Investigating Tank Mix Partners to Improve the Efficacy of Manuka Oil as a Herbicide for Organic Vegetable Production.

by
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ABSTRACT

INVESTIGATING TANK MIX PARTNERS TO IMPROVING THE EFFICACY OF MANUKA OIL AS A HERBICIDE FOR ORGANIC VEGETABLE PRODUCTION.

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Manuka oil (MO) is an essential oil derived from *Leptospermum scoparium*, a member of the Myrtaceae family. The active compound, leptospermone, exhibits pre (PRE) and post-emergent (POST) herbicidal activity. The objective of this project was to explore the PRE and POST efficacy of MO and investigate ways of enhancing its efficacy via tank mixes with adjuvants and/or surfactants or other organic herbicides. Field experiments were conducted on two morphologically different crops, at two different locations, and two planting dates. Efficacy was measured on the basis of visual efficacy and crop injury ratings, weed biomass, and crop yields. Growth room experiments were conducted to analyze the PRE efficacy of MO on green pigweed and large crabgrass. MO was tank mixed with surfactants to create four different herbicide combinations tested under different rain conditions. Weed emergence and weed biomass values were measured to determine efficacy. Results determined that MO had limited efficacy.

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Chapter 1.0 Introduction

Manuka oil is a plant derived essential oil from the manuka tree (*Leptospermum scoparium*), which is a member of the Myrtaceae family (Stefanello et al. 2011). Manuka trees are tropical trees that are related to the thin leafed tea tree (*Melaleuca alternifolia*), eucalyptus (*Eucalyptus sp.)* and bottlebrush tree (*Callistemon sp.*). Many trees in the Myrtaceae family produce essential oils that contain beta-triketones, which are released by the tree's roots into the surrounding soil to deter plant competition (Reichling et al. 2005). Dayan et al. (2007) believes that leptospermone is the primary beta-triketone responsible for the herbicidal properties of MO. Research by Dayan *et al.* (2011) found that MO soil applied at 1% (v/v) to growth room pots seeded with large crabgrass was able to have substantial PRE-emergent efficacy. Therefore it is believed that MO has soil residual activity and possesses PRE-emergent (PRE) herbicidal properties as well as POST-emergent (POST) herbicidal properties (Dayan et al. 2011; Dayan et al. 2007). These unique natural properties allow MO to exhibit herbicidal efficacy without undergoing synthetic manipulation, which meets the standards set out by the Canadian Organic Products Regulations (Canadian Agriculture Products Act 2009). This is important because there are currently very limited POST organic herbicides on the market and none with PRE herbicidal properties. Research to explore new potential organic herbicides is important to provide farmers with additional options to tackle crop protection issues. Research into organic agriculture and organic crop protection is extremely limited, especially when comparing it to the conventional sector. More research is needed on organic agriculture to support advances in the sector that are scientifically supported.

Two experiments were conducted to analyze the PRE and POST efficacy of MO either applied alone or tank mixed. The tank mix partners were either essential oils that comprise commercially available organic herbicides and/or surfactants. Field experiments were conducted in Ontario in the growing season of 2015 at two locations (Simcoe and Ridgetown), on two different crops (sweet corn and tomatoes) and at two planting dates (early and late). There were 10 tank mix partners; MO and Nu Film P, MO and yucca extract, MO and Agral 90, MO, clove and cinnamon oil, MO and citrus oil. These were tested against two controls (weedy and weed free) and a conventional industry standard (Callisto®). Applications were either applied PRE or POST and efficacy was determined based on visual weed efficacy, visual crop injury, final weed biomass, and crop yields. PRE efficacy was difficult to capture in the field experiments; therefore, a growth room experiment was conducted. The growth room experiment examined PRE efficacy on MO alone or with three other surfactants (Nu Film P®, Agral 90® or yucca extract) against one control and the conventional industry standard (Callisto®).

Chapter 2.0. Literature review

2.1. Herbicides

Herbicides are effectively used in agriculture, forestry, and urban gardening to eradicate weeds, shrubs and unwanted vegetation. The reason for using herbicides is their efficiency and affordability as compared to the labour costs of manual weed removal (Lanini and Strange 1994). It has been estimated that if the use of herbicides were discontinued in the United States, annual crop production would immediately decrease by about 20% (Timmons 2005). Considerable benefits can be derived through the use of herbicides, but if herbicides are not used properly, they can cause substantial damage to the crop, the environment and even to human health (Dumas et al. 2017). Crop damage can occur when proper label rates and application instructions are not followed. Environmental and human health issues are a concern for most conventional herbicides especially to non-target organisms or concentrated exposure to farm workers (Arcury et al. 2013; Preibisch and Binford 2007). All conventional herbicides are legally required to state the toxicology profile of the commercial product due to these inherent toxicology concerns. The Callisto label extensively reports the toxicology information for human and animal exposure by listing the lethal dosage values for aquatic and terrestrial life (Syngenta Canada 2016). Acute toxicity can occur with direct contact in bees at > 100 g/bee, Trout when exposed to > 120 ppm for 96 hours and Mallard Ducks (8-day dietary exposure) at >5,200 ppm (Syngenta Canada 2016).

Prior to the modern synthetic herbicide era, farmers had experimented with numerous concoctions and strategies to manage crop pests including weeds, insects, and pathogens. Much of the historical knowledge of primitive weed control is depicted in poems, artwork, and biblical verses. Luke 6:44 states, “For men do not gather figs from thorns, nor do they pick grapes from a briar bush.” This verse establishes the desire for certain plants and the disdain for unwanted weeds. Palaeolithic rock paintings in Spain and France that date back 40,000 years as well as numerous other rock paintings display many images of known highly valued plants, but are void of common weeds (Froese et al. 2013; McDonald and Veth 2012; Pike et al 2012). Many ancient coins and sculptures pay tribute to prized food or medicinal plants while associating undesirable weeds with bad omens and illustrate the historic struggle between humanity and weeds (Gray 2011; Howgego 1995). The historical struggle has been recorded, but the methods to control agriculture pests, particularly weeds, are more evident in recent times. Timmons (2005) noted that the use of homemade pesticides has been recorded in literature since at least the 1800’s. However, this is a difficult timeline to predict, as many farmers would lack an ability to properly record their management practices. Timmons (1970) also identified recorded evidence of agricultural weed management techniques as early as 1200 A.D. It is speculated that active weed control practices could go as far back as the Neolithic period when humanity switched from hunter-gathered to an agrarian society (Timmons 2005; Timmons 1970). Timmons (1970) stated that the majority of early weed control techniques were labour intensive and involved soil tillage, hand pulling and hoeing. Records of the more modern idea of applying an agent to control agricultural pests only traced back to the 19th century. In 1821, the London Horticultural Society described sulphur as the only specific remedy for treating mildew on peaches (Martin and Woodcock 1983) and mixtures with alcohol have been reported to be in early pesticide formulas (Timmons 2005; Timmons 1970). Similarly, records have indicated that farmers applied copper products to control powdery mildew on vines in France since at least 1848 (Ordish 1976; Martin and Woodcock 1983; Lang and Clutterbuck 1991) and many organic farmers still use copper-based products as fungicides (Nemecek et al. 2011; Schmid et al. 2011). Interestingly, DDT (dichlorodiphenyltrichloroethane) was originally synthesized in 1874, but it was not until the 1930's that it was utilized as a very effective insecticide (Abreu-Villaça and Levin 2017). Around 1890, copper sulphate was discovered to be a selective herbicide and was later replaced with ferrous sulphate (Lang and Clutterbuck 1991). Presently, the prominent organic selective herbicide for turf is Fiesta®, in which the active ingredient is chelated iron and its discovery is a result of the historical use of iron-based compounds as pesticides (Canadian Agriculture Products Act 2009; Gauvrit and Cabanne 1993). Many of these early pesticides established a framework for the development of more effective modern herbicides.

Modern synthetic herbicides revolutionized the agriculture industry shortly after World War II. This was because many scientific advances during the war were transferred into the agriculture industry after the end of the war. Nitrogen-based explosives provided the science and facilities to produce synthetic nitrogen fertilizers and weaponized aerial sprays paved the way for chemical herbicides (Rasmussen 2001). With the development of synthetic pesticides, the use of pesticides rose tremendously especially throughout the 1960's and 1990's (Appleby 2005; Nazarko et al. 2005). Timmons (1970) reported that from the beginning of the 20th century until

1969, 120 new conventional herbicides were commercially used. Appleby (2005) noted that from 1970 until 2005, 184 new herbicides were introduced with 30% and 40% of total herbicides begin introduced between the 1960-1970's and 1980-1990's, respectively. Tilman et al. (2002) reported that global pesticides imports increased from ~$1 billion (US) to over 11 billion (US) between 1960 and 2000. The increase in registered herbicides and the rapid adoption of their use is due to the tremendous benefit they provide the farmer. Oerke (2006) estimated that from 2001 to 2003 global crop losses from weeds were potentially 18-29% (wheat), 34-47% (rice) 37-44% (corn), 35-40% (soybeans), and 35-39% (cotton) for some of the most economically important crops. These values drop to 3-13% (wheat), 6-16% (rice), 5-19% (corn), 5-16% (soybeans), and 3-13% (cotton) when herbicides are incorporated into management practices. Applying herbicide is a much more cost effective and logistically feasible choice for farmers. Nazarko et al. (2005) discovered that herbicide applications can help farms reduced soil tillage, which has been linked to being more sustainable. Additionally, Gianessi (2013) estimated that farm cultivation and plow equipment required 4 and 17 times, respectively, more diesel fuel compared to a pesticide sprayer. It was also noted that in 2001 without herbicides to manage weeds in Japanese paddy fields it would require ~9 weeks of almost 2 million people performing manual labour to maintain yields. Lanini and Strange (1994) compared the economics of synthetic spray applications against manual hand weeding in *Capsicum annuum* and found that the high value of the crop was able to justify a larger threshold for weed management costs. The higher threshold would allow a limited amount of hand pulling. However, one application of a synthetic herbicide was able to provide returns equivalent to weeks of manual skilled labour. Effective pesticide sprays are able to reduce time, labour, and costs associated with weed management practices while minimizing yield loss.

Although synthetic pesticide provide operational benefits and yield protection these benefits come at a cost to the health and safety of people, particularly farm workers (Knobeloch et al. 2000; Landrigan and Benbrook 2015). Occupational exposure to pesticides is more easily recorded and often more severe compared to exposure to the consumer. Moebus and Bödeker (2015) and De Silva et al. (2006) reported in 1990 that annually 3 million global pesticides related poisoning incidents happened with 220 000 and 20 000 total deaths and unintentional death, respectively. Rosenstock et al. (1991) reported that of the fatal incidents the vast majority occur in developing countries and to migrant workers. Many agricultural workers in North America are migrant workers that face increasingly higher health risks due to cultural and financial barriers. Arcury et al. (2013) and Preibisch and Binford (2007) reported that Canada and the U.S. annually rely on 20,000 and 2.6 million migrant workers, respectively. These numbers do not include domestic agricultural workers, but represent the number of people at high risk of chronic and acute pesticide poisoning. Rosenstock et al. (1991) examined two groups of Nicaraguan farm workers, one with toxic exposure to organophosphate and one without (control). A series of neuropsychological tests were performed and concluded that the group exposed to organophosphates performed all sub-tasks at reduced levels compared to the control group. Delgado et al. (2004) performed a follow up study to Rosenstock et al. (1991) and speculated that there were some permanent motor and neurological impairment that was present in the exposed group that may be linked to central nervous system damage. Human health issues regarding pesticide exposure are global concern due to the toxic nature of the chemicals. Some pesticides act on biochemical processes that are common to many animals, plants and microorganisms, thus are a potential hazard to non-target organisms (Moorman 1989). The agriculture industry became very reliant on synthetic herbicides and started applying them excessively and grew reliant on a limited chemistry to protect their crops. Mechanization of the agriculture industry facilitated the systematic application of synthetic sprays and subsequently farmers started abandoning older farming practices (i.e. long-term crop rotations, cultivation techniques, and crop diversity, managing smaller farms, and sustainable tillage). The human and environmental impacts of this transition became evident with the 1930's dust bowl (Schubert et al. 2004), and the emergence of previously unknown human health conditions (Knobeloch et al. 2000). A physician in Iowa first described Blue Baby Syndrome in the 1940's and it was linked to nitrate contamination in the water supply from excessive applications of synthetic nitrogen fertilizers (Knobeloch et al. 2000). Abreu-Villaça and Levin (2017), Anwar (1997), Weisenburger (1993) and the Council On Environmental Health (2012) have identified the following health concerns due to pesticide exposure; acute and chronic neurotoxicity, reduced blood cell cholinesterase, cancers, birth defects, low birth rates, mental developmental issues, attention-deficit/hyperactivity, decreased lung function, pulmonary edema and other cardiovascular issues.

Newer pesticides and herbicide chemistries were developed to improve the selectivity and reduce the use of active ingredients. One significant technology development was biotechnology and genetically modified organisms (GMO). Biotechnology and the use of GMO crops was a natural progression away from the increasingly out-dated herbicide chemistries and helped to reduce the total volume of herbicide active ingredient applied. In a pesticide survey conducted on Ontario crops by the Ministry of Agriculture, Food and Rural Affairs between 1983 and 2008 reported that there was a reduction of 3.8 million kg of active ingredients of pesticides across all crops (Canadian Agriculture Products Act 2009). The adoption of GMO crops helped to alleviate the challenges that farmers faced while minimizing the negative human health and environmental effects of pesticides. Interestingly, the popularity of GMO's and the use of certain synthetic herbicides have resulted in herbicide (glyphosate) resistant weeds. In 2014, Heap (1997) reported there was recorded resistance to 152 different herbicides with the first reports starting around 1980. Nandula et al. (2005) and Johnson and Gibson (2006) state that glyphosate-resistant weeds require farmers to tank mix multiple MOA's, which increases the volume and cost of herbicides. It is speculated that this will start to increase the amount of herbicides applied on farmland in North America and counteract the benefits of the GMO technology (Nandula et al. 2005).

Overall, herbicides have provided many needed benefits to farmers by protecting yields and controlling weeds. Unfortunately, these benefits came at a cost to human health and have created more complicated issues with resistant weeds. Despite these negative consequences many farmer will still choose to apply herbicides because they are economically and logically feasible. The cost of weed control via herbicides is much lower than manual weeding and this is as true in organic production as it is in conventional production.

2.2. Potential for Use of Herbicides in Organic Agriculture

Organic agriculture has seen substantial growth in recent years with now over a million hectares of conventionally farmed land being converted to organic production in North America (Adl et al. 2011; Dayan and Duke 2010; Maggio et al. 2013). Weed competition is the single greatest economic challenge for organic producers (Dayan et al. 2011) and although organic production is based on a systems approach, herbicides still play a role as one weed management tool for organic farmers (Nazarko et al. 2005).

Herbicides that are currently available for organic production often are non-selective and non-systemic, intended to be applied POST, and they typically have negligible PRE efficacy (Dayan et al. 2011; Dayan and Duke 2010; Owens et al 2013). Most herbicides for organic production are considered contact herbicides providing a "burn down" effect that destroys the cuticular layer on young foliage causing tissue desiccation and plant death (Dayan et al. 2011; Tworkoski 2002). Compared to conventional herbicides natural and/or organic herbicides often have low efficacy that are more easily influenced by biotic and abiotic factors (weeds, crop, application timing, weather, and soil) (Abouziena et al. 2009; Dayan et al. 2011; Macias 1995; Oerke 2006; Tworkoski 2002; Xuan et al 2003). Biotic and abiotic conditions on a farm frequently change and make it challenging to achieve high efficacy when applying organic herbicides in agricultural settings. These herbicides have the potential to provide acceptable weed control, but under ideal conditions. Dayan et al. (2011) was able to reduce the chlorophyll and carotenoids of large crabgrass from 1.52 mg g^{-1} to 0.03 mg g^{-1} of leaf tissue when manuka oil was applied POST at 1% (v/v). PRE manuka oil applications (1% v/v) tests from Dayan et al. (2011) also significantly reduced the large crabgrass dry weight from 8.0 mg/plant to almost 0.0 mg/plant under controlled growth room conditions. Dayan et al. (2011) was able to show that MO had POST and PRE efficacy under controlled conditions; however, MO, unlike other organic herbicides, has systemic properties that would assist its efficacy. Most organic herbicides are strictly POST applied. Furthermore, testing efficacy under field conditions is likely to produce different results compared to controlled growth rooms. Abouziena et al. (2009) tested common organic herbicides (acetic acid, citric acid, garlic oil, corn gluten meal, and clove oil) on common agriculture broadleaves and grasses in Lake Alfred, FL. They tested early and late applied POST treatments and found that on broadleaves there was a significant reduction in efficacy when treatments were delayed. Abouziena et al. (2009) recorded that herbicide efficacy on *Morrenia odorata* was reduced by 86% (acetic acid 5% v/v), 75% (acetic acid 30% v/v), 75% (citric acid 10% v/v), 65% (citric acid 10% v/v and garlic oil 0.2% v/v), 10% (clove oil 45.6% v/v), and 25% (corn gluten meal 400g/m^2) when POST application was delayed by ~1 week. These treatments were also tested against six grassy weeds and often showed negligible efficacy for both early and late POST treatments. In order for many organic herbicides to provide season-long efficacy it requires a minimum of seamlessly timed POST applications followed by subsequent applications (Abouziena et al. 2009). This is because with POST applications only the apical meristems are destroyed, leaving the lateral meristems to regenerate (Cantrell et al. 2012; Dayan et al. 2011). This results in considerable volumes of product needing to be applied often. These volumes range from 50 to 1000 L of active ingredient per hectare (Dayan et al. 2011). Multiple applications can be logistically challenging and they can increase cost. The value of the crop and cost of the herbicide applications would have to be considered before justifying the incorporation of these herbicides into weed management practices (Cantrell et al. 2012). The search to develop organic herbicides with better efficacy is vital to facilitate organic farming and to encourage further growth in the organic industry.

2.3. Manuka Oil as a Herbicide

Manuka oil is a plant-derived essential oil that has historically been used as a traditional medicine (Stefanello et al. 2011). Manuka oil is distilled from the *Leptospermum spp.* A key source region for manuka oil is the East Cape of New Zealand (Owens et al. 2013; Stefanello et al. 2011). Studies by Stefanello et al. (2011) and Owens et al (2013) identified different triketones of manuka oil that may have herbicidal activity including; flavesone, iso-leptospermone, and grandiflorone (refer to Figure 2.1.). A study conducted by Owens et al. (2013) suggested that leptospermone extract had the most potent herbicidal activity and it worked by decreasing chlorophyll and carotenoid levels in plants significantly more than other triketones. Leptospermone has also been shown to be the primary HPPD inhibitor in manuka oil (Lee et al. 1996; Owens et al. 2013). Literature on flavesone, iso-leptospermone, and grandiflorone is limited, but according to Owens et al. (2013) a combination of the triketones increased the half-life of manuka oil from 15 days to 18 days and they suggested that triketone mixtures could be optimized for efficacy and persistence in soil.

2.4. Leptospermone

Leptospermone is believed to be the main compound in manuka oil that provides phytotoxic properties and thus the focus for its use as a herbicide (Owens et al. 2013). Published literature on additional benefits of leptospermone is limited and many medicinal or nutritional claims are anecdotal, meaning that more research is required to fully understand leptospermone as a herbicidal active ingredient. Reichling et al. (2005) discovered that manuka and tea tree oil aided the treatment of herpes simplex virus and it is believed that it may be due to beta-triketone compounds in the essential oils. Leptospermone has been reported to exhibit strong antiviral properties and aids in the treatment of herpes virus and the recovery of the infected skin (Carson et al. 2006; Kwon et al. 2013; Schnitzler et al. 2001). It was speculated that the beta-triketones functioned by blocking viral penetration and absorption into host cells and/or altering the viral membrane. Kwon et al. (2013) discovered that manuka oil helped skin rejuvenate and this process may also aid in the recovery of the skin during and after a herpes infection. Understanding how leptospermone interacts with viruses and in other biological capacities can help illustrate how the phytotoxic properties function in plants. Jeong et al. (2009) identified that *Leptosperomum scoparium* oil can be used to balance human intestinal bacteria, making it a potential alternative to conventional antibiotics. In addition, Kwon et al. (2013) reported that a 10%v/v manuka oil skin treatment significantly decreased skin damage severity in hairless mice exposed to intense UV-B rays. Not only did it decrease damage, but also the oil assisted the skin's natural protective mechanisms. The beta-triketones, particularly leptospermone, present in the manuka oil are believed to give manuka oil these properties.

2.5. Mesotrione

Mesotrione is a member of the benzoylcyclohexane-1,3-dione family of herbicides, which are derived from the natural phytotoxin, leptospermone. The phytotoxin is obtained from the Californian bottlebrush plant (*Callistemon chisholmii*) (Felix et al. 2007). Mitchell et al. (2001) explained that benzoylcyclohexandione can be divided into two distinct parts (the benzyl moiety and the dione moiety) that influence the herbicidal properties of the molecule. Changes in the electronic charges of the two distinct parts and the pH of soil aggregates provide the soil residual properties. The position and presence of the phenyl ring is also a key contributor to the herbicidal properties, which is illustrated in Figure 2.1. Benzoylcyclohexandiones are weak acids, and the combination of weak acidity and high water solubility is expected to allow for suitable uptake and translocation within plants (Felix et al. 2007; Philip and Wiebe 2002). Due to the functional effect of benzoylcyclohexandione, there is greater herbicidal efficacy since plants have greater difficulty in detoxifying the molecule (Felix et al. 2007; James et al. 2006; Mitchell et al. 2001; Philip and Wiebe 2002).

Mesotrione is the active ingredient used in the commercial herbicide Callisto used to control several dicotyledonous and monocotyledonous weeds and can exhibit both PRE and POST efficacy (Mitchell et al. 2001). According to Alferness and Wiebe (2002), mesotrione is absorbed by corn crops following foliar application and translocated primarily apoplastically, but also basipetally. Corn is able to rapidly metabolize mesotrione in comparison to susceptible weed species and this is what contributes to the selectivity of the herbicide (Alferness and Wiebe 2002; Mitchell et al. 2001). Mesotrione is registered in Canada and classified as a Group 27 or a beta-triketone herbicide that can be applied as a foliar spray (POST) or directly to the soil (PRE). It can be applied to corn, but not tomatoes because tomatoes are susceptible to mesotrione (Syngenta Canada 2016). Callisto controls many troublesome broadleaves species such as redroot pigweed (*Amaranthus retroflexus* L.), green pigweed (*Amaranthus powellii* S.), lamb's quarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medicus), wild mustard (*Sinapis arvensis* L.), vetch (*Vicia cracca* L.), and common ragweed (*Ambrosia artemisiifolia* L.) (Syngenta Canada 2016). Mesotrione can be soil applied because it has soil residual properties, which allow the active ingredient to persist in the soil and provide a longer window of protection against weeds (Mitchell et al. 2001; Torma et al. 2004). Similar to leptospermone, the active ingredient in manuka oil, mesotrione inhibits the 4-hydroxyphenyl-pyruvate dioxygenase (HPPD) enzyme, which is a non-heme iron-containing enzyme and halts caroteniod synthesis. It also disrupts the conversion of tyrosine to platoquinone and tocopherol, which leads to foliage bleaching (Dayan et al. 2007; Mitchell et al. 2001; Torma et al. 2004). Figure 2.1 shows the chemical structures of mesotrione and the similarities to the beta-triketones in manuka oil. Mesotrione was derived from leptospermone and in Figure 2.1 we see the differences in the molecular structures, particularly the relative position of the phenyl rings (Mitchell et al. 2001).

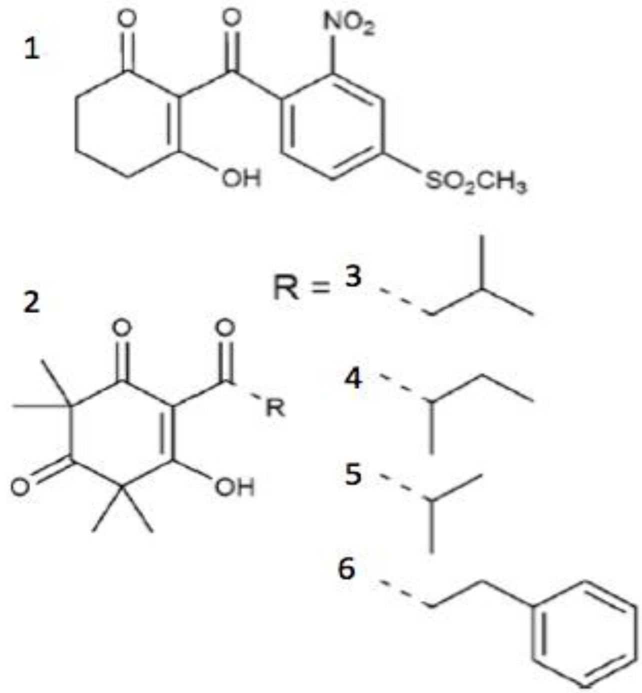

Figure 2. 1. Chemical structures of mesotrione (1) the base reference compound (2) leptospermone (3), isoleptospermone (4), flavesone (5) and grandiflorone (6) (adapted from Dayan et al. 2007).

In Figure 2.1, the chemical structures of leptospermone and mesotrione are displayed to help explain and provide a visual as to how and why these two chemicals react similarity. Shared residual herbicide properties between the two chemicals are due to the electronic charge of the structure in relation to the natural charge of soil (Maeghe et al. 2002). Mitchell et al. (2001) describes mesotrione as a weak acid, with a dissociation constant pKa of 3.12. There is pH dependency for proper water solubility and uptake of the chemical into plants. It is known that soil texture, soil pH, soil parent material, soil type, and rainfall can greatly influence the dissipation rate of residual herbicides (Dayan et al. 2007; Dayan et al. 2000; Maeghe et al. 2002). High performance liquid chromatography with ultraviolet detectors can be utilized to determine dissipation of mesotrione residuals in soil (Chen et al. 2012; Peng et al. 2007). Given the physicochemical similarities between mesotrione and leptospermone the techniques used to assess the dissipation of mesotrione may be able to be adapted to detect dissipation of leptospermone. Characteristics between the two chemicals may also help explain similarities in the mechanisms behind soil residual levels, plant uptake and phytotoxicity.

2.6. Essential Oils as Commercial Herbicides

Current literature on manuka oil or leptospermone is limited and literature pertaining to herbicidal properties is even more rare (results from comprehensive citation search engine, Web of Science). At the time of this study, extensive searches reveal less than five published peer-reviewed articles on the herbicidal properties of manuka oil. There has, however, been more published research on the herbicidal nature of other essential oils that may act in ways similar to manuka oil. Other essential oils that have been extensively researched are, but not limited to; cinnamon, thyme, garlic, citrus, clove and lemongrass seed oil (Abouziena et al. 2009; Cantrell et al. 2012). Many organic herbicide products rely on plant-based essential oils as the active ingredients because they cannot require synthetic manipulation; they usually rapidly degrade, are often classified as GRAS (Generally Recognized As Safe) and must meet organic certification in Canada (Tworkoski 2002). GRAS is a program in the United States (U.S.) that classifies certain food-based items as safe in relation to human health and does not require the typical U.S. Food and Drug Administration review before commercial release. There is some criticism regarding GRAS compounds because many have been grandfathered in before there were stringent regulations in place. Careful consideration of each compound is necessary to determine the overall human health and environmental safety. Compounds that are classified as GRAS in the U.S. can been used in organic farming. An example is yucca extract (Piacente et al. 2005). There are even some limited exceptions within the Pest Management Regulatory Agency (PMRA) that allow Canadians to experiment with compounds if they are GRAS classified (Health Canada 2016). However, some commonalities between essential oils and manuka oil are that they are all plant-derived complex essential oils, aromatic, with antibacterial, antiviral, antifungal and antioxydative properties. Table 2.1 lists some currently available organic herbicides in Canada and their active ingredients.

Table 2. 1. Comparing the inert and active ingredients of commercially available organic herbicide products in Canada. All data was retrieved directly from the labels on the manufacturer websites.

Commercial name	Company	Other/inert ingredient	Active ingredient
Weed Zap®	JH biotech Australasia Pty Lt d	Not disclosed	Clove oil (45%) Cinnamon oil (45%) (www.jhbiotech.com)
Avenger Organics	Cutting Edge Formulations, Inc.	Other proprietary ingredients, Emulsifiers.	New formula: d-Limonene (55%) Castor oil (5-15%) Old formula: d-Limonene (55%) (http://www.avengerorganics.com)
GreenMatch EX	Marrone Bio Innovations	Water, corn oil, glycerol esters, Potassium Oleate and Lecithin	Lemon grass oil (50%) (www.marronebioinnovations.com)
Proud 3®	Bio HumaNetics, Inc	Water, molasses, glycerin, wintergreen oil.	Thyme Oil (5.6%) (www.bhn.us)
EcoSMART® Organic Weed & Grass Killer	EcoSMART Technologies Inc.	Water, potassium oleate, sodium	2-Phenethyl Propionate (5%) Eugenol (5%) Sodium lauryl sulfate,

		bicarbonate and lecithins	surfactant (0.05%) (www.ecosmart.com)
Phydura®	Soil Technologies Corp.	Soybean oil, vinegar, and water	Clove oil (12%) Sodium lauryl sulfate, surfactant (8%) (www.soiltechcorp.com)
Fiesta® chelated iron*	Neudorff North America	Not disclosed	Iron HEDTA (FeHEDTA) (26.52% v/v)

Appleby (2005), Timmons (2005), Timmons (1970) and Gauvrit and Cabanne (1993) all stated that the first oil-based herbicides and surfactants were derived from petroleum products in the early 1900's. These products were often used as either the herbicide itself or as an adjuvant or surfactant. Over the years, different oils were experimented with and common oils became popular. With the assistance of modern scientific testing, the oils were analyzed to determine what characteristics about them made them suitable for certain applications (Timmons 2005; Timmons 1970; Gauvrit and Cabanne 1993). Gauvrit and Cabanne (1993) examined different oils for their ability to penetrate plant leaves and determined that certain oils were able to enter the plant sub-stomatically resulting in reduced transpiration, photosynthesis and chlorophyll content and increased respiration. Furthermore, most fluid oils were able to achieve certain levels of plant penetration by means of the stomata, but only the compounds that have known phytotoxic properties were found inside cells. The composition of the oil along with the presences of phytotoxic chemicals acts together as effective herbicides because the oil facilitates absorption and the phytotoxic compounds are able to find target sites inside the plant (Gauvrit and Cabanne 1993; Tworkoski 2002). Natural oil characteristics were analyzed by Tworkoski (2002) and Gauvrit and Cabanne (1993), and they determined that unsaturated oils with low-boiling points and molecular weights, exhibited herbicidal properties. Gauvrit and Cabanne (1993) identified a general rule that high aromatic oils are often herbicidal while low aromatic oils were more likely to have insecticidal properties. This is evident with the active ingredients column of Table 2.1, which lists oils with very strong aromatics. Many strong aromatic oils have become certified organic herbicide products.

Essential oil-based organic herbicides are limited in terms of use because of their high costs, limited efficacy and the fact that there are very few registered for use in Canada (Gauvrit and Cabanne 1993; Owens et al. 2013). The current available organic herbicides are often non-selective, non-systemic, POST with negligible PRE properties (Dayan et al. 2011; Dayan and Duke 2010; Owens et al. 2013). These products utilize a contact-based burn down method (Dayan et al. 2011) that causes tissue desiccation and necrosis (Tworkoski 2002). The herbicidal mechanism of these essential oils is not fully understood. Some research indicates that the essential oils alter the plant's cuticular layer by weakening the cellular membrane facilitating electrolyte leakage (Tworkoski 2002).

The MOA of manuka oil is not fully understood, but research suggests it does not utilize the same burn-down MOA as commercial synthetic herbicides (Tworkoski 2002). Work by Dayan et al. (2000, 2007, and 2011) suggests that manuka oil is more efficiently absorbed through roots than through foliage. It may be possible that certain compounds within manuka oil are performing vastly different tasks in relation to herbicidal activity, some causing direct-contact foliage damage and others more systemic damage. Spiering (2011) examined two organic herbicides (EcoSMART® Organic Weed & Grass Killer and Phydura®) that contained the same active ingredients and adjuvants, but produced significantly different plant-injury results. Spiering (2011) concluded that plants treated with Phydura suffered considerable foliage damage, but did not drop all their leaves whereas EcoSMART treated plants experienced complete defoliation. Interestingly, plants treated with EcoSMART showed a gradual increase in number of stems per plant despite the extremely weak appearance of the plants. The reasons for this are not understood, but there may be a relationship linking the concentrations of the phytotoxic compounds between the two herbicides. Review of the product labels and Material Safety Data Sheets (MSDS) revealed that the formulation of EcoSMART contained a concentration of the essential oils 2-phenethyl propionate (5%) and eugenol (5%) (EcoSMART Technologies 2009; Soil Technologies Corp. 2011). 2-phenethyl propionate and eugenol are both derived from clove oil and are often considered responsible for the herbicidal properties of clove oil (Gauvrit and Cabann 1993; Spiering 2011; Tworkoski 2002). In contrast, the active ingredient in phydura was clove oil (12%) in its whole form (Soil Technologies Corp. 2011). The exact percentage of 2-phenethyl propionate and eugenol in clove oil is unknown. In a similar study, Tworkoski (2002) determined the minimum concentration of eugenol required to cause significant plant injury to dandelions (*Taraxacum officinale* Weber ex Wiggers) was 8% (v/v). It may be possible that the phydura did not contain a sufficient concentration of the phytotoxic compound eugenol. Research by Gauvrit and Cabanne, (1993) indicated that the 12% concentration of clove oil contained a variety of compounds, which might not contain phytotoxic compounds able to penetrate cells and cause acute tissue damage. Isolating the herbicidal components of essential oils may be important in order to improve efficacy and assist in understanding the herbicidal MOA of essential oils.

Manuka oil contains a large variety of complex compounds with approximately 15-22% of these being triketones, which are the potential phytotoxic components of the oil (Dayan et al. 2007). The triketones can be further classified as leptospermone, isoleptospermone, flavesone and grandiflorone. Leptospermone is believed to be the most herbicidal component within manuka oil, but this may be due to its higher concentration compared to the other triketones. Dayan et al. (2007) proposed that isolating and concentrating leptospermone could significantly increase efficacy compared to manuka oil. This contrasts with research by Tharayil et al. (2008) who suggested that the soil activity and half-life of leptospermone was enhanced when applied in the multi-solute system within manuka oil. Limited published literature on this topic makes it difficult to determine what can enhance the efficacy of manuka oil and more research is needed. Isolating and examining the herbicidal components of manuka oil would better illustrate if efficacy was dependent on a specific phytotoxic compound or a synergistic effect of the components. The manuka oil used in the present study was specifically chosen because it contains a high concentration of triketones (31.0%) and leptospermone (19.17%) (http://www.manukaoil.com).

2.7. Herbicide Formulation and Improving the Efficacy of Manuka Oil

Optimal herbicide formulation is vital to efficacy (Buffington and McDonald 2006: Curran and Lingenfelter 2009; Jordan 1995; Jordan et al. 2011; Ramsdale and Messersmith 2002). Herbicide formulation is the processes of combining components, active ingredients and inert ingredients, to produce a commercially viable product. In the organic agriculture industry, essential oils comprise the majority of the active ingredients in herbicide products (Dayan and Duke 2010; Dayan et al. 2011). Historically, petroleum-based oils were used for pest management as both herbicides and adjuvants or surfactants (Gauvrit and Cabanne 1993). Research by Timmon (2005), Timmons (1970), Appleby (2005) and Gauvrit and Cabanne (1993) indicates that the early field use of petroleum-based oils was trial and error resulting in no consistent management practices at the time. Essentially every farmer used different oils and methods to control different pests and there was little scientific exploration regarding efficacy of the products. Despite the lack of consistency, the following published literature has shown that some oils have some level of efficacy either as herbicides or pesticides (Abouziena et al. 2009; Appleby 2005; Dayan and Duke 2010; Dayan et al. 2011; Gauvrit and Cabann 1993; Timmon 2005). In the mid-1900 the development of more effective active ingredients reduced the need for and usage of oils as pesticides (Appleby 2005; Timmons 2005; Timmons 1970). The biochemical and physical properties of the oils were reutilized to create adjuvants for the new synthetic pesticides, which was the beginning of modern pesticide formulation. This shift started a focus on searching for new sources of active ingredients for both conventional and organic agriculture. Macias (1995) outlined the main strategies used in the organic sector to discover new herbicides, which almost always involves discovering new phytotoxic compounds in nature, manipulating them and figuring out how to synthesise them for large-scale production. This strategy places the organic herbicides industry at a disadvantage because, by nature of the organic regulation standards, organic herbicides cannot be synthesized and must be similar to how they appear in nature (Canadian Agriculture Products Act 2009). Also the lack of research in the organic industry in comparison to the conventional industry has created a situation where conventional herbicide formulas are examined and improved annually (based on comprehensive literature search, Web of Science). This allows a natural progression of increasingly more effective conventional herbicides formulas where the organic industry still has a more primitive understanding and technology regarding the herbicide formulas available to them. In most cases, refined active ingredients result in better weed control; however, organic agriculture has limitations in terms of the methods allowed to enhance concentration of the active ingredient(s). These limitations restrict manipulation of the compound from its natural, and often less concentrated, form (Gauvrit and Cabanne 1993). The lack of proper formulation and the limitation on concentration forces organic farmers to apply substantial volumes of herbicides. Dayan et al. (2011) estimated that commercial organic herbicides are applied at a range of 50 to 500 L of active ingredient per ha^{-1} and in experiments conducted by O'Sullivan et al. (2013a) up to 1000 L ha^{-1} may be required for some products. These excessively high rates can be a significant cost issue for organic farmers and may cause logistical problems as well. Developing a scientific approach to the formulation of organic herbicides would lead to increased efficacy reducing the volume of herbicide needed.

Proper formulation has been shown to improve the efficacy of manuka oil. In experiments conducted by O'Sullivan et al. (2013a and 2013b) they showed a dramatic weed control increase from 20% (manuka oil 1% v/v) to 59% (manuka oil 1% v/v and Nu-Film P 1% v/v) with the addition the surfactant Nu-Film P®. The efficacy increased to 83% when Weed Zap® was added. A related study by O'Sullivan et al. (2014) confirmed that tank-mixing manuka oil with Green Match EX increased weed control and crop yields. Similarly, the addition of manuka oil to Weed Zap caused a moderate increase in weed control, but significant increases in crop yield

2.8. Adjuvants and Surfactants

The inert ingredient portion of commercial herbicide formulas are often adjuvants and/or surfactants, which modify the solution to assist the efficacy of the active ingredient. Abouziena et al. (2009) were able to use half of active ingredient of bentazon to control broadleaf weeds with the addition of adjuvants. Herbicide solutions often incorporate adjuvants in order to increase the penetrability, emulsification, adhering ability, shelf life, and absorption and reduce tank foaming and drift of the active ingredient (Abouziena et al. 2009; Buffington and McDonald 2006; Curran and Lingenfelter 2009; Jordon 1995). The majority of adjuvants are not molecularly classified in the way that most active ingredients are because they are inert and therefore, are not regulated in the same way (Curran and Lingenfelter 2009).

This allows companies to withhold their complete herbicide formulations, which is why only the active ingredients will be present on labels and Material Safety Data Sheets (MSDS). It is important to consider the addition of adjuvants when testing new active ingredients for efficacy against commercial products because some of the commercial products will be pre-formulated, such as Callisto. In the case of our study, manuka oil is the active ingredient (more specifically leptospermone) being tested against commercial organic herbicides (WeedZap® and Avenger® Weed Killer Ready-to-use Herbicide) and one conventional herbicide (Callisto®). These commercial products are pre-formulated with adjuvants and/or surfactants that assist their efficacy, which is why the appropriate adjuvants must be tank mixed with the manuka oil to optimize efficacy (Abouziena et al. 2009).

Activator adjuvants are a sub-category of adjuvants that includes surfactants, emulsifiers, wetting agents, stickers, spreaders and oils (Buffington and McDonald 2006; Curran and Lingenfelter 2009; Jordon 1995). They work by lowering the surface tension of water to provide a more homogenous connection between herbicide solutions and leaf tissue or soil aggregates (Curran and Lingenfelter 2009). Surfactant efficacy is quantified in dynes per centimeter and measured by the surface energy tension, with water having a value of 73 dynes/cm (Curran and Lingenfelter 2009). The average surfactant is able to reduce energy tension by a range of 30 to 50 dynes/cm significantly increasing the surfactant characteristics (Curran and Lingenfelter 2009; Jordan et al. 2011). Ramsdale and Messersmith (2002) reported that the addition of surfactant to 4 different active ingredients not only improved efficacy, but also allowed the spray volume to be significantly reduced while maintaining similar efficacy. Reduced spray volumes are favourable because they lower the volume of water needed, thus reducing the resources required to fill up the spray tank (Ramsdale and Messersmith 2002). The number of available organically certified surfactants are very limited with less than 7 listed on the Organic Materials Review Institute during the time of this experiment. Of these 7, some are not suitable tank mix partners and most have strict restrictions on when and how they can be applied making it difficult for organic farmers to utilize them (www.omri.org). More research into the use of organic surfactants as tank mix partners with organic herbicides should lead to greater efficacy and reduced costs for organic farmers.

2.9. Yucca Extract

Yucca extract is a natural compound that has GRAS status in the U.S., but is not yet available as an organic tank-mix option. If it were registered as an organically certified compound it would be classified as an activator adjuvant (wetting agent). Yucca extract is derived from the yucca (*Yucca schidigera* Roezl ex Ortgies) plant, which is a common ornamental plant belonging to the Agavaceae family, which is native to the Southwestern United States and Mexico (Piacente et al. 2005). It has been traditionally used by indigenous communities for generations and has only recently been used commercially. Yucca extract is unique because it has a natural wetting agent ability, which is attributed to the very high level of saponins (Kowalczyk et al. 2011). Currently, the commercial uses of yucca include; an additive of livestock feed to improve health and nutrition, a foaming agent in the cosmetic industry, and a nutritional supplement (Kowalczyk et al. 2011; Piacente et al. 2005).

Piacente et al. (2005) mapped the chemical composition of yucca extra and reported that it has tremendous potential to be used in the herbicide industry as an activating adjuvant.

2.10. Residual Effects of Pre-Emergence Herbicide in Soil

PRE-emergence (PRE) herbicides provide farmers with a longer range of protection from weeds because the herbicides persist in the soil for extended periods of time. PRE herbicides have soil residual properties killing newly germinated seeds in the top portion of the soil. It is generally accepted that rainfall is desirable after applying a PRE herbicide because it activates the product (Jordan et al. 2011; Knake et al. 1967; Menges 1963). Knake et al. (1967) recommended incorporating PRE herbicides if rainfall is not forecasted soon after application. They stated that the moisture from the rainfall is required to move the herbicide into the soil and near the root zone of future germinating weed seeds. According to Singh et al. (2014) PRE herbicides have been effective in crops such as corn. One very popular PRE herbicide that is used on corn is Callisto (Syngenta Canada 2016). A study conducted by Knezevic et al. (2013) established that sunflower plots sprayed with PRE applications of mesotrione had 25 to 37 days delay in weed emergence. These extra days without weeds can directly result in increased yields because there is less risk that early weed competition will outcompete the young crops. Research by Mitchell et al. (2001) and Knezevic et al. (2013) identified that the residual effects of herbicides are based on the theory that the physicochemical properties of the molecular compounds produce a polarity or an electronic charge that reacts with the soils. Residual impact of the PRE herbicides can be influenced by soil pH, soil texture and soil type (Mitchell et al. 2001; Knezevic et al. 2013). In addition, the PRE efficacy of herbicides can aid in the efficacy of subsequent POST herbicide applications resulting in drastically reduced weed pressure. Often in cornfields, there is virtually no weed pressure with PRE and POST applications of Callisto due to high efficacy rates. However, Callisto could never be used in an organic system because mesotrione does not meet organic regulation standards. There are some natural compounds that have been identified as having some level of PRE herbicidal properties. Compounds in manuka oil, specifically leptospermone, have been shown to have soil residual activity, which makes it an interesting option as a potential organic herbicide. More research is needed to identity and eventually commercialize organically certified herbicides that provide farmers with PRE efficacy.

2.11. Hypothesis and Objectives

Based on the unique PRE and POST efficacy characteristics of MO, we hypothesized that the PRE and POST efficacy of MO is enhanced when applied in tank mixture with adjuvants and surfactants or other certified organic herbicides. The objectives of this study are to explore ways to improve the efficacy of MO for use on organic farms, specifically tomatoes and sweet corn. Field experiments will use MO tanked mixed with different adjuvants, surfactants (yucca extract, Nu Film P and Agral 90) and/or other organic herbicides (citrus, clove and cinnamon oil) to determine control of common agriculture weeds including; redroot pigweed

(*Amaranthus retroflexus* L.), green pigweed (*Amaranthus powellii* S.), lamb's quarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medicus), wild mustard (*Sinapis arvensis* L.), and common ragweed (*Ambrosia artemisiifolia* L.) in Simcoe and Ridgetown, ON. Crop yields, total weed biomass weights, visual efficacy and crop injury rating will be assessed to determine tank mix efficacy. In addition, PRE efficacy of MO tank mixes will be further analyzed in growth room conditions on green pigweed (*Amaranthus powellii* S. Wats.) and large crabgrass (*Digitaria sanguinalis* L.). PRE efficacy was assessed by analyzing emergence percentage and total weed biomass. Growth room treatments will consist of MO tank mixed with adjuvants and surfactants (yucca extract, Nu Film P and Agral 90) to see if PRE efficacy can be enhanced under more controlled conditions.

Chapter 3.0. Field experiments: Improving the field efficacy of manuka oil using tank mixes with surfactants and commercial organic herbicides.

3.1 Abstract

Manuka oil (MO) was applied in combination with surfactants and organic herbicides for a total of ten different treatments. Three surfactants (Nu Film P, Agral 90, and yucca extract) and two essential oil-based organic herbicides (clove-cinnamon oil and citrus oil) were tank mixed with MO. Herbicide applications were analyzed against two checks (weedy and weed free) in eight unique scenarios to determine what tank mix options enhanced the PRE and POST efficacy of (MO). The eight unique scenarios included two locations (Simcoe and Ridgetown, ON), two crops (sweet corn and tomatoes) and two planting dates (early and late). Yield results and visual weed efficacy data indicated that MO had very weak PRE herbicidal properties in field scenarios. MO-based treatments inflicted minimal crop injury on the recently transplanted tomatoes and newly emerging corn. Crop injury ratings were negligible for almost all the MO based treatments later in the season when the crops were more robust. MO was able to provide good weed control when applied either solely or in tank mixes. Efficacy of MO increased when MO was used as a tank mix partner compared to MO applied alone.

3.2 Introduction

Surfactants and adjuvants as tank mix partners are known to improve the efficacy of many herbicides (Zhang et al. 2002; Abouziena et al. 2009; Buffington and

McDonald 2006). The value of tank mix partners for improving the efficacy of organic herbicides has been studied to a very limited extent and this is true for manuka oil (MO), which is a potent essential oil with proven herbicidal properties and potential soil residual activity (Dayan and Duke 2010; Maggio et al. 2013). MO is considered an acceptable product in terms of organic standards. The active compound in MO, leptospermone, is similar to the conventional active ingredient, mesotrione, which is found in the commercial herbicide Callisto (Owens et al. 2013). Both leptospermone and mesotrione have MOA's that work by inhibiting HPPD (Dayan et al. 2011).

In this study we considered organic (Nu Film P) and non-organic (Agral 90 and yucca extract) surfactants and adjuvants for enhancing the efficacy of MO for PRE or POST applications. Nu Film P was selected because it is a prominent certified organic surfactant available in North America. Agral 90 is the conventional industry standard wetting and spreading surfactant that is commonly used on non-organic farms. Yucca is a non-registered natural compound that has the potential to meet Canadian organic standards and become an OMRI registered product. Piacente et al. (2005) identified yucca extract as having a unique composition to be a potential organic surfactant. Yucca was selected for this experiment to test the potential surfactant qualities against the leading organic and conventional industry standard (Agral 90).

3.3. Materials and Methods

3.3.1. Experiments and Treatments

Experiments were conducted in 2015 to explore the efficacy of manuka oil for weed control in sweet corn and field tomato crops in Ontario. The experiments were arranged as a randomized complete block design with 12 treatments and 4 replications at 2 sites (Simcoe and Ridgetown) and with 2 planting dates (early and late). Three PRE and nine POST treatments were applied. The three PRE treatments were the following: PRE manuka (PRE MO) (1% manuka oil v/v); PRE manuka oil and Agral 90 surfactant (PRE MO+Agral 90) (1% manuka oil and 0.2% Agral 90); PRE Callisto (144 g ai ha^{-1}). The nine POST treatments were the following: POST manuka oil (POST MO) (2% manuka oil v/v); POST manuka, clove, and cinnamon oil (POST Manuka+Clove+Cinnamon) (2% manuka oil v/v and 5% clove and cinnamon oil v/v); POST manuka and citrus oil (POST MO+Citrus) (2% manuka oil v/v and 20% v/v d-limonene or citrus terpenes oil); POST manuka oil and Agral 90 surfactant (POST MO+Agral 90) (2% manuka oil v/v and 0.2% Agral 90 v/v); POST manuka oil and yucca extract (MO+Yucca) (2% manuka oil v/v and 0.132% v/v yucca extract); POST manuka and Nu Film P surfactant (POST MO+Nu Film P) (2% manuka oil v/v and 1% v/v Nu Film P organic surfactant) and POST Callisto and Agral 90 (POST Callisto) (144 g ai ha^{-1} and 0.2% Agral 90). Two checks were used to reference herbicidal efficacy and crop yield. The checks were Weedy (left unattended) and Weed Free (manually weeded).

3.3.2. Crops

Tomatoes and sweet corn were chosen based on the constraints of the experiments and because they are two morphologically different crops. Crops were planted at two different planting dates (early and late) at two sites (Simcoe and Ridgetown, ON). Location and location and soil characteristics are summarized in Table 3.1. Tomato planting dates represent the transplanting dates for seedlings that were started in greenhouses approximately 6 weeks prior to transplanting. Planting dates are detailed in Table 3.2. Simcoe tomatoes (*Solanumlycopersicum* L.) cv. Mountain Glory (Stokes, Thorold, ON) were planted in rows 4 m in length, with 1.5 m row spacing with 45 cm between each plant. Ridgetown tomatoes (*Solanumlycopersicum* L.) cv. LYPES (Cangrow, Alvinston, Ontario) were planted in rows with a length of 1.5 m and the width of 1 m and 25 cm spacing between plants. Simcoe and Ridgetown direct seeded sweet corn (*Zea mays saccharata)* cv. ZEAMS (Bonduelle, Strathroy, Ontario) at early and late planting dates listed in Table 3.2. Simcoe corn was planted in rows 4 m in length and 1.5 m in width. Ridgetown corn was planted in twin-rows 1.5 m in length and 1 m in width. Fields were not maintained as certified organic because synthetic fertilizers and other conventional products were applied to create an optimal environment for crops. Cultivation and fertilizer applications were based on management practices typical for the site. Fields in Simcoe were conventionally tilled with an offset disc on May 7th, 2015, (early-planted) and May 15th, 2015, (late-planted). Early-planted fields had Solubor (20.5% Boron) applied May 22nd and 34-0-0 fertilizer (150 kg ha^{-1}) applied May 26th. On May 25th, 2015, 34-0-0 (300 kg ha^{-1}) and 0-0-62 (80 kg ha^{-1}) fertilizer was worked into the soil with a S-tine cultivator for the late-planted fields. Row middles were managed with a narrow multivator to control strips of weeds that were in between the plots. Zampro (ametoctradin/dimethomorph), Ranman 400SC (cyazofamid), Presidio (fluopicolide), Bravo 500 (chlorothalonil) and Tattoo C (propamocarb/chlorothalonil) were applied at different times to control aggressive late blight in tomatoes. The late-planted fields required more applications compared to the early. The sweet corn fields in Ridgetown were conventionally tilled with an S-tine cultivator on May 7th, 2015 and nitrogen fertilizer (475 kg ha^{-1} of 19-19-19) was worked into the soil on May 8th, 2015. The tomato fields in Ridgetown were also cultivated prior to transplanting on May 29th, 2015 (early planted) and June 9th, 2015, (late planted).

3.3.3. Treatment Applications

PRE and POST herbicide treatments were applied using a CO_2-pressurized backpack sprayer at 207 kPa. The applicator distributed the solution at a constant walking pace and boom height of 50 cm. The PRE treatments were applied before the corn had emerged. Tomatoes were transplanted the day after the PRE application to avoid excessive foliage damaged of the seedlings. PRE MO and PRE MO+Agral 90 treatments were broadcasted once during the growing season at a solution application rate of 200 L ha^{-1} with AI11002VS (Simcoe) and ULD120-02 (Ridgetown) nozzles. A summary of the PRE and POST application dates for both sites are displayed in Table 3.3. PRE Callisto applications were broadcast once at the recommended label rates (144 g ai ha^{-1}). POST applications were applied a total of three times in two-week intervals at a solution application rate of 1000 L ha^{-1} (Simcoe) and 800 L ha^{-1}

(Ridgetown) with AI9508E (Simcoe) and ULD120-04 (Ridgetown) nozzles and with the use of a shroud. POST applications were direct sprayed along the rows as close as possible to the crop multiple times to achieve desired rates. Table 3.4 outlines the PRE and POST applications dates with rainfall occurrence within 48 hours to better determine if efficacy of treatments were influence by rainfall.

3.3.4. Visual Efficacy Ratings

Crop injury ratings (CIR) and weed efficacy ratings (WER) were conducted by visual assessment. CIR and WER were preformed on both the PRE and POST treatments approximately ten days after each application. PRE treatments, which were applied once, were assessed throughout the growing season to observe changing weed pressures. The researcher assessing the CIR and WER did the ratings from the posterior end of the plots where signage was not visible to ensure unbiased results. CIR and WER were assessed on a percent basis. For CIR, values over 11% are considered unacceptable in terms of crop injury. For WER, values of 100% represented total weed suppression (e.g. weed free check) and values of 0% represented total weed canopy coverage (e.g. weedy check). The weed free and weedy checks were used as guides in assessment of the other treatments.

3.3.5. Weed Biomass

Weed biomass was collected prior to crop harvest by placing one square quadrant with a length of 25 cm in each plot and removing all plant material within the borders. The quadrant was placed randomly by to prevent biased sampling of certain treatment groups. This was achieved by placing the quadrant down without looking at the plot. Individual weeds were then placed into sample bags and secured to prevent loss of plant material. Sample bags were labelled and transported to a drying room where they were dried for 48 hours at 80^{o} C and dry weights of samples were then measured and recorded. Due to methodological error, the Simcoe early weed biomass values for tomatoes and corn represent a combined value for the two crops.

3.3.6. Crop Yield

Corn in Simcoe was harvested by locating the centre of each plot and harvesting 1 m on each side of the midpoint. Both locations had buffer plots around the fields to reduce edge effects. The Simcoe tomatoes were harvested in a similar way. The entire 1.5-meter plots in Ridgetown for both corn and tomatoes were harvested for yield determinations. An average of 4.8 tomato plants and 12 corn plants were harvested from each plot at both locations. Total yield of sweet corn and tomatoes were hand harvested and yield was determined by taking the total fresh weight. Badly damaged or severely underdeveloped tomatoes and corn ears were discarded.

3.3.7. Statistical Analysis

Statistical Analysis System (SAS 9.4, SAS Institute, Cary, NC) was used to conduct all statistical analysis. Site and planting dates were analyzed separately and data was adjusted to t ha^{-1} for yield and kg m^{-2} for weed biomass. Initial variance

(ANOVA) analysis was conducted using ProcGlimmix. Block effects were considered random and treatments were considered fixed. For corn and tomato yield, a log link and a poisson distribution of errors was used with a heterogeneous error model for treatments. For weed biomass in both corn and tomato crops, a log link and a geometric distribution was used except for the late planting date in Ridgetown where an identity link and a Gaussian distribution of errors was used. Visual weed efficacy and visual crop injury were analyzed by dates. The effect of weed treatments was considered significant at $P \leq 0.05$ and Tukey's HSD test was used to test for significant differences between treatments. CIR and WER were analyzed using ProcGlimmix procedure with a log link beta distribution and Type III F-tests were used to determine significance between treatments.

3.4. Results

3.4.1. Weed Control Efficacy: Tomatoes

Although sites were not statistically contrasted; visual observations (data not shown) and total weed biomass (Table 3.5) showed that there was greater weed pressure in the tomato plots at Ridgetown compared to Simcoe. The late-planted tomato trials at Ridgetown had an average of ~50% more total weed biomass for all treatments compared to Simcoe. The weedy check treatments at Ridgetown had, on average, 177 g/m^2 (early) and 21 g/m^2 (late) more weed biomass than Simcoe, emphasizing the greater levels of base weed competition at Ridgetown versus Simcoe. Most of the treatments were not significantly different from the weedy check and therefore, did not provide significant efficacy as measured by weed biomass. This was more apparent at the Ridgetown site where the only treatment that resulted in significantly less weed biomass compared to the weedy check (excepting the weed-free control) was the MO+Clove+cinnamon treatment in both the early and late-planted tomato trials (Table 3.5). In the Simcoe trials, where weed pressure was less than at Ridgetown, there were more treatments that resulted in significant reductions in weed biomass; the majority Simcoe late-planted. For the late-planted trials weed pressure was greater in PRE MO+Agral 90, all the POST manuka oil treatments and POST Callisto. For the early-planted Simcoe trials it only the POST MO+Citrus and POST MO+Agral 90 had weed biomass that was significantly different from weedy check. All the PRE manuka oil treatments (PRE MO and PRE MO+Agral 90) did not significantly reduce weed biomass compared to the weedy checks except for late-planted Simcoe.

The visual efficacy ratings (Tables 3.7 and 3.8) relate to the weed biomass results because reduced control would mean more weed biomass. Furthermore, visual weed biomass is assessing the overall thickness of the weed canopy in each plot where the biomass values capture the weights of the weeds. These parameters might have different influences on different crops. Table 3.7 displays, at both locations, 6 PRE treatments (PRE MO, PRE MO+Agral 90 and PRE Callisto) had visual weed efficacy ratings that were significantly different and 12 were significantly the same as the weedy check. In Table 3.8 shows 11 PRE treatments were significantly different and 7 PRE treatments were not significantly different from weedy check. When only the PRE manuka oil treatments are considered Table 3.7 and Table 3.8 shows 3 and 7 PRE treatments (PRE MO and PRE MO+Agral 90) with ratings significantly different from weedy checks, respectively. For both early and late-planted tomatoes (Tables 3.7

and 3.8), 10 out of the 24 treatments had visual weed efficacy ratings that were significantly different from weedy checks indicating that a one-time application of PRE manuka oil does not provide adequate efficacy. Tables 3.7 show that the vast majority of the POST manuka oil treatments had visual efficacy ratings that were significantly different from the weedy check, with a few exceptions. These exceptions were for the 2nd observation in Simcoe for POST Callisto, 2nd observation in Ridgetown for POST MO and five treatments (POST MO, POST MO+Citrus, POST MO+Nu Film P, POST MO+Yucca and POST Callisto) for the 3rd observation in Ridgetown. POST Callisto was not significantly different from the weedy check twice. Two treatments that produced visual ratings that were consistently significantly different from the weedy check were POST MO+Clove+Cinnamon and MO+Citrus. POST MO+Clove+Cinnamon was rated at 90% or greater efficacy for 10 of 12 ratings and was significantly different from weedy check in all 12 observations (Tables 3.7 and 3.8) The PRE manuka treatments were consistently rated low (below 40 % in 18 out of 24 ratings).

3.4.2. Weed Control Efficacy: Corn Trials

In the sweet corn trials, there were not great differences in weed biomass between Simcoe and Ridgetown (Table 3.6), but there was a great difference in weed biomass between planting dates at both sites with much greater total weed biomass in the late versus the early-planted trials. The PRE manuka treatments (either with or without surfactant) did not provide a significant reduction in weed biomass in any of the four trials. PRE Callisto treatments were also not significantly different from the weedy for all planting dates and at both locations with an exception of Late-planted

Simcoe. Visual field observations indicated that Callisto killed many of the tomatoes and did not control purslane (*Portulaca oleracea* L.). In the late-planted trials at Ridgetown, all of the POST manuka treatments provided a significant reduction in weed biomass (Table 3.6). The same was not true at Simcoe. For three of the four trials, the POST MO+Citrus and POST MO+Clove+Cinnamon treatments had weed biomass values that were significantly different from the weedy checks.

Visual efficacy ratings for the corn trials closely resembled those for the tomato trials where the PRE manuka treatments were rated poorly compared to the POST manuka treatments (Tables 3.7, 3.8, 3.9, and 3.10). Table 3.9 shows that 41 POST ratings out of a total of 42 were significantly different from the weedy check. The exception was the 3rd observation in Ridgetown for POST Callisto. POST MO treatments consistently had visual weed biomass ratings that were significantly different from the weedy check. Table 3.10 shows 39 POST observations out of 42 were significantly different from the weedy checks. When considering only the POST MO treatments, 34 out of 36 observations had ratings that were significantly different from the weedy checks. In other words, 94% of the time POST manuka oil treatments produced visual efficacy ratings that were significantly different from the weedy checks. Additionally, as per the tomato trials, the POST MO+Clove+Cinnamon treatment was rated above 90% in every instance in both the early and late planted corn trials.

3.4.3. Crop Yield: Tomatoes

Weed pressure was not statistically analyzed between locations, but based on visual observations (data not shown), weed biomass results (Table 3.5), and visual weed efficacy ratings (Tables 3.7 and 3.8) there was greater weed pressure in Ridgetown. Published literature has extensively acknowledged the relation between weed competition and crop yield (Felix et al. 2007; Gianessi 2013; Khaliq et al. 2011; Knezevic et al. 2013; Oerke 2006). The greater weed pressure in the tomato trials at Ridgetown versus Simcoe may explain part of the reason why there was a great difference in the yields between locations (Table 3.11).

Visual crop injury ratings displayed on Tables 3.12 and 3.13 show the damage that PRE and POST Callisto had on the tomato plants. For these treatments, 4 of the PRE Callisto treatments and in 3 of the 4 POST Callisto treatments caused a significant decline in yield in contrast to the POST manuka oil treatments (Table 3.11). The yield results related somewhat to the efficacy results as measured by weed biomass. The PRE manuka treatments for both locations did not protect tomato yields and therefore had yields that were significantly the same as the weedy checks. When comparing the PRE manuka oil treatments to the weed free, 6 out of the 8 scenarios had tomatoes yields that were significantly different from the weed free. Only the late-planted Simcoe PRE MO and PRE MO+Agral 90 were significantly similar to the weed free. Table 3.11 shows that out of all the manuka oil POST treatments scenarios 46% had significantly similar yields to the weed free and 50% were not significantly different from the weedy checks. There was no treatment that consistently had yields that were different from the weedy. Lastly, PRE Callisto produced tomato yields that were numerically and significantly worse than the other treatments. Many of the POST manuka oil treatments did maintain tomato yields at levels not significantly different from the weed free checks. This was more the case at the Simcoe site than at the Ridgetown site, perhaps because of the greater weed pressure at Ridgetown.

3.4.4. Crop Yield: Corn Trials

Statistical analysis between locations was not performed, but based of off visual field observations (data not shown), total weed biomass values (Table 3.6) and visual efficacy ratings (Tables 3.9 and 3.10) sweet corn yields experienced higher weed pressure at Simcoe and generally had lower yields compared to Ridgetown (Table 3.14). For the late-planted trial at Ridgetown it was not possible to determine whether the treatments were beneficial to yields because there were no significant differences in yield between any of the treatments. POST manuka oil treatments had yields that were not significantly different from the weedy checks except for POST MO+Nu Film P and POST MO+Yucca in the early-plant trials. It was more likely that the Simcoe POST manuka oil treatments were likely to have yields that were significantly higher than the weedy checks. Early-planted Simcoe had three POST manuka oil treatments (POST MO, POST MO+Nu Film P and POST MO+Yucca) that had yields that were not significantly different from the weedy checks. Simcoe late-planted corn had POST manuka oil treatments that were 85% likely to have yields higher than the weedy checks. The only treatment that has yields not significantly different from the weedy checks was POST MO. This did not necessarily relate well to the weed efficacy results; however, somewhat in relation to the efficacy results.

There were two POST treatments (POST MO+Clove+Cinnamon and POST MO+Citrus) that, across both planting dates and locations, had yields that were significantly similar to the weed free. The PRE Callisto treatments resulted in no significant sweet corn yield loss for all four trials. Surprisingly, POST Callisto treatments did not have yields that were significantly different from the weedy checks.

Visual crop injury ratings were negligible throughout most of the growing season (Tables 3.15 and 3.16). There were some signs of crop injury in the early part of the season, but this did not reflected in the yields and zero signs of injury were present on mature plants.

3.5. Discussion:

The efficacy of Callisto can be influenced by soil characteristics. The Callisto label warns against applications on soils with soil organic matter higher than 10% (Syngenta Canada 2016). Dyson et al. (2002) reported that soil pH and organic matter content in soil could impact the overall efficacy of mesotrione. Dayan et al. (2011) suggested that there might be a relationship between manuka oil efficacy and soil organic matter content and/or clay content. Increasing soil pH can increase mesotrione degradation (Chaabane et al. 2008). The Ridgetown corn trials were on soils much higher in clay content and organic matter versus the Simcoe trials and soil pH levels were higher at Ridgetown than at Simcoe (Table 3.1). The PRE treatments for both early and late-planted tomatoes in Simcoe had higher yields compared to Ridgetown (Table 3.11). It may be possible that the higher soil pH in Ridgetown (Table 3.1) helped to speed the dissipation of leptospermum in the soil at a higher rate compared to Simcoe resulting in lower weed control efficacy (Table 3.7 and 3.8). The efficacy of Callisto did not seem to be influenced by the soil pH difference between the two locations compared to manuka oil. Mesotrione may have an efficacy that is able to buffer against higher soil pH changes.

Simcoe had higher weed diversity compared to Ridgetown and Ridgetown had more grass than broadleaf weeds (personal observations - data not shown). This may help to explain why there was limited tomato crop injury (Tables 3.12 and 3.13) at Ridgetown later in the season because the thick border of grassy weeds could have shielded the crop from the herbicide. Grain (Boydston and Williams 2005) and sweet corn (O'Sullivan et al. 2002) cultivars are able to metabolize PRE and POST mesotrione resulting in low crop injury (Table 3.15 and 3.16), reduced weed biomass (Table 3.6), and higher yields (Table 3.14). The PRE and POST Callisto treatments were infested with purslane (*Portulaca oleracea* L.), which is a short creeping weed species. This did not dramatically hinder corn yields, but tomatoes do not compete well with purslane and are unable to metabolize mesotrione (Felix et al. 2007). The result for the Callisto treatments in the tomato trials was high crop injury (Table 3.12 and 3.13), high weed biomass (Table 3.5), and lower yields (Table 3.11). These results agree with those of Felix et al. (2007).

The PRE treatments may have been impacted by rainfall. According to the Callisto label, minor levels of rainfall are beneficial for PRE applications, but not for POST (Syngenta Canada 2016). This may also be true for leptospermum. Heavy rain within 12, 24 or 48 hours may reduce the soil residual capacity in PRE treatments and reduce the contact time of the herbicide on foliage in POST treatments. According to weather data for our trials (see Appendix 7.12 to 7.16) eight application timings experienced rainfall within 48 hours (Table 3.4) and three application times experienced excessive rainfall (above 6.35 mm). These three were following the PRE applications for early-planted crops in Simcoe and Ridgetown and the first POST application in early-planted crops in Simcoe (Table 3.4). Table 3.4 lists all 8 treatment-timings that experienced rainfall within 48 hours of application of PRE or POST treatments. Simcoe experienced rainfall within 48 hours of application on 3 out of the 8 application timings. Ridgetown experienced 5 rainfall events within 48 hours out of 8 application timings (Table 3.4). Simcoe experienced 165 mm of rain in June, which was 85% above the historical average (see Appendix 7.13). This is important because there were 12 application timings in June between both locations (Table 3.3), which accounts for 38% of the total application times. Ridgetown experienced higher than average rainfall for May and June at 14.2% and 38.4% above historical averages, respectively (Appendix 7.18). Heavy rainfall following PRE applications for the early-planted crops on June 5th in Simcoe and May 29th in Ridgetown (Tables 3.2, 3.3 and 3.4) may have caused reductions in efficacy and lower yields (Tables 3.11 and 3.14). Otto et al. (2016) reported that, under certain field conditions, an average rainfall of 20 mm was adequate to cause traceable runoff of POST applications of herbicides and hinder herbicidal efficacy. The heavy rainfall events may have impacted PRE applications more than POST applications because there was more heavy rainfall within 48 hours of the PRE compared to the POST applications (Table 3.4). There is evidence that rainfall can either enhance or hinder the efficacy of mesotrione (Otto et al. 2016) and potentially leptospermum. Research by Dayan et al.

(2011) suggests that manuka oil, specifically leptospermone, might have weak soil residual activity that is affected by rainfall in a manner similar to mesotrione. Otto et al. (2016) reported an increase in crop injury ratings due to mesotrione following heavy rainfall. PRE manuka oil treatment results indicated that soil residual activity of PRE applied manuka oil may have been further weakened with heavy rainfall. PRE treatments for late-planted crops in Simcoe and Ridgetown did not receive significant rainfall within 48 hours of application. The lack of heavy rainfall events for the later-planted crops may have allowed the herbicides to remain in the soil longer and had a higher chance of making contact with weed species.

3.6. Conclusion

MO was able to provide some level of POST efficacy in comparison to weedy checks in all eight of the different scenarios, but it was not consistent. POST efficacy for MO was significantly higher compared to PRE efficacy. MO has lower efficacy when applied alone compared to when it is applied as a tank mix with other herbicides and surfactants. There was not necessarily a clear efficacy benefit among the eight different scenarios for adding other essential oils and surfactants. However, two POST treatments (POST MO+Clove+Ciinamon and POST MO+Citrus) seemed to be more consistent with their efficacy in the different scenarios. This may be to do the double MOA in the tank mixes, but more research is needed on this topic. Due to the fact that organic farmers have multifaceted weed management systems they might be able to incorporate MO tank mixes and achieve results that would be significantly similar to weed free conditions. This may help minimize labour time and costs. PRE efficacy of MO was very limited and did not provide weed control that was significantly different from the weedy checks. Given the high cost of MO and the low PRE efficacy, MO is not recommended as a PRE herbicide option on agricultural soils.

PRE and POST applications of MO caused negligible crop injury in tomatoes or sweet corn as long as the spray was directed between the rows and crop foliage contact was avoided. High rates and multiple applications of MO could help provide yields that are significantly higher compared to no herbicide applications or weed control techniques. MO treatments must be applied early and high application rates must be maintained throughout the growing season in order to manage weeds at acceptable levels. Problematic weeds, such as purslane, were not controlled by MO or other contact herbicides used in this experiment. Other weed management techniques, such as cultivation, may be the only way to control difficult weeds in an organic system. It is possible that an organic farmer may be able to adapt MO-based herbicides to their management practices if they had a high valued crop and were persistent with applications. High valued crops would better justify the cost of multiple applications and avoid hand weeding in areas that do not accommodate large cultivation equipment.

Table 3. 1. Field name, location, crop, planting dates, and soil characteristics for the experimental fields in 2015.

Field Name	Location	Crop	Planting date	Soil Texture	Clay %	Silt %	Sand %	Soil OM %	Soil pH
TES	Simcoe	Tomatoes cv. Mountain Glory	Early June 5th	loamy fine sand Wattford	4	16	80	1.3	6.4
CES	Simcoe	Corn cv. ZEAMS	Early June 4th	loamy fine sand Wattford	4	16	80	1.3	6.4
TER	Ridgetown	Tomatoes cv. LYPES	Early May 29th	Watford/Brady Series	20.9	29.5	49.6	8.2	6.8
CER	Ridgetown	Corn cv. ZEAMS	Early May 29th	loamy Watford/Brady Series	17	33.6	49.6	9.2	7.2
TLS	Simcoe	Tomatoes cv. Mountain Glory	Late July 11th	loam Bennington	10	43	47	1.9	6
CLS	Simcoe	Corn cv. ZEAMS	Late July 10th	loam Bennington	10	43	47	1.9	6
TLR	Ridgetown	Tomatoes cv. LYPES	Late June 11th	Watford/Brady Series	20.9	29.5	49.6	8.2	6.8

CLR	Ridgetown	Corn cv. ZEAMS	Late June 11th	loamy Watford/Brady Series	17	33.6	49.6	9.2	7.2

Table 3. 2. Location, planting and harvest dates for corn and tomato trials in 2015.

	Corn Planting Dates		Corn Harvest Dates	
	Early	Late	Early	Late
Simcoe	June 4th	July 10th	August 21st	September 25th
Ridgetown	May 29th	June 11th	August 24th	September 9th
	Tomato Planting Dates		Tomato Harvest Dates	
	Early	Late	Early	Late
Simcoe	June 5th	July 11th	August 21st	September 25th
Ridgetown	May 29th	June 11th	August 24th	September 9th

Table 3. 3. Date and times of PRE and POST treatment application for sweet corn and tomatoes at both locations (Simcoe and Ridgetown) for trials in 2015.

Simcoe					
Corn		PRE	POST 1	POST 2	POST 3
	Early	Jun 5th	Jun 26th	Jul 15th	Aug 5th
		11:00AM	10:30AM	9:00AM	10:15AM
	Late	Jul 10th	Jul 29th	Aug 12th	Aug 25th
		11:00AM	10:30AM	8:45AM	10:30AM
Tomatoes		PRE	POST	POST	POST
	Early	Jun 5th	Jun 26th	Jul 15th	Aug 5th
		11:00AM	10:30AM	9:00AM	10:15AM
	Late	Jul 10th	Jul 29th	Aug 12th	Aug 25th
		11:00AM	10:30AM	8:45AM	10:30AM
Ridgetown					
Corn		PRE	POST 1	POST 2	POST 3
	Early	May 29th	Jun 11th	Jun 24th	Jul 10th

		5:35PM	9:15AM	9:20AM	9:50AM
	Late	Jun 11th	Jun 24th	Jul 10th	Jul 22nd
		9:35AM	9:05AM	10:15AM	9:10AM
Tomatoes		PRE	POST	POST	POST
	Early	May 29th	Jun 11th	Jun 24th	Jul 10th
		5:20PM	8:20AM	8:40AM	9:00AM
	Late	Jun 11th	Jun 24th	Jul 10th	Jul 22nd
		8:50AM	8:20AM	9:25AM	8:45AM

Table 3. 4. Accumulative rainfall (mm) for 12, 24 and 48 hours after PRE and POST applications at each location in 2015.

Simcoe Corn and Tomatoes		Early			Late	
	12 hr	24 hr	48 hr	12 hr	24 hr	48 hr
PRE	9.8 mm	9.8 mm	9.8 mm	0 mm	0 mm	0.2 mm
POST 1	0 mm	6.6 mm	59.4 mm	0 mm	0 mm	0 mm
POST 2	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
POST 3	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm

Ridgetown Corn and Tomatoes		Early			Late	
	12 hr	24 hr	48 hr	12 hr	24 hr	48 hr
PRE	0 mm	11.4 mm	66.2 mm	0 mm	0 mm	0.3 mm
POST 1	0 mm	0 mm	0.3 mm	0 mm	0 mm	0 mm
POST 2	0 mm	0 mm	0 mm	0.2 mm	0.2 mm	0.2 mm
POST 3	0.2 mm	0.2 mm	0.2 mm	0 mm	0 mm	0 mm

Table 3. 5. The effect of various weed control treatments on weed biomass (g/m2) in tomato experiments at two locations (Simcoe and Ridgetown, ON) and two planting dates (early and late) in 2015.

Treatment	Simcoe		Ridgetown	
	Early	Late	Early	Late
	------------ (g/m^2) -----------		------------ (g/m^2) -----------	
PRE Manuka	52.1 *ab* [a]	153.1 *abc*	127.8 *a*	191.9 *ab*
PRE Manuka+Agral 90	51.5 *ab* [b]	74.3 *bc*	132.9 *a*	119.9 *ab*
PRE Callisto	72.3 *a*	187.3 *ab*	176.9 *a*	209.2 *a*
POST Manuka	26.8 *ab*	78.9 *bc*	96.5 *a*	121.2 *ab*
POST Manuka+Clove+Cinnamon	14.4 *ab*	7.2 *cd*	12.1 *b*	45.6 *b*
POST Manuka+Citrus	3.8 *bc*	40.8 *bcd*	61.4 *ab*	123.4 *ab*
POST Manuka+Agral 90	11.2 *b*	49.2 *bcd*	155.5 *a*	113.4 *ab*
POST Manuka+Nu Film P	26.5 *ab*	27.2 *cd*	81.0 *a*	153.5 *ab*
POST Manuka+Yucca	14.1 *ab*	39.6 *bcd*	95.2 *a*	155.5 *ab*
POST Callisto	19.5 *ab*	46.3 *bcd*	92.8 *a*	164.7 *ab*
Weedy	57.6 *ab*	228.9 *a*	234.2 *a*	250.2 *a*
Weed free	0.0 *c*	0.0 *d*	0.0 *c*	0.0 *c*

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

[b] Methodology error resulted in the early Simcoe weed biomass values to be a combined total between corn and tomatoes.

Table 3. 6. The effect of various weed control treatments on weed biomass (g/m^2) in sweet corn experiments at two locations (Simcoe and Ridgetown, ON) and two planting dates (early and late) in 2015.

	Simcoe		Ridgetown	
Treatment	Early	Late	Early	Late
	------------ (g/m^2) -----------		------------ (g/m^2) -----------	
PRE Manuka	52.1 *ab* [a]	142.5 *ab*	31.3 *abc*	74.9 *bcd*
PRE Manuka+Agral 90	51.5 *ab* [b]	131.7 *ab*	48.2 *ab*	162.4 *abc*
PRE Callisto	72.3 *a*	27.9 *bc*	3.8 *abcd*	194.6 *ab*
POST Manuka	26.8 *ab*	26.9 *bc*	10.6 *abcd*	13.3 *cd*
POST Manuka+Clove+Cinnamon	14.4 *ab*	19.8 *bc*	1.6 *cd*	0.0 *d*
POST Manuka+Citrus	3.8 *bc*	24.6 *bc*	1.1 *d*	0.0 *d*
POST Manuka+Agral 90	11.2 *b*	36.6 *abc*	0.0 *e*	11.4 *cd*
POST Manuka+Nu Film P	26.5 *ab*	36.2 *abc*	2.0 *bcd*	15.8 *cd*
POST Manuka+Yucca	14.1 *ab*	33.2 *abc*	14.8 *abcd*	12.8 *cd*
POST Callisto	19.5 *ab*	15.3 *c*	2.6 *abcd*	35.7 *cd*
Weedy	57.6 *ab*	238.8 *a*	65.3 *a*	289.9 *a*
Weed free	0.0 *c*	0.0 *d*	0.0 *e*	0.0 *d*

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

[b] Methodology error resulted in the early Simcoe weed biomass values to be a combined total between corn and tomatoes.

Table 3. 7. Visual herbicide efficacy ratings (%) of treatments on early-planted tomatoes at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

	Simcoe			Ridgetown		
Treatment	1st	2nd	3rd	1st	2nd	3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	60 *cde* [a]	11 *fg*	14 *e*	53 *cde*	14 *e*	13 *c*
PRE Manuka+Agral 90	50 *de*	10 *g*	9 *e*	18 *d*	8 *e*	4 *c*
PRE Callisto	84 *abc*	26 *efg*	28 *de*	87 *abc*	24 *de*	12 *c*
POST Manuka	78 *bc*	52 *cde*	67 *bc*	80 *bc*	46 *cd*	62 *ab*
POST Manuka+Clove+Cinnamon	94 *a*	93 *a*	92 *a*	90 *ab*	80 *a*	84 *a*
POST Manuka+Citrus	84 *abc*	94 *a*	95 *a*	95 *a*	74 *ab*	78 *ab*
POST Manuka+Agral 90	91 *ab*	87 *ab*	90 *ab*	85 *abc*	68 a*bc*	81 *a*
POST Manuka+Nu Film P	84 *abc*	80 *abc*	77 *abc*	74 *c*	59 *abc*	73 *ab*
POST Manuka+Yucca	84 *abc*	70 *bcd*	70 *bc*	77 *bc*	68 *abc*	74 *ab*
POST Callisto	81 *abc*	40 *def*	55 *cd*	50 *abc*	53 *bc*	49 *b*
Weedy	0 *e*	0 *fg*	0 *f*	0 *e*	0 *de*	0 *bc*
Weed free	100	100	100	100	100	100

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Table 3. 8. Visual herbicide efficacy ratings (%) of treatments on late-planted tomatoes at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

	Simcoe			Ridgetown		
Treatment	1st	2nd	3rd	1st	2nd	3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	26 *d* [a]	40 *bce*	26 *c*	50 *bcd*	14 *d*	12 *e*
PRE Manuka+Agral 90	28 *cde*	27 *c*	39 *bcd*	45 *cd*	10 *d*	12 *e*
PRE Callisto	87 *ab*	75 *ab*	39 *bcd*	88 *a*	50 *c*	41 *def*
POST Manuka	47 *bcd*	85 *a*	78 *a*	69 *abc*	75 *abc*	71 *bc*
POST Manuka+Clove+Cinnamon	92 *a*	91 *a*	93 *a*	82 *a*	94 *a*	94 *a*
POST Manuka+Citrus	63 *abcd*	89 *a*	88 *a*	75 *ab*	90 *ab*	88 *ab*
POST Manuka+Agral 90	76 *abc*	83 *ab*	87 *a*	73 *ab*	80 *abc*	83 *ab*
POST Manuka+Nu Film P	81 *ab*	84 *a*	74 *ab*	73 *ab*	77 *abc*	71 *bc*
POST Manuka+Yucca	81 *ab*	79 *ab*	83 *a*	71 *abc*	82 *ab*	63 *cd*
POST Callisto	67 *abcd*	86 *a*	93 *a*	71 *abc*	67 *bc*	61 *cd*
Weedy	0 *e*	0 *e*	0 *d*	0 *d*	0 *e*	0 *f*
Weed free	100	100	100	100	100	100

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Table 3. 9. Visual herbicide efficacy ratings (%) of treatments on early-planted corn at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

Treatment	Simcoe			Ridgetown		
	1st	2nd	3rd	1st	2nd	3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	64 *bc* [a]	11 *g*	11 *d*	51 *bcd*	55 *de*	29 *d*
PRE Manuka+Agral 90	44 *c*	9 *fg*	17 *cd*	35 *cd*	31 *e*	8 *d*
PRE Callisto	89 *ab*	36 *efg*	51 *bc*	81 *a*	47 *def*	62 *bc*
POST Manuka	78 *ab*	59 *cde*	62 *b*	86 *a*	84 *abc*	92 *a*
POST Manuka+Clove+Cinnamon	96 *a*	96 *a*	92 *a*	92 *a*	93 *a*	91 *a*
POST Manuka+Citrus	87 *ab*	90 *ab*	92 *a*	96 *a*	90 *ab*	92 *ab*
POST Manuka+Agral 90	88 *ab*	81 *abc*	90 *a*	90 *a*	90 *ab*	94 *a*
POST Manuka+Nu Film P	81 *ab*	77 *abc*	79 *ab*	88 *a*	69 *bcd*	86 *ab*
POST Manuka+Yucca	76 *b*	67 *bcd*	69 *ab*	90 *a*	86 *abc*	86 *ab*
POST Callisto	80 *ab*	44 *def*	70 *ab*	78 *ab*	64 *cd*	56 *cd*
Weedy	0 *c*	0 *h*	0 *d*	0 *d*	0 *f*	0 *cd*
Weed free	100	100	100	100	100	100

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Table 3. 10. Visual herbicide efficacy ratings (%) of treatments on late-planted corn at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

Treatment	Simcoe			Ridgetown		
	1st	2nd	3rd	1st	2nd	3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	26 *d* [a]	39 *bc*	29 *b*	75 *c*	40 *de*	45 *def*
PRE Manuka+Agral 90	28 *cde*	31 *b*	35 *bc*	78 *c*	29 *e*	30 *e*
PRE Callisto	87 *ab*	79 *a*	89 *a*	91 *ab*	62 *cd*	62 *cd*
POST Manuka	47 *bcde*	79 *a*	76 *a*	95 *a*	88 *ab*	87 *ab*
POST Manuka+Clove+Cinnamon	92 *a*	93 *a*	93 *a*	95 *a*	93 *ab*	95 *a*
POST Manuka+Citrus	63 *abcde*	87 *a*	93 *a*	97 *a*	96 *abc*	96 *a*
POST Manuka+Agral 90	76 *abc*	89 *a*	87 *a*	95 *a*	90 *ab*	88 *ab*
POST Manuka+Nu Film P	81 *ab*	85 *a*	73 *a*	93 *a*	91 *ab*	90 *ab*
POST Manuka+Yucca	81 *ab*	83 *a*	84 *a*	96 *a*	94 *a*	91 *ab*
POST Callisto	67 *abcde*	89 *a*	93 *a*	82 *bc*	69 *bcd*	77 *bc*
Weedy	0 *e*	0 *c*	0 *c*	0 *d*	0 *e*	0 *f*
Weed free	100	100	100	100	100	100

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Table 3. 11. The effect of various weed control treatments on tomato yields (t/ha) at two locations (Simcoe and Ridgetown, ON) and two planting dates (early and late) in 2015.

	Simcoe		Ridgetown	
Treatment	Early	Late	Early	Late
	------------ (t/ha) ------------		------------ (t/ha) ------------	
PRE Manuka	12.3 *bc*[a]	18.6 *abcd*	6.4 *bc*	8.4 *b*
PRE Manuka+Agral 90	5.6 *cd*	19.7 *abc*	2.3 *bc*	6.9 *b*
PRE Callisto	0.8 *d*	0.9 *e*	0.7 *c*	14.9 *b*
POST Manuka	16.0 *ab*	14.2 *bcd*	5.5 *bc*	10.0 *b*
POST Manuka+Clove+Cinnamon	19.3 *ab*	22.9 *ab*	14.2 *bc*	27.8 *ab*
POST Manuka+Citrus	18.3 *ab*	23.5 *ab*	25.5 *b*	9.4 *b*
POST Manuka+Agral 90	15.7 *ab*	23.9 *ab*	14.5 *bc*	24.0 *ab*
POST Manuka+Nu Film P	17.0 *ab*	22.2 *ab*	6.9 *bc*	11.6 *b*
POST Manuka+Yucca	12.7 *bc*	26.9 *a*	3.7 *bc*	8.4 *b*
POST Callisto	1.0 *d*	6.7 *de*	2.5 *bc*	18.6 *ab*
Weedy	3.9 *cd*	8.4 *cde*	4.3 *bc*	10.2 *b*
Weed free	23.2 *a*	24.2 *ab*	65.7 *a*	55.3 *a*

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Table 3. 12. Visual crop injury ratings (%) of treatments on early-planted tomatoes at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

Treatment	Simcoe 1st	Simcoe 2nd	Simcoe 3rd	Ridgetown 1st	Ridgetown 2nd	Ridgetown 3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	5 *b* [a]	NCI [b]	NCI	NCI	NCI	NCI
PRE Manuka+Agral 90	6 *b*	NCI	NCI	NCI	NCI	NCI
PRE Callisto	88 *a*	88 *a*	87 *a*	36 *ab*	NCI	78 *a*
POST Manuka	4 *b*	NCI	NCI	10 *b*	NCI	9 *c*
POST Manuka+Clove+Cinnamon	NCI	NCI	NCI	11 *b*	NCI	NCI
POST Manuka+Citrus	NCI	NCI	NCI	7 *b*	NCI	9 *c*
POST Manuka+Agral 90	NCI	NCI	NCI	10 *b*	NCI	21 *bc*
POST Manuka+Nu Film P	2 *b*	NCI	NCI	12 *b*	NCI	16 *bc*
POST Manuka+Yucca	NCI	8 *b*	8 *c*	9 *b*	NCI	13 *bc*
POST Callisto	37 *b*	37 *b*	45 *bc*	65 *a*	NCI	NCI

[a] Sites were analyzed separately because of significance of main effects and/or interactions by location represented in the columns. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

[b] Abbreviation: NCI, No crop injury, indicates that herbicide treatments did not cause injury to crops.

Table 3. 13. Visual crop injury ratings (%) of treatments on late-planted tomatoes at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

	Simcoe			Ridgetown		
Treatment	1st	2nd	3rd	1st	2nd	3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	NCI [b]	NCI	NCI	7 *ab*	NCI	NCI
PRE Manuka+Agral 90	NCI	NCI	6 *ab*	12 *ab*	NCI	NCI
PRE Callisto	80 *a* [a]	82 *a*	87 *a*	38 *a*	NCI	NCI
POST Manuka	NCI	NCI	15 *ab*	9 *ab*	NCI	NCI
POST Manuka+Clove+Cinnamon	NCI	NCI	6 *ab*	5 *ab*	NCI	8 *a*
POST Manuka+Citrus	NCI	NCI	6 *b*	6 *ab*	NCI	8 *a*
POST Manuka+Agral 90	NCI	NCI	5 *b*	6 *b*	NCI	6 *a*
POST Manuka+Nu Film P	NCI	NCI	NCI	8 *ab*	NCI	4 *a*
POST Manuka+Yucca	NCI	NCI	NCI	8 *ab*	NCI	NCI
POST Callisto	15 *a*	41 *a*	57 *bc*	9 *ab*	NCI	2 *a*

[a] Sites were analyzed separately because of significance of main effects and/or interactions by location represented in the columns. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

[b] Abbreviation: NCI, No crop injury, indicates that herbicide treatments did not cause injury to crops.

Table 3. 14. The effect of various PRE and POST weed control treatments on sweet corn yields (t/ha) at two locations (Simcoe and Ridgetown, ON) and two planting dates (early and late) in 2015.

Treatment	Simcoe		Ridgetown	
	Early	Late	Early	Late
	------------ (t/ha) ------------		------------ (t/ha) ------------	
PRE Manuka	9.3 *cdef* [a]	17.0 *bc*	24.9 *bc*	24.2 *a*
PRE Manuka+Agral 90	3.5 *ef*	16.8 *bc*	21.0 *c*	27.2 *a*
PRE Callisto	22.5 *ab*	32.8 *a*	27.3 *abc*	26.7 *a*
POST Manuka	8.1 *def*	21.1 *abc*	29.7 *abc*	30.0 *a*
POST Manuka+Clove+Cinnamon	21.0 *abc*	30.8 *a*	32.1 *abc*	28.8 *a*
POST Manuka+Citrus	17.1 *abcd*	23.2 *ab*	28.5 *abc*	26.4 *a*
POST Manuka+Agral 90	13.3 *bcde*	25.8 *ab*	30.9 *abc*	25.8 *a*
POST Manuka+Nu Film P	11.9 *bcdef*	24.1 *ab*	35.5 *ab*	30.4 *a*
POST Manuka+Yucca	8.7 *def*	25.6 *ab*	35.9 *ab*	30.9 *a*
POST Callisto	17.6 *abcd*	27.9 *ab*	31.7 *abc*	26.4 *a*
Weedy	1.2 *f*	10.7 *c*	20.8 *c*	21.5 *a*
Weed free	28.4 *a*	23.7 *ab*	38.6 *a*	30.8 *a*

[a] Site and planting date were analyzed separately because of significance of main effects and/or interactions. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Table 3. 15. Visual crop injury ratings (%) of treatments on early-planted corn at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

	Simcoe			Ridgetown		
Treatment	1st	2nd	3rd	1st	2nd	3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	4 *a* [a]	5 *ab* [b]	NCI[2]	3 *abc*	NCI	NCI
PRE Manuka+Agral 90	6 *a*	NCI	NCI	3 *bc*	NCI	NCI
PRE Callisto	3 *a*	NCI	NCI	3 *abc*	NCI	NCI
POST Manuka	3 *a*	5 *a*	NCI	12 *a*	NCI	NCI
POST Manuka+Clove+Cinnamon	5 *a*	5 *ab*	NCI	7 *abc*	NCI	NCI
POST Manuka+Citrus	6 *a*	6 *a*	NCI	11 *ab*	NCI	NCI
POST Manuka+Agral 90	4 *a*	4 *ab*	NCI	6 *abc*	NCI	NCI
POST Manuka+Nu Film P	7 *a*	NCI	NCI	6 *abc*	NCI	NCI
POST Manuka+Yucca	6 *a*	4 *ab*	NCI	9 *abc*	NCI	NCI
POST Callisto	4 *a*	3 *b*	NCI	2 *abc*	NCI	NCI

[a] Sites were analyzed separately because of significance of main effects and/or interactions by location represented in the columns. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

[b] Abbreviation: NCI, No crop injury, indicates that herbicide treatments did not cause injury to crops.

Table 3. 16. Visual crop injury ratings (%) of treatments on late-planted corn at two locations (Simcoe and Ridgetown, ON) in 2015. Observations were recorded in two-week intervals for each treatment.

Treatment	Simcoe			Ridgetown		
	1st	2nd	3rd	1st	2nc	3rd
	------------ (%) ------------			------------ (%) ------------		
PRE Manuka	NCI [a]	NCI [b]	NCI	5 *a*	NC	NCI
PRE Manuka+Agral 90	NCI	NCI	NCI	5 *a*	NC	NCI
PRE Callisto	NCI	NCI	NCI	5 *a*	NC	NCI
POST Manuka	NCI	NCI	NCI	15 *a*	NC	NCI
POST Manuka+Clove+Cinnamon	NCI	NCI	NCI	15 *a*	NC	NCI
POST Manuka+Citrus	NCI	NCI	NCI	2 *a*	NC	NCI
POST Manuka+Agral 90	NCI	NCI	NCI	16 *a*	NC	NCI
POST Manuka+Nu Film P	NCI	NCI	NCI	14 *a*	NC	NCI
POST Manuka+Yucca	NCI	NCI	NCI	18 *a*	NC	NCI
POST Callisto	NCI	NCI	NCI	NCI	NC	NCI

[a] Sites were analyzed separately because of significance of main effects and/or interactions by location represented in the columns. Means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

[b] Abbreviation: NCI, No crop injury, indicates that herbicide treatments did not cause injury to crops.

Chapter 4.0. Growth room experiment: Exploring the PRE-emergent efficacy of manuka oil as impacted by the addition of adjuvant or surfactant tank mixes.

4.1. Abstract

POST efficacy of manuka oil (MO) has been previously researched, but there are almost no studies on PRE efficacy of MO. This is a significant herbicidal characteristic for organic farmers because there are no registered PRE herbicides. When considering the PRE efficacy of potential organic herbicides, proper tank mix options as well as levels of rainfall are important. They are important because both are able to influence the efficacy of the active ingredient(s) in MO. Growth room experiments were conducted on green pigweed (*Amaranthus powellii* S. Wats.) and large crabgrass (*Digitaria sanguinalis* L.) seeded in a sandy growth media. Experiments were organized as a RCBD with simulated rain or no rain and four MO-based treatments tested against one control and one conventional industry standard (Callisto®). The four MO-based treatments were MO+Nu Film P, MO+Yucca, and MO+Agral 90. Total seed emergence counts (%) and weed biomass (mg/m^2) was calculated to determine efficacy. Results showed that MO exhibited limited PRE efficacy and the addition of surfactants enhanced the efficacy by decreasing weed biomass and emergence rates; however the results were not consistent.

4.2. Introduction

The addition of adjuvants and/or surfactants might be able to enhance not only the POST efficacy, but also the PRE efficacy of certain herbicides (Erbach and Lovely 1975). Currently, there are numerous tank mix options for commercial POST conventional herbicides and limited organic options. Buffington and McDonald (2006) and Koren (1972) stated that, the efficacy of PRE herbicides could be improved when applied with the proper tank mix partners and/or formulation. Many spreader-sticker surfactants have been reported to be able to stick to soil organic matter and plant residue on and in the soil (Buffington and McDonald 2006; Koren 1972; Tample and Hilton 1963). This has the potential to increase efficacy by keeping the active ingredients in the weed seed germination zone longer; however, plant residue is often elevated off the soil surface and can limit soil contact. Erbach and Lovely (1975) conducted experiments on different formulas of PRE herbicides to determine what formulas were ideal for increasing efficacy. They discovered that plant residue was often an issue on farms that practiced low or no-till. This was because the large plant residue was not incorporated into the soil leaving areas for herbicide product to be lost. This could be problematic because some organic farmers practice no-till and/or purposefully leave plant residue; however, it is very dependent on the management practices of the individual farmer. Erbach and Lovely (1975) noticed that granular herbicide performed better in a no-till systems, however, liquid applications were superior in almost all other tillage systems. MO is a plant derived essential oil and many essential oils have short shelf lives requiring proper storage away from light, air, and heat (Tisserand and Young 2013). Many essential oil producers claim that even under ideal shortage conditions, the essential oil starts to breakdown quickly, which could further decrease efficacy (Tisserand and Young 2013). On the contrary, Harpaz et al. (2003) and Karabagias et al. (2011) found that plant-based essential oils could prolong the shelf life of meat. Thyme and oregano oil were able to extend the shelf life of lamb by approximately 3 days (Karabagias et al. 2011) and drastically prolong shelf life of fish (Harpaz et al. 2003). The conflicting results illustrate the need for further research on essential oil-based organic herbicides such as MO. Knowing why essential oils have short shelf lives and identifying what constituents easily break down is important for understanding whether MO would be vulnerable to degradation

Herbicides that have PRE efficacy can have soil residual activity that allows the herbicide to be soil applied and has a prolonged period of weed protection (Dayan et al 2011; Kay and Mckell 1963; Kempen et al. 1963; Khaliq et al. 2011; Knake et al. 1967; Knezevic et al. 2013). This characteristic creates a benefit to farmers by providing more flexibility in management practises, can reduce weed pressure, and increase yields (Dayan et al 2011; Koren 1972; Lang and Clutterbuck 1991.). Manuka oil has been shown to have some PRE efficacy. Dayan et al. (2011) observed tiny bleached seedlings emerging after an application of 1% v/v manuka oil. This indicated that the MO stayed in the soil long enough to have a negative impact on the newly emerged seedlings. Their work concluded that MO displayed PRE efficacy at 1% v/v with a significant reduction in weed biomass, but had no noticeable effects on emergence rates. The study did not investigate the PRE efficacy of MO with rain simulation. It has been widely reported (Erbach and Lovely 1975; Otto 2016; Philip and Wiebe 2002; Quan 2015; Roggenbuck et al. 1993) that rainfall is important in activating PRE herbicides, which is why it was important to incorporate rain simulation in this experiment. On the contrary, heavy rainfall can increase crop injury and leaching by mobilizing the herbicide, which is why PRE applications should be planned when moderate levels of rain are in the weather forecast.

This study seeks to examine the PRE efficacy of manuka oil with three different surfactants (Nu Film P, yucca extract and Agral 90). It was hypothesized that the PRE efficacy of MO is enhanced when tank mixed with adjuvants and/or surfactants. Four MO treatments (MO, MO+Nu Film P, MO+Yucca and MO+Agral 90) were tested against a control and a conventional industry standard PRE herbicide (Callisto) on two weed species: green pigweed (*Amaranthus powellii* S. Wats.) and large crabgrass (*Digitaria sanguinalis* L.). Efficacy was determined based on total weed biomass (mg/m^2) and emergence (%) of green pigweed and large crabgrass.

4.3. Materials and Methods

4.3.1. Growth Room Experiment

Growth room experiments were conducted to test the PRE efficacy of manuka oil with four different surfactants (refer to Table 4.1). These with four manuka oil-based treatments were tested against a conventional industry herbicide standard (Callisto) and a control (no treatment). The experimental units were arranged as a randomized complete block design with herbicide combination and presence or lack of rain simulation as the treatments. There were three replications, two weed species and the experiment was repeated three times. Efficacy was tested on two morphologically different agricultural weeds, green pigweed (*Amaranthus powellii* S. Wats.) and large crabgrass (*Digitaria* *sanguinalis* L.). These were selected for the following reasons; they are common weeds in Southern Ontario; availability of published literature on the PRE efficacy of either mesotrione or leptospermone on large crabgrass and green pigweed; were available within the constraints of this experiment, and have relatively high germination rates (Dayan et al. 2011; Frick and Thomas 1992; Syngenta Canada 2016). Each experimental unit was a square black plastic pot measuring 8 cm^2 by 9 cm in height. These pots were filled with growth medium leaving a ~3 cm gap from the top. The growth medium was a 1:1:1 ratio of sand, fine vermiculite and turface (Turface® Athletics, PROFILE Products LLC). Growth medium was thoroughly mixed before pots were filled. Pots were watered until they reached total saturation and then allowed to drain for a day. Individual pots were then placed within an impermeable plastic tray. Ten seeds of either green pigweed or large crabgrass were placed on the growth medium surface in each pot and pre-moistened growth medium was evenly spread over the seeds to a depth of ~0.5 cm. Containers were left for 24 hours before treatments were applied.

4.3.2. Treatment Applications

A spray cabinet was used to administer the treatments. All pots that were to be treated with the same treatment were grouped together and placed on a holding tray inside the spray cabinet. The spray cabinet was calibrated to administer 1000 L ha^{-1} of spray solution for the manuka oil-based treatments and 200 L ha^{-1} for the Callisto treatment. The field rates were used for MO and Callisto was applied at recommended label rates. Application rates for the treatments are listed in Table 4.1. After treatment application, pots were moved to a growth room at 25°C with an 8/16 hour light/dark regime. For the simulated rain treatments the amount of simulated rainfall required was based on Callisto label information (Syngenta Canada 2016). Every three days the equivalent of 0.25 inches of rain was applied to the treatments. Pots receiving simulated rain received water applied evenly to the soil surface. Pots that would not receive simulated rainfall were watered at the base of each pot within the impermeable container. The experiments were run for five weeks.

4.3.3. Weed emergence counts and biomass

At the end of five weeks, the number of emerged weeds per pot were counted and recorded. The aboveground weed biomass was collected by harvesting the weeds at the soil surface, placing them into envelopes and allowing them to dry at 80^{o}C for 2 days. Dry weights were measured and recorded.

4.3.4. Statistical Analysis

Statistical analysis was conducted with Statistical Analysis System (Ver 9.4, SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Three key experiment factors were rain, treatment and run. The factors of rain were either simulated rain or no simulated rain. Treatment had 6 levels comprised of one control, one commercial standard (Callisto) and four manuka oil-based products. Run was the number of times the experiment was repeated. Biomass was expressed in mg m^{-2} and emergence data was expressed in percentage out of 10 seeds. Tukey-Kramer multiple range test (α=0.05) was used to determine significance differences between treatments and letter values were assigned to distinguish significance. Different letters represent significant difference. Data were pooled over experiment runs because run was not significant.

4.4. Results

4.4.1. Seedling emergence

There was a significant effect of presence or absence of rainfall on seedling emergence generally and so the results for the herbicide treatments are presented separately under either presence or absence of simulated rain (Table 4.2). For both green pigweed and large crabgrass the Callisto treatment had the greatest effect on seedling emergence compared to the control treatment.

For large crabgrass and green pigweed, the Callisto treatments provided a significant reduction in seedling emergence. PRE Callisto was the most effective PRE treatment with or without the presence of rain simulation. For green pigweed, the MO treatments generally provided some numerical reduction in seedling emergence, but were not significantly different from the control except for green pigweed with rain. MO-based treatments were no significantly different from the control under no rain conditions for both weeds. With simulated rain, all the MO treatments on large crabgrass had emergence percentages that were not significantly different from the control. Only green pigweed under with rain simulation had MO treatments that showed significantly different emergence. MO+Yucca, MO+Nu Film P and MO all had emergence percentages that were numerically and significantly lower than the control while MO+Agral 90 was numerically different from the control, but not significantly different for green pigweed with rain. There was substantive variability in the PRE herbicidal efficacy of MO treatments, which helps to explain why many of these treatments have great differences in means between treatments were not necessarily statistically different.

When simulated rain was applied to green pigweed, the manuka oil treatments that contained yucca extract or Nu Film P provided a numerically and statistically greater reduction in seedling emergence than to control (Table 4.2). MO+Agral 90 had emergence that was numerically smaller than the control, but not significantly (Table 4.2). The conventional surfactant could have increased the solubility of manuka oil in solution. The high solubility could result in the herbicide being flushed away from the germination zone of the weed species resulting in lower efficacy. This agrees with findings from Temple and Hilton 1963. Furthermore, a number of authors have published on how surfactants can increase leaching (Buffington and McDonald 2006; Curran and Lingenfelter 2009; Jordan et al. 2011; Koren 1972; Temple and Hilton, 1963) and this might help explain the results in this experiment.

4.4.2. Weed Biomass

The weed biomass results resemble the weed emergence results where for both green pigweed and large crabgrass the Callisto treatment provided excellent efficacy (Table 4.3). Between both weed species and rain or lack of rain simulation 44% of treatment were not significantly different from the control. The weed biomass results differ from the weed emergence results in that there were significant differences among treatments that were not significant for weed emergence. For green pigweed, when simulated rain was applied, all of the MO-based treatments resulted in significant reductions in biomass. For large crabgrass, two treatments (MO+Yucca and MO+Nu Film P) had significantly reduced biomass compared to control when simulated rain was applied and three treatments (MO+Yucca, MO+Nu Film P and MO) when simulated rainfall was not applied. These results agree with Dayan et al. (2011) who also found that large crabgrass biomass could be reduced with PRE applications of MO (1% v/v). Also notable was that between both rain scenarios the addition of yucca and Nu Film P surfactants had a 75% chance of producing weed biomass that was significantly different from control compared to 50% when MO was applied alone. When MO was applied with Agral 90 where was only a 25% chance of the biomass being significantly different from control. Jame et al. (2006) examined surfactants and adjuvants as tank mix options for POST applied mesotrione, but did not look at tank mix partners for PRE applications. It was reported that mesotrione was a weak acid that can readily be unavailable in the presence of high soil organic matter. This may also be true for leptospermone in manuka oil; however, there is no published research examining tank mix options for manuka oil to enhance PRE efficacy. The lack of supporting literature makes it difficult to conclude the advantages or disadvantages of included a surfactant as a PRE tank mix partner with MO. Our results show some supporting evidence towards using an organic surfactant, but further research is needed to explain why the conventional surfactant was less successful. The addition of simulated rain was more likely to result in the MO treatments having more treatments that were significantly different from the control compared to no rain.

4.5. Conclusion

Currently, no research has explicitly detailed if PRE efficacy can be enhanced for MO with the addition of adjuvants or surfactants. This growth room experiment provided further insight to the PRE herbicidal properties of manuka oil. In this experiment, manuka oil treatments displayed PRE efficacy by significantly reducing the total weed biomass of green pigweed and large crabgrass. Manuka oil also provided PRE control by significantly reducing the number of green pigweed seeds that germinated, but not the number of large crabgrass. Rain simulation seemed to have some positive impact on the efficacy of PRE manuka oil treatments depending on weed species, but the results were not completely consistent.

Table 4. 1. Growth room treatment lists and rates for PRE efficacy tests.

Treatment code	Manuka oil rate	Surfactant Trade Name	Surfacta Molecular Compound	Surfactant rate	Spray volume rate
MO	1 % v/v	-	-	-	1000 L ha^{-1}
MO+Y	1 % v/v	Yucca extract	Steroidal saponins (spirostanol and furostanol)	0.132% v/v	1000 L ha^{-1}
MO+NFP	1 % v/v	Nu Film P	Terpenic polymer (di-1-p-menthene)	1% v/v	1000 L ha^{-1}
MO+A90	1 % v/v	Agral 90	Nonylphenoxy polyethoxy ethanol	0.2% v/v	1000 L ha^{-1}
Callisto	Equivalence of 144g ai ha^{-1}	Agral 90	Nonylphenoxy polyethoxy ethanol	0.2% v/v	200 L ha^{-1}
Control	-	-	-	-	-

Table 4. 2. The effect of various soil applied weed control treatments and presence or absence of simulated rainfall on plant emergence (%) of green pigweed (*Amaranthu spowellii* S. Wats) and large crabgrass (*Digitaria sanguinalis* L.).

Treatment	Green pigweed		Large crabgrass	
	Rain	No Rain	Rain	No Rain
	------ (emergence %) ------		------ (emergence %) ------	
Control	76.7 *a*[a]	63.3 *abc*	66.4 *a*	78.6 *a*
Callisto	2.2 *d*	0.0 *d*	14.9 *b*	1.1 *b*
Manuka + Yucca	40.0 *bc*	55.6 *abc*	50.6 *a*	69.8 *a*
Manuka + Nu Film P	41.1 *bc*	52.2 *abc*	66.3 *a*	65.2 *a*
Manuka	33.3 *c*	61.1 *abc*	59.6 *a*	75.5 *a*
Manuka + Agral 90	52.2 *abc*	65.6 *ab*	60.7 *a*	77.7 *a*

[a] Rain or no rain were analyzed separately and means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Table 4. 3. The effect of various soil applied weed control treatments and presence or absence of simulated rainfall on biomass (mg/m^2) of green pigweed (*Amaranthu spowellii* S. Wats) and large crabgrass (*Digitaria sanguinalis* L.).

Treatment	Green pigweed		Large crabgrass	
	Rain	No Rain	Rain	No Rain
	----------- (mg/m^2) -----------		----------- (mg/m^2) -----------	
Control	39.4 *a*[a]	28.3 *a*	14.4 *a*	26.1 *a*
Callisto	0.0 *c*	0.0 *b*	0.0 *c*	0.0 *c*
Manuka + Yucca	12.7 *bc*	22.6 *a*	7.3 *b*	11.4 *b*
Manuka + Nu Film P	14.3 *b*	11.2 *ab*	9.4 *bc*	11.7 *b*
Manuka	9.5 *bc*	16.2 *a*	9.5 *ab*	13.9 *b*
Manuka + Agral 90	18.4 *b*	26.2 *a*	10.4 *ab*	17.2 *ab*

[a] Rain or no rain were analyzed separately and means within columns followed by the same letter(s) are not statistically different based on Tukey's multiple range test (α=0.05).

Chapter 5.0. General discussion

5.1. Organic versus Conventional

There is a large divide between the organic and conventional sector that needs to be mended. The public's perception of organic industry often results in associating organic food/agriculture as being positive and healthy and conventional as being flawed (Comer et al. 1999; Lee et al. 2013; Morgan and Murdoch 2000; Nelson 2001). Bad publicity surrounding GMO's and controversial documentaries about the agri-food industry have contributed to this negative perception. Morgan and Murdoch (2000) and Nelson (2001) outline the concept of organic versus conventional as well as the public's negative perception of GMO's and its impact on the perception of conventional agriculture. They both identified how GMO's and conventional agriculture currently have a negative perception among many groups of people in North American and Europe. Calvert (2006), McLagan and McKee (2012), and Whiteman (2010) have extensively analyzed and confirmed the socio-economic influence documentaries have on the public. Controversial documentaries about the agri-food industry not only tell a limited and narrow story, but also influence how the public perceives the industry. Although, using film to highlighting inherent flaws in the industry could be seen as a positive educational tool, it is not educational if the filmmakers are not properly informed about the subject. This had lead to consumers believing that organic food is superior to conventionally produced food even if there is no supporting research on the topic. Lee et al. (2013) tested labelling impact of organic food on consumers and found that when consumers ate organic food they perceive it to healthier (lower calories, lower fat and higher fibre) and were more willing to purchase products with organic labels, despite the price premium, even if there was no nutritional differences in the food. It should be noted that there are some aspects of organic food that would rank it better than conventionally produced food, but it needs to be assessed on a subject-by-subject bases (calories, fat content, pesticide residue, antioxidants, cadmium levels, etc.) as conventional can also be superior to organic. Ultimately, focusing on pinning organic agriculture against conventional is not conducive to addressing important on and off-farm issues in the agri-food industry. There are certain obstacles that organic farmers face that differ from the issues conventional farms deal with. Organic farmers tend to produce lower yields, have less crop protection options, complex crop rotations, fewer sources of certified inputs, limited organic farming information networks and rely heavily on mechanical cultivation or other cultural practices (Adle et al. 2011; Barański et al. 2014; Darnhofer et al. 2010; Dayan and Duke 2010; Dettmann and Dimitri 2010; Winter and Davis 2006). Sourcing organically certified seeds can often be a challenge for the average organic farmer. Barański et al. (2014) conducted a meta-analysis on organic crops and found that organic produce had higher antioxidants and lower cadmium on average compared to conventional crops. These results were linked to the use of organic fertilizers and soil amendments as well as the diverse varieties organic farmers have. Organic farmers tend to have more direct market sales eliminating the grocery retailer and their particular variety standards (Brown and Miller 2008; Cooley and Lass 1998; Hall and Mogyorody 2001). This allows the farmer to grow a wider range of crop varieties, which also contributes to higher antioxidant concentrations in their produce (Barański et al. 2014; Comer et al. 1999; Hall and Mogyorody 2001). Barański et al. (2014) also noticed that the soil amendment practices (using natural manure as opposed to synthetic fertilizers) on organic farms also contributed to the higher antioxidants and lower cadmium of vegetables. Lastly, organic farmers follow a different set of principles that commit them to health, ecology, fairness and care (see Figure 5.1). These principles help shape the organic industry. Conventional farmers do not necessarily share the same struggles or principles as organic farmers. A conventional farm or farmer is more likely to have more limited crop rotations, potential herbicide-resistant weeds, restrictions on crop and variety choices, larger communities and networks of knowledge sharing and they tend to be more economically driven (Darnhofer et al. 2010; Hall and Mogyorody 2001). These differences should be viewed as a source of collaboration between the sectors.

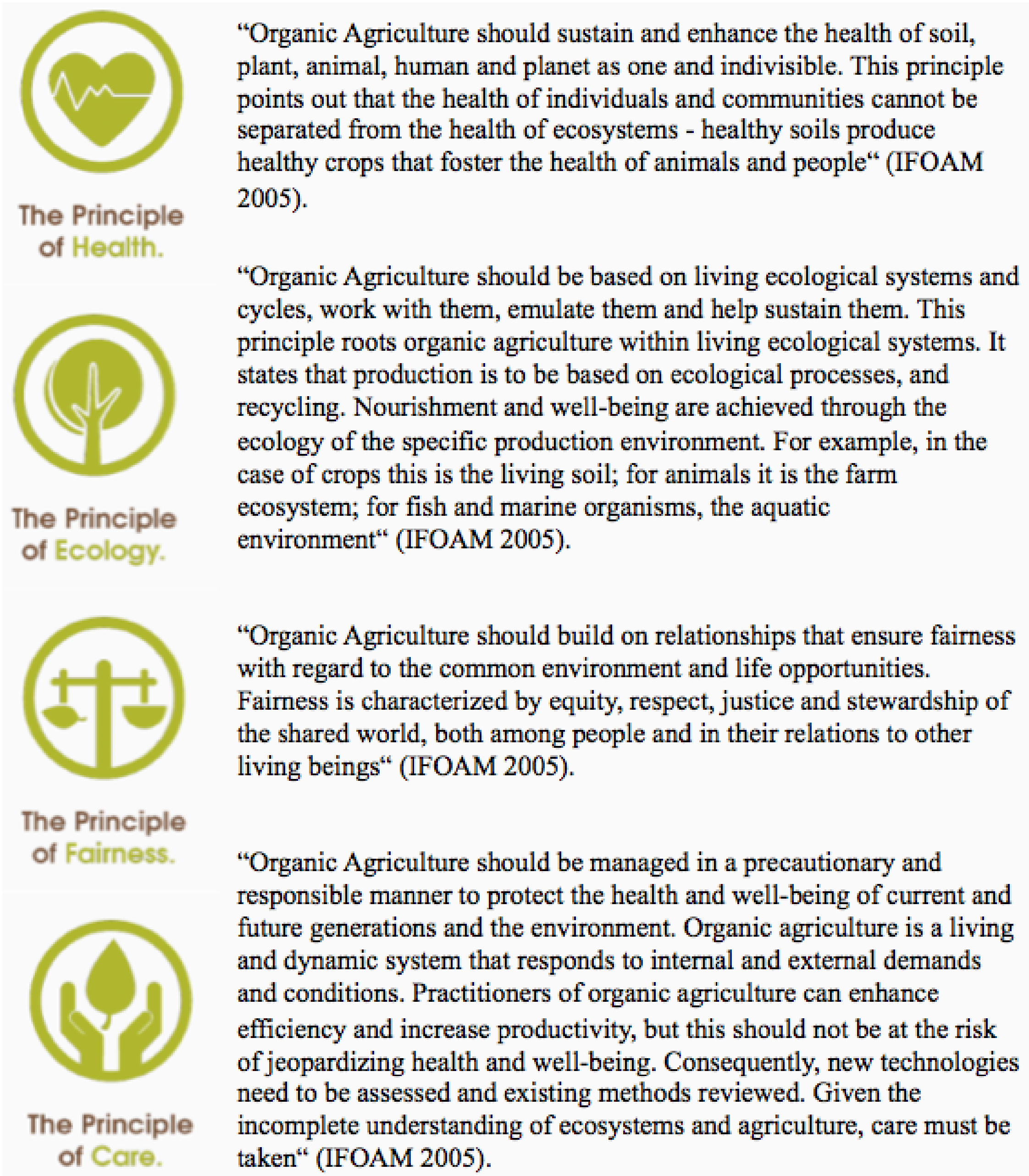

Figure 5 1. The four main principles of organic agriculture as described by IFOAM (2005) (image was modified from International Federation of Organic Agriculture Movements (IFOAM) 2005 and Darnhofer et al. 2009).

National and global matters such as food security, food distribution, human health, food safety, crop protection, environmental problems and water security are issues that concern all people. These are overarching struggles that impact both sectors as well as humanity. Consumers, farmers and researchers need to work together to address these issues and discover ideal solutions. Interestingly, organic farmers have adapted solutions that can address issues that conventional farmers face and vice versa. Organic farmers have mastered unique cultural practices (green manure, long crop rotations, modified cultivation equipment, fallow periods, unique mulches) to control weeds that could assist conventional farmers with herbicide-resistant weeds (Baskin and Baskin 1985; Campbell et al. 1991; Dayan et al. 2000; Gianessi and Reigner 2007; Mäder et al. 2002; Pimentel et al. 2005). The principles behind and the maintenance of soil on an organic farm could help a conventional farmer increase antioxidants and reduce cadmium levels in their produce (Barański et al. 2014). Increasing crop rotations can assist with crop protection by breaking annual weed cycles as well as hedging farm income (Graziani et al. 2012). Similarly, conventional farms can help organic farmers become more ecologically streamlined and economically focused. Comparing profitability between sectors can be challenging. Some of these challenges are outlined by Darnhofer et al. (2010) and Hall and Mogyorody (2001); they identifie the differences between organic farmers who transitioned to organic, new organic farmers, and large-scale organic operations that resemble conventional farms. Darnhofer et al. (2010) Hall and Mogyorody (2001) highlight distinguishable differences between organic and conventional farms in relations to their economic model and marketing efforts. Hall and Mogyorody (2001) reported that organic farmers tend to rank family health and environmental concerns above profits and finances when ask what motivated them to become organic farmers. This does not necessarily mean that organic farmers do not value economic stability, but that it is one of many parameters incorporated into farm managing decisions. Darnhofer et al. (2010) reported a large percentage of organic farms have started to adapt a more conventional economic model for their production system, which helped with profitability. The word ‘conventionalisation’ was developed to describe organic farms that start to resemble conventional farms due to a shift in the economic model (Darnhofer et al. 2010). Some critics view this as marring the organic industry. This is not necessarily the case because the merger of the two sectors is important to address the overarching struggles that impact all farmers. It is beneficial to have conventional farmers adapt certain ecological practices from organic farmers and for organic farmers to adapt a more economic business model if they choose. This creates a movement where both sectors begin to meet in the middle potentially creating a net benefit. With proper management this net benefit could result in a more ecological agri-food industry as a whole. Conventional farmers also have incredible science and business networks that organic farmers could emulate.

5.2. MO as a Potential Organic Herbicide.

Our studies showed that there is potential POST and PRE efficacy for MO. The PRE efficacy may not be feasible in a management setting unless a solution is found to increase the efficacy. The POST efficacy might be acceptable, but the value of the crop would have to justify the cost of multiple applications. More work is needed to find ideal application timings and tank mix partners to increase the efficacy to consistent acceptable levels. However, the MOA of MO is interesting for a natural product. The issue with adapting MO-based herbicides in an organic setting is that you start to run into the same issues that conventional farmers face with synthetic herbicides. The over reliance on herbicides leads to the development of resistant weeds. Weed resistance occurrence is positively correlated to increasing frequency and quantity of conventional herbicide applications (Beckie et al. 2014; Powles and Yu 2010). Theoretically, weeds could also develop a resistance to MO as a result of excessive applications. One of the main obstacles in trying to find blanket solutions to crop protection issues under an organic setting is that the organic principles and cultural practices are overlooked. Taking the exact same ideology from conventional farming and trying to apply it to organic farming is problematic because it overlooks the unique management practices on each organic farm. Many organic farms design their operations to be harmonious living organisms dealing with crop pests with very diverse management practices (crop rotations, variety selection and cultural practices). These elements are important when considering the efficacy of MO because MO most likely would not be adapted as the primary weed management practice, but rather one of many. Therefore, comparing the efficacy of MO to conventional products is not realistic. Upon certification, MO-based herbicides could be successfully adapted by organic farmers as one of their many weed control practices.

5.3. Discovering New Mode Of Actions From Nature.

Before the 1980's new mode of actions (MOA) were being discovered regularly, however, no new conventional herbicide MOA's have been discovered in over 25 years (Duke 2012). Herbicides have fared much worse than insecticides and fungicides where new MOA's have been discovered in recent years (Duke 2012). Historically, new MOA's have been discovered with the aid of natural sources such as mesotrione from the bottlebrush tree (Mitchell et al. 2001). Fortunately, the vast majority of living organisms on earth have not been studied in relation to their potential to yield new MOA's, and this is promising (May 1992). Additionally, as technology advances, researchers can reconsider species that have already studied. Duke (2012) and Dayan et al. (2000) reported that a lack of international protocols and collaboration has slowed the discovery of new MOA's. May (1992) stated that there is no international database that records the discovery of new species. The establishment of a database could be the start of developing a systematic approach to identifying new MOA's from previously or newly discovered species. May (1992) estimated that the number of different species on earth might vary by hundreds of millions. Mora et al. (2011) estimated that 86% of land dwelling and 91% of marine species are not yet catalogued. To say that new MOA's cannot be discovered from natural sources would, therefore, be naïve because of the huge gap of missing information. This is why research in organic agriculture relating to discoveries of natural products is so important for the industry for the environment and for humanity.

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Appendices

Appendix Table 7.1. Analysis of variance of sweet corn yield (t/ha) as affected by various weed control treatments, by date (early or late seeding) and by site (Simcoe versus Ridgetown).

Covariance Parameter	Estimate (t/ha)	Standard Error	Chi	P > chi
Block(site)	1.5340	2.1640	4.07	0.0437
Planting Date*block(site)	1.5782	2.1640	4.07	0.0437
Residual	25.7049	3.1641		
Effect	**Num DF**	**Den DF**	**F Value**	**Pr> F**
Treatment	11	132	14.77	<.0001
Planting date	1	6	14.49	0.0089
Planting Date*treatment	11	132	629.32	0.0196
Site	1	6	4.21	<.0001
Site*treatment	11	132	4.21	<0.0001
Site*planting date	1	6	39.62	0.0007
Site*planting date*treatment	11	132	1.11	0.3559

Appendix Table 7.2. Analysis of variance of tomato yields (t/ha) as affected by various weed control treatments, by date (early or late seeding) and by site (Simcoe versus Ridgetown).

Covariance Parameter	Estimate (t/ha)	Standard Error	Chi	P > chi
Block(site)	1.5819	4.0741	0.18	0.6724
Planting Date*block(site)	5.8736	7.5004	4.85	0.0277
Residual (Rig)	103.76	-		
Residual (Sim)	19.5452	3.3881		

Effect	Num DF	Den DF	F Value	Pr> F
Treatment	11	132	26.50	<.0001
Planting date	1	6	9.03	0.0239
Planting Date*treatment	11	132	1.91	0.0433
Site	1	6	0.00	0.9989
Site*treatment	11	132	11.44	<0.0001
Site*planting date	1	6	0.11	0.7544
Site*planting date*treatment	11	132	1.81	0.0582

Appendix Table 7.3. Analysis of variance of the comparison of PRE treatments and presence of simulated rain on the total biomass (mg/m^2) of green pigweed (*Amaranthus powellii* S. Wats) grown under growth room conditions (Guelph, 2015).

Treatment	Rain Trt	Treatment	Rain Trt	P value
MO+Agral 90[a]	No rain	Callisto	No rain	<.0001
MO+Agral 90	No rain	Callisto	Rain	<.0001
Callisto	No rain	Control	No rain	<.0001
Callisto	No rain	MO	No rain	0.0003
Callisto	No rain	MO+Yucca	No rain	<.0001
Callisto	No rain	Control	Rain	<.0001
Callisto	No rain	MO+Nu FilmP	Rain	0.0005
Control	No rain	Callisto	Rain	<.0001
MO	No rain	Callisto	Rain	0.0002
MO	No rain	Control	Rain	<.0001
MO+Nu FilmP	No rain	Control	Rain	<.0001
MO+Yucca	No rain	Callisto	Rain	<.0001
MO+Agral 90	Rain	Callisto	Rain	<.0001
MO+Agral 90	Rain	Control	Rain	0.0004
Callisto	Rain	Control	Rain	<.0001
Callisto	Rain	MO+Nu FilmP	Rain	<.0001
Control	Rain	MO	Rain	<.0001
Control	Rain	MO+Nu FilmP	Rain	<.0001
Control	Rain	MO+Yucca	Rain	<.0001

[a] All comparisons not shown were not significantly different according to Tukey-Kramer least square means multiple comparisons.

Appendix Table 7.4. Analysis of variance of the comparison of PRE treatments and presence of simulated rain on the total biomass (mg/m^2) of large crabgrass (*Digitaria sanguinalis* L) grown under growth room conditions (Guelph, 2015).

Treatment	Rain Trt	Treatment	Rain Trt	P value
MO+Agral 90[a]	No rain	Callisto	No rain	<.0001
MO+Agral 90	No rain	Control	No rain	0.0038
MO+Agral 90	No rain	MO+Agral 90	Rain	0.0035
MO+Agral 90	No rain	Callisto	Rain	<.0001
MO+Agral 90	No rain	MO+Yucca	Rain	<.0001
MO+Agral 90	No rain	Control	Rain	<.0001
MO+Agral 90	No rain	MO+Nu FilmP	Rain	0.0005
Callisto	No rain	Control	No rain	<.0001
Callisto	No rain	MO	No rain	<.0001
Callisto	No rain	MO+Nu FilmP	No rain	<.0001
Callisto	No rain	MO+Yucca	No rain	0.0003
Callisto	No rain	MO+Agral 90	Rain	<.0001
Callisto	No rain	Control	Rain	<.0001
Callisto	No rain	MO	Rain	0.0004
Callisto	No rain	MO+Nu FilmP	Rain	<.0001
Callisto	No rain	MO+Yucca	Rain	0.0019

Control	No rain	MO	No rain	<.0001
Control	No rain	MO+Nu FilmP	No rain	<.0001
Control	No rain	MO+Yucca	No rain	<.0001
Control	No rain	MO+Agral 90	Rain	<.0001
Control	No rain	Callisto	Rain	<.0001
Control	No rain	Control	Rain	<.0001
Control	No rain	MO	Rain	<.0001
Control	No rain	MO+Nu FilmP	Rain	<.0001
Control	No rain	MO+Yucca	Rain	<.0001
MO	No rain	Callisto	Rain	<.0001
MO	No rain	MO+Yucca	Rain	0.0048
MO+Nu FilmP	No rain	Callisto	Rain	<.0001
MO+Yucca	No rain	Callisto	Rain	<.0001
MO+Agral 90	Rain	Callisto	Rain	<.0001
MO+Agral 90	Rain	Control	Rain	0.0375
Callisto	Rain	Control	Rain	<.0001
Callisto	Rain	MO	Rain	<.0001
Callisto	Rain	MO+Nu FilmP	Rain	<.0001
Callisto	Rain	MO+Yucca	Rain	0.0001
Control	Rain	MO	Rain	0.0118
Control	Rain	MO+Nu FilmP	Rain	0.0106
Control	Rain	MO+Yucca	Rain	0.0004

[a] All comparisons not shown were not significantly different according to Tukey-Kramer least square means multiple comparisons.

Appendix Table 7.5. Analysis of variance of the comparison of PRE treatments and presence of simulated rain on seed emergence (%) of green pigweed (*Amaranthus powellii* S. Wats) grown under growth room conditions (Guelph, 2015).

Treatment	Rain Trt	Treatment	Rain Trt	P value
MO+Agral 90[a]	No rain	Callisto	No rain	<.0001
MO+Agral 90	No rain	Callisto	Rain	<.0001
MO+Agral 90	No rain	MO	Rain	0.0149
Callisto	No rain	Control	No rain	<.0001
Callisto	No rain	MO	No rain	<.0001
Callisto	No rain	MO+Nu FilmP	No rain	<.0001
Callisto	No rain	MO+Yucca	No rain	<.0001
Callisto	No rain	MO+Agral 90	Rain	<.0001
Callisto	No rain	Callisto	Rain	<.0001
Callisto	No rain	Control	Rain	<.0001
Callisto	No rain	MO	Rain	<.0001
Callisto	No rain	MO+Nu FilmP	Rain	<.0001
Callisto	No rain	MO+Yucca	Rain	<.0001
Control	No rain	Callisto	Rain	0.0004
Control	No rain	MO	Rain	0.0002
Control	No rain	MO+Nu FilmP	Rain	0.0069

Control	No rain	MO+Yucca	Rain	0.0047
MO	No rain	MO+Nu FilmP	No rain	<.0001
MO	No rain	MO+Yucca	No rain	<.0001
MO	No rain	Callisto	Rain	0.0013
MO	No rain	Control	Rain	0.0391
MO	No rain	MO	Rain	<.0001
MO	No rain	MO+Nu FilmP	Rain	0.0006
MO	No rain	MO+Yucca	Rain	0.0003
MO+Nu FilmP	No rain	MO+Yucca	No rain	<.0001
MO+Nu FilmP	No rain	Callisto	Rain	0.0032
MO+Nu FilmP	No rain	Control	Rain	0.0021
MO+Nu FilmP	No rain	MO	Rain	0.0008
MO+Nu FilmP	No rain	MO+Yucca	Rain	0.0360
MO+Yucca	No rain	Callisto	Rain	0.0022
MO+Yucca	No rain	Control	Rain	0.0069
MO+Yucca	No rain	MO	Rain	<.0001
MO+Yucca	No rain	MO+Nu FilmP	Rain	0.0125
MO+Yucca	No rain	MO+Yucca	Rain	0.0074
MO+Agral 90	Rain	Callisto	Rain	0.0030
MO+Agral 90	Rain	Control	Rain	0.0126
MO+Agral 90	Rain	MO	Rain	0.0176
Callisto	Rain	Control	Rain	0.0004
Callisto	Rain	MO	Rain	0.0111

Callisto	Rain	MO+Nu FilmP	Rain	0.0046
Callisto	Rain	MO+Yucca	Rain	0.0052
Control	Rain	MO	Rain	<.0001
Control	Rain	MO+Nu FilmP	Rain	<.0001
Control	Rain	MO+Yucca	Rain	<.0001
MO	Rain	MO+Nu FilmP	Rain	<.0001
MO	Rain	MO+Yucca	Rain	<.0001
MO+Nu FilmP	Rain	MO+Yucca	Rain	<.0001

[a] All comparisons not shown were not significantly different according to Tukey-Kramer least square means multiple comparisons.

Appendix Table 7.6. Analysis of variance of the comparison of PRE treatments and presence of simulated rain on seed emergence (%) of large crabgrass (*Digitaria sanguinalis* L) grown under growth room conditions (Guelph, 2015).

Treatment	Rain Trt	Treatment	Rain Trt	P value
MO+Agral 90[a]	No rain	Callisto	No rain	<.0001
MO+Agral 90	No rain	MO+Agral 90	Rain	0.0167
MO+Agral 90	No rain	Callisto	Rain	<.0001
MO+Agral 90	No rain	MO	Rain	0.0115
MO+Agral 90	No rain	MO+Yucca	Rain	0.0004
Callisto	No rain	Control	No rain	<.0001
Callisto	No rain	MO	No rain	<.0001

Callisto	No rain	MO+Nu FilmP	No rain	<.0001
Callisto	No rain	MO+Yucca	No rain	<.0001
Callisto	No rain	MO+Agral 90	Rain	<.0001
Callisto	No rain	Callisto	Rain	0.0105
Callisto	No rain	Control	Rain	<.0001
Callisto	No rain	MO	Rain	<.0001
Callisto	No rain	MO+Nu FilmP	Rain	<.0001
Callisto	No rain	MO+Yucca	Rain	<.0001
Control	No rain	MO+Agral 90	Rain	0.0195
Control	No rain	Callisto	No rain	<.0001
Control	No rain	MO	Rain	0.0139
Control	No rain	MO+Yucca	Rain	0.0006
MO	No rain	MO+Agral 90	Rain	0.0381
MO	No rain	Callisto	Rain	<.0001
MO	No rain	MO	Rain	0.0269
MO	No rain	MO+Yucca	Rain	0.0010
MO+Nu FilmP	No rain	Callisto	Rain	<.0001
MO+Yucca	No rain	Callisto	Rain	<.0001
MO+Yucca	No rain	MO+Yucca	Rain	0.0177
MO+Agral 90	Rain	Callisto	Rain	<.0001
Callisto	Rain	Control	Rain	<.0001
Callisto	Rain	MO	Rain	<.0001
Callisto	Rain	MO+Nu FilmP	Rain	<.0001

Callisto	Rain	MO+Yucca	Rain	<.0001
Control	Rain	MO+Yucca	Rain	0.0356
MO+Nu FilmP	Rain	MO+Yucca	Rain	0.0372

[a] All comparisons not shown were not significantly different according to Tukey-Kramer least square means multiple comparisons.

Appendix Table 7.7. Results of Analysis of variance Type-III for the seed emergence (%) of green pigweed (*Amaranthus powellii* S. Wats) grown under growth room conditions (Guelph, 2015).

Source	Degrees of Freedom	F Value	Pr > F
treatments	72	26.29	<.0001
rain	4	5.41	0.0805
rain*treatment	72	0.85	0.5834

Appendix Table 7.8. Results of Analysis of variance Type-III for the seed emergence (%) of large crabgrass (*Digitaria sanguinalis* L) grown under growth room conditions (Guelph, 2015).

Source	Degrees of Freedom	F Value	Pr > F
treatments	70	59.14	<.0001
rain	4	11.92	0.0260
rain*treatment	70	1.81	0.0739

Appendix Table 7.9. Results of Analysis of variance Type-III for the total weed biomass (mg/m^2) of green pigweed (*Amaranthus powellii* S. Wats) grown under growth room conditions (Guelph, 2015).

Source	Degrees of Freedom	F Value	Pr > F
treatments	72	27.55	<.0001
rain	4	0.91	0.3934
rain*treatment	72	2.10	0.0071

Appendix Table 7.10. Results of Analysis of variance Type-III for the total weed biomass (mg/m^2) of large crabgrass (*Digitaria sanguinalis* L) grown under growth room conditions (Guelph, 2015).

Source	Degrees of Freedom	F Value	Pr > F
treatments	70	45.96	<.0001
rain	4	47.73	0.0023
rain*treatment	70	4.59	0.0011

Appendix 7.12. Average daily rainfall (mm) for May (2015) in Simcoe and Ridgetown, ON.

Date	Simcoe	Ridgetown
01	12	0
02	11.9	0
03	14	0
04	17.3	0
05	12.1	-
06	15.5	0.2
07	17.6	-
08	20.1	0
09	21.3	0
10	21.8	0
11	21.6	4.4
12	11.8	0
13	6	0
14	7.4	0
15	12.2	0.5
16	17.2	0.3
17	20.2	0
18	22.3	0
19	11.3	0
20	9	0

21	12.1	0
22	6.5	0
23	7.7	0
24	14.5	0
25	21.7	0
26	22.5	13.6
27	21.6	4.9
28	18.1	0
29	18.8	0
30	20.7	11.4
31	9.4	54.8

Appendix 7.13. Average daily rainfall (mm) for June (2015) in Simcoe and Ridgetown, ON.

Date	Simcoe	Ridgetown
01	10.8	0
02	12.6	0
03	13.9	0
04	15.5	0
05	21.2	0
06	13.1	0
07	15.1	1.3
08	17.9	3.7

09	17.3	5.7
10	18.9	1.7
11	18.3	0
12	21.1	0
13	18.6	0.3
14	20.6	11.8
15	23.8	0
16	20	1.3
17	17.4	0
18	20.1	10.6
19	16.6	0
20	16.4	2.8
21	22.2	0
22	20.6	2.1
23	18.4	2.2
24	18	0
25	18.9	0
26	18.6	0
27	15	48
28	13	2.5
29	17.4	0
30	18.1	3.7
31	10.8	-

Appendix 7.14. Average daily rainfall (mm) for July (2015) in Simcoe and Ridgetown, ON.

Date	Simcoe	Ridgetown
01	17.9	0
02	16.2	0
03	15.5	0
04	16.8	0
05	19.6	0.2
06	21.1	0
07	20.9	7.5
08	16.3	0
09	18.2	9.7
10	18.4	0.2
11	19.8	-
12	20.8	0
13	21.6	0
14	21.6	10.8
15	17.3	0
16	16.4	0
17	21	1.9
18	25.1	0
19	23.2	0

20	20.9	0
21	20.7	0
22	17.8	0
23	18.9	0
24	20.4	0
25	22.8	30.2
26	22.4	0
27	23.6	0
28	23.7	0
29	24.2	1.5
30	23.5	0
31	21.5	-

Appendix 7.15. Average daily rainfall (mm) for August (2015) in Simcoe and Ridgetown, ON.

Date	Simcoe	Ridgetown
01	20	0
02	20.7	-
03	20.2	17.9
04	19.6	0
05	16.2	-
06	15.5	0
07	18.8	0

08	18.4	0
09	20	0
10	20	9.6
11	19.9	0
12	17	0
13	17.3	-
14	22.5	-
15	23.1	0
16	22.2	0
17	24.2	0
18	23.1	2.5
19	23.7	0
20	19.8	1.5
21	17.3	0
22	17	0
23	18	2.8
24	18.7	0
25	17.1	0
26	16.3	0
27	15	0
28	14.9	0
29	18.6	0
30	22.2	0

31	21.7	0

Appendix 7.16. Average daily rainfall (mm) for September (2015) in Simcoe and Ridgetown, ON.

Date	Simcoe	Ridgetown
01	21.5	0
02	23.3	0
03	24.7	2.9
04	22.8	0
05	22.9	0
06	24.3	0
07	24.6	0
08	25.4	0
09	19.7	1.7
10	16.9	0
11	15.6	3.1
12	13.2	0
13	11.6	0
14	14	0
15	17.3	0
16	19.3	0
17	18.8	0
18	19	0.6

19	15.7	30.9
20	12.4	0
21	13.1	0
22	14.5	0
23	15.8	0
24	17.4	0
25	19.1	0
26	16.1	0
27	15.4	-
28	18.8	0
29	17.3	5.6
30	13.7	0
31	21.5	.

Appendix 7.17. Average monthly temperature (°C) from May to September (2015) in Simcoe and Ridgetown, ON.

	Simcoe		Ridgetown	
	2015	Historical average	2015	Historical average
May	12.1[a]	13.2	16.2[b]	14.1
June	18.0	18.5	18.1	19.2
July	20.9	21.1	19.8	21.6

August	19.7	20.0	19.7	20.4
September	18.6	15.5	19.2	16.8

[a] Simcoe site weather data and historical averages retrieved from Weather Innovations Consulting LP.

[b] Ridgetown site data and historical averages retrieved from Weather Innovations Consulting LP.

Appendix 7.18. Total monthly rainfall (mm) from May to September (2015) in Simcoe and Ridgetown, ON.

	Simcoe		Ridgetown	
	2015	Historical average	2015	Historical average
May	78.9[a]	88.9[b]	90.1[c]	78.9
June	165	88.8	97.7	70.6
July	46	96.6	62	74.1
August	101	83.6	34.3	79.7
September	18	99.2	44.8	88.1
Total	330	368.2	328.9	391.4

[a] Simcoe site weather data retrieved from Weather Innovations Consulting LP.

[b] Data retrieved from Environmental Canada's historical records from 1981-2015 for Delhi, ON (closest weather station to Simcoe).

[c] Ridgetown site data and historical averages retrieved from Weather Innovations Consulting LP.