DIFFERENCES IN DENSITY MEASURES BETWEEN TREEAZIN® INJECTED AND NON-INJECTED ASH WOOD

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ABSTRACT

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Key Words: Systemic Insecticide, Density, Mechanical wood properties, Green Ash, Injection, Xylem, Phloem, Cambium.

This undergraduate thesis explores an observation made by the City of Thunder Bay's urban forestry department staff of TreeAzin injected Green Ash tree's limbs and branches failing shortly after being injected. The objective of the study was to determine whether or not TreeAzin injections were directly causing new woody growth to be less structural, resulting in failing limbs and branches. For this study, four Green Ash street trees were removed from the Northwood neighborhood of Thunder Bay, two injected and two not injected, to be tested for their mechanical properties. Due to COVID-19, and the inability to access testing facilities, only density measures for each tree could be obtained and tested. After statistical analysis of samples conditioned to 12% moisture content, it was observed that injected samples all had higher density values than not injected samples suggesting the failures were due to alternative factors. Alternative factors including environmental factors and quality of injection were discussed and concluded to be the likely cause of branch failures in Green Ash trees in the City of Thunder Bay.

CONTENTS

FIGURES

TABLES

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I would like to thank my thesis advisor Dr. Mathew Leitch for his assistance and continuous guidance throughout the completion of this undergraduate thesis as well as Robert Glover for conducting my analysis in the Lakehead University Wood Science Testing Facility (LUWSTF). I would also like to thank Robert Scott as well as Mike Dixon from the City of Thunder Bay for helping to coordinate the collection of and donation of testing material used for this undergraduate thesis as well as their knowledge of TreeAzin® injection history in Thunder Bay, Ontario. Finally, I would like to thank Dr. L. Hutchison for his detailed knowledge of urban forestry which was communicated to me through his fourth year Urban Forestry class at Lakehead University.

INTRODUCTION

Fraxinus pensylvanica, Green Ash is a commonly found urban street tree throughout much of its range and is present in abundance throughout the City of Thunder Bay's urban forest. The genus *Fraxinus* is the most commonly found genus in Thunder Bay's street tree inventory conducted by Davey Resource Group in 2011. Davey Resource Group found that there were 5243 trees from the genus *Fraxinus* throughout the city, many of which had been planted as monocultures along rights-of-way (Davey Resource Group 2011).

In 2002, *Agrilus plpanipennis,* Emerald Ash Borer, an invasive beetle from the family *Buprestidae* was detected for the first time in North America near the Detroit, Michigan and Windsor, Ontario border (Matsoukis 2020). The beetle is native to Asian countries and the Russian far east (NRCAN 2020). Since its discovery in North America, Emerald Ash Borer has spread to 35 states in the United States and 5 provinces in Canada, all on the eastern half of the two countries (USDA Forest Service n.d.). Along with its spread throughout nearly half of North America, Emerald Ash Borer has killed hundreds of millions of Ash trees since its arrival on the continent (Susich n.d.).

Numerous control measures have been considered and some have been implemented. Insecticide sprays such as bithenthrin and cyfluthrin could effectively control the adult populations of Emerald Ash Borer in individual trees. However, spraying individual trees is unfeasible in urban landscapes (Herms et al. 2014). Natural enemies of Emerald Ash Borer were considered, such as woodpeckers, who eat the larvae and instars of the beetle, interrupting the insect's lifecycle within the tree. This synergistic approach works best when used in conjunction with a systemic insecticide as the two control agents attack different stages of an insect's lifecycle (Barclay and Li 1991). Another method which has been adopted by the City of Thunder Bay is the use of a systemic insecticide called TreeAzin®.

TreeAzin® is produced from Neem tree seed extracts and is injected directly into the sapwood at the base of infected or susceptible Ash trees in order to control Emerald Ash Borer infestations (Bioforest n.d.). The Insecticide is taken up into the crown of Ash trees by way of tension-cohesion forces and spreads throughout the sapwood of the tree to attack the larval stages of the insect before they emerge to infect other trees. Ash trees in the City of Thunder Bay started were injected with the insecticide TreeAzin® in 2015 and 2016 by the first contractor and then subsequently a second contractor from Winnipeg (Scott 2020). Since 2017, injections have been completed by the second contractor and members of the City of Thunder Bay's urban forestry department annually.

OBJECTIVE

Recently, the City of Thunder Bay's Supervisor of Forestry and Horticulture, Mike Dixon, began to notice branch failures that did not have any apparent reason on trees which had been injected with TreeAzin®. This study will attempt to uncover whether or not there is a connection between the use of TreeAzin® insecticide and the observed failures of Ash trees. An injected tree and a non-injected tree of similar diameter at breast height (DBH) will be tested for their strength properties in order to determine whether there is a correlation between the injected insecticide and failures of

Ash. Oven dry density of the ring-porous Ash wood will be measured and tested to determine how the insecticide may cause changes in strength properties.

HYPOTHESIS

Ho: There will be decreased strength properties in Ash trees as a result of TreeAzin® Injections.

Ha: There is no decrease in strength properties in Ash trees when they are injected by TreeAzin®.

LITERATURE REVIEW

FRAXINUS PENNSYLVANICA Marsh.

Green Ash, (*Fraxinus pensylvanica* Marsh.) has been overplanted throughout North American cities and municipalities as the dominant street tree following the destructive force of Dutch Elm Disease (*Ophiostoma ulmi*). *Fraxinus pennsylvanica* is native to North America and can be planted or grown in cold hardiness zones 2 through 9 (Morton Arboretum n.d.). Green Ash is a species of tree which is found naturally in swamps, riparian zones, and moist uplands (Binnendyk 2017).

Since the arrival of Emerald Ash Borer, (*Agrilus plannipenis* L.) in Michigan, U.S.A. in 2002 (Raupp et al. 2006), it has spread north to the City of Thunder Bay (City of Thunder Bay 2016) where it has continued to colonize and kill private and municipal Ash trees. Green Ash is a highly adaptable species with remarkably fast growth rates, so its prevalence and value as an urban tree is quite high (Lane et al. 2016). Since the arrival of Emerald Ash Borer in Thunder Bay, ON., the City of Thunder Bay has implemented a proactive management plan which consists of 50% removal and 50% injection with the systemic insecticide TreeAzin® (Binnendyk 2017). This plan is intended to assist in limiting the spread of Emerald Ash Borer by decreasing the number of susceptible trees in the city.

Green Ash is a species with ring-porous wood meaning that there is an abrupt transition between the earlywood to latewood within each annual growth ring (Forest Products Laboratory 2010). Vessels, which only occur in hardwood species, participate in water conduction throughout the tree and are stacked upon one another to create a ‘column’ with perforation plates separating each ‘segment,’ or vessel (Wiedenhoeft

2013; Forest Products Laboratory 2010). The distinction between earlywood and latewood within each annual growth ring is made based on the size and distribution of vessel elements (Figure 1). In the earlywood portion of the annual growth, vessels are distinctly larger in diameter and occur at a lesser frequency (Forest Products Laboratory 2010). As the annual growth ring progresses from earlywood into its latewood xylem production, vessels become much smaller in diameter as well as denser and more fibrous (Forest Products Laboratory 2010). Ring-porous species have the highest density as well as strength for a moderately fast-growing tree (Government of Nova Scotia 2020). *Fraxinus* species are described as being a strongly ring-porous species and are more affected by changes in growth rate than those which have been categorized as weakly or semi-ring porous (Jagels 2006). The wood of Green Ash is hard, elastic, strong, brittle, and straight-coarse grained (Schoonover 1955).

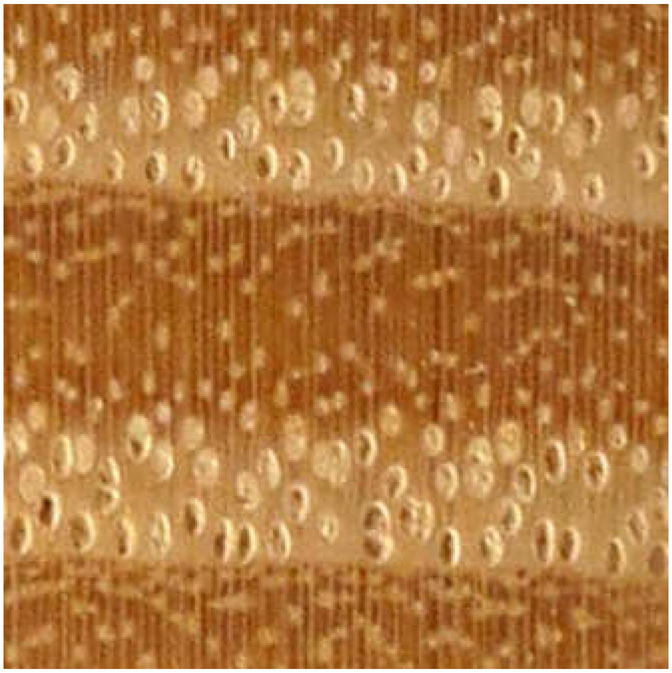

Figure 1. Green Ash’s earlywood and latewood distinction at 12 times magnification (Hobbithouse n.d.).

WOOD PROPERTIES

Physical properties of wood refer to the density, moisture content, and shrinkage. These properties influence the mechanical properties and strength of wood (Forest Products Laboratory 2010; Government of Nova Scotia 2020).

Green Ash has an average green moisture content of 58% which is a relatively low value when you consider that average green moisture content can range from 30% up to 200% in some species (Forest Products Laboratory 2010). The green moisture content is made up of both free water and bound water (Government of Nova Scotia 2020). Free water is the water which exists in cell lumens and other open spaces within the piece of wood whereas bound water is made up of water molecules which have penetrated cell walls and chemically bonded themselves to cellulose molecules within the cell wall (University of California n.d.; Government of Nova Scotia 2020; and Forest Products Laboratory 2010).

Density is a measure of the amount of solid wood that exists in any given sample of wood. A density value is determined for a piece or section of wood by measuring the amount of wood substance in a given volume of wood (Government of Nova Scotia 2020). Green Ash wood has a 12% oven dried density of 0.640739 g/cm^3 (Meier 2015).

Green Ash exhibits moderate shrinkage when compared to other hardwoods (Mullins and McKnight 1981). Shrinkage occurs when bound water is removed from the cell walls of woody cells. The shrinkage process is not influenced by the loss of free water from cells, which is the fastest way in which wood loses its moisture content (Government of Nova Scotia 2020). Shrinkage may occur on three different planes; radially, tangentially, and volumetrically and is measured as a percent change from green moisture content to oven-dry moisture content (Forest Products Laboratory 2010).

Shrinkage percentages for commonly planted urban trees including Green Ash are presented in Table 1 to show the variation amongst various trees.

Table 1. Shrinkage percentages expressed as percentage change from green to oven-dry moisture content for commonly planted urban trees.

Species	Tangential Shrinkage (%)	Radial Shrinkage (%)	Volumetric Shrinkage (%)
Fraxinus pennsylvanica	7.1	4.6	12.5
Quercus Rubra	8.6	4.0	13.7
Betula papyrifera	8.6	6.3	16.2
Acer saccharinum	3.0	7.2	12.0

Source (Meier 2015 Forest Products Laboratory 2010)

MECHANICAL PROPERTIES

Specific gravity, which is closely related to density, is the ratio of density of a substance to the density of water at a specified temperature, typically 4 degrees Celsius (Forest Products Laboratory 2010). Typically, as specific gravity increases, strength and stiffness increase as a result (Green 2001). Green Ash has a basic specific gravity value of 0.53 based on standard of oven dry weight and green volume (Meier 2015). At 12% moisture content, Green Ash has a specific gravity of .56 (Alden 1995).

The modulus of rupture (MOR) is a measure of a wood specimen's strength prior to rupturing. This measurement can be used to determine a wood species overall strength once dried to 12% moisture content and is expressed in pounds – force per square inch, or in megapascals (Meier 2015).

The modulus of elasticity (MOE) is a measure of a wood's stiffness, which is a good indicator of the wood's strength (Meier 2015; Forest Products Laboratory 2010). MOE is not a measure of ultimate strength and thus is not overly meaningful, however, can provide insight when it comes to comparison of different species of wood or different samples (Meier 2015).

The Janka Hardness Test measures the hardness of a species of wood by measuring the force required to embed a steel ball with a diameter of 11.28 mm halfway into the wood sample reported in Newton's (Forest Products Laboratory 2010; Meier 2015). This test is done both radially as well as tangentially and is generally expressed as the average of the two penetrations (Forest Products Laboratory 2010). A summary of Green Ash's mechanical properties as well as other commonly planted urban trees mechanical properties are presented in Table 2, showing the variability amongst some common urban tree's mechanical properties.

Table 2. Summary of various urban tree's, including Green Ash's mechanical properties values.

Species	Moisture Content	Specific Gravity	Modulus of Rupture (kPa)	Modulus of Elasticity (MPa)	Janka Side Hardness (N)
Fraxinus pennsylvanica	Green	0.53	66,000	9.653	3,869.76
	12%	0.56	97,000	11.446	5,337.60
Quercus rubra	Green	0.56	57,000	9,300	4,400
	12%	0.70	99,000	12,500	5,700
Betula papyrifera	Green	0.48	44,000	8,100	2,500
	12%	0.55	85,000	11,000	4,000
Acer saccharinum	Green	0.44	40,000	6,500	2,600
	12%	0.47	61,000	7,900	3,100

Source: (Meier 2015 Alden 1995 Forest Products Laboratory 2010)

SYSTEMIC INSECTICIDES

Systemic insecticides are applied to a portion of a plant or animal which then move throughout the plant's or animal's circulatory system to make the insecticide poisonous or toxic to the plant or animal (Government of Ontario 1987). Many water-soluble systemic insecticides have been developed to control infestations of Emerald Ash Borer. Various application methods such as; Trunk implants, Trunk injection, Soil drenching, and Basal spraying have been developed in conjunction with these systemic insecticides (Kuhns 2011).

Trunk injection can be done with two methods, drilling a hole and using a pressurized canister with a nozzle which is inserted into the drilled hole, or using a highly pressurized needle to inject the pesticide directly into the active sapwood of a tree (Kuhns 2011; Doccola et al. 2011). Determining the application method which is least harmful to the health of the tree being treated is an important step in order to minimize the potential for structural damage (Kuhns 2011; Government of Ontario 1987). Immediately, a system with a high-pressure injection can seem like the best option for reduced damage risks. However, these high-pressure injection methods may cause excess pesticide to accumulate between the bark and the cambium if the injection is conducted improperly (Kuhns 2011). Over application of a systemic insecticide may result in the death of treated plants by affecting the hydraulic forces within the tree or causing damage to essential growth enabling components of the tree (Government of Ontario 1987).

TREEAZIN®

TreeAzin® is a systemic insecticide that is injected into trees to control and manage certain insect pests of trees found in; forests, woodlots, urban landscapes, and residential landscapes (BioForest 2020). The class C pesticide is owned by the Canadian Forest Service (CFS) and was created with the assistance of BioForest (BioForest 2020). To produce the systemic insecticide, seed extracts from Neem tree's (*Azadiracta indica*) are used. The guarantee of the systemic insecticide is a 5% concentration of the active ingredient, Azadiracta. In Thunder Bay ON, TreeAzin® is being used by the City of

Thunder Bay’s urban forestry department and its contractors to inject Ash trees which are being attacked by Emerald Ash Borer. Approximately 1700 of the best trees in the city are selected to be injected, while others are to be removed and replaced (Rinne 2016).

Application of TreeAzin® systemic insecticide for hardwoods should be conducted from April (after bud burst) through to August in order for best results (BioForest 2020). Injection of TreeAzin® should only be conducted with BioForest’s “EcoJect” System, as the system is specifically designed for injection of TreeAzin® (BioForest 2020). Because TreeAzin® is a systemic insecticide, it must be injected directly into the active sapwood (Figure 2) (BioForest 2020). This is because the sapwood is where conduction of nutrients and water from the roots of the tree to the crown of the tree occurs. Injecting directly into the active sapwood permits the uptake and translocation of TreeAzin® Systemic insecticide throughout the crown of the tree (BioForest 2020; Herms et al. 2009). The uptake and translocation of TreeAzin® after being injected is quite rapid. If conducted correctly, and in the correct conditions, these processes are complete after approximately 48 hours (BioForest 2020; Herms et al. 2009).

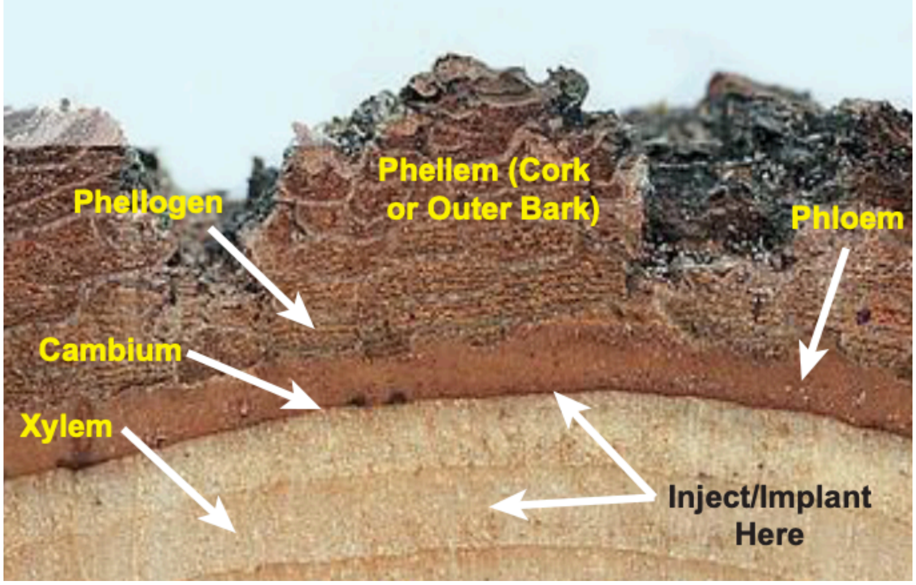

Figure 2. Anatomy of wood in cross-section showing active sapwood where injection should take place (Kuhns 2011).

The TreeAzin® pesticide label has an application rate of 2.0 ml /cm of diameter at breast height (DBH) for prophylactic treatments and 5 ml/cm DBH for Emerald Ash Borer attacked trees as well as trees over 30cm DBH (BioForest 2020). The injection process with the EcoJect system requires holes to be drilled through the bark and 1.5-2.0 cm into the active sapwood. A nozzle is then inserted into the hole, and a pressurized cannister inserted into the nozzle which displaces the insecticide into the sapwood of the tree. The holes drilled into the trunk of the tree should be 15-20 cm above the base of the tree, spaced at 15 cm spacing around the circumference of the tree, and drilled at 20-45 degrees downward (Scott while injecting Green Ash in summer 2019). Once the canisters are connected to their nozzles, it is necessary to observe and ensure that all of the insecticide is ejected from the canister prior to removing the nozzle and canister components from the base of the tree being injected (BioForest 2020 Scott while injecting Green Ash in summer 2019).

Uses For TreeAzin®

TreeAzin® can be used by licensed exterminators to control populations of a variety of insects that consume the tissues of trees in Canada (BioForest 2020). TreeAzin® has been approved for use on and successfully been used for control of insect species such as Emerald Ash Borer, Gypsy Moth, Tent Caterpillars, Spruce Budworm, Jack Pine Budworm, Leaf miners, and Sawflies which all pose risks to tree health (BioForest 2020).

IMPACT OF PESTICIDE INJECTION ON WOOD

Systemic insecticides injected directly into the trunk of a tree to control Emerald Ash Borer have the potential to cause long-term damage from the holes created during drilling if treatments are applied annually (Herms et al. 2009). These holes may allow for pathogens to enter, and eventual decay of wood if not covered by a grafting wax as suggested by the producers of TreeAzin® (BioForest 2020). Further, the use of application methods with high pressure injections systems similar to the EcoJect system may cause damage to the tree if the pressure exerted from the canister causes bark to bulge and separate from the cambium (Herms et al. 2009).

In a study conducted by Doccola et al. (2011), sixteen Green Ash trees in East Lansing, Michigan U.S.A. were selected to be injected with four different systemic insecticides to observe any potential cracking, oozing, or presence of decayed wood. The study was conducted on the basis that even small wounds created at injection sites had the potential to permit exposure of microorganisms including pathogens to the sapwood of injected trees. The four systemic insecticides used for this analysis were; ACECAP 97 (Acephate), TREE-äge (Emamectin benzoate), IMA-jet (imidacloprid), and Merit Tree

Injection (imidacloprid). Each of the trees were felled 1.1 to 3.8 years following injection treatment and sectioned to analyze each of the total 63 injection sites across the 16 selected trees. Contrary to Herms et al. (2009) suggestion that decay may be found in injection site wounds, Doccola et al. (2011) found that 76.1% of the wounds had completely healed over and displayed discolored, but rigid wood associated with the injection sites. The wounds which did not fully heal over were trees that had been heavily affected by Emerald Ash Borer prior to injection, leading to the lack of wound healing (Doccola et al. 2011).

MATERIALS AND METHODS

STUDY MATERIAL

Various Green Ash trees from throughout the Northwood neighborhood of the City of Thunder Bay were used to conduct chemical and mechanical analyses to study the differences in strength properties between TreeAzin® injected and non-injected trees. A total of two trees, one injected and one non-injected were studied.

STUDY AREA

The study was conducted in the City of Thunder Bay, Ontario which is located on the northern shore of Lake Superior (Figure 3). The trees collected for use in this study are all urban street trees which have been planted in front of residential buildings on quiet, low traffic streets. The injected and non-injected trees were removed from the Northwood neighbourhood of Thunder Bay.

Figure 3. The City of Thunder Bay's geographic location within Ontario, Canada. (World Map n.d.).

SAMPLE PREPARATION

The City of Thunder Bay's urban forestry and horticulture department conducted removals of each of the study trees as part of their regular maintenance operations. Tree truck crews removed trees and retained a section of the main stem of the tree for milling and further analysis. Injected trees that were removed were cut at the injection sites to expose the injection site and the associated wood development or damage caused by injection (Figure 4) at the Lakehead University Wood Science and Testing Facilities Portable Milling Location on the Universities campus.

Figure 4. Injection sites in cross-section of injected Green Ash from 627 Mohawk Crescent, Thunder Bay, Ontario (Dyer 2020).

Once trees had been felled and cut, the City of Thunder Bay's Urban forestry and horticulture department delivered the removed stem sections and branches to Dr. Leitch's portable milling site across Oliver road from Lakehead University, Thunder Bay for further processing. Dr. Leitch's portable milling students cut the logs to manageable sizes for property testing. Each of the samples was composed of clean wood from above the injection site for property testing.

STUDY DESIGN

The study is an impact evaluation type with trees selected being randomly decided upon by the City of Thunder Bay's tree removal operations. The injected Green Ash trees to be removed were accepted regardless of form and health prior to removal. Also, the non-injected Green Ash trees removed were selected in the same manner.

LAB ANALYSIS

Once samples were cut into manageable sizes, Dr. Leitch used a band saw located in the Lakehead University Woodworking Shop to cut wood cookies into small cubes which could be tested for their density. Sections of branch and stem were cut into the small cubic samples from both injected and non-injected trees. Each stem or branch cookie had its growth rings identified from 0-3 years old and 4+ years old to delineate the pre and post injection woody growth. Furthermore, individual density cubes were cut from each cookie from 0-3 years old and 4+ years old.

As samples were cut from each of the injected and non-injected cookies at varying ages, the samples were marked with their number and age range. These cubes were then placed in the Lakehead University Wood Science Lab's conditioning chamber (set at 65% relative humidity and 20°C) until conditioned to 12% moisture content. Once conditioned to 12% moisture content, Robert Glover measured each samples volume and weight to be used in density calculations (See Appendix A). The density calculations were completed through excel by dividing the samples weight by the volume of the sample. Each of the sample's densities were grouped together based on injected or non-injected, their origin from the tree, and age in excel to allow for statistical analysis. Due to restrictions from the COVID-19 pandemic, mechanical property testing was not able to be completed as students were not allowed access to university labs as the whole university was conducting courses online.

STATISTICAL ANALYSIS

With the density calculated for each of the samples organized according to whether they came from the injected tree or the non-injected tree, the branch or the base of the tree, and from 0-3 years or 4+ years, two-sample t-test assuming unequal variances were conducted to compare the densities of samples. Four t-tests were completed in excel using their data analysis pack in order to compare the following data sample groups. The t-tests conducted were two-tailed to determine whether the injection either increased density or decreased density.

1) Injected branch, 0-3 years old vs. Not-injected branch, 0-3 years old.
2) Injected base, 0-3 years old vs. Not-Injected base, 0-3 years old.
3) Injected branch, 4+ years old vs. Not-Injected branch, 4+ years old.
4) Injected base, 4+ years old vs. Not-injected base, 4+ years old.

It is hypothesized that there will be a decreased density measure in the samples treated with TreeAzin® injections when compared to the samples which were not treated with TreeAzin® injections, for each of the t-tests conducted. Below, the null hypothesis and alternative hypothesis are stated.

Ho: There will be decreased strength properties in Ash trees as a result of TreeAzin® Injections.

Ha: There is no decrease in strength properties in Ash trees when they are injected by TreeAzin®.

RESULTS

Four two-sample t-tests assuming unequal variances were conducted to compare the difference in densities for each of the sample groups. When comparing the mean densities for each of the sample groups, it is observed that the density of each injected sample group is higher than the counterpart for that specific t-test (Table 3). This shows that the average density of samples was higher across all the injected sample groups than that of the not-injected sample groups of the same age and section of tree they were cut from.

Table 3. Shows the mean density value for each of the sample groups tested.

Age	Test #	Sample Group	Mean Density (g/cm^3)
0-3 Yrs.	Test 1	1, Branch Injected	0.72008
0-3 Yrs.		2, Branch Not Injected	0.69430
0-3 Yrs.	Test 2	3, Base, Injected	0.62495
0-3 Yrs.		4, Base, Not Injected	0.57968
4+ Yrs.	Test 3	1, Branch Injected	0.69853
4+ Yrs.		2, Branch Not Injected	0.63943
4+ Yrs.	Test 4	3, Base, Injected	0.57978
4+ Yrs.		4, Base Not Injected	0.57914

Source: (Appendix A)

By conducting two-tailed t-tests, the results have the ability to show significant difference in both directions, either the injected was significantly different or the non-injected was significantly different. Having the ability to observe both tails in the distribution allows for a better understanding of any anomalies surrounding the impact which TreeAzin® injection may have.

The first t-test's results (Table 4) from the comparison between the injected branch from age 0-3 years old and the not injected branch from age 0-3 years old shows that t Stat is larger than negative t-Critical, two-tail and that t Stat is smaller than t

Critical two-tail. For test number one, the null hypothesis failed to reject, meaning there were no significant differences observed between the densities.

Table 4. Shows the results from the first t-test between the injected branch sample group and the non-injected branch sample group from age 0-3 years.

	1 Branch Injected	2 Branch Not Injected
Mean	0.720075404	0.694303677
Variance	0.001726602	0.000320793
Observations	13	4
Hypothesized Mean Difference	0	
df	13	
t Stat	1.76579212	
P(T<=t) one-tail	0.050444986	
t Critical one-tail	1.770933396	
P(T<=t) two-tail	0.100889972	
t Critical two-tail	2.160368656	

Source: (Appendix A)

The second t-test's results (Table 5) from the comparison between the injected base from age 0-3 years old and the not injected base from age 0-3 years old shows that t Stat is larger than negative t-Critical, two-tail and that t Stat is larger than t Critical two-tail. For test number two, the null hypothesis was rejected, meaning there was a significant difference observed between the densities. For this test, the mean density of the non-injected sample group was smaller than the base sample group which was injected.

Table 5. Shows the results from the second t-test between the injected base sample group and the non-injected base sample group from age 0-3 years.

	3, Base, Injected	4, Base, Not Injected
Mean	0.624952931	0.579679895
Variance	0.00154538	0.000255735
Observations	11	13
Hypothesized Mean Difference	0	
df	13	
t Stat	3.577349027	
P(T<=t) one-tail	0.001687496	
t Critical one-tail	1.770933396	
P(T<=t) two-tail	0.003374992	
t Critical two-tail	2.160368656	

Source: (Appendix A)

The third t-test's results (Table 6) from the comparison between the injected branch from the 4+ years sample group and the not injected branch from the 4+ age sample group shows that t Stat is larger than negative t-Critical, two-tail and that t Stat is smaller than t Critical two-tail. For test number three, the null hypothesis was rejected meaning there were significant differences observed between the densities. For this test, the mean density of the non-injected sample group was lower than the base sample group which was injected.

Table 6. Shows the results from the third t-test between the injected branch sample group and the non-injected branch sample group of ages 4+.

	1 Branch Injected	2 Branch Not Injected
Mean	0.698529771	0.639426773
Variance	0.000339761	0.000705016
Observations	9	5
Hypothesized Mean Difference	0	
df	6	
t Stat	4.420598683	
P(T<=t) one-tail	0.00223403	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.00446806	
t Critical two-tail	2.446911851	

Source: (Appendix A)

The fourth t-test's results (Table 7) from the comparison between the injected base from the 4+ years old sample group and the not injected base from 4+ years old sample group shows that t Stat is larger than negative t-Critical, two-tail and that t Stat is smaller than t Critical two-tail. For test number one, the null hypothesis failed to reject, meaning there were no significant differences observed between the densities.

Table 7. Shows the results from the fourth t-test between the injected base sample group and the non-injected base sample group of ages 4+.

	3 Base, Injected	4, Base Not Injected
Mean	0.579779393	0.579137427
Variance	0.000584402	0.000466777
Observations	17	16
Hypothesized Mean Difference	0	
df	31	
t Stat	0.080529369	
P(T<=t) one-tail	0.468166947	
t Critical one-tail	1.695518783	
P(T<=t) two-tail	0.936333893	
t Critical two-tail	2.039513446	

Source: (Appendix A)

DISCUSSION

Analysis of the data from each of the four sample groups of Green Ash wood shows the variability which can occur between urban trees. Urban trees are subject to numerous external factors which they have limited ability to control. Factors such as light availability, temperature variation, water availability, humidity level variation, and nutrient availability all have an impact on the mechanics of tree growth (VanDerZanden 2008). The four statistical tests which were conducted on the different sections of two trees show a significant difference between the injected 0–3-year-old stem and the non-injected 0–3-year-old stem groups as well as the injected 4+ year old branch and the non-injected 4+ year old branch groups. However, the significance of the two tailed t-test result shows that the density in the non-injected sample groups were significantly lower than the density of the injected sample groups. Although the results of these two tests suggest that there are significant differences, the results are indicative of increased strength values for the injected trees as the density measures are higher.

Finding that the densities and t-test results from each of the sample groups had varying results and measures suggests that injecting *Fraxinus* species with TreeAzin® systemic insecticide has little or no impact on the density of woody growth. The average density measure for Green Ash wood, as measured in various growing conditions, according to Meier (2015), is 0.640739 g/cm^3 at 12% moisture content, which is similar to each of the sample groups average densities in this study. The observed variation of measured densities could be attributed to their growing conditions as some trees grown in urban sites grow slower than trees grown in rural or woodlot sites (Quigley 2004). With the opposite effect, urban trees are often grown in full sunlight and begin to produce lateral branches to maximize leaf area and photosynthetic capacities earlier than their counterparts in forested landscapes (Cassens and Makra 2014). It is likely that the resulting variation in densities found in the study are attributed to variation in growing conditions or other growth affecting factors.

Urban soils have a very high variability which influences the health and growth of native trees (Pregitzer et al. 2016) which is likely a component of the variability found in the densities found in this study. Different genera of tree require different quantities and qualities of soil for urban tree planting (McGrath et al. 2019). Trees require an adequate amount of soil for access to air, water, and nutrients in order to achieve healthy growth. The quality of the soil also impacts the ability for urban trees to develop and grow properly and healthily. Heavily compacted soils or coarse soils may hinder the ability for healthy tree growth. Green Ash is a species which grows best on deep, moist, and medium to fine textured soils (Government of Canada 2020). It is possible that the variations seen within the density measures among the sample groups is attributed to variation in soil characteristics affecting tree growth. Green Ash grown on light-textured soils or dry sites often display reduced growth (Government of Canada 2020) which has the ability to increase the density of woody growth (Pretzsch et al. 2018). With the opposite effect, if Green Ash is grown in its optimal soils, growth should increase, and density should decrease (Pretzsch et al. 2018).

Water availability to trees is a factor which influences tree growth. With not enough water available to a tree, it may undergo stress and growth will be decreased depending on the species. On the contrary, when the soil around a tree is flooded with water, it may decrease tree growth depending on the species. The tolerance to flooding and drought stresses varies with each species and needs to be considered when deciding on a species to be planted in an urban setting (Hutchison 2020). Green Ash trees can tolerate flooding due to a variety of morphological adaptations such as increased lateral root penetration and decreased downward root penetration as well as succulent roots with more air spaces (Gucker 2005). However, if the stresses of flooding are combined with a limited quantity of quality soil for lateral root growth, Green Ash may not be able to adapt to the flood conditions adequately enough to tolerate flooding. Green Ash is also found in areas which experience periodic drought conditions, showing minimal effect on growth as long as drought conditions aren’t long-lasting (Gucker 2005). The inability for Green Ash to consistently adapt to changes in water availability when grown in an urban landscape could be explanatory for the variations in density measures found within this study.

Urban landscapes pose as a facilitator for nutrient availability stresses for urban trees (Hutchison 2020), Green Ash is no exception to that fact. When comparing a Green Ash planting location in an urban landscape to a forested landscape there are quite different nutrient availabilities exhibited influenced by a number of factors. Urban soils where there is poor hydration and or drainage associated with compaction, and smaller tree lawns due to increased paving limit the ability for required nutrients to be absorbed by trees (Marritz 2014). Macronutrients are required in order to facilitate healthy growth. A variation in the nutrient availability to the Green Ash used for this study may have caused a variation in density measurements if nutrient availability limited growth of the trees.

Thunder Bay as a city has had relatively consistent climate data over the past six years (2015-2020) as shown in Table 8, suggesting that the likelihood of varying growth conditions for the City of Thunder Bay has little to do with the impact on increased density measures for years 2018-2020 when compared to 2017 and older samples. The climate variable in Table 8 which could potentially have impacted the ability for Green Ash to produce more dense latewood fibres is the increased growing degree day variable (a measure of thermal heat accumulation) for the 2019 and 2020 years (ClimateAtlas n.d.). A summary of the climate data for 2015-2020 is shown in Table 8. The variation in densities could be attributed to the trees planting site and its microsites specific weather variables which may happen to be more optimal or less optimal for growth rates.

Table 8. Climate data for the City of Thunder Bay from year 2015 to 2020.

Climate Averages For Thunder Bay, ON						
Variable	2020	2019	2018	2017	2016	2015
Annual Precipitation (mm)	729.4	736.1	690.6	757.2	768.2	721.6
Length of Frost-Free Season (Days)	136.8	132.2	130.7	131.3	128.6	132.7
Day of Last Frost (Day of Year)	139.0	137.9	137.0	137.5	137.7	140.0
Day of First Frost (Day of Year)	275.8	270.1	267.7	268.8	266.3	272.7
Growing Degree Days (Base 10 degrees C)	856.2	883.0	807.4	835.4	826.3	807.5
Mean Temperature (Degrees C)	3.9	4.0	3.9	4.1	3.6	3.5

Source: (ClimateAtlas n.d.)

Trees grown in urban landscapes face external factors which impact their ability to grow and produce healthy woody growth. Soil quality, water availability and nutrient availability all have the ability to alter tree growth rates which have been shown by Pretzsch et al. (2018) to alter wood density.

Another potential explanation for the observed branch failures in the possibility of poor maintenance practices being conducted in the past to present. Many urban forestry departments within cities have cyclic pruning schedules (Hutchison 2020) which allow for routine assessment and pruning of trees when deemed necessary.

Systematic, and cyclic urban forestry tree pruning programs provide many benefits to cities such as lower long-term costs, enhanced public safety, reduced storm damage, as well as healthier and more attractive trees (Davey Resource Group 2011). Implementing cyclic pruning is suggested to work best at a four-to-five-year return cycle. Delaying pruning has been shown to lead to a decreased condition rating of trees, resulting in a lower appraised value of urban trees (Miller and Sylvester 1981). The City of Thunder Bay does not currently have a cyclic pruning schedule to help in providing these benefits, but rather schedules tree maintenance activities on an 'as-needed' basis (Davey Resource Group 2011). It is possible that the observed failures in branches are linked to the lack of a cyclic pruning schedule causing unhealthy and dangerous growth forms in urban trees. In order to minimize the potential for negative impacts such as branch failure in the future, a seven-year cyclic pruning schedule, based on neighbourhood or city blocks, was suggested to the City of Thunder Bay in 2011 by Davey Resource Group. Currently, with an aging tree population, the city's urban forestry program is focused on hazard tree removal, and necessary maintenance to maintain compliances with other city departments and utility companies.

TreeAzin® systemic insecticide injection methods require drilling into the tree, through the cambium and just into the xylem, or sapwood (Bioforest 2020). Improper tree injection practices can lead to tree wounding, which is known to have the potential for tree mortality, loss of tree vigour, as well as structural changes to forest structure (Loomis 1973; Walters et al. 1982; Reeves and Stringer 2011; Guillemette et al. 2008). It is possible that improper TreeAzin® injection practices where the cambium is damaged significantly caused improper compartmentalization formation and further damage to the tree.

Compartmentalization is the process of a wounded tree setting a boundary around any damaged tissue in order to resist the spread of injury and loss of normal tree functions such as water conduction (Smith 2006). If wounding due to improper injection was significant enough to impact the compartmentalization process, it is possible that essential tree growth functions may have been impacted, causing the beginnings of tree-mortality, loss of tree-vigour, or changes to the trees physical structure. Improper injection practice may be the cause for tree failure which has been observed in the City of Thunder Bay.

The process of cavitation or embolism occurs when gasses are present in the xylem vessels of a trees hydraulic system. Cavitation or embolisms can occur when xylem sap is under higher tension due to water stress, causing liquid water in the xylem to change into a gaseous state, which blocks xylem water flow to the tree's crown (Choat et al. 2018). A study by Dujesiefken et al. (1999) suggests that air embolisms may occur when boreholes are made into trees through the cambium. A borehole used to measure age and growth of trees or the quality of wood is quite similar to the size of the holes drilled into *Fraxinus* spp. to enable injection of TreeAzin®. With crown dieback and tree mortality as a result of air embolisms, it is possible that they caused mortality in sections of the crown which then failed and were observed by the Urban Forestry department in the City of Thunder Bay.

Although there have been observed failures in trees, it is likely that the failures are not attributed solely to TreeAzin® injection but rather a cumulation of stress factors which can threaten the health of urban trees. Urban trees which are subject to the most stress are roadside trees, these trees will be impacted the most in terms of the impacts of urbanization and existing environmental and abiotic stressors (Czaja et al. 2020). The health of urban trees is influenced by many factors which have the capability to negatively influence the health and longevity of their lives.

CONCLUSION

Urban *Fraxinus* spp. trees in the City of Thunder Bay have been threatened by Emerald Ash Borer since it's discovery in the region in 2015. The City of Thunder Bay began hiring contractors to inject *Fraxinus* spp. trees to prevent infestation of Emerald Ash Borer. After injections were completed, failures were observed in a number of injected *Fraxinus* trees throughout the city and the Supervisor of Forestry and Horticulture, Mike Dixon, questioned whether the failures were caused by the TreeAzin® injections.

This study analyzed the variations between injected and non-injected *Fraxinus pennsylvanica* trees wood densities prior to injection and after injection to determine if there was a significant variation which could cause a decrease in structural quality. It was found that TreeAzin® injections had no significant impact on the density of wood after injection. Alternative possibilities to explaining tree failures were explored in hopes of explaining the unknown cause for the observed failures. The negative impacts on trees such as environmental stresses, and anthropogenically caused stress have the ability to cause critical damages which may be the cause for observed tree failures.

Environmentally caused variabilities in growing conditions such as soil quality and quantity, water availability, and nutrient availability all have the potential to cause undesirable effects to the health of trees (Czaga et al. 2020). These negative effects on trees are more prevalent in urban landscapes than they are in natural forests (Hutchison 2020), and likely contribute to the observed tree failures in the City of Thunder Bay.

The benefits of a cyclical maintenance schedule have proven to provide a lower long-term cost to management, enhanced public safety, reduced storm damage, as well as healthier and more attractive trees (Davey Resource Group 2011). The possibility of a lack of cyclical maintenance practice throughout the City of Thunder Bay's urban forestry maintenance program is discussed as a possible cause for observed failures in injected *Fraxinus* spp. trees in the city. The management scheme focussing on the maintenance of high-risk hazard trees may be taking away from recognizing deteriorating tree health which may be causing the observed failures.

Injection quality was discussed and shown to be a potential for loss of essential tree growth functions (Smith 2006). *Fraxinus* spp. which have been injected compartmentalize the injection site so as to prevent infection or further damage. If compartmentalization does not occur, the numerous injection sites around the base of the tree have the potential to restrict essential tree functions and may be causal for tree mortality and failure (Smith 2006).

Since no significant findings were found to show that TreeAzin® injections caused a decrease in density of wood after injection, it is likely that the observed failure of injected *Fraxinus* spp. trees is caused by the cumulation of negative impacts which urban grown trees face. The cumulative effects of environmental and anthropogenic impacts facing *Fraxinus* spp. in the City of Thunder Bay along with the potential for injection methodology to harm healthy tree growth and form should be considered when managing for a healthy population of *Fraxinus* spp. throughout the City of Thunder Bay and other cities in the world being affected by Emerald Ash Borer.

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APPENDIX A

#1, Branch, Injected			
sample	weight	Volume	density
#1 0-3	4.2704	5.1	0.837333333
#1 0-3	4.8492	6.58	0.736960486
#1 0-3	7.1788	10.28	0.698326848
#1 0-3	8.0756	11.56	0.698581315
#1 0-3	9.9949	14.45	0.691688581
#1 0-3	8.9363	12.44	0.71835209
#1 0-3	5.5973	7.72	0.72503886
#1 0-3	9.9518	13.89	0.716472282
#1 0-3	5.3209	7.6	0.700118421
#1 0-3	5.9721	8.22	0.726532847
#1 0-3	3.2782	4.89	0.670388548
#1 0-3	4.9944	6.64	0.752168675
#1 0-3	5.7533	8.35	0.689017964
Average 1, 0-3	6.474862	9.055385	0.720075404
#1 4+	13.7902	19.93	0.691931761
#1 4+	11.7189	16.92	0.692606383
#1 TREATED OLI	4.6679	6.99	0.667796853
#1 TREATED 4+	14.4571	20.88	0.692389847
#1 4+	23.0598	32.87	0.701545482
#1 OLD	13.2015	17.9	0.737513966
#1 4+	14.7096	20.89	0.704145524
#1 4+1	20.7729	29.41	0.706320979
#1 4+T	14.1412	20.42	0.69251714
avergae 1, 4+	14.50212	20.69	0.698529771

#2, Branch, Not Injected			
sample	weight	Volume	density
#2 0-3	18.1427	26.79	0.677219112
#2 0-3	9.9999	14.19	0.704714588
#2 0-3	11.0538	15.48	0.714069767
#2 0-3	14.0602	20.64	0.68121124
Average 2, 0-3	13.31415	19.275	0.694303677
#2 MATURE	9.8939	14.85	0.666255892
#2 MATURE	13.0387	19.81	0.658187784
#2 NT 4+	27.9035	45.61	0.611784696
#2 NT MATURE	20.1404	30.95	0.650739903
#2 NT 4+	21.7402	35.63	0.610165591
Average 2, 4+	18.54334	29.37	0.639426773

#3, Base, Injected			
sample	weight	Volume	density
#3, 0-3	10.1713	18.32	0.555201965
#3, 0-3	11.8011	19.68	0.59964939
#3, 0-3	13.2688	21.94	0.604776664
#3, 0-3	12.286	17.51	0.701656196
#3, 0-3	12.1477	19.48	0.623598563
#3, 0-3	15.7662	25.51	0.618039984
#3 0-3	11.7371	18.65	0.629335121
#3 0-3	14.3961	22.91	0.628376255
#3 0-3	12.8367	20.76	0.61833815
#3 0-3	17.2679	25.28	0.683065665
#3 0-3	8.7947	14.36	0.61244429
Average 3, 0-3	12.77033	20.4	0.624952931
#3,4+	7.3051	13.89	0.525925126
#3,4+	7.7887	13.55	0.574811808
#3, 4+	8.0901	13.65	0.592681319
#3,4+	8.2588	13.69	0.603272462
#3,4+	7.8461	13.43	0.584221891
#3,4+	10.5208	17.61	0.597433277
#3,4+	22.2921	40.06	0.556467798
#3 4+	12.8256	23.18	0.553304573
#3 4+ #3 I	11.9238	21.32	0.559277674
#3 INJ #3 OLD	14.8527	24.24	0.612735149
#3 4+	9.2979	16.26	0.571826568
#3 4+	11.2344	19.42	0.578496395
#3 4+	7.308	13.16	0.555319149
#3 4+	10.9453	18.42	0.594207383
#3 4+	9.4151	16.3	0.577613497
#3 OLD	15.6726	25.77	0.608172293
#3 OLD	16.2938	26.69	0.610483327
Average 3, 4+	11.28652	19.44941	0.579779393

#4, Base, Not Injected			
sample	weight	Volume	density
#4 NI, 0-3	7.4812	12.76	0.58630094
#4 NI 0-3	7.9432	13.9	0.571453237
#4 NI 0-3	15.4454	27.27	0.566387972
#4 NI 0-3	5.1755	9.33	0.55471597
#4 NI 0-3	8.2633	13.84	0.597059249
#4 NI 0-3	7.7685	13.06	0.594831547
#4 NI 0-3	7.0965	12.15	0.584074074
#4 NI 0-3	9.493	16.12	0.588895782
#4 NI 0-3	9.2739	15.45	0.600252427
#4 NI 0-3	7.2076	12.89	0.559162141
#4 NI 0-3	9.4898	16.1	0.589428571
#4 NI 0-3	14.0149	25.18	0.556588562
#4 NI 0-3	10.0089	17.06	0.586688159
Average 4, 0-3	9.127823	15.77769	0.579679895
#4 NI, 4+	11.1349	18.6	0.598650538
#4 NI 4+	10.4617	18.2	0.574818681
#4 NI 4+	8.6584	15.79	0.548347055
#4 NI 4+	9.1552	15.52	0.589896907
#4 NI 4+	9.9928	16.89	0.591640024
#4 NI 4+	13.2077	23.16	0.570280656
#4 NI 4+	20.2798	33.58	0.603924955
#4 NI 4+	10.2606	18.38	0.558248096
#4 NI 4+	11.3778	21.33	0.533417722
#4 NI 4+	15.7044	26.59	0.590613012
#4 NI 4+	7.8819	13.39	0.588640777
#4 NI 4+	10.6145	18.1	0.586436464
#4 NI OLDER	11.5931	19.59	0.591786626
#4 NI OLDER	14.964	26.54	0.563828184
#4 NI OLDER	12.6859	20.67	0.613734881
#4 NI 4+	7.3501	13.08	0.561934251
Average 4, 4+	11.58268	19.96313	0.579137427

0-3 Years

t-Test: Two-Sample Assuming Unequal Variances

	1 Branch Injected	*2 Branch Not-Injected*
Mean	0.720075404	0.694303677
Variance	0.001726602	0.000320793
Observations	13	4
Hypothesized Mean Difference	0	
df	13	
t Stat	1.76579212	
P(T<=t) one-tail	0.050444986	
t Critical one-tail	1.770933396	
P(T<=t) two-tail	0.100889972	
t Critical two-tail	2.160368656	

t-Test: Two-Sample Assuming Unequal Variances

	3, Base, Injected	*4, Base, Not-Injected*
Mean	0.624952931	0.579679895
Variance	0.00154538	0.000255735
Observations	11	13
Hypothesized Mean Difference	0	
df	13	
t Stat	3.577349027	
P(T<=t) one-tail	0.001687496	
t Critical one-tail	1.770933396	
P(T<=t) two-tail	0.003374992	
t Critical two-tail	2.160368656	

4+ Years

t-Test: Two-Sample Assuming Unequal Variances

	1 Branch Injected	*2 Branch Not-Injected*
Mean	0.698529771	0.639426773
Variance	0.000339761	0.000705016
Observations	9	5
Hypothesized Mean Difference	0	
df	6	
t Stat	4.420598683	
P(T<=t) one-tail	0.00223403	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.00446806	
t Critical two-tail	2.446911851	

t-Test: Two-Sample Assuming Unequal Variances

	3 Base, Injected	*4, Base Not-Injected*
Mean	0.579779393	0.579137427
Variance	0.000584402	0.000466777
Observations	17	16
Hypothesized Mean Difference	0	
df	31	
t Stat	0.080529369	
P(T<=t) one-tail	0.468166947	
t Critical one-tail	1.695518783	
P(T<=t) two-tail	0.936333893	
t Critical two-tail	2.039513446	