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Bio-ionic Liquids as Adjuvants for Sulfonylurea Herbicides

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Abstract

Five bio-ionic liquids (BILs) with choline cations and fatty acid anions derived from pelargonic acid, glycerol tristearate, glycerol trioleate, canola oil, and coconut oil were synthesized and applied as spray adjuvants with three sulfonylurea herbicides: metsulfuron-methyl, iodosulfuron-methyl-sodium, and tribenuron-methyl. Physicochemical properties, including thermal stability, solubility, and surface activity, were determined, and the influence of these BILs on herbicidal efficacy was studied in greenhouse tests using four target weed species: common lambsquarters (*Chenopodium album* L.), cornflower (*Centaurea cyanus* L.), corn poppy (*Papaver rhoeas* L.), and oilseed rape (*Brassica napus* L.). BILs, particularly those with the oleic anion and anions derived from canola oil and coconut oil, greatly improved herbicidal activity. Addition of BILs to the spray solution significantly reduced the surface tension and contact angle of spray droplets and increased the area of herbicide deposit on the leaf surface.

Introduction

Sulfonylurea herbicides, discovered in the 1970s, bestowed a new standard in weed management. Chlorsulfuron and sulfometuron-methyl, first sold in 1981 and 1982, respectively, were the first commercialized compounds from this chemical family (Brown and Cotterman 1994; Russell et. al. 2002). In subsequent years, this chemical family has been intensively developed, and the agrochemical market currently offers more than 30 active ingredients (Herbicide Resistance Action Committee 2017). Compared with other chemical families of herbicides, sulfonylureas are applied at extremely low rates that are measured in several grams or several dozen grams per hectare. Moreover, sulfonylureas demonstrate excellent efficacy against many weed species, including difficult to manage weeds; high selectivity toward a wide range of cultivated plants; and low mammalian toxicity (Brown and Cotterman 1994; Green and Cahill 2003). Sulfonylurea herbicides inhibit acetolactate synthase, also called acetohydroxyacid synthase, an enzyme essential for biosynthesis of the branched-chain amino acids, isoleucine, leucine, and valine (Senseman et al. 2007). Herbicidal selectivity among plant species is based on differences in rates of metabolism and degradation of the active ingredient. Tolerant plants rapidly metabolize the herbicide (Russell et al. 2002).

Efficacy of foliar-applied herbicides can be significantly enhanced by adjuvants added to spray solutions (Wang and Liu 2007). Previous studies have shown the most effective additives to sulfonylurea herbicides are nonionic surfactants, crop oil concentrate, methylated vegetable oil, inorganic salts, and pH adjusters (Green and Cahill 2003; Nalewaja et al. 1995a, 1995b). Adjuvants increase efficacy of foliar-applied herbicides by improving droplet spread on plant surfaces, facilitating foliar uptake and translocation of the active ingredient into plant tissues and thus reducing application rates of active ingredients (Beck et al. 2012; Dong et al. 2015; Spanoghe et al. 2007; Zabkiewicz 2000). In recent years, new types of adjuvants have attracted the attention of researchers. These adjuvants include dimethylethanolamine-based esterquats and ionic liquid-type Gemini surfactants (Castro et al. 2014; Li et al. 2012).

In sustainable agriculture, a significant feature of plant protection is the use of environmentally safe materials that meet the principles of green chemistry. Such a trend is also currently emerging in the use of adjuvants. An adjuvant is identified as "green" when it is produced from natural, renewable raw materials and has a low effect on humans and the environment. Moreover, "green adjuvants" should not increase the toxicity of the active substance toward non-target organisms (Beck et al. 2012).

Ionic liquids (ILs) are compounds that fulfill many principles of green chemistry. These compounds are characterized by melting temperatures below 100 C (Wasserscheid and Welton 2008) and present unique properties directly linked to their structures. The proper combination of cation–anion coupling allows synthesis of ILs for a variety of different purposes. ILs are effective as

Table 1. Synthetic yield and physical properties of choline carboxylates.

	Choline nonanoate (**1**)	Choline stearate (**2**)	Choline oleate (**3**)	Choline canolate (**4**)	Choline cocoate (**5**)
Yield	98	92	90	93	94
Glass transition	−27	−45	—	—	−70
Crystallization temperature	2	38	−73	−79	−60
Melting point	19	23	−38	−38	−44
Temperature value which results in 5% mass loss	186	181	180	187	174
Temperature value which results in 50% mass loss	210	218	216	224	216

green solvents (Hajipour and Refiee 2015) and electrolytes (Galiński et al. 2006). Domagk was the first researcher to report the biological activity of ILs (quaternary ammonium salts) (Domagk 1935). Since then, a number of antimicrobial, antifungal, and antitumor ILs have been described (Messali 2015; Pernak et al. 2003; Shamshina and Rogers 2014; Zakrewsky et al. 2014). However, applications of bioactive ILs are not limited to medical purposes. ILs also increase efficacies of agricultural fungicides (Pernak et al. 2015b) and herbicides (Cojocaru et al. 2013; Pernak et al. 2011, 2013b). In addition, ILs express antifeedant activity toward stored product pests, thus protecting harvested crops during storage (Pernak et al. 2012, 2013a, 2015a). Recently, a new solution of ILs called double salt herbicidal ionic liquids was released (Choudhary et al. 2017). ILs can be synthesized from natural products (Klejdysz et al. 2016; Pernak et al. 2015a), leading to environmentally friendly, readily biodegradable products with high biological activity. Choline soaps have been described as ecofriendly compounds with high surface activity (Klein et al. 2008, 2013; Kunz et al. 2011). In this study, choline-based ILs with vegetable oils (rapeseed and coconut oil) as sources of anions are synthesized and then tested as effective and ecofriendly adjuvants for sulfonylurea herbicides.

Materials and Methods

Substrates, Herbicides, and Adjuvants Used

Choline hydroxide (46% in water), pelargonic acid (99%), glyceryl tristearate (technical), glyceryl trioleate (technical), canola oil, and coconut oil were purchased from Sigma-Aldrich (Poznan, Poland) and used without purification. Potassium hydroxide and solvents were purchased from Avantor (Gliwice, Poland) and used as obtained.

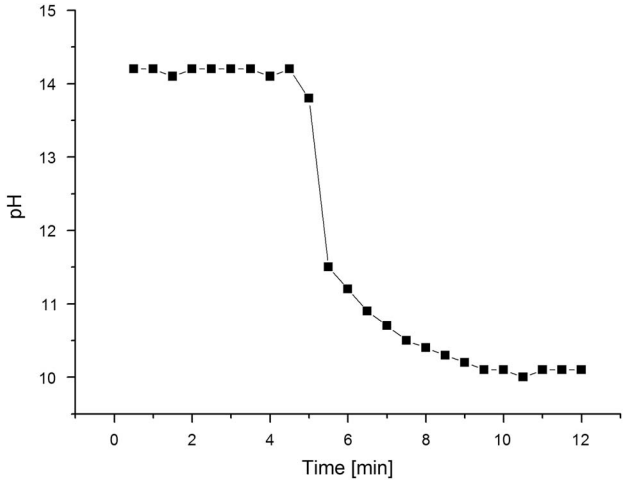

Figure 1. Changes of pH during synthesis of choline stearate (**2**).

The following herbicides were used in biological tests: metsulfuron-methyl (Galmet 20 SG, P.U.H. Chemirol, Mogilno, Poland, 20% ai), iodosulfuron-methyl-sodium (Huzar 05 WG, Bayer CropScience S.A., Lyon, France, 5% ai), and tribenuron-methyl (Granstar 75 WG, DuPont International Operations Sarl., Geneva, Switzerland, 75% ai). Actirob® 842 EC (Bayer, Warsaw, Poland, rapeseed oil methyl ester, 733 g L^{-1}) was used as an internal standard for tank-mixed adjuvants in the greenhouse studies.

Syntheses

Progress and conditions of all reactions were monitored using a Mettler-Toledo EasyMax 102 system (Mettler-Toledo, Greifensee, Switzerland).

Synthesis of Choline Nonanoate (1)

A 0.05 M aliquot of choline hydroxide (46% in water) was placed in a round-bottom flask and neutralized with a stoichiometric amount of pelargonic acid (99%). Reagents were mixed for 15 min, and then 15 ml of diethyl ether was added to remove excess pelargonic acid. After phase separation, the aqueous layer was isolated, and water was evaporated under vacuum. The obtained product was dried under vacuum at 60 C for 8 h.

Synthesis of Choline ILs from Triglycerides (2–5)

All reactions were completed according to a previously described procedure (Pernak et al. 2015a). A 0.03 M aliquot of choline hydroxide was mixed with 20 ml of 2-propanol in a round-bottom flask, and then 0.01 M of triglyceride or vegetable oil was added. Reagents were heated under reflux until the pH of the system

Table 2. Solubilities of prepared ionic liquids.

Solvent	ILs[a]				
	Choline nonanoate (**1**)	Choline stearate (**2**)	Choline oleate (**3**)	Choline canolate (**4**)	Choline cocoate (**5**)
Water	+	±	±	±	+
Methanol	+	+	+	+	+
Dimethyl sulfoxide,	–	–	±	±	–
Acetonitrile	+	–	±	±	+
Acetone	+	–	±	±	+
Ethyl acetate	–	–	–	–	–
Chloroform	+	+	+	+	+
Toluene	–	–	–	–	–
Hexane	–	–	–	–	–

[a]Solubility: +, complete; ±, limited; –, insoluble.

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Table 3. Surface parameters of the synthesized ionic liquids.[a]

ILs	CMC	γ_{CMC}	π_{CMC}		Γ_{max}	A_{min}	CA
	mmol L^{-1}	------mN m^{-1}------		pC_{20}	μmol m^{-2}	10^{-19} m^2	°
Choline nonanoate (**1**)	22.131	24.58	47.81	2.47	2.98	5.57	14.1
Choline stearate (**2**)	0.343	25.28	47.21	3.76	4.32	3.84	60.5
Choline oleate (**3**)	0.755	25.16	47.08	4.02	2.88	5.76	33.5
Choline canolate (**4**)	0.982	22.20	50.00	3.95	3.51	4.73	34.6
Choline cocoate (**5**)	0.421	24.72	47.66	4.06	5.18	3.20	41.2

[a]Abbreviations: γ_{CMC}, surface tension at the CMC; π_{CMC}, effectiveness of surface-tension reduction; pC_{20}, the negative logarithm of the surfactant concentration in the bulk phase required to reduce the surface tension of the water by 20 mN/m; Γ_{max}, maximum surface excess concentration; A_{min}, surface area occupied by the IL compound; CA, contact angle; CMC, critical micelle concentration; ILs, ionic liquids;

remained constant. The solvent was then evaporated under reduced pressure at 60 C, after which flask contents were dissolved in 15 ml of distilled water, placed in a separator, and washed three times with 10 ml of diethyl ether to remove unreacted triglyceride. After phase separation, the aqueous layer was isolated, and water was evaporated under vacuum. In the final step, the products were dried under reduced pressure (10 kPa) at 60 C for 24 h.

Nuclear Magnetic Resonance Analysis

The NMR spectra were recorded using a Mercury Gemini 300 spectrometer (Varian, Cambridge, UK) with tetramethylsilane as the internal standard operating at 300 MHz for ^{1}H NMR spectra and 75 MHz for ^{13}C NMR spectra.

Choline nonanoate (**1**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3H), 1.25–1.31 (m, 10H), 1.48–1.51 (m, 2H), 2.04–2.09 (m, 2H), 3.23 (s, 9H), 3.51–3.54 (m, 2H), 3.97–3.98 (m, 2H); ^{13}C NMR (75 Hz, $CDCl_3$): δ 13.9, 22.6, 26.8, 29.4, 29.6, 29.9, 31.8, 38.5, 54.0, 55.7, 67.8, 180.4.
Choline stearate (**2**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H), 1.26–1.32 (m, 28H), 1.51–1.55 (m, 2H), 2.08–2.12 (t, 2H), 3.28 (s, 9H), 3.59–3.61 (t, 2H), 4.01–4.03 (t, 2H); ^{13}C NMR (75 Hz, $CDCl_3$): δ 13.9, 21.7, 22.5, 24.9, 25.2, 26.8, 29.0, 29.5, 29.6, 31.8, 38.6, 55.8, 63.5, 68.0, 72.9, 180.3.
Choline oleate (**3**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H), 1.23–1.32 (m, 20H), 1.50–1.54 (m, 2H), 1.98–2.01 (m, 4H), 2.07–2.10 (m, 2H), 3.24 (s, 9H), 3.53–3.57 (m, 2H), 3.98–4.01 (m, 2H), 5.32–5.35 (m, 2H); ^{13}C NMR (75 Hz, $CDCl_3$): δ 14.0, 21.7, 22.6, 25.2, 26.8, 27.2, 29.2, 31.8, 38.6, 54.1, 55.8, 63.3, 37.9, 72.7, 122.6, 180.7.
Choline fatty acid anions isolated from canola oil, abbreviated as choline canolate (**4**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H), 1.23–1.35 (m, 18H), 1.50–1.54 (m, 2H), 1.98–2.03 (m, 4H), 2.07–2.10 (m, 2H), 3.25 (s, 9H), 3.55–3.57 (m, 2H), 4.00–4.02 (m, 2H), 5.32–5.35 (m, 2H); ^{13}C NMR (75 Hz, $CDCl_3$): δ 14.0, 21.7, 22.5, 26.9, 27.1, 29.2, 31.8, 38.6, 54.1, 55.8, 63.3, 67.9, 72.8, 127.8, 129.8, 180.5.
Choline fatty acid anions isolated from coconut oil, abbreviated as choline cocoate (**5**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H), 1.24–1.31 (m, 16H), 1.49–1.53 (m, 2H), 2.05–2.10 (m, 2H), 3.25 (s, 9H), 3.53–3.57 (m, 2H), 3.97–4.01 (m, 2H); ^{13}C NMR (75 Hz, $CDCl_3$): δ 14.0, 21.7, 22.5, 25.2, 26.8, 29.7, 31.8, 38.6, 54.1, 55.8, 63.3, 67.9, 72.8, 127.7, 129.6, 180.5.

Solubility

Solubilities of the prepared ILs were determined according to *Vogel's Textbook of Practical Organic Chemistry* (Furniss et al. 1989). Representative solvents were chosen and ranked by their Snyder polarity index value in descending order (water, 9.0; methanol, 6.6; dimethyl sulfoxide, 6.5; acetonitrile, 6.2; acetone, 5.1; ethyl acetate, 4.4; chloroform, 4.1; toluene, 2.3; hexane, 0.0). Tests were conducted at 20 C under ambient pressure. The term "complete solubility" refers to ILs (0.1 g of IL) that dissolve in 1 ml of solvent, while the term "limited solubility" refers to ILs (0.1 g of IL) that dissolve in 3 ml of solvent. The term "insoluble" refers to an IL (0.1 g) that does not dissolve in 3 ml of solvent.

Thermal Stability

Thermal transition temperatures were determined by differential scanning calorimetry using a Mettler-Toledo Stare DSC1 (Mettler-Toledo, Leicester, UK) unit under nitrogen. ILs (between 5 and 15 mg) were placed in aluminum pans and heated from 25 to 120 C at a heating rate of 10 C min^{-1}, cooled with an intracooler at a cooling rate of 10 C min^{-1} to −100 C, and then heated again to 120 C. Thermogravimetric analysis was performed using a Mettler-Toledo Stare TGA/DSC1 unit

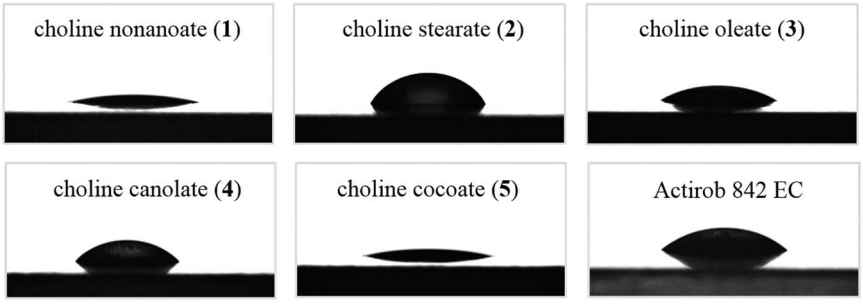

Figure 2. Drop shapes of ionic liquids on a hydrophobic surface (paraffin).

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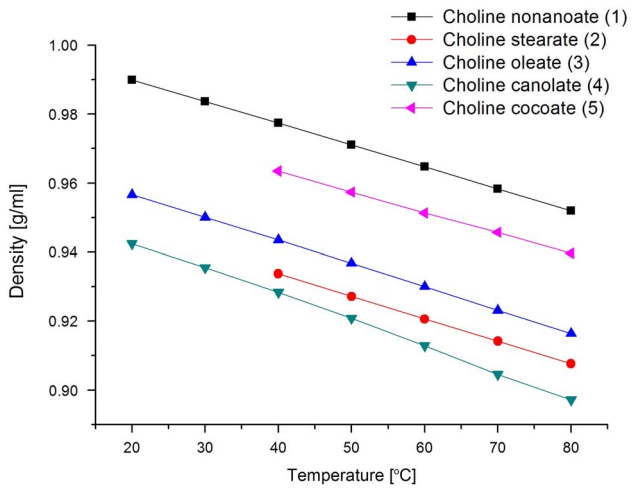

Figure 3. Densities of synthesized ionic liquids.

(Mettler-Toledo, Leicester, UK) under nitrogen. ILs (between 2 and 10 mg) were placed in aluminum pans and heated from 30 to 450 C at a heating rate of 10 C min^{-1}.

Surface Activity

Surface tensions were determined with the pendant-drop method (Berry et al. 2015). The measuring device was a DSA 100 analyzer (Krüss, Germany; accuracy ± 0.01 mN m^{-1}) set at 25 C (temperature controlled using a Fisherbrand FBH604 thermostatic bath [Fisher], with an accuracy of 0.1 C). Aqueous solutions of bio-ionic liquids (BILs) were freshly prepared using double-distilled water. The concentrations studied as initial samples were 0.01 mol L^{-1}. Ten samples of each compound were prepared for surface-tension measurements by serial dilution. At each step, 8 ml of the previous dilution was add to 8 ml of water. Each of the prepared samples was hand shaken vigorously to disperse the compound and was used within 1 h after preparation. The principle of the pendant-drop method involves the formation of an axisymmetric drop at the tip of a syringe needle. A CCD camera records an image of the drop (3 ml), and surface tension (mN m^{-1}) is calculated by analyzing the profile of the drop in accordance with the Laplace equation (Pernak et al. 2011). Values of the critical micelle concentration (CMC) and the surface tension at the CMC (γ_{CMC}) are determined based on analysis of linear regression of the intersection of the two lines drawn in the low- and high-concentration regions of the surface-tension curves (γ_{CMC} vs. log C curves) (Blesic et al. 2007). The contact angle of the sample above CMC is ascertained from the image of the drop on the examined surface (paraffin). After the actual drop shape and contact line are defined, the drop shape is adapted to fit a mathematical model used to calculate the contact angle. The most precise method to calculate this value is the Young–Laplace fitting (sessile-drop fitting), in which the entire drop contour is evaluated. After successful fitting of the Young–Laplace equation, the contact angle is determined as the slope of the contour line at the three-phase contact point (solid–liquid and liquid–air).

Herbicide Deposits

Droplets (2 µl) of spray solutions were applied manually with a micropipette to the upper surface of the first fully developed leaf of uniform oilseed rape (*Brassica napus* L.) plants at the 6-leaf stage. Immediately after the droplet dried, a 1 by 1 cm section of the treated leaf was removed, placed on aluminum stubs, and attached to a conductive carbon adhesive ribbon. Each sample was placed on a table, which was subsequently placed in a microscope chamber and cooled to −20 C. Pictures were recorded with a Hitachi S3000N scanning electron microscope under low vacuum (50 Pa) with a back-scattered electron detector.

Herbicidal Efficacy Evaluation

Seeds of *B. napus*, cornflower (*Centaurea cyanus* L.), common lambsquarters (*Chenopodium album* L.), and corn poppy (*Papaver rhoeas* L.) were planted into commercial peat-based potting material (Kronen, Cerekwica, Poland) in 0.5-L plastic pots. The resulting plants were grown in a greenhouse environment at 22 ± 2 C, 60% relative humidity, with a day length of 16 h for the duration of the test. Plants were watered as needed and thinned to 5 plants pot^{-1} within 16 d after emergence. Herbicides were dispersed in water and applied using a moving sprayer (APORO, Poznan, Poland) with a XR TeeJet® 110 02 VP flat-fan nozzle (TeeJet Technologies, Wheaton, IL, USA) delivering 200 L ha^{-1} spray mixture at 200 kPa operating pressure to plants at the 4-leaf stage (BBCH 14). Herbicide treatments consisted of: (1) metsulfuron-methyl at 4 g ai ha^{-1}, (2) iodosulfuron-methyl-sodium at 7.5 g ai ha^{-1}, and (3) tribenuron-methyl at 15 g ai ha^{-1}. Each herbicide was applied alone and with each choline fatty acid anion salt at 0.2% v/v, 0.4% v/v, or 0.8% v/v or with 0.75% v/v Actirob® 842 EC (17 total treatments for each herbicide). Plant responses were recorded by cutting plant shoots at surface level and recording the fresh weight of each harvested shoot separately (Sartorius BP 2000 S balance with 0.01 g precision; Sartorius, Göttingnen, Germany) at 3 wk after herbicide application. Means of fresh weights and percent reductions of fresh weights relative to fresh weights of untreated plants were compared to determine weed control of each treatment. Each experiment was conducted as a completely randomized design twice. Treatments containing metsulfuron-methyl on *C. cyanus* and tribenuron-methyl on *B. napus* were conducted in triplicate, while all other treatments consisted of four replications. Experimental variation was recorded as standard error of the mean (SEM). SEM values were calculated according to Equation 1:

$$SEM = s/n^{0.5} \quad [1]$$

where SEM is the standard error of the mean, s is the sample SD, and n is the number of samples.

Statistical Analyses

Each of the two series of greenhouse experiments was conducted in a completely randomized design. Each experiment for each herbicide consisted of 17 treatments that included five choline fatty acid anion salts (**1**–**5**) at three concentrations, a standard adjuvant (Actirob®), and a control with no added adjuvant (none). Each herbicide series contained three or four replications per treatment. Data were analyzed as a one-way ANOVA with a random series effect. A Fligner-Killeen median test of homogeneity of variances was conducted before ANOVA. Based on Fligner-Killeen analysis, some data were transformed with the Box-Cox transformation with the λ parameter. Tukey's multiple post hoc test ($\alpha = 0.05$) was used to compare treatments. The program package R v. 3.0.2 was used for calculations (R Core Team 2015).

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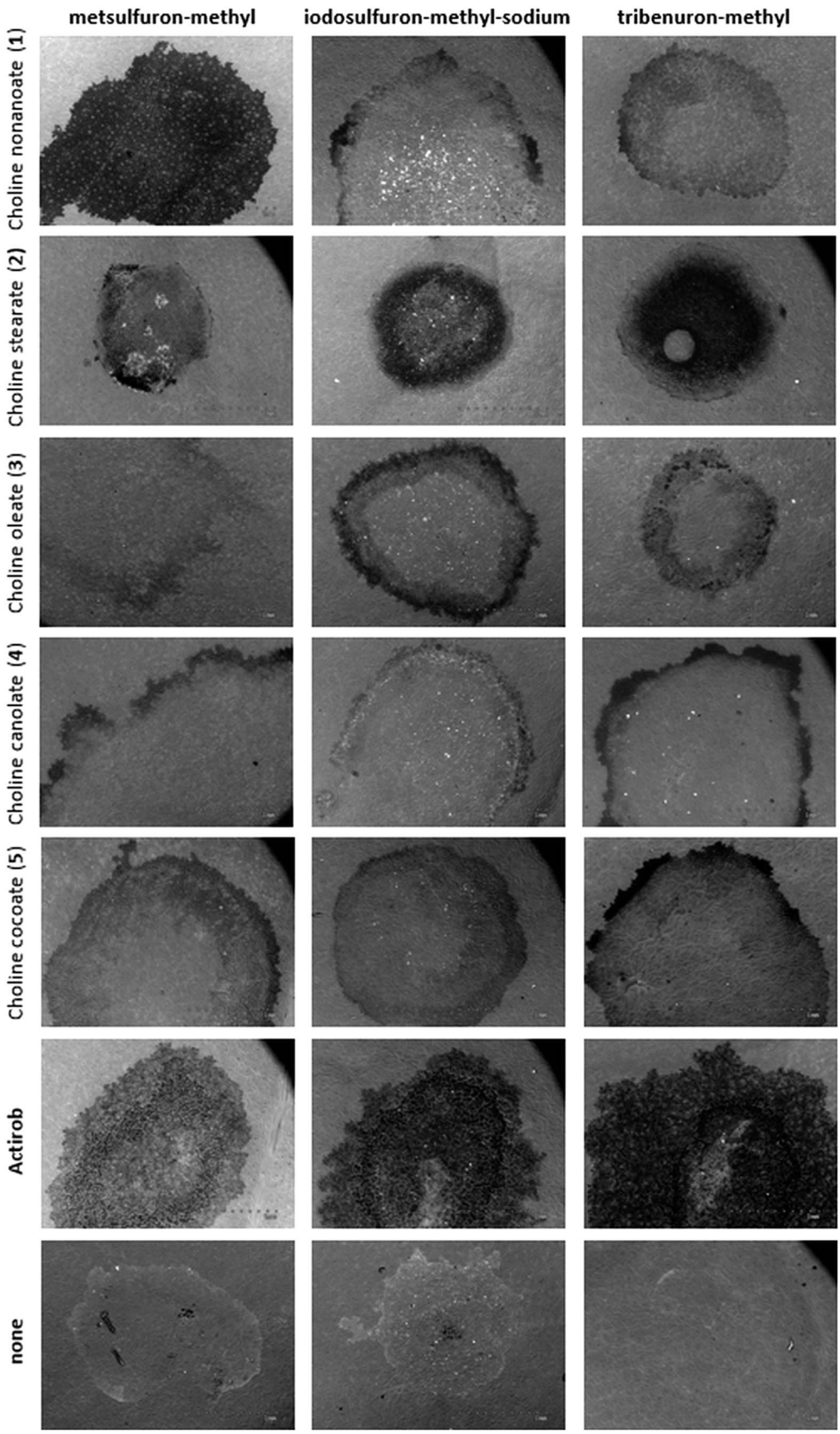

Figure 4. Scanning electron micrographs of herbicide deposits with adjuvants on leaves of oil-seed rape.

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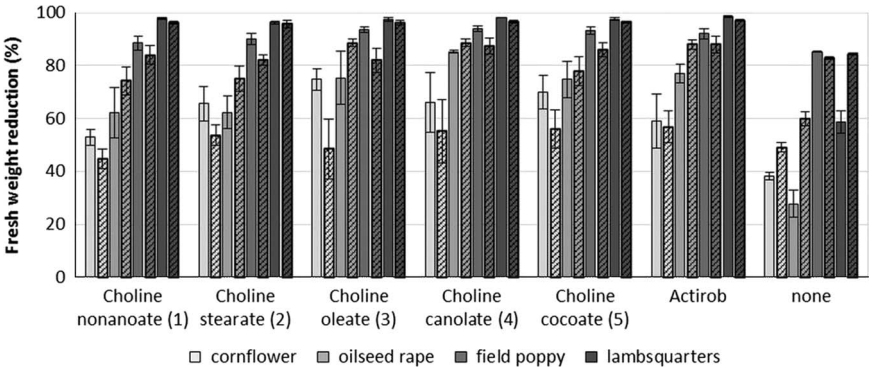

Figure 5. Reduction in fresh weight of plants treated with metsulfuron-methyl at 4 g ai ha^{-1} plus different bio-ionic liquids at 0.4% concentration or Actirob® at 0.75% concentration at 3 wk after herbicide application. Experiment 1, solid bars; Experiment 2, patterned bars. The error bars represent the SE of the treatment mean.

Results and Discussion

Synthesis and Characterization

Neutralization reactions were performed in the shortest time with the highest product yields. Hydrolysis reactions were completed with high yield after 10 to 15 min following the addition of triglycerides as shown in Figure 1.

Products were harvested with high yields (Table 1) and were characterized as high-viscosity liquids (choline nonanoate, **1**; choline oleate, **3**; choline canolate**,** **4**; choline cocoate, **5**) or solid (choline stearate, **2**) with melting points below 100 C. Glass transitions of compounds containing saturated anions (choline nonanoate, **1**; choline stearate, **2**; choline cocoate, **5**) were observed at temperatures from −70 C (choline cocoate, **5**) to −27 C (choline nonanoate, **1**). No glass transitions occurred for ILs **3** and **4**. Crystallization and melting temperatures varied from −79 C to 38 C and −44 C to 23 C, respectively. These obtained ILs can be described as thermally stable. No notable influence of the anion structure was observed. Temperatures of decomposition of 5% of the samples were determined to range from 174 to 187 C. The synthesized ILs are classified as room-temperature ionic liquids.

Chemical structures of the synthesized products were confirmed by analysis of ^{1}H and ^{13}C NMR spectra. Protons in the methyl groups of the cation generated a single strong signal in the range of 3.24 to 3.28 ppm. Methylene protons in position α to the quaternary nitrogen generated a signal at 3.51 to 3.61 ppm, while protons in position β occurred as a signal between 3.97 and 4.03 ppm. Signals originating from the anion were identified as peaks at 0.87 to 0.88 ppm for methyl protons and at 1.23 to 1.35 ppm for methylene groups, with two signals at 1.48 to 1.55 ppm and 2.04 to 2.12 ppm for protons in positions that are β and α to the carboxyl group, respectively.

Solubility

Solubility of the synthesized ILs was strongly influenced by their chemical structures (Table 2). All ILs are soluble in chloroform and methanol and express limited solubility in water. No ILs are soluble in hexane, toluene, or ethyl acetate. ILs with a longer alkyl chain in the anion (choline stearate, **2**; choline oleate, **3**; choline canolate, **4**) are less soluble in water than ILs with shorter alkyl substituents (choline nonanoate, **1**; choline cocoate, **5**).

Surface Activity

Aqueous solutions of ILs were investigated for their surface activities (Table 3). The following surface activity parameters were characterized: critical micelle concentration (CMC), surface tension at CMC (γ_{CMC}), effectiveness of surface-tension reduction (π_{CMC}), maximum surface excess concentration (Γ_{max}), and surface area occupied by the IL compound (A_{min}). Anion structure strongly influences the surface activity of the synthesized IL. The

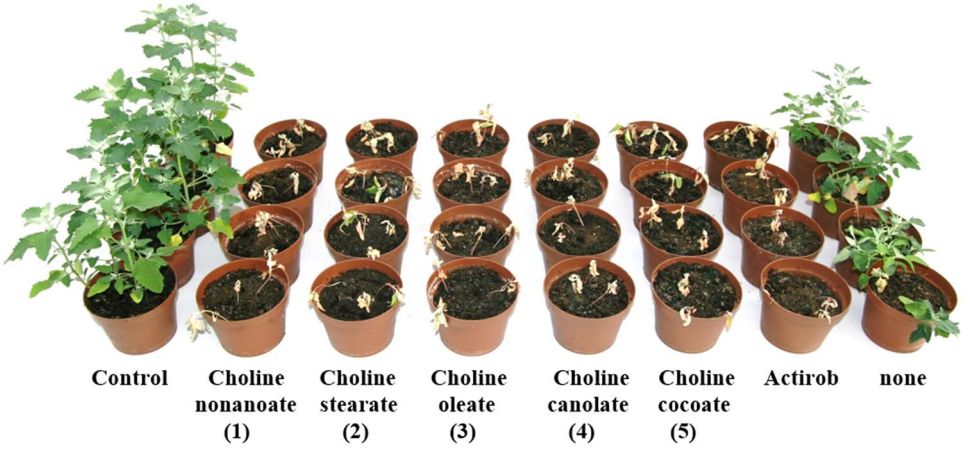

Figure 6. *Chenopodium album* response to metsulfuron-methyl, applied alone at 4 g ai ha^{-1} and with ILs at 0.4% concentration or with Actirob® at 0.75% concentration at 3 wk after herbicide application.

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Table 4. Fresh weights of plants treated with metsulfuron-methyl at 4 g ai ha^{-1} with and without adjuvants at 3 wk after treatment.

Tank-mix partner	Conc.[a]	Means of individual plant fresh weights[b]			
		Centaurea cyanus	*Chenopodium album*[c]	*Papaver rhoeas*	*Brassica napus*
	%	g			
Choline nonanoate (**1**)	0.20	11.180 ab	1.515 b	2.075 a	9.339 a
	0.40	11.988 ab	0.561 bc	1.818 a	5.010 bcde
	0.80	15.613 a	0.519 bc	1.710 a	5.424 bcde
Choline stearate (**2**)	0.20	9.538 ab	1.024 b	1.715 a	6.424 abc
	0.40	9.534 ab	0.740 bc	1.850 a	5.834 abcd
	0.80	11.263 ab	0.536 bc	1.453 a	5.170 bcde
Choline oleate (**3**)	0.20	9.559 ab	0.713 bc	1.716 a	3.456 cde
	0.40	9.296 b	0.568 bc	1.666 a	2.681 de
	0.80	10.194 ab	0.509 bc	1.228 a	2.717 de
Choline canolate (**4**)	0.20	8.901 b	0.484 bc	1.624 a	3.804 cde
	0.40	9.298 b	0.499 bc	1.270 a	2.106 e
	0.80	9.378 b	0.474 bc	1.308 a	3.229 cde
Choline cocoate (**5**)	0.20	8.081 b	0.651 bc	1.404 a	5.056 bcde
	0.40	8.796 b	0.549 bc	1.405 a	3.889 cde
	0.80	10.795 ab	0.469 bc	1.251 a	3.307 cde
Actirob®	0.75	9.786 ab	0.410 c	1.328 a	2.656 de
None	0.00	12.888 ab	4.698 a	2.088 a	8.553 ab
HSD		6.190	0.797		3.698
		General analysis–ANOVA			
		Centaurea cyanus	*Chenopodium album*[c]	*Papaver rhoeas*	*Brassica napus*
df		16; 118	16; 118	16; 118	16; 101
F		2.189	12.230	1.174	7.773
P value		0.0088	<2e-16	0.299	1.23e-11

[a]Abbreviation: Conc., concentration.
[b]Values followed by the same letter in the same column are not significantly different (P = 0.05).
Fresh weights of untreated plants were 17.443, 16.294, 12.914, and 16.294 g for *C. cyanus*, *C. album*, *P. rhoeas*, and *B. napus*, respectively.
[c]Data after transformation (λ = −0.061).

highest micelle concentration (CMC) was noted for choline nonanoate (**1**), which contains the shortest saturated alkyl chain in the anion. Elongating the saturated alkyl substituent results in a lower CMC value (choline stearate, **2**). An unsaturated bond (choline oleate, **3**) significantly decreases CMC relative to choline nonanoate. Results obtained for anions of natural origin (choline fatty acids from canola [**4**] and choline fatty acids from coconut oil [**5**]) confirm this relationship. The CMC for IL **5**, which consists of a mixture of short saturated substituents, places it between the results obtained for ILs **1** and **2**. For IL **4**, test results are consistent with predictions—rapeseed oil contains mainly oleic acid; however, small amounts of different acids, including saturated ones, are also present.

Contact angles were determined from analysis of the drop shapes of the ILs on paraffin (Figure 2). Choline nonanoate (**1**), with a contact angle of 14.1°, produces the lowest contact angle and provides the best wetting of paraffin. Choline stearate, with its longer saturated alkyl chain (**2**), significantly increases the contact angle to 60.5°. This trend of a longer alkyl chain increasing the contact angle is confirmed by test results for IL **5**, which consists of a mixture of saturated anions with moderate alkyl lengths. ILs with unsaturated anions (**3**, **4**) produce comparable contact angles of 33.5° and 34.6°, respectively. All synthesized ILs except IL **2** present better wetting properties than Actirob® (contact angle = 42.8°). The high contact angle (60.5°) for choline stearate (**2**) may be related to the longer saturated alkyl chain in the anion. This phenomenon was observed previously by Pernak and colleagues for herbicidal ILs with long alkyl chains in the cation (Pernak et al. 2017).

Densities of ILs were measured at temperatures ranging from 20 to 80 C (Figure 3). ILs 2 and 5 are highly viscous, so densities of these two ILs are measured at temperatures between 40 and 80 C. ILs with shorter alkyl chains (**1**, **5**) exhibit the highest densities. The presence of unsaturated bonds in ILs synthesized from glyceryl trioleate and canola oil (**3**, **4**) do not alter compound densities when compared with the IL with a saturated stearate anion (**2**).

Deposit of Herbicides—Scanning Electron Microscopy Studies

Leaves of *B. napus* are covered with a thick layer of well-developed epicuticular wax and are difficult to wet. ILs, when

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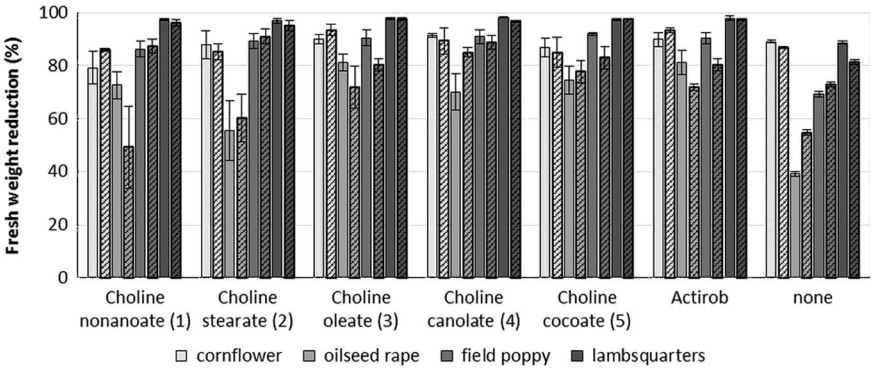

Figure 7. Reduction in fresh weight of plants treated with iodosulfuron-methyl-sodium at 7.5 g ai ha^{-1} plus different bio-ionic liquids at 0.4% concentration or Actirob® at 0.75% concentration at 3 wk after herbicide application. Experiment 1, solid bars; Experiment 2, patterned bars. The error bars represent the SE of the treatment mean.

Table 5. Fresh weights of plants treated with iodosulfuron-methyl-sodium at 7.5 g ai ha^{-1} with and without adjuvants at 3 wk after treatment.

		Fresh weight of weeds/mean values for treatments[b]			
Tank-mix partner	Conc.[a]	*Centaurea cyanus*	*Chenopodium album*[c]	*Papaver rhoeas*	*Brassica napus*[d]
	%	g			
Choline nonanoate (**1**)	0.20	5.605 ab	0.823 b	2.626 b	5.739 ab
	0.40	4.443 abc	0.550 b	2.478 b	5.008 abcd
	0.80	6.794 a	0.460 b	2.366 b	4.293 abcd
Choline stearate (**2**)	0.20	2.593 bc	0.738 b	3.000 b	4.628 abcd
	0.40	3.616 bc	0.640 b	1.833 b	5.475 abc
	0.80	4.245 abc	0.469 b	2.523 b	4.060 abcd
Choline oleate (**3**)	0.20	3.781 abc	0.575 b	2.359 b	2.738 bcd
	0.40	2.115 c	0.426 b	2.893 b	3.019 bcd
	0.80	3.650 bc	0.604 b	2.843 b	2.496 bcd
Choline canolate (**4**)	0.20	2.736 bc	0.511 b	2.389 b	3.228 abcd
	0.40	2.605 bc	0.444 b	1.934 b	2.948 bcd
	0.80	2.998 bc	0.436 b	2.226 b	2.689 bcd
Choline cocoate (**5**)	0.20	2.829 bc	0.740 b	2.254 b	4.041 abcd
	0.40	3.763 abc	0.426 b	2.479 b	3.098 abcd
	0.80	3.480 bc	0.473 b	2.183 b	2.296 cd
Actirob®	0.75	2.620 bc	0.483 b	2.473 b	2.296 d
None	0.00	3.255 bc	2.788 a	5.404 a	6.909 a
HSD		2.430	0.864	1.800	0.791
		General analysis–ANOVA in bottom stratum			
		Centaurea cyanus	*Chenopodium album*[c]	*Papaver rhoeas*	*Brassica napus*[d]
df		16; 118	16; 118	16; 118	16; 118
F		3.790	6.198	4.743	4.075
P value		1.23e-05	8.74e-10	2.5e-07	3.79e-06

[a]Abbreviation: Conc., concentration.
[b]Values followed by the same letter in a column indicate no significant difference between treatments. Fresh weights of untreated plants were 26.518, 18.214, 18.282, and 12.948 g for *C. cyanus*, *C. album*, *P. rhoeas*, and *B. napus*, respectively.
[c]Data after transformation ($\lambda = -0.182$).
[d]Data after transformation ($\lambda = -0.020$).

applied as herbicide spray adjuvants, significantly affect residual patterns of spray droplets on *B. napus* leaves (Figure 4). ILs consisting of choline oleate (**3**), choline canolate (**4**), and choline cocoate (**5**) produce droplet deposits with the largest spread area for all three herbicides and significantly change spray-droplet forms by creating coffee-ring deposit patterns. Ringlike deposits occur when the contact line of the droplet is pinned to the leaf surface. The liquid evaporates from the edge of the droplet and is replenished as liquid from the inner portion of the drop migrates toward the edge (Deegan et al. 2000). Adjuvants that produce a

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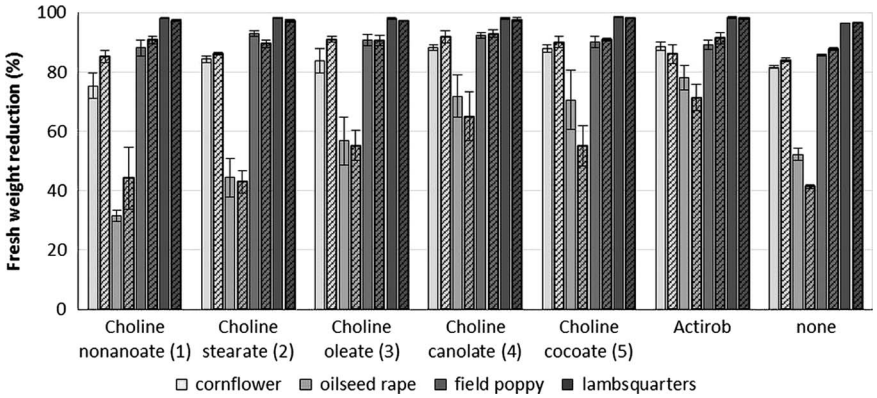

Figure 8. Reduction in fresh weight of plants treated with tribenuron-methyl at 15 g ai ha^{-1} plus different bio-ionic liquids at 0.4% concentration or Actirob® at 0.75% concentration at 3 wk after herbicide application. Experiment 1, solid bars; Experiment 2, patterned bars. The error bars represent the SE of the treatment mean.

large spread area with patterns that look like coffee rings are most effective in improving herbicidal activity. These test results suggest that ILs **3**, **4**, and **5** will maximize herbicidal activities of metsulfuron-methyl, iodosulfuron-methyl-sodium, and tribenuron-methyl.

Herbicidal Efficacy Trials

Adjuvants and Metsulfuron-methyl

Papaver rhoeas and *C. album* are most sensitive to metsulfuron-methyl. When averaged across both experiments, metsulfuron-methyl at 4 g ha^{-1}, applied alone, provides 84%, 71%, 48%, and 26% control of *P. rhoeas*, *C. album*, *B. napus*, and *C. cyanus*, respectively (Figures 5 and 6). The addition of IL adjuvant maintains or improves the herbicidal efficacy of metsulfuron-methyl. Herbicide combinations with all ILs at all three concentrations and with Actirob® significantly improve weed control activity by 18% to 24% on *C. album* (Table 4). In addition, herbicide combinations with ILs with most adjuvant concentrations and with Actirob® significantly increase weed control activity by 22% to 40% on *B. napus* (Table 4). Although not statistically significant, bioassay results for *C. cyanus* and *P. rhoeas* are consistent with bioassay results for *C. album* and *B. napus*. Overall, ILs consisting of choline oleate (**3**), choline plus fatty acids of canola oil (**4**), and choline plus fatty acids of coconut oil (**5**) are the most effective adjuvants. Choline nonanoate (**1**) and choline stearate (**2**) are slightly less effective in increasing the herbicidal activity of metsulfuron-methyl. The results show that ILs **3**, **4**, and **5**, at concentrations of 0.2% to 0.4%, are as effective as the commercial adjuvant, Actirob®, at 0.75%, for improving the herbicidal activity of metsulfuron-methyl. Improved herbicidal activities with ILs **3**, **4**, and **5** are consistent with predictions of improved herbicidal activity based on the large spread areas with patterns that look like coffee rings from the droplet-deposition assay.

Adjuvants and Iodosulfuron-methyl-sodium

Papaver rhoeas and *C. album* are most sensitive to iodosulfuron-methyl-sodium (Figure 7). When averaged across both experiments, iodosulfuron-methyl-sodium, applied alone at 7.5 g ha^{-1}, provides 88%, 85%, 70%, and 47% control of *C. cyanus*, *C. album*, *P. rhoeas*, and *B. napus*, respectively. The addition of IL adjuvant maintains or improves the herbicidal efficacy of iodosulfuron-methyl-sodium. When compared with iodosulfuron-methyl-sodium, applied alone without other herbicides, all adjuvants significantly increase the efficacy of the herbicide on *C. album* and *P. rhoeas* by 11% to 20% (Table 5). Although not significantly different, bioassay results for *C. cyanus*, and partially for *B. napus*, are consistent with those of *C. album* and *P. rhoeas*. In most cases, ILs consisting of choline oleate (**3**), canolate (**4**), and cocoate (**5**) are more effective than ILs consisting of choline nonanoate (**1**) and choline stearate (**2**). This trend is particularly noticeable for *B. napus*, the species least susceptible to iodosulfuron-methyl-sodium. ILs **3**, **4**, and **5**, at concentrations from 0.2% to 0.4% v/v, are as effective as Actirob® at 0.75% v/v for improving herbicidal activity of iodosulfuron-methyl-sodium. These bioassay results are consistent with bioassay results for metsulfuron-methyl and with predictions based on the spreading-droplet assay.

Adjuvants and Tribenuron-methyl

Chenopodium album, *P. rhoeas*, and *C. cyanus* are very sensitive to tribenuron-methyl. When averaged across both experiments, tribenuron-methyl at 15 g ha^{-1} provides more than 80% control of these weeds (Figure 8). No clear conclusions can be drawn from the *C. album*, *P. rhoeas*, or *C. cyanus* bioassay results, because herbicide use alone exhibits excellent efficacy. Responses of *B. napus* show the addition of ILs as adjuvants maintains herbicidal efficacy of tribenuron-methyl, and ILs **3**, **4**, and **5** significantly improve the herbicidal efficacy of tribenuron-methyl to a level similar to that of Actirob® (Table 6). Bioassay results for tribenuron-methyl are consistent with those for metsulfuron-methyl and iodosulfuron-methyl-sodium and with predictions based on the droplet-spread assay.

In summary, specific BILs such as choline oleate, choline plus fatty acid anions from canola oil, and choline plus fatty acid anions from coconut oil are viable candidates for a new class of bio-adjuvants for herbicides. These BILs improve POST herbicidal activities of the sulfonylurea herbicides metsulfuron-methyl, iodosulfuron-methyl-sodium, and tribenuron-methyl to levels similar to that of the methylated seed oil Actirob® 842 EC. Methylated seed soils and nonionic surfactants are currently the preferred adjuvants for sulfonylurea herbicides. BILs are a new type of adjuvant system with the advantage of being synthesized from renewable and sustainable natural products with extremely low levels of toxicity. This adjuvant system might be considered to be "green chemistry" in the herbicide industry. Opportunities to find the correct BIL to meet a particular adjuvant specification are good, because this particular group of compounds contains approximately 1,018 possible cation–anion combinations (Holbrey and Seddon 1999).

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Table 6. Fresh weights of plants treated with tribenuron-methyl at 15 g ai ha^{-1} with and without adjuvants at 3 wk after application.

		Fresh weight of weeds/mean values for treatments[b]			
Tank-mix partner	Conc.[a]	*Centaurea cyanus*	*Chenopodium album*	*Papaver rhoeas*	*Brassica napus*
	%	g			
Choline nonanoate (**1**)	0.20	3.471 ab	0.496 a	2.109 a	8.639 abcd
	0.40	4.44 ab	0.5 a	1.653 a	9.571 a
	0.80	5.116 a	0.453 a	1.731 a	8.29 abcde
Choline stearate (**2**)	0.20	4.323 ab	0.521 a	1.809 a	8.925 abc
	0.40	3.563 ab	0.506 a	1.483 a	8.666 abcd
	0.80	5.091 a	0.486 a	2.249 a	9.056 ab
Choline oleate (**3**)	0.20	3.859 ab	0.526 a	1.4 a	5.449 cdef
	0.40	2.799 ab	0.518 a	1.533 a	6.774 abcdef
	0.80	4.487 ab	0.453 a	1.4 a	4.799 ef
Choline canolate (**4**)	0.20	3.596 ab	0.631 a	1.728 a	5.785 bcdef
	0.40	2.304 b	0.47 a	1.188 a	4.864 ef
	0.80	3.546 ab	0.465 a	1.401 a	5.168 def
Choline cocoate (**5**)	0.20	3.916 ab	0.553 a	2.086 a	6.556 abcdef
	0.40	2.633 b	0.379 a	1.518 a	5.718 bcdef
	0.80	5.093 a	0.408 a	1.43 a	4.964 ef
Actirob®	0.75	3.239 ab	0.396 a	1.521 a	3.898 f
None	0.00	4.129 ab	0.724 a	2.108 a	8.178 abcde
HSD		2.43			3.57
		General analysis–ANOVA in bottom stratum			
		Centaurea cyanus	*Chenopodium album*	*Papaver rhoeas*	*Brassica napus*
df		16; 101	16; 118	16; 118	16; 118
F		3.061	1.494	1.978	6.690
P value		0.000325	0.113	0,0199	1.41e-10

[a]Abbreviation: Conc., concentration.
[b]Values followed by the same letter in a column indicate no significant difference between treatments. Fresh weights of untreated plants were 23.888, 21.048, 16.120, and 15.394 g for *C. cyanus*, *C. album*, *P. rhoeas*, and *B. napus*, respectively.

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References

Beck B, Steurbaut W, Spanoghe P (2012) How to define green adjuvants. Pest Manag Sci 68:1107–1110

Berry JD, Neeson MJ, Dagastine RR, Chan DYC, Tabor RF (2015) Measurement of surface and interfacial tension using pendant drop tensiometry. J Colloid Interface Sci 454:226–237

Blesic M, Marques MH, Plechkova NV, Seddon KR, Rebelo LPN, Lopes A (2007) Self-aggregation of ionic liquids: micelle formation in aqueous solution. Green Chem 9:481–490

Brown HM, Cotterman JC (1994) Recent advances in sulfonylurea herbicides. Pages 47–81 *in* Ebing W, ed. Chemistry of Plant Protection. Berlin: Springer-Verlag

Castro MJL, Ojeda C, Cirelli A (2014) Advances in surfactants for agrochemicals. Environ Chem Lett 12:85–95

Choudhary H, Pernak J, Shamshina JL, Niemczak M, Giszter R, Chrzanowski Ł, Praczyk T, Marcinkowska K, Cojocaru OA, Rogers RD (2017) Two herbicides in a single compound: double salt herbicidal ionic liquids exemplified with glyphosate, dicamba, and MCPA. ACS Sustainable Chem Eng 5:3261–6273

Cojocaru OA, Shamshina JL, Gurau G, Syguda A, Praczyk T, Pernak J, Rogers RD (2013) Ionic liquid forms of the herbicide dicamba with increased efficacy and reduced volatility. Green Chem 15:2110–2120

Deegan RD, Bakajin O, Dupont TF, Huber G, Nagel SR, Witten TA (2000) Contact line deposits in an evaporating drop. Phys Rev E 62:756–765

Domagk GA (1935) Eine neue Klasse von Desinfektionsmitteln. Deut Med Wochenschr 61:829–832

Dong X, Zhu H, Yang X (2015) Characterization of droplet impact and deposit formation on leaf surfaces. Pest Manag Sci 71:302–308

Furniss BS, Hannaford AJ, PWG Smith, Tatchell AR eds 1989) Vogel's Textbook of Practical Organic Chemistry. 5th ed. Harlow: Longman. Pp 1198–1204

Galiński M, Lewandowski A, Stępniak I (2006) Ionic liquids as electrolytes. Electrochim Acta 51:5567–5580

Green JM, Cahill WR (2003) Enhancing the biological activity of nicosulfuron with pH adjusters. Weed Technol 17:338–345

Hajipour AR, Refiee F (2015) Recent progress in ionic liquids and their applications in organic synthesis. Org Prep Proced Int 47:249–309

Herbicide Resistance Action Committee (2017) Global Classification Lookup. http://hracglobal.com/tools/classification-lookup/p3. Accessed: January 20, 2017

Holbrey JD, Seddon KR (1999) Ionic liquids. Clean Prod Proc 1:223–226

Klein R, Muller E, Kraus B, Brunner G, Estrine B, Touraud D, Heilmann J, Kellermeier M, Kunz W (2013) Biodegradability and cytotoxicity of choline soaps on human cell lines: effects of chain length and the cation. RSC Adv 3:23347–23354

https://doi.org/10.1017/wsc.2017.85 Published online by Cambridge University Press

Klein R, Touraud D, Kunz W (2008) Choline carboxylate surfactants: biocompatible and highly soluble in water. Green Chem 10:433–435

Klejdysz T, Łęgosz B, Czuryszkiewicz D, Czerniak K, Pernak J (2016) Biobased ionic liquids with abietate anion. ACS Sustainable Chem Eng 4:6543–6550

Kunz W, Maurer E, Klein R, Touraud D, Rengstl D, Harrar A, Dengler S, Zech O (2011) Low toxic ionic liquids: liquids catanionics, and ionic liquid microemulsions. J Disper Sci Technol 32:1694–1699

Li H, Yu C, Chen R, Li J, Li J (2012) Novel ionic liquids—type Gemini surfactants: synthesis, surface property and antimicrobial activity. Colloids Surf A 395:116–124

Messali M (2015) Eco-friendly synthesis of a new class of pyridinium-based ionic liquids with attractive antimicrobial activity. Molecules 20:14936–14949

Nalewaja JD, Praczyk T, Matysiak R (1995a) Salts and surfactants influence nicosulfuron activity. Weed Technol 9:587–593

Nalewaja JD, Praczyk T, Matysiak R (1995b) Surfactants and oil adjuvants with nicosulfuron. Weed Technol 9:689–695

Pernak J, Giszter R, Biedziak A, Niemczak M, Olszewski R, Marcinkowska K, Praczyk T (2017) Alkyl (C16, C18, C22) trimethylammonium-based herbicidal ionic liquids. J Agric Food Chem 65:260–269

Pernak J, Łęgosz B, Walkiewicz F, Klejdysz T, Borkowski A, Chrzanowski Ł (2015a) Ammonium ionic liquids with anions of natural origin. RSC Adv 5:65471–65480

Pernak J, Markiewicz B, Łęgosz B, Walkiewicz F, Gwiazdowski R, Praczyk T (2015b) Known triazole fungicides—a new trick. RSC Adv 5:9695–9702

Pernak J, Nawrot J, Kot M, Markiewicz B, Niemczak M (2013a) Ionic liquids based stored product insect antifeedants. RSC Adv 3:25019–25029

Pernak J, Niemczak M, Zakrocka K, Praczyk T (2013b) Herbicidal ionic liquid with dual-function. Tetrahedron 69:8132–8136

Pernak J, Sobaszkiewicz K, Mirska I (2003) Anti-microbial activities of ionic liquids. Green Chem 5:52–56

Pernak J, Syguda A, Janiszewska D, Materna K, Praczyk T (2011) Ionic liquids with herbicidal anions. Tetrahedron 67:4838–4844

Pernak J, Wasiński K, Praczyk T, Nawrot J, Cieniecka-Rosłonkiewicz A, Walkiewicz F, Materna K (2012) Sweet ionic liquids-cyclamates: synthesis, properties and application as feeding deterrents. Sci China Ser B 55: 1532–1541

R Core Team (2015) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. P 3551. http://www.R-project.org. Accessed: April 20, 2017

Russell MH, Saladini JL, Lichner F (2002) Sulfonylurea herbicides. Pestic Outlook 4:166–173

Senseman SA, ed (2007) Herbicide Handbook. 9th ed. Lawrence, KS: Weed Science Society of America. Pp 11–16

Shamshina JL, Rogers RD (2014) Overcoming the problems of solid state drug formulations with ionic liquids. Ther Deliv 5:489–491

Spanoghe P, Schampheleire M, Meeren P, Steurbaut W (2007) Review. Influence of agricultural adjuvants on droplet spectra. Pest Manag Sci 63: 4–16

Wang CJ, Liu ZQ (2007) Foliar uptake of pesticides—present status and future challenge. Pestic Biochem Phys 87:1–8

Wasserscheid P, Welton T (2008) Ionic Liquids in Synthesis. 2nd ed. Weinheim, Germany: Wiley-VCH

Zabkiewicz JA (2000) Adjuvants and herbicidal efficacy—present status and future prospects. Weed Res 40:139–149

Zakrewsky M, Lovejoy KS, Kern TL, Miller TE, Le V, Nagy A, Goumas AM, Iyer RS, Del Sesto RE, Koppisch AT, Fox DT, Mitragotri S (2014) Ionic liquids as a class of materials for transdermal delivery and pathogen neutralization. Proc Natl Acad Sci USA 111:13313–13318

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