2002 TAIWAN INTERNATIONAL SCIENCE FAIR

CATEGORY：Environmental Science

PROJECT TITLE：Bio-Active Plants

AWARD：Third Award

SCHOOL：Summerland Sceondary School

FINALISTS：Julia Lane

COUNTRY：Canada

Bio- Active Plants

Julia Lane
Summerland Secondary
Grade 10
Level 4
Experiment
Life Sciences

Bio-Active Plants

Julia Lane
S.S.S Grade 10
Level 4
Life science
Experiment

Introduction

My Science Fair project last year tested a local native plant for it's toxic effects on insects (fruit flies), bacteria, fungi, and viruses. The root of the plant *Lomatium dissectum*, had been used by the Salish People to control lice and other insect pests in horses and cattle. The root was also used to kill fish, which could then be harvested by women and children. The fish killed in this way were not harmful to eat as long as they were consumed soon afterward. I have found also that fishing with the aid of plant toxins was formerly very common in tropical Africa.

Lomatium dissectum, grows in dry rocky slopes in conjunction with Sagebrush. Many desert plants produce toxic substances that inhibit the growth of competing plants nearby. This adaptation is called Allelopathy. In a natural ecosystem, *Lomatium* does not kill fish because it does not grow beside creeks. But this raises the question of whether allelopathic plants growing outside their natural ecosystem are having a toxic effect on animal life. There are introduced invasive weeds that are allelopathic, such as Knapweed,growing near streams. And there are crop plants that are allelopathic – Rye and Alfalfa. Do these "natural herbicides" also kill fish? Walnut trees are allelopathic and compounds from Walnut kill fish.

If this effect does go beyond toxicity to other plants, it would be an important consideration to environmental guidelines for private land bordering on streams and rivers. The B.C. Ministry of Environment notes the importance of shade cover for spawning streams. It does not recognize the harmful effects of introduced plants. Yet, when we were purchasing supplies for our Koi Pond the pet supply company offered a list of *"Some of the Worst Plants to Have Around Koi"*. We do not know if the introduced allelopathic plants are poisoning fish or reducing fish stock by poisoning the food that fish need.

Science Fair rules do not allow any research that is expected to have any negative impact on vertebrate animals. Because of this, I have decided to test the effect of allelopathic plants on fruit flies (Drosophila). Fruit flies are similar to fish since, during larva and pupa forms, they live in direct contact with their culture media. I will also be testing the effect of allelopathic plants on Planaria (Turbellaria), an aquatic invertebrate.

Purpose

The purpose of this experiment is to test known allelopathic plants for toxicity to fruit fly and planaria. Canada science fair rules do not allow experiments on fish.

Hypothesis

Chemicals produced by the plants - Walnut, Wisteria, Alfalfa and Knapweed- are toxic to fruit flies and planaria.

Methods and Materials

Fruit Fly Experiment

Materials:

- Fruit Flies
- Culture Media
- Test Tubes
- Foam Stoppers
- Dissecting Microscope
- Petrie Dish
- Wisteria leaves
- Knapweed roots
- Alfalfa Pellets
- Walnut Extract

Method:

1. Culture fruit flies in order to have 2 male and 2 female for each vial.
2. For each plant treatment, set up 4 concentrations and 1 control with 5 repetitions as follows:
   walnut extract
   100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 0 ppm (control)
   wisteria leaves, knapweed roots, ground alfalfa pellets
   5 %, 2.5% , 1.25%, 0.6% , 0% (control). (percent by weight).
3. Place mixture in test tube with stopper and sterilize –
4. Place fruit flies from main culture in freezer for 1 – 2 minutes until the flies are inactive.
5. Empty inactive flies into petrie dish placed on a cold pack and, with the aid of a dissecting microscope, sort the flies for sex.
6. Place 2 males and 2 females in each test tube.
7. Place test tubes in a warm place (20 – 25 deg. C) to allow the flies to reproduce.
8. Count the offspring for each treatment by following the procedure in step 4.
9. Assess results by statistical analysis.

Planaria Experiment

Materials:

- Planaria
- Vials
- Aerator
- Water from host aquarium
- Lettuce leaves
- Wisteria leaves
- Knapweed roots
- Alfalfa Pellets
- Walnut Extract (Juglone)

Method:

1. The Lettuce Experiment was added to provide a control to test the effect of the leaf matter as opposed to plant secretions.
2. Hold Planaria in Aquarium equipped with aerator until ready to use.
3. For each plant treatment, using water from aquarium, set up 3 concentrations and 1 control with 4 replications as follows:
   walnut extract (Juglone)
   100 ppm , 10 ppm, 1 ppm and 0 ppm(control)
   wisteria leaves, knapweed roots, ground alfalfa pellets, and lettuce leaves
   10%, 1%, 0.1% and 0% (control) (percent by volume).
4. Measure 25 ml of each treament into vial and add 4 Planaria.
5. Aerate each vial for 10 seconds each day.
6. After 1 week count the number of live Planaria in each vial.

Results:

Fruit Fly Experiments

1. Most of the Fruit Fly Cultures became contaminated by mold and this had varying affects on the numbers of developing fruit flie larva.

2. Two Excell Programs were used to test the results for statistical significance. Using the Analysis of Variance Single Factor Test, the Knapweed and Alfalfa treatments had P values of 0.06 and 0.08. Using the z test to test the difference between means of concentrations used in these two experiments the results were as follows:

   Knapweed – the 2.5% and 5% treatments were statistically different from the control with probabilities 0.01 and 0.009.

   Alfalfa - the z test showed the means of the 2.5% and 1.25% treatments were different from the control (zero) with probabilities of 0.019 and 0.011.

Planaria Experiments

1. The Lettuce Experiment found no significant difference between treatments; the Lettuce did not kill the Planaria.

2. Walnut Extract (Juglone) killed all the Planaria at concentrations of 10 ppm and 100 ppm.

3. Alfalfa killed all the Planaria at concentrations of 1% and 10 % .

4. Wisteria and Knapweed killed all the Planaria at a concentration of 10 % .

5. Using the Excell Ananlysis of Variance for Single factor, four experiments showed significant differences between the means. The individual means that were significant are as follows:

Alfalfa:	0% compared to 1%	- significant
	0% compared to 10%	- significant
Wisteria:	0% compared to 1%	- significant
	0% compared to 10%	- significant
Knapweed:	0% compared to 1%	- significant
	0% compared to 10%	- significant
Walnut:	0% compared to 10 ppm	- significant
	0% compared to 100ppm	- significant

6. At lower concentrations, some vials had more than 4 Planaria at the end of 7 days. I think that these hatched from the aquarium water that was used for dilutions or that the planaria divided.

Data

Walnut

	replication				
treatm't	1	2	3	4	5
100 ppm	4/1	12/0	0/0	3/1	5/1
50 ppm	21/3	5/0	28/0	25/0	16/2
25 ppm	22/3	14/1	0/0	16/2	12/3
12.5 ppm	0/0	8/0	30/0	8/0	19/0
control	0/0	0/0	6/1	13/0	0/0

Wisteria

	replication				
treatm't	1	2	3	4	5
50%	0/0	0/0	0/0	0/0	0/0
25%	33/2	44/1	38/0	0/0	2/4
12.5%	27/0	22/0	38/0	31/0	0/0
6%	25/3	30/2	60/0	0/0	27/2
control	51/2	0/0	13/2	14/3	12/2

Alfalfa

	Replication				
treatm't	1	2	3	4	5
50%	0/0	0/1	1/0	24/5	50/25
25%	10/7	0/0	0/0	0/0	0/0
12.5%	0/0	0/0	13/0	7/3	0/0
6%	8/3	17/4	35/17	13/6	0/0
control	20/1	7/0	55/10	0/0	27/7

note: # of pupa 10/7 # of flies

Knapweed

	replication				
treatm't	1	2	3	4	5
50%	0/0	30/4	28/7	30/5	25/11
25%	30/4	0/0	17/6	24/4	22/12
12.5%	36/1	47/1	46/15	25/11	19/8
6%	25/3	30/2	60/0	0/0	27/2
control	7/0	29/6	45/3	32/5	49/7

Wisteria

	#1	#2	#3	#4	#5
0	8	0	9	7	33
0.6%	16	9	6	29	0
1.25%	18	37	16	17	6
2.5%	7	2	2	35	42
5%	0	0	0	0	13

Walnut

	#1	#2	#3	#4	#5
0	0	0	0	0	1
12.5 ppm	4	8	16	1	28
25 ppm	11	10	15	21	0
50 ppm	22	31	0	13	10
100 ppm	0	5	7	3	4

Alfalfa

	#1	#2	#3	#4	#5
0	8	1	8	34	20
0.6%	3	12	6	4	0
1.25%	1	4	0	5	0
2.5%	1	0	0	3	0
5%	1	15	32	0	11

Knapweed

	#1	#2	#3	#4	#5
0%	26	46	21	35	8
0.6%	18	19	21	27	10
1.25%	12	44	20	19	18
2.5%	21	13	7	13	0
5%	22	11	9	0	9

Table 1: The effect of Wisteria, Walnut, Alfalfa and Knapweed on Fruit Flies

Wisteria

	#1	#2	#3	#4
0	2	2	4	2
0.1%	3	3	3	2
1%	3	4	4	4
10%	0	0	0	0

Walnut

	#1	#2	#3	#4
0	5	4	3	5
1 ppm	3	4	3	4
10 ppm	0	0	0	0
100ppm	0	0	0	0

Alfalfa

	#1	#2	#3	#4
0	3	4	3	4
0.1%	6	4	2	3
1 %	0	0	0	0
10%	0	0	0	0

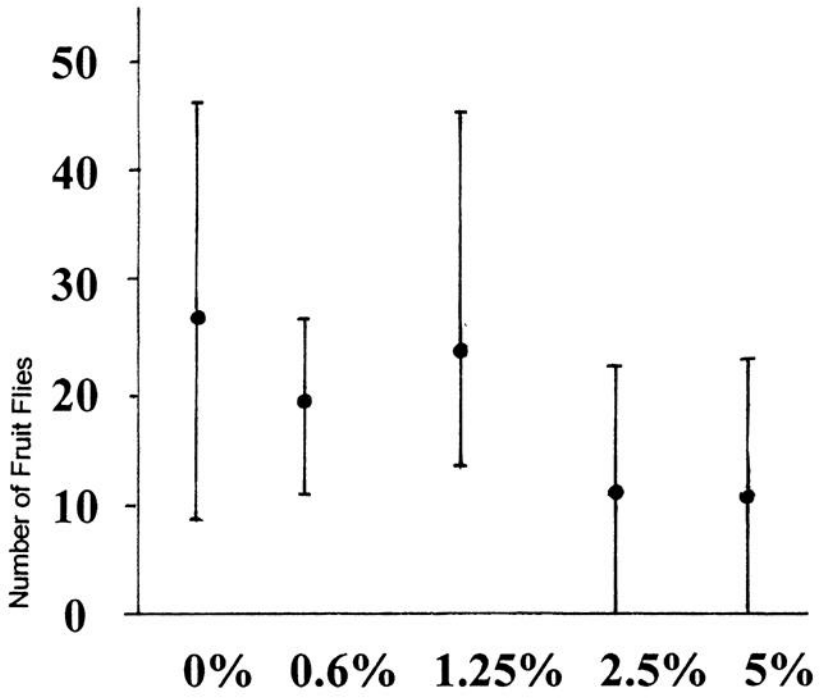
Knapweed

	#1	#2	#3	#4
0	4	3	3	3
0.1%	2	3	3	3
1 %	1	2	3	3
10%	0	0	0	0

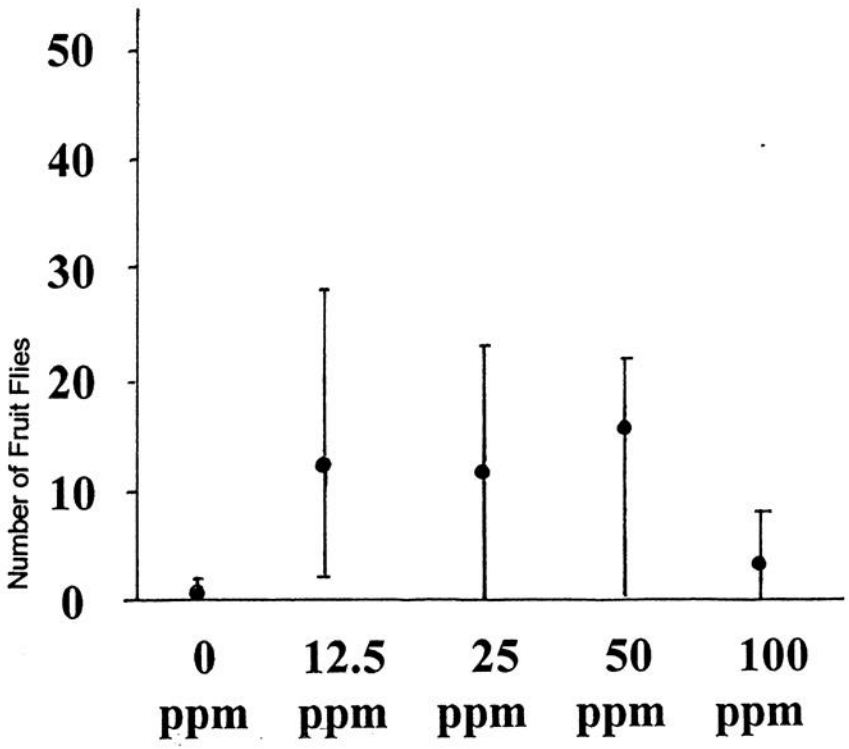
Lettuce

	#1	#2	#3	#4
0	6	4	5	4
0.1%	6	4	5	5
1 %	3	4	4	4
10%	3	4	4	5

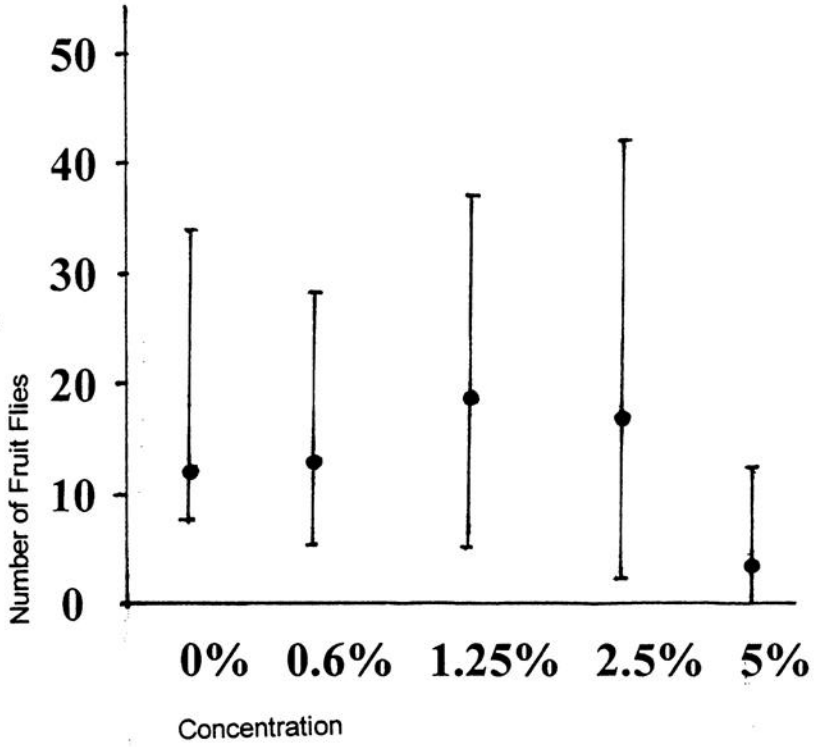
Table 2: The effect of Wisteria, Walnut, Alfalfa, Knapweed and Lettuce on Planeria.



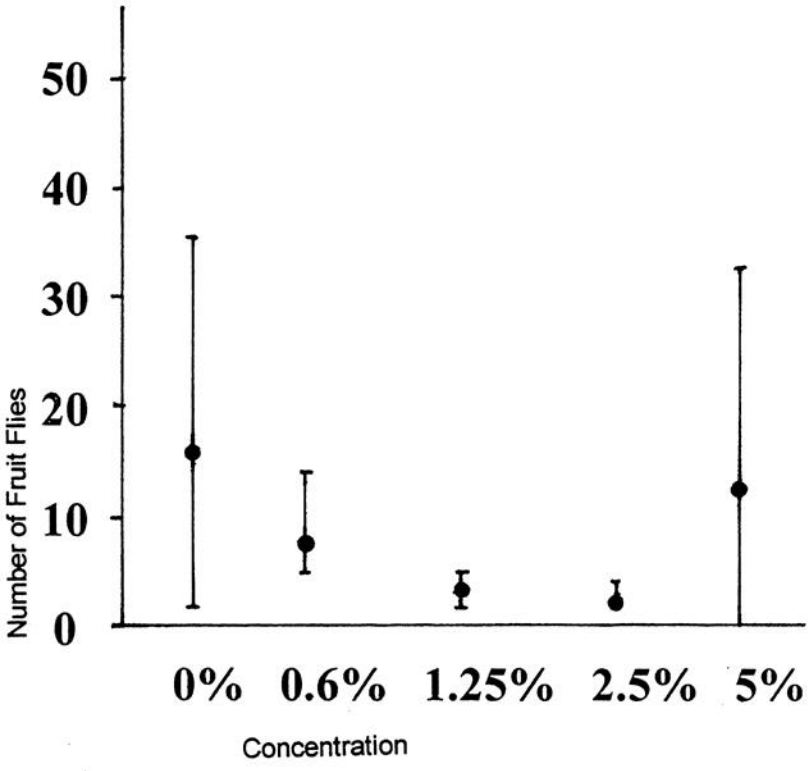
Dose Response of Knapweed on Fruit Flies



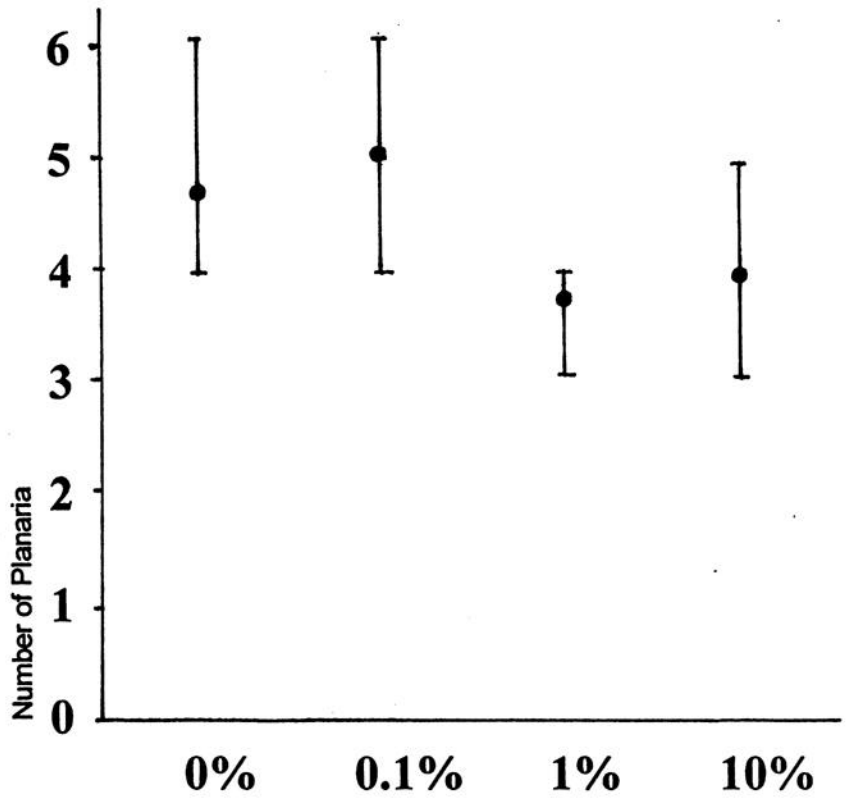
Dose Response of Juglone(Walnut) on Fruit Flies



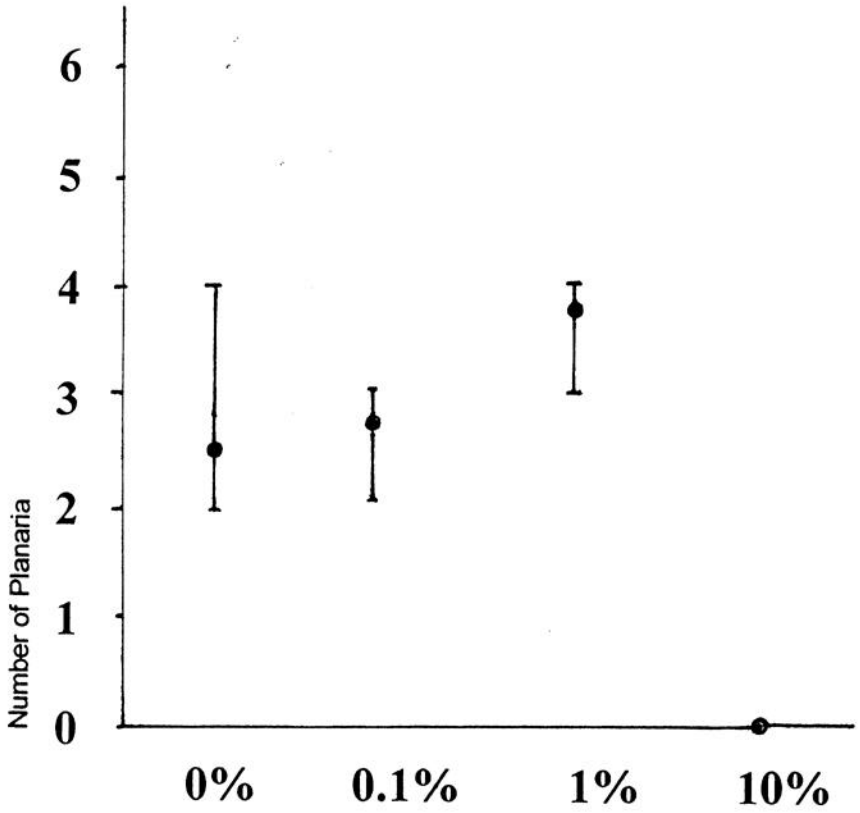
Dose Response of Wisteria on Fruit Flies



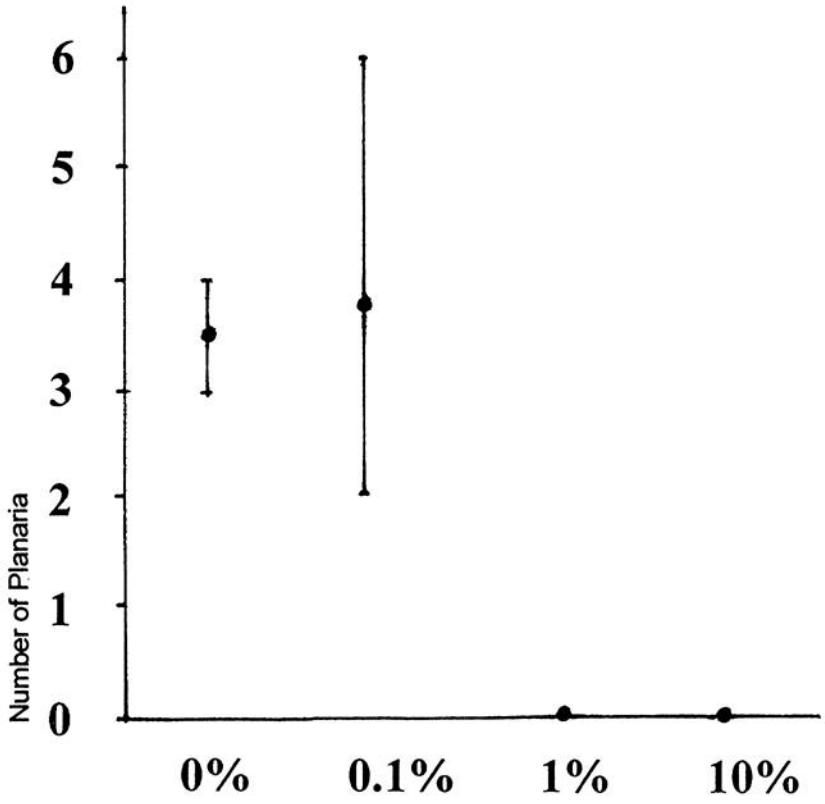
Dose Response of Alfalfa on Fruit Flies



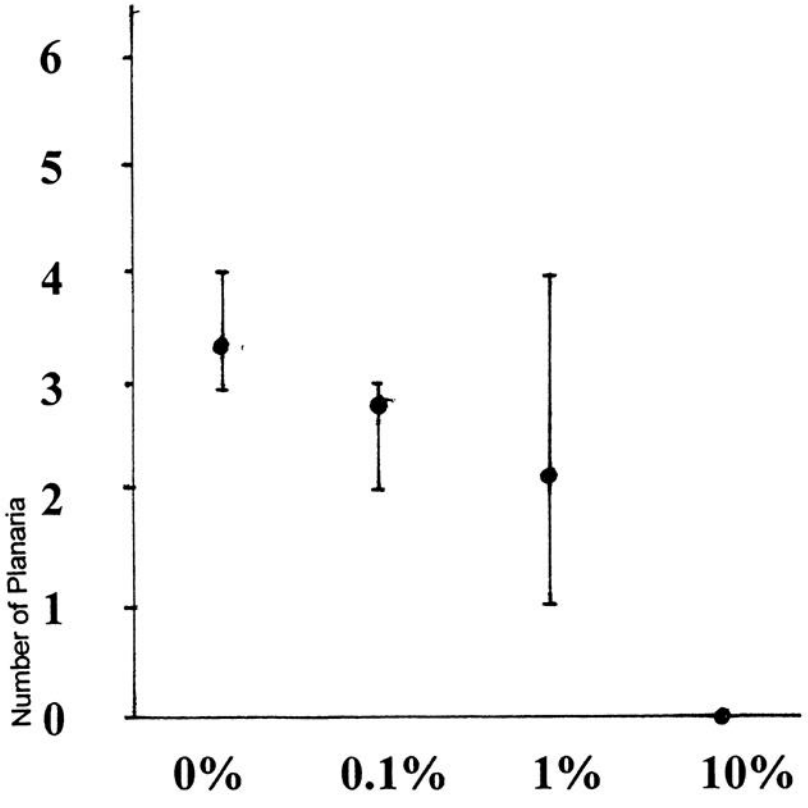
Dose Response of Lettuce on Planaria



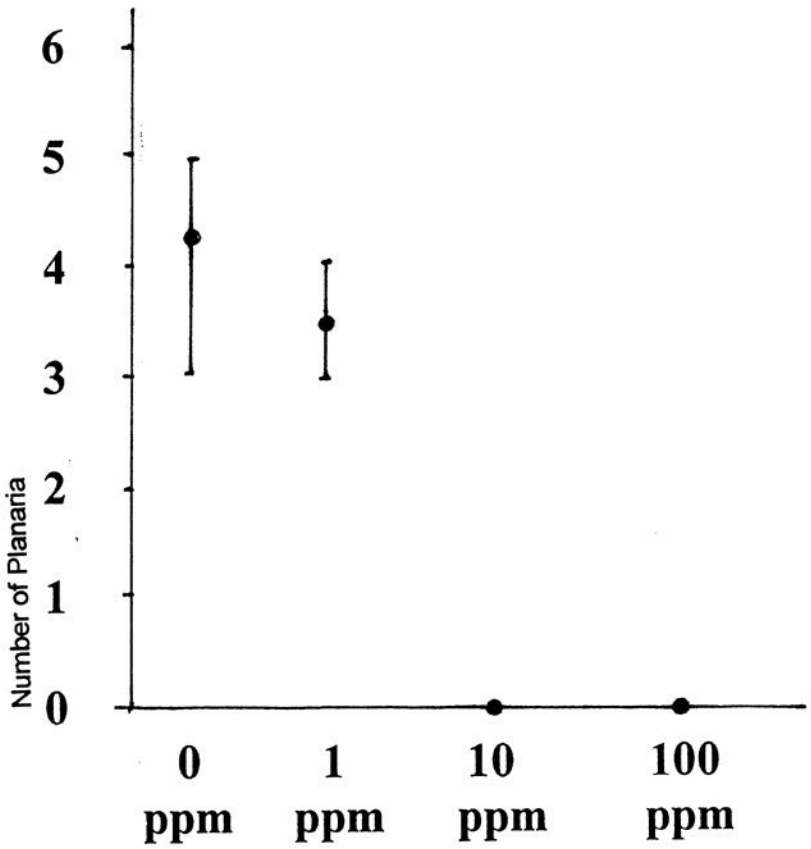
Dose Response of Wisteria on Planaria



Dose Response of Alfalfa on Planaria



Dose Response of Knapweed on Planaria



Dose Response of Juglone (Walnut) on Planaria

Walnut	
treatm't	average # of pupa & flies
100 ppm	7
50 ppm	21
25 ppm	15
12.5 ppm	21
control	4

Wisteria	
treatm't	average # of pupa & flies
50%	0
25%	14
12.5%	19
6 %	30
control	20

Alfalfa	
treatm't	average # of pupa & flies
50%	28
25%	21
12.5%	6
6%	3
control	21

Knapweed	
treatm't	average # of pupa & flies
50%	37
25%	32
12.5%	42
6%	32
control	37

Table 1: Effect of concentration on pupa & flies for Walnut, Wisteria, Alfalfa & Knapweed

Conclusions:

The fruit fly experiments didn't work very well because of the mold, but seem like a good way of testing for toxic effects of the allelopathic chemicals in the future because the fruit fly larva live and grow in close contact with the test chemical just like fish would in water containing the chemicals. Only the Alfalfa and Knapweed experiments gave significant results with reduced numbers of larva developing, compared to the control.

For the planaria experiments, the Walnut, Wisteria, Knapweed and Alfalfa Experiments showed significance but only some of the treatments in each experiment were significant, usually the highest concentration(s). For the Alfalfa, Knapweed and Wisteria experiments where dried powdered leaf or root tissue was added to the planaria water, the two highest concentrations were significant in all three experiments. Alfalfa had the strongest effect because no planaria survived even at the 1% concentration. With the walnut experiment, where the isolated allelopathic compound was used alone, the compound (Naphtha quinone) killed the planaria even at 10 part per million, a very low concentration although we found a scientific paper that says it sedates or kills fish even at 1 ppm.

The Lettuce Experiment that tested the effect of plant tissue as, opposed to allelopathic compounds, on Planaria showed that the plant tissue had no effect. This adds evidence that it is the chemical secretions from allelopathic plants that produces causes the planaria to die.

I conclude that allelopathic compounds from introduced crop plants and weeds that are known to produce these natural herbicides, should be looked at in more detail to see if they also are poisonous to fish and other organisms. The many large alfalfa hay fields, for example, could produce toxic compounds that leach into water and weaken or kill young salmon fry and other fish. Other crops that are allelopathic are barley, rye and wheat, and many of the worst new invasive weeds take over because of they are allelopathic. They all grow in fields along the banks of rivers like the Fraser where there are farms and fields with weeds like knap weed.

My father and I looked for reports of other experiments about the effects of allelopathic compounds on fish and found only two, one about walnut and another about the effects of alfalfa. Other allelopathic plants should be tested to see if they affect fish too.

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評　　語

本研究探討 allelopathic plants 對果蠅（Drosophila）之影響，由於果蠅在 larva 及 pupa forms 時會直接與 culture media 接觸，易於探討 allelopathic plants 濃度對其之影響。研究第二部分爲探討 allelopathic plants 對 Planaria （Turbellaria）之影響。實驗所得之 Dose Reponse 資料相當有用，可供生態控制工程之參考，但如 Wisteria 及 Alfalfa 對 Planaria 之劑量影響，分別出現 1% ~10% 及 0.1% ~1% 不明區帶狀況，值得進一步澄清試驗。