International Baccalaureate Diploma Programme

Extended Essay

Research Question:

"How would varying acidic watering solutions, simulating acid rainfall, affect the growth of purple loosestrife (*Lythrum salicaria*), in an attempt to assess the effectiveness of the acidic nature of southern Ontario precipitation as a control for the invasive growth characteristics of purple loosestrife?"

ABSTRACT

Lythrum salicaria, purple loosestrife, is an invasive plant that is affecting southern Ontario wetlands by reducing native plant diversity. Chemical and biological controls have been implemented to minimize its spread, but are only effective in certain situations and can cause hazardous side-effects. Due to the high density of urban-industrial regions in southern Ontario, acidic rainfall is very common.

This study investigates the effects of acidic watering solutions (pH 2, 3, 4, 5, and 7) on the growth of purple loosestrife during a four-week watering treatment, to examine whether acidic rainfall can potentially act as a growth control. Growth was assessed by measuring and calculating the percent change for the following indicators: stalk height, flower stalk height, number of flower buds, number of flowers, and leaf length. New growth was assessed after the exposure by counting new secondary shoots and flower stalks.

For each parameter recorded, there was a positive correlation between mean percent difference of growth and pH. Four growth indicators (flower buds, flowers, new secondary shoots, and new flower stalks) showed statistically significant correlation with watering solution pH (one-tailed test, $p<0.05$). All measured growth indicators showed positive growth when watered with solutions of pH 5 or 7. However, the highly acidic pHs of 2 and 3 resulted in reduced numbers of flowers and buds, with a reduction in stalk and flower stalk height as parts of the plants became brittle and fell off. In general, the sections of the plant that were actively growing during treatment were highly affected by acidic watering solutions of pH 4 or lower. The reduction in growth at pHs 2, 3, and 4 indicates that acidic rainfall can act as a potent control on the high growth rate of purple loosestrife, and may limit its invasive characteristics in urban-industrial regions.

(Word Count: 298)

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Many thanks and regards to my IB Year 1 and Year 2 Biology teachers, Ms. Mihaljevic and Ms. Kiaee.

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Last but not least, my many thanks to both my parents for their continued support and trust in me in pursuing this extended essay.

How would varying acidic watering solutions, simulating acid rainfall, affect the growth of purple loosestrife (Lythrum salicaria), in an attempt to assess the effectiveness of the acidic nature of southern Ontario precipitation as a control for the invasive growth characteristics of purple loosestrife?

TABLE OF CONTENTS

1. INTRODUCTION

Lythrum salicaria, commonly known as purple loosestrife, is a vibrant flowering plant which has always caught my attention during my summer trips to the Shakespearean Festivals in Stratford, Ontario. I have noticed that its population has begun to increase over the past few years and the diversity of the other plants in this area has decreased as purple loosestrife plants become more common. My interest in this plant is due to the fact that it is a very aggressive invader in many regions of North America, and its population is increasing in southern Ontario[1]. The areas where purple loosestrife has had the greatest invasion impact in Canada has been in southern Ontario, Nova Scotia, New Brunswick, and Quebec; it has also been found in nearby areas in the northeast United States[2]. Purple loosestrife and its ability to invade could pose a serious threat to the agricultural sector of the Niagara Belt, having disastrous economic consequences. I have seen it growing along our densely populated highway (401) in the industrial areas of southern Ontario, where the rainfall can be acidic due to pollution[3]. The current methods of controlling purple loosestrife growth are either insect-based or chemical herbicides, which pose potential problems of their own, such as limited effectiveness[4] and potential side-effects[5] [6]. However, if purple loosestrife is sensitive to being watered with acidic solutions, then the existing acidic rainfall in Southern Ontario may be effective as an additional

[1] White et al. (1993)
[2] Ontario Federation of Anglers & Hunters (2009)
[3] Environment Canada (2005)
[4] Blossey et al. (2001)
[5] Harris (1990)
[6] Thompson et al. (1987)

control, limiting the necessity of other, more dangerous control methods. Although some academic studies have been done that show that purple loosestrife seeds can germinate over a wide pH range[7], very little is known about the effects of varying pH on the growth of mature plants. Therefore, I will be investigating if acid rain can be used as an alternative control for the growth of purple loosestrife, by simulating acid rainfall via watering with acidic solutions and measuring the effects on plant growth.

[7] Thompson et al. (1987).

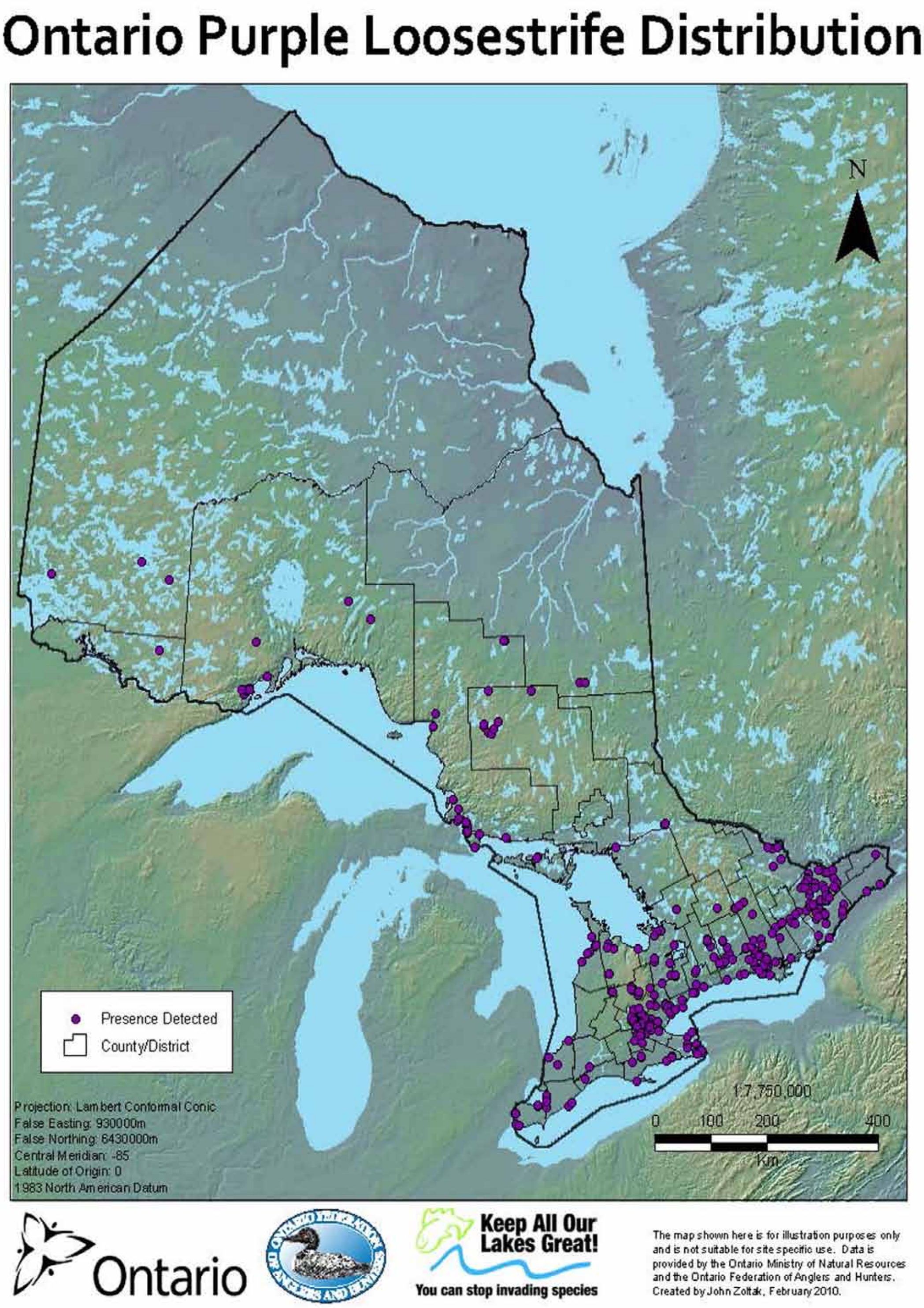
2. BACKGROUND INFORMATION

2.1 – Purple loosestrife: an invasive species

Many ecosystems exist in a delicately balanced state that can be adversely affected by the introduction of novel species. Occasionally, an alien species that has been transferred to a new ecosystem spreads swiftly, changing the landscape and threatening native plants and animals[8,9]. Species that are characteristic in their abilities to disperse and do damage to normally stable ecological systems are deemed "invasive species"[10]. An example of an invasive species is purple loosestrife (*Lythrum salicaria*). It originally grew across Europe and Asia, and was likely inadvertently transferred to North America[11]. Once established, it quickly spread through wetlands and waterways[12]. Its growth has now been reported in nine Canadian provinces and across America. Figure 1 shows purple loosestrife invasion sites in Ontario.

[8] Invasive Species Advisory Committee (2006)
[9] Thompson et al. (1987)
[10] Invasive Species Advisory Committee (2006)
[11] Thompson et al. (1987)
[12] Lakehead Region Conservation Authority (2008)

How would varying acidic watering solutions, simulating acid rainfall, affect the growth of purple loosestrife (Lythrum salicaria), in an attempt to assess the effectiveness of the acidic nature of southern Ontario precipitation as a control for the invasive growth characteristics of purple loosestrife?



13 Ontario Federation of Anglers & Hunters. (2009)

2.2 – Physiological & Ecological Characteristics of Purple Loosestrife

Purple loosestrife, from the Lythraceae family, is a perennial plant that grows up to 3m tall[14]. It exhibits a square stem and purple flowers along a tall flower stalk[15]. Mature plants can have 30-50 stems growing from a single rootstock and as up to 30 flower stalks that can produce approximately 2-3 million seeds annually, allowing it to spread very quickly[16,17]. Purple loosestrife also readily reproduces vegetatively through underground stems and many new stems can emerge from a single rootstock.

[14] Bender & Rendall (1987).
[15] Ontario Federation of Anglers and Hunters (2009)
[16] Ontario Federation of Anglers and Hunters (2009)
[17] Bender & Rendall (1987).

How would varying acidic watering solutions, simulating acid rainfall, affect the growth of purple loosestrife (Lythrum salicaria), in an attempt to assess the effectiveness of the acidic nature of southern Ontario precipitation as a control for the invasive growth characteristics of purple loosestrife?

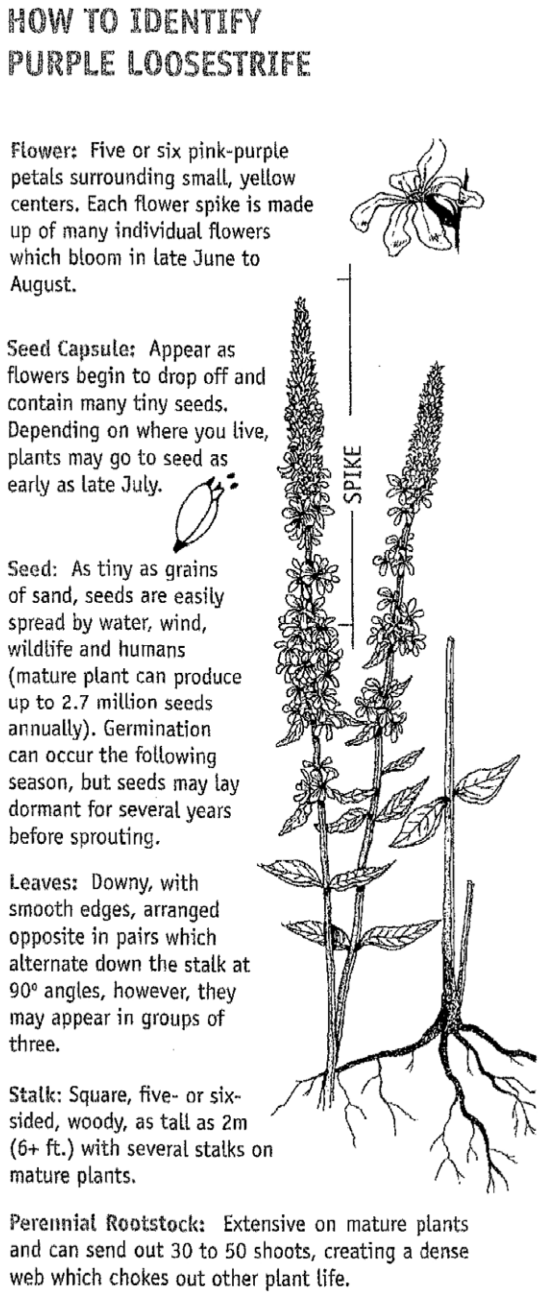


Figure 2 – Identifying Purple Loosestrife[18]

[18] Ontario Federation of Anglers and Hunters (2009)

2.3 – Potential Dangers of Purple Loosestrife Invasion

Purple loosestrife adapts readily to wetlands and even ecosystems with dry soil. After invasion, it forms a crowded monoculture of stems with thick rootstocks, outcompeting native plant species and established food sources for local fauna. Studies have shown that the presence of purple loosestrife in North American wetlands results in the decline of native populations such *as Lythrum alatum,* which negatively affects the habitat size of various bird species[19].

Since purple loosestrife can invade drier sites, concern is increasing as it becomes more common on agricultural land, encroaching on crops and pastures. In North America, approximately 190,000 hectares of land are affected by purple loosestrife, with can have millions of dollars worth of detrimental effects on agricultural and horticultural industries[20].

2.4 – Existing Biological Controls for Purple Loosestrife

A major dilemma in the control of purple loosestrife is that it has few natural predators[21], and also acclimatizes to both dry and wet environments[22]. The few insects used in its control belong to the Coleoptera order. (Table 1). Adult beetles and weevils feed on purple loosestrife plants, but the most damage is done by the larval stage insects[23]. The Coleoptera larvae preferably feed on purple loosestrife leaves and act as a control on the viability and growth of the plants[24].

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19 Blossey et al. (2001)
20 Ontario Federation of Anglers and Hunters (2009)
21 Swearingen (2009)
22 LaFleur (1996)
23 Heidorn & Anderson (1991)
24 Blossey et al. (1994)

However, the beetles and weevils can pose a threat themselves. At high enough populations they feed on native plants, damaging the local ecosystem and potentially exhibiting invasive characteristics[25,26]. Purple loosestrife can also be controlled manually by uprooting plants or cutting flower stalks before they seed, but this becomes infeasible for larger infestations. Glycophosate herbicides will destroy purple loosestrife, but they are potent non-specific herbicides that cause damage to all plants[27].

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[25] Harris (1990)
[26] Van Driesche & Bellows (1996)
[27] Thompson et al. (1987)

Table 1 - Insects used as biological controls for Purple Loosestrife[28].

Insect Type	*Scientific Name* **Common Name**	Appearance
Leaf beetle	*Galerucella calmariensis* **Black-margined loosestrife beetle** *Galerucella pusilla* Duftschmidt **Golden loosestrife beetle**	 Doug Landis, Michigan State University, Bugwood.org
Weevil	*Hylobius transversovittatus* Goeze **Loosestrife root weevil**	 Eric Coombs, Oregon Department of Agriculture, Bugwood.org
Weevil	*Nanophyes marmoratus* Goeze **Loosestrife flower weevil**	

2.5 – General Effects of pH Changes on Plant Growth

Plant growth is greatly influenced by alterations of pH in the root environment[29]. Yellowed leaf tissue due to lack of chlorophyll, reduced root growth, stunted shoot growth, poor flower development, and other symptoms associated with mineral ion function result from inappropriate pH conditions in the

[28] USDA Forest Service (2005)
[29] Arnon & Johnson (1942)

rhizophere[30,31,32,33]. These symptoms may stem from the influence of pH on ion solubility. Certain ions may become overabundant and toxic to plants at high concentrations of hydrogen ions or acidic conditions[34]. Acidic environments also have a direct effect on the permeability of root cell membranes or the functioning of root ion transporters, resulting in leakage of various ions from the roots or impairing root ion uptake[35,36,37,38].

2.6 – Acid Rain in Southern Ontario

Acid rain forms when nitrogen and sulfur oxides combine with atmospheric moisture to make nitric and sulfuric acids. Sulfur dioxide is a colourless gas released as a by-product of combusted fossil fuels containing sulfur[39]. Nitrogenous oxides are gaseous by-products caused by extreme high temperatures in chemical industries[40].

Both natural vegetation and crops are affected by acid rain. Plant roots are damaged, resulting in reduced plant growth or death. Soil nutrients are destroyed by acidity and microorganisms which release nutrients from decaying organic matter are killed, reducing available nutrients for plants[41]. Acid rain can also

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[30] Kochian et al. (2004)
[31] Findenegg et al. (2006)
[32] Hoagland (1917)
[33] Lucas & Davis (1961)
[34] Foy et al. (1978)
[35] Kochian et al. (2004)
[36] Yan et al. (1992)
[37] Raven & Smith (1975)
[38] Miyasaka et al. (1989)
[39] Campbell & Reece (2005)
[40] Pidwirny (2006)
[41] Islam et al. (1980)

damage the waxy layer on leaves, making the plant vulnerable to diseases. The cumulative effect means that even if the plant survives it can be weakened and unable to survive other climatic or ecological stresses[42].

Southern Ontario is a heavily industrialized and urban society, where the density of automobiles and factories leads to acidic rainfall[43]. There have been few published academic studies on the effect of pH on the growth of purple loosestrife. This study will examine the effects that acidic watering solutions have on the growth of mature purple loosestrife plants, to investigate whether acidic rain could potentially be a natural control to its rampant growth and spread. Since acidic rainfall is already present in the ecosystem[44], it could provide an alternative control for purple loosestrife growth without the potential problems of introducing insect controls or other chemical methods.

[42] Hutchison et al. (1986)
[43] Environment Canada (2005)
[44] Ontario Ministry of Agriculture, Food and Rural Affairs (1997)

3. AIM, HYPOTHESIS & VARIABLES

3.1 – Aim

To investigate the effects of acidic pH watering solutions (simulating acidic rainfall at pH 2, 3, 4, 5, and 7) on the growth of purple loosestrife (*Lythrum salicaria*), in an attempt to assess the effectiveness of the acidic nature of southern Ontario precipitation as a control for the invasive growth characteristics of purple loosestrife.

3.2 – Hypothesis

A review of literature indicated that changes in pH conditions away from a neutral pH damage plant structures and decrease growth. For this reason, I hypothesize that any acidic watering condition below a neutral pH will negatively affect the growth of *Lythrum salicaria*. Moreover, it is expected that the more acidic the solution, the more drastic the change in plant growth. I also hypothesize that the most acidic solutions (pH 2, 3, and possibly 4) will cause changes that will indicate that acid rain can be an effective control for the aggressive growth of purple loosestrife populations.

3.3 – Variables

Table 2: Independent, Dependent, Controlled Variables in Research Study

Independent:	The pH of the acidic solutions with which the purple loosestrife was watered.
Dependent:	Several indicators of the overall growth of purple loosestrife: stalk height, flower stalk height, the number of healthy buds and flowers, length of a representative leaf, the number of new secondary shoots formed and of new flower stalks formed, and the color of the leaves, stalk, flower stalk, and any other notable changes in appearance.
Controlled:	All plants were watered with the same amount of solution, 350 ml, the same time of the day at the same intervals. (biweekly on Saturday afternoon and Wednesday afternoon), with the same watering procedure (simulating rainfall) and the same size watering cans with identical streams. No external water came in contact with the plants because a tarp was set up over them. The same number of holes of approximately equal size was cut from each bucket. Plant buckets were placed on concrete so they would not come in contact with external soil or bugs. The same soil was used in every bucket and each bucket was filled to the same level with soil. The originating root clusters of the plants were kept intact, along with the plant as a whole. Plants had equal sun exposure. Plants were uprooted from the same area (Mississauga, ON, Highway 401 & Mavis Rd.) 5 trials were conducted to minimize random error. Plants and representative leaves (chosen on the same location of each plant) and flower stalks were tagged and labeled to avoid confusion. Plants were allowed to acclimatize to their new environments, while being watered with 350 ml of neutral water, to avoid any shocks to the plants not involving water acidity. Acidic solution controls: same stock solutions used, ratio of 2:1 sulfuric to nitric acid maintained, the beaker used to measure the amount of solution needed was rinsed and dried after every pH treatment.

4. METHOD DEVELOPMENT AND PROCEDURE

4.1 – Material List

Table 3 – List of materials assorted into 2 categories: apparatus and chemical reagents.

Apparatus	- Shovel (1) - 20L Buckets (25) - Drill and drill bit (1) - Commercial black earth (350L) - Watering cans of equal size (5) - 1L beaker (1) - 20L bucket with water for washing beaker (1) - Labels (75) - Sharpie marker (1) - Used for making acidic solutions: Funnel (1) 1L beaker (1) 100ml glass graduated cylinder (2) Rubber gloves & goggles (1) - hose for washing of any acid spills (1)
Chemical Reagents	- Stock solutions: 1L of concentrated sulfuric acid (10%) 1L of concentrated nitric acid (10%) - Working solutions (obtained from stock solutions): 16 L of solutions with pH 2, 3, 4, 6 and 7.

4.2.1 – Pre-experimental procedure

Purple loosestrife was found growing in dense clusters in Mississauga, Ontario (map shown in Appendix A). 25 plants were uprooted from this site and placed in 20L buckets. The existing soil and root ball was surrounded by commercial black earth, and the buckets had holes cut for drainage. Each bucket usually contained more than one stalk of purple loosestrife, constituting one entire plant. (explained in section 5.1). The buckets were placed on a concrete slab under a plastic tarp.

The plants were allowed to acclimate for 3 weeks while being watered with 350 ml of neutral water twice per week (approximately equivalent to local rainfall[45]). A watering can was used to water each plant from above, to simulate rainfall. Thirty litres of each pH solution was made using a Vernier pH probe and Vernier Labquest. The solutions were made with a 2:1 ratio of sulfuric acid to nitric acid, to simulate the components of actual acid rain, in accordance with previous studies done on simulated acid rainfall in southern Ontario[46,47]. The solutions were created in a well ventilated area while wearing rubber gloves and goggles.

4.2.2 – Experimental Procedure

After the acclimation period, each plant was watered (by watering can) with 350 ml of its respective solution twice per week. Measurements were taken after the acclimation period but prior to the beginning of acidic watering exposure; these measurements were listed as "initial". The following measurements were recorded: stalk height; flower stalk height, number of flower buds; number of blooming flowers (number of flowers); and length of a representative leaf (leaf length). A representative flower stalk and leaf on each plant were measured and tagged for identification after the exposure. After the four-week watering treatment, the same measurements were taken again, listed as "final". Finally, the number of new secondary shoots and new flower stalks were recorded, judged by a healthier appearance relative to old stalks and shoots. Each measurement was taken for each trial bucket, as shown in Appendix B.

[45] Turner (2009)
[46] Watmough et al. (1999)
[47] Hutchinson et al. (1986)

5. DATA COLLECTION & PROCESSING

5.1 – Methods of Data Collection

Purple loosestrife tends to grow in clusters of stalks, attempting to separate stalks can cause plant death due to disruption of the common root structure. For this reason, each "trial" represents a bucket that often contains multiple stalks growing from a single soil ball and root cluster. When taking measurements for each trial, all stalks in the bucket were measured individually, then averaged (see section 5.4 for sample calculations) to give the resulting value [e.g. Trial 1 (T1) of flower stalk heights]. Individual measurements are in Appendix B.

5.2 – Qualitative Data

Table 4: Qualitative Data for Appearance of Plants from each pH solution

pH	Appearance of leaf color		Appearance of stalk color		Appearance of flowers and buds		Other Changes	
	INITIAL	FINAL	INITAL	FINAL	INTIAL	FINAL	INTIAL	FINAL
7	Green	Dark green	Green-yellow	Green	Dark vivid	Purple	None	None
5	Green-yellow	Green	Yellow-green	Yellow-green (no change)	Light purple	Pink	None	None
4	Green	Green-yellow	Green	Green (no change)	Purple-pink	Light pink	Leaves healthy	Leaves beginning to wilt
3	Green	Yellow-green	Green-yellow	Yellow-green	Pink	Light brown	Flower stalk healthy	Flower stalk is dry/brittle
2	Green	Yellow-green	Green	Yellow-green	Purple	Dark brown	Leaves, flower stalk healthy	Flower stalk & leaves are weak/dry/brittle, falling off

Table 4 shows the resulting qualitative data as the appearance of various parts of each plant was assessed before and after the watering exposure.

5.3 – Quantitative Data

For each of the watering solution pHs (2, 3, 4, 5, and 7) there were five trials. In the following tables, the trials have been averaged to yield a mean percent difference for each pH. Tables 5-9 list the mean percent difference of the measurements, arranged according to pH. Percent difference was calculated because it exhibits a change in growth, allowing for better comparison of different trials and pHs, which have varying initial measurements.

Table 5: Mean Percent Difference of Stalk Height for each pH Solution

pH	Mean Percent Difference	Standard Error of Mean
7	5.7	1.36
5	10.7	3.3
4	2.0	0.76
3	4.7	2.9
2	-5.7	2.0

The stalk height of each plant was measured with a measuring tape, which had an error of ± 0.05 cm. The working solutions were created using a Vernier Labquest handheld computer and a Vernier pH sensor, which had an error ± 0.1[48].

Table 6: Mean Percent Difference of Flower Stalk Height for each pH Solution

pH	Mean Percent Difference	Standard Error of Mean
7	11.3	7.6
5	89.1	58.0
4	1.6	1.6
3	-12.9	16.6
2	-36.3	12.0

Flower stalk height was measured with a ruler, which had an error of ± 0.05 cm. The working solutions were created using a Vernier Labquest handheld computer and a Vernier pH sensor, which had an error ± 0.1[49].

[48] Vernier Software & Technology (2009)
[49] Vernier Software & Technology (2009)

Table 7: Mean Percent Difference of Number of Flower Buds for each pH Solution

pH	Mean Percent Difference	Standard Error of Mean
7	164.7	109.4
5	49.7	22.3
4	40.8	28.6
3	23.4	20.7
2	-36.3	12.0

The working solutions were created using a Vernier Labquest handheld computer and a Vernier pH sensor, which had an error ± 0.1[50].

Table 8 : Mean Percent Difference of Number of Flowers for each pH Solution

pH	Mean Percent Difference	Standard Error of Mean
7	20.9	6.5
5	2.2	2.0
4	-40.0	21.9
3	-31.4	18.3
2	-40.0	21.9

The working solutions were created using a Vernier Labquest handheld computer and a Vernier pH sensor, which had an error ± 0.1[51].

Table 9: Mean Percent Difference of Leaf Length for each pH Solution

pH	Mean Percent Difference	Standard Error of Mean
7	6.0	1.7
5	6.3	0.6
4	8.2	4.7
3	0.7	1.1
2	-7.5	5.8

Leaf length was measured with a ruler, which had an error ± 0.5 mm. The working solutions were created using a Vernier Labquest handheld computer and a Vernier pH sensor, which had an error ± 0.1[52].

Tables 10-11 contain the mean values for the measurements only taken after the watering exposure: number of new secondary shoots and new flower stalks.

[50] Vernier Software & Technology (2009)
[51] Vernier Software & Technology (2009)
[52] Vernier Software & Technology (2009)

Table 10: Mean Number of New Secondary Shoots for each pH Solution

pH	Mean New Secondary Shoots	Standard Error of Mean
7	2.7	0.6
5	1.4	0.3
4	1.4	0.8
3	1.3	0.2
2	0.7	0.3

The working solutions were created using a Vernier Labquest handheld computer and a Vernier pH sensor, which had an error ± 0.1[53].

Table 11: Mean Number of New Flower Stalks for each pH Solution

pH	Mean New Flower Stalks	Standard Error of Mean
7	1.0	0.4
5	1.2	0.6
4	0	0
3	0	0
2	0	0

The working solutions were created using a Vernier Labquest handheld computer and a Vernier pH sensor, which had an error ± 0.1[54].

5.4 – Processing of Tabulated Data

Sample calculations and explanations for the tabulated values from Section 5.3 will be shown in this section

5.4.1 – Calculation of Mean

The average of a data set can be calculated by the formula:

$$(T1_A + T1_B + T1_C + T1_{...}) / n$$

Where $T1_A$ equals a number in the data set of trial 1 and n equals the number of values in that set.

[53] Vernier Software & Technology (2009)
[54] Vernier Software & Technology (2009)

A sample calculation of averages for trial 1 (pH 3, initial) of flower stalk height is shown below:

Height of flower stalk 1: 15 cm
Height of flower stalk 2: 14 cm
Height of flower stalk 3: 14 cm
Average flower stalk height for T1 = (15 +14 +14) / 3
Average flower stalk height for T1 = 14.33 cm

5.4.2 – Calculation of Standard Error of the Mean[55]

For each growth parameter the mean percent difference was calculated. The standard error of each mean was then found, which is a measurement of the precision of the mean, or variability of the sampled measurements.
Standard error of the mean can be calculated via the formula:

$$SE = \sqrt{\left(\left(\Sigma(x_i - x_m)\right) / n\right)} / \sqrt{n}$$

Where x_i denotes each individual sample measurement, x_m denotes the mean of the measurements, and n denotes the number of samples.

A sample calculation of standard error for the mean percent difference of stalk height at pH 7 is shown below:

$$SE = \sqrt{\left(((2.4 - 5.7) + (9.0 - 5.7) + (9.0 - 5.7) + (5.5 - 9.7) + (2.1 - 5.7)) / 5\right)} / \sqrt{5}$$

SE = 1.364 %

[55] Samuels & Witmer (1999)

5.4.3 – Calculation of Percent Difference

Percent difference exhibits the difference between final and initial values as a percentage of the initial value.

$$\text{percent difference} = (x_2 - x_1)(100)/x_1.$$

A sample calculation for the stalk height for pH 7, trial 1 is shown below:

Mean Initial stalk height: 19 cm

Mean Final stalk height: 26.67 cm

% difference = $(x_2 - x_1)(100) / x_1$

% difference = (26.67 – 19) (100) / 19

% difference = 40.37 %

5.5 – Graphical Representation of Data

Figures 4-8 show the relationship between pH and the mean percent difference for each measurement. Figures 9-10 show the relation between the pH and the number of new shoots and flower stalks, as measured after the exposure. A regression line has been included for each graph (see section 5.6 for calculation). These graphs indicate how decreasing pH affects each part of the growing plant.

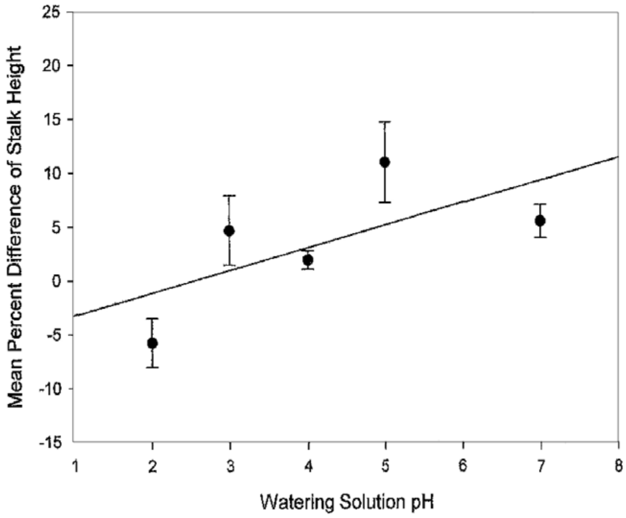

Figure 4 – Mean percent difference of stalk height vs. Watering Solution pH

Equation of Line of Regression: $y = 2.10x - 5.34$

Correlation Coefficient: 0.67 Statistically Significant Correlation: No

Coefficient of Determination: 0.45

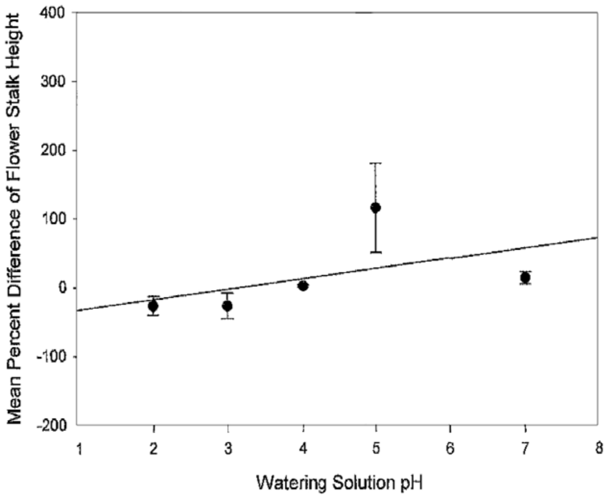

Figure 5 – Mean percent difference of flower stalk height vs. Watering Solution pH

Equation of Line of Regression: $y = 10.72x - 45.31$

Correlation Coefficient: 0.73 Statistically Significant Correlation: No

Correlation of Determination: 0.53

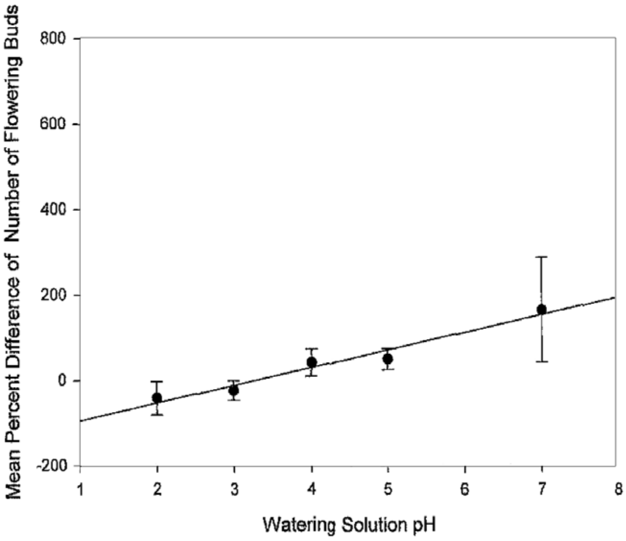

Figure 6 – Mean percent difference of number of flowering buds vs. Watering Solution pH

Equation of Line of Regression: y = 41.5x – 136.28

Correlation Coefficient: 0.98 Statistically Significant Correlation: Yes

Correlation of Determination: 0.96

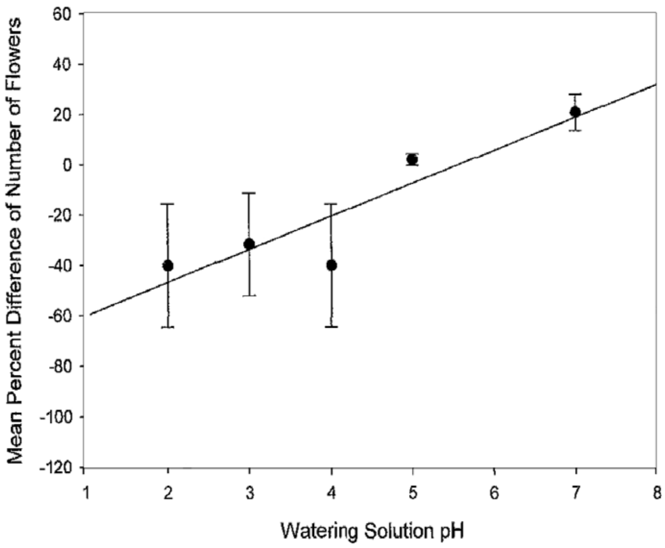

Figure 7 – Mean percent difference of number of flowers vs. Watering Solution pH

Equation of Line of Regression: y = 16.08x – 89.2

Correlation Coefficient: 0.95 Statistically Significant Correlation: Yes

Correlation of Determination: 0.89

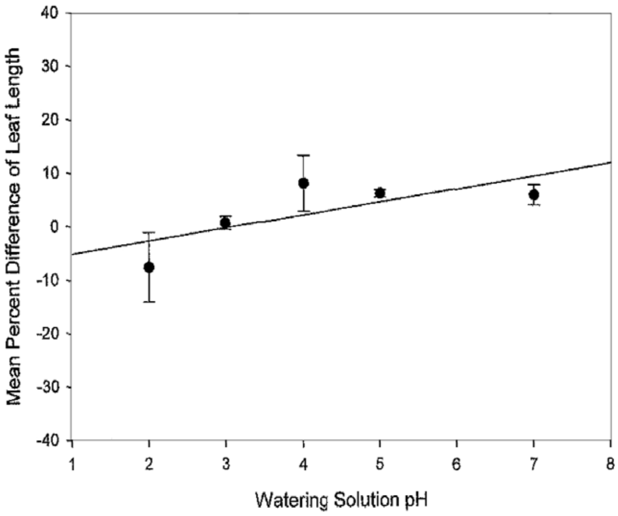

Figure 8 – Mean percent difference of leaf length vs. Watering Solution pH

Equation of Line of Regression: $y = 2.43x - 7.48$

Correlation Coefficient: 0.73 Statistically Significant Correlation: No

Correlation of Determination: 0.54

Figures 4-8 are scatter plots that all show a positive linear correlation between the mean percent difference of the measured growth indicators and pH. Two of these five graphs (number of flower buds, number of flowers) are statistically significant ($p<0.05$, see section 5.6 for example calculation) .

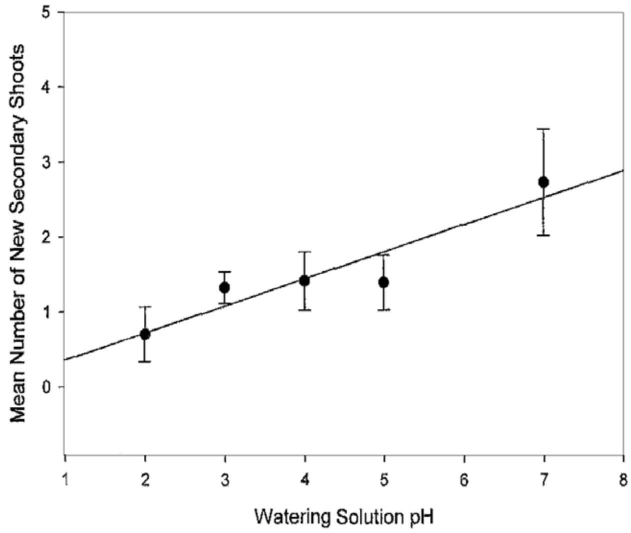

Figure 9 – Mean number of new secondary shoots vs. Watering Solution pH

Equation of Line of Regression: $y = 0.36x + 0.0039$

Correlation Coefficient: 0.94 Statistically Significant Correlation: Yes

Correlation of Determination: 0.88

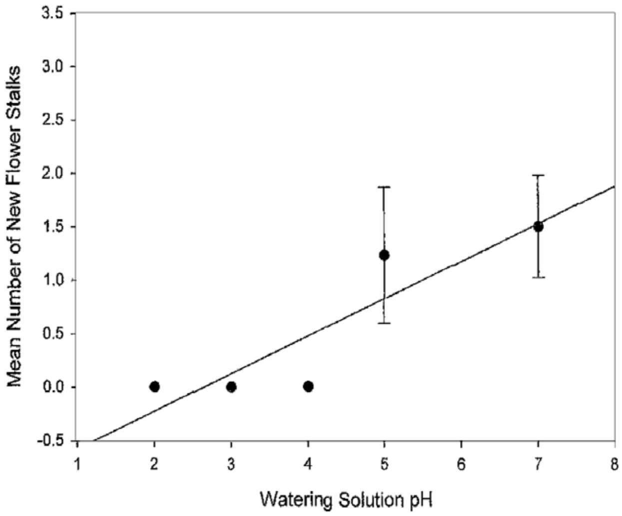

Figure 10 – Mean number of new flower stalks vs. Watering Solution pH

Equation of Line of Regression: $y = 0.35x - 0.93$

Correlation Coefficient: 0.89 Statistically Significant Correlation: Yes

Correlation of Determination: 0.80

Figures 9 and 10 are scatter plots that also show a positive linear correlation between pH and growth indicators. It should be noted that these graphs differ from figures 4 to 8 as they are not mean percent differences but rather absolute numbers. Both graphs are statistically significant ($p<0.05$).

Figures 11-15 are bar graphs of the mean percent differences for each of the measured metrics at a specific solution, to assess the total affect on plant growth at a given pH . The values for new secondary shoots and flower stalks are omitted from these figures, as these could not be taken initially.

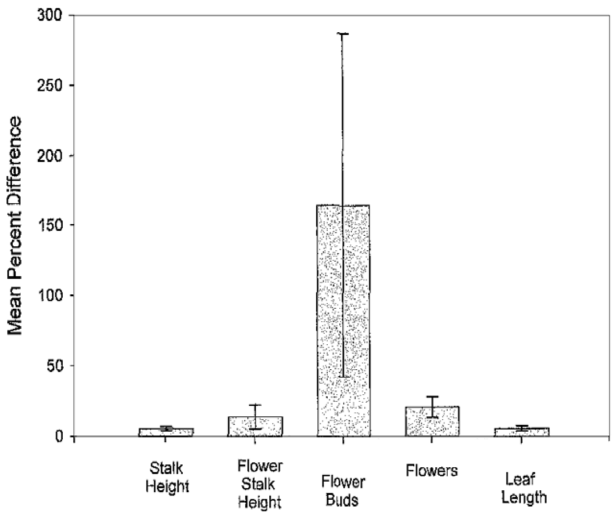

Figure 11 – Mean Percent Difference for Measured Values for Watering Solution of pH 7

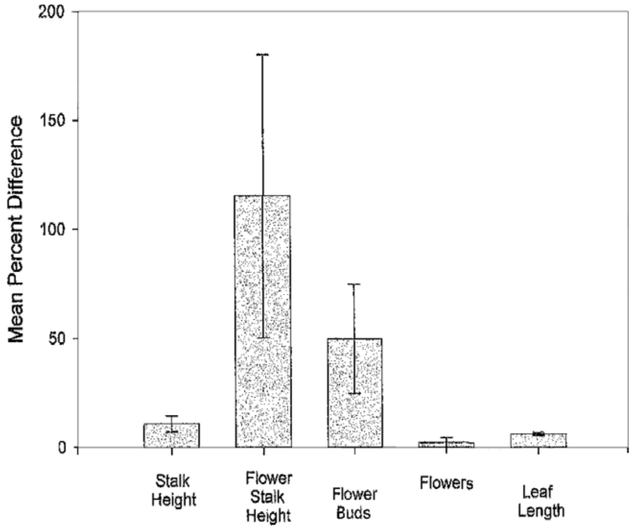

Figure 12 – Mean Percent Difference for Measured Values for Watering Solution of pH 5

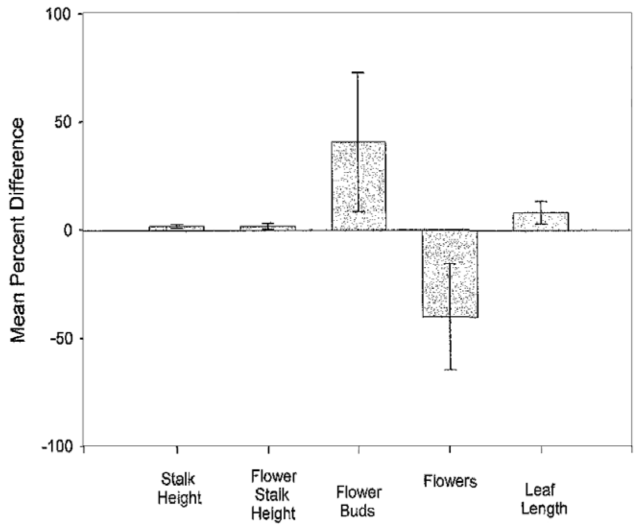

Figure 13 – Mean Percent Difference for Measured Values for Watering Solution of pH 4

How would varying acidic watering solutions, simulating acid rainfall, affect the growth of purple loosestrife (Lythrum salicaria), in an attempt to assess the effectiveness of the acidic nature of southern Ontario precipitation as a control for the invasive growth characteristics of purple loosestrife?

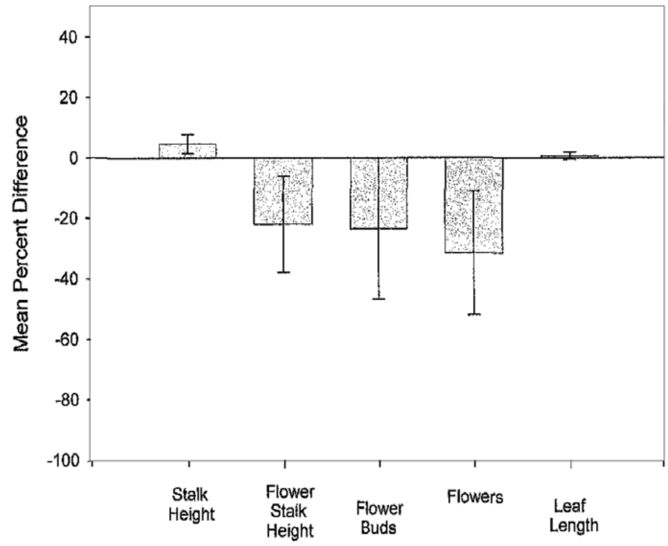

Figure 14 – Mean Percent Difference for Measured Values for Watering Solution of pH 3

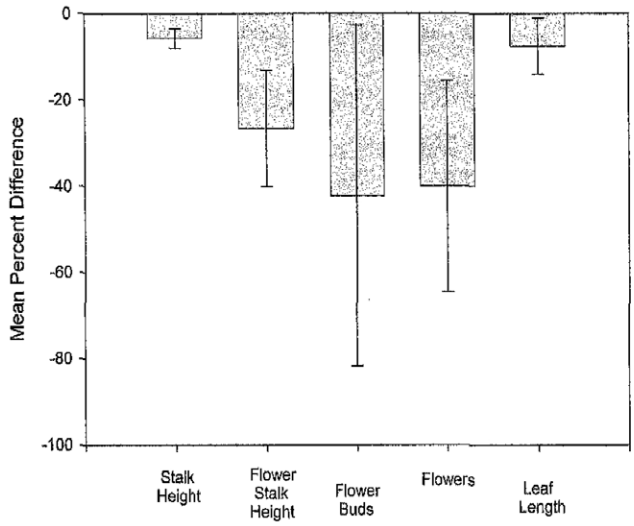

Figure 15 – Mean Percent Difference for Measured Values for Watering Solution of pH 2

n = 5, as there are five datapoints.

x_m, $y_{m,}$, represent the means of each set of values.

$x_m = \sum x / n = 21/5 = 4.2$ $y_m = \sum y / n = 17.4/5 = 3.48$

$S_{xy} = (\sum xy / n) - (x_m)(y_m) = (104.1/5) - (4.2)(3.48) = 6.204$

$S_{xx} = (\sum x^2/n) - (x_m)^2 = (103/5) - (4.2)^2 = 2.96$

For the regression line of the form y = ax + b:

$a = S_{xy}/ S_{xx} = 6.204/2.96 = 2.1$

$b = y_{m,} - (b)(x_m) = 3.48 - (2.1)(4.2) = -5.34$

Therefore, the regression line for the graph of Stalk Height vs. Watering solution pH (Figure 4) is $y = 2.1x - 5.34$

5.6.2 – Calculation of Correlation Coefficient[58]

The correlation coefficient (r), more accurately termed the Product-Moment correlation coefficient, indicates whether changes in water solution pH were correlated with changes in the growth metrics. A correlation of 1.0 indicates a perfect positive correlation, a value of 0 indicates no correlation whatsoever, and a value of -1.0 indicates a perfect negative correlation. An example calculation for the correlation of Stalk Height to Watering Solution pH (Figure 4).

The equation for r is:

$$r = S_{xy} /(S_x S_y)$$

From Section 5.6.1, $S_{xy} = 6.204$, and $S_{xx} = 2.96$

$S_x = \sqrt{S_{xx}} = \sqrt{2.96} = 1.72$

$S_{yy} = \sqrt{S_{yy}} = \sqrt{(\sum y^2 - ((\sum y)^2/n))} = \sqrt{(205.56 - (17.4)^2/5)} = 5.38$

$r = S_{xy} /(S_x S_y) = 6.204/((1.72)(5.38) = 0.67$

58 Samuels & Witmer (1999)

This represents a strongly positive correlation between stalk height and watering solution pH (indicated by an r value at 0.67 or above). In section 5.6.4, this value will be assessed for statistical significance.

5.6.3 – Calculation of Coefficient of Determination[59]

The coefficient of determination (r^2) measures how accurately the linear regression represents the data. It is simply the square of the correlation coefficient. A sample calculation for the regression analysis shown of Stalk Height vs. Watering Solution pH (Figure 4) as calculated in Section 5.6.1 is shown below

$$\text{Coefficient of determination} = r^2$$

From Section 5.6.2, r = 0.67.

$r^2 = (0.67)^2 = 0.45$

5.6.4 – Statistical Significance of Correlation Coefficient[60]

To measure whether the correlation indicated by the r value (section 5.6.2) is statistically significant, a one-tailed significance test was completed.
An example is shown below.

H_0: p = 0 (null hypothesis: there is no correlation between x and y variables)

H_1: p > 0 (alternative hypothesis: there is a positive correlation between x and y variables)

One tailed test at 5% ($p<0.05$), sample size = 5.

[59] Samuels & Witmer (1999)
[60] Samuels & Witmer (1999)

From the table of Product-Moment Correlation Coefficient critical values, the critical value at the 5% level is 8.054. If r is greater than the critical value, we can reject the null hypothesis in favor of the alternative hypothesis.

r = 0.67, which is less than 8.054.

Therefore H_0 is not rejected, and there is not a statistically significant correlation between stalk height and watering solution pH.

6. DISCUSSION

The measured results indicate that watering with acidic solutions can cause definite damage to the plant and limit its growth potential. Statistical analysis of the correlation between each growth measurement and the watering solution pH showed a statistically significant correlation for 4 of the 7 measurements. Number of flowering buds (Figure 6), number of flowers (Figure 7), number of new secondary shoots (Figure 9), and number of new flower stalks (Figure 10) all showed a statistically significant positive correlation with watering solution pH ($p<0.05$). It should also be noted that despite not being statistically significant, the remaining three measurements [stalk height (Figure 4), flower stalk height (Figure 5), and leaf length (Figure 8)] all showed positive correlation with watering solution pH. However, without statistical significance, it is difficult to draw reliable conclusions. Reasoning as to why there was a lack of significance is outlined below.

In addition to the information provided by Figures 4-10, the percent differences have been arranged per pH into bar graphs in Figures 11-15. Although statistical significance tests were not possible for these measurements, they still show a clear trend as the watering solution is acidified from neutral pH 7 to highly acidic pH 2. For pH 7 (Figure 11) and pH 5 (Figure 12), all measurements show a positive percent difference, indicating growth over the watering exposure. There is quite a bit of variability in some of the measurements, but overall, all values show positive growth, with some of it being quite a high percentage. This changes as the pH lowers, with the pH 4 solution (Figure 13) showing a negative percent difference for number of flowers. At pH 3 (Figure 14), number of flower buds and the flower stalk height first show negative growth over the exposure time, along with number of flowers. Finally, the most drastic effects are seen at pH 2 (Figure 15), where all the measured metrics show negative growth. From these changes it appears that essentially all aspects of the plant's growth can be affected by acidic watering at a low enough pH.

It should be noted that the fastest growing parts of the purple loosestrife plant were most affected by the acidic watering exposure. The number of budding flowers and the number of blooming flowers showed the steepest slopes for their linear regression lines (Figures 5 and 6), and both showed statistically significant correlation with changing pH. In addition, number of blooming flowers was affected at the least acidic pH, showing negative growth at pH 4. Since the watering exposure was done during the prime flowering season, these were actively growing and clearly susceptible to the acidic watering solution. In addition, the changes in measures of new growth, number of new secondary shoots (Figure 9) and number of new flower stalks (Figure 10), both showed statistically significant correlation as well. In contrast, the areas of the plant which would grow over the course of an entire season or many seasons, such as leaf length, flower stalks, and the total stalk, did not show statistically significant change. Since these parts of the plant grow over a longer term, the percent change exhibited would likely be lower, and we would expect them to grow more slowly overall, therefore being less susceptible to damage over the relatively short watering exposure. This results in the lack of statistical significance when correlating growth of these parts to changing pH. Qualitative data (Table 2) also support these findings, with flower color changing drastically from purple at pH 7 to dark brown at pH 2, while stalk and leaf color does not change as strongly, only becoming yellow-green at the most acidic levels.

In general, both the qualitative and quantitative changes seen over the course of the acidic watering exposure seem consistent with those discussed in the literature as responses to pH change. Yellowing of leaves indicates a loss of chlorophyll, likely caused by a lack of necessary ion uptake at the roots, due to changes in solubility at acidic pH. Proper ion balance is critical for plant growth as well, with the need for incorporation of ions into newly formed amino acids and DNA. Increasing acidity amplifies these effects, eventually reaching the drastic growth reduction seen in the purple loosestrife plants at pH 2. From all these measured changes, it is clear that acidic pH, similar in nature to what would be found in Southern Ontario (pH 4 or lower), strongly restricts the growth and potential for spread of purple loosestrife and can likely prevent it from reaching high densities in the local ecosystem.

7. EVALUATION, LIMITS, & FUTURE DIRECTION

Though an attempt was made to be as thorough as possible, this study was limited in both time and resources, and certain aspects would ideally have been different to achieve results that could be better analyzed and applied to real-world ecology. Chief among these was that due to a limited time in which the study was to be completed, the watering exposure was only 4 weeks in length. Judging from the results, an exposure that would last the entire growing season, or even multiple seasons, would be highly informative. It would be interesting to see the effects of slightly acidic solutions during long-term exposures, as well as seeing if the correlation between the growth of the stalk, flower stalk, and leaves and the pH solutions would become statistically significant on a longer time-scale. Another potentially beneficial study would be to look at the effects of the changing pH on the root structure of purple loosestrife plants. In this study, early attempts to disrupt the root cluster resulted in plant death, and time constraints and the number of available plants limited any further attempts. Ideally, more expertise could be gained with the plants to determine how to manipulate the root cluster without plant death. This would indicate the effects of pH on root growth, which past literature has shown to be highly susceptible to changing pH[61]. Another limitation of this study was the reliance on wild plants, which can vary in age and size. If plants could be grown from seed, these factors could be controlled. This also meant that the number of available plants was limited. Future studies could be aimed at exploring certain pH ranges in greater detail, especially in the pH 2-4 range where the results show that effects on growth become prominent. Increasing the number of replicates would also help reduce the variability of the samples, as outliers could be more easily identified. One of the limitations of the study results is the high variability of some of the measurements. This can be partially solved by an increase in the number of replicates, but some of this variance is also due to the variability of the initial measurements. This can cause the percent difference to be widely spread and

[61] Zieslin and Snir, 1989

therefore adversely affect the calculations. As stated, some of this is due to the natural variability of the wild plants, and cannot be easily avoided unless working directly with plants grown from seed. Finally, this study does not take into account the interactions that occur between purple loosestrife and other plants and animals in the ecosystem, an advanced study might attempt to simulate part of or the entire natural ecosystem and look at the effects of changing pH on the invasive nature of purple loosestrife.

8.0 CONCLUSION

This study offers conclusive, statistically significant data that the growth of purple loosestrife (*Lythrum salicaria)* is negatively affected by watering with acidic solutions that are highly similar to natural acid rain. There are statistically significant positive correlations between the growth of flowers (buds and open flowers) and new active secondary growth (shoots and flower stalks) as pH decreases from 7 to 2.

As the lower end of this range overlaps with the acidic rainfall that exists in Southern Ontario, it seems likely that existing populations of purple loosestrife in the region are being restricted in their spread by natural precipitation. This is a very interesting discovery, as acid rainfall is an existing part of the ecosystem, and while it has its own negative effects, it doesn't pose the potential dangers of the insect or chemical controls. Essentially, since acid rainfall is an inevitable outcome of the urban landscape of Southern Ontario, this is a somewhat positive effect as it gives natural protection to local ecosystems against invasion by purple loosestrife.

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