Herbicidal Activity of Slow-Release Herbicide Formulations in Wheat Stands Infested by Weeds

Natalia Zhila[1,2], Anastasiya Murueva[1], Anna Shershneva[1,2], Ekaterina Shishatskaya[1,2], Tatiana Volova[1,2,*]

[1]Institute of Biophysics SB RAS, Federal Research Center "Krasnoyarsk Science Center SB RAS", Akademgorodok, Krasnoyarsk, 660036, Russian Federation

[2]Siberian Federal University, 79 Svobodnyi Ave., Krasnoyarsk, 660041, Russian Federation

*Corresponding author. Tel.: +7 393 2494428; fax: +7 391 2433400

E-mail address: nzhila@mail.ru (Natalia Zhila)

ABSTRACT

This study addresses herbicidal activity of experimental formulations of metribuzin and tribenuron-methyl embedded in the degradable matrix of natural poly-3-hydroxybutyrate [P(3HB/MET) and P(3HB)/TBM] in stands of soft spring wheat (*Triticum aestivum*, cv. Altaiskaya 70) infested by weeds – white sweet clover *Melilotus albus* and lamb's quarters *Chenopodium album* – under laboratory conditions. Indicators of herbicidal activity were the density and weight of the vegetative organs of weeds measured during 30-day and 50-day experiments. Wheat crop yield was estimated as dependent on the method of herbicide delivery and the decrease in weed density. The experimental MET and TBM formulations showed pronounced herbicidal activity against the weed species used in the study. The effectiveness of the experimental formulations in inhibiting weed growth was comparable to and, sometimes, higher than that of the commercial formulations (positive control). The amount of the biomass of the wheat treated with the experimental herbicide formulations was significantly greater than that of the wheat treated with commercial formulations.

Keywords: metribuzin, tribenuron-methyl, poly-3-hydroxybutyrate, slow-release formulations, herbicidal activity, wheat, weeds

INTRODUCTION

Herbicides constitute the largest group of pesticides. They are extensively used to control thousands of species of weeds, which cause the most serious damage to farm crops. Weeds compete with crops for light, water, and nutrients. Therefore, the use of herbicides to control weeds is one of the most important approaches in modern high-performance agriculture. Application of herbicides, however, inevitably leads to high concentrations of chemical compounds in soils, not only posing hazard to human health but also causing the development of species resistant to the herbicides, threatening the stability of agroecosystems, and endangering the long-term soil fertility.

Much research effort has been devoted recently to creating new formulations and investigating their behavior in the environment. Research is aimed at developing less toxic and more selective pesticides and at reducing their application rates.

Some relatively recent studies describe encapsulation of herbicides in polymer materials. For instance, formulations of herbicides alachlor and norflurazon were prepared by encapsulating them in ethylcellulose.[1-2] However, there are few published data on this subject.

The major challenge in constructing slow-release pesticide formulations is to find proper materials to be used as a matrix and characterize them. Among materials investigated for this purpose are degradable polymers of various origins, including polyvinylchloride, alginates,[3] chitin, cellulose,[4] lignin/polyethylene glycol blends,[5,6] etc. Polymers of monocarboxylic acids – polylactides, polyglycolides, and polyhydroxyalkanoates (PHAs) – are also promising materials for constructing pesticide formulations. PHAs are microbial polyesters that are degraded in biological media by natural microflora to carbon dioxide and water under aerobic conditions and to water and methane under anaerobic ones. Production of this class of natural polymers is a rapidly developing branch of the industry of degradable bioplastics, and they are considered promising candidates for gradual replacement of synthetic polymers.[7] In a number of recent studies, our team has described experimental slow-release formulations of herbicides[8-10]

and fungicides[11-12] embedded in the degradable matrix of poly-3-hydroxybutyrate and their effects on weeds and plant pathogens – root rot agents – in laboratory soil microecosystems.

Although more research has recently focused on new-generation slow-release targeted herbicide formulations, published studies mainly describe methods of embedding herbicides and materials used as matrices. Very few data can be found on the herbicidal efficacy of new formulations and outcomes of studies conducted with weed-infested crops.

The purpose of this study was to investigate herbicidal activity of slow-release metribuzin and tribenuron-methyl formulations in wheat stands infested by weeds.

MATERIALS AND METHODS

Characterization of Herbicides

Two herbicides were used: metribuzin and tribenuron-methyl.

Metribuzin – (MET) [$C_8H_{14}N_4OS$] (State Standard Sample 7713-99 – the state standard accepted in Russia (Blok-1, Moscow) – has a systemic effect against a wide range of weeds infesting vegetable and grain crop stands; it both has a foliar action and can penetrate into plants through their roots; it is a pre-emergence and post-emergence herbicide. MET inhibits plant photosynthesis. The IUPAC name is 4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one. The CAS name is 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one. MET has the following main physicochemical properties: colorless crystals; molecular weight 214.3 g mol^{-1}; melting point 126.2 ºC; solubility at 20 ºC (g/L) in water - 1.2, in chloroform - 850, and in acetone - 820. Log K_{ow} 1.60. pK_a - 7.1.

Tribenuron-methyl – (TBM) [$C_{15}H_{17}N_5O_6S$] (State Standard Sample 8628-2004 – the state standard accepted in Russia (Blok-1, Moscow) – is a systemic herbicide that has a selective effect on a wide range of weeds; it is highly effective against weeds in different stages of their development (from the tillering phase to the formation of the second stem internode); it both has

a foliar action and can penetrate into plants through their roots. TBM has no aftereffects; it can be used in all types of crop successions. It is used to control broad-leaved weeds (poppy, chamomile, perennial Canada thistle, cruciferous plants, black bindweed, common chickweed, etc.) in cereals. TBM acts by inhibiting acetolactate kinase – the enzyme that catalyzes the synthesis of branched-chain amino acids (isoleucine and valine). The IUPAC name is methyl 2-[4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoylsulfamoyl]benzoate. The CAS name is methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl]benzoate. It has the following main physicochemical properties: light brown substance; solubility in water at 20°C (mg/L) – 2040; solubility in organic solvents at 20°C (mg/L): in dichloromethane – 250000, in acetone – 39100, and in ethyl acetate – 16300. Melting point (°C): 142. Decomposition temperature (°C): 175. Specific density (g/ml): 1.46. The lifespan in soil (in the field) is between 5 and 20 days, according to the data obtained in laboratory research in the European Union.

Production and Characterization of Poly-3-hydroxybutyrate [P(3HB)]

Polymer poly(3-hydroxybutyrate) – P(3HB) – the most common representative of PHAs – was used as a degradable polymer matrix for embedding the herbicide. The polymer was synthesized in the Laboratory of Chemoautotrophic Biosynthesis at the Institute of Biophysics SB RAS by using bacterium *Cupriavidus eutrophus* B10646, batch-cultured under strictly aseptic conditions in a 7.5-L BioFlo/CelliGen115 fermentor ("New Brunswic", U.S.), following the authors' procedure.[8] Polymer was extracted from bacterial biomass with chloroform, and the extracts were precipitated using hexane. The extracted polymer was re-dissolved and precipitated again 3-4 times to prepare homogeneous specimens.

Preparation of Slow-Release Metribuzin and Tribenuron-Methyl Formulations

Slow-release herbicide formulations were prepared using the procedure described in detail elsewhere.[8] P(3HB)/MET formulations were prepared as films and microparticles loaded

with MET at 25% (of the mass of the polymer matrix); P(3HB)/TBM formulations were prepared as microparticles loaded with TBM at 25% (of the mass of the polymer matrix).

Polymer films were prepared as follows: the P(3HB)/herbicide solution was poured into glasses, and then solvent evaporation occurred. We used 2 % (w/v) polymer solutions in chloroform. The films stayed under the bell glass for 24 h at room temperature, and then they were placed into a vacuum drying cabinet (Labconco, U.S.) for 3-4 days, until complete solvent evaporation took place. The films were then weighed on the analytical balance. The film thickness was measured with an EDM-25-0.001 digital micrometer (LEGIONER, Germany). Squares of 25 mm^2 in area (5 mm × 5 mm) were then cut from the film.

Polymer microparticles were prepared as follows: the polymer was precipitated from the solution into the sedimentation tank filled with the reagent in which P(3HB) did not dissolve (hexane), and, thus, crystallization occurred and polymer particles were formed. P(3HB) concentration in the chloroform solution was 10 % (w/v). A solution of the herbicide was added to the polymer solution; the system was mixed to achieve homogeneity, by using a Silent Crusher high-speed homogenizer (Heidolph, Germany). A Pumpdrive 5001 peristaltic pump (Heidolph, Germany) was used to drop the polymer/herbicide solutions into the sedimentation tank that contained hexane, where the polymer was crystallized and particles formed.

Analysis of metribuzin and tribenuron-methyl

Detection of metribuzin was performed by using gas chromatography. Measurements were done on the gas chromatograph equipped with a mass spectrometer (7890/5975C, Agilent Technologies, U.S.), using a capillary column, under varied temperature. The chromatography conditions were as follows: an HP-5MS capillary column, 30 m long and 0.25 mm in diameter; carrier gas – helium, flow rate 1.2 ml/min; sample introduction temperature 220 °C; initial temperature of chromatography – 150 °C; temperature rise to 310 °C at 10 °C per min; transfer line temperature – 230 °C, ion source temperature – 150 °C, electron impact mode at 70 eV,

fragment scan from m/z 50 to m/z 550 with a 0.5 second cycle time. The peak corresponding to metribuzin was detected by mass spectrometer. We used State Standard Sample 7713-99 – the state standard accepted in Russia: 99.7% pure. Calibration curve was prepared by using a wide range of concentrations of metribuzin in acetone (0.1-4.2 μg/μL). The range of linear detection was obtained for a wide variety of concentrations: between 0.1 μg/μL and 4.2 μg/μL. The standard error of the method was no more than 3%.

Detection of tribenuron-methyl, which is a low-volatility and thermally unstable compound, was performed by liquid chromatography, using an Agilent 1200 HPLC system with a diode array (Agilent Technologies, U.S.), an inline degasser, and a 20-μl injection loop.
The size of the XDB-C18 precolumn was 4.6 mm × 12.5 mm. The analytical column – Eclipse XDB-C18 – was 5 μм × 4.6 mm × 150 mm. The mobile phase was prepared by mixing acetonitrile and a 0.005 M solution of orthophosphoric acid, 45:55 (v/v). The eluent flow rate was 1 ml/min. Isocratic elution was performed. The temperature of the column was 30°C. Detection was done at a wavelength of 223 nm, with the slit width of 100 nm. The standard sample of tribenuron-methyl was State Standard Sample 8628-2004. Calibration curve was prepared by using a wide range of concentrations of tribenuron-methyl in acetone???? (0.1-500 μg/μl). The range of linear detection was obtained for a wide variety of concentrations: between 0.1-2.0 μg/μl and 50-500 μg/μl. Sensitivity of the method for detecting tribenuron-methyl by using the diode array was 0.1 μg/μl. The standard error of the method was 1.5%.

Laboratory Ecosystems with Higher Plants

Herbicidal activity of the experimental formulations was studied in laboratory ecosystems: 500-cm^3 plastic containers filled with 500 g field soil were used to grow higher plants. The agrogenically-transformed soil (collected at the village of Minino, the Krasnoyarsk Territory, Siberia, Russia) was placed into 250-mm^3 plastic containers (200 g soil per container). The soil was cryogenic-micellar agro-chernozem with high humus content in the 0-20-cm layer (7.9-9.6 %). The soil was weakly alkaline (pH 7.1-7.8), with high total exchangeable bases (40.0-45.2 mequiv/100 g). The soil contained nitrate nitrogen $N\text{-}NO_3$ – 6 mg/kg, and P_2O_5 – 6 and K_2O – 22 mg/100 g soil (according to Machigin).

Soft spring wheat (*Triticum aestivum*, cv. Altaiskaya 70) was used as the crop, and white sweet clover *Melilotus albus* and lamb's quarters *Chenopodium album* were the weeds.

The experimental herbicide formulations and seeds of wheat (100 g per 1 m^2), white sweet clover (24 g per 1 m^2), and lamb's quarters (20 g per 1 m^2) were simultaneously buried in

the soil. In the positive control, we treated soil with commercial formulations of metribuzin or tribenuron-methyl at the beginning of the experiment; the amounts of the herbicides applied corresponded to the recommended application rates – 0.015–0.025 kg/ha (the same concentrations as those of the active ingredients in the experimental formulations). Wheat stands infested by weeds but not treated with herbicides were used as negative control. Plants were grown in a Conviron A1000 environmental chamber (Canada) for 50 days. A six-step diurnal cycle of the temperature, lighting, and humidity was maintained: night – early morning – late morning – early afternoon – late afternoon – evening. The temperature was varied between 10°C at night and 18°C during the daytime in the first seven weeks of the experiment and between 14 °C and 22 °C in the following five weeks. Illumination was varied between 0 and 300 $\mu mol/m^2/s$, in 100 $\mu mol/m^2/s$ increments. The moisture content of the soil was no less than 50%.

Herbicidal Effects of P(3HB)/Metribuzin and P(3HB)/Tribenuron-Methyl Formulations

Wheat plants and weeds were photographed every week to monitor their state and growth. Indicators of the herbicidal activity of the experimental formulations were the time and the rate of death of the weeds, which were estimated from the amounts of their aboveground biomass and plant density per unit area. Indicators of the state of the wheat plants affected by weeds were their estimated productivity and measured aboveground fresh green biomass.

Statistical Analysis

Results were statistically processed using a standard software Microsoft Excel package, STATISTICA 8. Arithmetic means and standard deviations were determined using Student's t test. Results are given as X±m.

RESULTS

Herbicidal Activity of Experimental Slow-Release Metribuzin Formulations [P(3HB)/MET]

Herbicidal activity of slow-release metribuzin formulations, with the herbicide embedded in the degradable polymeric matrix of poly-3-hydroxybutyrate [P(3HB)/MET] shaped as films and microparticles, was studied in wheat stands infested with the weed – white sweet clover. The state of the wheat plants and results of weed suppression during the 50-day experiment are shown in Figure 1. Table 1 illustrates herbicidal effects of metribuzin delivered to plants in different formulations. In 10 days after sowing, the density of the white sweet clover plants and their biomass in the control (without application of the herbicide) reached 6537 plants/m^2 and 9.8 g/m^2, respectively. Those values were significantly higher than the corresponding values in the positive control (5185 plants/m^2 and 5.1 g/m^2) and treatments (4352-5556 plants/m^2 and 4.3-6.1 g/m^2), where the weed development was evidently inhibited. In 20 days after sowing, the effect was more pronounced; a great number of clover plants died, and their density dropped to 1481 and 1790 plants per m^2 in the ecosystems with P(3HB)/MET films and microparticles, respectively; in the positive control, the weed density was somewhat higher (2037 plants/m^2). The amount of clover biomass in the treatment groups had dropped to values that were an order of magnitude lower than the corresponding value in the negative control. At the end of the experiment, at Day 50, no white sweet clover plants were observed in any of the herbicide-treated ecosystems. It is important that the clover plant density and the amount of the weed aboveground biomass were significantly lower in the ecosystems with the two experimental formulations – P(3HB)/MET films and microparticles – than in the ecosystem with the commercial formulation.

Effective weed control caused an increase in wheat productivity. At Day 50, in the negative control group, with no herbicide application, wheat biomass increased to 135.5 g/m^2. That was considerably lower than in the herbicide-treated ecosystems (Table 1). The most noticeable effect was achieved by using experimental P(3HB)/MET formulations: the biomass of

the wheat vegetative organs reached 198.6 and 182.1 g/m^2 in the treatments with P(3HB)/MET films and microparticles, respectively. These values were higher than in the positive control (free herbicide) (168.6 g/m^2). These results suggest the effectiveness of using experimental formulations of metribuzin embedded in the matrix of degradable poly-3-hydroxybutyrate.

Herbicidal Activity of Experimental Slow-Release Tribenuron-Methyl Formulations [P(3HB)/TBM]

A similar experiment with wheat stands infested by the weed lamb's quarters (*Chenopodium album*) was performed to study the herbicidal activity of the systemic herbicide tribenuron-methyl (TBM) embedded in microparticles. Results of weed suppression and the state of the wheat plants during the 30-day experiment are shown in Figure 2 and Table 2.

In 7 days after sowing, in the negative control (with no herbicide application), the biomass and density of the weed reached 4815 plants/m^2 and 3.9 g/m^2, respectively. Those values were considerably higher than the corresponding values in the treatment group (585 plants/m^2 and 2.5 g/m^2) and positive control (926 plants/m^2 and 3.2 g/m^2). The effect of the free herbicide was somewhat weaker than that of the experimental formulations. At Day 20, most of the weeds were dead, and in the treatment group, their density dropped to 185 plants/m^2 and biomass to 1.1 g/m^2. Thus, the number of plants was higher by a factor of two than in the positive control, but the amounts of biomass in these two groups were comparable. At the end of the experiment, at Day 30, no lamb's quarters plants were observed in any of the herbicide-treated ecosystems; all weeds had been killed.

The decline in the density followed by the death of the *Chenopodium album* plants favored wheat growth and development. A noticeable effect was achieved by using both experimental and commercial herbicide formulations. In the wheat stands with P(3HB)/TBM microparticles and with free tribenuron-methyl, at Day 30, the amounts of the biomass of wheat vegetative organs were similar to each other, amounting to 117 and 119 g/m^2, respectively. That was significantly higher than in the negative control, where the presence of the weeds somewhat

inhibited wheat growth. At Day 30, wheat biomass in the negative control group (with no herbicide application) only increased to 50 g/m^2 (Table 2).

Thus, the study showed the effectiveness of using experimental formulations of two herbicides (metribuzin and tribenuron-methyl) embedded in the degradable matrix of poly-3-hydroxybutyrate in weed-infested wheat stands.

DISCUSSION

Herbicides are important and extensively used agrochemicals. Therefore, development and application of new-generation environmentally friendly and slow-release herbicide formulations is a fundamental task of agrochemists, biotechnologists, and plant breeders. The present study investigated the effectiveness of experimental slow-release formulations of metribuzin and tribenuron-methyl embedded in the degradable polymer matrix of the polymer of 3-hydroxybutyric acid in laboratory wheat stands infested by weeds.

Few published studies address the use of polyhydroxyalkanoates (PHAs) to construct environmentally friendly pesticide formulations, but recently, more research effort has focused on this approach. One of the first studies reported the use of films of poly-3-hydroxybutyrate as a matrix for embedding pesticides Ronilan and Sumilex, which effectively suppressed phytopathogenic fungus *B. cinerea*.[13] In a study conducted by our team, hexachlorocyclohexane and lindane were embedded in PHA to investigate polymer degradation kinetics and pesticide release into soil.[14-15] Suave et al. [16] reported encapsulation of pesticide malathion in microspheres of poly-3-hydroxybutyrate/poly(ε-caprolactone). Other authors reported encapsulation of pesticides ametrine and atrazine in microspheres of the 3-hydroxybutyrate/3-hydroxyvalerate copolymer.[17-18] In a more recent study, Prudnikova and co-authors [19] described embedding of herbicide Zellek Super in P(3HB/3HV) microparticles and films to prepare slow-release formulations. In our recent study, polymer P(3HB) was used as a matrix for slow-release formulations of the herbicide metribuzin; we constructed formulations of various shapes (films, microparticles, microparticles, and pellets) and studied metribuzin release kinetics

in laboratory soil microecosystems, showing the effectiveness of the formulations in inhibiting the growth of two model weeds: *Agrostis stolonifera* and *Setaria pumila*.[10] Kumar et al. [20] investigated different concentrations of metribuzin embedded in polyvinyl chloride, carboxy methylcellulose (CMC), and carboxy methylcellulose-kaolinite composite (CMC-KAO) in a field study, on a plot with wheat plants infested by various weeds; in the control, MET was used as a commercial formulation – emulsion used to spray young weeds. The authors showed that in 90 days after application, experimental MET formulations killed 76/1% of the weeds versus 57.14% in the control. That study also suggested that sensitivity of the weeds to metribuzin varied depending on their age.

The present study showed that in wheat stands, metribuzin embedded in P(3HB) effectively controlled white sweet clover, which was used as the weed. The herbicidal effects of both P(3HB)/MET films and microparticles were comparable to the effect of the commercial formulation in the early phases of the wheat stand development and superior to it in later phases (in more than 20 days after sowing).

An experimental formulation of another systemic herbicide – tribenuron-methyl, which was also embedded in the polymeric matrix of P(3HB) shaped as microparticles, was tested in wheat stands infested by the weed lamb's quarters, in the 30-day experiment. The first noticeable effects of the experimental formulation were observed at Day 7 of the experiment, and the number of weeds killed by the experimental formulation was by a factor of two greater than the number of the plants killed by the commercial formulation. This effect lasted for 20 days. This herbicide has received much less attention of the researchers. There are no data in literature on the use of TBM in slow-release formulations.

CONCLUSION

Thus, the study showed the effectiveness of using experimental formulations of two herbicides (metribuzin and tribenuron-methyl) embedded in the degradable matrix of poly-3-

hydroxybutyrate in weed-infested wheat stands. Results of this study suggest that degradable poly-3-hydroxybutyrate is a promising material for constructing pre-emergence slow-release herbicide formulations.

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FIGURE CAPTIONS

Figure 1. Photographs of wheat (*Triticum aestivum*) stands infested with white sweet clover (*Melilotus albus*) and treated with different P(3HB)/MET formulations: *a* – microparticles, *b* – films, *c* – positive control, *d* – negative control (untreated)

Figure 2. Photographs of wheat (*Triticum aestivum*) stands infested with lamb's quarters and treated with different P(3HB)/TBM formulations: *a* – negative control (untreated), *b* – P(3HB)/TBM microparticles (treatment), *c* – positive control, TBM

Table 1. Density and dry biomass weight of wheat (*Triticum aestivum*) and white sweet clover (*Melilotus albus*) plants in laboratory soil ecosystems treated with different formulations of the herbicide metribuzin.

Type of formulation	Dry biomass of wheat (g/m^2)			Density of wheat ($plants/m^2$)		
	10 days	20 days	50 days	10 days	20 days	50 days
Films	20.6±1.1	90.8±10.0	198.6±11.3	1852	1852	1852
Microparticles	24.5±1.9	107.9±8.3	182.1±9.9	1852	1852	1852
Control(-)	23.9±1.5	105.3±6.3	135.5±6.4	1852	1852	1852
Control (+)	23.1±1.9	101.4±7.5	168.6±10.3	1852	1852	1852
Dry biomass of clover plants (g/m^2)				Density of clover plants ($plants/m^2$)		
Films	4.3±0.2	1.9±0.1	-	4352±51	1481±37	-
Microparticles	6.1±0.4	1.3±0.1	-	5556±61	1790±32	-
Control(-)	9.8±0.4	14.7±0.4	90.5±6.4	6537±85	6537±85	6537±85
Control (+)	5.1±0.2	2.1±0.1	-	5185±77	2037±67	-

Table 2. Density and dry biomass weight of wheat (*Triticum aestivum*) and lamb's quarters (*Chenopodium album*) plants in laboratory soil ecosystems treated with different formulations of the herbicide tribenuron-methyl

Type of formulation	Dry biomass of wheat (g/m^2)			Density of wheat ($plants/m^2$)		
	7 days	20 days	30 days	7 days	20 days	50 days
Microparticles	22±1.5	95±5.6	117±7.9	1852	1852	1852
Control(-)	10±1.2	27±2.3	50±6.4	1852	1852	1852
Control (+)	23±1.7	94±8.5	119±10.0	1852	1852	1852
Dry biomass of clover plants (g/m^2)				Density of clover plants ($plants/m^2$)		
Microparticles	2.5±0.1	1.1±0.1	-	585±31	185±12	-
Control(-)	3.9±0.3	5.3±0.4	10.1±2.4	4815±141	4815±141	4815±141
Control (+)	3.2±0.2	1.3±0.1	-	926±59	370±24	-