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Weed Control Efficacy of Bio-Based Sprayable Mulch Films in Specialty Crop Systems

by

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A sprayable bio-based mulch film (biofilm) could present a more sustainable weed management tool for specialty crop producers than conventional plastic mulch films while also offering flexibility in application patterns and timing. From 2017 to 2019, six greenhouse trials and four field trials were conducted at the University of Nebraska-Lincoln to study the effects of biofilm application on weed suppression and crop yields. Multiple application rates (0.81 L/m^2 - 9.78 L/m^2) and application times (prior to weed emergence and post weed emergence) were tested. Corn starch, glycerol, keratin hydrolysate, corn gluten meal, corn zein, and isolated soy protein were evaluated as potential ingredients due to their ability to form biodegradable films or suppress weed growth as reported in other research. The efficacy of weed control displayed by biofilm solutions ranged from a promotion of weed biomass to 100% reductions relative to a non-treated, weedy control. The wide range of results was most likely attributable to solution viscosity: a greater efficacy of weed control was displayed when the viscosity was increased as this allowed a more cohesive layer to form on the soil surface. The most promising mulch film was displayed in the final field trial, to which biomass was reduced by greater than 97% when applied prior to weed emergence and by greater than 94% when applied post weed emergence. However, despite these findings, crop yields were not improved relative to a non-treated, weedy control in any of the field trials. Variables such as solution salinity and C:N ratio could play a role and need to be evaluated in future research trials.

Keywords: Sprayable mulch, bio-based, biodegradable, weed suppression, *Abutilon theophrasti*, specialty crop systems, crop yields, corn starch, keratin hydrolysate, corn gluten meal, corn zein, isolated soy protein

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1. INTRODUCTION

1.1. Weed Management in Specialty Crop Production Systems

Weeds hinder the cultivation of desired plants by competing for a finite supply of available resources, such as light, space, nutrients, and moisture. The inverse relationship between weed density and crop yield means that leaving weeds unmanaged in crop production systems can significantly limit yields (Swinton et. al 1994). Potential economic losses from poor crop yields are a major reason growers must employ effective strategies to prevent weed growth. This is particularly important in specialty crop production systems because they include crops grown for direct consumption, medicine, or aesthetics such as fruits, vegetables, ornamentals, trees, and shrubs. Two common weed management strategies in specialty crop production systems include herbicides and the use of plastic mulch films.

1.1.1. Herbicides

Herbicides are commonly used among crop producers to manage weeds because they are easy to apply and effective if used properly. They can be sprayed onto foliage or applied to soils. Chemicals in herbicides act by targeting plant specific biochemical pathways to inhibit cell division, photosynthesis, or amino acid production, or to mimic natural plant growth hormones thereby causing deformities (Ross and Childs 1995). However, negative consequences of herbicide application have been observed and include the development of resistant weed populations (Heap 2013), the accumulation of runoff in freshwater systems at concentrations exceeding safety levels (Goolsby et al. 1991), adverse effects on insect development (Dewey 1986), and even harmful effects on animals, including humans (Sterling and Arundel 1986; Rohr and Palmer 2009), though more extensive research is needed. Ultimately, non-chemical strategies could offer a safer and more sustainable approach to weed management in crop production systems.

1.1.2. Plastic Mulch Films

The use of mulch in crop production systems has a long history. Materials that have been used include paper, compost, straw, woodchips, and plastic mulch films. Plastic mulch films are one of the most commonly used items among specialty crop producers for weed management within crop rows because they are effective and inexpensive (Waggoner et al. 1960). Although, because plastic mulch films are only implemented within crop rows, some form of weed management is required in crop alleys and crop holes (Schonbeck 1998). Nonetheless, studies have shown that black plastic mulch film provides additional benefits to an agroecosystem including increased soil temperature through modification of the microclimate by altering the radiation balance (Tarara 2000; Ham et. al 1993), protection of soil from water and wind erosion (Jordan et al. 2010), retention of soil moisture and nutrients which reduce nitrate leaching (Qin et. al 2015), and pest management (Greer and Dole 2003; Csizinszky et al. 1995) – factors that ultimately promote increased crop yields.

Despite the beneficial effects from plastic mulch films, its use is not a sustainable agricultural practice. The most commonly used plastic mulch films are made of polyethylene, a synthetic polymer derived from petroleum – a nonrenewable resource. Furthermore, polyethylene is composed of nonpolar, saturated, high molecular weight hydrocarbons arranged in symmetrical chains, allowing for a high packing density. A high amount of energy is required to decompose this material, which is not suitable for microorganisms. For this reason, polyethylene is a non-biodegradable material and needs to be removed from fields following use. However, an adequate method of disposal is lacking. Strategies have included dumping in landfills, incineration, on-farm burning, and recycling, but these have a negative impact on the environment and are too expensive for farmers (Moore et al. 2016). According to the Environmental Protection Agency, the amount of plastic in landfills has increased from 390 thousand tons in 1960 to 26.82 million tons in 2017 (EPA 2019). Although agricultural films account for a small fraction of this total, affordable, sustainable alternatives are needed to reduce the rate of plastic usage and waste.

1.2. Sustainable Weed Management Strategies

Sustainable weed management strategies would provide safer options for weed control in specialty crop production systems. However, to be effective, a combination of non-chemical strategies should be used versus using one method alone (Swanton and Weise 1991). This practice is known as Integrated Pest Management (IPM). In IPM, non-chemical strategies include mechanical, biological, and cultural weed control methods; the use of chemicals to control weeds is considered a final option. For specialty crop systems, biodegradable mulches could be a key component of an IPM plan and there have been many studies that have evaluated a variety of ingredients for use as biodegradable mulch films.

1.2.1. Non-Chemical Weed Control

Most non-chemical weed management tools for specialty crop systems include mechanical weed control strategies. The most common methods such as tillage via hand tools or machine powered implements and mowing are effective short-term strategies, although they need to be done repeatedly to be successful over time. Alternative methods such as abrasive weeding (Wortman 2015), flame weeding (Datta and Knezevic 2013; Wszelaki et al. 2007), and hand-removal have also been used as non-chemical weed control in specialty crop systems.

Tilling the soil is a key component of many crop systems because it prepares the soil for the sowing of crop seeds. Interrow cultivation can also be used in an attempt to sever weed shoots and roots between crop rows. However, tillage can be disruptive to the soil structure, which can, over time, lead to soil erosion; tillage is also time consuming and can introduce previously buried weed seeds into the topsoil. Yet, studies done on the comparison of conventional tillage systems and no-till or reduced-till systems have shown that while reduced tillage can improve soil organic carbon, microbial activity, and soil structure in the upper soil layer, it does not result in improved crop yields (Mader and Baker 2011; Berner et al. 2008; Teasdale et al. 2007; Garcia-Franco et al. 2015). In fact, the global meta-analysis by Pittelkow et al. (2015) showed that crop yields decreased in the first 1-2 years in no-till systems and were only able to match yields for conventional tillage systems after 3-10 years. Thus, mechanical strategies should be used in combination with other non-chemical strategies.

Another form of non-chemical weed management is biological control, which relies on natural predators to target weeds. Sources of bioherbicides include fungi, bacteria, protozoa, and phytotoxic plant residues and extracts. This management strategy has been used as a long-term strategy to control invasive weeds by using natural enemies from the origin of the weed species. However, this approach could potentially introduce an additional invasive species to the ecosystem. Additional downsides of biological control strategies include a limited range of control (in theory only the target species is affected), strict environmental conditions required for application, small scale production, and short storage life. Biological control is not considered a suitable replacement for chemical herbicides, but can be used in an IPM plan (Boyetchko 1997).

One of the most important weed management practices is the prevention of the spread and introduction of weed species into new areas. Preventative strategies include cleaning equipment, controlling weeds before they reach their reproductive stage, and checking for weed seeds in organic amendments such as manure. Another cultural method of control is crop rotation. A sequence of crops that can provide varying patterns of resource competition, allelopathic interference, soil disturbance and mechanical damage can result in an unsuitable environment for the proliferation and dominance of a particular weed species (Liebman and Davis 2000). However, designing long-term crop rotations for the sake of weed management may not yield desirable economic returns. Another strategy is decreasing row spacing as a high crop density can enhance crop competitiveness by reducing the amount of light, soil moisture and soil nutrients available to weeds (Chauhan and Gill 2014). Intercropping can also increase crop competitiveness and utilizing crops with allelopathic properties can enhance its effectiveness (Nawaz et al. 2014). A similar effect can be seen with cover crops, which not only compete with weeds for light, nutrients, moisture, and space, but leftover residue can have a mulching and potential allelopathic effect (Blackshaw et al. 2001).

1.2.2. Biodegradable Mulches

Mulch can be an effective tool in cropping systems because it can increase soil temperature, protect soil from water and wind erosion, conserve soil moisture and nutrients, and manage pests. Materials that have been used as biodegradable mulches include fossil fueld-sourced polyesters such as poly(butylene succinate), poly(butylene succinate-co-adipate), and poly(butylene-adipate-co-terephthalate), as well as bio-based polymers such as polylactic acid, starch, cellulose, and polyhydroxyalkanoates (Kasirajan and Ngouajio 2012). Additionally, organic residues such as straw, woodchips, grass clippings, cover crop residue, leaves, shredded newspaper, and animal manure can be used as a mulch source. Biodegradable films have also become of interest in the food packaging industry, which relies heavily on plastic. Materials that have been used include aliphatic-aromatic copolymers, aliphatic polyesters, polylactide aliphatic copolymers, polycaprolactones, polylactic acid, polyhydroxyalkanoates, starch-based polymers, cellulose acetate, and keratin for example (Siracusa et al. 2008).

To qualify as biodegradable, the material in use must be degraded into carbon dioxide, water, and biomass by naturally occurring microorganisms such as bacteria, fungi and algae (Mooney 2009). For this reason, biodegradable mulches can be incorporated into the soil after a growing season, which is much more sustainable than conventional plastic mulch films. However, depending on the material used and soil and environmental conditions, the degradation time can range from six months to greater than 24 months (Li et al. 2014).

Most commercially available biodegradable mulch films for agricultural use are in a solid form on a roll to be applied in a similar fashion as conventional plastic mulches. However, a sprayable film approach would allow for easier application as well as flexibility in patterns and timing of application, plus sprayable machinery is widely used in agricultural practices to apply pesticides and nutrients. Multiple studies have been done on sprayable coatings for biodegradable mulch films to improve durability (Sartore et al. 2018; Schettini et al. 2012; Shogren 1999), but the ability to use a sprayable solution alone could be more beneficial.

1.2.3. Sprayable Liquid-Mulch Films

While the idea of a biodegradable liquid mulch film (biofilm) has been evaluated, there are many difficulties that remain in producing a successful product. Limitations to a liquid mulch film are often centered around low water resistance and weak mechanical properties. While a variety of ingredients have been evaluated in multiple studies, measurements such as efficacy of weed control, crop yield, soil moisture and temperature, and film degradation are common measurements. A range of biodegradable liquid mulch film studies were assessed prior to the selection of ingredients for this research.

Of the biofilm studies reviewed, not all showed promising results. Russo (1992) tested a black spray-on wood fiber based mulch applied at 98 ml/m^2 and measured its effect on the yield of eggplant (*Solanum melongena*), which were transplanted into the mulch. The mulch film degraded quickly upon application resulting in aggressive weed emergence as Russo reported spending an equal amount of time on mechanical weed control in treated and bare-soil control plots. Additionally, Russo reported that the mulch did not improve yield relative to the bare-soil control.

Poor mechanical properties, including quick degradation time and cracking in the film surface, are a problem commonly reported amongst biofilm studies. For example, Immirzi et al. (2009), reported that a sodium alginate-based spray mulch (sodium alginate is a polysaccharide obtained from seaweed) displayed cracks within the first month of application to which weeds were able to emerge from; after six months the film had degraded by 65%. However, unlike Russo, Immirizi found no differences in yield (strawberries) between the spray mulch, conventional black plastic mulch, or a straw mulch despite mulch degradation and weed emergence.

Similarly, Braunack et al. (2020) reported cracking in the spray film they tested. The sprayable biodegradable polymer membrane, an aqueous dispersion of polyurethane consisting of carboxylate, sulfonate and ammonium, contained 18% polymer solids, 2% hydroxyethyl cellulose solids, and 2% carbon black (to prevent photosynthetically active radiation from passing through to the soil, thereby suppressing weed germination). It was applied in bands in a glasshouse. Despite cracking in the mulch film surface, they reported finding soil water content to be improved at all application rates (0.25, 0.5, and 1.0 kg of liquid biofilm/m^2); and application at 1.0 kg/m^2 improved soil water content by 13.5-15.5% relative to an untreated control. Additionally, weed growth was suppressed at a level similar to conventional plastic mulch films, although weeds were still present after application at 0.5 kg/m^2 and 1.0 kg/m^2. Nonetheless, weeds were reduced relative to non-treated control pots. However, crop emergence was reduced by 85% when biofilm was applied at an application rate of 1.0 kg/m^2 when compared to lower application rates. As a result, they concluded that application should not be done over the planted seeds and instead in surrounding areas. Braunack et al. also concluded that a higher application rate produced a thicker band on the soil surface, which is likely more difficult for a seedling to penetrate.

Shen and Zheng (2017) also reported shrinking and drying within a couple of days using a solution with a base composition of corn, potato, wheat and cellulose applied in nursery pots at 0.5, 1.25, and 2.0 kg/m^2. The effect of shrinking left a gap between the film and the container wall from which weeds were able to emerge within. Nonetheless, they reported that total weed counts were reduced from 61-97%; higher application rates had a higher efficacy because they covered a greater volume of the container reducing the effect of film shrinkage. No negative effects were observed on hydrangea plant health as no difference was found between treated containers and a bare substrate control.

Massa et al. (2019) did not report cracking in the film surface, but they found that weed suppressive abilities were lost after the film degraded 10 months after transplanting. The solution tested was composed of organic fibers combined with an adhesive substance based on only polyvinyl alcohol with a degree of polymerization that makes it a solid hydro-compacting dust. The addition of water causes the adhesive to react and create a compacted composite organic “disk” on the surface of the pot. The solution was applied at 600 ml/pot, which corresponded to about 2.5 cm in thickness. This was reduced to roughly 1.5-2.0 cm after compaction. Prior to the degradation of the film, Massa et al. reported that the mulch reduced weed biomass by 74% compared to the untreated control and was not significantly different from a chemical control. Meanwhile, the dry weight of the transplanted selected test plants improved significantly relative to the untreated control.

Giaccone et al. (2018) also reported a successful mulch barrier on the soil surface until the film degraded three months following application. They tested a mulch spray containing 1.5 g of chitosan at 75% deacetylation degree dissolved in 100 ml of acetic acid solution (3% vol), with 1.5 g of polyglycerol, 1.5 g of cellulosic fibers and 0.2 g of carbon black on controlling weed growth in containers. The solution was applied to the soil surface around the base of shrub plants. For two months the mulch was successful at preventing weed emergence until it began to degrade, but no negative effects were found on the desired shrub. In the end they found the dry weight of weeds to be significantly lower in mulch plots than an untreated control and herbicide treated container (granular formulation of oxadiazon at 2%). They concluded that climatic conditions and the soil microbial community could be responsible for the quick degradation of the mulch.

The process by which biofilm degradation occurs was evaluated by Borrowman et al. (2020). The polymeric material used in their study was an aqueous suspension of a polycaprolactone based polyurethane developed by CSIRO. The solution was 20% by weight polymer solids, and 0.65% by weight methylcellulose - a biodegradable viscosity modifier. The pigmented polymer version also contained 4% by weight carbon black. Borrowman et al. reported that soil microbes were able to utilize the polymer as a carbon and energy source and that the polymer was biodegrading. They concluded that soil pH, percent soil organic matter, and polymer morphology (based on soil particle size) all could be important in controlling the rate of polymer biodegradation.

One reason for the weak mechanical properties reported for biofilms could have to do with the viscosity of solution. Adhikari et al. (2019) reports that most sprayable polymers are known to undergo wicking into soil due to their low viscosity and are consequently poor barriers for reducing soil water evaporation. They tested multiple viscosity modifiers in a water dispersible polyurethane solution with a 27 wt% polymer solid content. Addition of a modifier in the range of 2-8 wt% concentration sufficiently increased the viscosity and reduced soil wicking by 10-90%, depending on the source.

Xanthan gum was reported to produce the best results among the modifiers tested in terms of soil wicking, membrane formation, and tensile strength. It also reduced soil evaporation by more than 60% at a biofilm solution application of 0.58 kg/m^2. Furthermore, the natural structure of soil is not favorable for the formation of a uniform mulch barrier and so, the chance of a less viscous solution forming an effective barrier is less than that of a thicker, more viscous solution.

Other studies have also reported that an increase in film thickness is beneficial for enhancing the efficacy of weed control. For example, Warnick et al. (2006) tested a hydramulch containing shredded newspaper and gypsum that was applied as a 2 mm and 4 mm thick mulch. They found soil temperature under the hydramulch was 1-4 ^{o}C lower than that under polyethylene mulch and in the absence of rain, the hydramulch resulted in soil moisture levels that were 1-4% lower than in polyethylene mulched beds. As for weed control, they reported that broadleaf and grass weed densities with the hydramulch treatments were generally lower than the bare soil control and the suppression of broadleaf and grass weeds by hydramulch and polyethylene mulch was similar. However, weed species with strong penetrative abilities, such as yellow nutsedge (*Cyperus esculentus*), was difficult to control with a thin film layer. Thus, they concluded that mulches should be applied at no less than 4 mm thick.

Similarly, Claramunt et al. (2019) was able to conclude that a film with a higher tensile strength was more successful at preventing weed emergence. They tested 24 blends of hydromulch composed of paper pulp and either wheat straw, rice hulls, and substrate used for mushroom cultivation as fillers; or rice bran, white glue, sodium silicate, and powdered gypsum as agglomerating agents. The combination of paper pulp, wheat straw and gypsum was measured to have the highest tensile strength and stress resistance and also resulted in the highest level of efficacy as it was able to reduce weed seedling emergence by 85.7% to 92.9% relative to a bare soil control and, in general, the percentage of dead seedlings underneath was greater than that which passed through the barrier.

1.4. Bio-based Sprayable Mulch Film Components

In this study, a combination of the ingredients corn starch, corn gluten meal, corn zein, isolated soy protein, keratin hydrolysate, and glycerol were evaluated as bio-based materials for mulch film production. As discussed below, these ingredients have demonstrated the ability to be used in mulch film solution production or have exhibited weed suppressive abilities.

1.4.1. Corn Starch

Starch, one of the most abundant natural polysaccharides, is among the most plentiful bio-polymers (Carvalho 2008). It is biodegradable, renewable, and inexpensive, thus an attractive source for biodegradable film production. Starch consists of two glucose polymers: amylose and amylopectin. Amylopectin has a highly branched structure with short chains linked to them through alpha-1,4 glycosidic bonds and alpha-1,6 glycosidic bonds. Amylose is a linear structure of alpha-1,4 glycosidic bonds and behaves more similar to synthetic polymers (Molavi et. al 2015). The ratio of amylose to amylopectin and average molecular weight of the starch determine the quality of starch films (Sommerfeld and Blume 1992). Studies have shown that starches with a high concentration of amylose can produce films with a higher tensile strength and elongation (Lourdin et. al 1995). Corn starch is located in the endosperm of a corn kernel. The endosperm generally contains between 75-87% starch (Shukla and Cheryan 2001). Corn starch contains 28% amylose and 72% amylopectin (Swinkels 1985). The film forming properties of corn starch have been demonstrated in many studies as it is a popular source for the formation of biodegradable films for food packaging and agricultural mulches.

Alone, starch has film forming ability because of hydrogen bonds existing in its structure. However, because it is a hydrophilic compound, starch films are very brittle and greatly affected by the presence of moisture (Lloyd and Kirst 1963). To overcome the strong cohesive energy density of corn starch, a plasticizer can be added. A plasticizer promotes flexibility by reducing intermolecular H-bonding along polymer chains, which increases intermolecular spacing (Zhang and Han 2006). Glycerol, a colorless, odorless, viscous liquid present in the form of glycerides in all animal and vegetable fats is often used as a plasticizer in edible film production for food packaging (Janjarasskul and Krochta 2010). Nordin et al. (2020) showed that the addition of glycerol to corn starch films improved the thickness, decreased water solubility, increased flexibility, and increased thermal stability.

One of the first steps required in the formation for starch-based films is heating starch suspensions in an excess of water or another solvent able to form hydrogen bonding at high temperatures (65 to 100 °C depending on the type of starch) to provoke an irreversible gelatinization process (Jimenez et al. 2012). Gelatinization results in the loss of crystallinity, water absorption, and swelling within starch granules, which allows for amylose to be released (Carvalho 2008). Thermal gelatinization has been shown to produce thicker starch-based films with better mechanical properties (Romero-Bastida et al. 2005).

Mari et al. (2020) reported that a starch-based mulch film (in a solid state) performed better than potato starch, polylactic acid, and cellulosic fiber films when applied in a specialty crop system. The film maintained an efficacy of weed control similar to conventional plastic mulch and even improved the yield of pepper plants relative to polyethylene mulch. Furthermore, Waterer (2010) reported that corn starch-based mulches improved the average yield of sweet corn (*Zea mays*), zucchini (*Cucurbita pepo*), cantaloupe (*Cucumis melo var. cantalupensis*), pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*) over three growing seasons relative to conventional plastic mulch films. However, mulches degraded quickly, limiting their ability to control weeds throughout the growing season. Nonetheless, the film forming capabilities displayed by corn starch, in addition to being an inexpensive renewable resource, provide sufficient justification for its inclusion in a liquid spray mulch.

1.4.2. Keratin

According to the USDA, nine billion chickens were slaughtered in the United States in 2018. One of the byproducts of this industry is the feathers, many of which end up in the landfill. Recent work has been done in search of efficient and sustainable ways to use this waste product. For example, treated feather waste has shown potential as a source of fertilizer for agriculture as it can contain up to 15% total nitrogen (Joardar and Rahman 2017). Chicken feathers are a biodegradable, renewable, accessible, and inexpensive source of material, especially in Nebraska where there has been a recent growth in the poultry processing industry (Purdum and Koelsch 2018; USDA 2019). Additionally, the rich keratin content of feathers makes them of interest for biodegradable film production. Keratin proteins are fibrous structural proteins that compose the hair, feathers, nails, claws, and horns of vertebrates. Keratin has been studied as a biomaterial for biomedical applications (Rouse and Van Dyke 2010), as a bioplastic (Ramakrishnan 2018), and as a film for food packaging (Sanchez Ramirez 2017).

Keratin proteins in the different sources are quite different. Keratin proteins can be α- and β-keratins depending on the source. For instance, hair and wool contain α-keratin whereas chicken feathers are composed of β-keratin, which has 10 kDa molecular mass (Fujii and Li, 2008). Chicken feather is made of ≥90% crude keratin protein. Chicken feather is considered a promising raw material for preparation of protein-based biodegradable films. Feathers must be chemically treated to release keratin from rigid feather structure before used in film formulations. The disulfide bonds formed by cystine amino acids give high stability to keratin. These disulfide bonds and hydrogen bonds in the structure need to be broken to obtain a keratin-rich hydrolysate that could be used in film applications (Schrooyen et al., 2001; Virtanen et al., 2016). The chemical treatment involves dissolution of feathers in alkaline solution (pH 10-13) using reducing agents such as 2-mercaptoethanol, potassium cyanide, sodium sulfide, urea, sodium sulfate, etc. (Gupta et al., 2012).

Biodegradable films from chicken feathers are usually brittle. However, the addition of a plasticizing compound (e.g. glycerol) could significantly improve film properties. Tanabe et al. (2002) reported that, similar to starch, keratin films are very fragile alone and benefit from the addition of a plasticizer. Sanyang et al. (2015) reported that keratin-glycerol films have a lower tensile strength than starch-glycerol films and increasing the concentration of plasticizer further reduces tensile strength. However, a combination of the two could allow for a flexible film material with a high enough tensile strength to prevent weed emergence.

Few studies could be found on the use of keratin as a biodegradable mulch source, but its nitrogen content, film forming capabilities, and ease of acquisition suggest that it has potential. In this study keratin would be acquired via hydrolysis of chicken feathers in sodium hydroxide, which also prepares it in a liquid form beneficial for the mulch solution.

1.4.3. Corn Gluten Meal

Corn gluten meal is a byproduct of the corn wet-milling process. It has 60-65% protein composition. Corn gluten meal is commonly used for animal feeds. The use of gluten meal as a food additive is difficult because of its low water solubility and severely imbalanced amino acid composition (Zhuang et al., 2013). This material is considered a cost-effective alternative protein compared to other grain protein sources (e.g. wheat germ, soy meal and flax seed meal) that can be used directly as mulch material or as an ingredient in preparation of protein-based biodegradable mulch films.

Gioia and Guilbert (1999) prepared various films by blending corn gluten meal with polar plasticizers (water, glycerol) and amphiphilic plasticizers (octanoic and palmitic acids, dibutyl tartrate and phthalate, and diacetyl tartaric acid ester of mono-diglycerides). The plasticizing efficiency of the compounds was highly dependent on molecular weight and percent of hydrophilic groups in the plasticizers. Octanoic acid was found to be a promising plasticizer for preparation of biomaterials for agricultural applications.

Furthermore, Christians (1994) reported that corn gluten meal displays inhibiting effects on weed growth when applied as a pre-emergent herbicide, ultimately making it a viable candidate for a bio-based mulch film. Christians claimed that while corn gluten meal does not inhibit germination, it prevents the plant root structure from developing sufficiently, causing the plant to die from a lack of root growth. Bingaman and Christians (1995) found that corn gluten meal reduced plant survival, shoot length, and root development of 22 species of monocot and dicot weed species. While the cause of this was unknown, Liu and Christians (1996) isolated and studied compounds from corn gluten meal hydrolysate and found five dipeptides that were shown to have greater root-inhibiting activity than the crude extract of corn gluten hydrolysate. Nonetheless, corn gluten meal has been marketed as a natural weed preventer for vegetable gardens in the retail market (Preen; Lebanon Seaboard Corporation; Lebanon, PA, USA).

1.4.4. Corn Zein

Corn zein is the major storage protein in maize and is extracted from corn gluten meal. It comprises 45-50% of the protein in corn. Zein isolate is not used directly for human consumption due to its negative nitrogen balance and poor solubility in water (Shukla and Cheryan 2001). Nonetheless, corn zein has the ability to form tough, glossy, hydrophobic, greaseproof coatings that are resistant to microbial attack with excellent flexibility and compressibility, and desirable qualities for biodegradable films (Shukla and Cheryan 2001). Furthermore, blending corn zein with various materials (e.g. whey protein and phenolic acids) can improve flexibility and mechanical properties of the films. Much interest has been shown in using corn zein for biodegradable food packaging films. For example Aydt et al. (1991) reported that corn zein films had low tensile strength and were brittle, whereas Cho et al. (2010) reported that the addition of a corn zein layer to a soy protein isolate film increased the tensile strength and water barrier properties. Additionally, Zhang and Zhao (2017) reported that adding corn zein to a corn starch film decreased the water vapor permeability and water solubility. Few studies have investigated using corn zein films as an agricultural mulch. However, Parris et al. (2004) found that zein films increased the height and dry weight of tomato plants relative to a non-treated control as they helped retain soil moisture.

1.4.5. Isolated Soy Protein

Isolated soy protein is a byproduct of soybean oil milling and because soybeans are one of the most common crops grown in the United States, isolated soy protein is plentiful and accessible. Isolated soy protein is a potential material to replace petroleum-based polymers because of its biodegradability and availability (Schmidt et al. 2005). Isolated soy protein has been investigated heavily as a source for biodegradable film formation (Kim et al. 2002; Bradenburg et al. 1993; Rhim et al. 2000). However, application of soy protein isolate-based films is limited because of weak mechanical properties and high moisture sensitivity of the films. Blending soy protein isolate with a second material (starch, sorghum wax, etc.) can result in better films. The combination of soy protein isolate with starch noticeably improved mechanical and barrier properties of the films. The 70/30 w/w soy protein isolate/starch ratio resulted in best film (Soliman et al. 2007). Ghorpade et al. (1995) showed that tensile strength and elongation at break of the films prepared by blending soy protein isolate with poly(ethylene oxide) were increased by increasing the amount of poly(ethylene oxide) used in the formulations.

Soy protein has also demonstrated the ability to suppress weed growth. Hoagland et al. (2008) reported that application of soy meal increased the population of *Pythium spp.*, which consequently resulted in suppressed weed growth. However, they also reported that weed emergence was sometimes delayed rather than suppressed. Yang and Lu (2010) reported that the reason soy protein displays herbicidal potential might be caused by the free ammonia released by microbial activity under non-sterile conditions rather than by a specific peptide. Regardless, isolated soy protein shows promise for inclusion in a biofilm solution.

1.5. Objectives

The objective of this research was to identify a biofilm formulation capable of producing a solid mulch film on the soil surface that is effective at reducing weed emergence relative to an untreated control. Novel film formulations were first evaluated in a greenhouse, and promising formulations were advanced to field research trials. Biofilm effects on weed suppression and crop yields were quantified; the ideal biofilm would be capable of forming an impenetrable layer to prevent weed emergence while also improving crop yields relative to a non-treated, weedy control.

2. METHODS

2.1. General Laboratory Methods

Bio-based sprayable mulch films were prepared in the Industrial Agricultural Products Center laboratories at the University of Nebraska-Lincoln. The general procedure (Ali et al. 2004) for preparing a biofilm solution consisted of five steps:

1. Mix corn starch and keratin hydrolysate; bring to boil
2. Mix glycerol and seed meal protein (and water); add to starch-keratin solution; bring to boil and remove from heat
3. Add H_2SO_4 until pH is between 6.8 and 7.5
4. Blend to homogenize
5. Refrigerate at 4 °C for a minimum of 24 hours

Formulations were prepared in four-liter glass beakers and heat was applied by hotplates. Keratin hydrolysate was prepared by soaking 30 g of raw chicken feathers per L of 0.8 M NaOH for 48-72 hrs; remaining feather residue was removed via filtration through glass wool. The pH of the chicken feather hydrolysate is higher than 13 and applying a solution with a high pH to soil could limit the availability and plant root uptake of some essential nutrients (Alam et. al 1999). To circumvent this, H_2SO_4 was added until pH reached a level between 6.8 and 7.5 after the biofilm formulation was prepared. Quantities of ingredients used per L of biofilm solution were specific to the objectives of each iterative trial and are described in detail below.

2.2. General Greenhouse Research Trial Methods

Six research trials were conducted at the University of Nebraska-Lincoln between January 2017 and April 2019. In the greenhouse trials, the ingredients and method of preparation resulting in a film with ideal physical characteristics were identified and the effects of biofilm application on crop growth and suppression of monocotyledonous and dicotyledonous weed species were quantified. All trials were formatted in a factorial randomized complete block design; the selected treatment factors were specific to the objectives of each iterative trial and are described in detail below. A summary of the experimental layout for each trial can be found in Table 1.

In the greenhouse, black plastic pots (10 cm diameter; 12.5 cm depth) were filled with a steam pasteurized soil mix composed of vermiculite, sand, soil, and peat (1:1:1.2:2 ratio) to within 1.3 cm from the top. On the soil surface, 20 seeds of a selected weed species were placed and soil mix was added until pots were full to cover the seeds. Weed species commonly found in eastern Nebraska, velvetleaf (*Abutilon theophrasti*) and shattercane (*Sorghum bicolor*), were used as model dicot and monocot weed species, respectively. Weed seeds were stored in a refrigerator at 2.2 °C prior to use. Velvetleaf was stratified in a 70 °C water bath for 60 seconds before planting to break physiological dormancy (Ravlic et al. 2015). Biofilm solution was applied uniformly to pots by either a calibrated hand-pump sprayer or a graduated cylinder, in which case it was poured. Following the application of biofilm solution, pots were not watered for 24 hrs to allow solutions to dry and form a film. After 24 hrs, pots were watered to field capacity daily. A weedy control was present for both PRE (prior to weed emergence) and POST (after weed emergence, but prior to the formation of three true leaves) treatments. In trials where biofilm was applied as a POST treatment, the number of velvetleaf per pot, including in the control, was manually reduced to three plants to avoid crowding and to allow for a consistent baseline.

Aboveground weed biomass was collected from each pot approximately one month after weeds were planted by cutting plants at the soil surface. Before measuring the weight, biomass was stored in a paper bag in a dryer at 65.5 ◦C until a constant mass was achieved.

2.3. General Field Research Trial Methods

Four experimental trials (two in 2017 and two in 2019) were conducted at the University of Nebraska-Lincoln on the East Campus research farm. The purpose of these trials was to test the effects of a sprayable bio-based mulch film on weed suppression and crop yield under field conditions. Daily temperatures and precipitation events during the growing season are displayed in Figure 1 and Figure 2.

The experimental design and treatment factors were specific to the objectives of each trial and are described in detail below. A summary of the treatment factors can be found in Table 1. All trials contained four replications. Each replication (crop row) consisted of two control plots (weedy and weed-free) and biofilm treatments (specific to each trial and described below). Crops were planted in bare soil rows after the field had been tilled with a rotary tiller. Monocotyledonous and/or dicotyledonous weed species were present in both trials. Weed seeds were stored in a refrigerator at 2.2 °C prior to use (velvetleaf was stratified in a 70 °Cwater bath for 60 seconds). Biofilm application was done either using a calibrated hand-pump sprayer or a graduated cylinder, in which case it was poured. Watering was done in two to three hour increments via drip tape every 48 hrs under hot and dry conditions, every 72 hrs under cooler conditions, or every 48-72 hrs after a precipitation event. Aboveground weed biomass was collected before weeds matured to seed by cutting plants at the soil surface. Before measuring the weight, biomass was stored in a paper bag in a dryer at 65.5 °C until a constant mass was achieved.

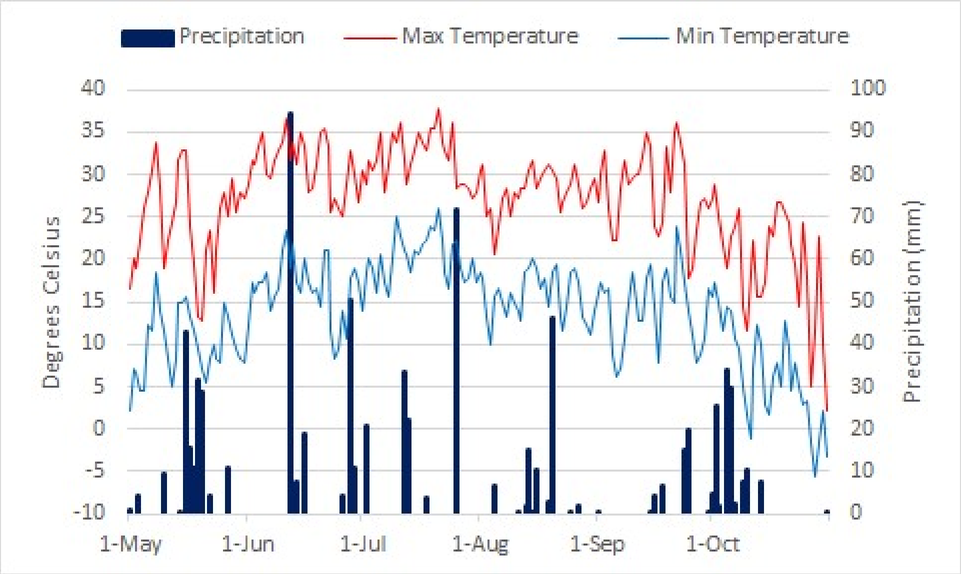

Figure 1. Daily maximum and minimum temperatures (◦C) and daily precipitation totals (mm) from 1 May 2017 to 31 October 2017 in Lincoln, NE, Lincoln Airport 68524. Retrieved from the High Plains Regional Climate Center (HPRCC).

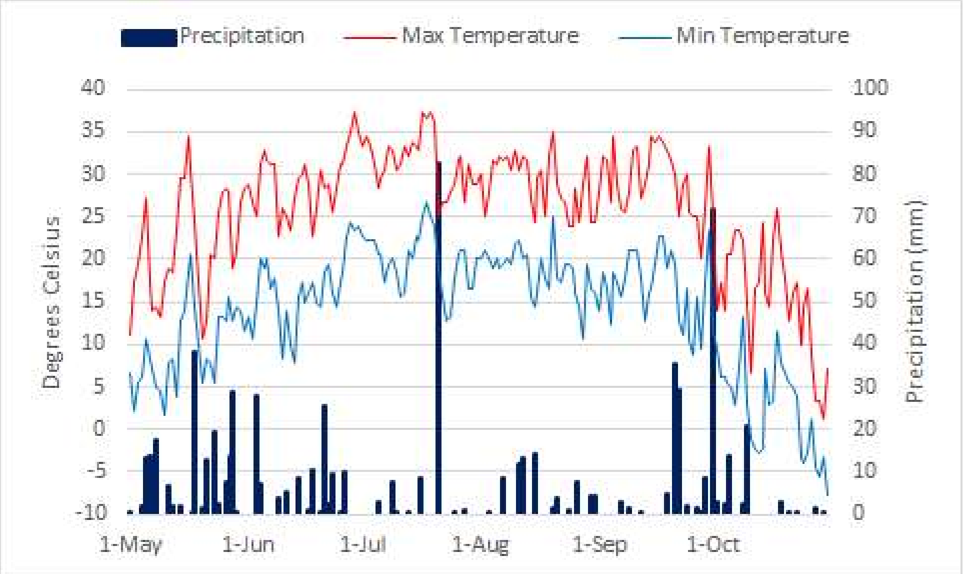

Figure 2. Daily maximum and minimum temperatures (◦C) and daily precipitation totals (mm) from 1 May 2019 to 31 October 2019 in Lincoln, NE, Lincoln Airport 68524. Retrieved from HPRCC.

2.4. Data Analysis

Analysis of variance was performed on aboveground weed biomass and crop yield data using the 'GLIMMIX' procedure in SAS (v9.4; SAS Institute, Cary, NC) to determine differences among experimental treatments. The experimental design was a randomized complete block (RCBD) with one or more treatment factors. Replicate blocks were treated as a random effect, while formulation, application rate, application time, and two-way and three-way interactions were treated as fixed effects. Differences among least squares means were determined using the Tukey-Kramer multiple comparisons test at a significance level of a $\alpha = 0.05$. Weed biomass was analyzed as a percentage reduced from a non-treated, weedy control. This data displayed a non-normal distribution and was analyzed as a beta distribution (Stroup 2015). To convert the data set to only contain values between zero and one, no weed reductions (0%) were recorded as 0.0001, whereas 100% reductions were recorded as 0.9999.

2.5. Individual Research Trial Methods

The following information contains the methods of bio-based sprayable mulch film research trials conducted at the University of Nebraska-Lincoln from 2017 to 2019 in chronological order, consistent with the iterative nature of the design process for innovation. A summary of all trials can be seen in Table 1.

2.5.1. GH Trial 1

The objective of this trial was to test the effects of a bio-based sprayable mulch film on weed suppression and crop growth in a controlled environment. One formulation was tested: corn starch (40.5 g/L), glycerol (128.6 ml/L), isolated soy protein (40.5 g/L) and water (810 ml/L). Peppers (*Capsicum annuum* cv. 'Carmen') were selected as a model crop and one plant was transplanted into each pot in January of 2017. Velvetleaf was the only weed species used in this trial. A non-treated, weed-free control and a non-treated, weedy control were present. This trial was formatted as an RCBD with four replications and one treatment factor, which was application rate. Rates included: 0.91, 1.81, 4.54, 9.08, and 18.15 L/m^2. Biofilm was applied to pots as a PRE 24 hrs after weed seeds were planted. Four weeks after application, the number of velvetleaf plants were counted and aboveground pepper biomass was collected.

2.5.2. Field Trial 1

The objective of this trial was to test the effects of the biofilm formulation from GH Trial 1 on weed suppression and crop yield in a specialty crop agroecosystem. This trial was formatted as an RCBD with one treatment factor, which was application rate. Rates included: 0.91, 1.81, 3.63, and 9.08 L/m^2.

In May of 2017, black plastic mulch beds and a line of drip tape were laid using a tractor with a plastic mulch layer implement. Tomatoes (*Solanum lycopersicum* cv. 'BHN 589') were started in 3 cm wide cell plug trays filled with Berger BM6 All-Purpose potting mix (containing peat moss, perlite, dolomitic and calcitic limestone, and non-ionic wetting agent standard fertilizer starter charge) in the greenhouse. In May, they were transplanted in the plastic mulch beds, with holes formed by a mechanical transplanter implement attached to a tractor, at 0.45 m spacing. There were ten plants per plot and seven plots per row. A non-treated, weed-free control and a non-treated, weedy control were present. On the same day, 20 seeds of velvetleaf were uniformly hand-sown in each planting hole per plot with the exception of the weed free control. Biofilm was applied as a PRE 24 hours later. On 27 July, aboveground weed biomass was collected from each plot. The number of tomatoes per plot and the total weight of tomatoes per plot were recorded across multiple harvest intervals beginning in July.

2.5.3. Field Trial 2

The objective of this trial was to test the effects of the biofilm solution from GH Trial 1 on weed suppression and crop yield in a field agroecosystem. Application rates included: 0.91, 1.81, 3.63, and 9.08 L/m^2. In August 2017, white plastic mulch beds and a line of drip tape were laid using a tractor with a plastic mulch-layer-implement. Broccoli (*Brassica oleracea*) seeds were started in 3 cm wide cell plug trays filled with Berger BM6 All-Purpose potting mix (peat moss, perlite, dolomitic and calcitic limestone, and non-ionic wetting agent standard fertilizer starter charge) in the greenhouse. In August, they were transplanted in the plastic mulch beds, with holes formed by a mechanical transplanter-implement attached to a tractor, at 0.45 m spacing. There were ten plants per plot and seven plots per row. A non-treated, weed-free control and a non-treated, weedy control were present. On the same day, 20 seeds of velvetleaf and 20 seeds of foxtail were uniformly hand-sown in each planting hole per plot, with the exception of the weed free control. Biofilm was applied 24 hours later at four rates. On 2 October, aboveground weed biomass was collected. On 26 October, the total fresh weight of broccoli per plot was recorded.

2.5.4. GH Trial 2

The objective of this trial was to test a formulation with a different protein composition (corn gluten meal and keratin hydrolysate) from the formulation used in the previous trials (isolated soy protein) in an effort to enhance the efficacy of weed suppression. The following formulation was tested in this trial: corn starch (6.36 g/L), glycerol (18.94 ml/L), corn gluten meal (19.90 g/L), and keratin hydrolysate (954.80 ml/L This trial was formatted as a factorial RCBD with four blocks and two treatment factors including weed species (velvetleaf and shattercane) and biofilm application rate (0.81 and 2.85 L/m^2). Velvetleaf and shattercane were planted on 5 June 2018; one bare soil control pot was present for each species. Biofilm was applied to pots as a PRE 24 hrs after seeds were planted via a calibrated hand-pump sprayer. Aboveground biomass was collected for each pot on 19 June.

2.5.5. GH Trial 3

The objective of this trial was to increase the viscosity of the formulation from GH Trial 2 in an effort to reduce soil infiltration to thereby improve the efficacy of weed control. The viscosity was increased by reducing the amount of keratin hydrolysate per L of solution by 40%. The resulting formulation was tested: corn starch (15.44 g/L), glycerol (30.65 ml/L), corn gluten meal (38.63 g/L), and keratin hydrolysate (926.88 ml/L). This trial was formatted as a factorial RCBD with four blocks and two treatment factors including weed species (velvetleaf and shattercane) and biofilm application rate (2.04 and 4.89 L/m^2). Velvetleaf and shattercane were planted on 2 July 2018; one bare soil control was present for each weed species. Biofilm solutions were applied as a PRE 24 hours later via a graduated cylinder. Rates were increased from GH Trial 2 in an effort to enhance weed suppression and accumulation of film solution on the soil surface. Aboveground biomass was collected for each pot on 13 July 2018.

2.5.6. GH Trial 4

The objective of this trial was to test a biofilm formulation capable of demonstrating ideal film forming properties (determined in the lab), due to the inability to form solid films on the soil surface in previous trials. Nine variations of biofilm solution were prepared in the lab in October of 2018. Solutions differed in the combination and amount of proteins used and are listed in Table 2 in the results. In a 13 cm diameter petri dish, 150 mL of each variation was poured. Dishes were stored at room temperature for two weeks before collecting observations on the amount of shrinking and cracking present as solutions dried. Recorded observations are also listed in Table 2. Two formulations displayed the least amount of cracking and thus were tested in a greenhouse trial.

This trial was designed as a factorial RCBD with four replications and three treatment factors. The first factor was formulation and these included:

1) corn starch (40.5 g/L), glycerol (128.7 ml/L), corn gluten meal (20.24 g/L), isolated soy protein (20.24 g/L), and water (811 ml/L).
2) corn starch (40.5 g/L), glycerol (128.7 ml/L), corn gluten meal (10.15 g/L), isolated soy protein (10.15 g/L), corn zein (10.15 g/L), keratin hydrolysate (374 ml/L), eggshell powder (12.2 g/L), and water (439 ml/L).

The second factor was application rate (2.04 and 4.89 L/m^2) and the third factor was application timing relative to weed growth stage. These included: 1) PRE (24 hours after weed seeds were planted); 2) POST - V0 (emerged plants, but prior to the formation of true leaves); and POST - V2 (plants with two true leaves). Velvetleaf was planted on 27 November 2018. Two controls were present: PRE and POST, in which the number of plants per pot was reduced to three to reduce the effects of crowding. Biofilms were applied via a graduated cylinder. Aboveground biomass was collected for each pot on 16 December 2018.

2.5.7. GH Trial 5

The objective of this trial was to eliminate water from the biofilm formulation to eliminate soil infiltration when applied as a solution and to reduce application volume required to achieve consistent weed efficacy. Formulations were prepared in a solid form using an extruder. The feeding part of the extruder was kept at 50 °C, while all other zones (e.g. the die) were maintained at 110 °C during extrusion (Ditudompo et al., 2016). The ~4 cm wide taffy-like strips (See Figure 3) obtained from the extrusion process were ground in a hammer mill and separated using a dry sieve into two sizes: larger than 1.05 mm and smaller than 850 µm. In order to determine the effects of the extrusion process itself, formulations were also applied in their raw forms.

This trial was designed as a factorial RCBD with four replications and three treatment factors. The first factor was formulation and included:

1) corn starch (375 g/kg), glycerol (250.5 g/kg), corn gluten meal (187.5 g/kg) and corn zein (187.5 g/kg)
2) corn starch (375 g/kg), glycerol (250.5 g/kg), corn gluten meal (187.5 g/kg) and isolated soy protein (187.5 g/kg)

The second factor was the method of preparation and granule size including: 1) extruded > 1.05 mm; 2) extruded < 850 µm; and raw powder. The third factor was application rate and included: 198.6, 497.0, and 997.8 g/m^2.

Velvetleaf was planted on 4 February 2019 and bio-based granules were applied as PRE treatments. Rates were determined based on equivalent starch and protein concentrations if prepared as a liquid biofilm solution (i.e. the amount of starch and protein applied to pots was identical even when the water content varied). Aboveground biomass was collected per pot on 25 February.

Figure 3. A bio-based formulation is produced from an extruder as a 4 cm wide strip. After the product dries it is ground into a powder.

2.5.8. GH Trial 6

The objective of this trial was to reduce the concentration of water per L of liquid biofilm solution due to the relatively poor efficacy of weed control displayed with dry formulations in GH Trial 5. Water was reduced to limit soil infiltration and promote film formation and reduce the application volume of a sprayable solution. Three formulations of varying water content were prepared and characterized as low, medium, and high viscosity. In contrast to previous trials, corn gluten meal was not included in these formulations due to its inability to become completely homogenized within the solution – it consistently accumulated at the bottom of the container when previously used.

This trial was designed as a factorial RCBD with four replications and two treatment factors. The first factor was viscosity level and treatments included:

1) Low viscosity: corn starch (40.91 g/L), glycerol (129.98 ml/L), keratin hydrolysate (515.97 ml/L), corn zein (13.96 g/L), isolated soy protein (13.96 g/L), and water (303.03 ml/L).
2) Medium viscosity: corn starch (47.55 g/L), glycerol (151.08 ml/L), keratin hydrolysate (599.76 ml/L), corn zein (16.22 g/L), isolated soy protein (16.22 g/L), and water (190.4 ml/L).
3) High viscosity: corn starch (58.74 g/L), glycerol (186.63 ml/L), keratin hydrolysate (740.88 ml/L), corn zein (20.04 g/L), and isolated soy protein (20.04 g/L).

The second factor was application timing and included PRE and POST (V2) applications.

Velvetleaf was planted on 28 March 2019. Biofilms were applied as a PRE 24 hours later and as a POST when velvetleaf was at the V2 stage via a graduated cylinder. Rates were standardized from 6.11 L/m^2 to deliver the same amount of starch and protein, but due to variable water content, application volume per pot was different (i.e. low viscosity was applied at 6.11 L/m^2, medium at 5.33 L/m^2, and high at 4.36 L/m^2). Aboveground biomass was collected per pot on 29 April.

2.5.9. Field Trial 3

The objective of this trial was to test a successful biofilm solution from ‘GH Trial 6’ in a field production system by quantifying the effects on weed suppression and crop yields. The ‘medium viscosity’ solution was selected because it produced a higher quality film on the soil surface than the ‘low viscosity’ and covered a greater soil surface area than the ‘high viscosity’ due to the inclusion of water in the formulation. In the greenhouse trial it was observed that all solutions contained aggregations of starch; it was hypothesized that a homogenous solution would result in greater film forming capabilities. Thus, the solution was blended to a uniform consistency prior to use. Unexpectedly, the viscosity of the solution appeared to be reduced in the process.

This trial was designed as an RCBD with four replications. Six treatments were tested including:

1) Non-treated, weed-free control
2) Non-treated, weedy control
3) PRE biofilm application at 4.07 L/m^2
4) PRE biofilm application at 8.15 L/m^2
5) POST-V3-4 biofilm application at 4.07 L/m^2
6) POST-V3-4 biofilm application at 8.15 L/m^2

Velvetleaf and shattercane were selected as the model weed species because they supplemented an existing weed seed bank that was observed to include velvetleaf, shattercane, palmer amaranth (*Amaranthus palmeri*), redroot pigweed (*Amaranthus retroflexus*), common purslane (*Portulaca oleracea*), dandelion (*Taraxacum officinale*) and common lambsquarter (*Chenopodium album*). Peppers (*Capsicum annuum* cv. 'Carmen') were selected as the model crop.

On 9 April 2019, pepper seeds were started in the greenhouse in 3 cm wide cell plug trays in Berger BM6 All-Purpose potting mix (contents include peat moss, perlite, dolomitic and calcitic limestone, and non-ionic wetting agent standard fertilizer starter charge). On 5 June, they were transplanted into the field in four rows. A 3 m bare soil alley served as a barrier between rows. Within a row, plants were spaced at 0.45 m. A set of five plants was designated as a plot and totaled 2.3 m in length. An area of 0.0675 m^2 (0.45 m by 0.15 m) was marked between pepper plants and an area of 0.03375 m^2 (0.225 m by 0.15 m) was marked outside the outermost plants in a plot. Together, they represented the space designated for biofilm application (see Figure 4). Between plots, 1.5 m of bare soil was left to serve as a boundary between treatments. Each row consisted of six plots.

On 6 June, in all plots except the weed-free control, a total of 80 velvetleaf seeds and 80 shattercane seeds were hand-sown and raked-in in the designated application area. On the same day, all plots received a fertilizer application of a diluted $Ca(NO_3)_2$ solution. On 7 June, PRE biofilm treatments were applied via a graduated cylinder. On 10 June, all peppers were replaced because the previous plants were damaged fertilizer burn. Screwdrivers were used to loosen the soil in the previous planting hole in an effort to minimize disturbance in areas where films had been applied. On 24 June, when velvetleaf was at the V3-V4 growth stage (3 or 4 true leaves, respectively), POST treatments were applied via a hand pump sprayer in a serpentine motion in an attempt to maximize leaf surface coverage. The sprayer was calibrated by measuring, in a graduated cylinder, the time required to release the desired volume.

On 24 July, aboveground velvetleaf and shattercane biomass was collected (combined) per plot within the biofilm band area. From 16 August to 11 October (one day prior to the first freeze) peppers were harvested once a week. The total and marketable yield (number and weight) of red peppers was collected per plot.

Figure 4. Bio-based sprayable mulch film applied on 7 June 2019 on the East campus research farm at the University of Nebraska-Lincoln. Solution was applied via a graduated cylinder within a 0.0675 m^2 area between pepper plants at two rates: 4.07 and 8.15 L/m^2 (pictured), prior to the emergence of velvetleaf and shattercane seedlings. A drip irrigation line was present in all crop rows.

2.5.10. Field Trial 4

The objective of this trial was to increase the viscosity of the biofilm solution relative to the solution applied in ‘Field Trial 3’ in an effort to enhance the film forming abilities and thus increase the efficacy of weed control. From the previous solution, water was removed and the concentration of starch was increased by 25%; the solution was still blended to achieve homogeneity. The resulting formulation was tested in a kale (*Brassica oleracea* var. *sabellica*) crop system and included: corn starch (72.81 g/L), glycerol (184.73 ml/L), keratin hydrolysate (733.32 ml/L), corn zein (19.84 g/L), and isolated soy protein (19.84 g/L).

This trial was designed as an RCBD with four replications. Five treatments were tested:

1) Non-treated, weed-free control
2) Non-treated, weedy control
3) PRE biofilm application at 4.07 L/m^2
4) PRE biofilm application at 6.11 L/m^2
5) POST-V3-4 biofilm application at 6.11 L/m^2

On 26 July 2019, kale seeds were started in the greenhouse in 3 cm wide plug cell trays filled with Berger BM6 All-Purpose potting mix (contents include peat moss, perlite, dolomitic and calcitic limestone, and non-ionic wetting agent standard fertilizer starter charge). On 19 August, they were transplanted into the field in four rows. A 3 m alley of bare soil served as a barrier between crop rows. Within a row, plants were spaced at 0.3 m. A set of seven plants was designated as a plot and totaled 2.1 m in length. An area of 0.09 m^2 (0.3 m by 0.3 m) was marked between kale plants and an area of 0.045 m^2 (0.15 m by 0.3 m) was marked outside the outermost plants in a plot. Together, they represented the space designated for biofilm application and totaled 0.63 m^2 (see Figure 5). Between plots, 1.5 m of bare soil was left to serve as a boundary between the five treatments.

Mustard (*Guillenia flavescens*) cover crop seed was used as a surrogate weed species to ensure uniform germination and establishment. On 27 August, in all plots except the weed-free control, a total of 80 mustard seeds were hand-sown and raked-in in the designated biofilm application area. On the same day, PRE biofilm treatments were poured in the designated area surrounding kale plants. On 12 September, POST treatments were uniformly poured in the outlined space by way of a serpentine motion when mustard was at the V3-V4 growth stage. On 18 October, aboveground kale and mustard biomass was collected per plot. Kale fresh weight was recorded while mustard was recorded as dried biomass per plot.

Figure 5. Bio-based sprayable mulch film applied on 27 August 2019 on the East campus research farm at the University of Nebraska-Lincoln. Solution was applied via a graduated cylinder within a 0.09 m^2 area between kale plants at two rates, 4.07 and 6.11 L/m^2 (pictured), prior to the emergence of mustard seedlings.

Table 1. Summary of all experimental trials conducted on bio-based sprayable mulch films at the University of Nebraska-Lincoln between January 2017 and October 2019, in chronological order.

Trial	Description	Biofilm Solution(s)*	Rate(s) (per m^2)	Application Time(s)**	Weed Species****
GH 1	Soy; Peppers	1) cs, gly, soy, water	0.91, 1.81, 4.54, 9.08, 18.15 L	PRE	VL
Field 1	Tomatoes	1) cs, gly, soy, water	0.91, 1.81, 3.63, 9.08 L	PRE	VL
Field 2	Broccoli	1) cs, gly, soy, water	0.91, 1.81, 3.63, 9.08 L	PRE	VL
GH 2	CGM+Ker	1) cs, gly, cgm, ker	0.81, 2.85 L	PRE	VL. SC
GH 3	CGM+Ker; Soy	1) cs, gly, cgm, ker 2) cs, gly, soy, water	2.04, 4.89 L	PRE	VL, SC
GH 4	Best Films from the Lab	1) cs, gly, cgm, soy, water 2) cs, gly, cgm, ker, soy, cz, egg, water	2.04, 4.89, 9.78 L	PRE, POST-V0, POST-V2	VL
GH 5	Extrusion; Dry Application	1) cs, gly, cgm, cz 2) cs, gly, cgm, soy	198.59, 496.97, 997.81 g	PRE	VL
GH 6	Viscosity***	1) cs, gly, cz, soy, ker (Low) 2) cs, gly, cz, soy, ker (Med) 3) cs, gly, cz, soy, ker (High)	Low-6.11, Med-5.33, High-4.36 L	PRE, POST-V2	VL
Field 3	Peppers	1) cs, gly, cz, soy, ker (Med)	4.07, 8.15 L	PRE, POST-V3-4	VL, SC
Field 4	Kale	1) cs, gly, cz, soy, ker (High)	4.07, 6.11 L	PRE, POST-V3-4	MUS

*cs=corn starch; gly=glycerol; cgm=corn gluten meal; cz=corn zein; soy=isolated soy protein; ker=keratin hydrolysis (from chicken feathers); egg=eggshell powder

**PRE=prior to weed emergence; POST=after weed emergence; V0=emerged weeds prior to forming true leaves; V2=weeds with 2 true leaves; V3=weeds with 3 true leaves; V4=weeds with 4 true leaves

***Low=303.03 ml water/L; Med=190.4 ml water/L; High=0 ml water/L

****VL=velvetleaf; SC=shattercane; MUS=mustard

3. RESULTS AND DISCUSSION

3.1. GH Trial 1

The biofilm formulation of corn starch (40.5 g/L), glycerol (128.6 mL/L), isolated soy protein (40.5 g/L) and water (810 mL/L) applied at 0.91 L/m^2 reduced the number of velvetleaf plants per pot (10 cm wide by 12.5 cm deep) by 93.75% ± 6.25%, which was not different from application at 1.81 L/m^2 (87.5% ± 7.22%, F[1,3] = 1.32, $p = 0.33$) or application at 4.54 L/m^2 and greater where the number of plants was reduced by 100% ± 0% (Figure 6).

Aboveground pepper plant biomass was different between treatments (F[6,18] = 11.20, $p < 0.0001$). Biomass in non-treated, weed-free control pots (1.17 ± 0.15 g) was higher relative to all other treatments ($p < 0.05$). A Tukey-Kramer comparison showed no difference in biomass between the non-treated, weedy control (0.71 ± 0.07 g) and biofilm application at 0.91 L/m^2 (0.69 ± 0.14 g, $p = 0.10$) and 1.81 L/m^2 (0.62 ± 0.08 g, $p = 0.10$), whereas biofilm solution applied at 4.54 L/m^2 (0.26 ± 0.08 g) and greater was approaching significance relative to the non-treated, weedy control ($p < 0.09$) (Figure 7).

Giaccone et al. (2018) found that a chitosan-based sprayable mulch film successfully reduced weed biomass relative to a non-treated control for two months before it began to degrade with no negative effects measured on desired ornamentals. Massa et al. (2019) found that liquid mulch reduced weed biomass by 74% relative to an untreated control while the dry weight of selected plants was increased compared to the control. In GH Trial 1, velvetleaf was also successfully reduced relative to a non-treated control, but crop plant biomass was reduced from a non-treated, weed-free control and even non-treated, weedy controls when applied at higher rates as plants were unable to withstand the solution application, indicating that the solution caused more damage than to weeds alone.

Results of GH Trial 1 suggest that biofilm applications made around the base of a crop at rates greater than 4.0 L/m^2 can damage crop plants either through direct contact (eg., burning) or through indirect plant-soil interactions (discussed later). To further understand the biofilm formulation and its response to environmental conditions, a research trial was conducted in a field environment.

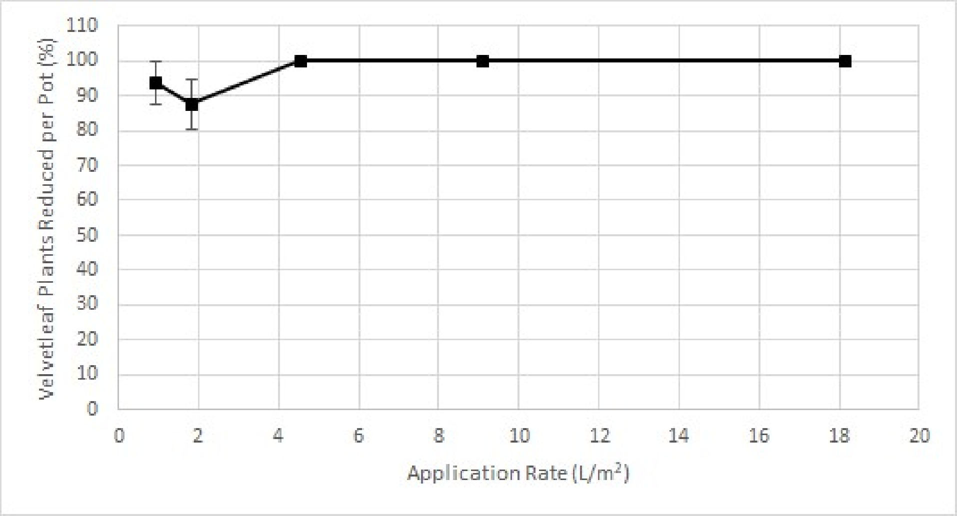

Figure 6. Mean percent reduction of velvetleaf plants per 10 cm wide by 12.5 cm deep pot relative to a non-treated, weedy control, ± one standard error of the mean, when biofilm solution was applied as a PRE at 0.91, 1.81, 4.54, 9.08, and 18.15 L/m^2. The research trial was completed from January to February of 2017 at the University of Nebraska-Lincoln in Agronomy and Horticulture Greenhouse 3 on East Campus.

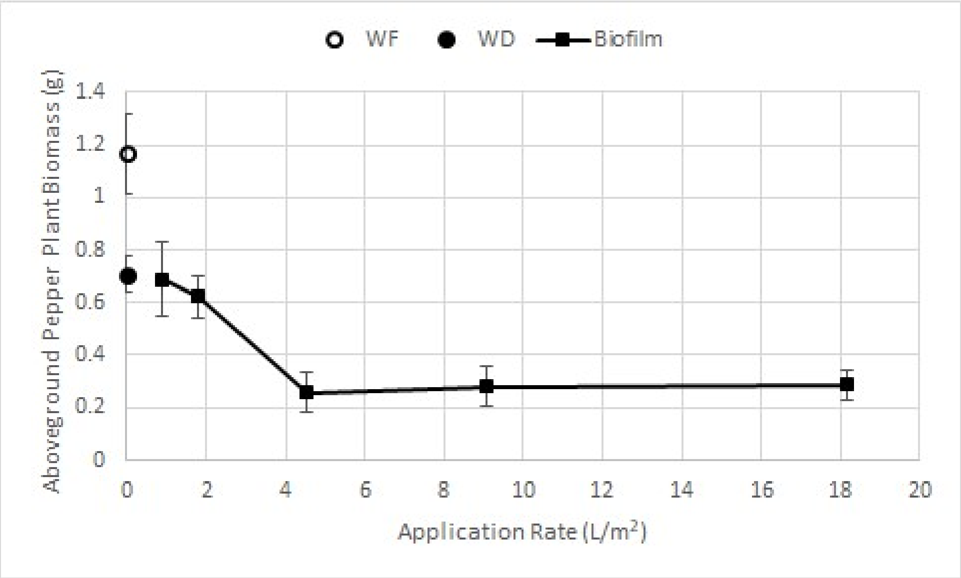

Figure 7. Mean dried aboveground pepper plant biomass (g) per 10 cm wide by 12.5 cm deep pot, ± one standard error of the mean, of a weed-free control, a weedy control, and PRE biofilm solution application at 0.91, 1.81, 4.54, 9.08, and 18.15 L/m^2. The research trial was completed from January to February of 2017 at the University of Nebraska-Lincoln in Agronomy and Horticulture Greenhouse 3 on East Campus.

3.2. Field Trial 1

The biofilm solution from GH Trial 1 applied at 3.63 L/m^2 and 9.08 L/m^2 reduced the number of velvetleaf plants per 4.5 m plot relative to a non-treated, weedy control by 16.50% ± 9.71% and 25.24% ± 22.50%, respectively. There was no difference in the percentage of plants reduced between application rates (F[3,9] = 1.47, p = 0.29) (Figure 8). There was no difference in tomato yields (g/plot) between a non-treated, weed-free control, a non-treated, weedy control, and biofilm treated plots (F[5,15] = 1.80, p = 0.17) (Figure 9).

The results of this trial showed that the biofilm solution did not perform the same in the field as it did in the greenhouse. In GH Trial 1, the number of velvetleaf plants per pot were reduced by greater than 80% relative to an untreated control, whereas in Field

Trial 1, the number of velvetleaf plants was only reduced relative to a non-treated, weedy control when biofilm was applied at 3.63 and 9.08 L/m^2 and only by 16.50% ± 9.71% and 25.24% ± 22.50%, respectively. The differences between trials can likely be attributed to the different environments. Warnick et al. (2006) reported that mulches should be no less than 4 mm thick to prevent weed penetration. However, film solutions were less successful at forming a solid, thick barrier on the soil surface and seemed to degrade faster than in the greenhouse. Immirizi et al. (2009) reported that the spray mulch they tested was able to retain its mulching effect for six months when the film had degraded by 65% even though cracks appeared within the first month and weeds were able to emerge. They found no differences in strawberry yields relative to conventional plastic mulch. To diversify the environmental conditions for testing this biofilm formulation, a second field trial was conducted in a fall broccoli crop.

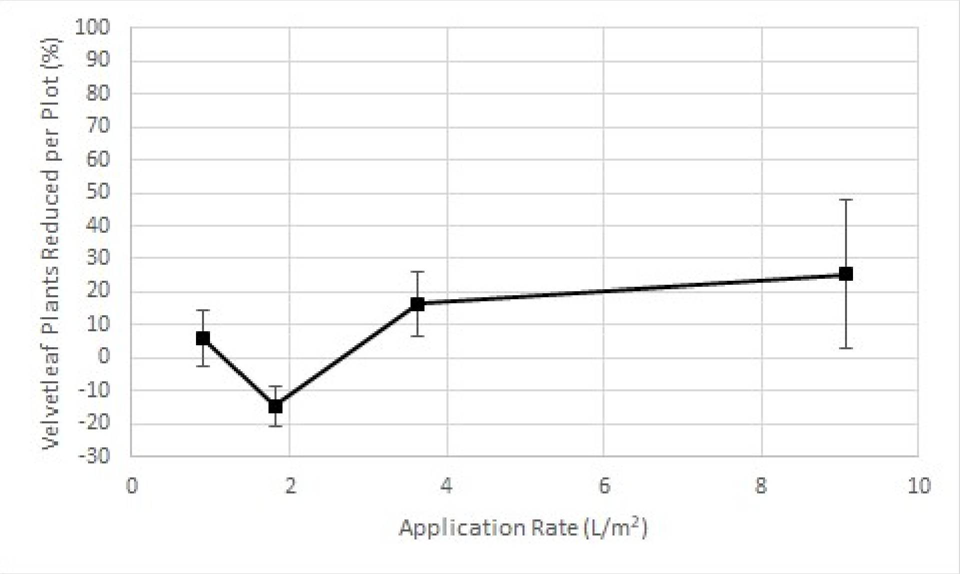

Figure 8. Mean percent reduction of velvetleaf plants per 4.5 m plot relative to a non-treated, weedy control, ± one standard error of the mean, when biofilm solution was applied as a PRE at 0.91, 1.81, 3.63, and 9.08 L/m^2. The research trial was completed from May to 27 July 2017 at the University of Nebraska-Lincoln on the East Campus research farm.

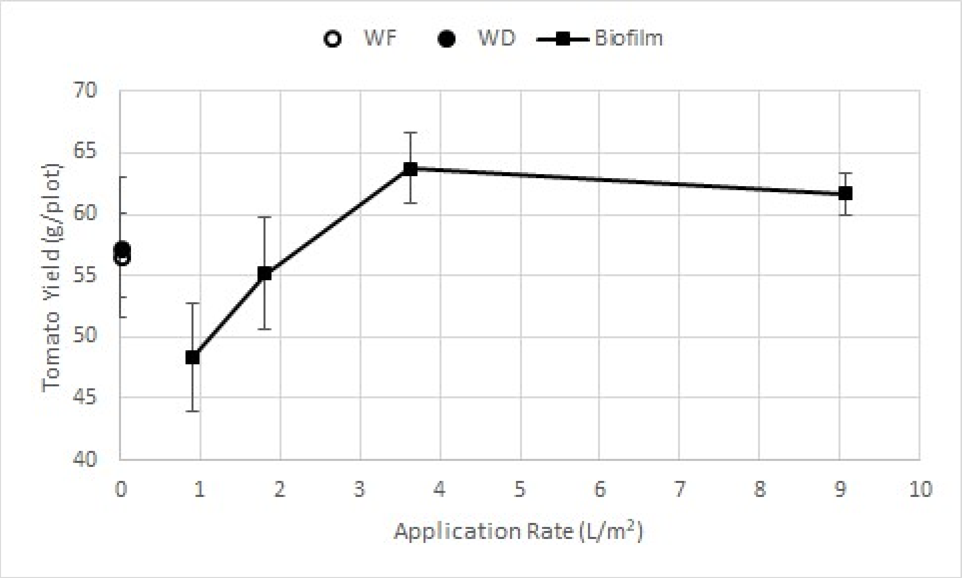

Figure 9. Mean tomato yield (g) per 10-plant plot, ± one standard error of the mean, of a non-treated, weed-free control, a non-treated, weedy control, and PRE biofilm solution application at 0.91, 1.81, 3.63, and 9.08 L/m^2. The research trial was completed from May to 27 July 2017 at the University of Nebraska-Lincoln on the East Campus research farm.

3.3. Field Trial 2

The number of velvetleaf plants per 4.5 m plot were reduced relative to a non-treated, weedy control at all biofilm application rates. Velvetleaf plants were reduced by 23.66% ± 4.77% when biofilm was applied at 0.91 L/m^2, by 19.35% ± 16.97% when biofilm was applied at 1.81 L/m^2 and by 74.19% ± 10.24% when biofilm was applied at 9.08 L/m^2. However, differences in weed suppression among application rates was only approaching significance (F[3,9] = 2.72, p = 0.11) (Figure 10). Similarly, differences in broccoli yield (kg per 10-plant plot) among treatments was only approaching significance (F[5,15] = 2.77, p = 0.06) (Figure 11).

Although velvetleaf plants were reduced compared to an untreated control, the efficacy of weed control did not reach the level measured in GH Trial 1. However, crop plants were not reduced as they were in GH Trial 1. A similar trend in yield was found between Field Trials 1 and 2. Given the inconsistent field performance of this biofilm formulation, it was determined that new formulations and subsequent greenhouse trials were needed.

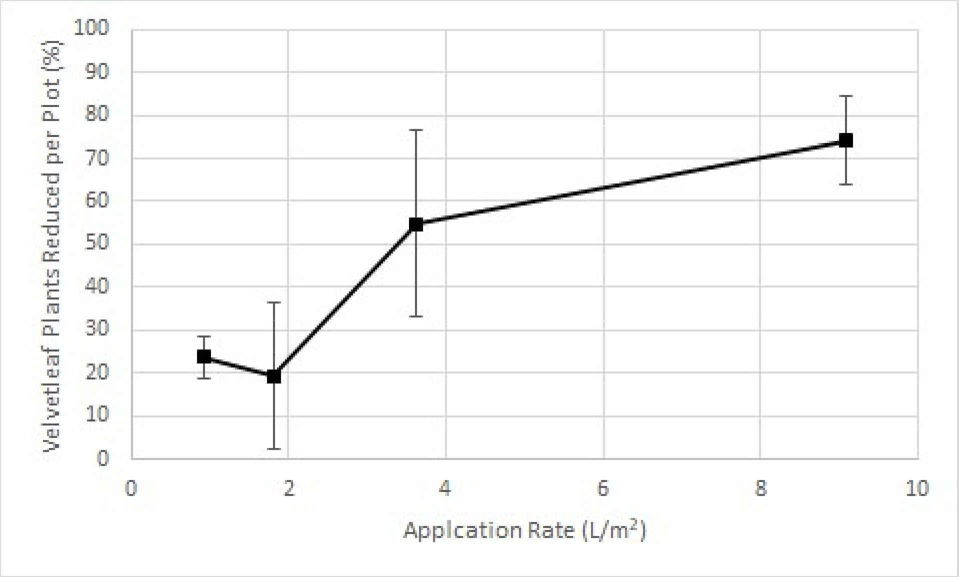

Figure 10. Mean percent reduction of velvetleaf plants per 4.5 m plot relative to a non-treated, weedy control, ± one standard error of the mean, when biofilm solution was applied at 0.91, 1.81, 3.63, and 9.08 L/m^2. The research trial was completed from August to 26 October 2017 at the University of Nebraska-Lincoln on the East Campus research farm.

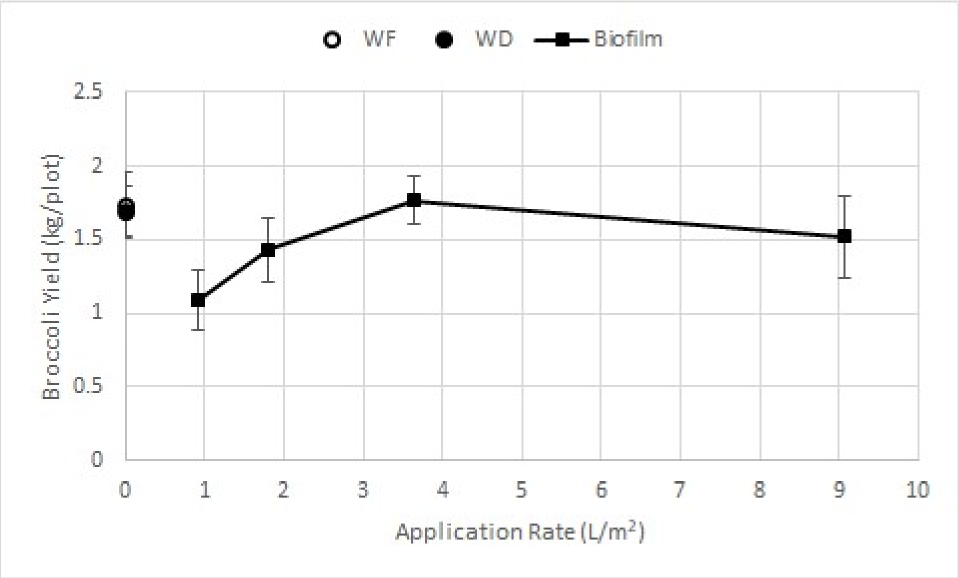

Figure 11. Mean broccoli yield (kg) per 10-plant plot, ± one standard error of the mean, of a non-treated, weed-free control, a non-treated, weedy control, and biofilm application at 0.91, 1.81, 3.63, and 9.08 L/m^2. The research trial was completed from August to 26 October 2017 at the University of Nebraska-Lincoln on the East Campus research farm.

3.4. GH Trial 2

The biofilm formulation of corn starch (6.36 g/L), glycerol (18.94 mL/L), corn gluten meal (19.90 g/L), and keratin hydrolysate (954.80 mL/L) applied as a PRE at 0.81 L/m^2 only reduced dried aboveground velvetleaf biomass by 6.63% ± 3.86% relative to a non-treated weedy control. In terms of biomass reduction, no difference was found between biofilm application rates (F[1,9] = 0.34, p = 0.57) or weed species (F[1,9] = 0.14, p = 0.71), and there was no interaction between rate and species (F[1,9] = 0.02, p = 0.90).

The lack of weed control from this formulation could likely be attributed to the inability to form a successful film on the soil surface, allowing weeds to emerge. Immirizi et al. (2009) also reported a spray mulch that displayed cracks within the first month, to which weeds were able to emerge through. To increase the solution efficacy, Warnick et al. (2006) and Adhikari et al. (2019) suggest that a thicker film layer on the soil surface increases its ability to prevent weed emergence. Due to the low viscosity of the solution tested in GH Trial 2 there was a high level of soil infiltration and minimal surface film formation. It was hypothesized that reducing the concentration of keratin hydrolysate, the only source of liquid in solution, could produce a more viscous film.

3.5. GH Trial 3

The biofilm formulation of corn starch (15.44 g/L), glycerol (30.65 mL/L), corn gluten meal (38.63 g/L), and keratin hydrolysate (926.88 mL/L) did not reduce any weed biomass relative to a non-treated, weedy control when applied as a PRE. Shattercane biomass was higher than velvetleaf biomass (F[1,9] = 5.76, p = 0.04), there was no difference in biomass between application rates (F[1,9] = 0.44, p = 0.52), and there was no interaction between rate and species (F[1,9] = 0.90, p = 0.37).

As discussed above, we hypothesized that increasing the viscosity of the film would lead to greater weed suppression. While the concentration of keratin hydrolysate was reduced in GH Trial 3, changes in weed suppression were not detected; in fact, velvetleaf biomass was increased relative to a non-treated, weedy control. This was again attributed to the lack of a cohesive mulch layer on the soil surface. Prior to another greenhouse trial, a variety of biofilm formulations were studied in the laboratory to determine the best physical properties and film forming capabilities produced from a combination of ingredients.

3.6. GH Trial 4

Lab exploration and tests showed that the five ingredient biofilm formulation of corn starch (40.5 g/L), glycerol (128.7 mL/L), corn gluten meal (20.24 g/L), isolated soy protein (20.24 g/L), and water (811 mL/L) displayed no cracking or shrinking and absorbed the highest amount of water. Thus, this formulation was selected for a greenhouse trial. An eight ingredient solution of corn starch (40.5 g/L), glycerol (128.7 mL/L), corn gluten meal (10.15 g/L), isolated soy protein (10.15 g/L), corn zein (10.15 g/L), keratin hydrolysate (374 mL/L), eggshell powder (12.2 g/L), and water (439 mL/L) was also selected for a greenhouse trial because it contained all considered ingredients and could be used to assess the effects of each (Table 2).

In the greenhouse, there was a difference in the percentage of dried aboveground velvetleaf biomass reduced per pot relative to a non-treated, weedy control (F[1,51] = 12.92, $p = 0.0007$). The eight ingredient solution reduced a greater amount of velvetleaf biomass than the five ingredient solution (Figures 12 and 13). There was also a difference in velvetleaf biomass reduction found between biofilm application times (F[2,51] = 77.77, $p < 0.0001$). A post hoc Tukey-Kramer comparison showed that PRE and POST-V0 application times were not different ($p = 0.70$), but both resulted in significantly greater reductions of velvetleaf biomass than the POST-V2 application time ($p < 0.0001$).

Although there was a difference in velvetleaf biomass reductions found between biofilm application rates (F[2,51] = 3.36, $p = 0.04$), the Tukey-Kramer test was not able to segregate among rates. Furthermore, no interaction was found between formulation and rate (F[2,51] = 1.77, $p = 0.18$), formulation and time (F[2,51] = 0.45, $p = 0.64$), time and rate (F[4,51] = 2.23, $p = 0.08$), or all three factors (F[4,51] = 1.95, $p = 0.12$).

Claramunt et al. (2019) reported that the mulch layers with the highest tensile strength and stress resistance out of the 24 blends tested were most successful in reducing seedling emergence. While those variables were not specifically measured in GH Trial 4, cracking, shrinking, and water absorption were. Unlike the findings of Claramunt et al. (2019), the solution that displayed the least amount of cracks and highest percentage of water absorbed in the lab did not perform the best in the greenhouse. While the five ingredient solution reduced velvetleaf biomass by greater than 50% at the lowest rate when applied as a PRE and POST-V0, the eight ingredient solution reduced velvetleaf biomass by greater than 70% at the lowest rate. The reason for this is unknown, but could be due to the inclusion of keratin hydrolysate in solution as it was observed to make the solution "stickier."

Furthermore, the results of this trial suggest that biofilm solution may not be able to control weeds once they develop two true leaves, but could control weeds at the cotyledon stage. When biofilm was applied as a POST, it was observed that the plant tissue displayed a bleaching effect where contact was made by the solution. This was also observed anecdotally through informal trials parallel to GH Trial 3 (Figure 14). A potential cause of this symptom could be due to a shift in water potential as a result of increased solutes, such as salt, within the biofilm solution. If the amount of salt buildup in solution was high enough it could warrant a lower water potential than that in the plant tissue (Nawaz et al. 2010). Thus, water would move from the plant to the area of higher solute concentration in an effort to pursue equilibrium. Leaf burn has been noted from foliar applied fertilizer solutions and can be more of a threat with increased salinity (Fageria et al. 2009). This could also be supported by the fact that the biofilm only worked where it contacted the plant, which suggests a contact - non-systemic - mode of action. The tissue in the area of application essentially burned off, but the plant was able to survive if enough of the surface remained uncontacted, such as those with two true leaves. The concentration of salt in solution should be measured to test this hypothesis.

Although the percentage of weed biomass reduced was greater than measured in GH Trial 2 and 3, adjustments to the formulation were still needed to improve efficacy. Similar to the findings of Immirzi et al. (2009) and Braunack et al. (2020), cracks became present as the solution dried on the soil surface and weeds were able to emerge, although they were unable to develop at a rate similar to the untreated control as they remained stunted throughout the trial. Due to the high level of cracking and soil infiltration displayed by biofilm solutions up to this point, we hypothesized that the formulation applied in a dry, granular form could allow for a solid layer to form on the soil surface with the potential to become a film when treated with water. This approach would provide the additional benefit of reducing application volume in a field scenario.

Table 2. Nine variations of biofilm formulation (with two replications) were prepared in the laboratory in October of 2018; 150 mL of each solution was poured in a 13 cm diameter petri dish and assessed for cracking and shrinking after a two week period. Water was added to the dish at 2.5 mL and after 12 hours excess water was decanted. Films were weighed before and after to determine the percent of water absorbed. All formulations contained corn starch, glycerol, and water. Two formulations were selected for a greenhouse trial (***).

Film Type*	Cracking**	Shrinking**	Water absorbed (%)
Soy-Zein	1	0	57.13 ± 0.18
Soy-Zein-Eggshells	1	1	60.20 ± 0.20
CGM-Soy***	0	0	60.94 ± 0.20
CGM-Zein	2	2	47.55 ± 0.19
CGM-Soy-Zein	2	0	46.64 ± 0.20
Ker-CGM-Zein	1	2	53.38 ± 0.20
Ker-CGM-Soy-Zein	2	0	37.96 ± 0.20
Ker-Soy-Zein-Eggshells	3	0	51.73 ± 0.20
Ker-CGM-Soy-Zein-Eggshells***	1	0	48.72 ± 0.20
*CGM=corn gluten meal; Zein=corn zein; Soy=isolated soy protein; Ker=keratin hydrolysate (from chicken feathers)			
**0=none; 1=low; 2=moderate; 3=high			
***was selected for a greenhouse trial			

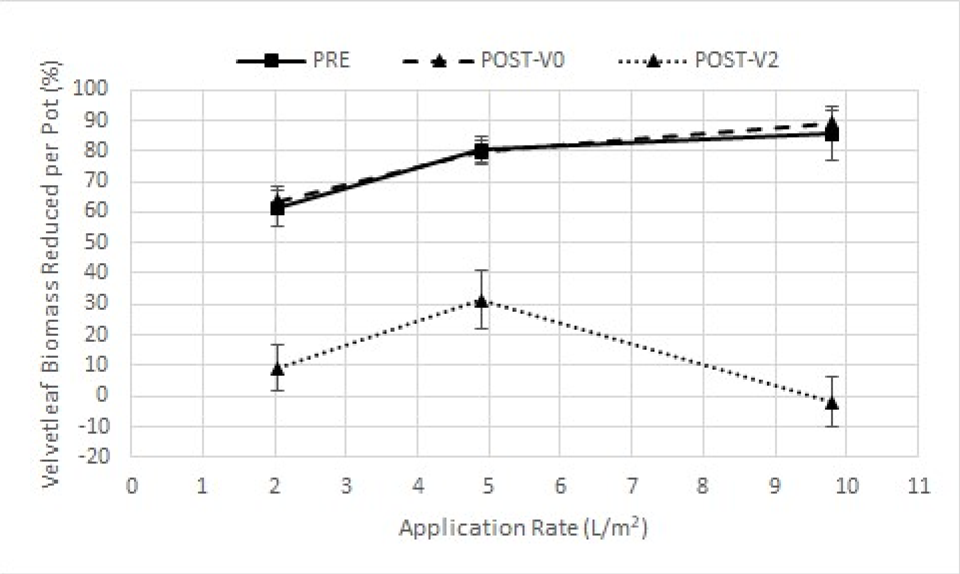

Figure 12. Mean percent reductions of dried aboveground velvetleaf biomass per 10 cm wide by 12.5 cm deep pot relative to a non-treated, weedy control, ± one standard error of the mean, when a five-ingredient biofilm solution was applied as a PRE, POST-V0, and POST V-2 at 2.04, 4.89, and 9.78 L/m^2. The research trial was completed from 27 November to 16 December 2018 at the University of Nebraska-Lincoln in Agronomy and Horticulture Greenhouse 3 on East Campus.

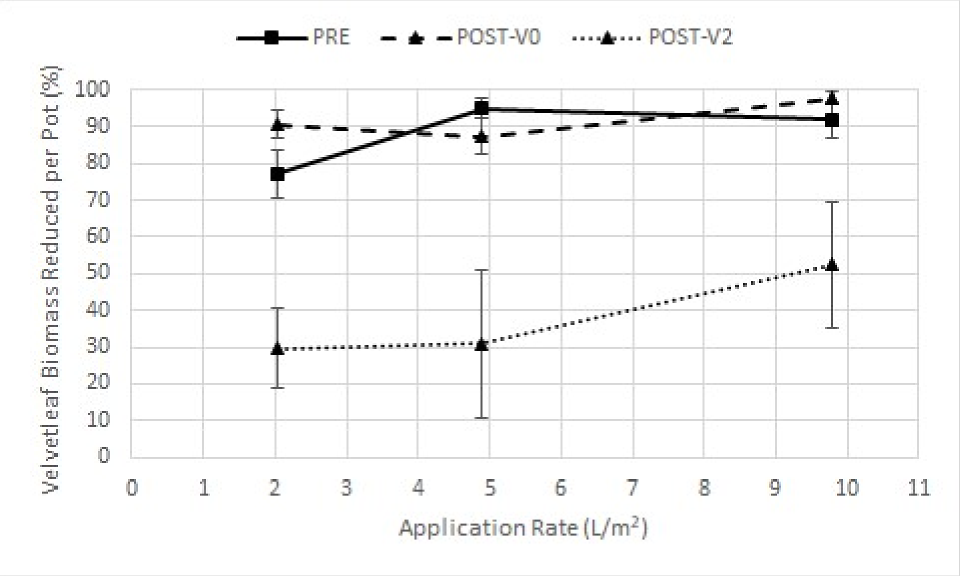

Figure 13. Mean percent reductions of dried aboveground velvetleaf biomass per 10 cm wide by 12.5 cm deep pot relative to a non-treated, weedy control, ± one standard error of the mean, when an eight-ingredient biofilm solution was applied as a PRE, POST-V0, and POST V-2 at 2.04, 4.89, and 9.78 L/m^2. The research trial was completed from 27 November to 16 December 2018 at the University of Nebraska-Lincoln in the Agronomy and Horticulture Greenhouse 3 on East Campus.

Figure 14. Velvetleaf leaf tissue bleaching as a result of biofilm contact after POST application.

3.7. GH Trial 5

The dry, extruded, and crushed bio-based formulations were found to be different in the percentage of dried aboveground velvetleaf biomass reduced per 10 cm wide by 12.5 cm deep pot relative to a non-treated, weedy control (F[1,51] = 14.62, p = 0.0004) (Figures 15 and 16). The formulation of corn starch (375 g/kg), glycerol (250.5 g/kg), corn gluten meal (187.5 g/kg) and corn zein (187.5 g/kg) reduced a greater percentage of velvetleaf biomass per pot than the formulation of corn starch (375 g/kg), glycerol (250.5 g/kg), corn gluten meal (187.5 g/kg) and isolated soy protein (187.5 g/kg). There was also a difference in biomass reductions between granule size (F[2,51] = 4.28, p = 0.02). A post hoc Tukey-Kramer comparison showed that the extruded products > 1.05 mm led to greater reductions in velvetleaf biomass than the raw, un-extruded, powder (p = 0.02).

No difference was found between application rates (F[2,51] = 2.54, p = 0.09). Additionally, there was no interaction between formulation and particle size (F[2,51] = 1.16, p = 0.32), between formulation and application rate (F[2,51] = 0.21, p = 0.81), between particle size and application rate (F[4,51] = 1.08, p = 0.38), or between all three factors (F[4,51] = 0.73, p = 0.57).

Christians (1994) reported that the application of corn gluten meal at 777 g/m^2 was sufficient to stop the establishment of creeping bentgrass and corn gluten meal showed the greatest inhibitory effects versus corn starch, dried corn germ, corn seed fiber, and cornmeal. Bingaman and Christians (1995) reported that corn gluten meal was only able to reduce velvetleaf relative to an untreated control by 35% when applied at 973 g/m^2, although velvetleaf was the least controlled species of the 22 tested, some of which were completely reduced: purslane, dandelion, green foxtail, and black nightshade. In general, the corn-based formulation in GH Trial 5 demonstrated similar reductions in velvetleaf biomass and resulted in a greater efficacy than the formulation containing isolated soy protein. However, applying the bio-based formulation in a dry granular form versus as a liquid reduced the efficacy relative to the formulations tested in GH Trial 4 by an average of 30%. This is likely because the dry form was unable to form a cohesive film on the soil surface and was thus unable to prevent weeds from penetrating the granular surface. To improve the efficacy while forming a mulch layer, results suggest that bio-based formulations should be applied as a liquid. Reducing the amount of liquid in solution would be tested based on the results of the previously mentioned Warnick et al. (2006) and Braunack et al. (2020) who concluded that a thicker mulch layer on the soil surface helps reduce seedling emergence.

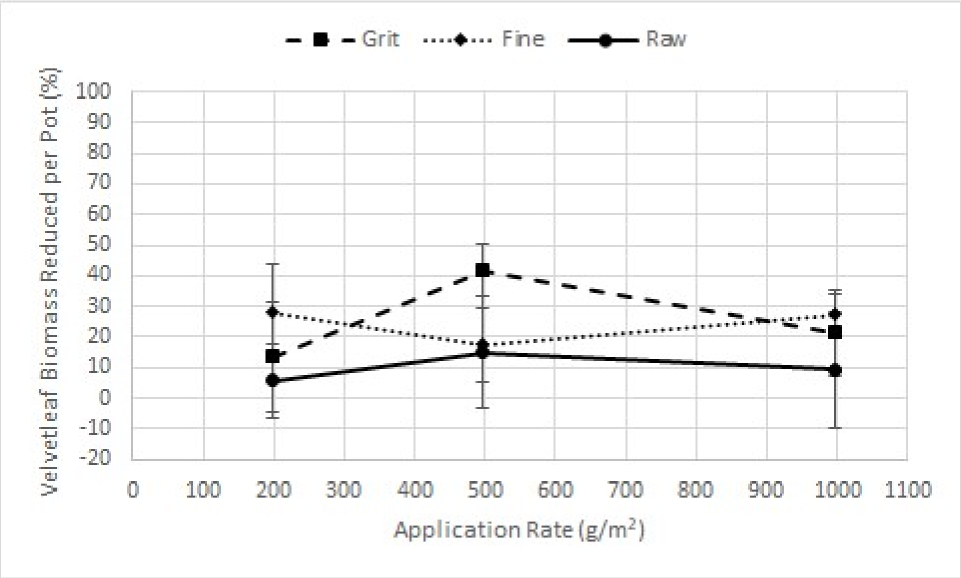

Figure 15. Mean percent reductions of dried aboveground velvetleaf biomass relative to a non-treated, weedy control, ± one standard error of the mean, when bio-based granules of corn starch, glycerol, isolated soy protein and corn gluten meal were applied as extruded products (Grit [> 1.05 mm] or Fine [< 850 µm]) or a raw powder at 198.6, 497.0, and 997.8 g/m^2 as a PRE. The research trial was completed from 4 February to 25 February 2019 at the University of Nebraska-Lincoln in the Agronomy and Horticulture Greenhouse 3 on East Campus.

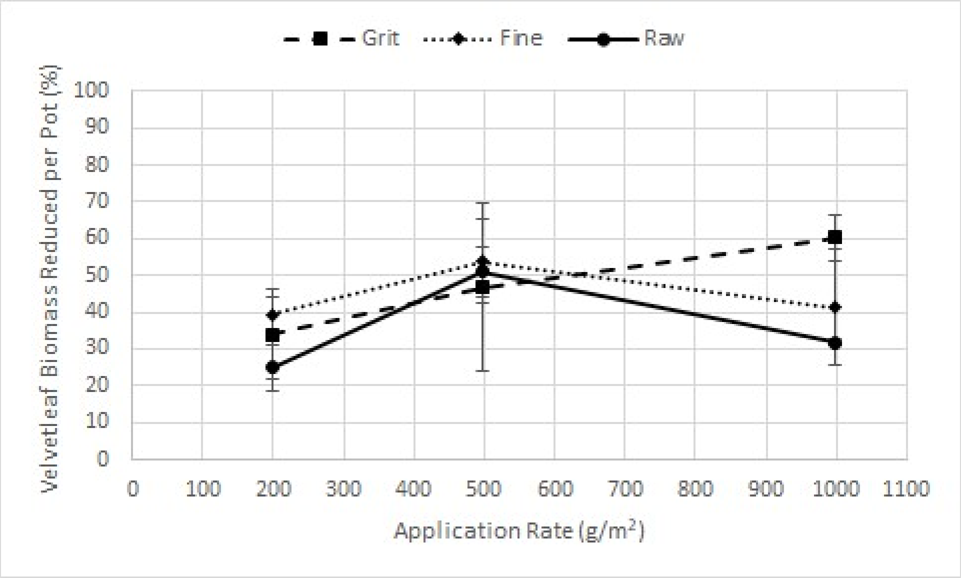

Figure 16. Mean percent reductions of dried aboveground velvetleaf biomass relative to a non-treated, weedy control, ± one standard error of the mean, when bio-based granules of corn starch, glycerol, corn gluten meal, and corn zein were applied as extruded products (Grit [> 1.05 mm] or Fine [< 850 µm]) or a raw powder at 198.6, 497.0, and 997.8 g/m^2 as a PRE. The research trial was completed from 4 February to 25 February 2019 at the University of Nebraska-Lincoln in the Agronomy and Horticulture Greenhouse 3 on East Campus.

3.8. GH Trial 6

Dried aboveground velvetleaf biomass was reduced relative to a non-treated, weedy control at a higher level when the biofilm solution of corn starch (40.91 g/L), glycerol (129.98 mL/L), keratin hydrolysate (515.97 mL/L), corn zein (13.96 g/L), isolated soy protein (13.96 g/L), and water depending on viscosity level (303.03 mL/L, 190 mL/L, or 0 mL/L) was applied as a POST-V2 versus as a PRE (Figure 17). There were no differences in biomass reduction between viscosity levels. When applied as a POST-V2, velvetleaf biomass was reduced by 100% ± 0% by the high and medium viscosity solutions and by 97.16% ± 2.84% by the low viscosity solution. When applied as a PRE, velvetleaf biomass was reduced by 90.69% ± 2.38% by the high viscosity solution, by 81.99% ± 6.73% by the medium viscosity solution, and by 83.38% ± 5.11% by the low viscosity solution.

Increasing the viscosity of the formulation relative to that tested in GH Trial 4 increased or maintained weed suppressive abilities by PRE application and increased the efficacy of POST-V2 application by greater than 50%. Adhikari et al. (2019), Warnick et al. (2006), and Braunack et al. (2020) all concluded that increasing the viscosity of solution to result in a more successful film formation on the soil surface was correlated with greater weed control as it prevented weed penetration. However, this theory may not explain the reason for reduced weed biomass from POST treatment. Nonetheless, the formulations tested in GH Trial 6 demonstrated the most promising film properties and greatest efficacy of the film formulations tested to this point and thus were tested in a field trial to measure how they respond to natural environmental conditions. The medium viscosity solution was the best candidate for a field trial because a greater volume of solution can be applied at the same ingredient concentration relative to the high viscosity solution while it is more viscous than the low viscosity solution.

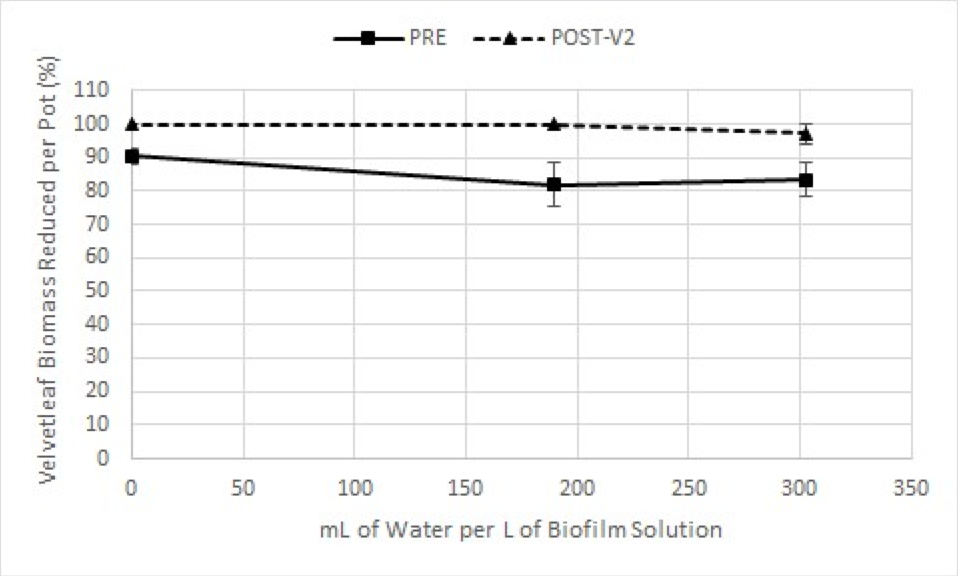

Figure 17. Mean percent reductions of dried aboveground velvetleaf biomass per 10 cm wide by 12.5 cm deep pot relative to a non-treated, weedy control, ± one standard error of the mean, when a low viscosity solution (303 mL water/L), a medium viscosity solution (190.4 mL water/L), and a high viscosity solution (0 mL water/L) were applied as a PRE and POST-V2 at a standardized rate of 6.11 L/m^2. The research trial was completed from 28 March to 29 April 2019 at the University of Nebraska-Lincoln in the Agronomy and Horticulture Greenhouse 3 on East Campus.

3.9. Field Trial 3

Dried aboveground velvetleaf and shattercane biomass per 2.3 m plot was only reduced when the formulation of corn starch (47.55 g/L), glycerol (151.08 mL/L), keratin hydrolysate (599.76 mL/L), corn zein (16.22 g/L), isolated soy protein (16.22 g/L), and water (190.4 mL/L) was applied as a PRE. Application at 4.07 L/m^2 reduced weed biomass by 22.19% ± 18.41% and application at 8.15 L/m^2 reduced weed biomass by 42.83% ± 12.60%. There were no differences in the amount of biomass reduced between application rates (F[1,9] = 0.05, p = 0.83) or application times (F[1,9] = 1.76, p = 0.22) and there was no interaction between rate and time (F[1,9] = 1.05, p = 0.33) (Figure 18).

There was a difference in pepper yields (kg) per five-plant plot between treatments (F[5,15] = 9.28, p = 0.0003) (Figure 19). A post hoc Tukey-Kramer comparison showed that yields in the non-treated, weed-free control plots (20.08 ± 1.16 kg) were higher than in all other treatment plots ($p < 0.005$). There was no difference in yield between the non-treated, weedy control plots and all biofilm treatment plots ($p > 0.2$).

The reduction in crop yields from biofilm application relative to a bare soil control are congruent with the findings of Russo (1992), who reported that a spray-on wood fiber based mulch degraded quickly, allowing weeds to emerge freely, which limited eggplant yields. The lack of a solid film formation on the soil surface due to the low viscosity of the solution tested in Field Trial 3, also allowed for competitive weed growth. As a result, pepper yields were not improved relative to an untreated, weedy control. Immirizi et al. (2009) reported that the solution they tested displayed cracks within the first month of application and weeds emerged within them, however the yields of strawberries were not different between treated and untreated plots. Adhikari et al. (2019) found that a low viscosity solution undergoes wicking and thus results in lower membrane formations, but increasing the viscosity of the solution alleviated these effects.

In this study, the viscosity of the solution was lower than anticipated because the solution was blended to achieve homogeneity and it was observed that the physical characteristics of the film changed as a result of the process. Thus, it was suggested that the viscosity of the solution be increased for another field trial. This would be achieved by removing excess water from the formulation and potentially increasing the concentration of corn starch as starch can be used as a thickening agent. The high viscosity solution (0 ml water/L) from GH Trial 6 would be re-evaluated for potential use in Field Trial 4.

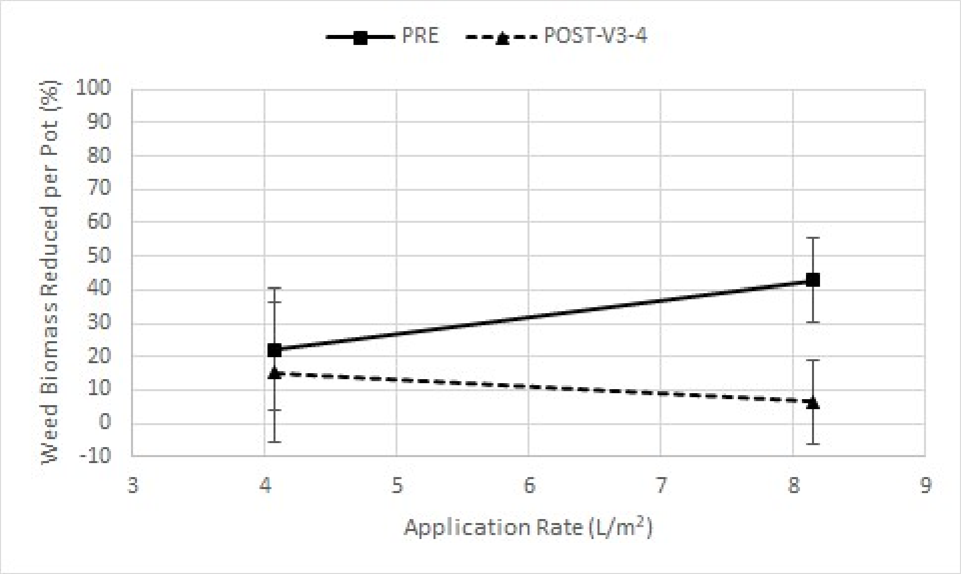

Figure 18. Mean percent reductions of dried aboveground velvetleaf and shattercane biomass per 2.3 m plot relative to a non-treated, weedy control, ± one standard error of the mean, when biofilm solution was applied as a PRE and POST-V3-4 at 4.07 and 8.15 L/m^2. The research trial was completed from 6 June to 11 October 2019 at the University of Nebraska-Lincoln on the East Campus research farm.

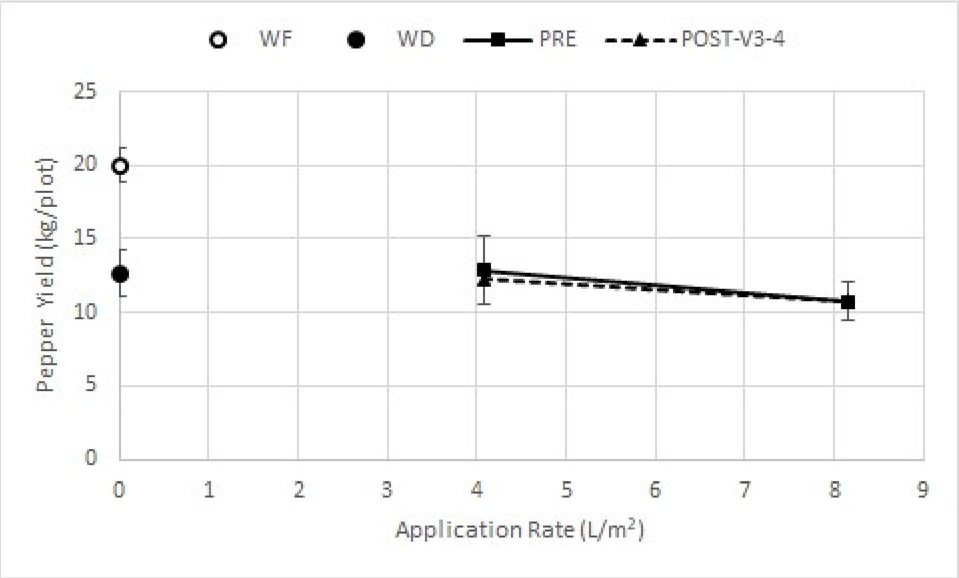

Figure 19. Mean pepper yield (kg) per 5-plant plot, ± one standard error of the mean, of a non-treated, weed-free control, a non-treated, weedy control, and PRE and POST V-3-4 biofilm solution application at 4.07 and 8.15 L/m^2. The research trial was completed from 6 June to 11 October 2019 at the University of Nebraska-Lincoln on the East Campus research farm.

3.10. Field Trial 4

The biofilm formulation of corn starch (72.81 g/L), glycerol (184.73 mL/L), keratin hydrolysate (733.32 mL/L), corn zein (19.84 g/L), and isolated soy protein (19.84 g/L) reduced dried aboveground mustard biomass per 2.1 m plot relative to the non-treated, weedy control by 98.74% ± 0.93% when applied as a PRE at 4.07 L/m^2, by 99.63% ± 0.37% when applied as a PRE at 6.11 L/m^2, and by 96.45% ± 2.40% when applied as a POST-V3-4 at 6.11 L/m^2. No difference was found in the percentage of velvetleaf biomass reduced between treatments (F[2,6] = 1.58, p = 0.28) (Figure 20), and no difference in kale yield (g/plant) was found between any of the treatments (F[4,12] = 2.32; p = 0.12) (Figure 21).

The removal of excess water along with a 25% increase in the concentration of corn starch in the formulation compared to that used in Field Trial 3 was enough to increase the viscosity to an effective level while still ensuring it could be applied as a liquid. As supported by the findings of Claramunt et al. (2019), Warnick et al. (2006), Braunack et al. (2020), Russo (1992), and Adhikari et al. (2019), a more viscous solution was more successful at forming a solid film layer on the soil surface, thus enabling the prevention of seedling emergence. The biofilm tested in Field Trial 4 reduced weed biomass by a greater percentage than reported in any of the literature on liquid mulch films. Claramnunt et al. (2019) reported a weed seedling reduction of 92.9%, Massa et al. (2019) reported a reduction in biomass of 74%, and Giaccone et al. (2018) reported that dry biomass was lower in sprayable mulch treated plots relative to a bare soil control and a herbicide treated plot. However, in Field Trial 4, kale yields were not increased in treated plots relative to a non-treated, weedy control, which was not the case for Massa et al. (2019) who found dry biomass of test plants to be improved from an untreated control. Giaccone et al. (2018) reported no difference in dry biomass between treated and untreated containers.

While an increased viscosity supports the results for the film used as a PRE, greater than 95% biomass reductions were recorded when biofilm was applied as a POST-V3-4. It was observed that plant tissue displayed a bleaching and shriveling effect when contacted by the biofilm solutions (Figures 14 and 22). As discussed in GH Trial 4, the concentration of salt in solution still needs to be measured to determine if a change in solute potential is present when biofilm solution makes contact with plant tissue; this could also have an effect on soil health and crop performance.

Despite the fact that weeds were controlled, kale yields were not improved relative to a weedy control. This could be attributed to the effect of nitrogen immobilization. This effect was also observed anecdotally in an unofficial greenhouse trial in which lettuce plants were subjected to the application of the low and high viscosity biofilm formulations from GH Trial 6 around the base of the plant on the soil surface. Lettuce plants displayed leaf burning and reductions in size relative to an untreated control upon application (Figure 23). However, after application of a 20-10-20 NPK general purpose fertilizer at 200 ppm, lettuce plants were able to recover and the final biomass was not different from the untreated control.

Nitrogen immobilization is a temporary effect that occurs when an organic substance which possesses a C:N ratio greater than approximately 20 is added to the soil. To break down organic matter, soil microbes need nitrogen and because the organic matter is lacking relative to the amount of carbon, microbes obtain nitrogen from an outside source which is usually plant available, thus depriving plants until the organic matter is decomposed. There is reason to believe this is a possible result from application of the biofilm solution because the majority of ingredients are carbon-based (e.g. corn starch and glycerol). The C:N ratio should be determined in solution to ultimately determine if this is the case. Potential adjustments to reduce this ratio could include increasing nitrogen in solution or in the field prior to application. Nonetheless, more trials should be conducted to study the effect of an adjusted formulation on crop yields.

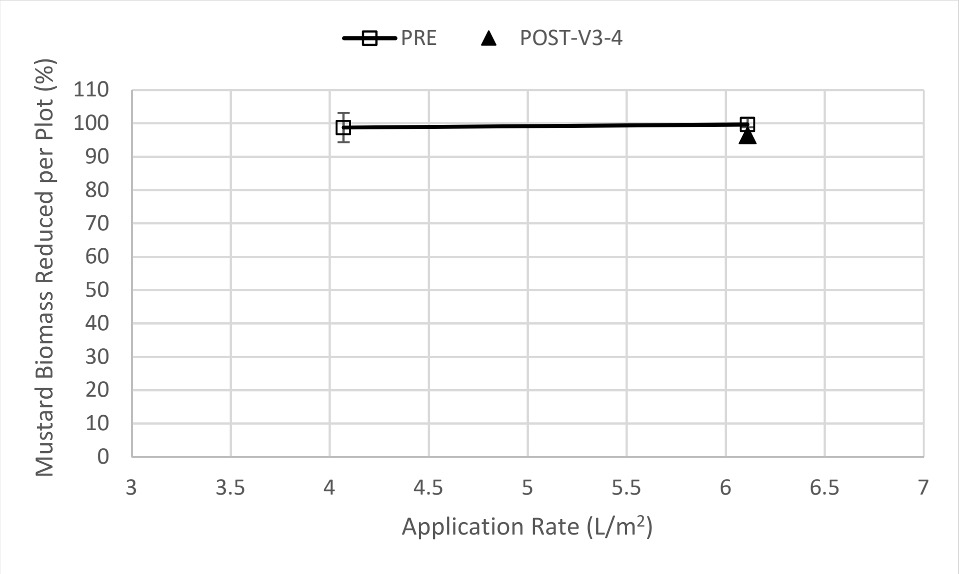

Figure 20. Mean percent reductions of dried aboveground mustard biomass per 2.1 m plot relative to a non-treated, weedy control, ± one standard error of the mean, when biofilm solution was applied as a PRE at 4.07 and 6.11 L/m^2 and as a POST-V3-4 at 6.11 L/m^2. The research trial was completed from 19 August to 18 October 2019 at the University of Nebraska-Lincoln on the East Campus research farm.

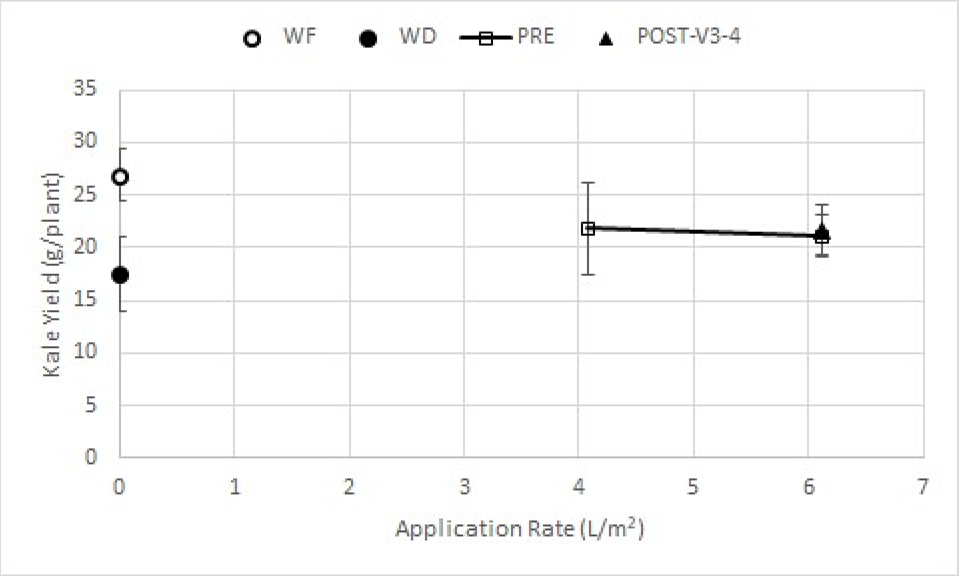

Figure 21. Mean kale yield (g/plant) per 7-plant plot, ± one standard error of the mean, of a non-treated, weed-free control, a non-treated, weedy control, and PRE biofilm solution application at 4.07 and 6.11 L/m^2 and POST-V3-4 biofilm solution application at 6.11 L/m^2. The research trial was completed from 19 August to 18 October 2019 at the University of Nebraska-Lincoln on the East Campus research farm.

Figure 22. Bleaching and weathering of velvetleaf plant tissue where contact was made by biofilm solution.

Figure 23. Effect of high and low viscosity biofilm solutions from GH Trial 6 on lettuce growth relative to an untreated control.

4. Conclusion

Biofilms show the potential to be used as a mulch for specialty crop systems due to their ability to suppress weed growth when applied as a PRE and even as a POST. In general, biofilms were most effective when the viscosity of the solution was increased. This is similar to findings in other research and can be attributed to the ability of a more viscous solution to form a cohesive layer on the soil surface. The formulation containing corn starch, glycerol, keratin hydrolysate, corn zein, and isolated soy protein tested in Field Trial 4 displayed the greatest ability to form an effective film layer. Additionally, when applied as a PRE, biomass was reduced by greater than 97% relative to a non-treated control and by greater than 94% when applied as a POST.

However, more research is needed to evaluate the effect of biofilms on crop yields as yields were not improved relative to a non-treated, weedy control in any of the field trials. As discussed above, solution salinity and the C:N ratio in solution could play a role and need to be evaluated prior to future research trials to gain a greater understanding of the effects biofilms could have on plant tissue and in soil interactions.

Furthermore, application of the formulation from Field Trial 4 at 4.07 L/m^2 in the designated 0.63 m^2 area would cost \$16.46/m^2 (using small-batch, research-grade ingredients) whereas the cost of conventional plastic mulch films area approximately \$0.50/m^2. The overall cost of the biofilm solution could potentially be reduced through the exploration of alternative protein sources (the cost of isolated soy protein and corn zein is \$22/kg and \$35/kg, respectively) or starch sources (the cost of corn starch is \$20/kg). Ultimately, prior to proposing biofilms as a weed management strategy for specialty crop producers, profitability needs to be measured and compared to conventional plastic films and other biodegradable mulches.

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