Biobased Sprayable Mulch Films Suppressed Annual Weeds in Vegetable Crops

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KEYWORDS. ***Abutilon theophrasti*, *Brassica oleracea* var. *italica*, *Brassica oleracea* var. *sabellica*, broccoli, *Capsicum annuum*, *Guillenia flavescens*, kale, pepper, shattercane, *Solanum lycopersicum*, *Sorghum bicolor*, tomato, velvetleaf, yellow mustard.**

ABSTRACT. **Biobased sprayable mulch (BSM) films are a potential alternative to herbicides, polyethylene plastic mulch film, and hand weeding for specialty crops. We developed a series of BSM films using locally available biomaterials [including corn (*Zea mays*) starch, glycerol, keratin hydrolysate, corn gluten meal, corn zein, eggshells, and isolated soy (*Glycine max*) protein] and tested their effects on weeds and crop yield during a total of seven greenhouse or field trials between 2017 and 2019 in Nebraska, USA. Application rates of BSM films applied in pots (greenhouse), planting holes in plastic film (field), or bed tops (field) ranged from 0.9 to 18.2 L·m^{-2}; they were applied before and after the emergence of weeds. Weed control efficacy was variable, and results of greenhouse pots were rarely replicated under field conditions. Increasing the viscosity of the final suspension tested [BSM7; a mix of corn starch (72.8 g·L^{-1}), glycerol (184.7 mL·L^{-1}), keratin hydrolysate (733.3 mL·L^{-1}), corn zein (19.8 g·L^{-1}), and isolated soy protein (19.8 g·L^{-1})] reduced weed biomass by more than 96% in field-grown kale (*Brassica oleracea* var. *sabellica*) when applied to bare soil bed tops before or after weed emergence, but kale yield in treated plots was not different from the weedy control. The results demonstrated the potential for postemergence applications of BSM films, which increase application timing flexibility for growers. Further research is needed to explore the effects of BSM films on soil properties and crop physiology and yield.**

Polyethylene plastic mulch film is commonly used for vegetable and small fruit production to help manage weeds, and biodegradable mulch films are being developed as a sustainable alternative to polyethylene (Tofanelli and Wortman 2020). Biodegradable mulches are made from biobased or biodegradable polymers and can be left in the soil to decompose after their useful life. Most manufactured mulch films are available as rolls with a fixed width and length that are applied with mechanical mulch layers. However, one alternative is a biobased sprayable mulch (BSM) film that can allow for greater flexibility in application patterns and timing (Filipović et al. 2020). For example, a BSM could be applied with modified spray application technologies (e.g., pumps and nozzles that can handle higher-viscosity BSM suspensions) in narrow bands within an established crop row and used in tandem with other between-row weed management tactics (e.g., tillage or flame-weeding). Another possible application of BSM is for management of weeds that emerge from within planting holes made in mulch films and fabrics. Plastic, biodegradable, and paper weed barriers are effective for suppressing weeds on specialty crop bed tops, but weed emergence through planting holes is common (Runham et al. 1998; Weber 2003). Without intervention, these weeds can reduce yield and contribute to the weed seedbank. Previous research suggested that ambient weeds [primarily pigweed (*Amaranthus* sp.) and foxtail (*Setaria* sp.)] left unmanaged in the planting hole of plasticulture vegetables, including tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*), reduced yield by nearly 33% (Wortman 2015).

Sprayable mulch films can be formulated from polysaccharides, proteins, and polyurethane polymers (Filipović et al. 2020). Previous research has focused on BSM films derived from the following: wood fiber (Russo 1993); shredded newspaper (Puka-Beals and Gramig 2021; Warnick et al. 2006); sodium alginate (Immirizi et al. 2009); a mix of corn (*Zea mays*), potato (*Solanum tuberosum*), wheat (*Triticum*

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
7.8125	fl oz/gal	mL·L^{-1}	0.1280
0.3048	ft	m	3.2808
0.0929	ft^2	m^2	10.7639
3.7854	gal	L	0.2642
40.7458	gal/ft^2	L·m^{-2}	0.0245
2.54	inch(es)	cm	0.3937
6.4516	inch^2	cm^2	0.1550
0.4536	lb	kg	2.2046
0.5425	lb/yard^2	kg·m^{-2}	1.8433
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274×10^{-5}
0.3052	oz/ft^2	kg·m^{-2}	3.2771
7.4892	oz/gal	g·L^{-1}	0.1335
33.9057	oz/yard^2	g·m^{-2}	0.0295
3.3906×10^4	oz/yard^2	mg·m^{-2}	2.9494×10^{-5}
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

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aestivum), and cellulose (Shen and Zheng 2017); chitosan and cellulose (Giaccone et al. 2018); blends of paper pulp, wheat straw, rice (*Oryza sativa*) hulls, and rice bran (Claramunt et al. 2020); aqueous mixtures of cotton (*Gossypium hirsutum*) and cellulose fibers, gums, starches, surfactants, and saponins (Masiunas et al. 2003); and aqueous dispersions of polyurethane and cellulose (Braunack et al. 2020a). Common challenges and limitations of these BSM films have included premature degradation, shrinking, drying, cracking, infiltration, or wicking into soil pore space, as well as insufficient weed suppression (Adhikari et al. 2019; Braunack et al. 2020a; Giaccone et al. 2018; Immirzi et al. 2009; Russo 1993; Shen and Zheng 2017). However, observed benefits of BSM films have included significant weed suppression (Braunack et al. 2020a; Claramunt et al. 2020; Giaccone et al. 2018; Shen and Zheng 2017; Warnick et al. 2006), increased soil moisture (Adhikari et al. 2019; Braunack et al. 2020a) and temperature (Braunack et al. 2021), and crop yield and plant growth comparable to polyethylene plastic mulch film or bare soil (Braunack et al. 2020b, 2021; Immirzi et al. 2009; Shen and Zheng 2017).

There are many possible biobased materials that can be included in BSM; however, generally, ideal ingredients are inexpensive, locally available, renewable, and contribute to desirable physical, mechanical, and agronomic properties of the film. Starch is one of the most common ingredients in solid biobased mulches and BSMs (Carvalho 2008). Corn starch is particularly valuable because of the high amylose concentration, which contributes to higher tensile strength and elongation in films (Lourdin et al. 1995; Swinkels 1985). Starch by itself is hydrophilic and brittle (Lloyd and Kirst 1963), but a plasticizer can be added to promote flexibility (Zhang and Han 2006). Glycerol, derived from 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 increased film thickness, flexibility, and thermal stability, and it decreased water solubility.

Chicken (*Gallus gallus domesticus*) feathers are a biodegradable, renewable, accessible, and inexpensive biomaterial, especially in Nebraska, where there has been recent growth in the poultry processing industry (Purdum and Koelsch 2018). Keratin, a fibrous structural protein found in chicken feathers, has bioplastic applications (Ramakrishnan et al. 2018). Chicken feathers are made of 90% or more crude keratin protein, but they must be chemically treated to release keratin from the rigid feather structure before use in film formulations (Schrooyen et al. 2001; Virtanen et al. 2016). Biodegradable films from chicken feathers are usually brittle; however, like starch, the addition of a plasticizer (e.g., glycerol) could significantly improve film properties (Tanabe et al. 2002).

Corn gluten meal is a cost-effective protein that has been successfully used to produce BSM films after mixing with water, glycerol, and other plasticizers (Di Gioia and Guilbert 1999). Corn gluten meal in the BSM may further contribute to weed suppression by chemically inhibiting root growth of at least 22 different weed species (Bingaman and Christians 1995; Christians 1991). Corn zein, which is extracted from corn gluten meal, can form a tough, flexible, hydrophobic film, particularly when blended with other biobased materials (Shukla and Cheryan 2001). Several studies have investigated the use of corn zein in solid films (Cho et al. 2010; Parris et al. 2004; Zhang and Zhao 2017) and found that zein-based solid mulch films increased tomato growth and conserved soil moisture. However, the use of corn zein in a sprayable film remains unexplored.

Isolated soy (*Glycine max*) protein has been investigated as an ingredient in biodegradable films (Kim et al. 2002; Rhim et al. 2000), but it is often limited by weak mechanical properties and moisture sensitivity of the films. However, mixing soy protein isolate (70%) with starch (30%) can improve the mechanical and barrier properties of the film (Soliman et al. 2007). Like corn gluten meal, soy protein isolate has been shown to suppress weeds by influencing microbial communities and free ammonia in soil (Hoagland et al. 2008; Yang and Lu 2010).

The overall goal of this research was to develop novel BSM films using locally available biomaterials that are optimized for weed suppression and vegetable crop performance. To accomplish this goal, we embarked on an iterative design process whereby BSM films were developed, tested on weeds and crops in the greenhouse and field, reformulated to address observed pitfalls, and tested again.

Materials and methods

General laboratory methods for preparing films. The BSM films of various formulations were prepared immediately before testing for weed efficacy in greenhouse and field trials. The general procedure (Ali et al. 2004) for preparing films included the following: mixing corn starch with water or keratin hydrolysate and boiling; mixing glycerol, protein source, and water; bringing the starch suspension to a boil again; adding concentrated sulfuric acid (H_2SO_4) to adjust the pH to between 6.8 and 7.5; blending to homogenize; and cooling at 4 °C for a minimum of 24 h before application.

Formulations were prepared in 4-L glass beakers, and heat was applied using hotplates. Keratin hydrolysate was prepared by soaking 30 $g \cdot L^{-1}$ of raw and clean chicken feathers in 0.8 M sodium hydroxide (NaOH) for 48 to 72 h. The remaining feather residue was removed via filtration through glass wool. The pH of the chicken feather hydrolysate is more than 13, and applying a suspension with a high pH to soil could limit plant availability of some essential nutrients; therefore, H_2SO_4 is added to the film formulation to adjust pH. Specific ingredients and quantities of each per liter of BSM suspension were specific to the objectives of each trial and are described in detail.

General greenhouse methods for testing films. Three greenhouse experiments were conducted between 2017 and 2019, at the University of Nebraska Plant Care Facility (Lincoln, NE, USA). All experiments used a randomized complete block design with four replications of each treatment, including a nontreated weedy control. Black plastic pots (diameter, 4 inches; depth, 5 inches) were filled with a steam-pasteurized soil mix composed of vermiculite, sand, pulverized top soil, and peat (1:1:1.2:2 ratio by volume) to within 0.5 inches from the top. Twenty seeds of velvetleaf (*Abutilon theophrasti*) or shattercane (*Sorghum bicolor*)—summer annual weeds common in eastern Nebraska, USA—were placed on the surface and covered with 0.5 inches of soil mix. Weed seeds had been previously stored in a refrigerator at 2 °C for less than 5 years before use. Velvetleaf seeds were stratified in a 70 °C water bath for 60 s before planting to break physiological dormancy (Ravlic et al. 2015).

Prepared BSM suspensions specific to each trial were applied uniformly to pots by either a calibrated hand-pump sprayer or a graduated cylinder. After the application of BSM films, the pots were not watered for 24 h to allow suspensions to dry and form a solid film. After 24 h, pots were watered to field capacity daily. A weedy control was included for both the before weed emergence (PRE) and after weed emergence (POST) treatments. During trials including BSM films applied as a POST treatment, weed seedlings were thinned to three per pot at the cotyledon stage before application. Emerged weeds were counted weekly. Aboveground weed biomass was measured in each pot ≈1 month after weeds were planted by cutting plants at the soil surface, drying at 65 °C to a constant weight, and weighing.

GENERAL FIELD METHODS FOR TESTING FILMS. Four field experiments were conducted at the University of Nebraska–Lincoln East Campus Research Farm in Lincoln, NE, USA, between 2017 and 2019 (lat. 40.84°N, long. 96.66°W). All trials were arranged in a randomized complete block design and included four replications of all treatments, including a weedy and weed-free control. Consistent with greenhouse trials, velvetleaf or shattercane seeds were used as model summer annual weeds and seeded (at densities specific to each trial) in each plot before BSM applications. Suspensions were applied (in the planting hole or banded in the row, depending on the trial) using a calibrated hand-pump sprayer or a graduated cylinder when the forecast excluded significant chances of precipitation for at least 24 h. After application, all crops were drip-irrigated regularly (between precipitation events) to prevent water-limiting crop stress (the drip line was positioned adjacent to the BSM application area). Weed-free control plots were hand-weeded weekly to remove all weeds, and no BSM was applied. Aboveground weed biomass was harvested before weeds produced mature seed by cutting plants at the soil surface, drying at 65 °C to a constant weight, and weighing. Key details of all greenhouse and field trials are summarized in Table 1 and described fully.

TRIAL 1 GREENHOUSE METHODS: PROOF OF CONCEPT TESTING. The objective of trial 1, which was conducted in Jan and Feb 2017, was to demonstrate the proof of concept for using a starch-based BSM to suppress annual weed emergence. The BSM1 formulation tested included the following: corn starch, 40.5 $g \cdot L^{-1}$; glycerol, 128.6 $mL \cdot L^{-1}$; isolated soy protein, 40.5 $g \cdot L^{-1}$; and water, 810 $mL \cdot L^{-1}$. Velvetleaf was the only weed species used in this trial. The application rate was the only treatment factor; these rates were 0.9, 1.8, 4.5, 9.1, and 18.2 $L \cdot m^{-2}$. The BSM was applied to pots within 24 h of seeding velvetleaf, and weeds were counted after 28 d.

TRIAL 2 FIELD METHODS: WEED CONTROL EFFICACY IN TOMATO. The objectives of trial 2 were to evaluate whether the benefits of BSM1 from trial 1 would translate from the greenhouse to the field and to determine crop tolerance to in-row applications of BSM1 in a plasticulture production system. The application rate was the only treatment factor; these rates were 0.9, 1.8, 3.6, and 9.1 $L \cdot m^{-2}$ (18.2 $L \cdot m^{-2}$ was eliminated after consideration of the results of trial 1).

Table 1. Experimental details of the seven trials, including environments, bio-based sprayable mulch (BSM) films, factors (BSM rates, viscosity, and timing), and species tested, and corresponding tables of results.

	Trial no.						
	1	2	3	4	5	6	7
Environment							
Greenhouse	x			x	x		
Field		x	x			x	x
BSM films tested (key ingredients)[i]							
BSM1 (1, 3, 4, 8)	x	x	x				
BSM2 (1, 2, 3, 4, 8)				x			
BSM3 (1, 2, 3, 4, 5, 6, 7, 8)				x			
BSM4 (1, 3, 4, 5, 6, 8)					x		
BSM5 (1, 3, 4, 5, 6, 8)					x	x	
BSM6 (1, 3, 4, 5, 6)					x		
BSM7 (1, 3, 4, 5, 6)							x
Multiple rates tested	x	x	x	x		x	x
Viscosity tested					x		
Timing tested							
Preemergence		x	x	x	x	x	x
Postemergence				x	x	x	x
Tested species							
Weeds							
Velvetleaf	x	x	x	x	x	x	
Shattercane						x	
Yellow mustard							x
Crops							
Tomato in plastic (holes)[ii]		x					
Broccoli in plastic (holes)			x				
Pepper without plastic						x	
Kale without plastic							x
Corresponding table of results	None	Table 2	Table 3	Table 4	None	Table 5	Table 6

[i] Key ingredients included the following: 1 = corn starch; 2 = corn gluten meal; 3 = isolated soy protein; 4 = glycerol; 5 = corn zein; 6 = keratin hydrolysate; 7 = eggshell powder; 8 = water.

[ii] Tomato and broccoli were grown in a plastic mulch film, with BSM films applied to the bare soil in the planting hole. Pepper and kale were grown without plastic mulch film, with BSM films applied to the area between plants within rows.

Raised beds [2.5 ft (width) × 5 inches (height)] were shaped and black plastic mulch films were laid with one line of drip tape beneath the plastic film for irrigation. 'BHN 589' tomato plants were started in the greenhouse in late March and transplanted to plastic film in early May 2017. Plants were in a single row on bed tops with 18-inch spacing between plants in a row. Each replicate plot contained 10 plants, and the planting holes—the 10- to 15-inch^2 bare soil area around each transplanted tomato—were the target of BSM application. Twenty velvetleaf seeds were sown within each planting hole (excluding the weed-free control) immediately after transplanting tomato, and BSM1 was applied as the PRE 24 h later. On 27 Jul (77 d after transplanting), velvetleaf weed density was measured in each plot. Tomato fruit were harvested on 3 Aug, 8 Aug, 15 Aug, 29 Aug, 5 Sep, and 20 Sep, when fruit reached the pink or light red color classification. Fruit was sorted and weighed as cull or marketable using United States number 3 grade as the minimum threshold for marketability (US Department of Agriculture, Agricultural Marketing Service 1991).

Trial 3 field methods: Weed control efficacy for broccoli (*Brassica oleracea* var. *italica*). The objective of trial 3 was similar to that of trial 2; however, we aimed to assess the tolerance of broccoli—a cool-season crop—to planting hole applications of the BSM1 formulation. Application rates were identical to those of trial 2. Raised beds were shaped and white plastic mulch film (used to avoid heat stress) was laid with a single line of drip tape beneath it for irrigation. 'Arcadia' broccoli seeds were started in the greenhouse and transplanted to plastic film on 2 Aug 2017. Plants were in a single row on each bed top, with 18 inches between plants in the row. Each replicate plot contained 10 plants and, as in trial 2, bare soil in the planting hole was the target of BSM applications. Procedures for seeding velvetleaf, applying the BSM, and irrigating were identical to those of trial 2. Weed density was measured on 2 Oct (61 d after transplanting), and broccoli was harvested on 26 Oct, sorted as cull or marketable using United States number 2 grade as the minimum threshold for marketability (US Department of Agriculture, Agricultural Marketing Service, 1959), and weighed fresh.

Trial 4 greenhouse methods: Weed control efficacy of two new films. A key observation during trials 1 to 3 was that the BSM1 suspension was quickly wicked or absorbed into soil, which potentially limited weed control efficacy. To remedy this, the objective of trial 4 was to test two new BSM formulations that demonstrated improved film-forming properties in the laboratory (e.g., reduced cracking and shrinking). This trial included three treatment factors, including formulation, application rate (2.0 and 4.9 $L{\cdot}m^{-2}$), and application timing (PRE, POST at the cotyledon stage, and POST at the two-leaf stage of velvetleaf). There were two BSM formulations. The first comprised a five-ingredient suspension (BSM2) of corn starch (40.5 $g{\cdot}L^{-1}$), glycerol (128.7 $mL{\cdot}L^{-1}$), corn gluten meal (20.2 $g{\cdot}L^{-1}$), isolated soy protein (20.2 $g{\cdot}L^{-1}$), and water (811 $mL{\cdot}L^{-1}$). The second comprised an eight-ingredient suspension (BSM3) of corn starch (40.5 $g{\cdot}L^{-1}$), glycerol (128.7 $mL{\cdot}L^{-1}$), corn gluten meal (10.2 $g{\cdot}L^{-1}$), isolated soy protein (10.2 $g{\cdot}L^{-1}$), corn zein (10.2 $g{\cdot}L^{-1}$), keratin hydrolysate (374.0 $mL{\cdot}L^{-1}$), eggshell powder (12.2 $g{\cdot}L^{-1}$), and water (439.0 $mL{\cdot}L^{-1}$). Velvetleaf biomass was harvested 19 d after seeding.

Trial 5 greenhouse methods: Effects of film viscosity on weed control efficacy. Reducing the water content and increasing the viscosity of BSM films were identified as potential strategies for mitigating BSM soil wicking and absorption observed during previous trials, and for reducing the total application volume and weight (e.g., to reduce transportation and fuel costs in the field). Therefore, the objective of trial 5 was to explore the effects of the water volume of the BSM suspension and subsequent changes in viscosity on weed control efficacy. Three new formulations were prepared using identical ingredients and variable amounts of water to achieve three levels of viscosity; each was applied at one of two timings (PRE and POST at the two-leaf stage of velvetleaf).

The low-viscosity formulation (BSM4) included corn starch (40.9 $g{\cdot}L^{-1}$), glycerol (130.0 $mL{\cdot}L^{-1}$), keratin hydrolysate (516.0 $mL{\cdot}L^{-1}$), corn zein (14.0 $g{\cdot}L^{-1}$), isolated soy protein (14.0 $g{\cdot}L^{-1}$), and water (303.0 $mL{\cdot}L^{-1}$). All ingredients in the medium-viscosity (BSM 5) and high-viscosity (BSM6) formulations were the same, except that water was reduced to 190.4 $mL{\cdot}L^{-1}$ in BSM5 and eliminated from BSM6. The application rates during this trial were standardized to deliver the same amount of starch and protein among treatments; because of the reduced water content with increasing viscosity, the application volume per pot was reduced as viscosity increased. As a result, the low-viscosity, medium-viscosity, and high-viscosity suspensions were applied at rates of 6.1 $L{\cdot}m^{-2}$, 5.3 $L{\cdot}m^{-2}$, and 4.4 $L{\cdot}m^{-2}$, respectively. Velvetleaf biomass was harvested 32 d after seeding.

Trial 6 field methods: Weed control efficacy for pepper. The objective of trial 6 was to test the medium viscosity BSM5 from trial 5 with the goal of reducing the application volume without sacrificing weed control efficacy or soil coverage (e.g., reduced flowability of the most viscous suspension could potentially reduce soil area covered). Additionally, we aimed to test the weed control efficacy of BSM5 applied in-row in a field crop without plastic mulch film. The BSM5 was applied at one of two rates (4.1 or 8.2 $L{\cdot}m^{-2}$) at two different application timings (PRE or POST at the three-leaf stage of velvetleaf and shattercane).

On 6 Jun 2019, 80 velvetleaf seeds and 80 shattercane seeds were hand-sown and raked into the 6-inch-wide in-row area of each plot. The PRE treatments were applied 7 Jun via a graduated cylinder in a 6-inch-wide band the length of each row yet to be transplanted. 'Carmen' pepper seeds were started in the greenhouse in April, and transplanted in the field on 10 Jun, with 18 inches between plants in a row. Each plot included a total of five plants. Care was taken to minimize BSM film disturbance in PRE plots during transplanting. On 24 Jun, when velvetleaf was at the three-leaf stage, POST treatments were applied via a calibrated hand-pump sprayer in a serpentine motion to maximize leaf surface coverage. On 24 Jul, aboveground velvetleaf and shattercane biomasses were collected from within the 6-inch in-row area of each plot (44 d after transplanting). Pepper fruit were harvested weekly when red between August and October, sorted as cull or marketable using United States number 2 grade as the minimum threshold for marketability (US Department of Agriculture, Agricultural Marketing Service 2005), and weighed fresh.

Trial 7 field methods: Weed control efficacy for kale (*Brassica oleraceae* var. *sabellica*). Despite efforts to increase viscosity in trial 5, BSM5 used in trial 6 did not reliably form a film on the soil surface under field conditions because of soil wicking and absorption. To address this pitfall, we increased the viscosity of the suspension further. The objective of trial 7 was to test the weed control efficacy of this new suspension for a fall-planted 'Winterbor' kale field production system without plastic mulch film.

Relative to BSM5, water was removed and the concentration of starch was increased, which resulted in a mix of corn starch (72.8 g·L^{-1}), glycerol (184.7 mL·L^{-1}), keratin hydrolysate (733.3 mL·L^{-1}), corn zein (19.8 g·L^{-1}), and isolated soy protein (19.8 g·L^{-1}). This formulation, BSM7, was applied PRE at rates of 4.1 or 6.1 L·m^{-2} or POST (three-leaf weed stage) at a rate of 6.1 L·m^{-2}.

Kale seeds were started in the greenhouse and transplanted to the field on 19 Aug 2019, with 1 ft between plants in a row and seven plants per 7.5-ft^2 plot. Yellow mustard (*Guillenia flavescens*) cover crop seed was used as a surrogate weed species to ensure uniform establishment and adaptation to fall growing conditions. On 27 Aug, 80 mustard seeds were hand-sown and raked into a 1-ft-wide in-row area between kale plants. The PRE treatments were applied the same day in a 1-ft-wide in-row band. The POST treatment was applied on 12 Sep using the same method as that during trial 6. Kale and mustard weed biomass were both harvested on 18 Oct (60 d after transplanting); kale was weighed fresh, whereas weeds were dried and weighed as described previously.

Data analysis. An analysis of variance of weed density (trials 1–3), weed biomass (trials 4–7), and crop yield (trials 2, 3, 6, and 7) response data was performed using the GLIMMIX procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) to determine differences among experimental treatments. Assumptions of the analysis of variance were checked using the UNIVARIATE procedure; additionally, a normal distribution was fit to all data. Replicate blocks were treated as a random effect in all analyses, whereas formulation, application rate, application time, and two-way and three-way interactions (depending on the trial design) (Table 1) were treated as fixed effects. The application rate and timing treatments were combined into a single factor for analysis during trial 7 because the treatment structure was unbalanced. Differences among least squares means were determined using the Tukey-Kramer multiple comparisons test at a significance level of $\alpha = 0.05$.

Results and discussion

Trial 1. The BSM1 application to greenhouse pots in trial 1 reduced velvetleaf emergence ($P = 0.002$), but there was no difference among any of the tested rates (data not shown). Weed suppression ranged from 93.8% ± 6.3% at the lowest rate (0.9 L·m^{-2}) to 100% at rates of 4.5 L·m^{-2} or more. Previous studies have demonstrated the potential for BSM films to reduce weed biomass and infestation by ≈70% to 85% [primarily sowthistle (*Sonchus* sp.)] (Giaccone et al. 2018; Massa et al. 2019), but the level of suppression (>93%) at rates as low as 0.9 L·m^{-2} observed here exceeded expectations.

Trials 2 and 3. Because of the greenhouse performance of BSM1 in trial 1, the same rates were tested in planting holes of plasticulture field tomato and broccoli production systems. However, velvetleaf density was not reduced by BSM1 application at any of the tested rates for tomato ($P = 0.24$). The BSM1 application had no effect on tomato yield compared with the controls ($P = 0.09$) (Table 2), but the tomato yield was not different between weedy and weed-free controls, which suggests that weed interference was not yield-limiting. In a broccoli plasticulture system, velvetleaf density in the planting hole was reduced by BSM1 at the highest rate ($P = 0.009$), and weed suppression generally improved with increasing BSM1 application rates up to the maximum of 9.1 L·m^{-2} (74.2% ± 10.2% reduction relative to the weedy control) (Table 3). However, there was no effect of BSM1 on broccoli yield compared with the weedy and weed-free controls ($P = 0.06$) (Table 3).

The reduced weed control efficacy of BSM films tested in the field environment may be attributed, in part, to differences between field soil application and greenhouse pot application. The surface roughness, including cracks in the soil, and grade were less consistent in the field (particularly within the planting hole), and the film was observed to preferentially flow into cracks and quickly absorb or wick into the soil. Another advantage of greenhouse pot applications is the presence of an outer wall or barrier to contain the suspension, which helps to minimize preferential flow and lateral spread of low-viscosity suspensions. Puka-Beals and Gramig (2021) used a steel bar to control the flow of a BSM in the field (to protect crop seedlings), but we did not make any attempt to contain the spread of BSM in this experiment. The result was a thin,

Table 2. Velvetleaf weed density and density reduction (relative to weedy control 77 d after transplanting) and marketable tomato yield after the application of biobased sprayable mulch (BSM) film suspension (BSM1: corn starch, glycerol, isolated soy protein, and water) at four rates (0.9, 1.8, 3.6, and 9.1 L·m^{-2}) during a 2017 field trial in Lincoln, NE, USA (trial 2).

	Mean (*SE*)		
Treatment[i]	Weed density (no./planting hole)	Weed density reduced (%)	Marketable tomato yield (kg/plot)[i]
Weed-free control	0		56.6 (3.4)
Weedy control	5.2 (0.3)		57.3 (5.8)
0.9 L·m^{-2}	4.9 (0.4)	5.8 (8.4)	48.4 (4.4)
1.8 L·m^{-2}	5.9 (0.3)	−14.6 (6.0)	55.2 (4.6)
3.6 L·m^{-2}	4.3 (0.5)	16.5 (9.7)	63.7 (2.9)
9.1 L·m^{-2}	3.9 (1.2)	25.2 (22.5)	61.6 (1.7)

[i] 1 L·m^{-2} = 0.245 gal/ft^2; 1 kg/37.5-ft^2 (3.48 m^2) plot = 0.2870 kg·m^{-2} = 0.9406 oz/ft^2.

and sometimes indetectable, surface barrier. Previous research has demonstrated the importance of a thick and solid surface membrane for preventing weed emergence (Immirizi et al. 2009; Warnick et al. 2006). The observed application and soil wicking challenges were also reported by Adhikari et al. (2019), who suggested increasing the BSM viscosity to improve performance.

Trial 4. Velvetleaf biomass was influenced by the BSM formulation type ($P = 0.001$), timing of application ($P < 0.001$), and application rate ($P = 0.04$), but not their interactions. Overall, the eight-ingredient BSM3 formulation was more effective than the five-ingredient BSM2 formulation. Earlier application (PRE and POST at the cotyledon stage) was more effective than later application (POST at the two-leaf stage). Application rates of at least 4.9 L·m^{-2} were slightly more effective than 2.0 L·m^{-2} (Table 4). The results of this trial demonstrated that applying BSM films over the top of emerged weed seedlings at the POST stage can be an effective weed management strategy, but only when weeds are small. Weed biomass was reduced 84.7% (±2.7%) when BSM films were applied on top of velvetleaf seedlings at the cotyledon stage, but biomass was reduced by only 25.3% (±6.0%) when applied at the two-leaf stage (Table 4). Previous reports of the weed control efficacy of BSM films have focused on preemergence applications (Braunack et al. 2020a; Claramunt et al. 2020; Giaccone et al. 2018; Shen and Zheng 2017; Warnick et al. 2006); however, our results suggest that growers may benefit from greater flexibility in the timing of application for the current BSM formulations.

Table 3. Velvetleaf weed density and density reduction (relative to weedy control 61 d after transplanting) and marketable broccoli yield after application of the biobased sprayable mulch (BSM) film suspension (BSM1: corn starch, glycerol, isolated soy protein, and water) at four rates (0.9, 1.8, 3.6, and 9.1 L·m^{-2}) in a 2017 field trial in Lincoln, NE, USA (trial 3).

	Mean (*SE*)		
Treatment[i]	Weed density (no./planting hole)	Weed density reduced (%)	Marketable broccoli yield (kg/plot)[i]
Weed-free control	0		1.74 (0.21)
Weedy control	4.7 (0.5) a[ii]		1.69 (0.18)
0.9 L·m^{-2}	3.6 (0.2) ab	23.7 (4.8)	1.09 (0.20)
1.8 L·m^{-2}	3.8 (0.8) ab	19.4 (17.0)	1.43 (0.22)
3.6 L·m^{-2}	2.1 (1.0) ab	54.8 (21.5)	1.77 (0.16)
9.1 L·m^{-2}	1.2 (0.5) b	74.2 (10.2)	1.52 (0.28)

[i] 1 L·m^{-2} = 0.245 gal/ft^2; 1 kg/37.5-ft^2 (3.48 m^2) plot = 0.2870 kg·m^{-2} = 0.9406 oz/ft^2.

[ii] Different letters within columns indicate significant differences among treatment groups using the Tukey-Kramer multiple comparisons test (α = 0.05).

Table 4. Velvetleaf weed biomass and biomass reduction (relative to weedy controls) measured 19 d after seeding and application of two different biobased sprayable mulch (BSM) film suspensions (BSM2 and BSM3) at three different timings [preemergence (PRE); postemergence (POST) for the cotyledon-stage of weeds; and POST for the two-leaf stage of weeds) and three rates (2.0, 4.9, and 9.8 L·m^{-2}) in the greenhouse (trial 4).

	Mean (*SE*)	
Treatment[i]	Weed biomass (mg/pot)[i]	Weed biomass reduced (%)
Formulation		
Weedy control, PRE	210 (39) a[ii]	
Weedy control, POST	160 (12) a	
BSM2[i]	75 (9) b	55.4 (5.8) b
BSM3[ii]	46 (9) c	72.5 (5.3) a
Timing		
Weedy control, PRE	210 (39) a	
Weedy control, POST	160 (12) a	
PRE	38 (7) c	81.9 (3.1) a
POST (cotyledon)	24 (4) c	84.7 (2.7) a
POST (two-leaf)	119 (10) b	25.3 (6.0) b
Rate		
Weedy control, PRE	210 (39) a	
Weedy control, POST	160 (12) a	
2.0 L·m^{-2}	77 (10) b	55.3 (6.3)
4.9 L·m^{-2}	54 (10) c	67.5 (6.5)
9.8 L·m^{-2}	51 (13) c	69.1 (8.0)

[i] BSM2 = corn starch, glycerol, corn gluten meal, isolated soy protein, and water. BSM3 = corn starch, glycerol, corn gluten meal, isolated soy protein, corn zein, keratin hydrolysate, eggshell powder, and water; 1 L·m^{-2} = 0.245 gal/ft^2; 1 mg/16-inch^2 (103.2 cm^2) pot = 96.8752 mg·m^{-2} = 0.0029 oz/yard^2.

[ii] Different letters within columns indicate significant differences among treatment groups using the Tukey-Kramer multiple comparisons test (α = 0.05).

Trial 5. Velvetleaf biomass was lower in all pots treated with BSM films compared with controls, but there was no difference among the BSM4, BSM5, and BSM6 formulations ($P = 0.37$) (data not shown). The lack of differences among these BSM films with variable viscosities suggests that application volume can be reduced to increase viscosity, without sacrificing weed control efficacy. The volume of BSM suspension and associated costs required and poor film-forming properties under field conditions are among the greatest challenges to developing a viable BSM (Braunack et al. 2021). However, these results, along with those of Adhikari et al. (2019), suggest that increasing the viscosity of BSM films may help to address these limitations. Weed control efficacy in trial 5 was also affected by the timing of application ($P < 0.001$), whereby the application at the two-leaf weed stage was more effective (99.1% ± 0.9% suppression) than the application before emergence (85.4% ± 2.9% suppression). The results from trial 5 support the conclusion from trial 4 that starch-based BSM formulations have the potential to enable postemergence weed control. Combined, these results suggest that the tested BSM suspensions may have herbicidal activity and provide physical suppression of weed emergence.

Trial 6. Total weed biomass was unaffected by the application rate ($P = 0.83$), timing ($P = 0.22$), and their interaction ($P = 0.33$). The lack of weed suppression from BSM6 resulted in pepper yield loss in all plots relative to the weed-free control ($P < 0.001$); there was no difference among BSM-treated plots and the weedy control (Table 5). Despite changes in the formulation, including increased viscosity, BSM6 exhibited the same preferential flow and rapid soil absorption that was observed with BSM1 in trials 2 and 3 for tomato and broccoli. As a result, the formulation was adjusted one final time to further reduce the water content and increase viscosity without losing the capacity for spray applications.

Table 5. Total weed biomass and biomass reduction (relative to weedy control 44 d after transplanting) and marketable pepper yield after the application of the biobased sprayable mulch (BSM) film suspension (BSM5: corn starch, glycerol, keratin hydrolysate, corn zein, isolated soy protein, and water) at two rates (4.1 and 8.2 L·m^{-2}) and timings [preemergence (PRE) and postemergence (POST)] during field trials in 2019 in Lincoln, NE, USA (trial 6).

	Mean (*SE*)		
Treatment[i]	Total weed biomass (g/plot)[i]	Weed biomass reduced (%)	Marketable pepper yield (kg/plot)[i]
Weed-free control	0		20.1 (1.2) a[ii]
Weedy control	630 (86)		12.7 (1.5) b
PRE, 4.1 L·m^{-2}	490 (116)	22.2 (18.4)	12.9 (2.3) b
PRE, 8.2 L·m^{-2}	360 (79)	42.8 (12.6)	10.8 (1.3) b
POST, 4.1 L·m^{-2}	534 (133)	15.3 (21.1)	12.3 (0.3) b
POST, 8.2 L·m^{-2}	590 (78)	6.4 (12.4)	10.8 (1.2) b

[i] 1 L·m^{-2} = 0.245 gal/ft^2; 1 g/7.5-ft^2 (0.70 m^2) plot = 1.4352 g··m^{-2} = 0.0423 oz/yard^2; 1 kg/7.5-ft^2 (0.70 m^2) plot = 1.4352 kg·m^{-2} = 2.6455 lb/yard^2.

[ii] Different letters within columns indicate significant differences among treatment groups using the Tukey-Kramer multiple comparisons test (α = 0.05).

Table 6. Mustard weed biomass and biomass reduction (relative to weedy control 60 d after transplanting) and kale yield after the application of biobased sprayable mulch (BSM) film suspension (BSM7; corn starch, glycerol, keratin hydrolysate, and isolated soy protein) at two rates (4.1 and 6.1 L·m^{-2}) and timing intervals [preemergence (PRE) and postemergence (POST)] during field trials in 2019 in Lincoln, NE, USA (trial 7).

	Mean (*SE*)		
Treatment[i]	Mustard biomass (g/plot)[i]	Mustard biomass reduced (%)	Kale yield (g/plant)
Weed-free control	0		269 (25)
Weedy control	91.1 (18.9) a[ii]		175 (35)
PRE, 4.1 L·m^{-2}	1.1 (0.8) b	98.7 (0.9)	218 (44)
PRE, 6.1 L·m^{-2}	0.3 (0.3) b	99.6 (0.4)	211 (20)
POST, 6.1 L·m^{-2}	3.2 (2.2) b	96.4 (2.4)	216 (24)

[i] 1 L·m^{-2} = 0.245 gal/ft^2; 1 g/7.5-ft^2 (0.70 m^2) plot = 1.4352 g·m^{-2} = 0.0423 oz/yard^2.

[ii] Different letters within columns indicate significant differences among treatment groups using the Tukey-Kramer multiple comparisons test (α = 0.05).

Trial 7. The application of BSM7 reduced yellow mustard biomass by as much as 99.6% ± 0.4% compared with the weedy control ($P < 0.001$) (Table 6, Fig. 1); however, there were no differences among the rates or timings tested. Despite the substantial mustard suppression, there was no detectable difference in kale yield among treatments ($P = 0.11$) (Table 6). When developing BSM films, lower-viscosity suspensions are often desired for their capacity to be sprayed, but our results are consistent with those of others who have observed improved agronomic performance and weed control efficacy of BSM films after viscosity and surface thickness of the film were increased (Adhikari et al. 2019; Braunack et al. 2020a; Claramunt et al. 2020; Russo 1993; Warnick et al. 2006). The level of weed suppression observed in trial 7 (exceeding greenhouse performances of BSM2, BSM3, BSM4, BSM5, and BSM6 in trials 4 and 5) is among the highest reported for BSM films, but this could have occurred because the surrogate weed yellow mustard is more susceptible to BSM films than wild weed populations. Claramunt et al. (2020) reported a weed seedling reduction of 85% to 93% for redroot pigweed (*Amaranthus retroflexus*), large crabgrass (*Digitaria sanguinalis*), prickly lettuce (*Lactuca serriola*), and common sowthistle (*Soncha oleraceus*). Massa et al. (2019) reported a reduction of 74% in weed biomass (primarily common sowthistle) and a dry weight increase of 11% in Japanese camellia (*Camellia japonica*). Measured benefits will vary greatly depending on environmental conditions and the species and density of the weed populations. Despite the weed-suppressive benefits, the lack of kale yield response will require further investigation. Several other studies have found that BSM films do not affect yield, despite changes in other agronomic properties. Braunack et al. (2021) found that BSM films increased the soil water content but did not affect tomato or watermelon (*Citrullus lanatus*) yield when compared with bare soil or plastic mulch film. Similarly, Braunack et al. (2020b) reported no difference in cotton yield when comparing BSM films to bare soil and a solid plastic mulch film.

The most likely explanation for the lack of yield benefits from BSM7, despite nearly complete weed suppression, is that mustard density and interference were insufficient to reduce kale yield (as evidenced by the lack of yield difference between weedy and weed-free controls). Therefore, future studies will need to test much greater densities of more aggressive weed species to fully characterize the potential benefits of BSM7. However, it is also possible that BSM7 may have immobilized nitrogen or increased soil salinity. Puka-Beals and Gramig (2021) tested the use of shredded newspaper and water hydromulch (carbon:nitrogen = 121:1) for direct-seeded carrot (*Daucus carota*) at a rate of 12.7 L·m^{-2}; they found that carrot yield was reduced by more than 50% in hydromulch plots despite excellent in-row weed suppression. The authors hypothesized that this yield loss was likely the result of nitrogen immobilization caused by the microbial degradation of the carbon-rich hydromulch. Biobased mulches are inherently carbon-rich; because of the direct contact with soil achieved with BSM films, there is greater potential for microbial degradation and immobilization of nitrogen under nitrogen-limiting conditions (Filipović et al. 2020). Another possible explanation for the yield results is that the BSM itself or its presence may contribute to increased soil salinity, which could limit water or nutrient

Fig. 1. Starch-based biobased sprayable films from trial 7 involving kale applied at a rate of 6.1 L·m^{-2} (0.15 gal/ft^2) before weed emergence (top) and 12 d after weed emergence (bottom) compared with the weedy control (middle) 18 d after weed emergence. All photos were obtained 18 d after weed emergence.

uptake. Braunack et al. (2021) observed increased salinity in BSM-treated plots; however, they suggested this was the result of less water infiltration (and related leaching of salts) caused by the physical presence of the BSM on the surface, not from the chemical properties of the film or its degradation byproducts.

Conclusions

Overall, the results of this study highlight the important relationship between BSM viscosity and weed control efficacy. Many of the early BSM films tested were not viscous enough to consistently form a weed-limiting barrier on the soil surface, particularly under field conditions. The results also demonstrate the potential for postemergence applications of BSM films, which could increase application timing flexibility for growers. Weed control efficacy of postemergence BSM applications was comparable to preemergence applications in most trials, particularly if the weeds were at the cotyledon growth stage. Further research of the effects of BSM films on soil chemical, biological, and physical properties is needed to determine why crop yield benefits are rarely proportional to other agronomic benefits. Additionally, future BSM development efforts should aim to increase cost efficiency because many of the biomaterials tested during this study (e.g., corn starch, zein, and isolated soy protein) and others are expensive, especially compared with polyethylene plastic mulch film (Braunack et al. 2021).

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