DEVELOPING AN INTEGRATED APPROACH FOR LIVERWORT MANAGEMENT IN CONTAINERIZED ORNAMENTAL PRODUCTION SYSTEMS

By

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ABSTRACT

Weed control is an important management area in containerized production of nurseries and greenhouses because the market value of ornamentals is determined primarily by their quality, appearance, and aesthetics. Liverwort (*Marchantia polymorpha*) is a notable problematic weed that exists in nursery and greenhouse conditions. It has a flattened thalloid body that forms a mat-like structure on the top of container media. It thrives well in propagation and container production environments with low ultraviolet radiation, high humidity and/or soil moisture, and high fertility. Hand removal of liverwort is a laborious, time-consuming, and costly operation. Not many herbicides are labeled for liverwort control inside greenhouses as most of them can cause severe phytotoxic damage to sensitive ornamentals. Hence, the goal of this research was to develop an integrated management approach for liverwort infestations in nurseries and greenhouses. Various non-chemical methods including organic mulching, strategic fertilizer placements and allelopathic properties of organic mulches; and chemical methods including pre- and post-emergence application of synthetic and organic herbicides were studied for their effects on liverwort control. Absorption and translocation of ^{14}C labeled radioactive 2,4-D and indaziflam in liverwort thallus were assessed by liquid scintillation spectrometry and phosphor imaging**.** Results indicate that mulching with rice hull and pine bark provided an excellent liverwort control and no phytotoxicity to *Hosta spp*. varieties 'Curly Fries' and 'Pandora Box'. Thicker layers of mulching with cocoa hull and hardwood mulches caused phytotoxicity to 'Curly Fries' and 'Pandora Box', respectively. The sub-dressing and dibble placements of controlled-release fertilizers improved growth of dicot Begonia and monocot Dracaena while minimizing the fresh biomass of liverwort in containerized production. Agar impregnated with maple leaf and shredded cypress mulch based allelopathic extracts showed maximum suppression of liverwort gemmae germination in growth chamber studies. Whereas allelopathic extracts from pine bark and hardwood mulches were most effective for liverwort control under greenhouse conditions. The post-emergence application of glyphosate and 2,4-D at 1X rate and indaziflam at 2X and 3X rates were effective in controlling liverwort. Also, pre-emergence application of indaziflam provided complete inhibition of liverwort gemmae germination and establishment. In the ^{14}C absorption and translocation studies, the total recovery of ^{14}C radiolabeled 2,4-D ranged from 63-80% while it ranged from 49-80% for ^{14}C radiolabeled indaziflam. Phosphor imaging of the translocation samples of liverwort thallus displayed higher movement of 2,4-D as compared to indaziflam in liverwort thallus. The organic herbicide

treatments of WeedPharm and Scythe at 2X rate and Avenger at 1X rate provided season-long liverwort control. Overall, a proper utilization of multiple tactics including cultural methods (mulching and strategic fertilizer placement), allelopathy, and chemical methods (pre- and postemergence herbicides) can lead a way to develop an effective integrated control program for liverwort in nurseries and greenhouses.

This dissertation is dedicated to Almighty, Mum-Dad and Panjab.
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CHAPTER 1: LITERATURE REVIEW

The work presented in this chapter is part of the final publication:

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Abstract

Common liverwort (*Marchantia polymorpha*) is a primitive, spore-bearing bryophyte that thrives in containerized ornamental crop propagation and production environments. It is one of the major weed problems in container nurseries and greenhouses because it competes with ornamental plants for soil/growing medium, nutrients, water, space, and oxygen within the container. As a result, its presence can reduce the overall quality and market value of the ornamental crop. Once established in nurseries and greenhouses it spreads rapidly due to its ability to propagate both asexually and sexually. Currently, no effective method of controlling common liverwort in container production systems are available as significant knowledge gaps exist. Therefore, research is needed to determine if organic mulches (types, depths, moisture holding capacity, and particle size), biopesticides, and strategic placement of fertilizers within containers suppress or inhibit common liverwort growth and development. Additionally, newer chemicals (both synthetic and organic) and combinations need to be tested on different growth stages of common liverwort. The objective of this review is to summarize previous and current research related to common liverwort control in container production and identify areas where additional research is needed either to improve current control methods or to develop new ones.

Keywords: Fertilizer placement, herbicides, *Marchantia polymorpha*, organic mulches, thallus structure, weed competition

Chemicals: acetic acid (Weed Pharm), ammonium nonanoate (Axxe®), d-limonene (AvengerAg®), flumioxazin (BroadStar™, SureGuard®), hydrogen peroxide (Zerotol®), oxadiazon (Ronstar®), oxyfluorfen (GoalTender®, Goal®), pelargonic acid (Scythe™), quaternary ammonium chloride (GreenShield®, Physan20™, Triathlon®)

The 2013 estimated economic contribution of the ornamental green industry in the United States was $136 billion in sales revenue. In terms of employment and gross domestic product (GDP) contributions, the production of nursery, greenhouse, and floriculture crops alone created 240,809 jobs and contributed over $20 billion towards GDP (Hodges et al., 2015). Weed control is important for horticultural crops because weed competition for light, nutrients, water, and space causes reductions in crop growth and yield. In addition, weeds can harbor insects, pests, diseases, and pathogens resulting in further reduction of market value of the crops. In restricted growing environments, such as container plant production, weeds reduce marketability and crop growth by up to 60% (Fretz, 1972). A single redroot pigweed (*Amaranthus retroflexus*) or large crabgrass

(*Digitaria sanguinalis*) plant in a 2.4 L nursery pot reduce Japanese holly (*Ilex crenata*) plant growth by 40% to 60% over a season (Fretz, 1972). A large number of eclipta (*Eclipta alba*) and prostrate spurge (*Euphorbia supina*) weeds reduced shoot dry weight of container grown azalea (*Rhododendron eriocarpum*) and barberry (*Berberis thunbergii*) plants (Berchielli-Robertson et al., 1990). Weed control in container nursery production is often the highest production cost encountered by nursery growers, often exceeding $4000 per acre (Case et al., 2005; Mathers, 2003). Several products registered for use on established ornamentals can be injurious to newly established plants and hence weed control is a challenge in the production of all nursery plants and especially problematic in propagation (Fausey, 2003). The large variety of ornamental species in nursery production presents a challenge for herbicide manufacturers, as each plant must be tested under different conditions before adding it to an herbicide label (Mervosh and Ahrens, 1998). As a result, growers are often left with limited weed control strategies for ornamentals (Fausey, 2003). There are also few herbicides that are labeled for use inside greenhouses due to volatility and potential for crop injury. Injury can occur from spray drift if fans are operating at the time of herbicide application or can occur from herbicides that are volatile as vapors can accumulate in enclosed greenhouses and injure the crops (Smith, 2019). According to Norcini et al. (1996), the lowest reported use of herbicides was within greenhouses in comparison with field plantings and nursery container production.

Several difficult to control weeds including higher plants [broadleaves (dicotyledons), grasses (Poaceae), and sedges (Cyperaceae)] as well as primitive plants such as algae, liverworts, and mosses have spread in nurseries and greenhouses throughout the United States (U.S.) at an alarming rate (Fausey, 2003), and despite heavy expenditures, crops incur losses of billions of dollars, annually (Loux et al., 2019). Liverwort is a primitive plant with over 6,000 species naturally occurring in moist temperate regions throughout the world (Crum, 1991) with common liverwort being the most common in greenhouses and container nurseries (Marble et al., 2017). Common liverwort spreads rapidly in nurseries and greenhouses due to its ability to propagate both asexually and sexually (Ross and Puritch, 1981). It is not uncommon for ornamental liners infested with liverwort such as common liverwort to be produced in one region of the country, transported to another for finishing, and shipped again to retail (Fausey, 2003). Thus, containers can acquire and disseminate liverwort at each point of transfer. No effective method of controlling common liverwort in container production systems currently exist. Limited research evaluating

the effects of fungicides, disinfectants, and insecticides on common liverwort have been published (Chase, 2000; Chase and Osborn 1984; Hammett, 1976). However, varying interpretations of the results have not proven effective for growers (Fausey, 2003). In most of the cases, application of one product or method is not enough to control common liverwort successfully and may require integration of two or more approaches. The focus of this review is to synthesize previous and current research pertaining to common liverwort control in container nurseries and greenhouses and to discuss and/or identify areas where additional research is needed either to improve existing control methods or to develop new ones.

Morphology of common liverwort

Liverwort is a nonvascular, primitive, spore-bearing bryophyte which belongs to the Marchantiaceae family (Durand, 1908) and is more closely related to lower group plants such as algae, mosses and ferns than to higher group plants (Altland, 2003; Svenson, 1997). This is the second largest phylum of bryophytes as there are 5,000 to 7,500 species of liverworts all over the world (Soderstrom et al., 2016; Von Konrat et al., 2010). Common liverwort belongs to the thalloid complex of liverworts, which include about 5% of all liverworts, and has often been used to represent the model morphology of liverworts (Budke et al., 2018). The thickness of the thallus is 0.3 to 0.6 mm at the midrib region and gradually becomes thinner towards the margin (Shimamura, 2015). The thallus is dorsiventral with a broad laminar surface for maximum interception of light (Raven et al., 1999). In common liverworts, photosynthesis occurs in a defined cell layer on the dorsal surface (Budke et al., 2018). On the lower surface there are rhizoids and scales, which absorb moisture and anchor the plant body to the substrate (Budke et al., 2018). The rhizoids are in localized areas over the whole ventral surface of the thallus (McConaha, 1941), and also grow down the grooves in the gametophore stalks (Bell, 1992).

There are two phases in the common liverwort life cycle, sporophytic and gametophytic stages. When common liverworts are exposed to cool temperatures ranging between 10 to 15 °C, the sexual structures or fruiting bodies develop (Newby, 2006) and the sporophytic life cycle begins. In this sporophytic stage, antheridia produced on stalked antheridiophores, fertilize the archegonia which are also borne on stalked archegoniophore to produce spores (Newby, 2006). Common liverwort is a dioecious plant as the male and female gametangia are produced on separate plants (Budke et al., 2018) (Fig. 1.1). The male gametangia are located on the upper surface of the gametophores whereas, the female archegonia are inverted and hang downward

(Budke et al., 2018). Irrigation or rainwater mainly facilitate sperm dispersal although no studies have been conducted to determine whether animals or insects may contribute to their dispersal (Budke et al., 2018). The archegonia are fertilized by antheridia to produce sporangia. Each sporangium then give rise to a spore mother cell which produce four tetrahedral spores (Durand, 1908).

Spores germinate to give rise to the gametophytic life cycle. Spore germination depends on light (Heald, 1898) and requires day lengths of 10 h or longer (Nakazato et al., 1999). During the gametophytic life cycle, the plant propagates asexually by producing gemmae within cup like structures called gemmae cups (Newby, 2006) (Fig. 1.2). Numerous gemmae, produced by each gemmae cup, are released to the immediate surroundings when they come in contact with irrigation or rainwater (Svenson, 1997). A single gemma can give rise to one or two clonal plants after contact with moist soil or media (Saha et al., 2020). Fragmentation is another method of asexual propagation in common liverwort (Svenson, 1997). The common liverwort sporophytes are dependent on the gametophytes for water and nutrition. Each sporophytic structure is composed of a foot (which is embedded in the female gametophyte), a seta, and a capsule (Shimamura, 2015). The capsules contain diploid elaters, which are intermixed with the haploid spores (England, 2007). Elaters provide nutrition for the new developing spores, facilitate response to changing moisture and humidity, and help in spore dispersal (Kremer and Drinnan, 2003; Schuster, 1966).

Common liverwort as a major weed in container nurseries and greenhouses

Historically common liverwort was reported as a weed in cooler regions of the northeast and Pacific northwest regions of the United States. (Newby, 2006). However, common liverwort is one of the major weeds in container nurseries and greenhouse operations nationwide, as it competes with the ornamental plant for soil/growing medium, nutrients, water, space, and oxygen within the container (Fig. 1.3). Vegetative growth occurs most rapidly at temperatures between 18 to 22 °C (O'Hanlon, 1926) and thus thrives in propagation and container production environments that have low ultraviolet (UV) radiation, high humidity and/or soil moisture, and high fertility (Newby et al., 2006). In container plant production, common liverwort infestations use nutrients and water intended for the crop, impede water movement into the root-zone and reduce crop market value (Svenson, 1997) and overall quality of the ornamental. Therefore, controlling common liverwort in nursery and greenhouse container ornamental production is extremely important.

Common liverwort can form a thick mat, covering not only the container media surfaces (Altland et al., 2007) but also on walkways and poorly drained areas under greenhouse benches and on nursery ground cover, especially in the presence of overhead irrigation. Competition from weeds can limit root volume of containerized crops. Similar to other weeds, common liverwort can provide a habitat for pests and potential pathogens such as fungus gnats (e.g., *Bradysia*), snails (e.g., *Helix*), slugs (e.g., *Deroceras*), and a host of microbial threats such as basal rot (*Fusarium oxysporum*) and damping-off (*Pythium aphanidermatum*) (Svenson et al., 1997). Additional costs to control these pests, combined with production losses resulting from their activity, can reduce profit margins.

The main limitation for common liverwort control inside the greenhouse is the lack of herbicide options since most are not labeled for greenhouse use. On the other hand, in nursery container production, herbicides used at higher rates needed for common liverwort control can cause phytotoxicity to sensitive ornamental plants and can have residual effects as well. Hand removal of common liverwort is very laborious, time-consuming, and costly as it forms a mat like structure on top of the container medium. In order to remove the rhizoids, approximately an inch of the media needs to be removed from the container and the medium must be subsequently replaced. Currently, flumioxazin is one of the synthetic herbicides that has been labeled for common liverwort control and is popular among commercial growers. However, flumioxazin and other potential organic and synthetic herbicides have not been tested on different common liverwort growth stages. The efficacy of these chemicals may vary according to different growth stages of common liverwort.

Overview of non-chemical control of common liverwort

Organic mulching. In general, top dressing with organic mulches such as pine bark, pine straw, and hardwood mulch reduce weed growth in nursery container production (Llewellyn, 2003; Saha et al., 2019a). Saha et al. (2019a) quantified the effect of herbicide combinations with pine bark, pine straw, and hardwood chip mulches on weed control for container production. They reported an 88% to 100% reduction of large crabgrass and garden spurge (*Euphorbia hirta*) in containers with a combination of herbicide and mulch with depths of 1 inch or greater. According to Svenson (1997), fast-drying mulches such as European hazelnut (*Corylus avellana*) shells, rice (*Oryza sativa*) hulls, and pumice on the container media surface can suppress common liverwort growth to some extent. Altland and Krause (2014) reported that top dressing containers in the

greenhouse with rice hull mulch can reduce common liverwort growth. Rice hulls applied at a depth of 0.6 cm showed 2.5% and 20% common liverwort coverage at 4 and 8 weeks after potting (WAP), respectively. In contrast, rice hulls at depths of 1.3 and 2.5 cm showed 0% common liverwort coverage at 4 and 8 WAP. However, no research has been conducted to determine how different organic mulch types, depths, particle sizes and their moisture-holding capacity can impact common liverwort growth in nursery containers and in greenhouses.

Fertilization practices. Common liverwort growth is correlated with increasing nitrogen levels (Svenson, 1998). Nitrogen application rates less than 75 parts per million (ppm) slow down common liverwort establishment (Svenson, 1997). However, nitrogen less than 75 ppm is usually not sufficient for growing ornamental crops. For example, poinsettia (*Euphorbia pulcherrima*), bedding plants, and geranium (*Geranium*) require 250, 200, and 250 ppm of nitrogen, respectively, for optimal growth (Cox, 1997) whereas, some commercial growers often deliver 125-150 ppm of nitrogen to bedding plants and geraniums. By altering the placement of controlled-release fertilizers (CRFs) within containers, growth of traditional weeds can be decreased. For example, incorporation of CRFs in the substrate increased spotted spurge (*Euphorbia maculata*) germination by 77% to 183% compared to top dressing, subdressing, dibbling, and no fertilizer (Saha et al., 2019b). Subdressed CRFs were placed at a depth of 7.6 cm below the surface of the media as a layer. Whereas, dibbled CRFs were placed in a small pocket at a depth of 7.6 cm below the surface of the media. Both subdressing and dibbling reduced seed production by 63% and 92% for large crabgrass and spotted spurge, respectively (Saha et al., 2019b). Strategic placement of CRFs can increase nutrient availability to the crop but not to the smaller weed seeds that are introduced at the top layer. This method has been effective in controlling general broadleaf and grass weed species such as eclipta (*Eclipta prostrata*), spotted spurge, and large crabgrass. Altland (2004) recommended incorporating or dibbling CRFs as a method to reduce common liverwort growth compared to topdressing. However, additional research is required to determine if CRF placement and depths in the container can control common liverwort and not negatively influence crop growth and whether requires more labor than the popular top dressing method.

Irrigation practices. Given that common liverwort growth is promoted by moist conditions, containerized crops should be irrigated according to soil moisture content instead of following fixed irrigation schedules and rates (Altland, 2004). Additionally, air circulation should be improved around the container surface to decrease localized relative humidity (Altland, 2004).

Overhead irrigation can cause more splashing of water and increase dispersal of the gemmae than drip or flood floor irrigation systems. In a study conducted by Svenson (1998), high irrigation frequency resulted in a 100% common liverwort coverage on the container media, whereas only 59% common liverwort coverage was observed with low irrigation frequency. Therefore, cultural practices that reduce the moisture content of the container media can help reduce common liverwort growth (Newby, 2006). Svenson and Deuel (2000) found increased common liverwort coverage in containers with daily irrigation compared to low (every 3 day) irrigation across a range of surface mulch treatments that included hazelnut shells, oyster shells, and copper-treated geotextile discs. They also recommended the use of sub-irrigation instead of overhead irrigation to reduce common liverwort growth. Clemens et al. (1991) compared different irrigation methods including capillary, ebb-and-flow and overhead, and reported that greater common liverwort presence on the compost surface was the main problem with capillary systems.

Effects of light and shading. Light has significant effects on common liverwort growth and reproduction. It has been observed that light intensities of 370 to 555 $\mu mol \cdot m^{-2} \cdot s^{-1}$ promote vegetative growth of common liverwort whereas higher light intensities inhibit it (Mache and Loiseaux, 1973). More gemma cups (asexual structures) are produced under a short photoperiod of about 8 h than under a long photoperiod of 17 to 18 h (Voth and Hamner, 1940). In contrast, high light intensity, long day condition and natural diffused daylight can accelerate female sexual structure, archegoniophore formation (Terui, 1981). Even the spore germination is light dependent (Heald, 1898) and requires light for 10 h or longer (Nakazato et al., 1999). Greef et al. (1971) reported that senescence in common liverwort is also controlled by phytochrome and photoperiods of white light. The green tissue of mature common liverwort gets bleached significantly when placed in continuous darkness for 4 d but remained green when given daily 1 h photoperiods of white light (De Greef et al., 1971). The bleaching was taken as a measure of senescence because a breakdown of cell organelles and cytoplasm accompanies loss of chlorophyll in the bleached tissue (De Greef et al., 1971). So, from vegetative growth, formation of reproductive structures to senescence, most of the phases in common liverwort life cycle are affected by either light quality or quantity. Inside greenhouses, ornamental crop canopies can produce enough shade on the container media surface to promote common liverwort growth (Svenson et al., 2001). Altland and Krause (2014) quantified how the canopy of containerized 'Radrazz' rose (*Rosa*) influenced common liverwort growth. Under a rose canopy, there was 13%, 40%, and 99% common liverwort

coverage of the container surface at 1, 4, and 8 WAP. In contrast, there was only 6%, 21%, and 48% common liverwort coverage on container media at 1, 4, and 8 WAP in the absence of canopy shading. This research provides evidence that canopy shading can increase common liverwort growth in comparison to no shading. Since very little research has been conducted on the effects of shading on common liverwort growth and development, more research is required in this area.

Overview of chemical control of common liverwort

Chemical control of common liverwort by preemergence herbicides was suggested in 1979 (Elmore et al., 1979). Benzylkonium chloride and cinnamic aldehyde (sold as Cinnacure™; Pro-Guard, Suisun City, CA) have shown some success in controlling common liverwort, however these products can sometimes injure ornamental crops depending on the season (Svenson, 1997). Herbicides containing flumioxazin, oxadiazon, or oxyfluorfen are effective for preemergence common liverwort control (Fausey, 2003; Svenson, 1998). Fausey (2003) reported that under controlled-environment conditions, common liverwort can be managed to some extent with flumioxazin, oxyfluorfen, acetic acid, pelargonic acid and oxadiazon. In addition to postemergence control of common liverwort, flumioxazin, oxadiazon and oxyfluorfen also had residual activity when applied to potting media. In comparison of granular and sprayable formulations of flumioxazin, oxadiazon and oxyfluorfen, the control of established common liverwort was greater with sprayable than with granular formulations. The granular and sprayable formulations of flumioxazin provided greater pre and postemergence control of common liverwort as compared to granular or sprayable formulations of oxadiazon and oxyfluorfen. All three of these herbicides are known as "protox" or protoporphyrinogen oxidase (PPO) inhibitors and belong to the Weed Science Society of America (WSSA) Group 14 herbicides. According to Marble et al. (2017), preemergence herbicides of WSSA Group 14 may provide some level of common liverwort suppression depending on nursery conditions, but further research is required.

Stamps and Chandler (2004) reported that a granular form of sodium carbonate peroxyhydrate (TerraCyte®; BioSafe Systems, LLC, East Hartford, CT), can provide excellent postemergence common liverwort control after 2 weeks of treatment. However, Altland et al. (2003) reported poor to moderate control of common liverwort with TerraCyte applied at a rate of 728.5 $kg \cdot ha^{-1}$ and was injurious to certain perennial crops. Although some synthetic herbicides have been tested to control common liverwort, many of these products are phytotoxic to

ornamental plants. A list of synthetic herbicides/ chemicals that have shown some degree of common liverwort control are provided in Table 1.1.

Herbicide efficacy can vary from region to region or depend on environmental factors and weed species population (Varanasi et al., 2016; Waltz et al., 2004). For example, glyphosate efficacy can vary on broadleaf weeds such as velvetleaf (*Abutilon theophrasti*) with application time of day (Waltz et al., 2004). Velvetleaf control was consistently greater with glyphosate applications during the day compared with at night, regardless of constant air temperature and relative humidity, dew absence or presence, or leaf blade orientation with natural light-dark movements or a fixed horizontal position (Waltz et al., 2004). In a series of experiments, Newby et al. (2007), showed that granular pre-emergence herbicide efficacy on common liverwort control varied by location; flumioxazin and oxadiazon provided the most effective control in Alabama, U.S., whereas flumioxazin and oxyfluorfen + oryzalin provided the most effective preemergent common liverwort control in Oregon, U.S. This variation in herbicide efficacy for common liverwort control as reported by Newby et al. (2007) might be due to different environmental conditions/cultural practices in two different locations. More in-depth research is required to determine the underlying causes for such variation in herbicide efficacy for common liverwort control.

Another chemical, quinoclamine (Gentry®; Chemtura Corp., Middlebury, CT) has shown 96% postemergence control at 2 days after treatment (DAT) and 94% control at 45 DAT to mature common liverwort (Altland et al., 2003). Altland et al. (2008) also studied the response of common liverwort to the herbicide quinoclamine, in a medium containing pine bark. Quinoclamine provided preemergent control of gemmae propagules as well as contributed to postemergent control of established common liverwort. In a simulation of preemergent activity of the herbicide, hydroponically grown common liverwort and germinating gemmae were subjected to increasing concentrations of quinoclamine. Phytotoxicity to both gemmae and plants was obtained with a minimal herbicide concentration of 4 to 6 $mg \cdot L^{-1}$. In a later study, Altland et al. (2011) studied the differential response of common liverwort tissues to post-applied quinoclamine. The archegonial receptacles (female) were more tolerant of quinoclamine than either antheridial receptacles (male) or thalli (leaflike structures). The doses that resulted in 50% control of the population (I_{50}) of antheridial receptacles and juvenile thalli were 1.60 and 1.27 $kg \cdot ha^{-1}$, respectively. The I_{50} of archegonial receptacles exceeded 10.45 $kg \cdot ha^{-1}$. After application of radiolabeled quinoclamine,

absorption of ^{14}C was lower in archegonial receptacles than in either antheridial receptacles or thalli. The tolerance of archegonial receptacles to quinoclamine could be partially attributed to reduced absorption, which happened due to limited pore size and lesser pore area of the archegonial receptacles.

Khadduri (2011) reported the effect of essential oils or distilled plant extracts in common liverwort control in container nursery production during three seasons of trials. Sporatec™; Brandt Consolidated, Springfield, IL (formerly sold as Sporan™; EcoSMART Technologies Inc., Franklin, TN) is a product that consists of rosemary, clove, and thyme oil. This product was tested on a common liverwort and moss-infested crop of western redcedar (*Thuja plicata*) seedlings (Khadduri, 2011). The results from this trial showed 91% common liverwort control 9 d after treatment. However, there was significant damage to the redcedar plants, and the common liverwort re-established within 14 d of knockdown. Other organic products such as pelargonic acid, acetic acid products, d-limonene, and ammonium nonanoate have shown some suppression of common liverwort but these compounds often require repeated applications and can cause severe damage to ornamentals crops (Table 1.2).

Graham and Dixon (2012) reported that the maintenance of a small residual aqueous ozone (O_3) concentration during the distribution of irrigation water to the crop has the potential to offer some level of common liverwort control. They conducted experiments to evaluate contact time thresholds and application frequencies suitable for common liverwort management. Contact times between 0.84 and 1.68 $mg \cdot L^{-1} \cdot min$ with three applications per week reduced common liverwort growth and fecundity. Chemical treatments that are commonly used to reduce spore loads of common liverwort, moss, and algae are quaternary ammonium chlorides, sold as products such as GreenShield® (Whitmire Micro-Gen Research Laboratories, St Louis, MO), Physan 20™ (Maril Products Inc., Tustin, CA), and Triathlon® (OHP Inc., Mainland, PA). Other chemicals include hydrogen peroxide (ZeroTol®; BioSafe Systems LLC, Hartford, CT) and chlorine bleach, which are used as disinfectants for controlling algae, common liverwort, and moss (Smith, 2007). These disinfectant chemicals are mostly suitable for non-crop targets. If they come in contact with the sensitive ornamental plants, severe injury can occur.

Knowledge gaps and future research areas

Although initial research has been conducted with mulch materials such as rice hull and hazelnut shells, more research is required to determine which type of organic mulch material can

provide acceptable common liverwort control. Impacts of mulch depth, particle size, aging and moisture holding capacity of various organic mulch materials needs to be investigated. Based on such data, recommendations specific to both nursery and greenhouse operators for controlling common liverwort can be made. Organic mulch extracts with allelopathic properties can be used for weed management because they can act as natural herbicides or biopesticides (Saha et al., 2018). These natural products possess complex structures, can readily decompose, and contain different modes of action compared with synthetic herbicides (Dayan et al., 1999; Duke et al., 1997, 2000). Hence, these natural chemicals can act as alternatives to synthetic herbicides for controlling weeds in case of herbicide-sensitive ornamentals. (Marble et al., 2015). To our knowledge, no research has been published on how different mulch extracts with allelopathic properties can control common liverwort in greenhouse and nursery container production. Since there is a limitation to using synthetic herbicides within greenhouses and inside closed structures, these natural products (organic mulch extracts) may act as potential biopesticides for common liverwort control.

Altland (2004) made a recommendation of incorporating or dibbling CRFs at a depth of 7.6 cm to reduce common liverwort growth. However, there is no research report or data available on strategic placement of fertilizer in the container that can control common liverwort effectively. Research is required to determine whether subdressing or dibbling of controlled release fertilizer can be an effective method of suppressing common liverwort growth in comparison to popular practices of topdressing and incorporation. The right depths for subdressing and dibbling need to be determined. In addition, further studies need to focus on how fertilizer placement can impact common liverwort growth rate and reproduction cycles and influence competitiveness with ornamental crops.

Some attempts have been made previously to control common liverwort with both synthetic and organic chemicals/ herbicides, but more research is required in this area because in many cases, the results varied from region to region and with environmental conditions. Research needs to focus on how different synthetic and organic chemicals can affect different growth stages of common liverwort (sexual structures and vegetative body), and how different combinations of newer herbicides (preemergence and postemergence activity) at different application rates can affect common liverwort growth. In particular, different combination of preemergence herbicides containing active ingredients of PPO inhibitors need to be tested on common liverwort growth

stages. Dimethenamid-P is another herbicide that potentially suppresses common liverwort and requires an in-depth study (Marble et al., 2017). Identifying the group of chemicals, determining their phytotoxic effects to ornamentals, costs involved in their applications, and application rates and timing that can provide control of common liverwort will help the billion-dollar green industry in the U.S. to improve productivity and profit margins. Hence, there is need to conduct further research on both non-chemical and chemical methods for controlling common liverwort in container nurseries and greenhouse operations.

Tables and figures

Table 1.1. Synthetic herbicides/ chemicals evaluated for common liverwort control.

Active ingredient	Trade name	Greenhouses and other enclosed structures	Notes
Diquat	Reward®; Syngenta Crop Protection, Greensboro, NC.	No	Has shown postemergence control of common liverwort but requires repeated applications with surfactant (Marble et al., 2017).
Flumioxazin	BroadStar™; Valent U.S.A. LLC, Walnut Creek, CA.	No	BroadStar can be applied to container production and is less effective than the sprayable formulation (SureGuard).
	SureGuard®; Valent U.S.A. LLC, Walnut Creek, CA.	Yes (only inside empty greenhouse)	SureGuard has shown both pre and postemergence control of common liverwort (Marble et al., 2017).
Oxadiazon + prodiamine	RegalStar II®; Regal Chemical Company, Alpharetta, GA.	No	Has shown some preemergence common liverwort control and can be used in container production in nurseries (Marble et al., 2017).

Table 1.1 (cont'd)

Oxyfluorfen	GoalTender® & Goal®; Dow AgroSciences LLC, Indianapolis, IN.	No	Has residual activity when applied to container media (Fausey, 2003). Granular formulation less effective and slower to provide control than sprayable formulations. Have both pre and postemergence activity (Marble et al., 2017).
Oxyfluorfen + oryzalin	Rout®; ICL Specialty Fertilizers, Summerville, SC.	No	Can be used in container production only. Has shown preemergent common liverwort control but effect varies from region to region depending on environmental conditions (Newby et al., 2007).
Oxyfluorfen + oxadiazon	Regal OO Herbicide®; Regal Chemical Company, Alpharetta, GA. Double O™; Nufarm Americas Inc., Alsip, IL.	No	Labeled for use in ornamental plants production in containers and has shown suppression of common liverwort (Marble et al., 2017).
Oxyfluorfen + pendimethalin	OH2®; Everris NA Inc., Dublin, OH.	No	Labeled for use in ornamental plants production in containers and has shown suppression of common liverwort (Marble et al., 2017).

Table 1.1 (cont'd)

Oxyfluorfen + prodiamine	Biathlon®; OHP Inc., Mainland, PA.	No	Labeled for use in ornamental plants production in containers and has shown suppression of common liverwort (Marble et al., 2017).
Quarternary ammonium chloride	GreenShield®; Whitmire Micro-Gen Research Laboratories, St Louis, MO. Physan20™; Maril Products Inc., Tustin, CA. Triathlon®; OHP Inc., Mainland, PA.	Yes (only on hard surfaces)	These products are disinfectants and can be used in nurseries as well to control common liverwort, algae and moss. Contact with sensitive ornamental plants can cause severe injuries.
Sodium carbonate peroxyhydrate	TerraCyte®, BioSafe Systems, LLC, East Hartford, CT.	Yes	Can be used in container production (Saha et al., 2020) and suppress mature common liverwort (Altland et al., 2003). However, it can cause severe injury to certain perennial plants if granules become trapped in/on plant foliage (Altland et al., 2003; Marble et al., 2017).

Table 1.2. Organic herbicides/ chemicals evaluated for common liverwort control.

Active ingredient	Trade name	Greenhouses and other enclosed structures	Notes
Combination of rosemary, clove, and thyme oil	Sporatec™; Brandt Consolidated, Springfield, IL.	Yes	Sort-term postemergence control (90%) but common liverwort re-established within 2 weeks (Khadduri, 2011).
Pelargonic acid	Scythe™; Gowan Company, Yuma, AZ.	Yes [z]	Requires repeated applications for effective control of common liverwort (Marble et al., 2017).
Cinnamic aldehyde	Cinnacure™; Pro-Guard, Suisun City, CA.	Yes	Acts as contact herbicide and has shown some suppression of common liverwort but may cause sporadic injury to ornamental crops depending on the environmental conditions (Svenson, 1997).
Acetic acid	Many products available	Yes[z]	Repeated applications are needed for effective postemergence control of common liverwort. Can be used in nursery container production.

Table 1.2 (cont'd)

Ammonium nonanoate	Axxe®; BioSafe Systems, LLC, East Hartford, CT.	Yes[z]	Broad spectrum herbicide that requires repeated applications for effective postemergence control of common liverwort. Can be used in nursery container production (Marble et al., 2017).
d-limonene	AvengerAg®; Avenger Organics, LLC, Gainesville, GA.	Yes[z]	Non-selective contact herbicide that may require repeated application for effective postemergence control of common liverwort. Can be used in nursery container production (Marble et al., 2017).

[z] May cause damage to ornamentals if applied directly. Spot application is suggested.

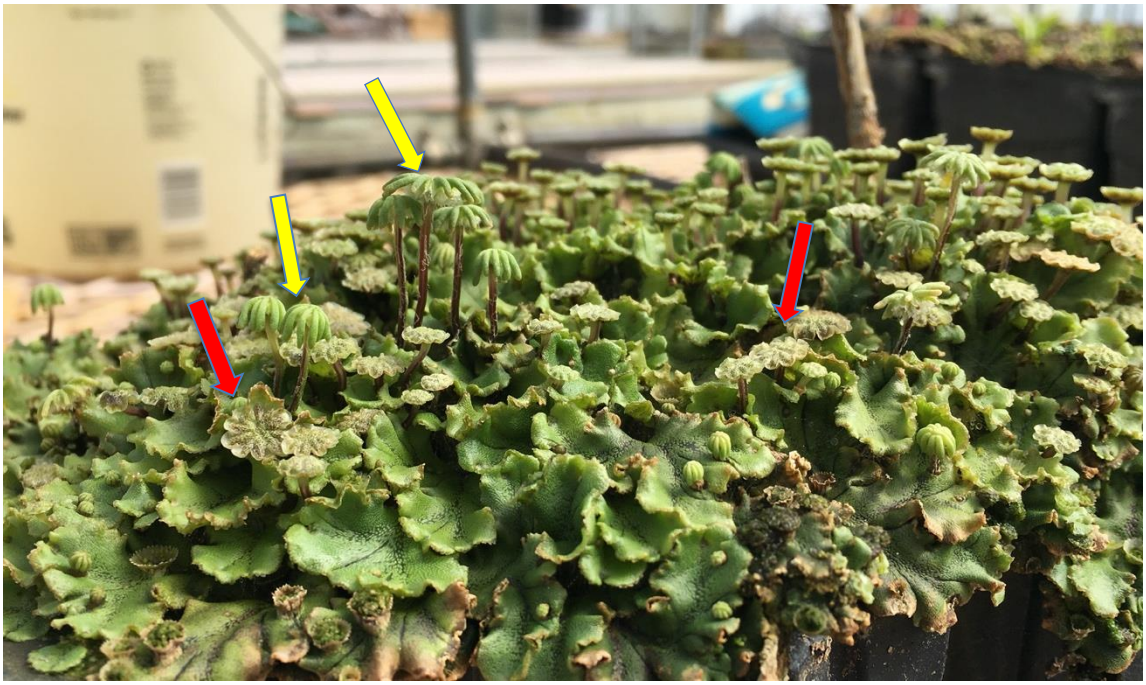

Figure 1.1: The red arrows showing the male antheridia borne on stalked antheridiophores and the yellow arrows showing the umbrella-like female archegonia borne on stalked archegoniophores on separate thalli of common liverwort under a greenhouse condition.

Figure 1.2: Gametophytic life cycle of common liverwort is represented by circular gemma cups containing numerous gemmae.

Figure 1.3: Common liverwort forming a mat and growing on the container media inside a greenhouse operation. It is competing with the ornamental plant for media, water, nutrients, space, and oxygen and reducing the overall quality of the ornamental crop.

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CHAPTER 2: PREEMERGENT LIVERWORT CONTROL IN CONTAINER-GROWN ORNAMENTALS BY ORGANIC MULCHING

Abstract

Liverwort (*Marchantia polymorpha*) competes with the ornamental plants for soil, nutrients, water, and space within the containers in nursery and greenhouse production, resulting in reduction of quality, aesthetic, and market value of the ornamentals. There are some herbicide options labeled for liverwort control, but they cause plant phytotoxicity and raise environmental concerns from off-target movement. Hand weeding of liverwort is laborious, time-consuming, and expensive. Other non-chemical liverwort control methods could improve the overall quality and profitability in container nursery production. This experiment was conducted to assess the impact of different organic mulch types, depths and their moisture holding capacity on preemergent liverwort control and determining phytotoxicity of organic mulch materials in greenhouse container production. The moisture holding capacity and percent moisture retention of four different organic mulch materials [rice hull (RH), cocoa hull (CH), pine bark (PB) or red hardwood (HW)] were determined in a laboratory experiment. A greenhouse experiment was conducted where two varieties of *Hosta* spp. namely, 'Curly Fries' and 'Pandora Box' were potted and mulch type of either RH, HW, CH, or PB was applied on top of the substrate in each container at a depth of 0.63, 1.27, 2.54, or 5.08 cm. A control set without any mulch materials was also included. Gemmae of common liverwort were applied over the mulch materials and the substrate (for control) after one day and was continued bi-weekly. Percent of container surface covered by liverwort thalli was visually estimated at 2, 4, 6, 8, 10, and 12 weeks after treatment (WAT). Fresh weight of the thalli was recorded at 12 WAT. Growth indices of the *Hosta* spp. were recorded at the beginning and end of experiment to assess the phytotoxic effect of the mulch materials. Results indicated that CH mulch retained highest amount of moisture among all mulch types. RH and HW mulch at depths of 1.27 cm or more, provided excellent liverwort control whereas, CH provided least control in 'Curly Fries'. All mulches at depths of 1.27 cm or more showed excellent liverwort control for 'Pandora Box'. Depth of 5.08 cm of CH and HW mulches caused reduction in growth of 'Curly Fries' and 'Pandora Box', respectively. The RH and PB mulches at depths of 1.27, 2.54, and 5.08 cm provided an excellent liverwort control and no reduction in growth of plants.

Keywords: Liverwort, Organic mulches, Moisture retention, *Hosta spp.*, Ornamental production

Introduction

Common thalloid liverwort (*Marchantia polymorpha*) is a major problematic weed in nursery and greenhouse container production systems (Svenson et al., 1997). It is a difficult to

control weed species and has spread throughout the United States (Fausey 2003). Liverwort is particularly a problem where there is low temperature (10-15°C for reproductive growth and 17-22°C for vegetative growth), low ultraviolet (UV-B) radiation, high moisture, and high fertility, (Newby et al., 2006). It can rapidly reproduce, both sexually by spores (male anthrediophores and female archegoniophores) and asexually by gemmae that are produces in specialized structures known as gemmae cups and by fragmentation (Altland et al., 2003; Svenson et al., 1997). Liverwort colonies form a mat-like structure on the soil surface, impeding water and nutrient flow to the root zone of the ornamentals. As a result, the growth and quality of the ornamental plants is affected, reducing their market value (Svenson et al., 1997). Liverwort control by herbicide application may result in phytotoxicity to sensitive ornamental plants and can have residual effects on environment. Whereas, hand removal of common liverwort is a laborious, time-consuming, and costly operation. In addition, weeding by hand can result in removal of the upper substrate and top-dressed fertilizer from the container. This disrupts plant root growth in the upper layers and adds to production costs.

An alternative approach that avoids these problems is the use of organic mulches. Various experiments in the past have reported good weed control by mulching with different materials at varying depths. Non-living mulches may be organic or inorganic, that are either the by-product of industrial manufacturing processes or are specifically manufactured for their purpose (Arentoft et al., 2013). Particle mulches such as bark chips, finer wood particles, and crop wastes help in weed cover reduction, and are non-phytotoxic to ornamentals and other crops (Bond and Grundy, 2001). Top dressing the growing media in containers with organic mulches like pine bark, pine straw, cocoa hull, cereal straw, and hardwood chips is a common practice in nursery production to reduce weed growth (Llewellyn et al., 2003; Saha et al., 2019; Kazemi and Safari ,2018). Mulching has several additional benefits such as: increasing soil moisture; reducing soil loss and compaction; moderating soil temperatures; improving soil nutrition; reducing salt and pesticide contamination; improving plant establishment and growth; reducing occurrence of diseases; and improving aethestics of landscapes. The type of mulch to be used should be carefully chosen as some mulches may lead to soil acidification, allelopathic activity on plants, competition from living mulches like grasses, flammability of mulch materials, contamination from pathogens or weed seeds and nutrient deficiency (Chalker-Scott, 2007).

Sarkka and Tahvonen (2020) reported effective control of liverwort in nursey plants such as highbush blueberry, black currant, and rhododendron using *Sphagnum* moss and blackcurrant stem pieces. Liverwort control by *Sphagnum* moss alone ranged from 78-99% while blackcurrant stem pieces provided complete control. For roses, Altland and Krause (2014) reported complete liverwort control for 8 weeks with a 1.3 or 2.5 cm (0.5 or 1.0 in) depth of parboiled rice hulls, with no adverse impact. Rice hulls applied at a depth of 0.6 cm showed 2.5% and 20% liverwort coverage on growing media at 4 and 8 weeks after potting (WAP), respectively. Svenson et al. (1997) reported that fast-drying mulches such as European hazelnut (*Corylus avellana*) shells, rice (*Oryza sativa*) hulls, and pumice on the container media surface can suppress liverwort growth. A depth of 1.3 cm of hazelnut and oyster shell mulches resulted in reduced liverwort growth (Svenson 1998). Saha et al. (2019) quantified the effect of different herbicide combinations with organic mulch materials such as pine bark, pine straw, and hardwood chips on weed control in container production system. There was 88% to 100% reduction of large crabgrass (*Digitaria sanguinalis*) and garden spurge (*Euphorbia hirta*) in containers with mulch depths of 1 inch or greater along with herbicide combination.

Limited research has been conducted so far to determine how different organic mulch types, depths, and their moisture-holding capacity can impact common liverwort growth in container production. Therefore, the main objectives of this experiment were to assess the moisture-holding capacity of different organic mulch materials [rice hull (RH), cocoa hull (CH), pine bark (PB) or red hardwood (HW)] and to evaluate their impact on preemergent liverwort control and phytotoxicity on container-grown ornamentals.

Materials and Methods

Laboratory experiment: The moisture-holding capacity and percent moisture retention of four mulch materials was determined in a laboratory experiment conducted at the Department of Horticulture, Michigan State University in summer 2020. Following the methods described in Saha (2019), two-piece plastic Buchner funnels (12.7 cm inner diameter, 6.6 cm tall) were filled with 5 cm of either rice hull (RH), cocoa hull (CH), pinebark (PB) or red hardwood (HW) mulch materials (Figure 2.1). The weight of the mulch was first determined, and these weights were used to uniformly apply the same mass of each mulch material to replicate funnels. The volume of water to be added was determined in advance based on an irrigation depth of 1.02 cm (0.4 inches). Each mulch-filled funnel was weighed (W_i) and placed over a 900 mL glass jar and 171 mL of water

(equivalent to 1.02 cm of irrigation) was added. The water passing through the mulch layer was collected in a glass jar. The funnels were weighed after the irrigation ceased and funnels stopped dripping (W_0), and 1 h, 4 h, and 24 h after irrigation (W_1, W_4, and W_{24}, respectively). The volume of water passing through the mulch was measured (V). Following formulas were used to calculate water retention (%) (Saha, 2019):

a) The percentage of water not retained by the mulch was calculated as: $[V \div (W_0 - W_i + V)] \times 100$.
b) The amount of water retained in the mulch layer at 1 h, 4 h, and 24 h was calculated as $W_1 - W_i$, $W_4 - W_i$, and $W_{24} - W_i$, respectively.
c) The percentage of water retained by the mulch at 1 h, 4 h, and 24 h was calculated by the formula: $[(W_{1to\ 24} - W_i) \div W_i] \times 100$.

The experiment was conducted in a completely randomized design with four mulch types and three time intervals. There were four replications per mulch material. All data were analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) to determine the effects of mulch type for water retention (%). Before analysis, the data was inspected to ensure that the assumptions of ANOVA are met, and data was log transformed because it did not meet the normality assumptions. When ANOVA results revealed significant effects, mean comparisons were performed using Tukey's honest significant differences (HSD) test. All the effects were considered significant at alpha=0.05, to separate out the means. The experiment was repeated, and the combined data was analyzed to separate out the means.

Greenhouse experiment: A greenhouse experiment was conducted at the Horticulture Teaching and Research Center, Michigan State University, Holt, MI in summer 2020 to assess the impact of organic mulch type and depth on preemergent control of liverwort and phytotoxicity on container-grown ornamentals. In this experiment, 3.78 L (1 gallon) nursery containers were filled with standard commercial soilless media containing 70% peat moss, 21% perlite, and 9% vermiculite (Suremix, Michigan Grower Products Inc., Galesburg, MI). Controlled release fertilizer (CRF) Osmocote® [N: P: K 17-5-11 (8 to 9 months)] (ICL Specialty Fertilizers, Dublin, Ohio) was incorporated at the highest labeled rate according to the manufacturer's recommendation of 35 grams per gallon container. The experiment was conducted in two replicates using *Hosta* 'Curly Fries' and 'Pandora' respectively. Ornamental plants were obtained from a cooperating liner nursery (Walter Gardens, Zeeland, MI). For the first round of experiment,

'Curly Fries' was potted in 3.78 L pots. One day after potting, RH, HW, CH, or PB mulch (Figure 2.1) was applied on top of the substrate in each container at a depth of 0.63 cm, 1.27 cm, 2.54 cm, or 5.08 cm. A control set without mulch was also included. Containers were irrigated with approximately 1.02 cm (0.4 inches) of water via overhead sprinklers. After 1 or 2 days, liverwort (*Marchantia polymorpha*) gemmae were applied over the mulch or the substrate (for control) in each container. For gemmae collection, gemmae cups were scraped off vigorous liverwort stock plants and put into a bowl of tap water, thus releasing gemmae upon separation from their clumps (Altland and Krause, 2014). A plastic spoon was used to apply approximately 5 ml (1 tsp) water from the bowl, which contained gemmae, across the surface of each container. The containers were completely randomized after gemmae application. Gemmae were applied bi-weekly to each container. All containers received irrigation daily of approximately 1.02 cm via overhead sprinkler.

The initial growth indices of the *Hosta* plants, calculated as an average of the plant height and two widths, were recorded 1 day after the initial gemmae application. The percentage of container surface covered by liverwort thalli was visually estimated at 2, 4, 6, 8, 10, and 12 weeks after treatment (WAT). Fresh weight of the thalli was also recorded at 12 WAT. The final growth indices of the *Hosta* spp were recorded at 12 WAT (end of the experiment).

The percent increase in growth index of plants was calculated using the following formula at the end of the experiment:

$$\% \text{ Increase in growth index} = 100 \times \frac{(Final\ growth\ index - Initial\ growth\ index)}{Initial\ growth\ index}$$

The experiment was conducted in a completely randomized design as a 4 x 4 factorial treatment arrangement with four mulch types and four mulch depths. There were four single-pot replications per treatment. All data were analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) to determine the effects of mulch type and depth and the interactions of these variables, on data collected for various experimental parameters.

Before analysis, the data was inspected to ensure that the assumptions of ANOVA are met, and data was log transformed where needed. The replications were considered as random effects while mulch type and depth were considered fixed effects. When ANOVA results revealed significant effects, mean comparisons for fixed factors were performed using Tukey's honest significant differences (HSD) test. All the effects were considered significant at alpha=0.05, to separate out the means.

Results and Discussion

Laboratory experiment: There was a significant difference in water retention at different time-points ($p<0.05$) for all the mulch types studied. After 1 hour of initiation of the experiment (adding water to the mulches), CS mulch retained the highest amount of water (91.2%), while PB allowed maximum amount of water to infiltrate and retained only 9.1% of water applied. Similarly at 4 and 24 hours, CS retained maximum quantity of water (89.7% and 85.8%, respectively) and PB retained least amount of water (7.7% and 3.8%, respectively) (Table 2.1). For all the mulch types, out of the whole amount lost through infiltration, maximum was observed within first hour and it was slower for other time points observed. For example, within PB – 90.9% water passed through the mulch by 1 hour, and only 1.4% and 3.9% infiltrating by 4 hours and 24 hours, respectively. Similarly, for HW and RH, the amount of water infiltrating through the mulches by one hour was 82.8% and 84.9% respectively. Altland et al., (2016) studied the moisture retention and effects of rice hull mulch on controlling weeds in container production. It was found that increasing mulch depth reduced weed seed germination and establishment for both bittercress (*Cardamine hirsuta*) and creeping woodsorrel (*Oxalis corniculata*). Also, rice hull was compared to peat moss and pine bark for their moisture retention abilities. It was found that rice hull retained lesser amount of moisture as compared to other mulches which turned out to be the primary mechanism of controlling weed seed establishment above the mulch layer. Kader et al (2019) conducted studies on effects of rice straw and newspaper mulching on soil moisture and temperature regimes, moisture availability and water use efficiency in soybean. It was found that mulching improved soil moisture availability, reduced soil water consumption, and improved water use efficiency by 25-47% in comparison to un-mulched soil. In a previous study, Zhang et al (2023a) studied the water holding capacity and water permeability properties of organic mulches (bran, grass and newspaper) for their effects on moisture retention in soil and crop growth. It was found that bran possessed highest water retention and beneficial for water retention under sprinkler or drip irrigation. Also, the water holding capacity was found to be directly related to amount of water absorbed and water immersion (application) time. Zhang et al (2023b) also studied the impact of organic mulching with bran, grass and newspaper in greenhouse tomato production, and found that it helped in regulating soil moisture and temperature, water use efficiency and improved crop yields. Zribi et al., (2015) studied the effectiveness of various organic (pine bark, wheat straw, vine pruning residues, geotextile) and inorganic mulches for evaporation control and found that

pine bark had lower evaporation rate than other organic mulches. This could be a beneficial attribute of the mulch for reducing water loss and improving moisture retention for plant growth. For best weed control results, a coarse textured mulch of particle size 0.63-1.9 cm (0.25-0.75 inches), with a low water holding capacity should be considered (Wilen 2018).

Greenhouse experiment: The growth index (%) of Hosta spp. variety Curly Fries was significantly affected by the interaction of mulch types and depths ($p<0.05$). The growth index of plants was found to be higher in the RH (324.3%) and HW (316.1%) mulch applications at a depth of 5.08 cm. Within the CS and RH treatments, there were differences observed in the plant growth index at various depths. For CS, there was maximum suppression of growth when mulch was applied as a thicker layer (5.08 cm). The plants recorded lower growth index (153%) as compared to other treatments, thereby indicating a phytotoxicity caused by thicker layer of mulch application on the top of the substrate that limited the growth of Curly Fries. In contrary, for RH, the 5.08 cm depth of mulch resulted in highest growth index, while the 2.54 cm depth caused reduction in plant growth (Table 2.2). Kazemi and Safari (2018) examined the effectiveness of various organic and inorganic mulches including pine needles, wood chips, volcanic stone (scoria) and polyethylene on growth characteristics of flowering zinnia (*Zinnia elegans*). Mulching with pine needles and wood chips helped to attain higher water use efficiency, and improved growth and shoot fresh and dry weights of the plants. The flowering time increased by 6 days and the time to first flowering reduced with the application of mulches. Amoroso et al., (2010) studied the effect of application of biodegradable mulches to container-grown giant arborvitae 'Martin' (*Thuja plicata*). It was found that mulching resulted in improving plant growth, moderating substrate temperature, and improving water content availability, alongside reducing weed growth in containerized production. Poudel and Witcher (2022) studied the effect of pine pellet, rice hull, paper pellet and vermiculite mulching propagation of butterfly bush (*Buddleja davidii*), crape myrtle (*Lagerstroemia indica*) and hydrangea (*Hydrangea paniculata*) cuttings. The rice hull mulch resulted in slight reduction (less than 50%) of root volume and length of crape myrtle cuttings. Marble et al., (2019) compared pine bark, shredded hardwood, pine sawdust, applied alone or in combination with plastic film and paper slurry mulch, in container nursery production. The results of this experiment showed 64-91% weed reduction by the organic mulch application, which was equivalent to control provided by plastic mulch. The mulch application significantly reduces the hand weeding time and weed biomass as compared to non-treated control. In another experiment, Khamare et al., (2023) tested

various stratified substrates composed of pine bark, additionally mulched with rice hull on the top, on their effects on growth of an ornamental *Hibiscus rosa-sinensis*, and nursery weeds liverwort and bittercress. It was found that mulching the pine bark substrate with rice hull was highly effective for controlling bittercress and liverwort in nursery containers. Richardson et al., (2008) studied pine bark mini-nuggets applied at depths of 0, 1.5 or 3 inches, for their effects on oxalis and bittercress control in nursery containers planted with *Gardenia jasminoides*, *Lagerstroemia indica*, *Hydrangea quercifolia* and *Ternstroemia gymnanthera*. There was no detrimental effect recorded on the growth of the ornamentals with mulching and mulching at depth of 3 inches provided season-long control of weeds.

The effect of mulch application was also significant in limiting liverwort thallus coverage in the pots containing *Hosta* spp. variety 'Curly Fries' from 2-12 WAT. From 2-10 WAT, all the mulch treatments were significantly different from the control, but not different amongst themselves. The mulch applications were able to limit the liverwort growth to under 15% until 10 WAT, as compared to control, that had 76% liverwort coverage on the top of the containers. At 12 WAT, the mulch treatments were significantly different from the control as well as amongst themselves. The RH, HW and PB mulches provided best control of liverwort (had 5,7 and 12 % liverwort coverage) as compared to control which had 81% of container surface covered with liverwort thallus (Table 2.3). Out of all the depths of mulching, all the depths performed equally until 10 WAT, but they always provided significantly better than the untreated control pots. There was a significant difference in depths of mulches applied at 12 WAT (Table 2.4). The 5.08 cm depth of mulching provided a season-long liverwort coverage control and continued to provide excellent control until the end of this experiment. The 1.27 and 2.54 cm depths of mulching also provide a good (>80%) season long (12 weeks) control of liverwort coverage, whereas the 0.63 cm depth of mulching was least effective towards the end of the experiment. Arentoft et al (2013) studied the weed-suppressing effects of spruce bark (*Picea spp*.) mulch and cocoa husk (*Theobroma cacao*) mulch applied at 0, 2.5, 5, 10 and 15 cm in apple (*Malus spp*.) orchard. It was found that lesser thickness of bark was needed to reduce weed biomass by 50% at 60 days after establishment than that of cocoa mulch. However, the cocoa mulch performed better later in the season than bark mulch in limiting weed growth (75 and 90 days after establishment). Massa et al., (2019) analyzed a hydro-compacting organic fiber mulch in containerized production and found that it reduced weed presence by 70%, and improved plant performance of camellia

(*Camellia japonica*), cupressus (*Cupressus sempervirens*) and photinia (*Photinia fraser* 'Red Robin'). Yunus et al., (2023) reported that rice husk mat applied at 8 mm depth can be used for weed control in nursery polybags. They found that applying rice husk reduced coverage, emergence and biomass of *Cyperus distans*, *Ageratum conyzoides* and *Eleusine indica* weeds. Bartley et al., (2017) studied the effects of different tree-based mulches and their depths on control of eclipta (*Eclipta prostrata*), spotted spurge (*Chamaesyce maculata*) and long stalked Phyllanthus (*Phyllantus tenellus*) in nursery production. The mulches included ground whole loblolly pine (*Pinus taeda*), eastern red cedar (*Juniperus virginiana*), sweetgum (*Liquidambar styraciflua*) applied at 1-, 2- or 4-inches depth; and were also compared to the effects of herbicide dimethenamid-p. Results showed that mulching at 1 inch depth reduced fresh weight of weeds by 82-100%, 30 days after application. The effects of mulching were still significant until 168 days after application, providing 90-100% reduction in spotted spurge fresh weight, when the herbicide treatments had lost all its efficacy in comparison.

For *Hosta* spp. variety 'Pandora Box', neither the mulch applications nor the different depths of mulches applied had an effect on the growth indices of the plants ($p>0.05$). However, the liverwort thallus coverage on the top of container surfaces in pots containing 'Pandora Box' was significantly affected by mulching from 2-12 WAT. All the mulch treatments were significantly different from control but not amongst themselves from 2-12 WAT. AT 12WAT, the liverwort coverage in all the mulch treated pots was less than 10% while it was 94% in the untreated control containers (Table 2.5). Various depths of the organic mulch applied were also significantly effective in limiting liverwort thallus coverage on the top of container surfaces from 2-12 WAT ($p<0.05$) (Table 2.6). Any depth of mulch application was significantly different from untreated pots from 2-12 WAT. At 2, 4 and 6 WAT, the liverwort coverage at either of mulch depths was <10%, while it was 21%, 29% and 46% for untreated control pots at these bi-weekly intervals, respectively. At 8, 10 and 12 WAT, the 1.27 cm, 2.54 cm and 5.08 cm depths provided almost complete inhibition of liverwort, and 0.63 cm depth provided >80% control; in comparison to control pots which had 74%, 85% and 94% liverwort coverage at 8,10 and 12 WAT, respectively (Table 2.6). The growth index for *Hosta* spp. variety 'Pandora Box' was recorded non-significant for the effect of type or depth of mulch applied ($p>0.05$). Altland and Krause (2014) studied the effect of parboiled rice hull mulch applied at depths of at 0, 0.25, 0.5 and 1 inches, on controlling liverwort and hairy bittercress (*Cardamine flexuosa*) in container nursery production of single rose

(*Rosa spp*. 'Radrazz'). There were no adverse effects on growth and quality parameters of ornamental crop and the weed establishment was significantly reduced with increasing depth of rice hull mulch. Sarkka and Tahvonen (2020) utilized sphagnum moss and stem pieces of blackcurrant as mulches for controlling liverwort in blackcurrant, highbush blueberry and rhododendron production. The liverwort control ranged from 78-100% after application of these mulches and there was no significant difference observed in the depth or coarseness of mulch layers.

The liverwort fresh weight obtained from the containers at 12 WAT where it was growing in competition with either Curly Fries or Pandora box, was also significantly affected by the mulch treatments applied over the top of the container surface ($p<0.05$). In case of Curly Fries, RH and HW were most effective in minimizing liverwort establishment, thus producing least fresh weight of liverwort thallus (0.8 and 0.9 gm, respectively). The PB (2.9 gm) and CS (9.2 gm) treatments also recorded significantly lower liverwort thallus fresh weight in comparison to control (24.3 gm). For Pandora Box containers, liverwort fresh biomass recorded for various mulch treatments ranged only from 1-2%, as compared to control (26.2%) (Table 2.7). Poudel and Witcher (2022) studied the effect of pine pellet, rice hull, paper pellet and vermiculite mulching on control of large crabgrass (*Digitaria sanguinalis*), bittercress (*Cardamine 36ornicu*), mulberry weed (*Fatoua villosa*) and creeping woodsorrel (*Oxalis 36orniculate*). It was found that pine pellets and paper pellets applied at 0.5-inch depth reduced the growth of all four weed species.

Overall, the results from this experiment indicate that the application of RH or HW mulches at a depth of 1.27 cm or more outperformed other mulches and improved the growth of 'Curly Fries' in addition to providing excellent liverwort control. In contrary, the CS mulch provided least liverwort control and caused reduction in growth of Hosta spp., 'Curly Fries'. However, for 'Pandora Box', all the mulches provided promising control of liverwort, but the HW mulch caused reduction in its growth indices. For the moisture retention capabilities of the mulches, CS retained maximum amount of water, which is not suitable for containerized production, as it will lead to promotion of liverwort gemmae germination and establishment on the top layer of mulch. Therefore, the RH and PB mulches at depths of 1.27, 2.54, and 5.08 cm are recommendable for an excellent liverwort control with no reduction in growth of 'Curly Fries' and 'Pandora Box' varieties of *Hosta* spp. Growers need to consider mulch costs, stability in container (decomposition

rate), availability and good source of material, and labor costs for the mulch application in addition to their weed control benefits.

Tables and figures

a)  b) 

b)  d) 

Figure 2.1: Organic mulches: a) Pine bark, b) Cocoa shell, c) Red hardwood, and d) Rice hull.

Table 2.1: Percent moisture retention in organic mulches (pine bark, cocoa shell, red hardwood, and rice hull) at 1, 4 and 24 hours.

Moisture retention (%)				
Mulch type	1 hour	4 hours	24 hours	p value
Pinebark (PB)	9.1Ca*	7.7Cab	3.8Cb	0.0057
Cocoa shell (CS)	91.2Aa	89.7Aa	85.8Ab	0.0045
Red hardwood (HW)	17.2Ba	16.2Bab	12.4Bb	0.0110
Rice hull (RH)	15.1B	14.0B	11.3B	NS
p value	<0.0001	<0.0001	<0.0001	

*Means followed by different capital letters in column for time interval for each mulch or small letters in the row which compare a specific mulch material across different time intervals are significantly different according to Tukey's honest significant differences (HSD) test, at alpha=0.05.

NS, S*: Non-significant and significant at a = 0.05, respectively.

Table 2.2: Percent increase in growth index of *Hosta spp*. variety 'Curly Fries' growing in presence of liverwort; as affected by application of organic mulches (pine bark, cocoa shell, red hardwood, and rice hull) at different depths (0.63 cm, 1.27 cm, 2.54 cm, or 5.08 cm).

Percent increase in growth index of *Hosta spp*. variety 'Curly Fries'					
Mulch type	Depth of mulch applied				
	0.63 cm	1.27 cm	2.54 cm	5.08 cm	p value
Pinebark (PB)	185.5	279.18	223.37	252.55AB	NS
Cocoa shell (CS)	230.9ab*	313.8b	303.0b	153.0Aa	0.0111
Red hardwood (HW)	224.5	241.4	271.7	316.1B	NS
Rice hull (RH)	238.1ab	269.2ab	162.1a	324.3Bb	0.0253
Control	163.2	163.2	163.2	163.2A	NS
p value	NS	NS	NS	0.0020	

*Means followed by different capital letters in column for different depths for each mulch type or small letters in the row which compare a specific mulch material across different depths of mulches applied are significantly different according to Tukey's honest significant differences (HSD) test, at alpha=0.05.

NS, S*: Non-significant and significant at a = 0.05, respectively.

Table 2.3: Liverwort coverage (%) on top of container surface from 2-12 weeks after treatment (WAT) as affected by application of different organic mulches (pine bark, cocoa shell, red hardwood, and rice hull) in containers having *Hosta spp*. variety 'Curly Fries'.

Liverwort coverage (%) on the top of container surface bi-weekly						
Treatment	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	16a*	22a	41a	64a	76a	81a
Pinebark (PB)	0b	2b	4b	7b	9b	12bc
Cocoa shell (CS)	0b	0b	2b	5b	15b	29b
Red hardwood (HW)	0b	1b	3b	4b	6b	7c
Rice hull (RH)	0b	0b	1b	2b	3b	5c
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Percentages followed by the same letter are not significantly different within a column at p=0.05.

Table 2.4: Liverwort coverage (%) on top of container surface from 2-12 weeks after treatment (WAT) as affected by application of organic mulches at different depths (0.63 cm, 1.27 cm, 2.54 cm, or 5.08 cm) in containers having *Hosta spp*. variety 'Curly Fries'.

Liverwort coverage (%) on the top of container surface bi-weekly						
Treatment	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	16a*	23a	41a	64a	76a	81a
0.63 cm	1b	3b	6b	11b	18b	24b
1.27 cm	0b	0b	1b	6b	11b	19bc
2.54 cm	0b	0b	0b	2b	4b	8bc
5.08 cm	0b	0b	0b	0b	0b	1c
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Percentages followed by the same letter are not significantly different within a column at p=0.05.

Table 2.5: Liverwort coverage (%) on top of container surface from 2-12 weeks after treatment (WAT) as affected by application of different organic mulches (pine bark, cocoa shell, red hardwood, and rice hull) in containers having *Hosta spp*. variety 'Pandora Box'.

Liverwort coverage (%) on the top of container surface bi-weekly						
Treatment	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	21a*	29a	46a	74a	85a	94a
Pinebark (PB)	0b	2b	2b	4b	6b	9b
Cocoa shell (CS)	0b	0b	1b	2b	5b	9b
Red hardwood (HW)	0b	1b	2b	4b	4b	5b
Rice hull (RH)	0b	0b	1b	3b	5b	7b
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Percentages followed by the same letter are not significantly different within a column at p=0.05.

Table 2.6: Liverwort coverage (%) on top of container surface from 2-12 weeks after treatment (WAT) as affected by application of organic mulches at different depths (0.63 cm, 1.27 cm, 2.54 cm, or 5.08 cm) in containers having *Hosta spp*. variety 'Pandora Box'.

Liverwort coverage (%) on the top of container surface bi-weekly						
Treatment	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
0 (Control)	21a*	29a	46a	74a	85a	94a
0.63 cm	0b	2b	5b	11b	15b	20b
1.27 cm	0b	0b	0c	2c	4c	8c
2.54 cm	0b	0b	0c	0c	0c	1c
5.08 cm	0b	0b	0c	0c	0c	0c
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Percentages followed by the same letter are not significantly different within a column at p=0.05.

Table 2.7: Liverwort fresh biomass (grams) recorded at 12 weeks after treatment (WAT) as affected by application of different organic mulches (pine bark, cocoa shell, red hardwood, and rice hull) in containers having *Hosta spp*. variety 'Curly Fries' and 'Pandora Box'.

Liverwort fresh biomass at 12 WAT (gm)		
Treatment	Curly Fries*	Pandora Box
Control	24.3a	26.2a
Pinebark (PB)	2.9bc	2b
Cocoa shell (CS)	9.2b	2b
Red hardwood (HW)	0.9c	1b
Rice hull (RH)	0.8c	1b
p value	<0.0001	<0.0001

*Percentages followed by the same letter are not significantly different within a column at p=0.05.

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CHAPTER 3: LIVERWORT GROWTH, REPRODUCTION, AND COMPETITIVENESS WITH ORNAMENTALS IN CONTAINER PRODUCTION AS INFLUENCED BY STRATEGIC FERTILIZER PLACEMENT

Abstract

Liverwort (*Marchantia polymorpha*) is a problematic weed for nurseries and greenhouses, and there are currently no effective control methods. This study assessed liverwort growth, reproduction, and its competitiveness with ornamental plants in response to strategic placement of a controlled-release fertilizer (CRF) [Osmocote [17-5-11 (8 to 9 months)]. We investigated the effects of fertilizer placement (top dress, incorporation, sub-dress, and dibble) on liverwort growth and reproduction. CRFs were sub-dressed or dibbled at depths of 2.5, 5.1, or 7.6 cm. In each container, the top of the medium was inoculated with liverwort gemmae and the percent of the container surface covered by liverwort thalli was estimated visually after 2, 4, 6, 8, 10, and 12 weeks. At 12 weeks, gemma cups were counted and after approximately 28 weeks, the number of sexual reproductive structures and total fresh biomass of liverwort were recorded. The lowest liverwort growth, gemmae formation, and number of archegoniophores occurred when CRFs were incorporated with a dibble at 7.6 cm or sub-dressed at 7.6 cm. We also investigated the effects of CRF placement on liverwort competitiveness with a dicot begonia and a monocot dracaena. CRFs were either top dressed, sub-dressed, or dibbled all at a depth of 7.6 cm. Approximately one week after planting the ornamentals and gemmae application, liverwort was thinned to contain 0, 3, or 9 gemmalings per container. Percent increase in growth index of the ornamental plants, and the fresh mass of liverwort and ornamental plants were recorded at the end of the experiment. Results indicated that CRF sub-dressing and dibbling applied at 7.6 cm were the most effective in reducing liverwort coverage, while the highest coverage was in containers with CRF incorporation. Sub-dressing and dibbling CRFs also improved the growth of both begonia and dracaena while minimizing the fresh biomass of liverwort in containerized production. Therefore, the strategic fertilizer placements were effective for controlling liverwort growth in ornamental container production considering both the quality of ornamentals and their competitiveness with liverwort.

Keywords: Bryophyte, controlled-release fertilizer, CRF, ornamental, *Marchantia polymorpha*, sub-dressing.

Introduction

The common liverwort (*Marchantia polymorpha*) is thalloid spore-bearing bryophyte that belongs to the family *Marchantiaceae* (Durand, 1908; Budke et al., 2018). It grows by developing a flat thalloid structure and the lower surface of the thallus consists of rhizoids and scales, that assist in moisture and nutrient absorption and anchoring it to the growing medium (Budke et al.,

2018). The life cycle of liverwort consists of sporophytic (sexual) and gametophytic (vegetative) stages (Newby, 2006). In the sporophytic stage, sperm cells from antheridia (male sexual structure) produced on stalked antheridiophores fertilize the egg cell of archegonia (female sexual structure) borne on stalked archegoniophore, resulting in the production of spores (Newby, 2006). The gametophytic or asexual life cycle begins with germination of spores formed in the sporophytic stage. Spore germination is dependent on light availability (Heald, 1898) and day lengths of 10 h or more are required for germination (Nakazato et al., 1999). The cup like structures called gemmae cups are formed on the surface of thallus during the gametophytic life cycle, in which the plant propagates asexually by producing propagules called gemmae (Newby, 2006; Simpson, 2019). Numerous gemmae are released from each gemmae cup to the immediate surroundings by splashing with irrigation or rainwater (Svenson et al., 1997).

Liverwort spreads rapidly in nurseries and greenhouses due to its ability to propagate both asexually by gemmae and sexually by spores (Ross and Puritch, 1981). Liverwort is considered a weed for ornamental container production in nursery and greenhouse operations as it thrives environments with low ultraviolet (UV) radiation, high humidity, soil moisture, and fertility (Newby et al., 2006). It competes with ornamental plants for soil/growing medium, water, nutrients, space, and oxygen within the container and it obstructs movement of water into the root-zone. Ultimately it reduces the quality and market value of ornamental crops (Svenson et al., 1997).

Not many herbicides are labeled for use in the greenhouse environment and hand weeding of liverwort is a laborious and time-consuming task. Additionally, in container nursery production, herbicides must be applied at higher rates to control liverwort, which can cause phytotoxicity to sensitive ornamental plants and can have residual effects on the environment.

Therefore, it is important to study alternative methods of liverwort management. Strategic placement of controlled release fertilizers (CRFs) is a non-chemical physical method of weed control, which refers to applying/ placing CRFs to containerized plants in a manner different than the traditional incorporation or mixing of fertilizer within the substrate; or simply top-dressing the medium with the fertilizer (Di Tomaso, 1995). Alternative methods of fertilizer placement that can influence weed management include dibbling (placing in a pocket directly below the root zone at a depth of few cm) or sub-dressing the CRF (placing the CRF in a uniform layer a few cm below the root zone) (Stewart et al., 2018). They can reduce weed growth by limiting their access to

nutrients, by increasing the nutrient availability to crops as well improving their competitive capability (Nkebiwe et al., 2016; Di Tomaso, 1995). These fertilizer placement methods have been shown to reduce weed growth in several production systems (Marble et al., 2015). In a study conducted by Khamare et al. (2023), the growth of bittercress (*Cardamine hirsuta*) and liverwort increased in top-dressed (application of fertilizer in a layer on the top of the substrate) containers in comparison to incorporated (uniformly mixing the fertilizer with the medium) containers. Saha et al. (2019) reported that CRF dibbling and sub-dressing resulted in reduced growth and reproduction of eclipta (*Eclipta prostrata*), large crabgrass (*Digitaria sanguinalis*) and spotted spurge (*Euphorbia maculata*) as compared to the industry standard practice of CRF incorporation or top-dressing. Incorporation of CRFs in the medium increased germination of spotted spurge from 77% to 183% in comparison to top dressing, sub-dressing (7.6 cm below the surface of the media), dibbling (in a small pocket 7.6 cm below the surface of the media), and no fertilizer application. Both sub-dressing and dibbling reduced seed production by 63% and 92% for large crabgrass and spotted spurge, respectively. Using these alternative methods of fertilizer placement such as banding or placing the fertilizer near the root zone has been shown to provide a competitive advantage in agronomic (Chauhan and Ahugho, 2013; Mashingaidze et al., 2012) and ornamental crops (Fain et al., 2003), as compared to traditional methods of fertilizer application. In another study, Altland et al. (2004) found that CRF dibbling reduced weed germination, in comparison to incorporation and top-dressing methods. In another container study, Broschat and Moore (2003) reported reduced weed growth with the application of CRFs in the form of a layer beneath the root zone (modified dibble) in comparison to top-dressing and incorporation. Dibble CRF placement also resulted in faster plant establishment and superior plant quality (Meadows and Fuller, 1983).

Liverwort growth is directly correlated with increasing nitrogen (N) levels (Svenson, 1998). Its establishment slows down at N application rates of <75 mg L^{-1} (Svenson et al., 1997) but this amount of N (l<75 mg L^{-1}) is usually not sufficient for growth of ornamental crops. For example, poinsettia (*Euphorbia pulcherrima*), zonal geranium (*Pelargonium zonale*) and ivy geranium (*Pelargonium peltatum*) require 250, 250 and 250 mg L^{-1} of nitrogen, respectively, for optimal growth (Cox, 1997). So, altering the placement of CRFs within containers, can aid in reducing the growth of traditional weeds. However, additional research is required to determine if CRF placement and varying depths can help to control liverwort in container production and not negatively influence crop growth. Therefore, this study was undertaken to evaluate the

effectiveness of various methods of CRF placement methods including incorporation, sub-dressing, dibbling, and sub-dressing on liverwort control in containerized greenhouse production.

Materials and methods

The experiments were conducted in a greenhouse with polycarbonate sidewalls and a double layer polyethylene roof at the Michigan State University Horticulture Teaching and Research Center in 2021 and 2022. The minimum, maximum, and average daily temperatures were 21.0, 26.6, and 23.8 °C. The study was conducted in a greenhouse as it allowed for temperature and irrigation management and reduced pest pressure and weed competition as compared to outdoor production (Gallina et al., 2023).

CRF placement experiment 1. Containers were filled with 70% peat moss, 21% perlite, and 9% vermiculite (Suremix, Michigan Grower Products Inc., Galesburg, MI) standard medium. Controlled-release fertilizer (CRF), Osmocote® [N: P: K 17-5-11 (8 to 9 months)] (ICL Specialty Fertilizers, Dublin, Ohio) at the manufacturer's recommended highest labeled rate (35 grams per 3.8 L or 1 gallon container). Each 5.7 L container received 52.5 grams of CRF. Strategic CRF placement in the containers included: top dressing, sub-dressing, medium incorporation, and dibbling. CRFs were top-dressed to the top layer of the substrate after the container was filled with the with the previously mentioned medium. Medium CRF incorporation of consisted of thoroughly mixing the CRF with the medium and then filling the container. For sub dressing, the CRF was added at three different depths of 2.5, 5.1, or 7.6 cm from the top. For the dibble method, the CRF was placed into small pockets at three different depths of 2.5, 5.1, and 7.6 cm from the top. Lastly, the untreated control did not receive any fertilizer.

After 1 or 2 d, gemmae of common liverwort were applied bi-weekly to the top of the medium in each container. Overhead sprinklers inside the greenhouse provided all containers approximately 1.02 cm of irrigation. The percentage of container surface covered by liverwort thalli was visually estimated at 2, 4, 6, 8, 10, and 12 weeks after treatment (WAT). At 12 weeks, the number of gemma cups (asexual reproductive structures) produced on the liverwort thallus were counted in each container. The liverwort thalli were allowed to continue to grow and monitored at a regular basis to identify the development of sexual reproductive structures. After, approximately 28 weeks, the number of sexual reproductive structures (male: antheridiophores and female: archegoniophores) was recorded in each container to determine any differential responses. At the end of the experiment, the total liverwort fresh biomass was recorded.

The experiment was conducted in a completely randomized design and there were six single-container replications per treatment. The experiment was repeated twice and the data from both replications in time was pooled for statistical analysis. All data were analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct an Analysis of Variance (ANOVA). The replications were considered as random effects and CRF placement methods were considered fixed effects. When ANOVA results revealed significant effects, mean comparisons for fixed factors were performed using Tukey's honest significant differences (HSD) test to separate out the means. All the effects were considered significant at alpha=0.05.

Liverwort competitiveness experiment 2. In this experiment, 3.8 L containers were filled up with previously mentioned commercial soilless media. CRF Osmocote® [N: P: K 17-5-11 (8 to 9 months)] (ICL Specialty Fertilizers, Dublin, Ohio) was applied at the highest labeled rate according to the manufacturer's recommendation of 35 g 3.8 L container. CRF placement in the containers included the four previously mentioned strategic fertilizer placement methods. For sub dressing and dibbling, only the 7.6 cm depth was used as it was the most effective based on the results from experiment 1. *Begonia* and *Cordyline indivisa* were planted after the fertilizer was added to the medium. Containers were irrigated daily with approximately 1.02 cm of water via overhead sprinkles inside the greenhouse.

After 1 or 2 d, gemmae of common liverwort were applied on top of the medium in each container. One week after planting, gemmalings were thinned to contain 0, 3, or 9 per container. Different densities of gemmalings per container was considered a significant treatment factor. Another treatment factor considered was the presence or absence of ornamental plants. Hand weeding was done to ensure that no other weeds were growing in the control or treatment containers.

Growth indices of the ornamental plants were recorded at the initiation and conclusion of the study (2 and 12 weeks after planting) by averaging the length and two widths of each plant. Percent increase in growth index of plants was measured using the following formula:

$$\% \text{ increase} = 100 \times \frac{(Final - Initial)}{Initial}$$

At 12 weeks after planting, liverwort thalli and ornamental plant shoot fresh biomass were recorded.

The experiment was conducted in 4 × 3 × 2 factorial arrangement with four fertilizer placement methods, three gemmae densities, and the presence or absence of an ornamental crop

in a completely randomized design. There were four single-container replications per treatment. All data were analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct an Analysis of Variance (ANOVA). The replications were considered as random effects and fertilizer placement methods, gemmae densities, and ornamental presence or absence were considered fixed effects. When ANOVA results revealed significant effects, mean comparisons for fixed factors were performed using Tukey's honest significant differences (HSD) test. All the effects were considered significant at alpha=0.05, to separate out the means.

Results and Discussion

Experiment 1

The effect of fertilizer placement method significantly influenced ($p < 0.0001$) liverwort % coverage on the top of the container surface from 2-12 WAT (Table 3.1). CRF sub-dressing and dibbling applied at 7.6 cm were the most effective in reducing liverwort coverage, while the highest coverage was in containers with CRF incorporation. At 12 WAT, % liverwort coverage in containers that had CRFs sub-dressed at 7.6 cm, dibbled at 7.6 cm, or the control were 39%, 34, and 22%, respectively. CRF incorporation had the highest coverage (99%), followed by sub-dress at 2.5 cm (91%), dibble at 2.5 cm (70%) and top-dressing (65%). These treatments were less effective at influencing liverwort coverage, in comparison to other previously mentioned treatments and the control. Notably, all the fertilizer placement methods with the exception of CRF incorporation had up to 30% liverwort coverage until 6 WAT (Table 3.1). Sub-dress and dibble with CRF below the top of the medium provided a significant control of liverwort. This was primarily because liverwort rhizoids do not penetrate the medium beyond depths of 1 to 2 cm. The liverwort thallus anchors itself to the top layer of the growing medium with its rhizoids and scales (Budke et al., 2018) that mostly utilize moisture and nutrients from the surface of the medium. In a previous study, it was reported that when CRF top-dressing and incorporation were applied at same rate to containers, CRF incorporation rapidly released nutrients compared to top-dressing (Hoskins et al., 2014). Similar results were observed by Khamare et al. (2020), who indicated that sub-dressing at a depth of 7.5 cm effectively reduced the growth of eclipta by 50% in comparison to top-dressing. However, sub-dressing at shallower depths (2.5 or 5 cm) did not have any effect on growth. In addition, when eclipta was competing with the ornamental plants, sub-dressing at 7.5 cm reduced the growth of little leaf Boxwood (*Buxus microphylla*) and glossy privet (*Ligustrum lucidum*). This study also emphasized that the depth of sub-dressing should be based

on the liner size of the nursery plants, to prevent delays in production time. Altland and Fain (2003) showed that weed control with CRF incorporation and top-dressing increased linearly with increasing rates of herbicide. However, for dibbling, there was significant weed control even without any application of herbicide. In a separate study, Altland et al. (2004) found that dibbling provided more than 85% control of prostrate spurge (*Euphorbia prostrata*), common groundsel (*Senecio vulgaris*), and oxalis (*Oxalis spp.*) as compared to lower rates of weed control with CRF incorporation and top-dressing. Many weeds that impact container grown ornamentals have small seeds do not grow beyond cotyledonary stage if the amount of nutrient availability is limited (Wada 2005). Fain et al. (2003) reported a reduction in the growth of eclipta by 44% with dibbling as compared to top-dressing.

In the current study, the number of gemmae cups and antheridiophores were also significantly influenced by the CRF placement method (Table 3.2). At 12 WAT, the highest number of gemmae cups were recorded with CRF incorporation (284) and top-dressing (231), while the dibbling treatment had a lower number, ranging from 90-151. The control had the lowest number of gemmae cups recorded (55). In contrast, for the number of antheridiophores recorded, dibbling at 5.1 cm and sub-dressing at 7.6 cm resulted in higher numbers (259 and 228, respectively), followed by the control and the other sub-dressing and dibbling treatments. Whereas top-dressing resulted in only 63 male structures. The fresh biomass of liverwort thallus in containers was also significantly influenced by CRF placement. It was highest when CRFs were top-dressed (168) and incorporated (167), as compared to other treatments and the control. The fresh biomass of liverwort for the untreated control was 11 g, which was followed by dibble at 7.6 cm (32 gm) and sub-dress at 7.6 cm (38 gm) (Table 3.2). It was recorded higher in top-dress and incorporation, when the fertilizer was available to liverwort rhizoids in their in immediate vicinity, as compared to sub-surface types of fertilizer application methods (sub-dressing and dibble). A previous study conducted by Altland and Fain (2003) reported that weed shoot dry weight was 60% lesser in the containers where the controlled-release fertilizers were dibbled as compared to incorporation and top-dressing. Saha et al., (2019) reported that sub-dressing type of fertilizer placement reduced seed production in eclipta (*Eclipta prostrata*), spotted spurge (*Eclipta maculata*) and large crabgrass (*Digitaria saguinalis*) by 94%, 92% and 63%, respectively. Also, there was no seed production for these weeds in case of dibbling. There was also a reduction in

fresh biomass of Eclipta, spotted spurge, and large crabgrass by 90%, 85% and 81%, respectively, in sub-dressing as compared to top-dressed and incorporation placements.

A possible explanation for the higher number of antheridiophores recorded for dibbling and sub-dressing could be that under nutrient stress conditions, liverwort may tend to put more reproductive structures, to ensure its multiplication for subsequent generations. CRF placement method did not influence the number of archegoniophores ($p>0.05$). This was likely due to the fact thar archegoniophores usually appear later than the antheridiophores in the liverwort sexual reproduction cycle, and the present study was terminated before all the archegoniophores may have appeared. The age of liverwort, photoperiod and light intensity inside the greenhouse could have influenced the number of female reproductive structures. These factors were not controlled in the study as they were out of the scope of this research objectives. Mache and Loiseaux (1973) found that light intensities ranging from 370 to 555 $\mu mol \cdot m^{-2} \cdot s^{-1}$ promoted vegetative growth of liverwort whereas higher light intensities impeded it. More asexual reproduction (gemmae production) occurs at shorter daylengths (~8 h) than under longer daylengths (17 to 18 h) (Voth and Hamner, 1940). Long days, high light intensities, and natural diffused daylight promote formation of antheridiophores (Terui, 1981). Future experiments for longer durations and under controlled environmental conditions may help to elucidate the response of liverwort to different fertilizer placement methods in term of number of archegoniophores.

Experiment II

The interaction of fertilizer placement method and liverwort gemmae density significantly ($p <0.05$) influenced growth index and fresh biomass of the dicot *Begonia* spp. (Table 3.3). At a liverwort gemmae density of 3, top-dressing, dibbling at 7.6 cm and sub-dressing at 7.6 cm provided a significantly higher % increase in growth index (393, 310 and 169%, respectively) as compared to CRF incorporation, where it was only 61%. However, when the liverwort gemmae density increased to 9, the % increase in growth index was maximized with CRF incorporation (472%), followed by dibbling at 7.6 cm (341%). Per cent increase in growth index of begonia was significantly lower (77%) with sub-dressing at 7.6 cm and 90% with top-dressing treatments. In the containers that received CRF incorporation, the % increase in growth index was maximum when gemmae density was highest (472%). While for top-dressing, it was highest when gemmae density was 3 (393%), followed by dibble at 7.6 cm (310%). For begonia, it was observed that lower weed pressure (liverwort gemmae density 3), strategic CRF application (top-dressing,

dibbling at 7.6 cm and sub-dressing at 7.6 cm) resulted in higher growth indices than incorporation. The highest % increase in growth index for begonia was recorded under the liverwort gemmae density of 9 and CRF incorporation, followed by dibbling at 7.6 cm (341%). Within incorporation treated pots, the growth index was highest at higher weed pressure (liverwort gemmae density 9), while for top-dress treatments it was highest at lower weed pressure (liverwort gemmae density 3). Broschat and Morre (2003) reported that dibbling or sub-dressing had an increased or no effect on the growth of Chinese hibiscus (*Hibiscus rosa-sinensis*), plumbago (*Plumbago auriculata*), and downy jasmine (*Jasminum multiflorum*). In contrast, Marble et al. (2012) reported no difference in the growth of gumpo azaleas (*Azalea* × hybrid 'Gumpo White) among the incorporation, top-dressing and dibbling treatments. Altland and Fain (2003) reported a reduction in growth index of azalea (*Rhododendron* spp.) with CRF incorporation versus dibbling. Also, the growth index of holly (*Ilex aquifolium*) was greater in containers that had CRFs dibbled as compared to those that received CRF incorporated or top-dressing. Meadows and Fuller (1983) reported that better quality of azalea (*Rhododendron* spp.) cultivars were produced from dibbling of fertilizers as compared to incorporation.

The fresh biomass of begonia was significantly different among the fertilizer placement methods when gemmae density was highest (Table 3.3); 128, 983, 765, and 560 g with sub-dressing at 7.6 cm, incorporation, dibbling at 7.6 cm, and top-dressing, respectively. There were also significant differences observed within the incorporation treatment for various liverwort gemmae densities. The maximum fresh biomass recorded was when the gemmae density was the highest (983 g), as compared to gemmae densities of 0 and 3 (Table 3.3). The highest fresh biomass was recorded when CRFs were incorporated, dibbled, and top-dressed, at higher weed pressure (gemmae density 9), possibly indicating that there was competition between liverwort and the ornamental for obtaining nutrients as the fertilizer was available for the plant. Also, considering the size of the container and plants, the fibrous roots of begonia were able to reach a depth below the top of the container, which enabled them to utilize nutrients from deeper placement zones (dibble and sub-dress) as compared to liverwort which has a shallow rhizoid system. Khamare et al. (2023) studied the effect of top-dressing versus incorporation with mulched or stratified medium on growth of hibiscus 'Snow Queen', and bittercress (*Cardamine flexuosa*) and liverwort. Top-dressing generally increased growth of both weeds as compared to incorporation. Surface CRF application and/or dibbling provided better shoot growth in comparison to incorporation for sweet viburnum

(*Viburnum odoratissimum*) azalea (*Rhododendron obtusum*) 'Hinodegiri', and golden privet (*Ligustrum × vicaryi*) (Cobb, 1985; Conover and Poole, 1985; Blessington et al., 1981), whereas incorporation produced superior quality plants for gardenia (*Gardenia jasminoides*) 'Radicans' (Cobb, 1985).

For the monocot dracaena (*Cordyline indivisa*), the percent increase in growth index was non-significant for effects of fertilizer placement methods and liverwort gemmae densities (Table 3.4). However, there were significant differences observed in the fresh biomass at the end of the experiment with different CRF placement methods and gemmae densities. When there was no weed pressure (gemmae density 0), the maximum fresh biomass recorded when CRFs were sub-dressed at 7.62 c (962 g) as compared to other treatments. When the gemmae density was 3, the biomass of dracaena receiving a CRF top-dressing and incorporation were 748 g and 530 g, respectively, in comparison to sub-dressing at 7.6 cm and dibbling at 7.6 cm (397 g and 265 g, respectively). Of the fertilizer placement methods investigated, the fresh mass of dracaena was influenced most by top-dressing and sub-dressing at 7.6 cm and by varying liverwort gemmae densities (Table 3.4). For containers that were top-dressed, the maximum fresh biomass of dracaena was recorded when the gemmae density was 3 (747.9 g), while for sub-dressing it was highest when there was no liverwort gemmae present (961.8 g), followed by subdressing at 7.6 cm when gemmae density was 9 (681.1 g). Similar to begonia, dracaena have a fibrous root system which allows them to grow well below the container surface. Broschat and Morre (2003) reported that sub-dressing (layering beneath the medium surface) or dibbling had an increased or no effect on the growth of bamboo palms (*Chamaedorea seifrizii*), Fishtail palms (*Caryota mitis*), Areca palms (*Dypsis lutescens*), Alexandra palm (*Archontophoenix alexandrae*), foxtail palm (*Wodyetia bifurcata*) and Macarthur palms (*Ptychosperma macarthurii*). Layering resulted in higher shoot dry mass than incorporation for Alexandra palm and foxtail palm. Out of all the species studied, only Areca plams performed best with incorporated fertilizer.

The analysis of variance of the effects of fertilizer placement method (F), gemmae density (D) and presence/absence of ornamental (O) on the fresh biomass of liverwort (grams) at 12 WAT, for both ornamental crops is presented in Table 3.5. For the begonia, there was a significant interaction between F and O as well as between D and O, for their effect on the fresh biomass of liverwort ($p<0.05$). For dracaena, there was significant 3-way interaction between F, D, and O for their effect on the fresh biomass of liverwort at the end of the experiment ($p<0.05$) (Table 3.5).

In the case of begonia, there was no significant difference in liverwort fresh mass under different fertilizer placements and in presence of the ornamental plant ($p>0.05$), but it was significantly different in absence of ornamental plant ($p<0.05$). When there was no ornamental present, the lowest liverwort fresh mass was recorded when the CRF was sub-dressed at 7.6 cm and dibbled at 7.6 cm (6.5 g and 9.1 g, respectively) in comparison to incorporation and top-dressing, which had a higher liverwort fresh mass (14.4 g each). Each of the fertilizer placement methods also had a significant influence on liverwort fresh biomass between the presence or absence of the ornamental plant ($p<0.05$). Liverwort fresh biomass was significantly higher under all the fertilizer placement methods when the ornamental plants were not present and there was no competition for the resources within the container (Table 3.6). No differences in liverwort fresh biomass under different fertilizer placements was recorded at the end of the experiment when liverwort was growing in a container with begonia. This indicates that in the plant-plant competition, neither of the fertilizer placements was promoting liverwort growth and the ornamental plant was able to utilize the fertilizer as compared to liverwort. However, the fresh mass of liverwort was highest when there was no ornamental plant present, and with surface CRF application methods (top-dress and incorporation). In a previous study by Altland and Fain (2003), they reported that weed shoot dry mass was 60% lower in containers where CRFs were dibbled as compared to incorporated and top-dressed.

Similarly, for the interaction of gemmae densities and ornamental (present/absent), there was no significant difference in liverwort fresh mass under liverwort gemmae densities in presence of ornamental plant ($p>0.05$), but it was significantly different in the absence of an ornamental plant ($p<0.05$). When the ornamental plants were absent and only the liverwort was growing in the container, the highest liverwort fresh mass was recorded when the gemmae density was highest (22.5 g). Liverwort gemmae densities (3 and 9) also had significant influence on liverwort fresh mass between the presence or absence of the ornamental plant ($p<0.05$). Liverwort fresh mass was significantly higher under all the liverwort gemmae densities when the ornamental plants were not present and there was no competition for the resources within the container (Table 3.7).

For dracaena, there was a significant interaction between fertilizer placement methods, gemmae densities, and presence/absence of ornamental for their effect on the fresh biomass of liverwort at the end of the experiment ($p<0.05$) (Table 3.8). In the presence of an ornamental, there were significant differences observed in liverwort fresh mass under different fertilizer treatments

when the gemmae densities were 3 and 9. The maximum recorded liverwort fresh mass was observed with CRF incorporation for the gemmae densities were 3 and 9 (1.8 g and 3.3 g, respectively). Similarly, different fertilizer placement strategies influenced liverwort fresh mass recorded at the end of the experiment, under the liverwort gemmae densities of 3 and 9, when there was no ornamental plant. The highest liverwort fresh mass were recorded under both the gemmae densities of 3 and 9 and CRF top-dressing (13.5 g and 40.9 g, respectively), followed by incorporation (10.7 g and 16.6 g, respectively) and sub-dress at 7.62 cm (8.1 g and 9.0 g, respectively). There were also significant differences within the incorporation, top-dressing and sub-dressing at 7.6 cm treatments, for different liverwort gemmae densities, when the ornamental plants were not present ($p<0.05$) (Table 3.8). In the case of liverwort growing alongside dracaena, liverwort was competitive for nutrient uptake among different fertilizer placement methods. Based on the higher liverwort fresh biomass obtained from incorporation treatment at higher gemmae densities, it seemed to be competitive and utilize significant amount of nutrients in presence of dracaena. In another situation, when there was no competition from ornamental plant (ornamental plant absent), liverwort fresh biomass was recorded higher than the above situation, as expected. It was higher in the surface fertilizer application method (top-dressing), followed by incorporation – which were the application methods providing maximum availability of nutrients for liverwort growth. Berchielli-Robertson et al. (1990) reported that competition from weeds growing in containers significantly reduces crop growth. Out of different fertilizer placements, dibbling and top-dressing resulted in the highest quality plants as compared to incorporation. Stewart et al. (2018) also mentioned that plant response to fertilizer placement methods is species-specific, thereby underlining the need of conducting these studies for various ornamental plants for developing specific recommendations.

Overall, the results indicate that sub-dressing at 7.6 cm and dibbling at 7.6 cm are effective fertilizer placements for controlling liverwort growth in ornamental container production considering both the quality of ornamentals and their competitiveness with liverwort. These treatments are also promising for improving the overall growth of the begonia and dracaena considered in this study. Future studies need to focus on any possible toxic effects of dibbling, as placing a significant amount of fertilizer right below the roots may have a detrimental effect on the ornamental plants' root zone.

Tables

Table 3.1: Liverwort coverage (%) on top of the container surface from 2 to 12 WAT as affected by different fertilizer placement methods (Incorporation, top dress, dibble at 3 different depths, and sub-dress at 3 different depths).

Liverwort coverage (%) on top of container surface bi-weekly						
Fertilizer placement method	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Incorporation	11 a*	24 a	66 a	89 a	97 a	99 a
Top dress	5 bc	13 bc	29 b	48 bc	62 b	65 b
Dibble 2.5 cm	7 b	13 bc	25 bc	51 bc	65 a	70 b
Dibble 5.1 cm	3 cd	8 cd	15 cd	26 de	39 cd	44 cd
Dibble 7.6 cm	2 d	6 d	11 d	22 e	31 d	34 de
Sub-dress 2.5 cm	7 b	15 b	31 b	60 b	83 a	91 a
Sub-dress 5.1 cm	3 cd	10 cd	21 bcd	40 cd	56 bc	62 bc
Sub-dress 7.6 cm	2 d	5 d	10 d	21 e	31 d	39 de
Control	2 d	6 d	11 d	18 e	23 d	22 e
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*Percentages followed by the same letter are not significantly different within a column at p=0.05.

Table 3.2: Number of gemmae cups, male structures, female structures, and fresh mass of liverwort (grams) recorded at 12 WAT as affected by different fertilizer placement methods (Incorporation, top dress, dibble at 3 different depths and sub-dress at 3 different depths).

Fertilizer placement method	No. of gemmae cups	No. of male structures	No. of female structures	Fresh biomass (g)
Incorporation	284 a*	131 ab	1	167 a
Top dress	231 a	63 b	0	168 a
Dibble 2.54cm	124 bc	197 ab	1	107 ab
Dibble 5.08cm	151 ab	259 a	0	61 bcd
Dibble 7.62cm	90 bc	222 ab	1	33 d
Sub-dress 2.54cm	139 ab	170 ab	0	137 ab
Sub-dress 5.08cm	195 ab	224 ab	0	66 bc
Sub-dress 7.62cm	129 ab	228 a	0	38 cd
Control	55 c	124 ab	0	11 e
p-value	<.0001	0.0012	NS	<.0001

*Percentages followed by the same letter are not significantly different within a column at p=0.05.

Table 3.3: Growth index (% increase) and fresh mass (g) of begonia as influenced by interaction between fertilizer placement methods (Incorporation, top dress, dibble 3 inches and sub-dress 3 inches) and liverwort gemmae density (0, 3, and 9).

Fertilizer placement method	Growth index (% increase)				Fresh biomass (gm)			
	Gemmae density				Gemmae density			
	0	3	9	Significance	0	3	9	Significance
Incorporation	306 a*	61 Bb	472 Aa	0.0009	626 ab	125 Bb	983 Aa	0.0258
Top dress	203 ab	393 Aa	90 BCb	0.0124	471	969 A	560 AB	NS
Dibble 7.6 cm	287	310 AB	341 AB	NS	781	518 AB	765 AB	NS
Sub-dress 7.6 cm	269	169 AB	77 C	NS	411	496 AB	128 B	NS
Significance	NS	0.0085	0.0005		NS	NS	0.0221	

*Means followed by different capital letters in column for gemmae density for each fertilizer placement methods or small letters in the row which compare a specific fertilizer placement method across gemmae densities are significantly different according to Least significant difference (a = 0.05).

NS, S*: Non-significant and significant at a = 0.05, respectively.

Table 3.4: Growth index (%) and fresh mass (g) of dracaena as influenced by interaction between fertilizer placement methods (Incorporation, top dress, dibble 3 inches and sub-dress 3 inches) and liverwort gemmae density (0, 3, and 9).

Fertilizer placement method	Growth index (% increase)				Fresh biomass (gm)			
	Density				Density			
	0	3	9	Significance	0	3	9	Significance
Incorporation	49	69	62	NS	510 B*	530 AB	423	NS
Top dress	78	44	65	NS	425 Bab	748 Aa	328 b	0.0235
Dibble 7.6 cm	36	65	57	NS	113 B	265 B	339	NS
Sub-dress 7.6 cm	56	74	66	NS	962 Aa	397 ABb	681 ab	0.0035
Significance	NS	NS	NS		0.0001	0.0219	NS	

*Means followed by different capital letters in column for gemmae density for each fertilizer placement methods or small letters in the row which compare a specific fertilizer placement method across gemmae densities are significantly different according to Least significant difference (a = 0.05).

NS, S*: Non-significant and significant at a = 0.05, respectively.

Table 3.5: Analysis of variance of the effects of fertilizer placement method (F), gemmae density (D) and presence/absence of ornamental (O) on the fresh mass of liverwort (g) at 12 WAT, for the begonia and dracaena.

Liverwort fresh weight (g)					
Effect	Degrees of freedom (df)	begonia		dracaena	
		F value	p value	F value	p value
F	3	5.19	0.0036	44.59	<.0001
D	2	57.43	<.0001	121.30	<.0001
O	1	162.87	<.0001	276.21	<.0001
F × D	6	1.98	0.0873	24.67	<.0001
F × O	3	5.42	0.0028	40.13	<.0001
D × O	2	55.78	<.0001	91.63	<.0001
F × D × O	6	2.17	0.0631	23.55	<.0001

Table 3.6: Liverwort fresh mass (g) at 12 WAT as influenced by interaction between fertilizer placement methods (Incorporation, top dress, dibble 7.6 cm and sub-dress 7.6 cm) and presence/absence of the ornamental plant begonia.

Liverwort fresh mass (gm)			
Fertilizer placement method	begonia		Significance
	Present	Absent	
Incorporation	0.1 b*	14.4 Aa	<0.0001
Top dress	0 b	14.4 Aa	<0.0001
Dibble 7.6 cm	0 b	9.1 Ba	<0.0001
Sub-dress 7.6 cm	0.2 b	6.5 Ba	0.0007
Significance	NS	<0.0001	

*Means followed by different capital letters in column for ornamental plant presence or absence for each fertilizer placement methods or small letters in the row which compare a specific fertilizer placement method across presence or absence of plant are significantly different according to Least significant difference (a = 0.05).

NS, S*: Non-significant and significant at a = 0.05, respectively.

Table 3.7: Liverwort fresh mass (g) at 12 WAT as influenced by interaction between gemmae density (0, 3, 9) and presence/absence of the dicot ornamental plant begonia.

Liverwort fresh mass (g)			
Density	begonia		Significance
	Present	Absent	
0	1	0 C	NS
3	0.1 b*	10.8 Ba	<0.0001
9	0.2 b	22.5 Aa	<0.0001
Significance	NS	<0.0001	

*Means followed by different capital letters in column for ornamental plant presence or absence for each gemmae density or small letters in the row which compare a gemmae density across presence or absence of plant are significantly different according to Least significant difference (a = 0.05).

NS, S*: Non-significant and significant at a = 0.05, respectively.

Table 3.8: Liverwort fresh mass (g) at 12 WAT as influenced by interaction between fertilizer placement methods (Incorporation, top dress, dibble 7.6 cm and sub-dress 7.6 cm), liverwort gemmae density (0, 3 and 9) and presence/absence of the monocot ornamental plant dracaena.

Liverwort fresh mass (g)								
Fertilizer placement method	dracaena present				dracaena absent			
	Density			Significance	Density			Significance
	0	3	9		0	3	9	
Incorporation	0 c*	1.8 Ab	3.3 Aa	<0.0001	0 b	10.7 ABa	16.6 Ba	<0.0001
Top dress	0	0.8 AB	1.0 B	NS	0 c	13.5 Ab	40.9 Aa	<0.0001
Dibble 7.6 cm	0	0.6 B	0.4 B	NS	0	4.7 B	5.1 C	NS
Sub-dress 7.6 cm	0	0.2 B	0.2 B	NS	0 b	8.1 ABa	9.0 Ca	0.0017
Significance	NS	0.0091	<0.0001		NS	0.0098	<0.0001	

*Means followed by different capital letters in column for gemmae density for each fertilizer placement methods or small letters in the row which compare a specific fertilizer placement method across gemmae densities are significantly different according to Least significant difference (a = 0.05).

NS, S*: Non-significant and significant at a = 0.05, respectively.

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CHAPTER 4: ASSESSING THE ALLELOPATHIC PROPERTIES OF ORGANIC MULCHES ON LIVERWORT CONTROL IN CONTAINERIZED ORNAMENTAL PRODUCTION

Abstract

Liverwort (*Marchantia polymorpha*) is one of the major weed problems in ornamental crop production as it reduces the overall quality and aesthetic value of the crop. The major limitation of liverwort control is lack of organic and synthetic herbicides options labeled for use inside enclosed structures such as greenhouse and their potentiality to cause injury. The current research was undertaken to study the effectiveness of allelopathic properties of six different organic mulch materials including rice hull (RH), cocoa hull (CH), pine bark (PB), maple leaf (ML), shredded cypress (SC) and red hardwood (HW) for liverwort control. Mulch extracts were prepared by the modified EPA 1312 synthetic precipitation procedure and were used to impregnate agar media at an increasing dose at 1X (2 mL), 2X (4 mL), 3X (6 mL), and 4X (8 mL) rates. The liverwort gemmae were sterilized and 10 gemmae were transferred to the culture medium in each petri dish. These petri dishes were maintained inside a growth chamber at 20 °C and under a light intensity of 72 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for a 16-$h \cdot d^{-1}$. Data was recorded for number of gemmae germinating in each petri dish after 1 week and gemmae surviving at the end of the experiment (after 2 weeks). In a greenhouse, mulch extracts were applied to containers filled with standard substrate and amended with controlled release fertilizer for assessing the liverwort control. The RH, HW, CH, ML, SC, PB mulch extracts or no extract (control) were applied to each of the container uniformly at 1X (15 mL), 2X (30 mL), 3X (45 mL), or 4X (60 mL) rates. Vigorous liverwort plants were placed amidst the experimental pots to serve as a source of gemmae, simulating the natural conditions of a commercial greenhouse. The percentage of substrate surface covered by liverwort thalli was visually estimated bi-weekly for 10 weeks. Fresh biomass of the thalli and number of gemmae cups in each pot were also recorded at the end of the experiment. After 1 week in the growth chamber, ML followed by SC, PB and RH extracts showed maximum suppression of liverwort gemmae germination. At 2 weeks, ML applied at either of the rates provided complete inhibition of liverwort growth. In the greenhouse, all the mulch extracts were able to provide complete liverwort control for the first two weeks. All the mulches and rates of applications were significantly different from the control after 6, 8 and 10 weeks. PB and HW mulches showed excellent liverwort control and minimum fresh biomass of liverwort after 10 weeks as compared to other mulches. Hence, the allelopathic potential of the organic mulches can be a promising option for biopesticidal control of liverwort, and a component of integrated liverwort management.

Future work needs to focus on identifying the allelochemicals responsible for the biopesticidal activity in these organic mulches.

Keywords: Allelopathy, biopesticides, organic mulch, liverwort, greenhouse production

Introduction

Common liverwort (*Marchantia polymorpha*) is a nonvascular, spore-bearing bryophyte in the family Marchantiaceae (Durand, 1908); more closely related to 'lower' plants such as algae, mosses, and ferns than to higher group plants such as angiosperms and gymnosperms (Altland, 2003; Svenson et al., 1997). In the northeast and pacific northwest of the United States, it is considered one of the most problematic weed species in containerized greenhouse and nursery production. Liverwort propagates by both asexual (gametophytic) and sexual (sporophytic) means of reproduction. Sporophytic reproduction occurs at cooler temperatures (10 to 15 °C), due to the development of sexual structures - antheridia (male) and archegonia (female). The sperm cells from antheridia fertilize the egg cells of archegonia, resulting in the formation of diploid, sexual spores. Gametophytic reproduction occurs at warmer temperatures (18 to 22 °C) and results from the formation of gemma cups which contain numerous gemmae within them (Newby, 2006, O'Hanlon, 1926). Gemmae are small clumps of dispersable somatic cells that are able to regenerate liverwort thallus after they are scattered. Rain and overhead irrigation systems in nurseries and greenhouses can mediate the spread of the spores and gemmae by splashing water which spread the infestation in surrounding areas (Svenson et al., 1997). Liverwort thrives best in environments with high humidity and/or soil moisture, low ultraviolet radiation, and high fertility (Newby et al., 2006). In containerized production, liverwort competes with ornamental crops for water, nutrients and other resources intended for the crop, obstructs water and fertilizer movement into the root-zone and reduces market value and overall crop quality (Svenson et al., 1997). Therefore, controlling liverwort in containerized greenhouse and nursery production systems is important.

The major limitation for liverwort control in greenhouses is the limited number of organic or synthetic herbicide options labeled for use in enclosed structures. Additionally, few studies have quantified the efficacy of herbicides or biopesticides on liverwort control in nursery and greenhouse operations due to low finanical incentives for chemical companies. Furthermore, chemical herbicides are generally not labelled for use within enclosed greenhouses as they can volatilize and cause severe injuries to sensitive ornamentals. Therefore, allelochemicals or

biopesticides can be a promising option and an effective, sustainable, and environment-friendly substitute to chemical weed control.

Many plants produce allelochemicals, which are secondary metabolites such as terpenoids, hydroxylated aromatic compounds, and phenolics that are produced through various metabolic pathways that effect the growth of other plants, either intentionally or not. (Duryea et al., 1999; Singh et al., 2003, Farooq et al., 2020). These chemicals often act as germination or growth inhibitors for surrounding plants, providing a competitive advantage for the plant that produces the allelochemical (Farooq et al., 2020, Hadacek, 2002, Jabran 2017, Jabran et al., 2015). Various organic mulches have been identified that possess allelopathic properties such as pine bark, shredded wood chips, black walnut wood chips, red maple leaves and shredded cypress (Rathinasabapathi et al., 2005, Duryea et al., 1999, Henschke and Politycka, 2016, Stein, 1988). Santos et al., (2013) identified the presence of phenolic compounds - catechin, quinic acid, gallic acid, protocatechuic acid, and chlorogenic acid; that were responsible for allelopathic nature of eucalyptus hardwood. Pine bark mulch possess allelopathic properties due to the presence of monoterpenes, pinenes, camphene, and carene that can inhibit germination and growth of weed seeds (Harman-Ware et al., 2016). Therefore, allelochemicals have the potential to act as natural herbicides or biopesticides and can be used for weed management in ornamental crop production. Using these natural products instead of synthetic chemicals could be beneficial because of their ability to readily decompose and the lack of volitization or drift. In addition, many allelochemicals have novel modes of action, thus providing alternative sites of action for weed control. This can help in tackling herbicide resistance issues that are becoming ever more prevalent in weedy populations. (Duke et al., 1997, Dayan et al., 1999, Marble et al., 2015). Previous studies have focused on allelopathic effects of mulch extracts for controlling broadleaves weeds or grasses but not on lower plants such as liverwort. The objective of this research was to assess the allelopathic effects of different organic mulch materials on liverwort gemmae germination and its growth.

Materials and Methods

***Mulch extract preparation*:** During the summer and fall 2022, six different mulch materials including rice hull (RH), cocoa hull (CH), pine bark (PB), maple leaf (ML), shredded cypress (SC) and red hardwood (HW) were used to obtain mulch extracts. Mulch extracts were prepared by following the modified EPA 1312 synthetic precipitation procedure (www.epa.gov). Mulch materials were crushed and ground to $\leq$ 9.5 mm in size. An extraction fluid was prepared

by adding a 60/40 weight percent mixture of concentrated sulfuric and nitric acids to deionized ASTM type II water maintaining a pH of 4.2 ± 0.05 with a pH meter (Accumet® Portable Laboratory, Fisher Scientific, Waltham, MA). The extraction fluid was added to extraction bottles containing mulch materials in 20:1 (extraction fluid:mulch) ratio. The extraction bottle was then allowed to rest for 18 h, and thereafter the extract was filtered out through a qualitative filter paper. This liquid extract was collected separately for RH, HW, CH, ML, SC and PB mulch materials.

***Laboratory and growth chamber experiment*:** The mulch extracts obtained above were used to impregnate agar media each at increasing doses (1X, 2X, 3X, and 4X rates). The agar culture media was prepared by adding 23 g nutrient agar in 1L of room temperature distilled water and sterilized in autoclave at a steam pressure of 0.004 kilogram square meter (121 ^{o}C) for 20 minutes (Consolidated Sterilizer Systems autoclave, Boston, MA). As the media cooled down to ~50 ^{o}C, the mulch extracts of either RH, HW, CH, ML, SC and PB mulches were added to the solution at either 1X (2 mL mulch extract in 25 mL media), 2X (4 mL mulch extract in 25 mL media), 3X (6 mL mulch extract in 25 mL media), and 4X (8 mL mulch extract in 25 mL media) rates. According to methods outlined in Saha (2019), 2 mL of mulch extract was added to 25 mL agar media in 47 mm diameter petri dishes for studying allelopathic effects of organic mulches on broadleaf weed seed germination. Therefore, 2 to 8 mL of mulch extracts were applied to each plate. Approximately 25 mL of media containing the mulch extract was transferred to sterile petri plates under the laminar air flow (Nuaire laminar Flow Products, Plymouth, MN), to obtain mulch extract impregnated agar media. The liverwort gemmae were collected from stock plants maintained in a greenhouse. The gemmae were collected from the gemmae cups using forceps and transferred to a vial containing a few drops of distilled water. For sterilization of the gemmae, another vial was filled with 1:30 bleach (Clorox, Oakland, CA) water solution, with a small amount of detergent added in order to break the surface tension of the floating gemmae and wash them thoroughly. This solution was quickly added to the first vial, and the contents were poured back and forth a few times. It was allowed to stand nearly 5 minutes and then the bleach solution containing gemmae was poured into a funnel lined with filter paper. The gemmae collected in the filter paper were then rinsed with 100 mL of distilled water, to obtain sterile gemmae to be transferred to the culture medium (Miller, 1964). Ten gemmae were transferred to the culture medium in each petri dish containing mulch-extract impregnated agar media at an increasing dose. All petri dishes containing gemmae were maintained inside a 0.42 m^2 growth chamber (Percival

Scientific, Inc, Perry, IA);. Cool-white fluorescent lamps (Philips model F17T8/TL741-17 watt, Cambridge, MA) mounted 20 cm above each shelf provided an average Photosynthetic Photon Flux Density (PPFD) of 72 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the 16-h photoperiod for 3 weeks. The air temperature above the petri plates was 20 ℃ during the light/dark period. The petri plates were observed for liverwort germination and vegetative growth.

Data was recorded for number of gemmae germinating in each petri dish after 1 week and germination percentage was calculated using the following formula:

(Number of gemmae germinated/ total number of gemmae applied) ´100

After 2 weeks, the number of gemmae surviving were counted and survival percentage was calculated using the following formula:

(Number of gemmae surviving/ total number of gemmae applied) ´ 100

The experiment was conducted in 7 × 4 factorial treatment arrangement with seven treatments of mulch extracts including control, and 4 rates of application in a randomized complete block design on fours helves of a growth chamber. There were four petri plate replications per treatment. Combined data from run 1 and 2 were analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) to determine the effects of mulch extracts, and their rates of application; and the interactions of these variables on data collected for various experimental parameters. The replications were considered as random effects and mulch extracts, and their rates of application were considered fixed effects. When ANOVA results revealed significant effects, mean comparisons for fixed factors were performed using Tukey's honest significant differences (HSD) test. All the effects were considered significant at alpha=0.05, to separate out the means.

***Greenhouse experiment*:** The experiment was conducted in a double-sided polyethylene and polycarbonate greenhouse, in fall of 2022 at the Michigan State University Horticulture Teaching and Research Center. The air temperature ranged from 21℃ to 26.6 ℃, with an average temperature of 23.8 ℃. The greenhouse study allowed for all conditions such as temperature, irrigation, pest pressure, other weed competition to be controlled and only the treatments to be held accountable (Gallina et al., 2023). The mulch extracts obtained in the laboratory experiment were utilized in the container liverwort control study. Square plastic (767 mL) containers (East Jordan Plastics Inc., East Jordan, MI), 10.5 cm (width) × 11.4 cm (height), were filled with commercial soilless media containing 70 % peat moss, 21 % perlite, and 9 % vermiculite (Suremix, Michigan

Grower Products Inc., Galesburg, MI) and amended with 7-5-11 controlled release fertilizer (Osmocote 8 to 9 month, ICL Specialty Fertilizers, Dublin, OH) at the manufacturer's labeled medium rate of 7.1 g/L. Then either RH, HW, CH, ML, SC or PB mulch extracts were applied to uniformly over the top of substrate at 1X (15 mL), 2X (30 mL), 3X (45 mL) or 4X (60 mL) rates. These extracts were applied bi-weekly until the end of the experiment. To simulate a greenhouse environment, liverwort plants were placed between the pots to serve as a source of inoculation to spread the gemmae by overhead irrigation. Overhead irrigation of 1.2 cm was applied daily. A control set without any mulch extract application was included as well.

The percentage of substrate surface covered by liverwort thalli in each container were visually estimated after 2, 4, 6, 8 and 10 weeks based on a scale 0 to 100 % (where 0 % was no liverwort coverage and 100 % was liverwort coverage on the substrate surface). After 10 weeks, the number of gemmae cups and fresh biomass of the liverwort thalli were recorded. Liverwort thalli from each pot were separated from the substrate and placed into individual paper bags and weighed.

The experiment was conducted in 7 × 4 factorial treatment arrangement with seven treatments of mulch extracts including control, and 4 rates of application in a randomized complete block design. There were four single-container replications per treatment. All data were analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) to determine the effects of mulch extracts, and their rates of application; and the interactions of these variables on data collected for various experimental parameters. The replications were considered as random effects and mulch extracts, and their rates of application were considered fixed effects. When ANOVA results revealed significant effects, mean comparisons for fixed factors were performed using Tukey's honest significant differences (HSD) test. All the effects were considered significant at alpha=0.05, to separate out the means.

Results and Discussion

Effects of allelopathic properties of organic mulch extracts on liverwort gemmae germination and growth in agar-impregnated media

The main effects of mulch extracts ($P < 0.0001$) and rate of application ($P < 0.0001$) significantly impacted liverwort gemmae germination (Table 4.1 and 4.2). The ML extract reduced the germination of gemmae the most (25.6 %) when compared to other mulch extracts and the control (Table 4.1). Across all mulch extracts, higher rates of application were generally more

effective at reducing liverwort germination than lower rates. Liverwort germination was 46.9 %, 34.4 %, 34.4 %, and 30.2 % for the 1X, 2X, 3X, and 4X rates of application, respectively, compared to the control which had 80 % gemmae germination (Table 4.2). After 2 weeks, significant interactions were observed for the mulch extract and the rates of application at reducing the survival of liverwort gemmae (Table 4.3). All the mulch extracts applied from 1X to 4X rates were significantly influential in limiting liverwort survival below 50 %, in comparison to control that had 73.7 % survival. The ML extract at all rates, SC extract at the 2X rate and RW at the 4X rate provided 100 % suppression of liverwort growth. All other mulch extracts applications provided a marginal control ranging from 5 to 48 % (Table 4.3).

Due to the potential presence of various allelopathic compounds, the mulch extracts from maple leaves, shredded cypress, pine bark, red hardwood and rice hulls provided inhibition of liverwort gemmae germination and suppressed its growth over time. These compounds are known to be phytotoxic to weed seeds and have helped to achieve improved weed control efficacy in previous studies (Dordevic et al., 2022, Khamare et al., 2022, Farooq et al., 2020; Iqbal et al., 2020). Saha et al. (2018) hypothesized that β-pinene and camphene could be the potential allelochemicals present in pine bark mulch and responsible for inhibiting germination and growth of weed seeds. A research trial conducted by Rathinasabapathi et al., (2005) has shown that mulch eluates from shredded wood chips of red cedar (*Juniperus silicicola*), red maple (*Acer rubrum* L.), neem (*Azadirachta indica*), swamp chestnut oak (*Quercus michauxii*), and magnolia (*Magnolia grandiflora* L.) highly inhibited germination and growth of seeds in a lettuce bioassay. The inhibition of growth of hypcotyl and radicle were higher under the application of black walnut wood chips (*Juglans nigra* L.). Inhibitory allelochemicals were also found to be present in leaves of red maple (*Acer rubrum* L.) apart from their activity in the wood chips. Duryea et al., (1999) reported that the allelopathic effects of pine bark and cypress mulches were due to some hydroxylated aromatic compounds that inhibited lettuce seed germination in a standard germination test. In a study conducted by Henschke and Politycka (2016), the application of pine bark mulch released some phenolic compounds in the soil. This adversely affected the growth and flowering of ornamental grasses during first year of the study, but the effect diminished during the consecutive year. Another study by Stein (1988) concluded that mulch leachate from red maple leaves inhibited weed seed germination for pigweed (*Amaranthus retroflexus*) and morning glory (*Ipomoea purpurea*). Li et al., (2021) conducted the laboratory experiments to study the

allelopathic effects of Chinese mugwort (*Artemisia argyi*) on plant and weed seeds. They found that water soluble extracts containing the allelochemicals significantly reduced seed germination in incubator conditions. In addition, they were effective at reducing germination of weeds in chrysanthemum field with no adverse effect on the plant growth. Further RNA-Seq analysis indicated the suppression was due to multi-target and multi-path inhibition, in addition to the inhibition of chlorophyll synthesis, the key mechanism causing inhibition of weed seeds germination.

Effects of allelopathic properties of organic mulch extracts on growth of liverwort gemmae in containers

The interaction among the mulch extract types and rate of application were reported nonsignificant $P \geq 0.05$) for liverwort thallus coverage in containers from 4 to 10 weeks, fresh biomass of thallus, and number of gemmae cups; Therefore, results are presented based on the main effects (Table 4.4 and 4.5). All of the mulch extracts and rates tested provided 100 % liverwort control for the initial two weeks. After 6 weeks, there was 4.0 % liverwort coverage on containers receiving PB mulch extract, followed by containers receiving SC, HW, ML and RH mulch extracts, while the CS extract was the least effective (Table 4.4). After 8 weeks, CS and RH provided minimal control, while all other mulch extracts were effective at controlling liverwort growth and limiting the percent liverwort coverage over the top of substrate to 13 to 21 %. At the end of the experiment, HW and PB mulch extracts were the most effective, limiting the liverwort coverage to 26 to 31 %, followed by SC (41 %) and ML extracts (46 %) (Table 4.4).

The number of gemmae cups recorded was influenced by the type and application rate of mulch extracts. The containers that received PB (24.9 %) extract had the least number of gemmae cups, followed by the containers receiving HW (33.2 %) and SC (39.1 %) mulch extracts. The CS extract minimally effected the production of gemmae cups. Similarly, the fresh biomass of liverwort thallus was lowest (2.6 g) when containers were treated with PB extract providing the minimal fresh biomass (2.7 g), followed by HW (3.2 g) and SC (4.9 g). The fresh biomass of liverwort thallus in containers treated with CS mulch extracts was highest (9.3 g), compared to control (5.6 g) and all other mulches (Table 4.4).

Liverwort percent coverage was impacted by the various rates of mulch extract applications when compared with the control, but they were not different amongst themselves after

4, 6 and 8 weeks. All the rates of application had no effect on gemmae cup production and recorded fresh biomass of liverwort thallus at the end of the experiment (Table 4.5).

From our results, it is evident that the rates of mulch extract applications mostly showed a linear response for liverwort control. The higher rates of application proved to be more effective than the lower rates for most mulch extract materials, except rice hull, where a lower rate was more effective. This could be because the specific type of organic mulch extracts at higher application rates may have opposite or complimentary effects on seed growth or have no further effect on inhibition of seed germination. Another possible explanation could be that lower rates of application undertaken in the current study (1X, 2X or 3X) be the most effective rates of mulch extracts for causing the inhibition/suppression of liverwort. Therefore, future studies need to be done to validate the definite effects of rates of application of specific types of organic mulch extracts and identifying the underlying chemical compounds causing reduction of liverwort gemmae germination and growth.

The application of organic mulch derived allelochemical extract had a quite significant impact on controlling liverwort gemmae germination and growth, specifically early in the experiment, when all the mulch extracts were equally effective in their activity. Their activity declined over time, and liverwort gemmae were able to utilize available resources and establish themselves. However, different mulch extract treatments and rates of application performed markedly better in comparison to the control. Previous studies have shown that several phenolic acids and similar compounds having allelopathic properties are present in pine bark and needles and in soils from pine tree communities (Lee and Monsi, 1963; Kil and Yim 1983; Node et al., 2003). Allelopathic extracts of red pine needles (*Pinus densiflora*) have been shown to inhibit the root and shoot growth of various weeds such as cress (*Cardamine hirsuta*), ryegrass (*Lolium perenne*), large crabgrass (*Digitaria sanguinalis*), barnyard grass (*Echinichloa crus-galli*) and timothy-grass (*Phleum pratense*). Also, increasing the doses of these extracts led to increased inhibition of root and shoot growth of weeds (Kato-Noguchi et al., 2009). Nektarios et al., (2005) reported that phenolics present in pine needles and straw of Aleppo pine (*Pinus halepensis*) inhibited growth of bermudagrass and tall fescue. Our results align with these previous research results which show that pine tree products (bark, straw, needles etc.) contain allelochemicals that can control weeds. Allelopathic properties of rice have been reported to have an adverse impact on development and establishment of weeds, which could be used as a biochemical tool for

integrated weed management (Serra Serra et al., 2021, Rahaman et al., 2022). Nikolai et al., (1998) reported that the maple (*Acer spp*.) and oak (*Quercus spp*.) tree leaf litter could be mulched into the turfgrass without any deleterious effects on turfgrass quality and color. Further, Kowalewski et al., (2009) found that the application of leaf litter from silver maple (*Acer saccharinum*), red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), high sugar content-sugar maple, and red oak (*Quercus rubra*) as mulch was effective in reducing the incidence of common dandelion (Taraxacum officinale) populations in Kentucky Bluegrass (Poa pratensis) stand. The application of these mulches caused 80 % and 53 % reduction in common dandelion counts after one and two applications, respectively. Rathinasabapathi et al., (2005) also found an allelopathic potential of red cedar wood chips significantly suppressed Florida beggarweed (*Desmodium tortuosum*) in greenhouse, as compared to gravel-mulching and non-mulch control.

In the laboratory study, the ML and SC extracts were most impactful in reducing the germination and growth of liverwort gemmae in agar-impregnated with mulch extracts. However, in the greenhouse study, PB and HW, followed by ML and SC provided good results in comparison to other mulch extract types and the control. The slight reduction in activity of ML and SC mulch extracts in the greenhouse study could be due to various reasons. Some possible explanations could be that inside the greenhouse, the overhead irrigation system could be leaching down the allelochemicals, or the allelochemicals could be binding to the substrate, or they might be getting decomposed by microbial activity in the substrate.

In conclusion, the mulch extracts of PB, HW, ML, and SC possess allelopathic properties that can reduce or suppress liverwort growth and gemmae germination. These allelochemicals could be a promising option to be included in the integrated liverwort management in containerized greenhouse production. The current research could serve as a valuable source of information for future research projects in this area as there is lack of research and insufficient supporting data for liverwort control. Further research is also needed to study other potential organic mulches for their allelochemical properties, evaluating their phytotoxic effects on ornamental plants and to identify the specific chemicals present in these extracts which are responsible for the allelopathy as well as their commercialization as bioherbicides for liverwort control inside greenhouse conditions.

Tables

Table 4.1: Liverwort gemmae germination (%) in petri plates containing agar media impregnated with organic mulch extracts (Cocoa shell, maple leaf, pine bark, red hardwood, rice hull, and shredded cypress) after 1 week.

Mulch type	Gemmae % Germination after 1 week
Control	80.0 a*
Cocoa shell (CS)	60.0 b
Rice hull (RH)	52.0 b
Red hardwood (HW)	43.6 b
Pine bark (PB)	49.8 b
Shredded cypress (SC)	44.8 b
Maple leaf (ML)	25.6 c
p value	<0.0001

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

Table 4.2: Liverwort gemmae germination (%) in petri plates containing agar media impregnated with organic mulch extracts at four different rates (1X, 2X, 3X and 4X) after 1 week.

Rate of application	Gemmae % Germination after 1 week
Control	80.0 a*
1X	46.9 b
2X	34.4 bc
3X	34.4 bc
4X	30.2 c
p-value	<0.0001

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

Table 4.3: Liverwort gemmae survival (%) in petri plates containing agar media impregnated with organic mulch extracts at four different rates at 2 weeks.

Mulch types x Rate	Gemmae survival at 2 weeks (%)			
	1X	2X	3X	4X
Cocoa shell (CS)	30bc*	42.5b	43.8b	18.8
Rice hull (RH)	23.8bc	28.8bcd	46.3b	31.3
Red Hardwood (HW)	26.3bc	6.3cd	16.3bc	0
Pine bark (PB)	47.5b	33.8bc	18.8bc	20
Shredded cypress (SC)	30bc	0d	5d	6.3
Maple leaf (ML)	0c	0d	0d	0
p value	0.0041	0.0001	<.0001	0.3690
Note: Gemmae survival (%) in control was 73.7a				
* Percentages followed by the same letter are not significantly different within a column; p=0.05.				

Table 4.4: Liverwort thallus coverage (%) after 4, 6, 8, and 10 weeks, fresh weigh of thallus (grams) after 10 weeks and number of gemmae cups after 10 weeks in containers treated with organic mulch extracts (Cocoa shell, maple leaf, pine bark, red hardwood, rice hull and shredded cypress).

	Liverwort coverage (%)				Number of gemmae cups	Fresh biomass (g)
Mulch types	4 weeks	6 weeks	8 weeks	10 weeks		
Control	5.8	12.0 a*	37.5 a	58.8 ab	50.3 ab	5.6 bc
Cocoa shell (CS)	2.4	9.8 a	35.2 a	71.5 a	65.2 a	9.3 a
Rice hull (RH)	1.8	9.1 ab	24.4 ab	62.8 ab	58.1 ab	6.6 ab
Red Hardwood (HW)	1.7	5.6 ab	16.0 b	31.7 c	33.2 cd	3.2 bc
Pine bark (PB)	1.3	4.0 b	13.8 b	26.9 c	24.9 d	2.7 c
Shredded cypress (SC)	1.6	5.6 ab	18.1 b	41.4 bc	39.1 bcd	4.9 bc
Maple leaf (ML)	1.8	8.0 ab	21.3 ab	46.2 bc	48.3 abc	5.4 bc
p-value	0.8089	0.0157	0.0002	<0.0001	<0.0001	<0.0001

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

Table 4.5: Liverwort thallus coverage (%) at 4, 6, 8, and 10 weeks, fresh biomass of thallus (grams) at 10 weeks and number of gemmae cups at 10 weeks in containers treated with organic mulch extracts at four different rates (1X, 2X, 3X and 4X).

Rate	Liverwort coverage (%)				Fresh biomass (g)	Number of gemmae cups
	4 weeks	6 weeks	8 weeks	10 weeks		
Control	5.7a	12.0a	37.5a	58.7a	5.6	50.3
1X	0.6b*	5.9b	20.4b	45.5a	5.3	43.5
2X	1b	6.3b	20.4b	44.9a	5.2	47.4
3X	0.9b	6.2b	15.1b	43.8a	5.2	40.6
4X	0.5b	4.5b	13.8b	40.6a	5.4	42.0
p-value	<0.0001	0.0004	<0.0001	0.1506	0.9964	0.5096
* Percentages followed by the same letter are not significantly different within a column; p=0.05.						

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CHAPTER 5: SYNTHETIC HERBICIDES FOR LIVERWORT CONTROL IN CONTAINERIZED PRODUCTION AND ASSAY FOR ABSORPTION AND TRANSLOCATION OF ^{14}C HERBICIDES IN LIVERWORT

Abstract

Liverwort is a thalloid, branched, ribbon-like bryophyte that lacks distinct stems, leaves, and roots. It thrives well in conditions providing ample moisture and fertility in nursery and greenhouse conditions. Hand pulling of liverwort thallus and rhizoids can be expensive and time-consuming. Growers mostly rely on chemical weed control using various pre-emergent (PRE) and post-emergent (POST) herbicides supplemented with hand weeding for weed management in containerized production in outdoor nursery condition. In this experiment, to study the efficacy of POST herbicides, 2,4-D amine weed killer, glyphosate (Roundup Pro concentrate) and indaziflam were applied at the rates of 1.42 liter per acre, 0.77 liter per acre and 0.55 liter per acre for 1X rate. In addition, the 2X and 3X application rates were also applied. Indaziflam was also evaluated for its PRE activity on liverwort control. In a laboratory experiment, absorption and translocation studies of ^{14}C labeled radioactive 2,4-D and indaziflam in liverwort thallus were assessed by liquid scintillation spectrometry and phosphor imaging. Results indicated that POST application of glyphosate and 2,4-D at 1X provided an excellent control of liverwort as least amount of liverwort fresh biomass were recorded at the termination of the experiment. The POST application indaziflam was effective in controlling liverwort at 2X and 3X rates and recorded no liverwort coverage or fresh biomass for later part of the study. Indaziflam applied PRE at either of the rates was highly effective and provided complete inhibition of liverwort gemmae germination and establishment. In the ^{14}C absorption and translocation studies for radiolabeled 2,4-D and Indaziflam, less than 80% recovery of applied herbicide applied was observed. The total recovery of ^{14}C radiolabeled 2,4-D ranged from 63-80% for different times of sample collection while it ranged from 49-80% for ^{14}C radiolabeled Indaziflam. Phosphor imaging of the translocation samples of liverwort thallus displayed higher movement of 2,4-D as compared to Indaziflam in liverwort thallus.

Keywords: Liverwort, glyphosate, ^{14}C radiolabeled herbicide, 2,4-D, indaziflam, container production

Introduction

Weed control is an important management area in containerized production of nurseries and greenhouses. Unlike other production areas, ornamental crop production has zero-tolerance to weeds, because the market value of ornamentals is determined by their appearance and aesthetics. Occurrence of weeds can significantly reduce quality, growth, and marketability of ornamental

crops. Hand removal of weeds is an effective way of post-germination weed control, but the labor cost for that could be over $4000 per acre (Vial, 2017; Neal 2018; Marble 2021; Pickens et al 2021). Weed control is also time consuming as nearly 1/4th of nursery growers spend more than 100 hours per month for this task (Marble 2021). Therefore, growers rely on chemical weed control using various pre-emergent (PRE) and post-emergent (POST) herbicides. Chemical herbicides application is an economic and effective way for weed management as compared to hand weeding and various non-chemical methods of weed management (Saha 2019). Growers mostly use PRE herbicides because of limited number of POST herbicides labeled for greenhouse and nursery production and due to the tendency of POST applied herbicides to cause injury to sensitive ornamentals (Saha 2019). For an herbicide to have effective results, it is important to choose the appropriate herbicide for the target weed, apply herbicides at right time, and following label instructions for recommended application rate.

Commonly occurring weeds in nursery and greenhouse production include broadleaves (dicotyledons), grasses (Poaceae), and sedges (Cyperaceae). Apart from these, liverwort (*Marchantia polymorpha*) is a notable problematic weed that exists in nursery and greenhouse conditions (Altland et al., 2011). Liverwort belongs to a broad plant group 'Bryophyta', which the second most diverse group of plants after angiosperms (Graham and Gray, 2001; Goffinett et al., 2001; Shaw and Renzaglia, 2004). It thrives well in almost all the conditions required for ornamental crop production but does best in propagation and container production environments with low ultraviolet (UV) radiation, high humidity and/or soil moisture, and high fertility (Newby et al., 2006). They lack vascular connections and use diffusion pathway to obtain hydration and nutrition (Carriqui et al., 2019). Liverwort spreads rapidly in nurseries and greenhouses due to its ability to propagate both asexually by gemmae and sexually by spores (Ross and Puritch, 1981). The overhead irrigation system present in the nursery and greenhouse production systems boosts the spread of asexual gemmae of liverwort. Also, in the sexual reproduction phase of liverwort, sperm dispersal is mainly facilitated by irrigation water or rainwater (Budke et al., 2018). It forms a mat like structure on the top of container media and impedes the irrigation water and fertilization to reach to the root zone of the ornamentals. Based on authors' personal observations, liverwort gemmae are the main source of propagation during summer season. The sexual structures appear in cooler periods of the year and they co-exist with gemmae cups in those periods. An archegoniophore contains approximately seven million viable spores, which remain viable for up

to 1 year from its release (O'Hanlon 1926). A typical 1-gallon container may have 50-100 archegoniophores (personal observation).

The main limitation for liverwort control inside the greenhouse and containerized production systems is the lack of herbicide options since most of the herbicides are not labeled for use in greenhouse environment. Also, in container nursery production, herbicides applied at higher rates to control liverwort, could cause phytotoxicity to sensitive ornamental plants and could have residual effects on environment. Hand removal of common liverwort is very laborious, time-consuming, and costly task as the mat like structure formed on the top of container medium must be removed. And while removing the rhizoids, approximately an inch of the media needs to be removed from the container and the medium must be subsequently replaced. Currently, flumioxazin (WSSA group 14 – Protoporphyrinogen Oxidase or PPO inhibitor) is one of the synthetic herbicides that has been labeled for common liverwort control in greenhouses when there is no ornamental plant present inside the greenhouse and is popular among commercial growers (Saha et al., 2020; WSSA/HRAC, 2024). Glyphosate (Roundup) can also be used inside greenhouses when there are no plants present (Altland et al., 2003). Glyphosate is a Weed Science Society of America (WSSA) group 9 herbicide (WSSA 2014), that acts by inhibiting Enolpyruvyl Shikimate Phosphate Synthase (EPSPS). This leads to reduced levels of aromatic amino acids (phenylalanine, tryptophan, and tyrosine) that are needed for cell wall, protein and secondary metabolite synthesis. Inhibition of EPSPS causes shikimic acid pathway deregulation, that results in disruption of plant carbon metabolism (Velini et al., 2009). 2,4-D is a systemic broadleaf synthetic herbicide in WSSA group 4 that mimics natural auxin compounds at molecular level in plants. It causes abnormal and elongated growth, senescence, and death of plants (Song 2014; WSSA 2014). Indaziflam, a newer selective contact preemergence herbicide introduced by Bayer Crop Science, is a cellulose biosynthesis inhibitor (CBI) in WSSA group 29. It is used for preemergent control of grasses and broadleaf weeds (WSSA/HRAC, 2024; Brabham et al., 2014). Altland et al., (2003) have compared the efficacy of three different POST herbicides on liverwort control in presence or ornamentals in a retractable roof greenhouse, with roof open all the time. Flumioxazin, quinoclamine (an algaecide for algae and moss control in paddy fields in Japan); and Terracyte (granular sodium carbonate peroxyhydrate) that oxidizes cell membranes of organisms. It was found that quinoclamine provided excellent POST liverwort control (89-96%, with higher control at higher rates) and no effects seen on ornamentals tested. Newby et al., (2007) studied the

effect of various granular and liquid herbicides on PRE control of liverwort in container nursery system. They concluded that granular herbicides (flumioxazin, oxyfluorfen, oxadiazon + oryzalin) and sprayed quinoclamine provided effective control of liverwort. There are not many studies conducted on evaluating the efficacy of herbicides having different modes of actions. There are chances of weeds developing herbicide resistance by repeated application of same herbicide over several years. Therefore, it is integral to test the efficacy of other chemicals with different modes of action to avoid herbicide resistance. This experiment was conducted to evaluate the efficacy of POST glyphosate, indaziflam and 2,4-Dicholorphenoxyacetic acid (2,4-D) amine and PRE Indaziflam application to control common liverwort in containerized production systems.

There have also been very limited studies on absorption and translocation of herbicides within the liverwort tissues. The ^{14}C-sucrose acropetal translocation demonstrated by Rota and Maravola (1975) indicates the presence of metabolism transport mechanism in liverwort thalli, despite the lack of any vascular connection. Diffusion is the primary mechanism in liverwort for facilitating cell-to-cell water and nutrient movement (Carriqui et al., 2019). We hypothesized that this would be a mechanism for translocation (if any) of herbicides in liverwort. Altland et al., (2011) reported that POST quinoclamine controlled liverwort but had differential response when applied to thalli, male receptacles, and female receptacles. The absorption of ^{14}C after application of radiolabeled herbicide was lower on archegonial receptacle than antheridial receptacle or thalli. This was further explained by scanning electron microscopy of the structures, showing that female receptacles had smaller stomatal pores than other structures studies. In another study, Altland et al., (2007) reported that after ^{14}C-quinoclamine application, 70% of total amount applied was recovered after 9 hours of application. Despite lack of vascular tissue, ^{14}C was readily translocated across the tissues and tended to accumulate near margins. There is no information available on behavior of 2,4-D (a systemic herbicide) and indaziflam (a contact herbicide) in the liverwort thallus. Hence, this study also focuses on assessing absorption and translocation of ^{14}C labeled radioactive 2,4-D and indaziflam in liverwort.

Materials and Methods

1. Greenhouse experiment

The experiments were conducted in a greenhouse, whose walls were made of double-sided polyethylene and the roof made of polycarbonate, at Michigan State University (MSU) Horticulture Teaching and Research Center located at 3291 College Rd, Holt, MI, 48842 in 2023.

The temperature inside the greenhouse was controlled, with a minimum temperature of 21 °C, maximum temperature of 26.6 °C and average temperature of 23.8 °C. The temperature inside the greenhouse was maintained by air circulating fans in summer. The weather at the time of herbicide application was clear and sunny. The average air temperature was 16 °C, air humidity 70%, wind speed 1mph and precipitation 0 inch. Greenhouse studies were used because it allowed for all conditions such as temperature, irrigation, pest pressure, other weed competition to be controlled and only the treatments to be held accountable (Gallina et al, 2023). All the herbicide applications were done outside the greenhouse, at MSU Horticulture Teaching and Research Center. A carbon-dioxide (CO_2) backpack sprayer (custom built by Bellspray R&D sprayer Inc., Opelousas, LA) calibrated to deliver 252.55 liters/hectare using an 8004 flat-fan nozzle (TeeJet Technologies, Wheaton, IL) at a pressure of 206.843 kilopascals was used for spraying. The pots were relocated into the greenhouse after the restricted entry interval (REI) for each herbicide was met.

a. Postemergence application of glyphosate, 2,4-D and indaziflam.

Square plastic (767 mL) containers (East Jordan Plastics Inc., East Jordan, MI), 10.5 cm (width) × 11.4 cm (height), were filled with commercial soilless media containing 70% peat moss, 21% perlite, and 9% vermiculite (Suremix, Michigan Grower Products Inc., Galesburg, MI) and amended with 7-5-11 controlled release fertilizer (Osmocote 8 to 9 month, ICL Specialty Fertilizers, Dublin, OH) at the manufacturer's labeled medium rate of 7.1 g/L. Containers were irrigated approximately 0.4 inches of irrigation via overhead sprinkles inside the greenhouse daily. After 1 or 2 days, gemmae of common liverwort (*Marchantia polymorpha*) were applied. Gemmae were collected by first scaping gemmae cups of vigorous common liverwort stock plants and releasing the gemmae into a 250 ml bowl of tap water were separated out from their clumps (Altland and Krause, 2014). A plastic spoon was used to apply approximately 5 ml (1 tsp) water from the bowl, which contained approximately 20-25 gemmae, across the surface of each container (Altland and Krause, 2014). The liverwort was allowed to establish and grow for two weeks and develop ~15% liverwort coverage on top of the substrate. A post-emergence (POST) application of synthetic herbicides was done after two weeks from potting. The synthetic herbicides 2,4-D amine weed killer, glyphosate (Roundup Pro concentrate) and indaziflam were applied POST at the rates of 1.42 liter per acre, 0.77 liter per acre and 0.55 liter per acre, as per the manufacturers recommendation for 1X rate (Table 5.1). In addition, the 2X and 3X application rates were also applied. A control set that received no herbicide application was also included. The bi-weekly

application of gemmae was continued until the end of the experiment. All pots were taken out from the greenhouse for the post-emergence herbicide spray applications at the rates given above. The pots were moved back to the greenhouse after the restricted entry interval (REI) for the herbicides was met.

For data collection, visual estimation of percent control of liverwort was done starting the next day after the treatment and continued at 2, 4, 6 and 8 weeks after treatment (WAT). The fresh weight of liverwort was determined for each pot at the end of the experiment. Liverwort thalli from each pot were separated from the substrate and placed into individual paper bags and weighed.

The experiment was conducted in a completely randomized design as a 4 x 3 factorial treatment arrangement with four herbicide treatments (including untreated control) and three rates of application. There were six single-pot replications per treatment. The data was analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) to determine the effects of herbicide treatments, and their rates of application; and the interactions of these variables on data collected for various experimental parameters. The replications were considered as random effects and herbicide treatments, and their rates of application were considered fixed effects. When ANOVA results revealed significant effects, mean comparisons for fixed factors were performed sing Tukey's Honest Significant Difference (HSD). All the effects were considered significant at alpha=0.05, to separate out the means.

b. Preemergence application of indaziflam.

For pre-emergence (PRE) herbicide application, the pots were prepared in a similar manner as explained above. For indaziflam application, all pots were moved outside the greenhouse where indaziflam was sprayed over the top of container substrate at the rate of 0.55 liter per acre as per the manufacturers recommendation (1X). In addition, the 2X and 3X application rates were also applied. The pots were moved back to greenhouse after completion of REI. A control set that received no herbicide application was also included. The next day, gemmae of common liverwort (*Marchantia polymorpha*) were applied following the same procedure as in experiment mentioned above. The bi-weekly application of gemmae was continued until the end of the experiment. For data collection, visual estimation of percent control of liverwort was done bi-weekly for 8 weeks. The fresh weight of liverwort was determined for each pot at the end of the experiment. Liverwort thalli from each pot were separated from the substrate and placed into individual paper bags and weighed.

The experiment was conducted in a completely randomized design as a 4 x 3 factorial treatment arrangement with four herbicide treatments (including untreated control) and three rates of application. There were six single-pot replications per treatment. The data was analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) to determine the effects the rates of application of Indaziflam. When ANOVA results revealed significant effects, mean comparisons for rates of herbicide were performed sing Tukey's Honest Significant Difference (HSD). All the effects were considered significant at alpha=0.05, to separate out the means.

2. Assay for absorption and translocation of ^{14}C radiolabeled 2,4-D and indaziflam within liverwort.

To initiate this protocol, liverwort was first grown within a greenhouse setting. All specimens were then relocated to a growth chamber approximately 3 days prior to herbicidal application for acclimatization. The liverwort thalli were treated with a mix of cold (commercial herbicide at manufacturer's recommended rate) and hot ^{14}C labeled herbicide. For ^{14}C radiolabeled 2,4-D and Indaziflam application, the liverwort thallus was administered with a desired radioactivity concentration in small droplets via pipetting to avoid runoff from the leaf's surface. Previous protocols by Figueiredo et al., (2018) and Shyam et al., (2022) described a total of 200,000 dpm plant^{-1} ^{14}C radiolabeled 2,4-D applied in 10 x 1 µL of 20,000 dpm solution. In the current experiment, 100,000 dpm plant^{-1} ^{14}C radiolabeled 2,4-D or Indaziflam was applied in 10 x 1 µL of 10,000 dpm solution, on account of limited herbicide availability. Thus, 10 droplets of 1 µL each, for a total radioactivity applied to a small part of thallus per plant of 100,000 dpm or 1.7 kBq administered to the thallus. Dosage of herbicide for rest of the thallus was complemented to the field recommended dosage (1.42 liter per acre for 2,4D and 0.55 liter per acre for Indaziflam; in carrier volume of 102.2 liters per acre) with an application in a bench track sprayer (DeVries Manufacturing Inc., Hollandale, MN) prior to radiolabeled herbicide application. In this instance, target thallus area to be treated with radiolabeled herbicide were covered in aluminum foil prior to application within the bench track sprayer.

Based on the herbicide labels recommendations, this experiment also utilized adjuvant crop oil concentrate at a rate of 0.1%. After these steps, treated plants were returned to the growth chamber. Plants were harvested at the desired time points: 6, 24, and 72 hours after treatment (HAT). Each biological replicate was separated into a tissue paper to contain absorbed and translocated

herbicide separately. The samples were wrapped in a single layer of Kimwipe (Kimtech Science ™ Kimwipes ® by Kimberly-Clark, Irving, TX). For collecting absorption sample, the treated thallus was severed with a pair of scissors. It was washed twice with 5 mL of wash solution (10% methanol and 0.05% Tween 20) for 60 seconds in a 20 mL scintillation vial, to remove unabsorbed herbicide. The wash solution containing radiolabeled herbicide was collected twice separately in two vials. Radioactivity within these thallus rinsates was measured using liquid scintillation spectrometry by adding 15 mL high flash-point liquid scintillation cocktail Ultima Gold™ (Revvity, Waltham, MA). The dissected and washed thallus were followed by individually wrapping in Kimwipes, packed into brown paper envelopes, and dried in an oven at 45°C for a period of 72 hours. The remaining part of thallus from each biological replicate, which contained any translocated herbicide was also washed in a similar manner and individually wrapped in Kimwipes in a way that thallus stayed flat for scanning and imaging the translocation of radiolabeled herbicide. These were then packed into brown paper envelopes and dried in an oven at 45°C for a period of 72 hours.

After adequate drying, the translocation samples were placed in Phosphor screen films by Amersham Typhoon (General Electric company, Boston, MA) for 3-4 days. These screens were then scanned in Amersham Typhoon Biomolecular Imager (serial number: 67210108), using Amersham Typhoon Scanner Control software 1.1.07, with a 25 μm pixel size. The images obtained were processed and quantified on ImageQuant™ TL Toolbox v8.1 (GE Healthcare Life Sciences).

Total radioactivity absorbed and translocated in the liverwort thallus samples was quantified by combusting samples using a biological oxidizer (Perkin Elmer Sample Oxidizer Model 307, Shelton, CT). The samples were combusted at O_2 pressure of 50 psig and N_2 pressure of 60 psig. The liverwort tissues wrapped in Kimwipes/ paper was placed in COMBUSTO-CONES (Revvity, Waltham, MA) and combusted for 45 seconds. Permafluor E+ and Carbo-sorb E were used as solvents for collecting ^{14}C and metering pumps of these solvents were set to dispense 10 ml solvent each from their respective reservoirs.

The radiolabeled herbicide obtained from each sample was collected in 20 mL scintillation vials containing Permafluor E+ and the Carbo-sorb E. These vials were subjected to Liquid scintillation counting in Liquid scintillation spectrometer [Perkin Elmer (now Revvity) Liquid Scintillantion Analyzer Tri Carb® 4910 TR, Waltham, MA). The scintillation counts were

processed by QuantaSmart™ software designed by Perkin Elmer for Tri Carb® Liquid Scintillation Analyzer. The radioactivity present in each vial was displayed in disintegrations per minute (dpm).

Total radiolabeled herbicide recovery was determined as:

$$\%\ Recovery\ = R_W \times \frac{100}{R}$$

$R\ =$ Total radioactivity applied (dpm)

$R_W\ =$ Radioactivity (dpm) recovered in (wash solution + absorption + translocation)

This laboratory experiment was conducted in a completely randomized design with time as a fixed factor for both ^{14}C radiolabeled 2,4-D and Indaziflam. Each time point had three single-plant replications. The experiment was repeated twice, and the data was pooled for analysis. The data was analyzed by PROC MIXED in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) using the general linear model. The Tukey's Honest Significance Difference (HSD) at alpha=0.05, was used to separate out the means, with all the effects considered significant at alpha=0.05.

Results and Discussion

Effects of postemergence herbicide applications: The interaction among the herbicide treatments and their different rates of application had a significant effect ($p<0.05$) on liverwort thallus coverage (%) occurring on the top of container surface for the entire duration of the experiment (Table 5.2). The liverwort coverage at the time of the POST applications was ~15% in all the experimental pots. Most notable impact was visible from the next day of application of herbicides where, 2,4-D treatment completely terminated liverwort at 1X rate, and 2X and 3X rate treated pots had 5-10% liverwort coverage; glyphosate eliminated liverwort at 1X and 3X rates but had 10% liverwort coverage in 2X treated pots; pots treated with 3X rate of indaziflam had only 5% coverage while those of 1X and 2X had 11.0% and 14.0% coverage, respectively. The liverwort coverage in all the treated pots was lower than untreated pots which showed 17.5% liverwort coverage. At 2 WAT, 2,4-D treated pots provided excellent control at all the different rates of application and glyphosate treated pots continued to provide complete suppression of liverwort growth. However, the difference in various rates of application of 2,4-D and glyphosate were reported non-significant ($p>0.05$). The 2X and 3X rates of indaziflam performed better, resulting in complete inhibition of liverwort, but 1X had lower efficacy in comparison and showed

25% liverwort coverage. All the rates and treatments had a significantly different effect than control, which recorded 59.5% liverwort coverage at 2 WAT. Similarly at 4 WAT, 2,4-D and glyphosate treated pots had less than 10% liverwort coverage. Also, indaziflam at 2X and 3X rates completely inhibited liverwort growth in comparison to control which had 83.5 % liverwort coverage by that time point. At 6 WAT, 1X rate of 2,4-D provided best control of liverwort (13.5%) coverage, all rates of glyphosate provided significantly better control (17-27% coverage), and 2X and 3X rates of indaziflam provided 100% control, as compared to untreated pots, which had profuse liverwort coverage (92.8%). Similarly, at the end of the experiment (8 WAT), 1X rate of 2,4-D had only 8% liverwort coverage, all rates of glyphosate had less than 40% coverage, and indaziflam at 2X and 3X rates had no liverwort coverage, as compared to control which showed nearly full coverage (96.6%). The fresh biomass (grams) recorded at the end of the experiment was also reported significantly different ($p<0.05$) for interaction of different herbicide treatments and their rates of application (Table 5.2). Out of the pots treated with 2,4-D, the 1X rate recorded lowest liverwort fresh biomass (20.1 gm). Also, glyphosate treatment at 1X resulted in lowest liverwort biomass (17.23 g). indaziflam at 2X and 3X rate had no living liverwort tissue recorded at the end of the experiment. In comparison to all the treated pots, the untreated pots showed extensive liverwort growth and recorded 60.81 grams of liverwort fresh biomass. Newby et al., (2006) studied the effects of different rates between 1.8 and 7.6 kg ai/ha of quinoclamine, in two different spray volumes (1019 or 2037 liters/ ha) on liverwort control in nursery containers. It was found that the POST control was >90% when liverwort infestation was light (≤25% over the substrate) and higher rates or herbicide in combination with surfactant were effective when the infestation was ≥60%. At highest labeled rate (7.6 kg ai/ ha), POST control of liverwort ranged from 96-100% after 14 days of treatment. Egorov et al., (2021) tested various PRE and POST herbicides: Goal 24% EC (oxyfluorfen), Stomp 33% EC (pendimethalin), Velpar 90% (hexazinone), Pledge 25% Wettable powder (flumioxazin), Mogeton 25% wettable powder (Quinoclamine), Anchor-85% (sulfometuron methyl) and Granstar 75% (tribenuron-methyl) for moss and liverwort control in containerized production of pine and spruce seedlings. The results showed Mogeton provided excellent PRE control and Velpar, Mogeton, Granstar, and Anchor-85 provided long-term effective liverwort control with no phytotoxicity to the seedlings, while Goal, Stomp, Pledge and their mixtures were phytotoxic to the seedlings.

Effects of preemergence indaziflam application: The PRE application of indaziflam at all the different rates of application was significantly effective in controlling liverwort through the duration of the experiment ($p<0.05$) (Table 5.3). The liverwort coverage recorded in different treatments of indaziflam were significantly different from the control but no amongst themselves at all bi-weekly time points. For different rates of application, the liverwort coverage ranged from 0-2.7% at 2 WAT, 0-16.7% at 4 WAT, 0-29% at 6 WAT and 0-31.2% at 8 WAT in comparison to untreated pots which recorded 10.5, 51.7, 78.5 and 91.2 per cent liverwort coverage eon the top of the container surface at corresponding points of time. The 2X and 3X rates were highly effective as they showed a complete inhibition of liverwort throughout the period of study (Table 5.3). Similarly, the fresh biomass of liverwort thallus recorded at the end of the experiment was 12.03 g for 1X and no living tissue detected for 2X and 3X rates of application of indaziflam, which were significantly lower than that recorded for control (45.3 g). In a previous study like this, Svenson et al., (1997) reported that PRE applied oxyfluorfen provided fair, oryzalin and isoxaben provided fair to good, and oxadiazon provided fair to very good control of liverwort. Wilson and Hughes (1985) also found that PRE applied oryzalin provided 99% and oxadiazon provided 76% control of liverwort, when applied at 9-week intervals. Fausey (2003) reported 100% and 74% pre-emergence liverwort control with flumioxazin sprayed as 50% water dispersible granules, after 35 and 60 days of treatment, respectively. In another study, Altland et al., (2008) reported that PRE applied quinoclamine at minimal rates of 4-6 mg L^{-1} caused phytotoxicity to both liverwort thallus and gemmae in a hydroponic growing system.

Absorption and Translocation: The amount of radioactivity (dpm) recovered in the liverwort samples treated with ^{14}C radiolabeled 2,4-D collected at 6, 24 and 72 hours after treatment were not significantly different from each other ($p>0.05$) (Table 5.4). The total recovery of ^{14}C ranged from 63-80% for different times of sample collection. Out of the total amount of recovered ^{14}C, 9.3% was absorbed at 6 HAT, 9.5% at 24 HAT, and 15.2% at 72 hours of treatment. The per cent of herbicide translocated was 5.1%, 4.7% and 2.7% at 6, 24 and 72 HAT, respectively. Most of the radiolabeled herbicide was recovered in wash solution – 85.4%, 85.7% and 90.7% for the three consecutive times of sample collection, respectively.

Conversely, the total radioactivity (dpm) recovered in the liverwort samples, the radioactivity (dpm) recovered in wash solution and absorption of ^{14}C at 6, 24 and 72 HAT were significantly different from each other ($p>0.05$) (Table 5.5). The per cent ^{14}C recovered from the

radiolabeled Indaziflam in liverwort thallus was maximum at 6 HAT (79.23%), while it remained 49% at both 24 and 72 HAT. The percentage of radioactivity absorbed out of total radioactivity recovered was: 24.9% at 6 HAT, 55.7% at 12 HAT, and 69.4% at 72 HAT; indicating that the absorption increased with increasing time. This is also evident from the amount of radioactivity recovered in the wash solution, maximum recovered at 6 HAT (57248 dpm) and significantly lower amounts recovered at later time intervals (19483 and 14387 dpm, at 24 and 72 HAT, respectively). The translocation of ^{14}C in the liverwort thallus ranged from 0.5-5% out of the total recovered radioactivity, at different time intervals, which were not significantly different from each other.

All the radiolabeled herbicide treatments recorded less than 80% recovery in the current experiment. Further research needs to be conducted for identifying possible reasons for incomplete recovery of ^{14}C, such as volatility of herbicides; unique anatomy of liverwort as it contains pores analogous to stomata in plants and lacks guard cells that facilitate pore closure (Doyle 1970); and testing different rates as well as spray volumes and pressures of herbicide application. Altland et al., (2007) studied quinoclamine applied at three spray volumes and three rates of application, for POST control of liverwort in nursery production system. They reported that high spray volume of >935 L ha^{-1} was effective for liverwort control. In addition, they studied absorption and translocation of ^{14}C-quinoclamine application in liverwort tissues. They observed that 67% of total amount applied was recovered after 9 hours of application. Also, ^{14}C was readily translocated across the tissues despite lack of any vascular connections in liverwort thallus and the radiolabeled herbicide tended to accumulate near margins. In a later study by Altland et al., (2014), it was found that application of 1.60 and 1.27 kg. ha^{-1} resulted in 50% population being controlled (I_{50}) for antheridial receptacles and juvenile thalli, respectively. The amount of herbicide required for archegonial receptacles was significantly higher (exceeded 10.45 kg ha^{-1}). The absorption of ^{14}C after the application of radiolabeled quinoclamine was also higher in antheridia and thallus as compared to archegonial receptacles. They recovered 93 to 100% of radiolabeled herbicide applied to different types of liverwort tissues. The scanning electron microscopy of these structures revealed that antheridia and thallus had larger stomatal pores and higher overall pore area than archegonia which could be a possible reason for lower absorption in archegonia.

Phosphor Imaging: The phosphor imaging by Amersham Typhoon Biomolecular Imager for translocation of ^{14}C radiolabeled 2,4-D in liverwort displays visible movement of herbicide in

liverwort thallus (Figure 5.1, 5.2 and 5.3). The final volume of the quantity of material in the image and maximum pixel intensity in the images were recorded non-significant for 6 and 24 HAT. However, the percentage of total volume of respective samples on the phosphor imaging screens, containing liverwort samples treated with ^{14}C radiolabeled 2,4-D was higher at 6 HAT and 24 HAT (12.67% and 12.3%), indicating more translocation at these time points, as compared to 72 hours, which recorded only 8.37 % of total volume of translocated herbicide (Table 5.6). This ImageQuant™ also agrees with the data obtained in Table 5.4 where the amount of radioactivity (dpm) recovered for translocation was more in 6 HAT (4100 dpm), followed by 24 HAT (3494) and 72 HAT (1764).

On the other hand, for Indaziflam, which is relatively less mobile than 2,4-D, the phosphor imaging shows no or very little translocation of ^{14}C radiolabeled Indaziflam in liverwort thallus (Figure 5.4, 5.5 and 5.6). Little movement is observed at 74 HAT (Figure 5.6), which is supported by data from ImageQuant™ showing higher percentage of total volume of ^{14}C on the phosphor imaging screens as compared to the samples from 6 and 24 HAT (Table 5.7). These observations, are however, inconsistent with the amount of radioactivity (dpm) recovered in translocation samples for liverwort thallus in Table 5.5, at different time intervals.

Conclusion

In conclusion, POST application of glyphosate and 2,4-D at 1X provided an excellent control of liverwort. Notably, the POST application of the otherwise PRE herbicide Indaziflam, was effective in controlling liverwort at 2X and 3X rates. Indaziflam applied PRE at either of the rates was highly effective in controlling liverwort. POST herbicide applications may be useful to control liverwort when done before the appearance of reproductive structures (vegetative or sexual) and PRE application can be a valuable preventive tool for liverwort in nursery and greenhouse container production. However, these herbicide applications cannot be done inside closed structures like greenhouses, glasshouses, or covered hoop houses. From absorption and translocation studies, less than 80% recovery of herbicide applied has been observed. It is speculated that liverwort may not be equally efficient in retaining herbicide as in higher plants. Future work needs to focus on liverwort biology, behavior of chemical herbicide in the liverwort tissue, and possible occurrence of metabolism of herbicides in liverwort tissues, in order to explain partial recovery of herbicides and to ensure adequate performance of herbicides.

Tables and figures

Table 5.1: Synthetic herbicide common names, signal word, REI (Restricted Entry Interval), and application rates.

Product trade name	Common name	Signal word	REI (hours)	Rate used in trial
2,4-D amine weed killer	2,4-D	Danger	48	1.42 liters per acre (1X)
Round Up Concentrate	glyphosate	Caution	4	0.77 liters per acre (1X)
Marengo	indaziflam	Caution	12	0.55 liter per acre (1X)

Table 5.2: Liverwort thallus coverage (%) at next day after the spray and after 2, 4, 6, and 8 WAT; and fresh biomass of thallus (grams) at the end of the experiment in containers applied post-emergence with synthetic herbicides (2,4-D, glyphosate and indaziflam) at 1X, 2X and 3X rates.

Herbicide	Rate	Liverwort coverage (%)					Fresh biomass (g)
		Next day after Spray	2 WAT	4 WAT	6WAT	8WAT	
2,4-D	1X	0a*	0	5.0	13.5c	8c	20.1
	2X	5.8a	2.0	8.0	28bc	23bc	21.9
	3X	9.5a	1.0	10.0	33b	36b	34.8
	p value	0.001	NS	NS	0.0356	0.0253	NS
Glyphosate	1X	0b	0	4.0	17.0	17.0	17.23c
	2X	10.0a	0	9.0	26.0	27.0	34.67b
	3X	0b	0	10.0	27.0	40.0	36.37b
	p value	<0.0001	NS	NS	NS	NS	0.0121
Indaziflam	1X	11.0a	25.0b	45.0b	58.0b	51.0b	49.51ab
	2X	14.0a	0c	0c	0c	0c	0c
	3X	5.0b	1.0c	0c	0c	0c	0c
	p value	0.0018	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Control		17.5a	59.5a	83.5a	92.8a	96.6a	60.81a
Herbicide × Rate		0.0012	0.0003	<0.0001	<0.0001	<0.0001	<0.0001

* Percentages followed by same letter are not significantly different within a column; p=0.05.

Table 5.3: Liverwort thallus coverage (%) at 2, 4, 6, 8, and 10 WAT; and fresh biomass of thallus (grams) at the end of the experiment in containers applied pre-emergence with indaziflam at 1X, 2X and 3X rates.

Rate	Liverwort coverage (%)				Fresh biomass (g)
	2 WAT	4 WAT	6WAT	8WAT	
1X	2.7b*	16.7b	29b	31.2b	12.03b
2X	0b	0b	0b	0b	0b
3X	0b	0b	0b	0b	0b
Control	10.5a	51.7a	78.5a	91.2a	45.3a
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

Table 5.4: Amount of radioactivity (disintegrations per minute) recovered in wash solution, absorption and translocation samples; and total ^{14}C recovery (%) from liverwort thallus treated with radiolabeled 2,4-D, at 6, 24 and 72 Hours after treatment (HAT).

	Amount of radioactivity recovered				
Time (HAT)	Recovered in wash solution (dpm)	Absorbed (dpm)	Translocated (dpm)	Total recovered (dpm)	Recovery (%)
6	68127	7486	4100	79723	79.72
24	63156	6999	3494	73649	73.65
72	57707	9696	1764	63590	63.59
p value	NS	NS	NS	NS	NS

Table 5.5: Amount of radioactivity (disintegrations per minute) recovered in wash solution, absorption, and translocation samples; and total ^{14}C recovery (%) from liverwort thallus treated with radiolabeled Indaziflam, at 6, 24 and 72 Hours after treatment (HAT).

	Amount of radioactivity recovered				
Time (HAT)	Recovered in wash solution (dpm)	Absorbed (dpm)	Translocated (dpm)	Total recovered (dpm)	Recovery (%)
6	57248a*	19224c	455	76927a	79.23a
24	19483b	27554b	2417	49454b	49.45b
72	14387b	34253a	718	49358b	49.36b
p value	<0.0001	<0.0001	NS	<0.0001	<0.0001

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

Table 5.6: The normalized volume (raw volume minus background volume) of the quantity of material in the image, Maximum pixel intensity in the images, the percentage of total volume of respective samples on the phosphor imaging screens, containing liverwort samples treated with ^{14}C radiolabeled 2,4-D.

	ImageQuant™ output for samples treated with ^{14}C radiolabeled 2,4-D		
Time (hours)	Final volume	Pixel Intensity	Per cent
6	1575	0.08	12.67a*
24	1473	0.12	12.3a
72	1089	0.05	8.37b
p value	NS	NS	0.0462

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

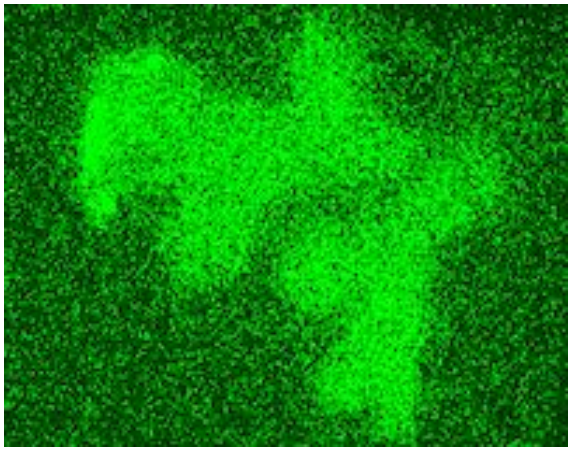
a)  b) 

Figure 5.1: Images for translocation of ^{14}C radiolabeled 2,4-D in liverwort harvested 6 HAT. (a) Original liverwort sample collected after 6 hours; (b) Corresponding phosphor image of the liverwort sample obtained from phosphor imaging by Amersham Typhoon Biomolecular Imager.

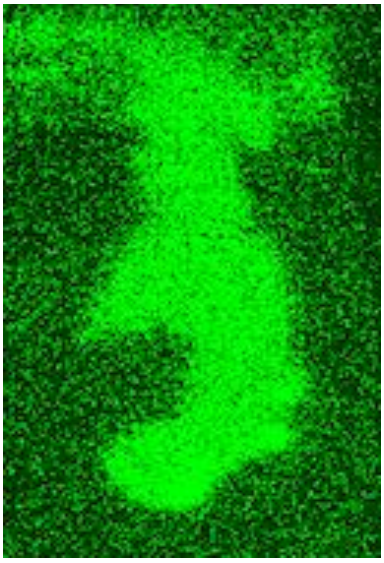
a)  b) 

Figure 5.2: Images for translocation of ^{14}C radiolabeled 2,4-D in liverwort harvested 24 HAT. (a) Original liverwort sample collected after 24 hours; (b) Corresponding image of the liverwort sample obtained from phosphor imaging by Amersham Typhoon Biomolecular Imager.

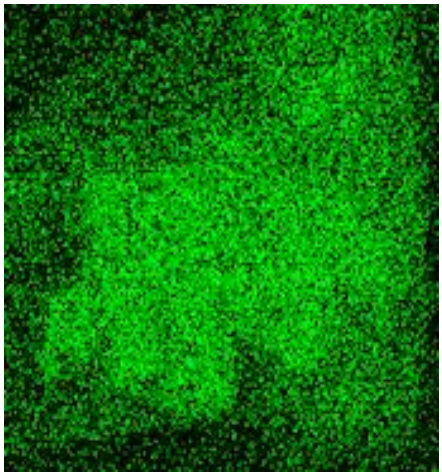
a)  b) 

Figure 5.3: Images for translocation of ^{14}C radiolabeled 2,4-D in liverwort harvested 72 HAT. (a) Original liverwort sample collected after 72 hours; (b) Corresponding image of the liverwort sample obtained from phosphor imaging by Amersham Typhoon Biomolecular Imager.

Table 5.7: The normalized volume (raw volume minus background volume) of the quantity of material in the image, Maximum pixel intensity in the images, the percentage of total volume of respective samples on the phosphor imaging screens, containing liverwort samples treated with ^{14}C radiolabeled Indaziflam.

	ImageQuant™ output for samples treated with ^{14}C radiolabeled Indaziflam		
Time (HAT)	Final volume	Pixel Intensity	Per cent
6	459b*	0.01	10.37b*
24	674b	0.01	10.96b
72	1042a	0.03	12.03a
p value	NS	NS	0.0064

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

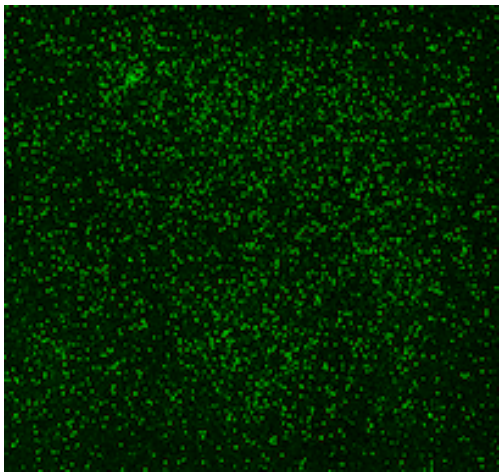
a)  b) 

Figure 5.4: Images for translocation of ^{14}C radiolabeled Indaziflam in liverwort harvested 6 HAT. (a) Original liverwort sample collected after 6 hours; (b) Corresponding image of the liverwort sample obtained from phosphor imaging by Amersham Typhoon Biomolecular Imager.

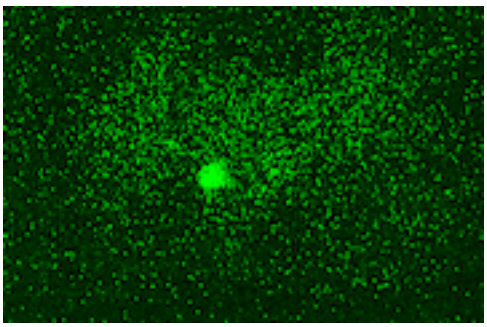
a)  b) 

Figure 5.5: Images for translocation of ^{14}C radiolabeled Indaziflam in liverwort harvested 24 HAT. (a) Original liverwort sample collected after 24 hours; (b) Corresponding image of the liverwort sample obtained from phosphor imaging by Amersham Typhoon Biomolecular Imager.

[Note: It is suspected that the little bright green hotspot visible in (b) is the point where herbicide was applied directly, or the herbicide travelled a short distance by mini runoff. This area was probably missed while dissecting the thallus while obtaining sample for absorption study for the herbicide applied.]

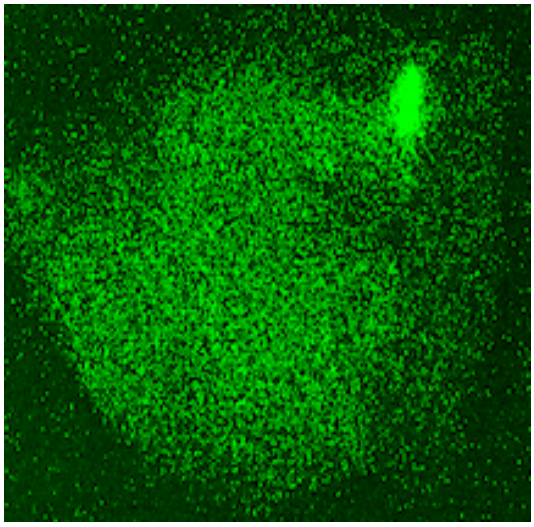
a)  b) 

Figure 5.6: Images for translocation of ^{14}C radiolabeled Indaziflam in liverwort harvested 72 HAT. (a) Original liverwort sample collected after 72 hours; (b) Corresponding image of the liverwort sample obtained from phosphor imaging by Amersham Typhoon Biomolecular Imager.

[Note: It is suspected that the little bright green hotspot visible in (b) is the point where herbicide was applied directly, or the herbicide travelled a short distance by mini runoff. This area was probably missed while dissecting the thallus while obtaining sample for absorption study for the herbicide applied.]

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CHAPTER 6: EVALUATING THE EFFECTIVENESS OF ORGANIC HERBICIDES ON LIVERWORT CONTROL

Abstract

There is growing interest among growers, general public, and even researchers regarding organic or natural-based herbicides for weed control. Liverwort (*Marchantia polymorpha*) is a commonly occurring weed problem in container nurseries and greenhouse operations and it competes with the ornamental plant for resources within the container. There are limited number of chemical herbicides labeled for liverwort control in greenhouse container production. Moreover, the potential effects of synthetic herbicides on non-target species, public health and environment, highlight the need of testing prospective alternative organic products for liverwort control. The aim of this study was to evaluate the efficacy of three different organic herbicides – Avenger AG (70% d-limonene), Scythe (57% Pelargonic acid, 3% fatty acids) and Weed Pharm (20% acetic acid), for liverwort control in containerized production systems. The herbicides were applied at 1X and 2X rates to nursery containers filled with standard substrate and amended with controlled release fertilizer for assessing the post-emergent liverwort control. Percent of substrate surface covered by liverwort thalli was visually estimated bi-weekly until 10 weeks. Fresh biomass of the thalli in each pot was also recorded at the end of the experiment. The organic herbicide treatments were able to limit liverwort coverage under 30% as compared to control (98%). WeedPharm and Scythe application at 2X and Avenger application at 1X rates recorded minimum liverwort fresh biomass. These organic chemical products can be a promising component for an integrated liverwort control program in containerized ornamental production. Further research is needed to evaluate more organic based substances and their mixtures; as well as to optimize their rates, timings and frequency of application in weed management programs under different crop production environments.

Keywords: Organic herbicide, d-limonene, Pelargonic acid, Acetic acid, Liverwort, Weed control

Introduction

Weed control continues to be one of the biggest challenges in greenhouse and nursery containerized ornamental crop production. Weeds are a significant problem in the specialty production systems because they compete for resources within the containers and thereby, reduce the quality of ornamental plants. Apart from employing various non-chemical methods such as tilling, hoeing, hand weeding, mulching and solarization, growers may choose to apply herbicides as a part of an integrated/ sustainable weed management program. Many of these non-chemical methods (such as tilling, solarization, and hoeing) are applicable to the field production system

only and do not fit with the container production. There are a few chemical herbicides labeled for use in nurseries and greenhouses. However, there are concerns related to chemical herbicides such as their potential effects on non-target species, water quality and public health. Also, these herbicides may be injurious to sensitive ornamental plants. Therefore, organic herbicides could be a potential and appealing alternative to synthetic herbicides in nursery and greenhouse container production systems. Organic herbicides are the ones that are produced from chemicals that occur naturally and are not synthetically manufactured in a laboratory (Kate 2020). These herbicides are less toxic, non-residual, easily decompose, and have lesser side effects on environment, plants, and soil. There are several organic herbicides available in market such as WeedZap (45% clove oil and 45% cinnamon oil), Weed Pharm (20% acetic acid), C-cide (5% citric acid), Scythe (57% Pelargonic acid), Avenger (70% d-limonene), GrrenMatch (55% d-limonene or citrus oil), GreenMatch EX (50% lemongrass oil) and Matratec (50% clove oil) (Kate 2020; Shaffer 2022). Using these herbicides is a choice of gardeners and home-owners because of potential health effects of synthetic herbicides (Shaffer 2022).

Organic herbicides are mostly contact herbicides and must be applied over the whole plant in high volumes for effective weed control (Gale Perez 2023; VanTine et al., 2003). Applying them in addition with an organically accepted adjuvant such as Nu Flim 17, Nu Film P, Natural Wet and Silwet ECO spreader could increase the effectiveness of these herbicides. They are non-selective and must be targeted towards the weed (Kate 2020; Wilen 2018; Rossman 2012). Symptoms appear rapidly on contacted plants within an hour on a warm day (Neal and Senesac, 2018). They are post-emergent herbicides and kill the plants that are already present and have no residual activity on weeds emerging later. That necessitates re-application of organic herbicides for continuous weed control as the weeds emerge later. Also, they are able to kill small (2-4 inch) annual broadleaf weeds, but for perennial weeds, they kill the tops of plants, and the plants may recover quickly from their undamaged roots, after the effect of applied herbicides has diminished. In addition, these are more effective at controlling smaller weeds (at their cotyledonary or first true leaf stages) in comparison to well-established and grown-up weeds. So, the effectiveness of these herbicides varies with weed size, weed type and presence of optimal environmental conditions such as temperature (above 75 °F) and sunlight (Kate 2020; Wilen 2018; Shaffer 2022).

Liverwort (*Marchantia polymorpha*) is an important weed of containerized nursery and greenhouse production systems, generally occurring in cooler regions of the northeast and Pacific

northwest regions of the U.S. (Newby, 2006). It is a spore-bearing bryophyte in family Marchantiaceae (Durand, 1908). It propagates both sexually (by antheridiophores and archegoniophores); and asexually by gemmae which quickly disperse around with irrigation or rainwater (Budke et al., 2018). In container plant production, liverwort infestations occur as a mat-like structure formed by its thalloid body over the top of the container. It uses water and nutrients intended for the crop, obstruct movement of water and fertilizer into the root-zone and reduce market value of crop and overall quality of the ornamental plants (Svenson et al., 1997).

Khadduri (2011) reported the effect of essential oils or distilled plant extracts for liverwort control in container nursery production during three seasons. Sporatec™; Brandt Consolidated, Springfield, IL (formerly sold as Sporan™; EcoSMART Technologies Inc., Franklin, TN), a product that consists of rosemary, clove, and thyme oil, was tested on liverwort and moss-infested crop of western redcedar (*Thuja plicata*) seedlings. There was 91% control of liverwort 9 days after treatment. However, there was significant damage to redcedar plants, and the liverwort re-established within 14 d of knockdown. Loddo et al (2023) evaluated the efficacy of pelargonic acid on several weed and found variable response among monocot and dicot weeds. The grass weeds were less sensitive to pelargonic acid while the dicots had variable sensitivity, ranging from lower sensitivity in ladysthumb (*Persicaria maculosa*) and velvetleaf (*Abutilon theophrasti*) to higher sensitivity in tall fleabean (*Conyza sumatrensis*) and black nightshade (*Solanum nigrum*). Travlos et al (2020) compared the efficacy of different pelargonic acid products and essential oil products for control of streile oat (*Avena sterilis*), rigid ryegrass (*Lolium rigidum*) and cleaver (*Galium aparine*). They found that a mixture of lemongrass oil and pelargonic acid resulted in 77% reduced dry weight of rigid ryegrass; the mixture of manuka oil and pelargonic acid provided 96% reduction in dry weight of sterile oat; and pelargonic acid products also caused 97% reduction in dry weight of cleaver plants, while a mixture of manuka oil and pelargonic acid completely eliminating cleaver.

Other organic products such as pelargonic acid, acetic acid products, d-limonene, and ammonium nonanoate have shown some suppression of liverwort but these compounds often require repeated applications and can cause severe damage to ornamentals crops. There has been limited research carried out on effectiveness of organic herbicides on liverwort control in containerized greenhouse production. In a project focused on developing an integrated liverwort management program at Michigan State University, we considered evaluating the effectiveness of

organic herbicides on liverwort control that could be a potential alternative to synthetic herbicides or an added component in the weed control program. We evaluated various non-chemical methods such as organic mulching, strategic fertilizer placement and allelopathy; as well as synthetic herbicides including glyphosate, 2,4-D and indaziflam for liverwort control. Organic herbicides could serve as a sole choice of weed management for home gardeners or additional tool for integrated weed management programs. With an intention to provide a resource on possible organic herbicide options for liverwort control this experiment was undertaken to evaluate the efficacy of various organic herbicides at different rates for postemergence liverwort control in containerized production systems.

Materials and Methods:

***Greenhouse* experiment:** The experiment was conducted in a double-sided polyethylene and polycarbonate greenhouse, in summer 2023 and repeated in fall 2023 at the Michigan State University Horticulture Teaching and Research Center. The greenhouse study allowed for all conditions such as temperature, irrigation, pest pressure, other weed competition to be controlled and only the treatments to be held accountable (Gallina et al., 2023). Square plastic (767 mL) containers (East Jordan Plastics Inc., East Jordan, MI), 10.5 cm (width) × 11.4 cm (height), were filled with commercial soilless media containing 70% peat moss, 21% perlite, and 9% vermiculite (Suremix, Michigan Grower Products Inc., Galesburg, MI) and amended with 7-5-11 controlled release fertilizer (Osmocote 8 to 9 month, ICL Specialty Fertilizers, Dublin, OH) at the manufacturer's labeled medium rate of 7.1 g/L. The containers were irrigated approximately 0.4 inches of irrigation via overhead sprinkles inside the greenhouse daily. The gemmae of common liverwort (*Marchantia polymorpha*) were applied on the top of container surface 2 weeks before the first organic herbicide treatment. Gemmae were collected by scaping gemmae cups of vigorous common liverwort stock plants and releasing the gemmae into a 250 ml bowl of tap water where they separated out from their clumps. A plastic spoon was used to apply approximately 5 ml (1 tsp) water from the bowl, which contained approximately 20-25 gemmae, spread uniformly across the surface of each container (Altland and Krause, 2014). After two weeks of applications of gemmae, all containers were brought outside the greenhouse for the herbicide applications. The weather at the time of herbicide application was clear and sunny. The average air temperature during spraying was 16 °C. The air humidity noted on the day was 70%, wind speed 1mph and precipitation 0 inch. Organic herbicides that were applied to liverwort containing pots included,

WeedPharm (20% acetic acid), Avenger AG (70% d-limonene), and Scythe (57% Pelargonic acid, 3% fatty acids). WeedPharm (1:2), Avenger (1:7) and Scythe (5% solution) were applied at the rate at the rate of 200 gallons/acre as per the manufacturers recommendation (1X) (Table 6.1) with a carbon dioxide (CO_2) backpack sprayer (custom built model by Bellspray R&D sprayer Inc., Opelousas, LA) calibrated to deliver 252.55 liters/hectare using an 8004 flat-fan nozzle (TeeJet Technologies, Wheaton, IL) at a pressure of 206.843 kilopascals. In addition, the 2X application rates were also applied (400 gallons/acre). All herbicides were non-selective and contact type herbicides. The pots were moved to greenhouse after the restricted entry interval (REI) for the herbicides was met. The REI for Avenger AG was 4 hours, Scythe was 12 hours and Weed Pharm was 48 hours (Table 6.1). In the first run of the experiment (summer 2023), the post-emergent application of herbicides was done after 14 days of beginning of the experiment (potting and gemmae application); and in the second run (fall 2023) they were applied twice – after 14 and 28 days of beginning of the experiment. Application of gemmae was also continued bi-weekly to each of the containers. For data collection, visual estimation of percent control of liverwort was done bi-weekly for 8 weeks. The visual estimation was based on a scale ranging from 0% to 100% (0% no liverwort coverage and 100% complete coverage). At the end of the experiment (8 weeks), liverwort thalli from each container were scrapped off with a pair of forceps from the surface of the substrate and placed into individual paper bags and weighed for recording the fresh weights.

The experiment was conducted in a completely randomized design as a 4 x 2 factorial treatment arrangement with four treatments and two rates of application. There were six single-pot replications per treatment. The data will be analyzed by PROC GLIMMIX in SAS (Ver. 9.4, SAS Institute, Cary, NC) to conduct the Analysis of Variance (ANOVA) and Tukey's HSD at alpha=0.05, to separate out the means. All the effects were considered significant at alpha=0.05, to separate out the means.

Results and Discussion

In the summer 2023 trial, there was no significant difference among the organic herbicide treatments and their rates of application ($p>0.05$) (data not shown). The single application of organic herbicides was not effective for suppressing liverwort growth or providing a long-lasting control. However, in the fall 2023 trail, when the organic herbicide was applied twice, the effects were recorded significant on reducing liverwort coverage and fresh biomass of liverwort in this experiment ($p<0.05$) (Table 6.2). There was no liverwort establishment and growth in the pots

treated with Scythe and Weed Pharm (both 1X and 2X) until 6 weeks after the first application and 4 weeks after the second treatment. There was 17.5% liverwort coverage in pots that received Avenger AG at 6 weeks after the first herbicide application. At 8 weeks, Avenger AG at 1X, Scythe at 1X and 2X, and Weed Pharm at 1X and 2X rates provided excellent control of liverwort as compared to control. Similarly at 10 weeks, for 1X application rates of all the organic herbicides and 2X rates of Scythe and Weed Pharm provided best results as compared to control. When applied at 2X rate, Avenger AG treated pots had notable amount of liverwort coverage (28.3%) as compared to Scythe (2.8%) and Weed Pharm (3.7%) but was significantly lower than untreated pots which had 98.8% liverwort coverage. The fresh biomass of liverwort was also significantly influenced by the application of organic herbicides as compared to untreated pots ($p=0.05$) (Table 6.2). In 1X rate of application, all the treatments recorded lower fresh biomass; 15.2 g for Avenger AG, 22.6 g for Scythe and 24 g for Weed Pharm, as compared to control which recorded 40.1 g. At 2X rate, Weed Pharm treatment provided best results in reducing liverwort fresh biomass. Weed Pharm treated pots recorded 11 g of liverwort fresh biomass in comparison to Scythe (13 g) and Avenger AG (28.7 g).

The results of the study indicate that liverwort appears to be susceptible to all the products applied, which is clear from extensive control achieved by application of these herbicides. The results of these study agree with various previous findings on the effects of organic herbicides for weed control. Fausey (2003) studied the efficacy of pelargonic acid and various synthetic herbicides for liverwort control in containerized ornamentals and reported that post-emergent pelargonic acid application provided 95-100% control of liverwort. However, it had no activity when applied before the establishment of liverwort. Ogbangwor and Sochting (2022) tested the effect of pelargonic acid product (Finalsan) on 24 different weed species (including both dicots and monocots) and found that there was a significant foliar damage and reduction in fresh biomass in all the weeds, however, for achieving a stronger weed control in grass weeds, it must be applied to younger weeds or be applied at higher application rates. Fogliatto et al (2018) conducted greenhouse trials to study weed control efficacy (for *Viola tricolor*, *Digitaria sanguinalis* and *Cyperus esculentus*) and crop (corn and rice) selectivity for two organic products (pelargonic acid and vinegar). It was found that the crop biomass was not affected by these applications but weed biomass and density showed more than 90% reduction in both the crops. Another study by Kanatas et al (2022) reported that pelargonic acid had a knock-down effect against johnson grass (*Sorghum*

halepense) and barnyard grass (*Echinochloa crus-galli*) and resulted in 89% and 45% reduction of biomass for johnsongrass and barnyard grass, respectively, as compared to untreated plants. Sani (2022) applied eight different acetic acid concentrations to straggler daisy and Bermuda grass and found that 1-2% acetic acid provided more than 90% weed control. Abouziena et al (2017) compared efficacy of various organic products (acetic acid, citric acid, clove oil) in several weed species, and found that citric acid (5%) + garlic (2%), and acetic acid (30%) provided more than 95% control of younger broadleaf weeds at 2-4 leaf stage and only acetic acid (30%) was effective on narrowleaf weeds at 2-4 leaf stage when applied early post-emergence. Later application at 4-6 leaf stages significantly reduced the efficacy of these herbicides. Kang (2011) studied the herbicidal effect of naturally- developed d-limonene on velevetleaf (*Abutilon theophrasti*), Indian jointvetch (Aeschynomene indica) and barnyard grass (*Echinochloa crus-galli*) in greenhouse conditions and star cucumber weed (*Sciyos angulatus*) in field conditions. The foliar application of 100 and 200 kg a.i. per hectare of the herbicide eliminated the weed causing wilting and burndown of leaves and stems, in greenhouse conditions 3 days after treatment but had no effect as pre-emergence treatment. In field, 70-140 a.i. per hectare of the product effectively controlled 5-20 leaved star cucumber weed. Fagodia et al (2017) compared the effectiveness of *Citrus aurantifolia* essential oil and its primary component limonene for their weed control capabilities. It was found that the *Citrus aurantifolia* essential oil application significantly inhibited germination of barnyard grass (*Echiochloa crus-galli*), wild oats (*Avena fatua*) and canary grass (*Phalaris minor*) at rates of ≥1.5 mg/mL, ≥1.0 mg/mL and ≥0.75 mg/mL, respectively, while limonene had lesser effect. The limonene application however reduced percent germination and other parameters studied such as dry weight, seedling growth and total chlorophyll content. Shaffer (2022) suggested application of organic herbicides as 'lower concentrations at higher spray volumes (e.g. 10% herbicide concentration in 70 gallons $acre^{-1}$) versus 'higher concentrations at lower spray volumes (e.g. 20% herbicide concentration in 35 gallons $acre^{-1}$), for effective results.

Overall, the results of this study lead to a conclusion that the utilization of organic herbicides can be a valuable addition to integrated / multi-tactic weed management programs for sustainable weed management. Weed Pharm (20% acetic acid) and Scythe (57% Pelargonic acid, 3% fatty acids) at 1X and 2X rates, and Avenger AG (70% d-limonene) at 1X provided best control of liverwort in the present study. However, these products should be less relied upon as a stand-

alone tactic as the weed control by these herbicides is not residual and systemic. Their application at early stages of weed growth is recommended for achieving satisfactory weed control.

Tables

Table 6.1: Organic herbicide active ingredients, signal word, REI, application rates and price per liter.

Product name	Active ingredient	Signal word	REI	Price	Rate used in trial
Avenger AG	70% d-limonene	Caution	4 hours	$78 per 3.72 L	1:7 (Avenger:Water) @ 200 gallon/acre
Scythe	57% Pelargonic acid, 3% fatty acids	Warning	12 hours	$82 per 3.72 L	5% solution @ 200 gallon/acre
Weed Pharm	20% acetic acid	Caution	48 hours	$45 per liter	1:2 (Weed Pharm:Water) @ 200 gallon/acre

Table 6.2: Liverwort thallus coverage (%) at 2, 4, 6, 8, and 10 weeks, and fresh biomass of thallus (grams) at 10 weeks in containers treated with organic herbicides (Avenger AG, Scythe, and Weed Pharm) at 1X and 2X rates.

	Liverwort coverage (%)										Fresh biomass (grams)	
WAT	2W		4W		6W		8W		10W			
Treatment/Rate	1X	2X	1X	2X	1X	2X	1X	2X	1X	2X	1X	2X
Avenger AG	0b*	20a	0b	21.7b	0b	17.5b	1.7b	36.7b	2.5b	28.3b	15.2b	28.7ab
Scythe	0b	0b	0b	0c	0b	0c	3.7b	1.7c	6.5b	2.8c	22.6b	13bc
Weed Pharm	0b	0b	0b	0c	0b	0c	6.8b	2.2c	8b	3.7c	24ab	11c
Control	23.3a	23.3a	63.5a	63.5a	78.8a	78.8a	89.2a	89.2a	98.8a	98.8a	40.1a	40.1a
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0041	0.0001

* Percentages followed by the same letter are not significantly different within a column; p=0.05.

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