Pyroligneous Acid Effects on Seed Germination, Plant Growth, Female/Male Sex Ratio, and Yield of Cucumber (*Cucumis sativus* L.)

by

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ABSTRACT

Pyroligneous acid has shown great promise as a biostimulant for many agronomic crops by stimulating seed germination, plant growth, and yield. However, most of the studies did not determine effect of different modes of application on plant growth and yield performance. This study focused on applying PA at differing rates (0%, 2.5%, 5%, and 10%), durations (0, 3, 6, 9, 12 hours), and frequencies (never repeated, biweekly, and monthly) to evaluate its impact on germination, early seedling root growth, plant growth, sex ratio, and yield of cucumber (*Cucumis sativus* L.). Rate and soaking time significantly ($P < 0.05$) improved germination and root growth. Seeds soaked in lower concentrations (i.e. 2.5% and 5%) for longer soaking periods (i.e. 9 and 12 hours) showed the greatest germination and early root growth response compared to control treated seeds and seeds soaked in 10% PA. Monthly applications of 2.5% and 5% PA led to significantly ($P < 0.05$) highest female/male flower ratios compared to control treatments which led to significantly ($P < 0.05$) improved fruit setting and the highest calculated yield values. Improving growth and yield parameters in cucumber helps support the case that pyroligneous acid can serve as a useful product in the field of agriculture from both an economic and global population perspective.

LIST OF ABREVIATIONS USED

ABA- Abscisic acid

ATP- Adenosine triphosphate

AdoMet- S-adenosyl-L-methionine

ACC- 1-Aminocyclopropane-1-carboxylic acid

ACCS- 1-Aminocyclopropane-1-carboxylic acid

Ca- Calcium

CaC_2- Calcium carbide

$CaCl_2$- Calcium dichloride

CO_2- Carbon dioxide

cm- Centimetre

Cu- Copper

cm^3- Cubed centimetre

m^3- Cubed metre

c.v.- Cultivar

°C- Degree Celsius

DNA- Deoxyribonucleic acid

C_2H_4- Ethylene

GR- Germination Rate

ha- Hectare

HPS- High pressure sodium

hrs- Hours

HCN- Hydrogen cyanide

kg- Kilogram

LSD- Least significant difference

L- Litre

Mg- Magnesium

Mn- Manganese

MGT- Mean germination time

MTA- 5'-Methylthioadenosine

m- Metre

ml- Millilitre

mm- Millimetre

mmhos/cm- Millimhos per centimetre

min- Minutes

M-monthly

N- Nitrogen

n- Never/unrepeated

O_2- Oxygen

ppm- Parts per million

PA- Pyroligneous acid

P- Phosphorus

PEG- Polyethylene glycol

K- Potassium

pH- Potential of hydrogen

s- Seconds

cm^2- Squared centimetre

m^2- Squared metre

t- Tonnes

H_2O- Water

Zn- Zinc

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Chapter 1.0: Introduction

1.1 Thesis Overview

Cucumber (*Cucumis sativus* L.) is an angiosperm and a member of the *Cucurbitaceae* family, which is also referred to as cucurbit. This family consists of approximately 120 genera and about 850 species (Abbey, 2016). Other notable members of the Cucurbit family are watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita*), and zucchini *(Cucurbita pepo*). Cucurbits are typically monoecious plants which simply put means that each unique plant develops and grows male and female biological structures (Irish & Nelson, 1989). Cucumbers originated in India and have been domesticated now for a minimum of 6000 years (Whitaker & Davis, 1962; Staub *et al.,* 1999). Today, they are the second highest cultivated cucurbit after watermelon (Singh *et al.,* 2018). Cucumbers are globally consumed as a fruit. In Canada alone between 2014 and 2018, anywhere in the range of 61,000 to 74,000 tons of cucumber were produced each year. This led to 59,000 to 73,000 tons of cucumber being marketed per year. Within this time frame the marketed cucumbers in Canada generated between 32 million and 40 million dollars of farm income (Statistics Canada, 2019). Cucumber popularity can be attributed to their inherent nutrition and health benefits including weight loss and aiding with hydration (Mousavizadeh *et al.,* 2010; Alsadon *et al.,* 2016). It is also a valuable source of vitamins A, C, K, and B6 as well as minerals like magnesium (Mg), potassium (K), phosphorus (P), copper (Cu), and manganese (Mn) (Vimala *et al.,* 1999; Singh *et al.,* 2018).

One important limitation of the production of cucumbers and many other plants is germination success and early seedling growth. Successful germination is the first step towards producing a healthy plant and providing maximum value in the case of cucumbers and other agronomic crops. With this in mind, ensuring improved germination (success and rate) of cucumber seeds along with early seedling growth would benefit the growers as it would allow for a smoother transition from seed to harvestable plant. Another issue related to cucumber production is the likelihood of an imbalanced sex ratio of the plant. Typically, in cucurbits the male flowers develop and open first, followed by alternating sequence of female and male flowers leading to large male to female sex ratios (Ghani *et al.,* 2013). When the female flower count is low, it leads to a decreased fruit yield. The sex ratio of Cucurbits has been shown to be influenced by several factors including genotype, nutrition, several physical factors such as light and temperature, and most predominantly chemical growth regulators (Abbey, 2016). If the cucumber plant sex ratio could be purposely altered so that there is a greater balance between male and female flowers, then cucumber production could be increased to more readily meet the increasing consumers' demand and generate a greater economic return for growers.

One potential solution to the sex ratio imbalance and possibility for improving germination and early seedling growth in cucumber plants is an organic liquid called pyroligneous acid (PA). Pyroligneous acid, also known as wood vinegar, is one of several by-products of pyrolysis of plant biomass. Pyrolysis is a strategic method that can reduce greenhouse gas emissions compared to historical disposal methods of plant biomass and has been primarily done to produce charcoal (Grewal *et al.,* 2018). Pyroligneous acid is derived from the condensed smoke produced during this process. As an up-and-coming product, PA has been used in agriculture as a bio stimulant for aiding plant growth and seed germination and for a multitude of other purposes including management of microbial pathogens and diseases, insect pests, and weeds (Grewal *et al.,* 2018). The complex chemical makeup of PA (elemental composition, acetic acid, phenols, esters, aldehydes, etc.) may allow it to form a close relationship with growth regulators and there is a reason to believe that this is a large contributing factor as to why PA has shown to improve plant development (Grewal *et al.,* 2018). Plant growth regulators have been linked to cucumber sex ratio alteration, as well as having major roles in seed germination and seedling growth.

Therefore, the main objectives of this study were to determine the influence PA has on seed germination, early seedling root growth, male:female sex ratio, and cucumber fruit yield. If PA can successfully improve germination parameters and the balance of sex ratios in cucumber, then an increase in fruit yield could be achieved leading to increased farm revenue.

1.2 Thesis Objectives & Organization

The hypothesis tested in this study was:

- Pyroligneous acid application will improve seed germination, plant growth, sex ratios, and fruit yield in cucumber plants.

The Specific Objectives of this study were to:

- Evaluate the effect of different rates and frequencies of PA application on the germination of cucumber seeds.
- Determine the effect of different rates and frequencies of PA application on radicle/early root development of cucumber seedlings.
- Investigate the effect of different rates and frequencies of PA application on growth, sex ratios, and fruit yield in cucumber plants.

This thesis consists of five chapters, including this introductory chapter (Chapter 1.0). Chapter 2.0 dives into the scientific background and previous studies that will help support the basis for this research. This includes information on cucumber botany, growth, and development, as well as factors that influence flower sex development in cucumber. This chapter also includes background information on seed germination and seed priming, as well as plant growth regulators such as ethylene. It also discusses PA and its success in agriculture studies and the research gaps. Chapter 3.0 investigated the effect of differing PA application rates and seed soaking times on cucumber seed germination parameters. Then analyzes the effect of these PA soaking treatments on early seedling root growth. In Chapter 4.0 a field experiment was performed during which PA was applied to cucumber plants at varying application frequencies and rates and the effects on plant growth, sex ratio, and fruit yield were determined. The thesis is concluded in Chapter 5.0 along with suggestions for future research considerations.

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Chapter 2.0: Review of Literature

2.1 Cucumber Botany

Cucumber (*Cucumis sativus* L.) plants are a fruit bearing angiosperm and a member of the family Cucurbitaceae (Abbey, 2016). They are climbing annual plants that grow under specific conditions (Okoli, 1984). Cucumber plants can be identified as a vine plant having structural tendril organs for support (Rubatzky & Yamaguchi, 1997; Abbey, 2016). They have long and coarse stems with leaves that contain three to seven lobes and have an alternate growing pattern (Ojo, 2016). Abbey (2016) reviewed that cucumber plants are often monoecious which means they develop both male and female flowers on the same plant. However, occasionally cucumber plants have alternative sex and flower development patterns involving different male, female, and bisexual flower ratios. The details of alternative development patterns will be discussed later in this document. Cucumber flowers are commonly yellow with five petals that enclose either the male (anther and filament) or female (ovary, style, and stigma) sex organs. Pollen is produced in the anthers of the male flower, and then is transferred by external forces such as bees, wind, or manual pollination from the male flowers to the female flowers. The pollen fertilizes the female flower by attaching to the stigma then forming and traveling down pollen tubes in the female style to receptive ovules. The pollen then fertilizes the ovule and forms an embryo. The pollinated female flowers will produce green skinned, spiny, elongated fruits that have a small curvature and tapered shape (Figure 2.1). The flesh of the fruit is a light green colour and often contains copious amounts of seeds. The fruits can grow up to 50 cm in length depending on the cultivar if left to fully mature (Ojo, 2016).

Figure 2. 1. Freshly sliced cucumber fruit.

2.2 Growth and Development

Cucumber is a warm season plant and therefore, highly frost sensitive. In Canada, cucumber seeds are mostly started (i.e. germination and seedling establishment) in indoor facilities prior to transplanting in the field in spring. Germination ordinarily takes 3-5 days but can be fast-tracked and homogenized using seed priming methods. The seedlings are grown indoors for 4-5 weeks before transplanting (Ojo, 2016). If seeds are to be directly seeded, it is essential that soil and air temperatures are at an optimum level during both the day (21-32°C) and night (15-21°C) (Bai *et al.,* 2016). Cucumber plants growth and performance are optimized in moist, organic rich soils with a neutral pH. Flower development begins 40-45 days post sowing of the seeds (Ojo, 2016). Cucumber plants are not self-pollinators, and as such they rely heavily on external factors to promote pollination, with the primary source of pollination being bees (Kishan *et al.,* 2017). Fruits are commonly harvested in an immature state for marketing purposes about 2-4 weeks after flowering. Consistent harvesting allows for increased opportunity for further fruit development (Ojo, 2016). Cucumber plants can grow up to 2 m tall and 5 m long. Plants grown in an outdoor environment will typically die if exposed to unfavourable conditions such as unfavourable temperatures, drought, flooding, or salt stress. Unfavourable conditions can also lead to diseases such as powdery mildew, downy mildew (*Pseudoperonospora cubenis*), and target leaf spot (*Corynespora cassiicola*) (Ojo, 2016).

2.3 Factors Influencing Cucumber Plant Growth & Fruit Quality

Plant growth and fruit quality is extremely important for the fresh market. When plant growing conditions are optimized for fruit-bearing plants it ensures the optimum plant health, productivity, and harvest quality of the plant. Fruit quality can be defined as the shape, texture, color, taste, and nutrient value of the harvested fruit (Mousavizadeh *et al.,* 2010). The best quality fruits are what buyers are most likely to consider and will always generate the greatest amount of income. Therefore, it is crucial that marketable fruits are grown under the appropriate and the best recommended growing conditions for greater fruit yields, desirable fruit quality, and maximum economic returns.

2.3.1 Edaphic Factors

The soil in which cucumber plants are grown has a significant impact on the growth of the plant. Soils should be fertile and have a neutral pH (6.5-7.5) and have all the appropriate macro and micro-nutrients either previously existing in the soil or be provided via fertilizer application (Singh *et al.,* 2017). This should include phosphorus, potassium, nitrogen, calcium, magnesium, sodium, sulfur, aluminum, iron, manganese, copper, and zinc. A general recommendation of 700kg/ha application has been suggested for cucumber, but it is best to refer to a soil evaluation for specific nutritional needs (Ojo, 2016). Soil should also always be within suitable field capacity levels required for the crop, never soaked or too dry to provide optimum water availability to the plant during growth and development (Singh *et al.,* 2017).

2.3.2 Climatic Factors

Cucumber plants are best grown in the summer months in Canada. This means temperature is a critical factor for cucumber growth (Marcelis & Hofman-Eijer, 1993; Alsadon *et al.,* 2016). It has been shown that cucumber growth and yield can transpire when growing temperatures are between 15º and 32°C but optimized growth and yield occurs between 25-32°C (Alsadon *et al.,* 2016; Ojo, 2016). In addition to temperature, sunlight or lighting in general is a major factor influencing plant and fruit development in cucumber plants (Särkkä *et al.,* 2017). Cucumber fruit yield and plant growth were optimum when exposed to complete sunlight for a minimum of 8 hrs a day (Särkkä *et al.,* 2017). In another study, cucumbers c.v. Toploader grown under artificial lighting conditions comprising a combination of high-pressure sodium (HPS) and light emitting diode (LED) recorded the highest yield and fruit quality during winter months due to optimized light use efficiency and heat (Särkkä *et al.,* 2017). Post-harvest storage temperature also strongly influences cucumber quality. It was shown that cucumber fruit quality based on color and texture was maintained for the longest period when kept at 13°C and a relative humidity of 95% (Schouten *et al.,* 2002; Ojo, 2016). Fruit water content, soluble sugar content, and in turn flavour are also important when evaluating cucumber quality (Huang *et al.,* 2009).

2.3.3 Management Factors

It is well established that management factors such as irrigation, fertilization and weeding have a significant impact on cucumber plant productivity and fruit quality. Cucumber plants require adequate irrigation for growth and fruit development (Şimşek *et al.,* 2005). Studies showed that when watering levels were decreased, the transpiration rate of the plant was reduced leading to a reduction in nutrient uptake and partitioning throughout the plant (Şimşek *et al.,* 2005; Rahil and Qanadillo, 2015). Excess watering can also lead to decreased fruit yield and quality, which can be attributed to the dilution of solutes in the fruit (Roh & Lee, 1996). It is important to determine the optimum water crop requirement so that fruit yield and quality can be optimized (Rahil & Quandillo, 2015) determined that an irrigation level of 70% of the total evapotranspiration of the crop gave the best cucumber fruit yield and quality.

It is also crucial to provide cucumber plants with the proper nutrition during the active growth and development stage so that the fruits can be at their utmost metabolic performance. Fertilizer application is essential for favourable plant growth and fruit quality (Eifediyi & Remison, 2010; Adebayo *et al.,* 2016). Nitrogen – phosphorus - potassium (NPK) compound fertilizer application has been shown to increase cucumber fruit growth and quality in terms of fruit weight, girth, length and colour. Cucumber plants treated with 10 t/ha of farmyard manure and 400 kg/ha inorganic fertilizer (NPK 20:10:10) showed over a 1.50 kg increase in fruit weight per plant and over 20 t/ha increase in fruit yield which was 166% higher than the control (Eifediyi & Remison, 2010). Micronutrients at smaller intake levels are also important for improving cucumbers plants growth and fruit yield (Adrees *et al.,* 2015). Fertile soils that are properly weeded provide the plant with the optimum soil conditions for plant growth, plant health, and fruit development (Putnam & Duke, 1974).

2.4 Seed Germination

Seed germination is the crucial first step in the growth and development of a mature plant. Germination can be broken down into three distinct phases. These include imbibition, metabolic activation, and cell elongation with radicle protrusion (Lutts *et al.,* 2016; Ali & Elozeiri, 2017). The seed actively absorbs water during the imbibition stage of seed germination. This increase in water content in the seed triggers the second phase of germination. During the second phase, seed water content remains at a constant level and metabolic processes commence that prepare the seed to complete the germination process. The metabolic processes include, energy metabolism, oxidation regulation, cell cycle activation, seed reserve mobilization and hormonal activation, among others. This leads into the final phase of the germination process with a second uptake of water, where cell elongation, weakening of the outer seed coating, and radicle protrusion occurs (Armin *et al.,* 2010; Lutts *et al.,* 2016).

2.5 Seed Priming Process and Benefits

Seed priming is a pre-sowing technique used to increase seed vigor (Sadeghi *et al.,* 2011). Priming techniques promote increased germination percentages and rates as well as synchronized germination among many seeds compared to unprimed seeds. This means priming can be beneficial at increasing yields under non-stressful and stressful conditions alike (Di Girolamo & Barbanti, 2012; Lutts *et al.,* 2016). Seed priming is a controlled hydration procedure followed by a dehydration and possible storage of seeds. This allows seeds to experience the first two phases of germination (imbibition and metabolism activation) prior to pausing the germination process before radicle protrusion occurs (Hussain *et al.,* 2015). The seeds can then be stored in this active state without sacrificing seed integrity at differing times depending on the cultivar (Di Girolamo & Barbanti, 2012). The primed seeds will experience accelerated germination and increased seedling vigor when these seeds are placed under the proper germination conditions again. This can be linked to a reduced imbibition lag timing, germination promoting metabolite development, DNA repairing, antioxidant system activation, reserve mobilization, and seed structure modifications (Kubala *et al.,* 2015; Hussain *et al.,* 2016). Positive results due to seed priming is dependent on several factors such as, soaking duration, temperature, light presence, seed species and condition, as well as priming technique (Hussain *et al.,* 2006; Di Girolamo & Barbanti, 2012; Ibrahim, 2019).

There are several different modes of performing the seed priming process which include hydropriming, osmopriming, hormopriming, and solid matrix priming (Sharma *et al.,* 2014; Lutts *et al.,* 2016). Classification of the priming technique is based on the priming solution used to hydrate the seeds. For example, hydropriming utilizes distilled water, osmopriming utilizes osmotic solutions such as polyethylene glycol (PEG), hormopriming deals with plant hormone solutions, and solid matrix priming seeds are treated with matrixes such as vermiculite or other water-absorbent polymers (Sher *et al.,* 2019). Therefore, the success rate of seed priming can also depend on the priming solution utilized. Nevertheless, each of these methods have shown positive germination, seedling establishment, and yield results for several different plants (Lutts *et al.,* 2016).

2.6 Early Root Development

Early seedling development occurs immediately after radicle protrusion during seed germination. Good root development is critical as they allow for plants to gain easy access to water and nutrients within the rhizosphere. Fast and early root development improves the capability of a plant to absorb nutrients and water when these resources are in short supply (Wang *et al.,* 2016). Early root development can be attributed to plant growth regulator interactions as well as seed reserved nutrients which are mobilized during the seed germination process (Ichie *et al.,* 2001; Farooq *et al.,* 2010 Lutts *et al.,* 2016). Once the reserves are depleted, further root development will rely on external nutrient sources in the soil. Maximizing root development prior to this allows for increased capabilities of the roots once this stage is reached and the seedlings are transplanted (Blunk *et al.,* 2019).

2.7 Sex Determination in Cucumber

2.7.1 Genetic Factors

Genetically, there are two major genes that influence sex ratio in cucumbers. The *F* gene and the *M* gene. The *F* gene is generally responsible for the development of female flowers whereas the *M* gene determines if the flower is unisexual or bisexual (Shiber *et al.,* 2008). Together these two genes will determine the phenotypic line of the cucumber flowers. That is, monoecious (*Mff)*, gynoecious (*MF)*, hermaphroditic (*mmF*), or andromonoecious (*mmff*) (Shiber *et al.,* 2008; Miao *et al.,* 2010; Abbey, 2016). An important third gene that has also been linked to sex expression in cucumber plants is the CS-ACS1 gene, which is responsible for producing an enzyme that leads to the production of the female promoting growth regulator, ethylene (Shiber *et al.,* 2008; Lee *et al.,* 2018). Ethylene biosynthesis and mode of function will be discussed further in sections 2.7.4 and 2.8.

2.7.2 Climatic Factors

Cucumber plants are susceptible and highly influenced by the physical environment they are grown in. Some common physical factors that can influence sex ratio and yield of cucumber plants are the temperature and light while relative humidity and wind speed can affect pollination. It has been shown that lower day and night temperatures tend to promote the development of female flowers in cucumber plants (Maynard & Hochmuth, 2007; Miao *et al.,* 2010; Abbey, 2016). This is because cucumber plants grown in lower temperatures have been shown to have increased ethylene production (Miao *et al.,* 2010). The opposite is true for the dominance of male flower emergence. When cucumber plants are subjected to temperatures above 30°C in the daytime and above 18°C in the night the male to female sex ratio increased (Abbey, 2016). As for light exposure, it has been shown that shorter day lengths (i.e. 9 hrs or less) promote the growth and development of female flowers while male flower development seemed to be stimulated when cucumber plants were exposed to longer photoperiods (Wang *et al.,* 2014; Abbey, 2016).

Furthermore, based on the research done by Miao et al. (2010) and Lai et al. (2018), it was determined that shorter photoperiods (< 8 hours) and lower temperatures (12-18°C) cause the levels of ethylene and sugars in the shoot apex to increase compared to longer photoperiods (12-16 hours) and higher temperatures (24-32°C). Furthermore, the exogenous application of ethylene and sugars (sucrose and glucose) have shown to be positively correlated with increased female flower development (Miao *et al.,* 2010).

2.7.3 Soil Factors

There are many chemical factors in the soil that also influence sex ratios in cucumber plants. These chemical factors include mineral nutrients. In terms of mineral nutrients, nitrogen potassium and calcium have been shown to strongly influence the sex of cucumber plants. Nitrogen is commonly found in fertilizer products and has a major importance for plant growth as it is a major component of chlorophyll and amino acids within the plant (Fageria & Baligar, 2005; Singh *et al.,* 2018). Increasing the rate of nitrogen application on cucumber plants has been shown to decrease female flower development (Abbey, 2016; Singh *et al.,* 2018). It is elucidated that as the plant is exposed to and takes up more nitrogen, the growth of the plant becomes the focal point of development, leaving reproductive development as secondary. As a result, the typical sexual development occurs and there is large male to female sex ratios (Singh *et al.,* 2018). On the other-hand, potassium does not only play an important role in photosynthesis and enzyme activation in plants but also found to increases female flower development in Cucurbits such as pumpkin plants (Agbaje *et al.,* 2012; Abbey, 2016). Calcium has been linked to improving ethylene levels which in turn, promotes female flower development (Shakar *et al.,* 2016 [a]; Shakar *et al.,* 2016 [b]). Organic amendments such as vermicompost also have been shown to promote early female flower development in cucumber plants (Singh *et al.,* 2018).

2.7.4 Plant Growth Regulators

Growth regulators and their interactions are said have the ultimate control over cucumber flower sex expression. There are several plant regulators or hormones that have been shown to have an influence on Cucurbit sex expression and sex ratio including ethylene, gibberellins, and auxins (Ghani *et al.,* 2013). Ethylene has the primary role in increasing female flower production and development in Cucurbits plants including cucumber. Exogenous application of ethylene on monoecious cucumber plants increased female flower development (Miao *et al.,* 2010; Abbey, 2016). It has been shown ethylene triggers a response factor that degrades the DNA that codes for the anther, a male sex organ (Ikram *et al.,* 2017). Endogenous ethylene is synthesized by an enzyme, 1-Aminocyclopropane-1-carboxylic acid synthase (ACCS) that is produced by the CS-ACS1 gene (Lee *et al.,* 2018; Pawełkowicz *et al.,* 2019). Gynoecious plants contain a second copy of this gene (CS-ACS1G) and monoecious plants with higher rates of female flowers contain higher levels of ethylene than other sexual lines of cucumber plants (Ikram *et al.,* 2017). On the other hand, gibberellins have been shown to inhibit the expression of the CS-ACS1 gene and therefore, increases the production and development of male flowers (Miao *et al.,* 2010; Abbey, 2016). Auxins are another major group of hormones that promote femaleness in Cucurbits by enhancing the production of ethylene (Iwahori *et al.,* 1970; Abbey, 2016; Wang *et al.,* 2018).

2.8 Ethylene

2.8.1 Ethylene Biosynthesis

Ethylene is a plant growth regulator with many roles in plant development. It has a simple chemical structure (C_2H_4) and is a gaseous growth regulator. As mentioned by Schaller

2012, ethylene is often considered to be the “aging” hormone due to its roles in accelerating developmental actions such as fruit ripening and senescence (Schaller, 2012). The biosynthesis of endogenous ethylene is conducted via a pathway involving enzymes S-adenosyl-L-methionine (AdoMet) synthetase, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, and ACC oxidase. Together these enzymes are able to catalyze the pathway of ethylene biosynthesis by producing methionine and ACC and converting them to ethylene (Kende, 1993; Yamamoto *et al.,* 2018). AdoMet is synthesized via a molecule of methionine and a molecule of ATP by AdoMet synthetase. AdoMet is then converted to ACC via ACC synthase. During this process 5’-methylthioadenosine (MTA) is also produced as a by-product. MTA can be recycled into more methionine via multiple enzyme pathways entitled the Yang Cycle. This allows for further ethylene biosynthesis. During the final step of biosynthesis, ACC is converted to ethylene by ACC oxidase via the following reaction: $ACC + O_2 \rightarrow C_2H_4 + CO_2 + HCN + 2\ H_2O$ (Kende, 1993; Schaller, 2012; Arc *et al.,* 2013). Ethylene has been one of the most highly studied plant growth regulators due to its important roles in many plant and crop developmental processes. Along with other growth regulators (auxins, cytokinins, gibberellins, abscisic acids), ethylene has strong primary influences on leaf growth, flower development, and fruit ripening (Iqbal, *et al.,* 2017).

2.8.2 Ethylene Role in Seed Germination

According to Mu et al. 2003, PA has shown promotional effects on germination and early radicle growth by stimulating growth-regulators release within the seed (Mu *et al.,* 2003). The interactions of growth-regulators play a significant role on stimulating and preventing seed germination (Linkies & Leubner-Metzger, 2012; Miransari & Smith, 2014). Three growth regulators that have been highly studied in regards with their effects on germination are gibberellins, abscisic acid (ABA), and ethylene. Gibberellins have been linked to promoting germination, whereas ABA has shown roles in preventing germination by promoting seed dormancy (Linkies & Leubner-Metzger, 2012). Ethylene seems to have a more complex role in the germination process but with the help of using mutants, it has been suggested that ethylene production and release has been linked to the breaking of seed dormancy and improving stress tolerance (Matilla & Matilla-Vázquez, 2008; Miransari & Smith, 2014; Shah *et al.,* 2020). It has been demonstrated that seeds with increased vigour produce increased levels of ethylene compared to seeds with low vigour (Matilla & Matilla-Vázquez, 2008). Ethylene signaling and metabolism prior to germination has been shown to act as an antagonist against ABA and its roles in inhibiting germination. For example, when CaC_2 (calcium carbide) was applied to cucumber seed it improved germination rates and percentages and this success was linked to the greater ethylene production during the imbibition stage of germination (Shakar *et al.,* 2016). When dormancy is broken, germination can occur when conditions are favourable.

2.8.3 Ethylene Role in Sex Expression of Cucumber Flowers

Ethylene is of such great interest to researchers because it has been shown to promote femaleness in cucumber plants (Wang *et al.,* 2010; Abbey, 2016; Pan *et al.,* 2018). Sex expression has been well studied in cucumbers and as mentioned earlier there are several factors that have been shown to influence sex expression in cucumber plants. These include genetic factors, climatic factors, soil factors, along with plant growth regulators such as ethylene. Each of these additional factors have an impact on syntheses of ethylene and alternative growth regulators production. Because of this it is said that plant growth regulators are the true underlying factor impacting flower sex expression in cucumbers.

The mechanism of how ethylene promotes female flower development has been understudied for years. It has recently become an increased focus for researchers as understanding this mechanism may hold further information on how to increase cucumber yields in the near future. In flowering plants, sex determination typically occurs via the selective development interruption of either male or female sex organs (Guo *et al.,* 2010). It is now understood that ethylene promotes female flower development via the degrading of anther coding DNA. Ethylene binds to receptors such as *CsETR1* in the endoplasmic reticulum which are regulated by a MADS-box gene. The corresponding signal is then passed on via a negative factor *CscTR1*. This causes the up regulation of a DNase which results in the damage of the DNA coding for the anther in flowers (Wang *et al.,* 2010; Ikram *et al.,* 2017).

2.9 Uses of Pyroligneous Acid in Agriculture

2.9.1 Description of Pyroligneous Acid

Pyroligneous acid is an organic liquid derived from smoke (Mathew & Zakaria, 2015; Grewal *et al.,* 2018). It is one of several by-products of anaerobic thermal decomposition of plant biomass by a process called pyrolysis (Mathew & Zakaria, 2015). Pyrolysis is becoming more popular because it can serve as an alternative method to valorize biomass waste from the wood and forestry industries into industrial and domestic products (e.g. biochar, biodiesel, graphene and PA) rather than simply burning it or placing it in a landfill (Mohan *et al.,* 2006). Thus, pyrolysis increases waste diversion from landfills and significantly reduce greenhouse gas emissions. Pyroligneous acid is a reddish-brown liquid formed from condensed vapors and is composed of a variety of chemical compounds found in plants, primarily, cellulose, hemicellulose, and lignin (Mathew & Zakaria, 2015). Pyroligneous acid is a complex mixture that consists of many oxygenated compounds (Souza *et al.,* 2012; Mathew and Zakaria, 2015). This variety of compounds found in PA leads to the potential of an array of uses. It has been suggested that by-products of smoke such as PA perform similarly to growth regulators and can also trigger mechanisms that alter the existing levels and ratios of essential plant growth regulators such as auxins and cytokinins in plants like geranium (Senaratna *et al.,* 1999). These findings explain why PA can function as a biofertilizer or plant growth regulator.

Pyroligneous acid has been closely studied mainly in Asia due to the large tar (an additional by-product of pyrolysis) production in Asian countries but also has been considered in many other countries around the world (Souza *et al.,* 2012). There have been several agricultural studies that use PA for a multitude of reasons. It has been studied as a product for promoting seed germination, aiding growth and development (biostimulant), as an antimicrobial agent, and as an insecticide as well as, more recently proposed to have herbicidal properties (Grewal *et al.,* 2018).

2.9.2 Pyroligneous Acid Effect on Seed Germination

Pyroligneous acid has been shown to stimulate seed germination (Jäger *et al.,* 1996; Mu *et al.,* 2003; Van Staden *et al.,* 2008). It is believed that PA is a smoke extract that sends a cue to dormant seeds that promotes their germination process. Pyroligneous acid interacts with growth regulators such as gibberellins and ethylene which aid in promoting germination and radicle growth (Van Staden *et al.*, 2008; Linkies & Leubner-Metzger, 2012; Miransari & Smith, 2014). There was a study conducted showing that PA made from bamboo biomass positively affected seed germination in lettuce (*Lactuca sativa*), chrysanthemum species (*Chrysanthemum*), honewort (*Cryptotaenia canadensis*), and watercress (*Nasturtium officinale*). The PA stimulated seed germination and radicle growth of the geminated seeds at rates of dilution 10^4-10^7 times with distilled water (Mu *et al.,* 2003). However, it was suggested that because of the low pH of PA (≤ 3), higher levels of application (diluted 10^2-10^3 times) will cause a severe reduction or inhibit seed germination (Mu *et al.,* 2003; Grewal *et al.,* 2018).

2.9.3 Pyroligneous Acid Effect on Plant Growth & Yield

There have been growth studies showing that PA application has aided growth and yield of tomatoes (*Solanum lycopersicum*) (Mungkunkamchao *et al.,* 2013), rock melon (*Cucumis melo var. cantalupensis*) (Zulkarami *et al.,* 2011), lettuce (*Lactuca sativa*) (Chen *et al.,* 2016), and rice (*Oryza sativa*) (Polthanee *et al.,* 2015; Kang *et al.,* 2012) among others. Tomatoes grown with soil applied with PA had increased plant fruit yields and weights along with a significant increase in soluble solutes in the tomatoes (Mungkunkamchao *et al.,* 2013). Rock melons treated with a 10% PA application showed increased growth and flower development which lead to increased fruit yield (Zulkarami *et al.,* 2011). Pyroligneous acid was able to render an appropriate soil pH to mimic growth of lettuce when grown with other chemical application (Chen *et al.,* 2016). Along with this, rice grain yield was significantly increased when applied with a PA distilled 300 times and cow manure mixture (Polthanee *et al.,* 2015). It is suggested that the increased growth and yield of plants following PA application is due to the nutrient value of PA and furthermore, the coordinated interactions between PA and plant growth hormones such as cytokinins, gibberellins and ethylene. PA application has either stimulatory or inhibiting effects on these hormones that lead to increased plant growth, development, and yield (Zulkarami *et al.,* 2011). Currently, the concentrations of PA in different studies are often quite different. It has shown success at rates from 1:800 up to 10-20% (Zulkarami *et al.,* 2011; Mungkunkamchao *et al.,* 2013). This is due to different biomass sources being used to make the PA along with the experimenting of different rates with the anticipation of finding an optimum application level.

2.9.4 Pyroligneous Acid Effects on Sexual Development and Reproduction

Overall, there have been fewer studies on the direct effects of PA on sex development and reproduction in Cucurbits. Zulkarami et al., (2011) made a brief note on how PA had positively influenced flower growth in rockmelon. They assumed that if PA is having a positive effect on plant growth, fruit production, and vegetable yield on rockmelon and other plants from other studies as well then it is also positively influencing the sexual development and activities of these same plants. Further studies are needed to better understand the direct effect of PA on sex determination and fruit formation in plants as proposed in this research.

2.10 Summary

Based on the literature reviewed, PA has served as a beneficial biostimulant by improving growth and yield of plants, along with promoting seed germination. The literature showed that exogenous application of growth regulators has an effect on sex ratio just like the endogenous counterparts. So, if the chemical agents in PA act similarly or promote the release of one or some of these growth regulators it was postulated that PA will have an influence on sexual determination and sex ratio of cucumber flowers. Pyroligneous acid has also shown to increase growth of various plants, including some cucurbits. Therefore, it was theorized that PA will aid cucumber growth and development as well. The purpose of this study was to test these theories and begin the process of sequential investigation to determine if and how PA will affect cucumber seed germination and radicle/early root growth, male:female sex ratios, plant growth, and fruit production.

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Chapter 3.0: Pyroligneous Acid Effect on Cucumber Seed Germination and Early Root Growth

3.1 Abstract

Seed germination and early seedling establishment are crucial stages of plant growth and development. Pyroligneous acid (PA), an organic waste from pyrolysis has been shown to improve seed germination and plant growth of some plants but rarely used to prime cucurbit seeds and seedling establishment. A controlled environment study was conducted to assess PA application rates (0%, 2.5%, 5%, 10%) and soaking times (0, 3, 6, 9, 12 hours) on cucumber (*Cucumis sativus* L. cv. Straight Eight) seed germination and early seedling root growth. Rate and soaking time significantly ($P < 0.05$) impacted germination and root growth. Seeds soaked in lower concentrations (i.e. 2.5% and 5%) for longer soaking periods (i.e. 9 and 12 hours) showed the greatest germination and early root growth response compared to control treated seeds and seeds soaked in 10% PA. The 9-hr 2.5% treatment had the earliest mean germination time at 3.3 days, which was earlier than all control treatments by 0.42-1.34 calculated days. Further research will be needed to understand PA's impact on antioxidant response and seedling vigour compared to non-soaked seeds. The 12-hr 5% treatment had the best response in terms of root length (38.0 cm), surface area (11.3 cm^2), and length/volume (32.2 cm/m^3) which was 3.89-19.36 cm, 0.98-3.71 cm^2, and 1.90-14.84 cm/m^3 greater than controls respectively. In conclusion, PA was successful at promoting seed germination and early root growth, but further studies should be conducted to fully understand and build upon PA's potential as a priming agent for early cucumber development.

3.2 Introduction

Successful seed germination is critical for early establishment, seedling vigor and primary developmental steps for plants. As such, different methods for stimulating seed germination have been extensively studied for many years and the knowledge on this subject is continuously expanding (Lutts *et al.,* 2016; Hussain *et al.,* 2019). Seed priming is one of the most studied and was proven to be efficient at stimulating seed germination in many different plant species (Sadeghi *et al.,* 2011; Mouradi *et al.,* 2016; Hussain *et al.,* 2019). Unfortunately, one of the current limitations of seed priming is the absence of a single method that is optimum for all plant species (Sharma *et al.,* 2014; Barbosa *et al.,* 2016). Seed priming techniques include hydropriming, osmopriming, and biopriming with many alternating variables such as soaking agent, time, rates, and lighting (Lutts *et al.,* 2016; Waqas *et al.,* 2019). Often germination success is reliant on the plant species and the seed priming technique (Hussain *et al.,* 2006; Lutts *et al.,* 2016). This means that there are several seed priming variables yet to be explored for many plant species and the potential for further optimizing a crops germination success is something that can be built upon with increased knowledge and improved techniques.

Cucumber (*Cucumis sativus* L.) is a warm season dicotyledonous plant with a relatively shallow tap root system. The primary functions of cucumber roots are to deliver water and minerals to the plant while also securing the plant in its growing medium (Leskovar & Stoffella, 1995; Wang *et al.,* 2016; Illina *et al.,* 2018). Cucumber roots are exceedingly sensitive to stresses such as salt, cool temperatures, drought, and contaminants which in turn diminishes the health of the entire plant (Reyes *et al.,* 1994; Du *et al.,* 2010; Jóźwiak & Politycka, 2019). Due to these sensitivities, ensuring healthy root growth is critical for cucumber seedling establishment and increased productivity. Plant root development relies heavily on germination success as it begins shortly after germination and is greatly dependent on nutrient reserves and plant growth regulators that have been activated during the germination process (Agele *et al.,* 2010; Blunk *et al.,* 2019). In cucumbers the dominant taproot begins growing first shortly after germination, followed by the development of horizontal lateral roots which are derived directly from the apical meristem of the primary root, forming the overall root system (Ilina *et al.,* 2012; Mu *et al.,* 2016; Ilina *et al.,* 2018; Kiryushkin *et al.,* 2019). In addition to germination, early root growth is critical for plants as it sets the precedent for further plant development and yield potential, while early root morphological traits can help contribute to a plant's ability to prevail through physiologically altering stresses and stand establishment (Leskovar & Stoffella, 1995).

Pyroligneous acid (PA), a liquid by-product of pyrolysis of woody plant biomass has shown to be a successful biostimulant that is a cheap and environmentally friendly alternative to many growth promoting products (Grewal *et al.,* 2018). PA is a complex mixture of chemicals and consists of many oxygenated compounds (Souza *et al.,* 2012; Mathew and Zakaria, 2015). The variety of compounds found in PA leads to the potential of an array of uses and because of this, its importance in agriculture is continuously growing. PA has shown success in improving germination in plants such as rock melon and lettuce (Zulkarami *et al.,* 2011; Grewal *et al.,* 2018). This is likely due to its chemical makeup and strong interactions with growth regulators. PA interacts with growth regulators such as gibberellins and ethylene which aid in promoting germination and radicle growth (Van Staden *et al.*, 2008; Linkies & Leubner-Metzger, 2012; Miransari & Smith, 2014). The impact PA has on germination success has been strongly studied and supported. However, there have been limited studies that describe the effect PA priming has on early seedling growth and establishment after analyzing its impact on germination.

The main objective of this work was to evaluate the potential of PA as a priming agent for cucumber seeds by evaluating its effect on germination and early seedling root growth. Based on previous research, it is hypothesized that PA will improve germination and early seedling root growth due to its source of chemicals and interactions with plant growth regulators. This research will provide an initial building block for understanding which variables; namely application rate and seed soaking times, provide improved germination and seedling root development. Successful germination and seedling establishment are the first steps toward growing and providing a reliable and high-quality crop. If PA soaking shows promising implications for improving cucumber seed germination and allows seedlings to have advantageous root morphological characteristics in early development, this would increase the likelihood of producing healthy mature plants with improved growing ranges and yield potential.

3.3 Materials & Methods

3.3.1 Location & Materials

This experiment was conducted in the Compost and Biostimulant Laboratory in the Department of Plant, Food, and Environmental Sciences, Dalhousie University in March 2020. Cucumber seeds cv. Straight Eight were purchased from Halifax Seed, NS for this experiment. The PA used in this experiment was produced and provided by Proton Inc., California, USA. Seed pouches were CYG seed pouches purchased from Mega International, Minnesota, USA.

3.3.2 Seed Priming Method & Treatments

The seed priming treatments conducted were focused on two variables: PA rate and seed soaking time. The experiment was done completely randomized with a 4x5 factorial design. The four PA application rates used were 0% (distilled water), 2.5%, 5%, and 10% PA. The soaking times were 0 (control), 3, 6, 9, and 12 hrs. Prior to the priming treatment, all the cucumber seeds were surface disinfected with 75% ethanol for 30 s which was immediately followed by a rinse in distilled water for 30 s. The seeds were then blotted and dried prior to priming (Mouradi *et al.* 2016). The seeds were soaked in 20 ml solution of PA contained in 5 cm cylindrical plastic containers in a fume hood at room temperature and no lighting (Ghassemi-Golezani & Esmaelpour, 2008). The 0-hour (control) treatments were done by placing the seeds in the individual solution and were removed after approximately 5 s. There were 24 seeds primed per treatment. After the allotted soaking times, the seeds were removed from solution, rinsed with distilled water for 30 s, and laid out then dabbed with paper towel to dry for 30 min. Once this was done, the seeds were stored in sealed envelope pouches in refrigerator (4°C) overnight. The germination experiment began the following day.

3.3.3 Germination Conditions

After 12 hours of storage, the seeds were carefully placed in the designated slits of germination pouches at evenly spaced intervals. This was followed by the addition of 15 ml of distilled water, which was enough to moisten the seeds in the pouches. The pouches were then stored in randomized order standing upright in dark drawers at room temperature (~20°C) for 7 days post-sowing. Additional distilled water was added whenever the pouches were found to be visibly dry.

3.3.4 Germination Measurements

The seeds were in the pouches for 7 days post-soaking. Each day during this time, the seeds were observed, and germination counts were conducted. Germination was defined as radicle protrusion through the seed coating (at least > 1 mm) (Krishnasamy & Seshu, 1989). Germinated seed counts, and time of seed germination data were used to calculate germination percent (%), germination rate (GR), and mean germination time (days). Germination % was calculated as germination %= (Ni/Nt) x100. Where Ni is the number of germinated seeds over the seven-day period and Nt is the total number of seeds (Ellis & Roberts, 1981; Bijanzadeh & Egan, 2018). Germination rate was calculated as the germination % at different times. Mean germination time (MGT) was calculated as MGT= $(\Sigma DN_t/\Sigma N)$, where N_t is the number of seeds germinated on day D, D is the day post-sowing and N is the total number of seeds germinated (Ellis & Roberts, 1981).

3.3.5 Early Root Measurements

Following the 7-day post-sowing germination period, morphological data was acquired from the early developed roots of 8 normal chosen Straight Eight cucumber seedlings from each of the treatments. This root data was obtained by utilizing the WINRHIZO (Reagent Instruments Inc., Canada) scanner. This scanner software provides detailed grey scale images of the seedling roots (Figures 3.3-3.6), as well as provides an extensive amount of quantitative data including: root length (cm), root surface area (cm^2), root volume (cm^3), root diameter (mm), root length/volume (cm/m^3), and root tips/forks counts. Roots were measured, scanned, and analyzed from the base of the hypocotyl to the tips of the primary and lateral roots (Beedi *et al.,* 2018). This data was plotted and visualized on Microsoft Excel (Microsoft Corporation, Washington, USA) using the WINRHIZO software add on: XLRHIZO.

3.3.6 Statistical Analysis

This experiment was arranged in a completely randomized design with four replications per seed priming treatment. A 2-way analysis of variance (ANOVA) was used to determine significance in treatment differences at $P \leq 0.05$. Separation of means was also done using Fisher's least significant difference (LSD) method at alpha = 0.05 when the ANOVA showed a significant difference at $P \leq 0.05$. The statistical analysis was performed using MINITAB version 18 (Minitab Inc., Pennsylvania, USA) and Microsoft Excel was used to plot all graphs.

3.4 Results & Discussion

3.4.1 Cucumber Seed Germination

The non-distilled PA chemical analysis is shown in Table 3.1. The analysis indicated a wide variety of chemicals in the PA. In particular, there were high levels of potassium (13.47%), calcium (34.66%), and zinc (5.47 ppm), low levels of nitrate (0.35%) and phosphorus (2.86%), and a pH of 3.05. The chemicals present in the PA were used to help explain results seen in this experiment and corresponding experiments (Chapter 4.0). The Straight Eight cucumber seeds showed successful germination percentages for all treatments (83.3-100%) per pouch depending on treatment (Table 3.2a and Table 3.4). It should be stated that PA treatments (rate and soaking time) did not significantly ($P > 0.05$) effect germination percentage. However, when considering just PA rate, the 2.5% rate had significantly ($P < 0.05$) greater germination success (over an 8% improvement) compared to seeds soaked in distilled water (Table 3.2a). All seeds soaked in 2.5% PA showed 100% germination success regardless of soaking time and 5% PA was similarly successful with 100% success up to 6 hours soaking (Table 3.2 and Table 3.4). In addition to this, soaking time had a significant effect on both final germination count ($P=0.006$) and mean germination time ($P=0.00$), while there was significant interaction effect from rate and soaking time on mean germination time as well (Table 3.4). Figure 3.1 shows that seeds that were not primed took longer on average to reach final germination levels (less synchronized) than seeds that had been soaked in lower PA rates for 6-12 hours. The 10% PA treatments, especially for longer soaking periods, showed less germination success with lower germination percentages and later mean germination times than those treatments soaked in lower rates of PA (2.5% and 5%). This may be due to increased stress or a toxic effect on the seed from the higher concentration of complex chemicals causing high generation of reactive oxygen species causing these seeds to invest more energy (ATP) into the antioxidant system and activity. If the antioxidant system within a seed is over-stressed it could lead to delayed or failed germination (Ali & Elozeiri, 2017). In addition to this, the higher concentration may not have had the same influence on the germination-promoting growth regulators as the lower concentrations as typically lower concentrations of hormones or hormonal promoting substances show most success (Mu *et al.,* 2003). Along with having 100% germination success, the 9-hour 2.5% treatment had the earliest mean germination time at a calculated 3.3300 days (Table 3.4 and Figure 3.2). Although this was not significantly ($P > 0.05$) earlier than all seeds that were not primed, there may be additional priming benefits (reserve and antioxidant activation) occurring within the PA primed seeds that would not be as prominent in the non-soaked seeds. Future research will be needed to assess these claims on seedling vigor.

When seeds have an earlier mean germination time without sacrificing germination success (percentage) it allows for farmers and growers alike to have flexibility in sowing time and provides the ability to save on money, product, and time. When seeds have less synchronized germination times and/or lower germination success rates it causes non-uniform development and in turn negative impacts on yield (Barbosa *et al.,* 2016). Successful germination is the first step towards having a premier product for consumption and/or for sale. So, if the product can be improved, along with having to spend less money on growing products altogether, this should lead to an overall economic benefit for farmers and growers alike.

Table 3. 1. Chemical analysis of non-distilled PA produced by Proton Inc. and utilized as a biostimulant on cucumber (Cucumis sativus cv. Straight Eight) seeds.

Analysis Performed	Non-distilled PA
Phosphorus %	2.86
Potassium %	13.47
Calcium %	34.66
Magnesium %	1.64
Copper ppm	0.01
Zinc ppm	5.97
Boron ppm	<0.02
Cadmium ppm	0.073
Chloride ppm	370.00
Conductivity umhos/cm	2120.00
Chromium ppm	0.03
Iron ppm	1.26
Manganese ppm	0.60
Sodium %	5.73
Nickel ppm	0.46
Nitrate-N %	0.35
Lead ppm	0.06
Sulfate ppm	89.95
pH	3.05
Alkalinity ppm	162.71
Arsenic ppm	-

Table 3. 2a. Mean germination counts (per pouch) of cucumber (Cucumis sativus cv. Straight Eight) seeds based on PA application rate (%).

PA Application Rate	N	Mean Germination Count (per pouch)
2.50%	24	6.0 ^{a} (100%)
5.00%	24	5.8 ^{ab} (96.7%)
Control	24	5.5 ^{b} (91.7%)
10.00%	24	5.5 ^{b} (91.7%)

* Means sharing the same letter within each column are not significantly different at the 5% level of significance. PA- pyroligneous acid

Table 3. 3. Two-way analysis of variance (ANOVA) P-values acquired for main and interaction effects (P=0.05) of PA rate (%) and soaking time (hrs) on germination time of seeds of cucumber (Cucumis sativus cv. Straight Eight).

Model Terms	Mean Germination Time P-Value
Rate (%)	0.074
Soaking Time (hrs)	0.000
Rate * Soaking Time	0.000

*PA- pyroligneous acid.

Table 3. 4. Mean germination counts, percentages, and germination time for cucumber (Cucumis sativus cv. Straight Eight) seeds treated with varying rates of PA for differing soaking times calculated seven days post-sowing.

Treatment	Germination	Germination Percentage	Mean Germination Time
0 hrs 0%	6.0 ^{a}	100	4.0825 ^{bc}
0 hrs 2.5%	6.0 ^{a}	100	3.7500 ^{cdef}
0 hrs 5%	6.0 ^{a}	100	3.7500 ^{cdef}
0 hrs 10%	6.0 ^{a}	100	3.7500 ^{cdef}
3 hrs 0%	5.5 ^{ab}	91.7	3.9250 ^{cde}
3 hrs 2.5%	6.0 ^{a}	100	4.1650 ^{bc}
3 hrs 5%	6.0 ^{a}	100	3.9975 ^{cd}
3 hrs 10%	6.0 ^{a}	100	4.0000 ^{cd}
6 hrs 0%	5.0 ^{b}	83.3	4.6675 ^{a}
6 hrs 2.5%	6.0 ^{a}	100	3.9975 ^{cd}
6 hrs 5%	6.0 ^{a}	100	3.7500 ^{cdef}
6 hrs 10%	5.0 ^{b}	83.3	3.9425 ^{cde}
9 hrs 0%	5.5 ^{ab}	91.7	3.5425 ef
9 hrs 2.5%	6.0 ^{a}	100	3.3300 ^{f}
9 hrs 5%	5.5 ^{ab}	91.7	3.9075 ^{cde}
9 hrs 10%	5.0 ^{b}	83.3	4.8000 ^{a}
12 hrs 0%	5.5 ^{ab}	91.7	4.1650 ^{bc}
12 hrs 2.5%	6.0 ^{a}	100	3.5850 ^{def}
12 hrs 5%	5.5 ^{ab}	91.7	4.0975 ^{bc}
12 hrs 10%	5.5 ^{ab}	91.7	4.4400 ^{ab}

* Means sharing the same letter within each column are not significantly different at the 5% level of significance. PA- pyroligneous acid.

According to Mu et al. (2003), PA promotes germination and early radicle growth by stimulating growth regulators in the seed. Two of the main growth regulators that have shown to be crucial for initiating and promoting the germination process in seeds are ethylene and gibberellins (Chang *et al.,* 2010; Miransari & Smith, 2014; Bogatek & Gniazdowska, 2018). The PA used for this experiment had substantial quantities of calcium and potassium (Table 3.1). It has been shown that Ca^{2+} has roles in activating growth regulators such as ethylene as well as triggering stress response in seeds (Raz & Fluhr, 1992; Ludwig *et al.,* 2005; Ali & Elozeiri, 2017). Along with this, increased K+ concentration has been shown to improve reserve mobilization, including increasing α-amylase activity for reducing sugars which as a process has been linked to improved germination and seedling vigor (Farooq *et al.,* 2006). Ethylene production and release has been linked to the breaking of seed dormancy and improving stress tolerance (Miransari & Smith, 2014; Shah *et al.,* 2020). When CaC_2 (calcium carbide) was applied to cucumber seeds it improved germination rates and percentages with the success being linked to the greater ethylene production during the imbibition stage of germination (Shakar *et al.,* 2016). Ethylene has been shown to counteract the effects of abscisic acid (ABA) by regulating ABA metabolism which in turn assists in breaking seed dormancy (Corbineau *et al.,* 2014).

Gibberellins have also been strongly linked to seed germination. During early germination stages, the embryo releases gibberellins into the endosperm. This triggers the activation of several genes that release hydrolytic enzymes which are critical for mobilizing seed reserves. Mobilized seed reserves provide the nutrients needed for completing germination and allowing for early seedling growth (Groot *et al.,* 1988; Anil & Rao, 2001; Betts *et al.,* 2020; Gou *et al.,* 2020). Along with stimulating ethylene production, it has also been suggested that Ca^{2+}

presence is related to gibberellin production and along with calcium dependent protein kinases serve as a signal for reserve mobilization (Anil & Rao, 2001; McCubbin *et al.,* 2004; Farooq *et al.,* 2010).

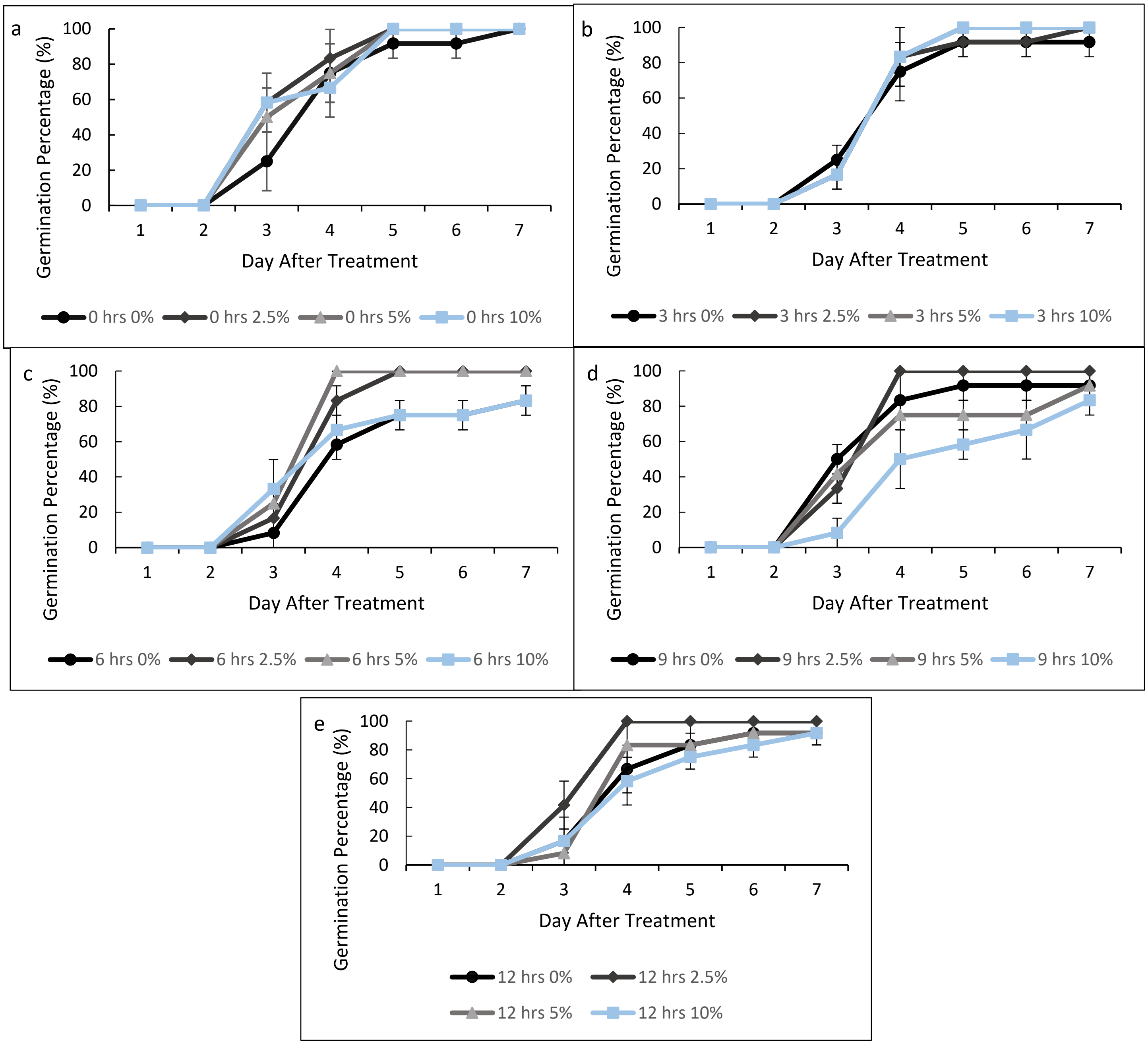

Figure 3. 1. Germination rates shown across seven-day period for cucumber (*Cucumis sativus* cv. Straight Eight) seeds primed based on soaking time (a- 0 hrs, b- 3 hrs, c- 6 hrs, d- 9 hrs, e- 12 hrs) and PA application rates. PA- pyroligneous acid.

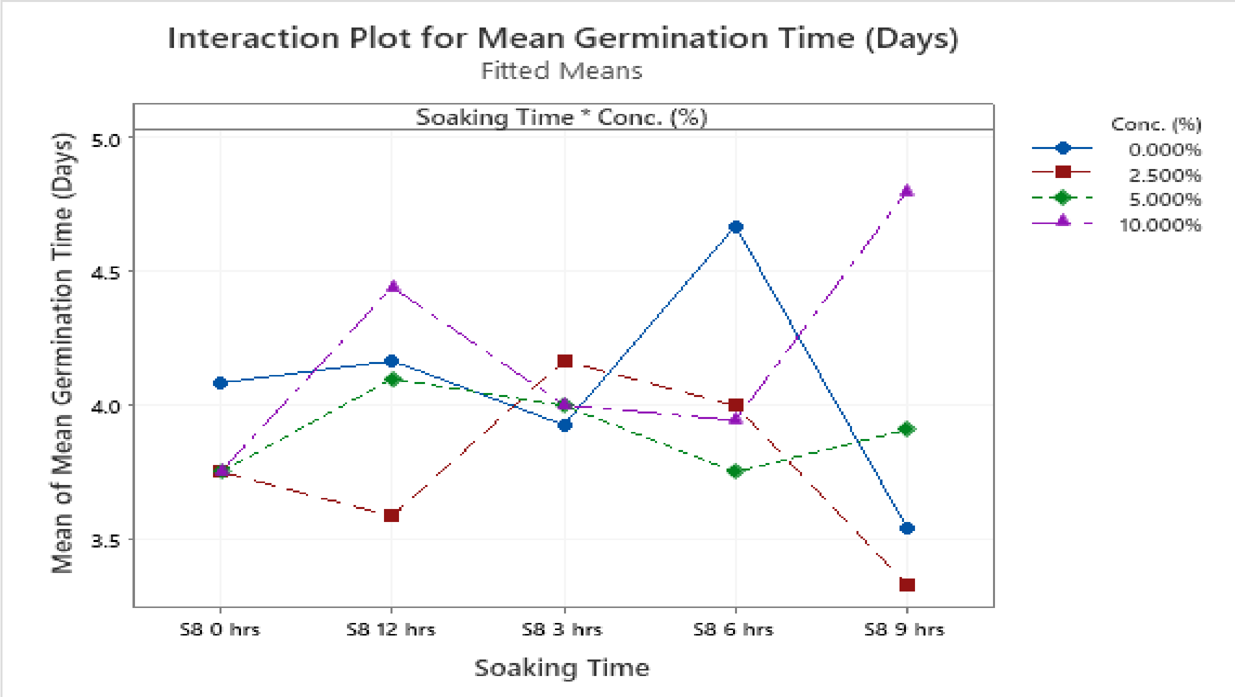

Figure 3. 2. Mean germination time (days) for cucumber (Cucumis sativus cv. Straight Eight) seeds displayed as affected by the interaction of different PA application rates and soaking times.

Soaking the seeds in PA would have triggered the steps of germination by altering the water potential of the seeds (Lutts *et al.,* 2016). Along with this, metabolic processes would have been stimulated via the activation of plant growth regulators within the seed partly due to the chemical attributes of the PA. As for why the 2.5% PA treatment at longer soaking times showed the most promising results for the cucumber seeds, this could be due to its likely close tie to growth regulators within the seeds. It has been shown that lower concentrations of plant growth regulators or plant growth regulator stimulating products have greater promotional effects on both seeds and plants (Mu *et al.,* 2003; Maliang *et al.,* 2020). In addition to this, Chang et al 2010 showed that increasing imbibition (soaking times) from two to twelve hours in $CaCl_2$ solution increased ethylene production in cucumber seeds and sequentially this led to improved germination (Chang *et al,* 2010). It is also likely that longer soaking periods allowed for seeds to complete the imbibition stage of germination and transition into the metabolic activation stage of gemination (Ruttanaruangboworn *et al.,* 2017). This can help explain why the longer soaking times increased germination success compared to those seeds soaked for shorter periods of time in the same rates of PA. These seeds were given longer activation times for metabolic processes such as plant growth regulator activation and synthesis. Further research will be needed to understand the additional priming benefits (reserve and antioxidant activation) PA has on cucumber seeds during germination along with the interactions PA soaking has on growth regulators within seeds and how this can be translated to germination success. Longer soaking times and alternative cucumber cultivars should also be studied to identify if further germination benefits can be achieved using PA as a priming agent.

3.4.2 Cucumber Seedling Root Development

Straight Eight seedlings root morphological data (Table 3.5 and Table 3.6) showed varying degrees of growth among all treatments. Overall root length values were between 18.6745-38.0024 cm. The highest mean root length being the 12 hours 5% treatment and the shortest being the 0 hours 0% treatment. The 12 hours 5% treatment had significantly ($P < 0.05$) greater root length than many of the control treatments and produced nearly 4 cm longer roots than the 12-hour 0% treatment, which had the longest roots from a control treatment. Overall, the root lengths were greater in those treatments that were soaked in the lower concentrations of PA compared to those soaked in water or in the 10% concentration for longer soaking times. Shorter soaking times (3 hours) in 10% PA produced longer roots as well (34.0456 cm), which may be attributed to less exposure to the high concentration of complex mixture of compounds in the PA. Root length data translated in close association with the root surface area data. The 12 hours 5% treatment (11.3030 cm^2) was again significantly ($P < 0.05$) greater than all control treatments aside from the 12 hours in distilled water treatment. As well as, longer soaking periods (9 and 12 hours) of the lower PA concentrations (2.5% and 5%) showed the larger root surface areas compared to those seedlings that had been soaked in these rates for shorter time frames or the longer soaking periods with 10% PA. Larger root surface areas are beneficial because it increases the absorption capability of the roots within the rhizosphere (Blunk *et al.,* 2019). According to Mu et al., 2003, promotional effects of PA for germination and early radicle growth was seen with the use of low concentrations and concluded that the lower concentrations were more successful at stimulating growth regulators within the seeds (Mu *et al.,* 2003). This may explain the results acquired in this experiment.

Root diameter did not follow the same pattern as root length and root surface area. The 10% PA and 0% (distilled water) treatments led to larger root diameters. For each soaking period aside from 0 hours, the 10% PA treated seedlings had the largest root diameters. With this being said, there were many lower rate PA treatments that were not significantly different than the highest response for the cucumber seedling root diameters. Decreases in diameter were to be expected in those seedlings with greater lengths and surface areas because as roots elongate, plants typically optimize the ratio between length and surface area to weight to allow for the best uptake capabilities without over investing carbohydrate for root development (Wu *et al.,* 2016). In terms of root volume, there was significant differences ($P < 0.05$) amongst treatments. For all soaking times aside from 12 hours, the PA treated seeds showed greater root volume values than those soaked in distilled water. The 12 hours 5% treatment produced the largest root length/volume response and was over 6.5 cm greater than the highest response in non-soaked treatments (0 hours). There were also minimal significant differences in root tip and fork counts amongst treatments (data not shown).

Table 3. 5. Effect of PA rate and seed soaking time on cucumber (Cucumis sativus cv. Straight Eight) seedlings' average root length, diameter, and surface area.

Treatment	Root Length (cm)	Root Diameter (mm)	Root Surface Area (cm^2)
0 hrs 0%	18.6745 [f]	0.670867 [ab]	7.5946 [e]
0 hrs 2.5%	23.3588 [def]	0.577300 [bc]	8.3185 [de]
0 hrs 5%	21.3721 [ef]	0.667867 [ab]	8.8773 [bcde]
0 hrs 10%	30.9125 [abcde]	0.356300 [d]	8.2169 [de]
3 hrs 0%	21.0046 [ef]	0.591567 [abc]	7.7851 [e]
3 hrs 2.5%	31.3128 [abcde]	0.641667 [abc]	10.4648 [ab]
3 hrs 5%	30.8698 [abcde]	0.673667 [ab]	8.4963 [de]
3 hrs 10%	34.0456 [abcd]	0.699733 [a]	9.9695 [abcd]
6 hrs 0%	26.8057 [bcdef]	0.635367 [abc]	8.5852 [cde]
6 hrs 2.5%	31.1474 [abcde]	0.584333 [abc]	9.7028 [abcd]
6 hrs 5%	31.6044 [abcde]	0.612233 [abc]	9.6393 [abcd]
6 hrs 10%	27.1057 [bcdef]	0.681333 [ab]	9.6393 [abcd]
9 hrs 0%	23.4403 [cdef]	0.542367 [c]	8.8011 [bcde]
9 hrs 2.5%	28.5496 [abcdef]	0.629867 [abc]	8.5979 [cde]
9 hrs 5%	34.7663 [ab]	0.577300 [bc]	9.7790 [abcd]
9 hrs 10%	23.6677 [cdef]	0.648967 [abc]	8.3566 [de]
12 hrs 0%	34.1103 [abc]	0.619933 [abc]	10.3251 [abc]
12 hrs 2.5%	34.1282 [abc]	0.568467 [bc]	9.6901 [abcd]
12 hrs 5%	38.0024 [a]	0.571900 [bc]	11.3030 [a]
12 hrs 10%	19.7440 [f]	0.671033 [ab]	8.9789 [bcde]

*Values represent means from six chosen seedlings for each treatment.

**Means sharing the same letter within each column are not significantly different at the 5% level of significance. PA- pyroligneous acid.

Table 3. 6. Effect of PA rate and seed soaking time on cucumber (*Cucumis sativus* cv. Straight Eight) seedlings' average root volume and root length per volume.

Treatment	Root Volume (cm^3)	Root Length/Volume (cm/m^3)
0 hrs 0%	0.0726667 ^{abcde}	21.2403 ^{bcd}
0 hrs 2.5%	0.0543333 ^{cdef}	21.1009 ^{bcd}
0 hrs 5%	0.0766667 ^{abcde}	22.0224 ^{bcd}
0 hrs 10%	0.0260000 ^{f}	25.8209 ^{abc}
3 hrs 0%	0.0513333 ^{def}	18.2694 ^{cd}
3 hrs 2.5%	0.0740000 ^{abcde}	23.0234 ^{abcd}
3 hrs 5%	0.0850000 ^{ab}	24.2898 ^{abcd}
3 hrs 10%	0.0966667 ^{a}	25.6161 ^{abc}
6 hrs 0%	0.0546667 ^{cdef}	17.3880 ^{cd}
6 hrs 2.5%	0.0703333 ^{abcde}	26.0322 ^{abc}
6 hrs 5%	0.0853333 ^{ab}	28.6748 ^{ab}
6 hrs 10%	0.0580000 ^{bcde}	15.7705 ^{d}
9 hrs 0%	0.0483333 ^{ef}	20.8473 ^{bcd}
9 hrs 2.5%	0.0800000 ^{abcd}	25.4762 ^{abcd}
9 hrs 5%	0.0780000 ^{abcde}	28.9658 ^{ab}
9 hrs 10%	0.0593333 ^{bcde}	18.2447 ^{cd}
12 hrs 0%	0.0896667 ^{a}	30.3332 ^{ab}
12 hrs 2.5%	0.0680000 ^{abcde}	27.0099 ^{abc}
12 hrs 5%	0.0830000 ^{abc}	32.2321 ^{a}
12 hrs 10%	0.0580000 ^{bcde}	17.2424 ^{cd}

*Values represent means from six chosen seedlings for each treatment.

**Means sharing the same letter within each column are not significantly different at the 5% level of significance. PA- pyroligneous acid.

It was important to monitor early root morphology in this study because having improved early root growth is critical for seedling establishment as it allows for roots to permeate through several types of soil to gain access to water and nutrients along with improving absorption capabilities (Wang *et al.,* 2016; Blunk *et al.,* 2019). Cucumber plants have a shallow root system compared to other plants (Mu *et al.,* 2016; Xu *et al.,* 2017). Therefore, it is important to maximize root morphological traits where possible to allow for increased exploration and uptake capabilities. This could prove extremely beneficial in scenarios where cucumber plants are being grown in stressful environments where water and/or nutrients are limiting in the soil. Although, there were less significant differences among treatments than expected, a favourable pattern for PA treatments was distinguishable for lengths, surface area, and length/volume. In addition to this, it is very possible that the root morphological analysis may have shown even greater differences for PA treated seeds compared to controls at seven days post sowing if the seedlings had been transferred from the seed pouches to soil-filled pots with readily available water and nutrients. According to Blunk et al., 2019 newly emerged seedlings use seed reserves as a source of nutrients for approximately the first four days post-sowing to aid with germination and early growth (Blunk *et al.,* 2019). There is greater energy turnover and utilization of nutrients in primed seeds leading to accelerated depletion of reserves. This is needed to promote early and successful germination in addition to early seedling growth (Lutts *et al.,* 2016; Mouradi *et al.,* 2016). With limited nutrient reserves available after the first few days, this may have also played a contributing factor as to why the root morphological results had fewer significant differences than expected. So based on this knowledge, it can therefore be assumed that the seed reserves of these cucumber seedlings were completely utilized prior to the end of the seven-day measuring period leaving the seedlings with only access to water in the pouches for the remaining time. If the seedlings were given external nutrients, the PA treatments may have followed their early germination with further improved root/seedling growth compared to the controls and there may have been greater significant differences among these treatments. This serves as an area of interest to explore further and would provide additional information that would strengthen the results acquired from this study.

The patterns seen for root development in this experiment may be due to Zinc and calcium levels in the PA. As shown by de la Rosa et al., 2013 lower concentrations of Zn^{2+} influenced early root development in cucumber seedlings by affecting auxin production which has been positively linked to root elongation (Li *et al.,* 2007; de la Rosa *et al.,* 2013). Calcium influxes into pericycle cells have been shown to promote lateral root development even in auxin receptor mutants (Richter *et al.,* 2009; Overvoorde *et al.,* 2010). Auxins promote cell division and impact cellular patterning in root systems (Overvoorde *et al.,* 2010). It has also been stated that although zinc is an essential element for plant growth, higher concentrations can hinder the plants development and root's ability to uptake nutrients (de la Rosa *et al.,* 2013). This may be another reason why the 10% PA treatments for longer soaking times showed decreased results in terms of early root development in cucumber seedlings. It is also possible that hydrogen peroxide, a common reactive oxygen species associated with stress could be serving as a signal for auxin production and therefore increased root development (Li *et al.,* 2007). Hydrogen peroxide levels commonly increase in seeds that have undergone priming and serve as a signal for antioxidant system activation and strengthening (Lutts *et al.,* 2016). This, along with improved seed reserve mobilization may explain why the 12-hour 0% treatment also showed favourable results. As seen in Figures 3.3-3.6, seedlings that underwent any form of priming (PA or water) showed obvious improvements in terms of additional lateral root development and lengths compared to seedlings from non-soaked seeds. Another possibility for explaining improved root development is the production and release of ethylene from PA priming. Ethylene has shown to activate auxin biosynthesis and response (Růžička *et al.,* 2007; Fei *et al.,* 2017; Qin & Huang, 2018). It is likely that PA stimulated ethylene production within the seeds (as explained earlier) which in turn can regulate auxin biosynthesis and thus improve root development. Agele et al, 2010 stated that accelerated root development of rice (*Oryza sativa)* seedlings was due to PA impacting auxin levels. It is feasible that any one of or a combination of these mechanisms contributed to increased auxin levels which in turn led to improved seedling root development in this experiment. Further research will be needed to understand the influence and interactions PA has with plant growth regulators within the seeds and developing seedlings of cucumber and how this may impact early root development.

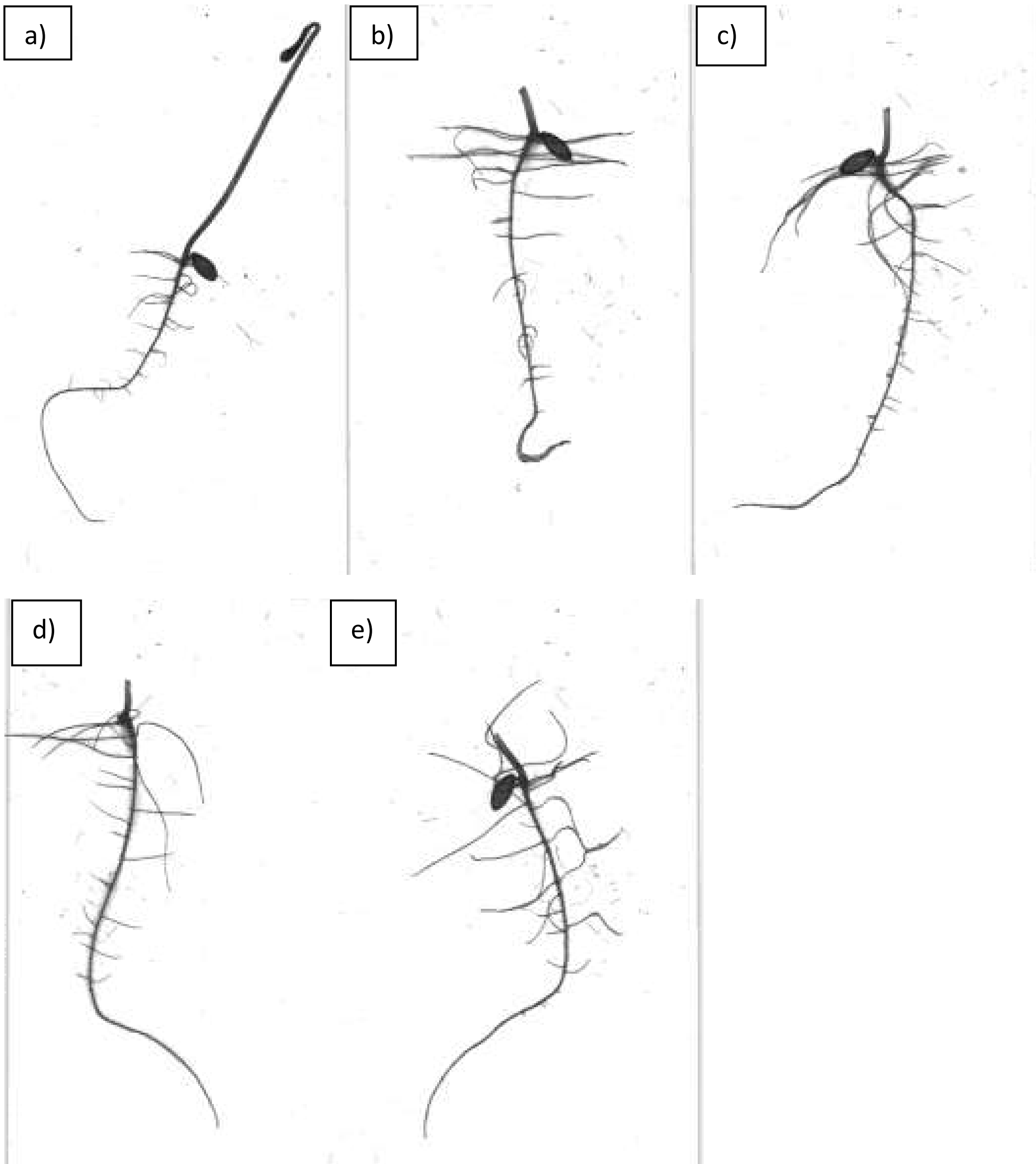

Figure 3. 3.WINRHIZO scanned analysis of early seedling growth for cucumber (*Cucumis sativus* cv. Straight Eight) seeds that had been soaked in 5% PA (a)- 0 hours, b)- 3 hours, c)- 6 hours, d)- 9 hours, and e)- 12 hours). From these scanned images, early root growth was highlighted and measured. PA- pyroligneous acid.

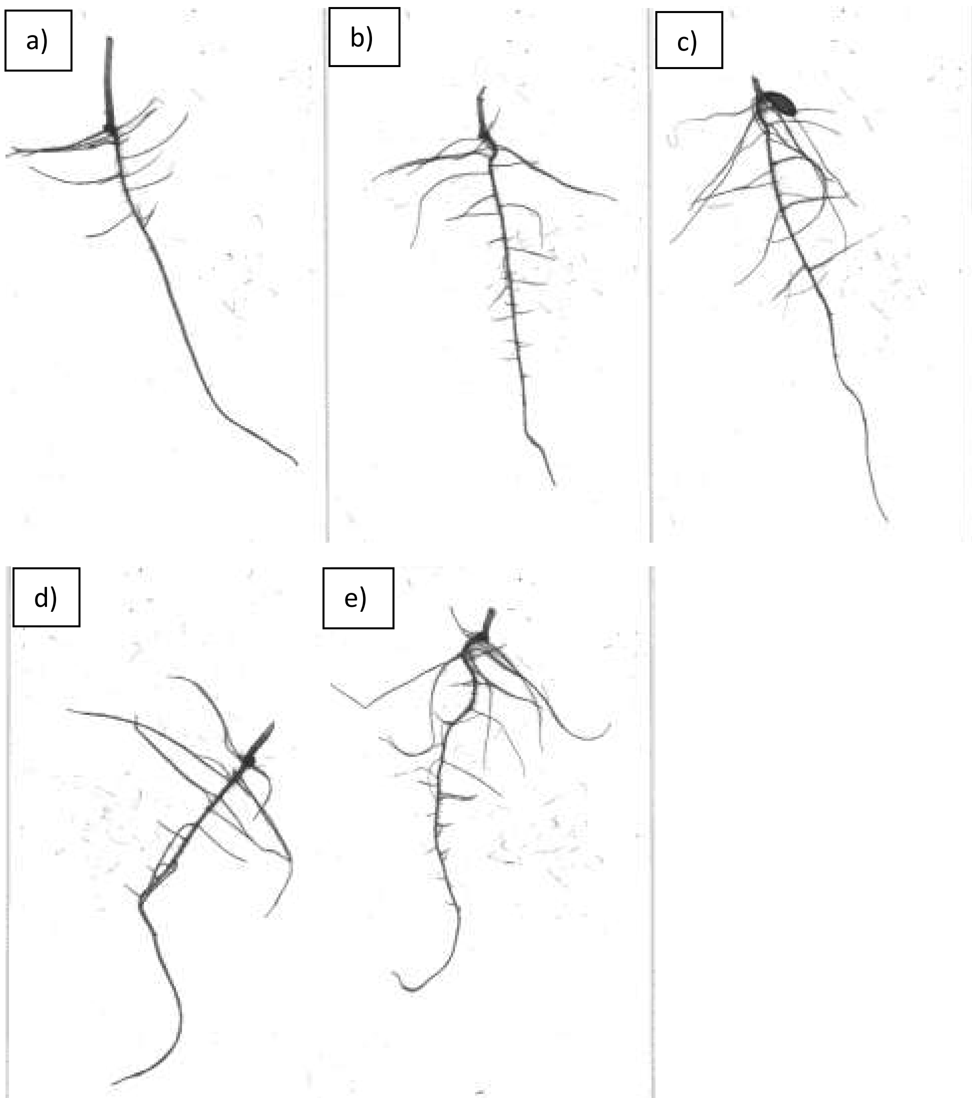

Figure 3. 4. WINRHIZO scanned analysis of early seedling growth for cucumber (*Cucumis sativus* cv. Straight Eight) seeds that had been soaked in 2.5% PA (a)- 0 hours, b)- 3 hours, c)- 6 hours, d)- 9 hours, and e)- 12 hours). From these scanned images, early root growth was highlighted and measured. PA- pyroligneous acid.

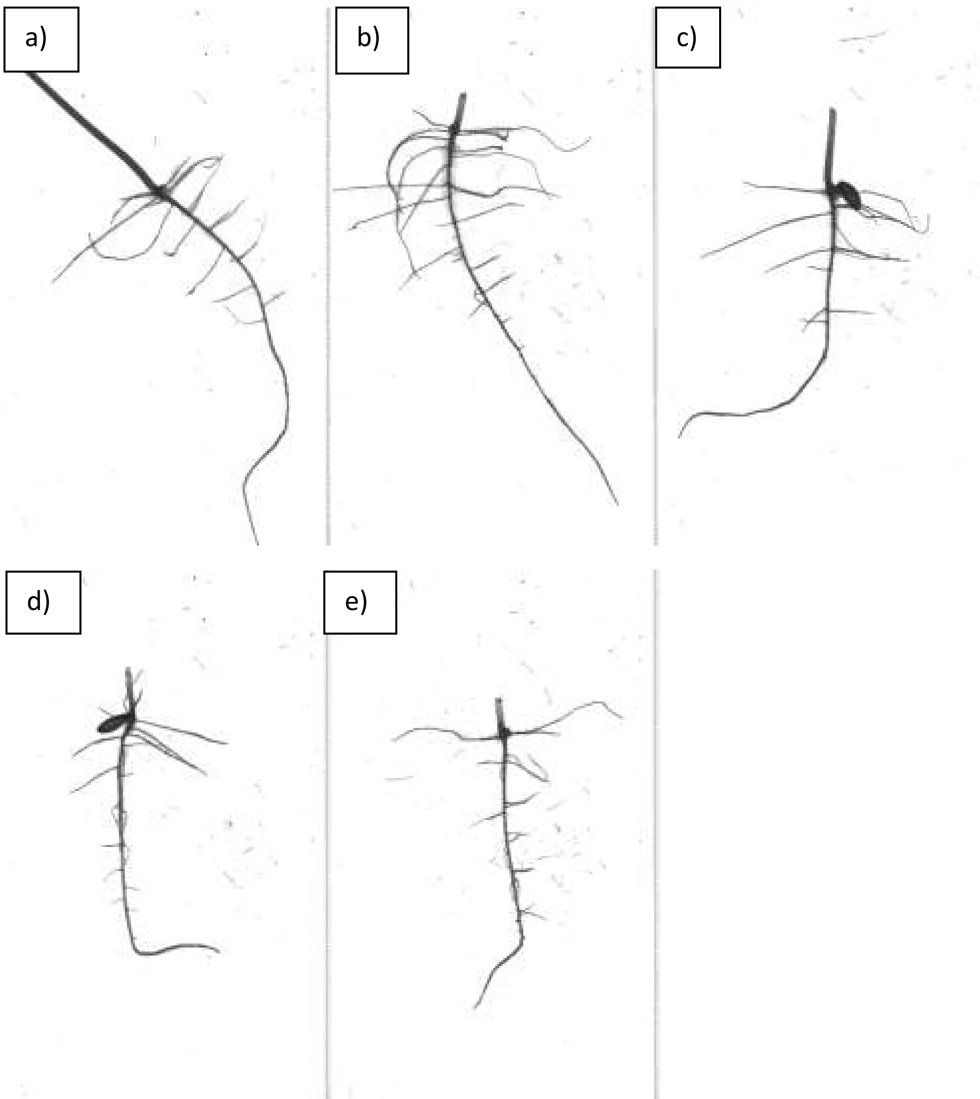

Figure 3. 5. WINRHIZO scanned analysis of early seedling growth for cucumber (*Cucumis sativus* cv. Straight Eight) seeds that had been soaked in 10% PA (a)- 0 hours, b)- 3 hours, c)- 6 hours, d)- 9 hours, and e)- 12 hours). From these scanned images, early root growth was highlighted and measured. PA- pyroligneous acid.

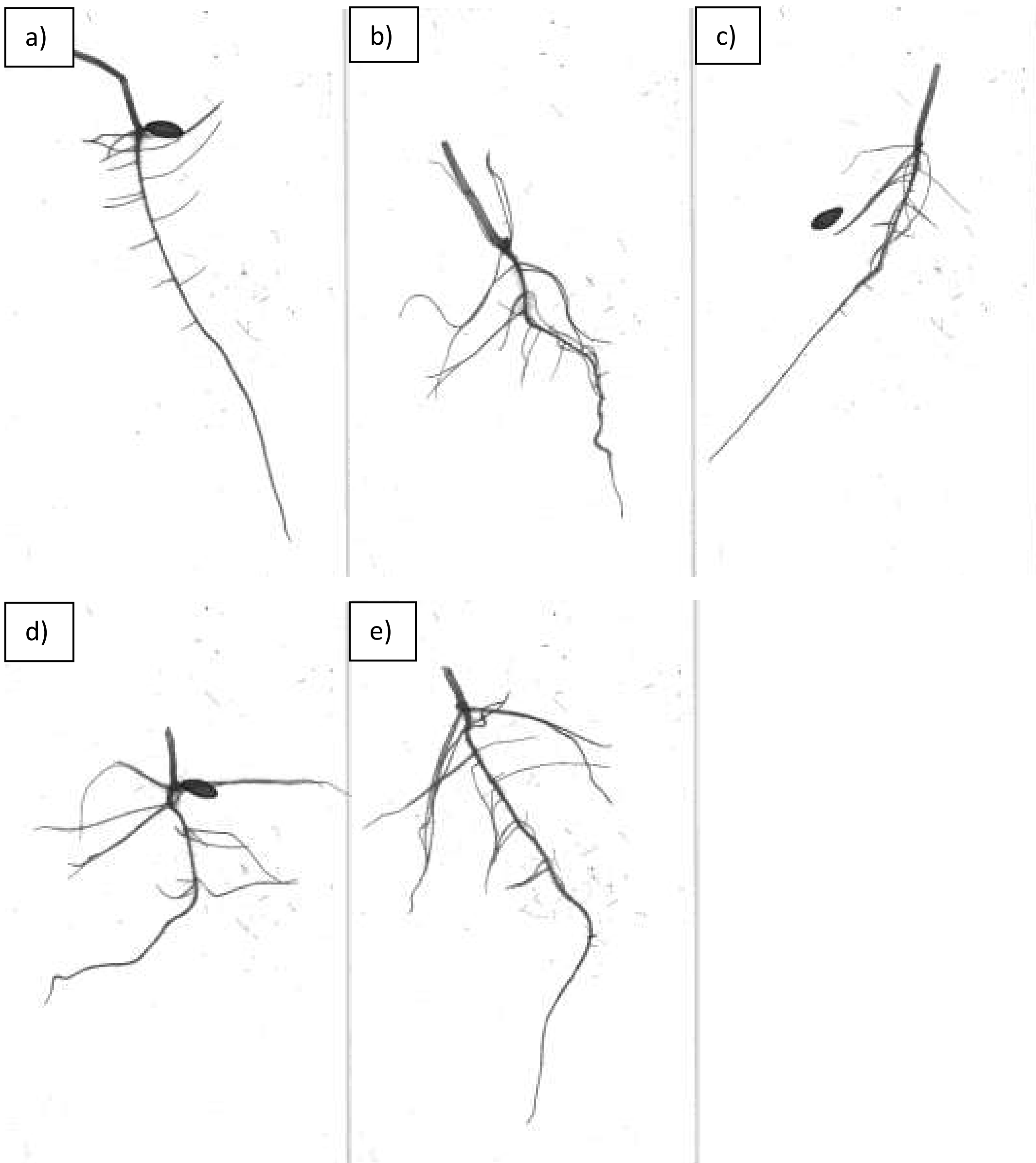

Figure 3. 6. WINRHIZO scanned analysis of early seedling growth for cucumber (*Cucumis sativus* cv. Straight Eight) seeds that had been soaked in 0% PA (distilled water) (a)- 0 hours, b)- 3 hours, c)- 6 hours, d)- 9 hours, and e)- 12 hours). From these scanned images, early root growth was highlighted and measured. PA- pyroligneous acid.

3.5 Conclusion

After analyzing all germination data for cucumber seeds that underwent different PA soaking treatments it was concluded that seeds soaked in lower rates such as 2.5% for longer soaking times (i.e. 9 hrs and 12 hrs) were the most successful PA treatments to provide improved germination. In particular, the 9-hour 2.5% treatment had perfect germination success, as well as the earliest mean germination time, which was significantly ($P < 0.05$) earlier than all control treatments soaked in distilled water besides the 9-hour treatment which did not have as successful germination percentage. The calculated mean germination time for the 9-hour 2.5% treatment was also 0.45-0.78 days faster than those seeds that were not soaked. In addition to this, it is theorized that the PA treated seeds would have further benefits associated with priming including improved reserve and antioxidant activation compared to those seeds that were not primed.

A similar pattern to the germination results was seen with the early root development as well. A pattern in which longer soaking periods (i.e. 9 and 12 hours) at lower PA rates (i.e. 2.5 and 5%) showed improved results. The 12-hour 5% treatment had the greatest response in terms of root length, surface area, and length/volume. This is likely due to the interactions and stimulating features PA had with plant growth regulators within the seeds along with improved seed reserve mobilization due to the priming process and chemical features of the PA. Increased root length values led to smaller root diameters which has shown to be a common trend in plant roots. Based on previous research, it is highly likely that seed reserves in these seedlings would have been greatly depleted at the end of the seven-day experiment time frame and that this may have contributed to less significant differences among treatments. Due to this, it is theorized that increased significant differences would have occurred if seedlings would have been moved into more favourable growing conditions, including transplanted into soil, giving them an external source of nutrients.

When considering both germination and early seedling root growth, the results from this research would suggest a recommendation of 9 or 12 hours soaking in 2.5% or 5% PA as a beginning point for growers or for further research. PA being an organic product can be used as a cheaper and more environmentally friendly method for priming cucumber seeds. In addition to this, evaluating the impact PA has on plant metabolic processes during and post germination could only help fortify the reasoning as to why PA should be chosen as a priming agent for cucumber seeds over other methods. This study may help build and expand the use of PA in agriculture as it has shown promise that PA can improve cucumber germination and early root growth, both of which are essential processes that contribute to successful seedling establishment and in turn, lead to prosperous crop maturation and yield.

3.6 References

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Chapter 4.0: Pyroligneous Acid Effect on Plant Growth, Sex Ratio, & Yield of Field-Grown Cucumber

4.1 Abstract

Typically, the development of cucumber (*Cucumis sativus* L.) flower is skewed towards maleness, which can limit the potential yield of the plant. A field study was carried out to determine the effects of varying concentrations (0%, 2.5%, 5%, 10%) and frequencies (unrepeated, biweekly, and monthly) of foliar applications of pyroligneous acid (PA) on cucumber cv. Straight Eight plant growth, female/male sex ratio and fruit yield. All the PA treatments showed increased number of female flowers relative to males compared to the controls ranging from 5.23-18.02% increase. Monthly applications of 2.5% and 5% PA led to significantly ($P < 0.05$) highest female/male flower ratios compared to control treatments with 41.7% and 40.1%, respectively. This was an approximately 10-17% increase in female flowers compared to the control treatments. The 2.5% monthly treatment gave the highest fruit count per plot at 13, which was significantly ($P < 0.05$) higher than the control treatments. Both the 2.5% and the 5% monthly treatments increased cucumber fruit yield by over 15,000 kg/ha compared to 3763.5 -9345.0 kg/ha for the control treatments. Although 2.5% or 5% monthly application of PA increased fruit yield, further field studies will be required to optimize and validate PA application rate and frequency prior to recommendation.

4.2 Introduction

Many species of cucumber (*Cucumis sativus* L.) have developmental patterns that place limitations on their yield potential. The primary contributing factor to the low yield in cucumber and the other species of cucurbits is the imbalanced sex ratio of their flowers. According to Abbey (2016), cucumber plants are most classified as monoecious, which conveys that the plant develops both male and female flowers. There are other less common sexual lines as well including gynoecious (i.e. only female flowers present), androecious (i.e. only male flowers present), hermaphroditic (i.e. bisexual flowers only), or andromonoecious (i.e. male flowers and bisexual flowers present) but these are rare developmental patterns (Abbey, 2016). Regarding the typical monecious cucumber plant, the frequent issue that arises is that there is an imbalanced sex ratio in favour of male flowers. Typically, in cucurbits the male flowers develop and open first, followed by alternating sequence of female and male flowers which often leads to these imbalanced sex ratios (Ghani *et al.,* 2013). In some cucumber varieties there have been recordings of male:female sex ratios of up to 15-30:1 (Miao *et al.,* 2010; Abbey, 2016). These large male:female sex ratios lead to inevitable lower cucumber fruit set and yields as monecious cucumber plants rely on cross pollination for fruit development (Abbey, 2016). These yield limitations have the potential to reduce farm revenue and along with the ever-increasing need for food security are putting stress on farmers.

Plant growth regulators have been deemed the overall underlying control of plant growth and development including sex determination in cucurbits (Ghani *et al.,* 2013; Abbey, 2016). Related to this, pyroligneous acid (PA) has been shown to influence production and release of many plant growth regulators (Staden *et al.,* 2000). Ethylene may be the most important plant growth regulator for balancing the sex ratios of cucumber plants because it has been linked to female flower development in cucurbits (Wang *et al.,* 2010; Abbey, 2016; Ikram *et al.,* 2017;

Pan *et al.,* 2018). This is due to ethylene causing a cellular response in which deoxyribonucleic acid (DNA) coding for the anther is damaged by a downstream DNAse which is produced when ethylene binds to receptors such as CsEtr1 leading to an overall increase in female sex organ development (Wang *et al.,* 2010; Ikram *et al.,* 2017). Gynoecious cucumber plants (only develop female flowers) have two copies of the CS-ACS1 gene, which has critical roles in ethylene biosynthesis (Shiber *et al.,* 2008; Lee *et al.,* 2018). Also, when cucurbits have been treated with gaseous ethylene or ethylene releasing chemicals such as ethephon this has led to increased female flower development (Rudich *et al.,* 1972; Ando *et al.,* 2001; Chen *et al.,* 2016). Auxins, another female promoting growth regulator has shown to have crosstalk with and positively influence ethylene production (Abts *et al.,* 2017; An, *et al.,* 2020).

Pyroligneous acid is a complex mixture that consists of many compounds including acetic acid, aldehydes, ketones, alcohols, phenols, and a complex mixture of chemicals and elements (Grewal *et al.,* 2018). This complex chemical makeup leads to several agricultural benefits including stimulating germination, improving soil physical and chemical properties, and pests and disease management (Grewal *et al.,* 2018). In addition to these benefits, PA has also been linked to improved plant growth and yield of several plant species such as rock melon (*Cucumis melo*; Zulkarami *et al.,* 2011) and tomato (*Solanum lycopersicum* L.; Mungkunkamchao *et al.,* 2013). It was therefore, hypothesized that PA would improve female flower development which would in turn, improve cucumber yield. This study evaluated the effect of PA application rate (0, 2.5, 5, and 10%) and frequency (unrepeated, biweekly, and monthly) on cucumber plant growth, flower development, and yield.

4.3 Materials and Methods

4.3.1 Location & Materials

The experiment began in the Dalhousie University Faculty of Agriculture greenhouse in June 2019. The greenhouse provided growing conditions for seedlings that were to be transplanted to a field setting. These included daily and night temperatures between 18-32°C and approximately 12 hours of natural light. Cucumber (cv. Straight Eight) seeds were purchased from Halifax Seed, Halifax, NS, Canada and used in this study. The PA used in this experiment was acquired from Proton Inc., California, United States. Pro-Mix BX (pH range: 5.5-6.5, electrical conductivity: 1.3-2.0 mmhos/cm, air porosity: 17-22% by volume, water holding capacity: 700-900% by weight) was used as the potting medium. All pots and seed trays were acquired from Walmart and CO-OP Truro, NS, Canada.

The experiment was continued at the Dalhousie Demonstration Garden, Bible Hill, NS, Canada (45°23′ N, 63°14′ W) during July-September 2019. The soil in which the cucumbers were transplanted was a Pugwash sandy loam and could be classified as OrthicHumo-Ferric Podzol according to the Canadian soil classification (Webb *et al.,* 1991). The previous crop grown in the experimental site was ryegrass (*Lolium multiflorum*) for multiple growing seasons.

4.3.2 Greenhouse Seedlings

Six 36-cell seed trays were prepared by filling each tray with Pro-Mix BX. There was one seed sowed per cell for a total of 216 seedlings raised. The cucumber seedlings were grown for four weeks in the Dalhousie greenhouse. During this time, seedlings were watered daily and given a 2.5 ml per 4L mixture of synthetic fertilizer (nitrogen-phosphorus-potassium 24-8-16) weekly. The PA was not applied to the cucumber seedlings while in the early stages of development in the greenhouse. Once the seedlings reached the four true-leaf stage, they were hardened in an exposed outdoor greenhouse alongside the demonstration garden for five days before being transplanted.

4.3.3 Field Configuration & Treatments

In the field, the cucumber seedlings were arranged in a randomized complete block design with four blocks. There was a total of 12 treatments and 48 plots (4 PA rates x 3 frequency of application x 4 replications) measuring 1 m x 2 m (Figure 4.1).

Figure 4. 1. Straight Eight cucumber plants grown in the Dalhousie demonstration garden to test pyroligneous acid effect on growth, development and fruit yield.

Each of the 48 plots contained four plants (16 plants per treatment) that were grown 30 cm apart with a metre of free space between plots. There were four different rates of PA applied at three different application frequencies in the factorial design with 4 blocks. Plants in each plot were randomly assigned a PA application rate and frequency so that all combinations of experimental units were included. The application rates were: 0% (deionized water), 2.5%, 5%, and 10% as a foliar application (sprayed until runoff) using handheld pump sprayers. The frequencies of PA application were either biweekly (BW), monthly (M), or unrepeated (N) which were given one single spray of the assigned rate on the day the plants were first sprayed as the control. PA application was done until harvesting began. The plants were continuously watered every other day *via* an auto-controlled sprinkler system in the field and the soil was also supplied with an organic fertilizer (nitrogen- 90 kg/ha and potassium at 40 kg/ha) by working it into the top four to six inches of the soil prior to planting. The field was weeded manually each day throughout the course of the experiment. The cucumber plants were grown for an additional six weeks in the field before harvesting began.

4.3.4 Data Collection

4.3.4.1 Soil Sampling & Climate Evaluations

Field soil samples (n=5) were taken from 0-20 cm in five random locations of the experimental plot in the demonstration garden then mixed to form a composite sample. The soil samples were sent to the Nova Scotia Department of Agriculture Laboratory Services in Truro, NS for chemical nutrient analysis. The soil analysis was done prior to transplanting seedlings and after the field experiment concluded. The concentration of plant available nutrients in the soil was determined by an inductively coupled plasma optical emission spectrometer (ICP-OES-725, Agilent, California, USA). During the course of the experiment, daily temperatures, and weather conditions were recorded.

4.3.4.2 Measuring Growth & Yield Response

Throughout the growing process in the field weekly plant measurements and counts were conducted. These values included plant heights, stem girths, and leaf sizes that were measured using a caliper, meter stick, and measuring tape. Chlorophyll a and b as well as anthocyanin concentrations were determined from each fourth leaf using a SPAD meter (Spectrum Technologies Inc., Aurora, USA) and ACM-200 Plus anthocyanin meter (OPTI-SCIENCES, Hudson, USA) following the method found in Zhou et al. (2016). Total flower counts were conducted each week while also noting the number of male and female flowers on each plant. Male and female flowers can be differentiated based on appearance. At the distal end of the cucumber female flower is an obvious embryonic fruit present while male flowers grow with a thin stem (Malepszy & Niemirowicz-Szczytt, 1991). The sex ratios per plant were calculated as [Sex Ratio%= # of male flowers/ # of female flowers]. These measurements were conducted every week until the first harvest was conducted six weeks post-transplanting.

Harvesting began six weeks after the plants were transplanted to the field. For the first harvest, all cucumber fruits that were at the edible harvesting age (≥10 cm in length) were harvested. This process was done continuously for 10 days, after which a final harvest was done. For this final harvest when local temperatures were dropping, all the fruits from the cucumber plants were collected and the experiment was concluded. The harvested fruits were immediately brought to the lab, cleaned, and measurements were taken. These measurements included fruit counts for each treatment, fruit fresh weights measured with an analytical balance, as well as fruit lengths, and fruit diameters done using a ruler and caliper. Fruit weights were converted to yield by first calculating weights for each treatment as grams per 2 m^2 and then extrapolating these values to kg/ha. The fruits were then sent to the Nova Scotia Department of Agriculture Laboratory services for tissue chemical analysis. The harvested data along with the weekly growth measurements were used to run statistical analysis for this experiment.

4.3.5 Statistical Analysis

Statistical analysis for this experiment was done using MINITAB Version 18 (Minitab Inc., Pennsylvania, USA) and Microsoft Excel (Microsoft Corporation, Washington, USA) was used to generate graphs and tables. The significant differences or otherwise of the measured mean values were assessed by using a two-way analysis of variance (ANOVA) at $P \leq 0.05$. Separation of means was also done using Fisher's LSD method to determine significant differences among the calculated means at alpha=0.05 when the ANOVA suggested $P \leq 0.05$.

4.4 Results & Discussion

4.4.1 Climate & Soil Condition

Weather conditions during the experiment were typical for Nova Scotia summer months. Temperatures varied from 16°C-39°C during the day and from 12°C-28°C during the nights. The optimum temperatures for cucumber growth have been determined as 21°C-32°C (day) and 15°C-21°C (night) (Bai *et al.,* 2016). Precipitation totals for the growing period were approximately 110 mm of rain. Plants were not irrigated on days with precipitation above 5mm. Warm, sunny weather with 12 hours of sunlight is optimal for growth, pollination, and yield of cucumber. Based on these recommendations, the cucumber plants development should have not been hindered by weather conditions throughout this experiment.

Relevant data acquired from the soil analysis indicated a soil pH of 6.08, nitrogen % of 0.09, phosphorus (P_2O_5) level of 1294 kg/ha, potassium (K_2O) level of 306 kg/ha, 2179 kg/ha of calcium, 313 kg/ha of magnesium, < 16 kg/ha of sodium, and 12 kg/ha of sulfur. Additional trace elements were also detected in the quantities of ppm. Based on these results a recommendation of 90 kg/ha of nitrogen and 40 kg/ha of potassium (K2O) was made for optimum soil chemical conditions to grow cucumber plants. Overall, based on established optimal growing conditions, the climate and adjusted soil conditions would have been satisfactory for healthy cucumber growth.

4.4.2 Plant Growth & Flower Development

Over the first four weeks post-transplanting growth patterns (Figure 4.2) and final plant height (Figure 4.3) results showed that there were no significant ($P > 0.05$) differences amongst treatments. The non-significant results occurred despite PA having a great number of chemical compounds and had shown to be a successful growth promoter in many other studies (Mu *et al.,* 2006; Zulkarami *et al.,* 2011; Grewal *et al.,* 2018). This may be due to differences in application method, PA product, and/or plant species. All plants had similar growth rates per week and the highest average plant height at the final measuring period was 5% PA unrepeated plants at 89.0 cm while the least average plant height was recorded by the 2.5% PA biweekly treated plants at 82.56 cm (Figure 4.3). It was noted that although PA did not significantly ($P > 0.05$) influence plant growth, the yield and quality of fruits will be most valuable for farmers to increase revenue.

In addition to plant height, there were also no significant differences ($P > 0.05$) among treatments for measured leaf sizes or stem girths (Table 4.1). There were also no significant differences in chlorophyll and anthocyanin measurements at $P > 0.05$ (Table 4.2). This implies that there were no changes in the role of chlorophyll in photosynthesis and no obvious stress being placed on the plants due to PA application (Genty & Meyer, 1995; Chalker-Scott, 1999; Kovinich *et al.,* 2015).

Table 4. 1. Two-way analysis of variance (ANOVA) P-values acquired for main and interaction effects (P=0.05) of rate (%) and frequency of pyroligneous acid application on average leaf size and stem girth measurements of cucumber (Cucumis sativus cv. Straight Eight) plants.

Model Terms	Leaf Size P-Value	Stem Girth P-Value
Rate (%)	0.726	0.428
Frequency	0.514	0.659
Rate * Frequency	0.874	0.772

Table 4. 2. Two-way analysis of variance (ANOVA) P-values acquired for main and interaction effects (P=0.05) of rate (%) and frequency of pyroligneous acid application on average chlorophyll and anthocyanin contents of cucumber (Cucumis sativus cv. Straight Eight) plants.

Model Terms	Chlorophyll Reading P-value	Anthocyanin Reading P-value
Rate (%)	0.842	0.639
Frequency	0.903	0.396
Rate * Frequency	0.549	0.536

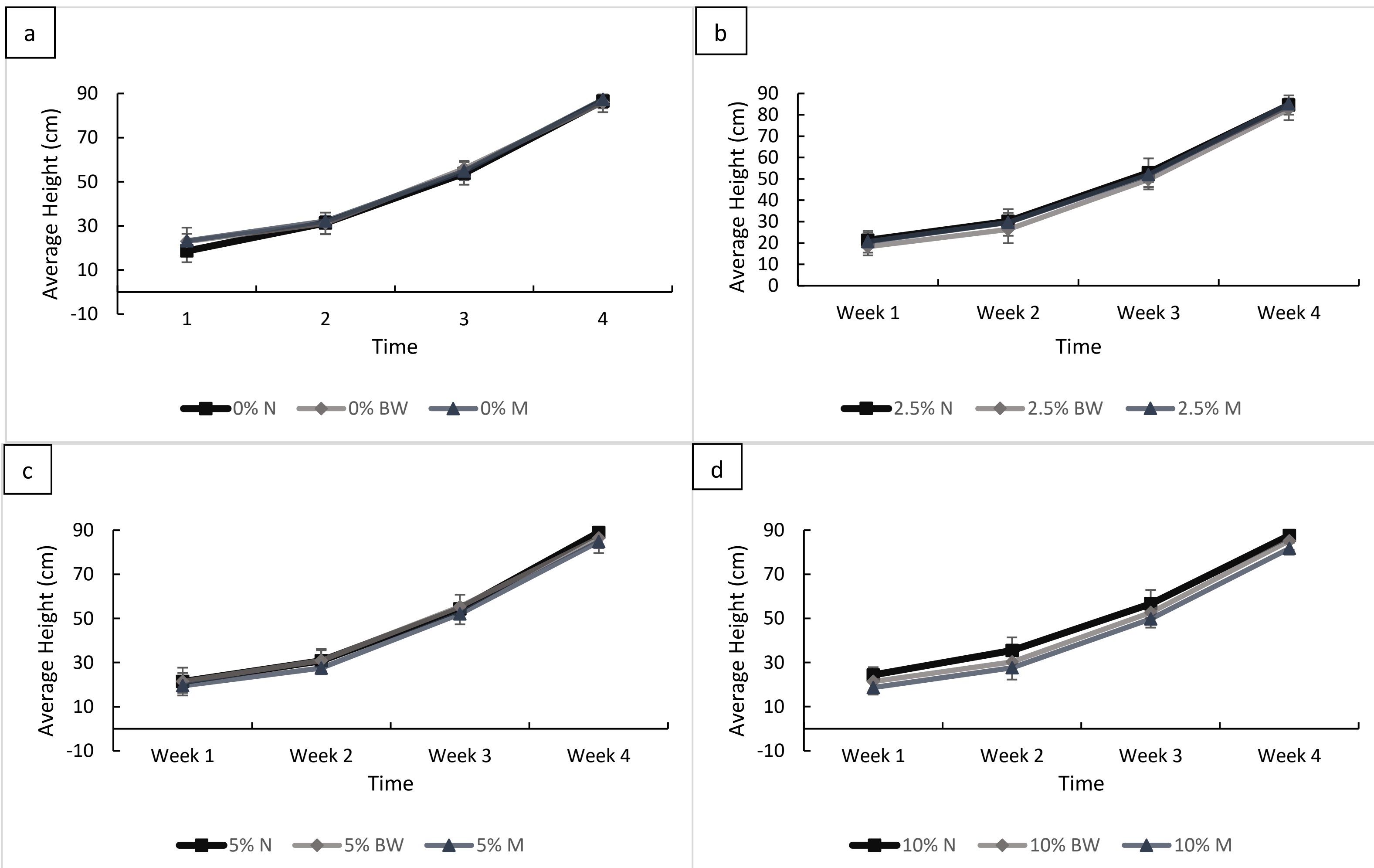

Figure 4. 2. Cucumber (*Cucumis sativus* cv. Straight Eight) plant height over a four-week growing period as affected by varying rates and frequencies of pyroligneous acid (PA) application (a-0%, b-2.5%, c-5%, d-10%). N-Unrepeated, BW- Biweekly, M-Monthly.

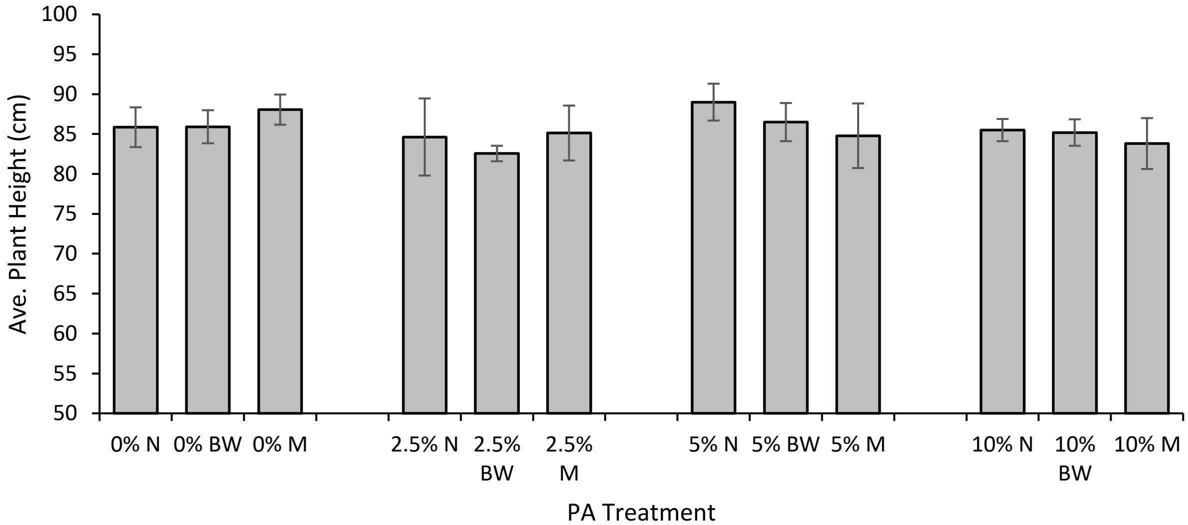

Figure 4. 3. Mean plant height at final harvest of cucumber (*Cucumis sativus* cv. Straight Eight) plants treated with differing PA concentrations and frequencies grown under field conditions. PA- pyroligneous acid, N- unrepeated, BW- biweekly, M- monthly.

Flower development during the six-week growing period gave compelling results (Figure 4.4 & Table 4.3). Cucumber plants followed the typical flower development pattern i.e., male flowers first followed by alternating pattern of female and male flowers. However, as indicated in Figure 4.4, total female flower counts, and percentages were improved on plants treated with PA compared to those that served as controls. The effects of application rate ($P=0.002$) and frequency ($P = 0.00$) and interaction ($P = 0.00$) significantly impacted female flower percentage at $P = 0.05$. The 10% biweekly treatment produced the least female to male flowers (36.3%) for all PA treatments, which was still 5.8% higher than the highest response for the control treatments which was 30.5% for the 10% never repeated plants. The 2.5% monthly and 5% monthly treatments showed the highest female flower percentages with 41.47% and 40.1% respectively. These were significantly ($P < 0.05$) higher female flower percentages than all the control treatments which had female flower percentages in the range of 23.45%-30.5%. These results indicated a greater response to lower rates compared to the higher rates and controls and the monthly applications showed improvements compared to biweekly and unrepeated treatments. Cucumber plants with increased female flower counts have the capacity for improved fruiting and plant yield.

Table 4. 3. Mean female flower percentages based on PA application rate applied to field cucumber (*Cucumis sativus* cv. Straight Eight) plants. PA- pyroligneous acid.

PA application rate	N	Mean
5%	16	35.39 [a]
2.5%	16	34.77 [a]
10%	16	34.51 [a]
control	16	25.81 [b]

** Means sharing the same letter within each column are not significantly different at the 5% level of significance.

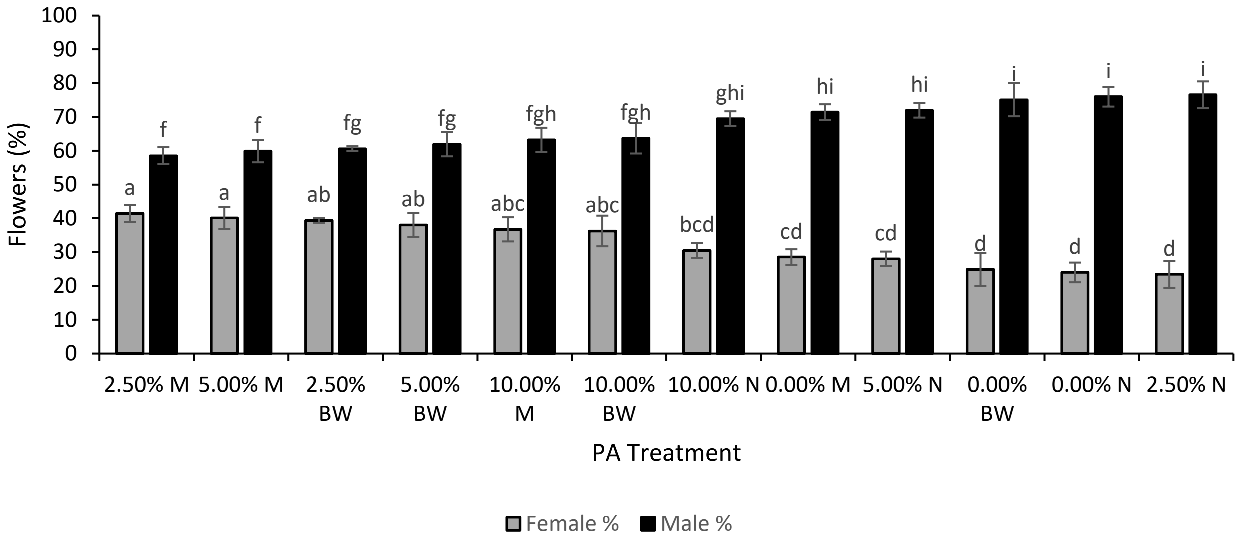

Figure 4. 4. Mean female flower percentage (%) compared to total mean male flower % for cucumber (*Cucumis sativus* cv. Straight Eight) plants treated with different rates of PA at differing frequencies under field conditions. PA- pyroligneous acid, M- monthly, BW- biweekly, N- unrepeated.

Female flower development in cucurbits has been highly studied and has been greatly linked to the plant growth regulator ethylene (Wang *et al.,* 2010; Abbey, 2016; Pan *et al.,* 2018). This is due to a downstream response from ethylene receptor binding which includes the release of a DNAse which damages anther-coding DNA allowing for further female sex organ development. Pyroligneous acid has also been shown to have close interactions with and stimulating effects on plant growth regulators such as ethylene, auxins, and gibberellins (Staden *et al.,* 2010; Zulkarami *et al.,* 2011; Zhai *et al.,* 2015; Grewal *et al.,* 2018). Male flowers were still the dominant sex in all treatments in this experiment, indicating that gibberellins were still strongly influencing flower development (Gupta & Chakrabarty, 2013). However, based on previous knowledge of plant growth regulators along with the results observed in this study, it can be surmised that foliar application of PA, especially at lower rates (2.5 and 5%) had positive influence on ethylene production in the cucumbers which would have influenced the biological response of increased female flower production. In addition to this, although no fruit dropping and minimal flower dropping was observed in this study it's important to consider that ethylene has roles in fruit ripening and senescence of leaves and flowers (Iqbal *et al.,* 2017). This could have an impact on flower and fruit counts. Further work will be required to confirm the impacts PA has on ethylene production and its biological response in cucumber plants.

Pyroligneous acid is much cheaper and more practical to apply to cucumber plants than gaseous ethylene and many of its derivatives (Shakar *et al.,* 2016a). Furthermore, one propitious theory as to why PA promoted ethylene production and in turn, female flower other than plant growth components was the chemical makeup and in particular, the considerable quantity of calcium in the PA used in this study (Table 4.4). Calcium has been strongly associated with endogenous ethylene production in plants including cucumber. According to Shakar et al. (2016) calcium carbide (CaC_2) treated seeds enhanced germination and root growth of cucumber seedlings with success being strongly correlated to ethylene production. They also showed that lower concentrations of CaC_2 promoted the development of early female flowers in cucumbers, which increased total fruit yield potential (Shakar *et al.,* 2016b). This is similar to the results of this study in that the 2.5% and the 5% PA had the highest response compared to higher rates and control treatments. Ferguson (1983) deemed calcium a stimulant for aminocyclopropane-1-carboxylic acid (ACC) dependent ethylene in mung bean (*Vigna radiata*) and cucumber plants. Furthermore, Yu *et al*. (2019) demonstrated that calcium played a major role in endogenous ethylene production and triggering of ethylene signal transduction pathway. Another possibility is that applying PA increased auxin production in the plants, which directly impacted ethylene production (Ayankanmi & Agele, 2010). Auxins are closely tied to ethylene production because the CSACS1 gene has shown to be inducible by auxins (Trebitsh *et al.,* 1987; Yamasaki *et al.,* 2000). It has also been suggested that auxin treatments have stimulated ethylene production by ensuring that ACC is used for ethylene production rather than malonyl-ACC (Abts *et al.,* 2017). Although the mechanism is not completely clear, this study has shown that PA can promote female flower production in cucumber plants. Like typical hormonal treatments and previous PA studies, the results also showed that PA is most effective at lower concentration (Mu *et al.,* 2003; Shakar *et al.,* 2016b). Further analysis on ethylene concentration in cucumber plants after PA application will need to be conducted to fully support the suggested theory.

Table 4. 4. Chemical analysis of non-distilled PA produced by Proton Inc. and utilized as a biostimulant on cucumber (Cucumis sativus cv. Straight Eight) seeds.

Analysis Performed	Non-distilled PA
Phosphorus %	2.86
Potassium %	13.47
Calcium %	34.66
Magnesium %	1.64
Copper ppm	0.01
Zinc ppm	5.97
Boron ppm	<0.02
Cadmium ppm	0.073
Chloride ppm	370.00
Conductivity umhos/cm	2120.00
Chromium ppm	0.03
Iron ppm	1.26
Manganese ppm	0.60
Sodium %	5.73
Nickel ppm	0.46
Nitrate-N %	0.35
Lead ppm	0.06
Sulfate ppm	89.95
pH	3.05
Alkalinity ppm	162.71
Arsenic ppm	-

4.4.3 Fruit Harvest

The improved female flowers on cucumber plants by PA is a promising and appealing phenomenon for improving fruit set, yield and economic output. The pivotal concept of monoecious cucumber plants producing greater yields in this frame of study was to provide a greater balance to the flower sexes by increasing female flower production. However, diminishing male flower percentage to an excessive degree may also have a negative impact on yield due to incapability to sufficiently pollinate the crop (Arancibia *et al.,* 2017). For that reason, although there were obvious improvements in female flower development, it was important to continue this study into the fruit development stage. As theorized, the two treatments with significantly ($P < 0.05$) highest female flower percentages (i.e., 2.5% monthly and 5% monthly applications) led to an increase in fruit counts per plot after harvest. The 2.5% and 5% monthly treatments led to an average of 13 cucumbers more that was harvested per plot, a value that was significantly ($P < 0.05$) greater than control treatment (Table 4.6). This implies that improved proportion of female flower positively impacted fruit production. Consequently, PA application would be a much cheaper and simpler method to achieve such an outcome rather than purchasing expensive gynoecious seed varieties and planting a mixture of these and monoecious plants as suggested in the literature (Arancibia *et al.,* 2017).

Table 4. 5. Two-way analysis of variance (ANOVA) P-values acquired for main and interaction effects (P=0.05) of rate (%) and frequency of pyroligneous acid application on cucumber (Cucumis sativus cv. Straight Eight) plants harvested fruits measured weight, length, and diameter.

Measured Fruit Quality	**Rate (%) P-value**	**Frequency (%) P-value**	**Rate * Frequency P-value**
Weight (g)	0.893	0.167	0.678
Length (cm)	0.582	0.214	0.622
Diameter (cm)	0.880	0.241	0.648

Table 4. 6. Average harvested fruit counts per plot, mean fruit weights, lengths, and diameters for fruits harvested from cucumber (*Cucumis sativus* cv. Straight Eight) plants treated with differing rates and frequencies of PA.

Treatment	Fruit Count	Fruit Weight (g)	Fruit Length (cm)	Fruit Diameter (cm)
0% N	7.00 ^{de}	179.91 ^{a}	13.63 ^{a}	4.35 ^{a}
0% BW	6.00 ^{e}	226.45 ^{a}	14.69 ^{a}	5.05 ^{a}
0% M	7.00 ^{de}	250.55 ^{a}	15.59 ^{a}	4.92 ^{a}
2.5% N	4.00 ^{f}	172.40 ^{a}	14.78 ^{a}	4.52 ^{a}
2.5% BW	10.00 ^{b}	239.50 ^{a}	15.58 ^{a}	4.59 ^{a}
2.5% M	13.00 ^{a}	234.91 ^{a}	15.90 ^{a}	4.86 ^{a}
5% N	8.00 ^{cd}	234.66 ^{a}	15.95 ^{a}	4.69 ^{a}
5% BW	9.00 ^{bc}	201.98 ^{a}	14.39 ^{a}	4.61 ^{a}
5% M	13.00 ^{a}	247.10 ^{a}	15.61 ^{a}	5.19 ^{a}
10% N	8.00 ^{cd}	231.37 ^{a}	14.95 ^{a}	4.97 ^{a}
10% BW	10.00 ^{b}	216.44 ^{a}	14.98 ^{a}	4.58 ^{a}
10% M	8.00 ^{cd}	250.06 ^{a}	15.63 ^{a}	4.93 ^{a}

* Means sharing the same letter within each column are not significantly different at the 5% level of significance. PA- pyroligneous acid, N- unrepeated, BW- biweekly, M-monthly.

There was no significant difference ($P > 0.05$) in the harvested cucumber fruit weights for the different treatments (Table 4.5 & Table 4.6). The fruit weights ranged from 179.91 g for the 0% untreated to 250.55 g for the 0% monthly treatment. Although there was no significant ($P > 0.05$) difference, the 10% monthly treatment produced the highest fruit weight on average compared to all the other PA treated plants. Cucumber fruit lengths and diameters were all similar across treatments as P values calculated from the ANOVA were $P > 0.05$ (Table 4.6). These results suggested that the increase in fruit bearing on the plants treated with PA did not come with any sacrifices in terms of fruit physical size.

Cucumber fruit yield was estimated from the fresh fruit weight and fruit setting values for each treatment (Table 4.7). The significantly ($P < 0.05$) highest fruit yields were from the 2.5% and 5% monthly PA treatments with 15,210 kg/ha and 16,002.7 kg/ha, respectively, which were

5,865.1 and 6,657.7 kg/ha to 11,446.6 and 12,239.2 kg/ha higher than the control treatments, respectively. This can be attributed to the increased fruit setting from the PA treated plants. According to Government Canada, slicing cucumber yields are typically in the range of 6000-20,000 kg/ha and according to Statistics Canada, field cucumber yields were approximately 9412 kg/ha in Nova Scotia (Statistics Canada, 2012). The calculated yields for PA treated plants in this experiment were greater than this calculated Nova Scotian yield and given a longer harvesting period, it is likely the calculated yields from the PA treated plants would have surpassed the upper values of average Canadian calculated yields as well. Along with the significant differences that were detected between treatments at P = 0.05, PA treated plants had higher calculated yields. The lowest calculated yield being 3763.5 kg/ha for the 2.5% PA unrepeated plants. This data reinforces the importance and significance that improved sex ratios can have on cucumber production and potential economic returns.

Table 4. 7. Average calculated yield (kg/ha) of cucumber (*Cucumis sativus* cv. Straight Eight) fruit based on harvest numbers collected from cucumber plants that were treated with varying rates and frequencies of PA.

Treatment	Yield (kg/ha)
0% N	7463.3 ^{b}
0% BW	6968.9 ^{b}
0% M	9236.4 ^{ab}
2.5% N	3763.5 ^{b}
2.5% BW	11000.8 ^{ab}
2.5% M	15210.1 ^{a}
5% N	8827.4 ^{ab}
5% BW	8753.5 ^{ab}
5% M	16002.7 ^{a}
10% N	9345.0 ^{ab}
10% BW	10897.0 ^{ab}
10% M	9793.7 ^{ab}

*Means sharing the same letter are not significantly different at the 5% level of significance. PA- pyroligneus acid, N- unrepeated, BW- biweekly, M- monthly.

4.5 Conclusion

The results of the present study showed that PA has a positive impact on female flower production at the proper rate and frequency, significantly ($P < 0.05$) improving the proportion of female flowers compared to control treatments. These results should be reproduced in further studies to fully strengthen these claims. Based on previous knowledge on ethylene's impact on female flower production (Ando *et al.,* 2001; Wang *et al.,* 2010; Abbey, 2016; Pan *et al.,* 2018), it is suggested that the foliar PA application during this experiment stimulated the cucumber plant's ethylene production either directly or perhaps through crosstalk with additional plant growth regulators such as auxins. The 2.5% and 5% monthly treatments generated the significantly ($P < 0.05$) highest female flower percentage with a 10% increase compared to the control response. The increased proportion of female flowers augmented the number of fruits at harvest in addition to improving fruit yield. Due to this, 2.5% and 5% monthly foliar applications of PA would serve as recommended treatments moving forward with further research on PA impact on cucumber flower sex development and the potential to improve fruit yield. Pyroligneous acid has been shown as a promising biostimulant for plant growth in other studies, it was confirmed in this study. Even with no significant impact by PA application on plant growth parameters, female flower development, fruit setting, and fruit yield were significantly improved which could potentially have a positive impact on economic returns. The improved female flower development and yield results from this study were of the most interest because it provided the emergence to a potential solution to solving an economically important issue in monoecious cucumber varieties. It also demonstrated another impactful use for PA in agriculture.

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Chapter 5.0: Conclusion & Recommendations

5.1 Overview

Pyroligneous acid (PA) has shown great promise as a biostimulant for many agronomic crops through stimulation of seed germination, plant growth, and yield (Zulkarami *et al.,* 2011; Mungkunkamchao *et al.,* 2013; Grewal *et al.,* 2018). These effects have been mainly attributed to the complex chemical nature of PA and its capability to interact with, and to stimulate numerous plant growth regulators (Staden *et al.,* 2010; Grewal *et al.,* 2018). The current study evaluated the impacts of different rates and soaking times of PA had on seeds of cucumber cv. Straight Eight in terms of germination success and early seedling root growth. These same rates were also applied to maturing cucumber plants to evaluate the impact it had on growth, female/male flower sex ratio, and plant yield. As is the case for many hormonal treatments, PA application in this study showed its greatest success at lower rates. The PA treatments improved seed germination, seedling root growth, female/male sex ratios, and fruit development. Therefore, the research objectives were met in this study. Improvements in plant development, fruit setting, and subsequent yields should allow for considerable positive impact in the cucumber fruit market. It was also theorized that seeds that were primed with PA would have improved antioxidant response, vigor, and other beneficial properties associated with seed priming and it was theorized that if the seedlings were transplanted in a soil medium providing the plant with external nutrients, the root morphological differences among treatments would have been more significant.

As scientific research continues to focus on methods to improve crop yields to maintain pace with the ever-growing population, new techniques and materials will be implemented. Pyroligneous acid has tremendous promise to have global implications in the near future. The main limitation currently with PA is lack of knowledge and consistency with product. However, as the quantity of credible studies using PA as a successful biostimulant increases, so too will the interest of companies and funding. The pyrolysis method will be utilized beyond primarily for the formation of charcoal and PA will become one of the main products. PA can provide a cheaper and environmentally friendly method that can replace current plant growth stimulating methods. This research was done in hopes of not only improving yield potential and economic value of cucumber but also to add knowledge to the field of agriculture as a whole. Additional information on PA's impact in the world of agriculture will only continue to ensure that PA has the best chance of becoming a well known and methodical biostimulant.

5.2 Future Recommendations

Future considerations that can add to the knowledge gained from these experiments include but are not limited to:

- Evaluate and gain further knowledge on mechanisms behind the interactions and promoting properties PA has on plant growth regulators throughout all stages of cucumber plant development.
- Assess the impact PA seed priming has on antioxidant response and seedling vigor. This can be tested by growing the plants in several types of unfavourable conditions and comparing them to plants grown in favourable conditions.
- As suggested in Chapter 3.0, grow seedlings under nutrient sufficient conditions after seed reserves have depleted to evaluate hypothesis that root growth would show further improvements and benefits from PA-soaked seeds.
- Evaluate the effects of PA application throughout the entire cucumber plant development (from seed to maturity) to identify if benefits are amplified.
- Investigate alternative PA rates and application methods in field grown cucumber.

- In addition to fruit yield, further assess how PA application impacts fruit quality (appearance and nutritional).
- Extend PA application into alternate cultivars of cucumber and other species of cucurbit.

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