UBC Social Ecological Economic Development Studies (SEEDS) Student Report

The Control of Giant Hogweed (*Heracleum mantegazzianum*) and Canada Thistle(*Cirsium arvense*) by the Organic Herbicides Ecoclear (Acetic Acid) and TopGun (Pelargonic Acid)

Lanie Fung

University of British Columbia
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Research Supervisor: DR. SANTOKH SINGH

Department of Botany Faculty of Science University of British Columbia

CONTENTS

Abstract

Organic herbicides have been found to be effective in weed control. Topgun and Ecoclear are both organic herbicides, but differ in their active ingredients. Topgun is a fatty acid based herbicide and contains the active ingredient pelargonic acid; while Ecoclear is vinegar based herbicide with the active ingredient being acetic acid. Both of these substances work by drying out the vegetation it contacts and destroying the waxy cuticle of the leaf. Through protein analysis we found that the proteins Rubisco and Light Harvesting Complex were proteins degraded by the herbicides. The proteins were degraded in a two stage process where the plasma membrane was first destablized and broken, then Reactive Oxygen Species (ROS) oxidized the proteins, causing them to be degraded (Fukuda *et al.*, 2004). Over a three week period, all Canada Thistle plants and Giant Hogweed plants were seemingly eradicated. Also, higher concentrations of herbicide were correlated with faster plant death.

1.0 Introduction

Organic herbicides are not a new invention. Acetic acid, or vinegar, has been used in vegetation control for centuries. Hydrocarbon oils, or fatty acids, have been repeatedly used as herbicides for the past 50 years. However, as environmental awareness has grown, and as the concern for the impacts of chemical herbicides on plant, animal, and human health has grown, there has been a larger movement in the usage of organic herbicides.

Pelargonic Acid is an organic herbicide used on terrestrial plants and is a post-emergent herbicide, meaning it can only be used on the aerial portion of plants. It works best on herbaceous (non-woody) plants and works by drying out the vegetation it contacts by destroying the waxy cuticle of the weed. It has the chemical formula $C_9H_{18}O_2$, consisting of a nine carbon chain terminating in a carboxylic acid (Coleman and Donald, 2006). Pelargonic acid occurs naturally in members of the family Geraniaceae. It is considered to have low toxicity and low environmental impact as well as leaving no residual activity.

Topgun is a nonselective weed controller that contains the active substance pelargonic acid (nonaoic acid). (Non-selective means that any parts of the plant that come into contact with the herbicide will be killed.) It is completely decomposed by bacteria in the soil within a few days. The product is based on organic fatty acids (C8, C9, C10- main fraction C9, 368g/L). It has been approved by the EPA in the United States and is also approved in Canada by the British Columbian government. The organic fatty acid dissolves the cell walls of vegetation after which cells of the plant leaks and the plant dries out.

Acetic acid is another weed controller and works as a burn down, non-selective herbicide. It is often used in driveways, side of roads, and industrial sites. Acetic acid solutions of 10-20% provide greater than 80% control of most immature weeds (Evans *et al.*, 2009). However, the cost of applying acetic acid is more than 10 times greater than the cost of using the synthetic herbicide glyphosate, which has proven efficacy on roadside vegetation (Ivany, 2010).

Acetic acid is also only able to kill the aerial portions of plants and does not control the parts underground. Plants may therefore re-sprout from the root system after a few days or weeks (Evans *et al.*, 2009). Ecoclear is the acetic acid based weed control product used in this experiment).

Canada Thistle is a perennial plant in the Asteraceae family. It is typically found along roadsides and rocky environments. Canada Thistle has established itself as a prevalent weed on the UBC campus as well as around the world. It reproduces through seeds and also through root regeneration, which is why they are so hard to manage. They have spiny leaves, purplish flowers when in bloom, and grow to an average of 1.2m in height.

Hogweed is a dangerous and toxic weed that has overrun Canada, the United States, as well as Europe. This pervasive and invasive plant is native to Asia, and was first brought overseas as an ornamental (Ministry of Agriculture, 2012). Giant Hogweed is a large hairy perennial herb with strong taproot and fibrous roots (Caffrey, 2001). Its stems and stalks excrete a clear watery sap, that upon exposure, sensitizes human skin to UV radiation. Exposure to sunlight could result in severe burns leading to painful blisters and dark scars (Ministry of Agriculture, 2012).

The objective of this study is to compare the effects of different concentrations of TopGun and Ecoclear on Canada Thistle and Giant Hogweed. Lower concentrations of Ecoclear and Topgun are tested on Canada Thistle because 100% concentrations have been shown to be effective in managing vegetation. However, using 100% solutions as a long term solution for weed control is not cost effective. Therefore, testing lower concentrations will enable us to find a balance between efficacy and economy.

2.0 Materials and Methods

Three treatments were used in the Giant Hogweed Experiment.

1. 100% TopGun
2. 100% Ecoclear
3. 100% 50/50 Mixture

Three Hogweed plants were treated with the herbicides, and pictures were taken at t=1, 5, 11, 14 days.

In addition to control, six treatments were used in the Canada Thistle Experiment:

1. Soapy base solution (control)
2. 50% Topgun (50TG)
3. 25% Topgun (25TG)
4. 50% Ecoclear (50EC)
5. 25% Topgun (25 TG)
6. 50% 1:1 Mixture made of Topgun and Ecoclear at 100% concentration (50M)
7. 25% 1:1 Mixture made of Topgun and Ecoclear at 50% concentration (25M)

Each plot is sprayed with one herbicide treatment. Leaf samples were taken three times through the experimental period at t=1, 7, and 14 days. 0.03g of leaves were ground using a mortar and pestle. 30uL of sample buffer was added to prevent the leaf samples from further degrading. The sample were boiled for 5 minutes along with molecular markers, which were boiled for 1 minute. Afterwards, the samples were microcentrifuged for 2 minutes at high speed.

The Electrophoresis SDS-Page Apparatus was set up and 10uL of each of the samples were placed in each well. The gels were run for an hour, when stained overnight. The next day, they were placed in destaining solution for 3-4 hours.

3.0 Results

3.1: Effects of Organic Weedicides on Hogweed

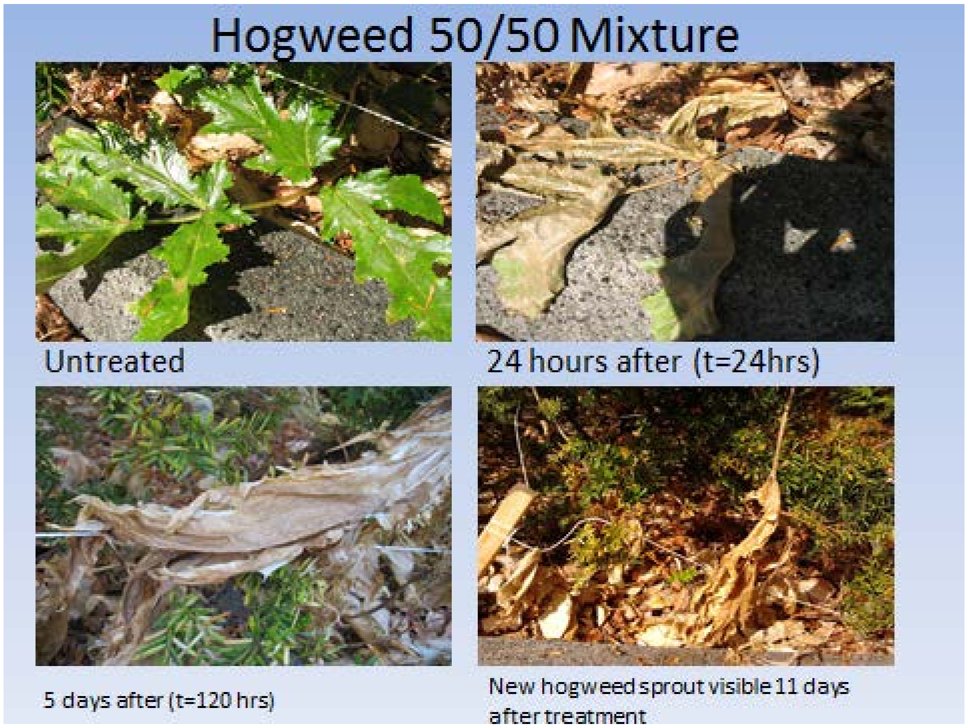

Figure 1: Hogweed 50/50 Treatment at t=1, 5, and 11 days. Plants were sprayed with 50% 1:1 Mixture made of Topgun and Ecoclear at 100% concentration.

Figure 2: Hogweed 50/50 Treatment. Respray at t=0, 1, and 2 days. Plants were sprayed with 50% 1:1 Mixture made of Topgun and Ecoclear at 100% concentration.

Figure 3: Hogweed 50/50 Treatment. Respray at t = 14 days. Plants were sprayed with 50% 1:1 Mixture made of Topgun and Ecoclear at 100% concentration.

3.2: Effects of Organic Weedicides on Protein Profiles in Leaves of Canada Thistle

SDS-Polyacrylamide gel electrophoresis (SDS-Page), is a technique used to view proteins taken from plant leaves. Each column is a treatment. The columns on the far right and far left are the molecular weight markers (MW), and the lines, also called bands, in the columns display known protein weights in kDa. The heavier proteins (protein bands) are closer to the top, and the lighter proteins are on the bottom.

Bands at the same level show the same proteins. A darker and thicker band indicates a high abundance of the protein, which shows that the proteins are intact and not broken down. A lighter and thinner band indicates a low abundance of the protein, and means that the proteins are most likely degraded.

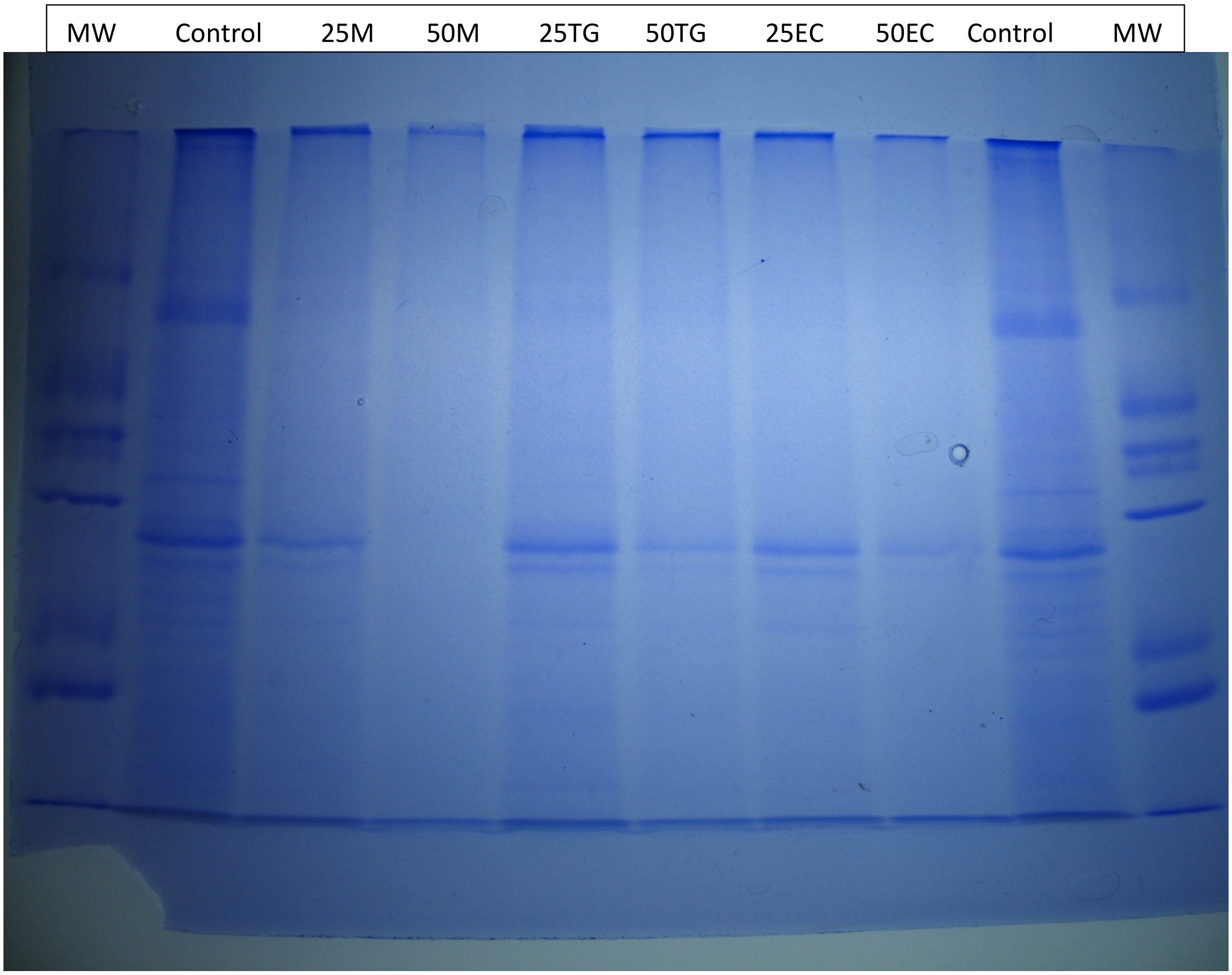

Figure 4: A SDS-PAGE gel showing leaf protein profiles from leaf extracts obtained from Canada thistle leaves one day after treatment with organic weedicides ((see details of treatments and their abbreviations in the Materials and Methods section above).

As seen in Figure 4, the Rubisco band at 56kDa is only visible in the control.
A band at 23kDa seen in control and all treatments except 50M and lighter in 50TG and 50EC

MW: Molecular Weight Markers
25M: 50% Concentration of 1:1 ratio of Topgun and Ecoclear
50M: 100% Concentration of 1:1 ratio of Topgun and Ecoclear
25TG: 25% Concentration Topgun

50TG: 50% Concentration Topgun
25EC: 25% Concentration Ecoclear
50EC: 50% Concentraion Ecoclear

Figure 5: A SDS-PAGE gel showing leaf protein profiles from leaf extracts obtained from Canada thistle leaves 7 days after treatment with organic weedicides (see details of treatments and their abbreviations in the Materials and Methods section above).

As seen in Figure 5, the band at 56kDa (Rubisco Large Subunit) is missing from 25M, 50 TG, 25EC, 50EC.

Also, the band at 23Da (LHC-IIb/c) missing from 25M, 50M, 50TG, 50EC, and very light in 25EC.

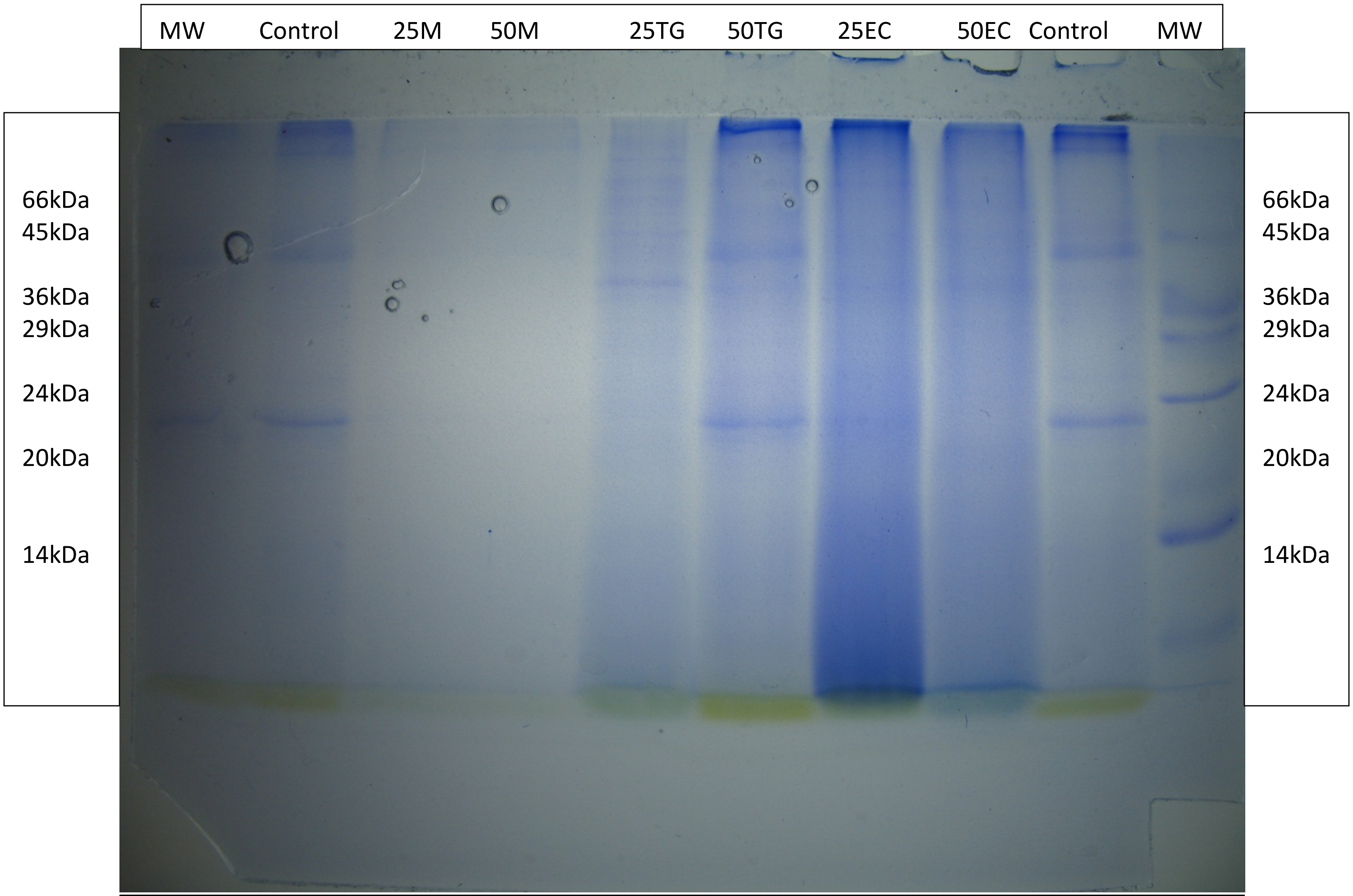

Figure 6: A SDS-PAGE gel showing leaf protein profiles from leaf extracts obtained from Canada thistle leaves 14 days after treatment with organic weedicides (see details of treatments and their abbreviations in the Materials and Methods section above).

No protein bands are visible in 25M, 50M, 25EC, and 50EC. The band at 23Da (LHC-IIb/c) is missing from 25M, 50M, 25TG, 25EC, and 50EC. The Rubisco band is seen at 56kDa only in 25TG.

The clear columns of 25M and 50M mean that all proteins have totally degraded, and are too small to be seen on the gel.

The columns that are a smear of blue mean that the proteins has broken down into pieces, but are still large enough to be resolved onto the gel.

4.0 Discussion

The most significant result in the Giant Hogweed experiment was found when using a 50/50 mixture on Hogweed. As seen in Figure 1, regrowth was visible 11 days after the initial treatment, showing that the plant is able to store most of its sugars in roots and nutrients underground for long periods of time, and can therefore survive without photosynthesizing for a limited time before sprouting a new shoot (Davies *et al.*, 1985).

In the Canada Thistle experiment, the two protein bands that are consistently found in the control and are degraded at different levels in the treatments are Rubisco (56kDa) and Light Harvesting Complex (26kDa). In addition, the 50% concentration herbicides degraded the proteins faster than the 25% herbicides. Rubisco is the most abundant protein on earth, and consists of up to 50% of proteins in leaves (Feller *et al.*, 2008). Chloroplast proteins, like the Light Harvesting Complex (LHC), are also broken down relatively quickly during senescence, including senescence brought on by abiotic factors (Feller *et al.*, 2008). As proteins are further degraded, the fragments become undetectable on stained SDS-Page gels.

Chloroplasts are a major site of protein degradation and ribulose-1,5-bisphosphate carboxylase (Rubisco) is quickly degraded during senescence and stress (Khanna-Chopra, 2012). The process of protein degradation is initiated by Reactive Oxygen Species (ROS) and involves proteolytic enzymes such as cysteine and serine proteases (Wada *et al.* 2009). Leaf cysteine proteases specifically break down Rubisco. The binding of oxidized Rubisco protein to the chloroplast envelope creates Rubisco vesicular bodies (RVB). These vesicles contain cysteine proteases and remove proteins marked for degradation by ROS (Wada *et al.* 2009).

Fukuda *et al.* (2004) found that plants treated with Pelargonic acid fatty acids showed soaking and wilting symptoms within an hour after treatment, as well as discoloration and necrosis of the leaves. After 1 or 2 days, the plants were killed. Complete plant death was observed 3 days after treatment. Only a few plants displayed regrowth within 15 days. The rapid herbicidal effect and "soaking" of the plants strongly suggests that fatty acids directly damage the membrane of the plants.

The action of pelargonic acid consists of two steps. First, there is the movement of the fatty acid into the cellular membrane, destabilizing it and therefore causing cell component leakage (Fukuda *et al.*, 2004). Fatty acid can easily move past the cell membrane because of their similarity of form to the phospholipids that make up the plasma membrane. Because of their polar heads, and because of their amphiphilic structure (molecules with a hydrophilic head and a hydrophobic tail), they similar in structure to the phospholipids in the bilayer, and are therefore able to move between the phospholipids of the cell membrane as well as through the membranes of the organelles in the cell. They move into the plant cells, and at the same time, negatively affect the structural stability and the protein carriers in the membrane, and cause the leakage of all cell components into the cytosol and out of the cell membrane (Fukuda *et al.*, 2004).

Secondly, large quantities of reactive oxygen species (ROS), like superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen are generated from light energy and oxidation reactions that occur with reactive chloroplast components that have strong photooxidative potential (Khanna-Chopra, 2012). This initiates the degradation of many chloroplast proteins and cell components. ROS further damage the cell by causing irreversible damage to important proteins like Rubisco and other chloroplast proteins. In general, an increased production of ROS oxidizes proteins, lipids, and DNA, resulting in lipid peroxidation, cellular damage and cell death as the antioxidants in the leaf are reduced (Lederer *et al.*, 2004).

The action of how vinegar, or acetic acid degrades chloroplast proteins is similar to how pelargonic acid works. The hydrogen ions from acetic acid move into the intracellular space through a concentration gradient through proton pumps. The high concentration of acid inside the cell causes the plasma membrane to be unstable and break. Once inside the chloroplasts and the mitochondria, the hydrogen ions can prevent the production of ATP as they reduce the proton gradient that allows energy to be formed and be used in the cell. In addition, reactive oxygen species to enter the cell and react with the reactive photosynthetic molecules in the chloroplasts, causing the cell proteins to degrade and lead to cell and plant death through oxidation of cellular components as well as through redox status changes (Lederer *et al.*, 2004).

Under low stress circumstances, a plant has antioxidants and other ROS fighting enzymes that can help maintain the cell structure and integrity by preventing the destruction of important proteins in the plant cell. However, herbicides overwhelm the plant and its defences, and therefore are able to effectively eradicate weeds (Lederer *et al.*, 2004). This may be the reason why the higher concentration herbicides (50%) were able to degrade proteins faster than the 25% (lower) concentration herbicides.

5.0 Conclusion

One of the downsides with using burn-down herbicides like Ecoclear and TopGun is that regrowth can occur if the plant has stored enough energy in its roots. However, in this study, there was no regrowth in the Canada Thistle over the three week experimental period. Regrowth may not have occurred because of the approaching winter season. The plants are, in a way, hibernating and storing whatever carbohydrates they have made through photosynthesis into their roots that will last the through the winter (Davies *et al.*, 1985). Until next spring, when plants start sprouting again, we will not know if the Canada Thistle or Hogweed plants have been completely eradicated.

Based the results of this study, I recommend using 25% Ecoclear on smaller, younger weeds. At 25% concentration, it worked the best out of all the other treatments. At 50% concentration, I would recommend using a 1:1 mixture of Topgun and Ecoclear. It is a stronger solution and works extremely well in removing smaller weeds.

6.0 Acknowledgments

Special thanks to Mr. Jarnail Mahroke and Dr. Santokh Singh for the abundance of advice and support throughout this project, as well as the use of the laboratory and equipment. I would also like to thank Brenda Sawada of UBC SEEDS for coordination of this project. Also, my special thanks to all the volunteer students in Dr. Singh's lab who helped me at the various stages of this project.

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