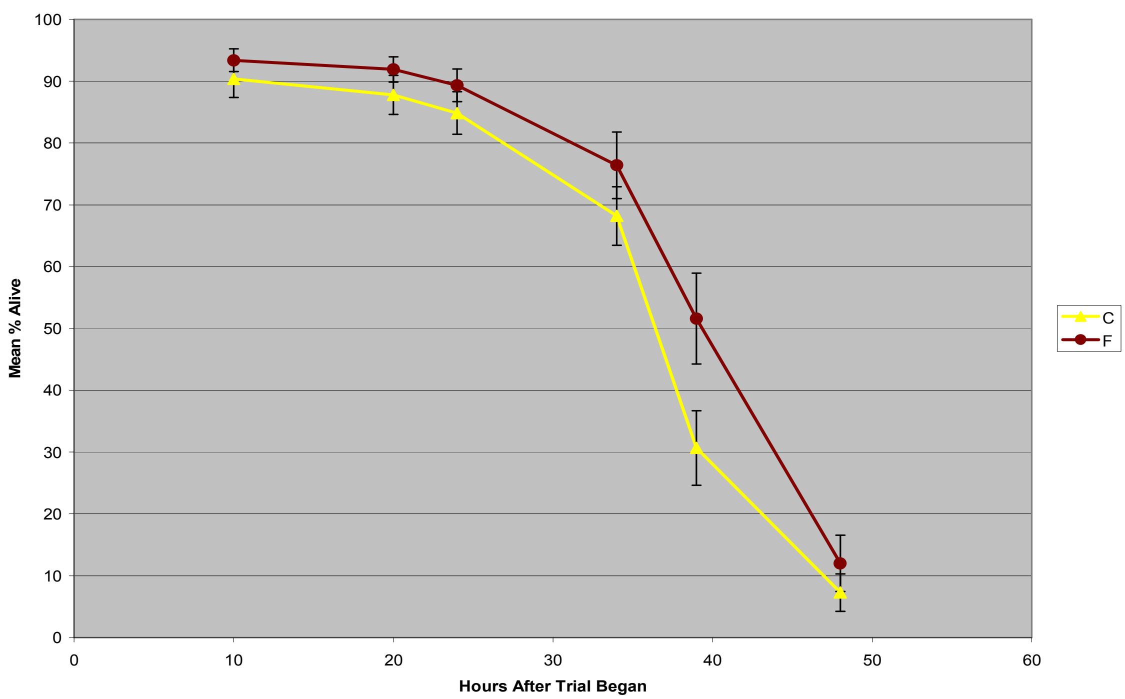

Figure 6. Total survivorship of planktonic shrimp for treatment C and control F over time. Error bars show standard error.

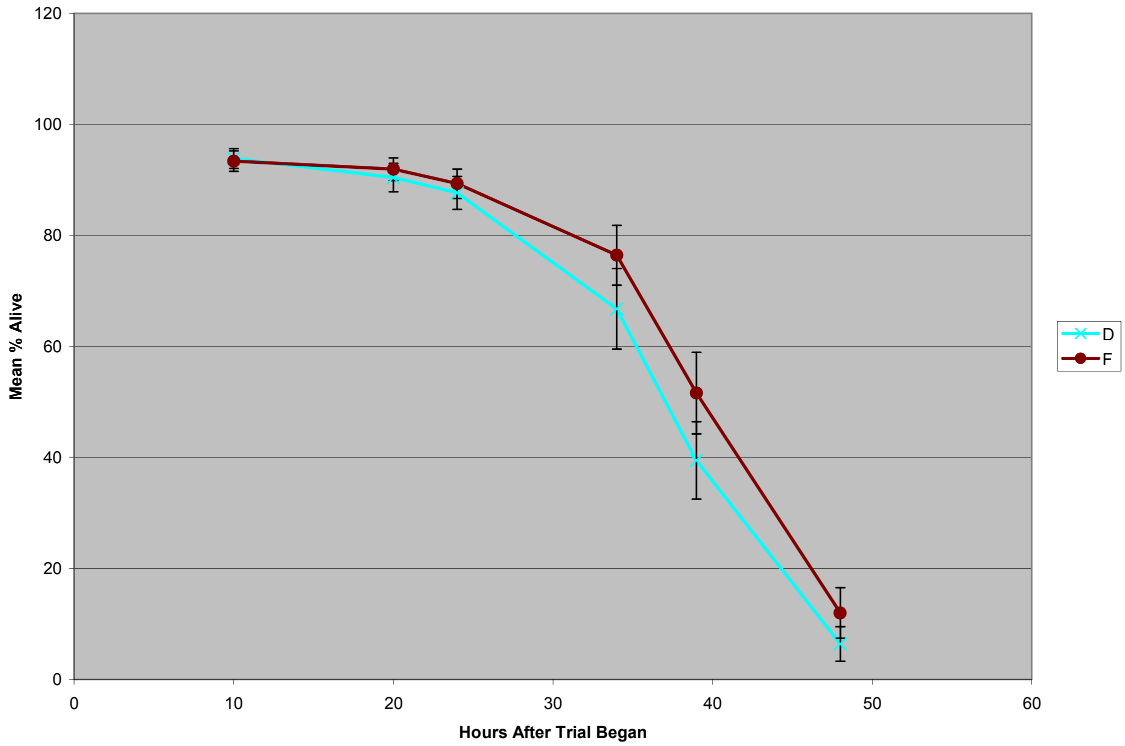

Figure 7. Total survivorship of planktonic shrimp for treatment D and control F over time. Error bars show standard error.

Decreased Effects on Survivorship of Water Passed Through Soil Sample A With Time From Contamination

A trend was observed showing that the effects of treatment A decreased as the time after making the water sample increased before it was used in a trial. However, the results from using water one day after it was run through the soil do not follow this trend (Figure 8). There is a significant difference between results from day 2 and day 4 and between day 2 and day 1 after running the water through the soil sample (Table 5).

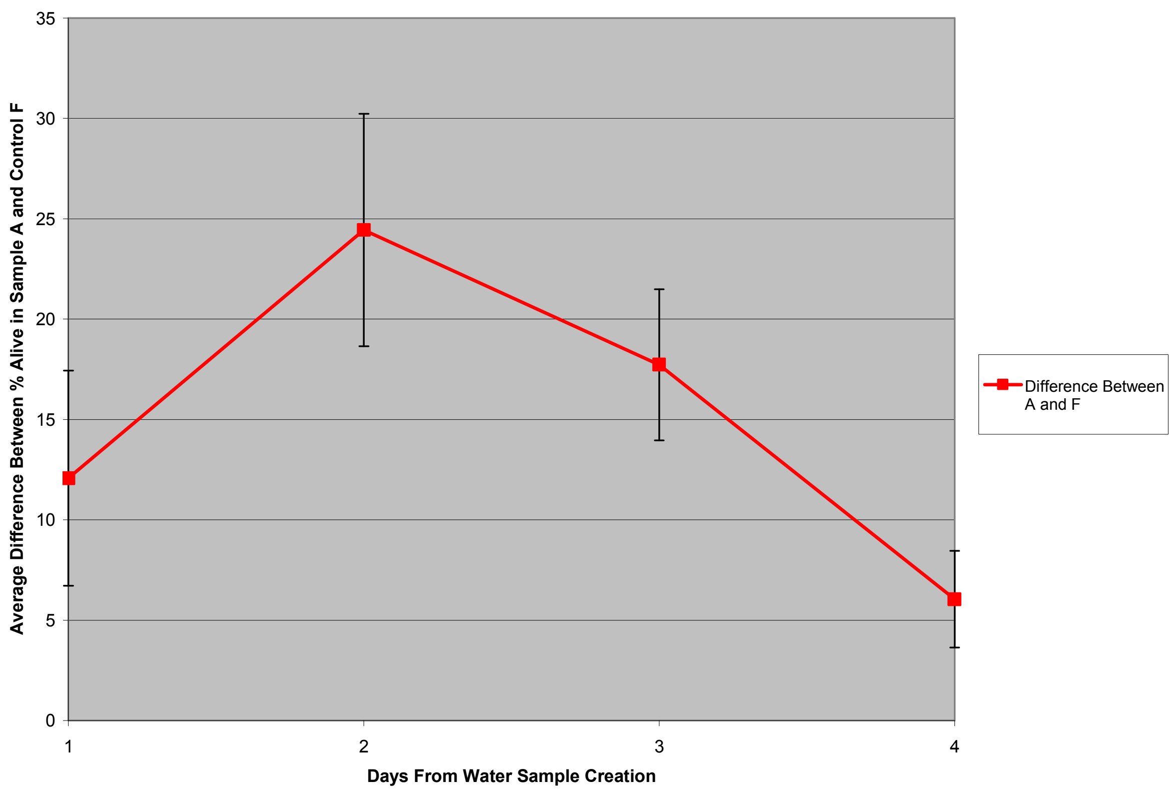

Figure 8. Average difference between % alive in dish A and dish F over time. In this case time is the number of days after the water sample had been that it was used, with a minimum of 1 and a maximum of 4. The error bars represent the standard error

Table 5. Mean differences between % alive in dish A and dish F for trials conducted 1, 2, 3 and 4 days after water was contaminated.

DAY	MEAN
1	27.622054 ± 5.365134 A
2	17.792797 ± 5.791423 A
3	11.646333 ± 3.764413 A B
4	5.115865 ± 2.406637 B

Note: Means are ± standard errors. Means followed by a different letter are significantly different based on Tukey-Kramer HSD test using JMP software.

DISCUSSION

Effects on Survivorship

It appears that simply exposing planktonic shrimp to runoff from any soil will negatively affect the shrimps' survivability. However, this effect is compounded when the plankton are exposed to runoff from soil sample A. A is the only treatment that results in a significantly reduced survivorship in the plankton compared to control E. E controls for effects due simply to the water having passed through soil. This shows that something in soil sample A, something that leaches through the soil or runs off its surface into water, has a deleterious effect on plankton survivability. This supports the commonly held notion that insecticides are more acutely harmful to animals than herbicides (Klassen 2008). The insecticide Tamaron is used on lettuce fields at the School of Agriculure, is toxic to aquatic organisms (Juarez and Sanchez 1989) and is expected to be present in marine ecosystems due to runoff from agriculture (Méndez 2008). Gramoxone, an herbicide, was also used solely on lettuce, however it has extremely low movement in soil and breaks down fairly quickly (Table 1) and so is less likely to contaminate water. Another possible explanation for these results is that herbicides had not been sprayed on pineapple fields for 6 months before soil collection in the case of the "alternative" fields and 1-3 months before soil collection in the case of the conventional fields. Therefore the chemicals used on the pineapples had a long time to break down and get washed away by rain.

Characteristics of the soil in which the crops are grown certainly have an effect on which agricultural chemicals end up in water systems, how fast they get there, and how much. Helene Tolliver (1997), a previous student in the class, looked at soil characteristics of several different pineapple plantations of varying ages, and determined that the soil of all of them has a high clay composition and therefore high water and nutrient holding ability. The lettuce field, on the other hand, has tilled soil that is much less compacted. It also looks richer and darker, likely due to the addition of fertilizers. This may mean that more pesticides are more likely leach out of soil from the lettuce fields than from the pineapple fields.

Decreased effects on survivorship of water passed through soil sample a with time from contamination

My results show inconclusively that there is a trend toward the effects of treatment A decreasing with increased time the contaminated water was sitting in a jar. This indicates that whatever is affecting the survivability of the shrimp breaks down fairly quickly in water. The results of this aspect of the study should be inconclusive, because I was unable to know how long after application of pesticides I was collecting soil.

Conclusion

This study shows that agricultural chemicals used on lettuce fields at the School of Agriculture on Moorea have the ability to leach into water systems, and also have a deleterious effect on planktonic shrimp found in Moorea's lagoons. These results appear to be related to the presence of Tamaron and/or Gramoxone in the soil samples (although other factors related to the media investigated may be causing the decreased survivorship of the shrimp). This means that growing lettuce and other crops using certain pesticides may impact marine ecosystems because decreasing survivability of plankton means decreasing a food source at the bottom of the food chain, which may have a cascading effect up trophic levels. This study also indicates that the chemical harming the shrimp may break down in water fairly quickly. The results of this study have important implications for agricultural practices. They highlight the importance of lessening use of pesticides and also of using pesticides that break down quickly. These results also indicate that growing crops away from streams and rivers, and keeping time between pesticide application and heavy rainfall, would be helpful in preserving the health of marine ecosystems. A more extreme measure that would help keep pesticides out of estuaries and lagoons is to direct runoff into holding ponds, where pesticides may degrade before entering the ocean. Before concrete advice can be given to farmers on how to mitigate effects of pesticides on aquatic ecosystems, more rigorous research must be conducted. Laboratory studies should be conducted to determine exactly which pesticide is affecting plankton and at what concentrations effects are seen. Field testing is necessary to determine what concentrations of pesticides are likely to get into streams, rivers, lagoons, estuaries etc because it is possible that the levels getting in these water systems are not high enough to have an impact on aquatic life.

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