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Biological effects of the free and embedded metribuzin and tribenuron-methyl herbicides on various cultivated weed species

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ABSTRACT

The present study addresses the herbicidal activity and biological effects of the metribuzin (MET) and tribenuron-methyl (TBM) herbicides used to control various weed species (*Amaranthus retroflexus, Sinapis arvensis,* and *Leucanthemum maximum*). The effects of the free herbicides and the herbicides embedded in granules of degradable polymer poly-3-hydroxybutyrate [P(3HB)] blended with birch wood flour were compared. Metribuzin, regardless of the form, caused 100% mortality of the three weeds by day 21. The herbicidal activity of tribenuron-methyl was lower than that of metribuzin, but the embedded TBM was superior to the free herbicide in the length and strength of its action on the weeds. Both metribuzin forms dramatically decreased the main parameters of fluorescence: maximum quantum yield of photosystem-II [$Y(II)_{max}$], maximum quantum yield of non-photochemical quenching [$Y(NPQ)_{max}$], and maximum rate of non-cyclic electron transport [ETR_{max}] and concentrations of chlorophyll *a* and *b*. The effect of the embedded TBM on the photosynthetic activity of the weeds was lower in the first two weeks of the growth of herbicide-treated plants but lasted longer than the effect of the free TBM and increased over time. Embedding of metribuzin in the matrix of degradable blend did not decrease its herbicidal activity.

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Metribuzin; tribenuron-methyl; degradable P(3HB); weed growth inhibition; photosynthetic activity

Introduction

Herbicide applications inevitably result in excessively high levels of chemicals in soil, posing health risks, causing some plant species to develop resistance to the herbicides applied, presenting threat to agroecosystems, disturbing their stability, and endangering long-term soil fertility. Because herbicides are used at enormous scale, playing a substantial part in agriculture, the development and application of new-generation environmentally safe herbicide formulations is a priority for agrochemists, biotechnologists, and horticulturists. A current research focus is to develop less toxic and more selective pesticides and reduce application rates.

Much research effort has been devoted recently to decreasing the risk of uncontrolled distribution and accumulation of pesticides in the biosphere by developing environmentally safe new-generation controlled release pesticide formulations in which the active ingredient is either coated by a layer or embedded in a matrix of a biodegradable material. The main condition for constructing such formulations is the availability of appropriate materials with the following properties:

1. compatibility with the environment and global biosphere cycles, i.e. degradability;
2. safety for living organisms and their nonliving environment;
3. long-term presence (for weeks and months) in the natural environment and controlled degradation followed by formation of nontoxic products;
4. chemical compatibility with pesticides;
5. processability by available methods compatible with processes of production of agrochemicals.

Materials investigated as candidates for constructing a matrix for embedding pesticides include synthetic, non-degradable polymers such as polystyrene, polyacrylamide, polyethylene acrylate, polyamide, polyurethane, and polycyanoacrylate. Studies published in recent years reported investigations of degradable materials that can be decomposed by soil microflora without producing more contaminants, with release of chemicals occurring gradually. These are such materials as cellulose, agarose, dextran, carrageenan, starch, chitosan, alginate, protein-containing gelatin, and albumin.[1] The shortcomings of these natural polymers are their low mechanical strength and rapid hydrolysis in liquid media, which is an obstacle to preparing sustained-release formulations of agrochemicals.

Among biodegradable materials, special attention is given to biopolymers synthesized by microorganisms in biotechnological processes such as polyhydroxyalkanoates (PHAs). These polymers are thermoplastic, mechanically strong, and slowly degradable in biological media.[2] As these polymers

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decompose via truly biological degradation and do not undergo hydrolysis in liquid media, the products made from them may function, e.g. in soil, for months. The rates of release and delivery of the active ingredient can be varied within a wide range by controlling the degradation rate of the PHA matrix, by using products of different shapes that contain different amounts of preparations. The available literature data on using PHAs to construct environmentally safe pesticide formulations are limited. However, poly-3-hydroxybutyrate [P(3HB)] – the best studied and most commonly used PHA – has been successfully used to construct slow-release pesticide formulations.[2] Metribuzin formulations were prepared in the form of films, microgranules, and microparticles based on P(3HB).[3] Those formulations were found to be effective herbicides in experiments with weed model.[4]

They were also used to control weeds in the wheat stands.[5] In order to make PHA polymers, whose cost is rather high now, more easily accessible and less expensive, research was conducted in which fungicides were embedded in matrices of degradable P(3HB) blended with natural materials (peat, clay, and birch wood flour).[6,7]

One of the challenges in developing slow-release pesticide formulations is maintaining the biological efficacy of the active ingredient loaded into the matrix. Therefore, it is necessary not only to develop the process of constructing slow-release herbicide formulations but also to study their biological activity in weed control relative to the herbicidal activity of the free active ingredient. Plants affected by herbicides and suffering photosynthetic stress should be investigated to estimate the state of their photosynthetic apparatus, chlorophyll fluorescence parameters, and the content of chlorophyll-protein complexes. Herbicides were found to slow down photosynthesis rate, decrease the contents of green pigments and carotenoids, inhibit CO_2 assimilation, and impair plant nutrition and growth.[8]

These damages are caused not only by herbicides aimed at photosynthesis (atrazine, metribuzin, etc.),[9] but also, as reported in recent studies.[10] By such herbicides as glyphosate and imazethapyr, whose main targets are acetolactate synthase and reactions of synthesis of branched-chain amino acids.

The purpose of the present study was to investigate the biological efficacy of the slow-release metribuzin and tribenuron-methyl herbicides loaded into the matrix of degradable poly-3-hydroxybutyrate blended with birch wood flour in controlling different weed species, taking into account weed mortality dynamics and inhibition of photosynthetic activity.

Materials and methods

Herbicides

Two herbicides with different modes of action were studied: metribuzin (MET) and tribenuron-methyl (TBM).

Metribuzin [4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one] is a systemic selective herbicide of the class of 1,2,4-triazines, having a broad spectrum activity against some dicots and grass weeds. MET has a long-lasting effect, acting via both leaves and soil. The mode of action is based on inhibiting the Hill reaction (water photolysis) and photosynthetic electron transport between primary and secondary electron acceptors in Photosystem II. MET effectively protects soybean, maize, cereal, potato, and tomato crops from annual dicots and grass weeds.

Tribenuron-methyl [methyl ester of 2-(6-methyl-4-methoxy-1,3,5-triazin-2-yl(methyl) carbamoylsulfamoyl) benzoic acid] is a systemic selective herbicide of the sulfonylurea family. The mode of action is based on inhibiting acetolactate synthase, which takes part in biosynthesis of branched-chain amino acids (valine, leucine, and isoleucine), causing a decrease in the levels of these amino acids in plant tissues followed by disruption of protein and nucleic acid synthesis. TBM effectively protects cereal crops from dicots and grass weeds.

The metribuzin and tribenuron-methyl herbicides used in experiments with plants were supplied by Xi'anTai Cheng Chem Co., Ltd (China); the content of the active ingredient in metribuzin was 97.2% and in tribenuron-methyl – 95.5%

Materials for embedding herbicides

P(3HB) polymer samples were synthesized using the *Cupriavidus eutrophus* B10646 strain and proprietary technology.[2] Polymer was extracted from cells with chloroform, and the extracts were precipitated using hexane. The extracted polymers were re-dissolved and precipitated again 3–4 times to prepare homogeneous specimens. The polymer had the following properties: degree of crystallinity 75%, melting point 176 °C, thermal decomposition temperature 287 °C, molecular weight (M_w) 590 kDa, and polydispersity index 5.8.

A natural material, wood flour, was used as filler. Wood flour was produced by grinding wood of birch (*Betula pendula* Roth) using an MD 250-85 woodworking machine ("StankoPremyer" Russia). Then it was dried at 60 °C for 120 h until it reached constant weight, and 0.5 mm mesh was used to separate the particle size fraction; degree of crystallinity 26%; onset of thermal decomposition 220 °C.

Experimental herbicide formulations

The polymer and wood flour were pulverized by impact and shearing action in ultra-centrifugal mill ZM 200 (Retsch, Germany). To achieve high fineness of polymer grinding, the material and the mill housing with the grinding tools were preliminarily cooled at −80 °C for about 30 min in an Innova U101 freezer (NEW BRUNSWICK SCIENTIFIC, U.S.). Grinding was performed using a sieve with 2-mm holes at a rotor speed of 18000 rpm. The fractional composition of the polymer and filler powders was determined using vibratory sieve shaker AS 200 control (Retsch, Germany). Then, polymer powder was mixed with the filler powder in benchtop planetary mixer SpeedMixer DAC 250 SP (Hauschild Eng., Germany); the blend time was 1 min, and the speed was 1000 rpm.

Table 1. Chemical characterization of soil (0–20 cm).

Parameter	Value
pH_{H2O}	7.2
Humus, %	10.7
Hydrolytic acidity, mmol/100 g	0.75
Total absorbed bases, mmol/100 g	71.0
Cation exchange capacity, mmol/100 g	71.8
Base saturation, %	99.0
Nitrate nitrogen, mg/kg	16.0
Ammonium nitrogen, mg/kg	10.1
Labile phosphorus, mg/kg	239.0
Exchangeable potassium, mg/kg	110.7
Exchangeable calcium, mmol/100 g	27.2
Exchangeable magnesium, mmol/100 g	4.3
Silt and clay content, %	64–65
Including: sand, %	35–36
Silt fraction, %	36–39

Herbicide granules were prepared using polymer paste wetted with ethanol and mixed with birch wood flour and the herbicide in screw granulator Fimar (Italy). The formulations contained the following percentages of the components: P(3HB)/wood flour/herbicide — 50/30/20 (wt.%). The solid granules were 3 mm in diameter and 4 to 6 mm long. In the positive control, herbicides were applied to the soil as solutions at concentrations equal to the concentrations applied with granules.

Weeds

The following plant species were used as weeds: red-root amaranth (*Amaranthus retroflexus*) – a widespread annual spring herb, infesting tilled crops and grain crops (wheat, rye, oat, barley, and corn) and occurring in kitchen gardens; field mustard (*Sinapis arvensis*) – an annual plant, heavily infesting spring grain fields; max chrysanthemum (*Leucanthemum maximum*) – a perennial taproot herb, growing 80 cm tall, infesting perennial grasses and various annual crops, mainly grain crops.

Metribuzin is a broad-spectrum herbicide, effective against red-root amaranth and field mustard. Tribenuron-methyl suppresses broadleaf weeds, including various mustard varieties.

Cultivation and evaluation of parameters of weeds affected by herbicides

Weeds were grown in laboratory soil microecosystems in a 35-day experiment. The soil was collected at the field laboratory of Krasnoyarsk State Agrarian University, in the vicinity of Krasnoyarsk (Russia). It was meadow-chernozem soil, whose soil profile was similar to the profile of chernozem soils with a thick humus-rich layer and loose granular structure. The soil was neutral, with low hydrolytic activity and high contents of nitrogen, labile phosphorus, and exchangeable potassium (Table 1).

The soil was collected from a plot that had not been treated with pesticides, including metribuzin and tribenuron-methyl. The seeds for the experiments were taken from the collection of the Department of Terrestrial Ecosystems of the Siberian Federal University. Seeds had been collected in the vicinity of the city of Krasnoyarsk, in a natural forest (mixed birch-pine forest), 20 km away from the city. This area has never been used as agricultural land and no pesticides have been used there. Agricultural land is 30–40 km away from the seed collection site. Thus, the plants grown in the experimental systems could not have developed resistance to these herbicides, and the effect of the herbicides was their true biological effect.

Soil was placed into 500-cm^3 plastic containers (400 g soil per container), and plant seeds were sown in the soil (150 g seeds per 1 m^2). Granules with embedded herbicides were placed in close-meshed gauze bags and buried in the soil at a depth of 1.5–2.0 cm simultaneously with sowing. The application rates of MET and TBM corresponded to the recommended application rates of these herbicides: 400 g/ha and 20 g/ha, respectively.[11] In the positive control, solutions of unembedded herbicides were added to soil at rates recommended for field application and corresponding to herbicide concentrations in granules. The herbicides were preliminarily dissolved in distilled water at room temperature on a shaker until complete dissolution was achieved, and 100 ml of the solution was added to the soil in each container before seed sowing. The target concentrations of herbicides embedded in the matrix were achieved by varying the amounts of the granules buried in the soil. Plants were grown in an environmental chamber (Fitotron-LiA-2, Russia). The temperature, lighting, and soil moisture content were controlled in the six-step mode: "night – early morning – late morning – early afternoon – late afternoon – evening". The temperature was varied between 10 °C by night and 18 °C by day in the first seven weeks of the experiment and between 14 °C by night and 22 °C by day in the following five weeks. Lighting was varied between 0 and 300 µmol/m^2/s, in 100 µmol/m^2/s increments. The lowest soil moisture content was 50%.

Samples for analysis were collected weekly. Other indicators of the state of the weeds were the time when the highest mortality rate was achieved and plant density (the number of plants per 1 m^2). Weed plant mortality was monitored by counting the number of the dead and living weed plants in a container. At each time point, plants in three containers were counted in each experiment. To study photosynthesis reactions and determine pigments, three leaves per plant were collected from at least three plants per container.

Biological efficacy of the embedded herbicides was estimated using corrected percent mortality, C_{cor}, derived from modified Abbot's formula,[12] which shows the decrease in the number of weeds caused by application of herbicides (percent of the initial infestation or the control) corrected for the control:

$$C_{cor} = 100 - \frac{\frac{B_0}{A_0} * a_k}{b_k} * 100 \quad (1)$$

where:

A_0 is the number of weed plants per m^2 to determine the initial infestation rate in the treatment;

B_0 – the same in the second and following counts;

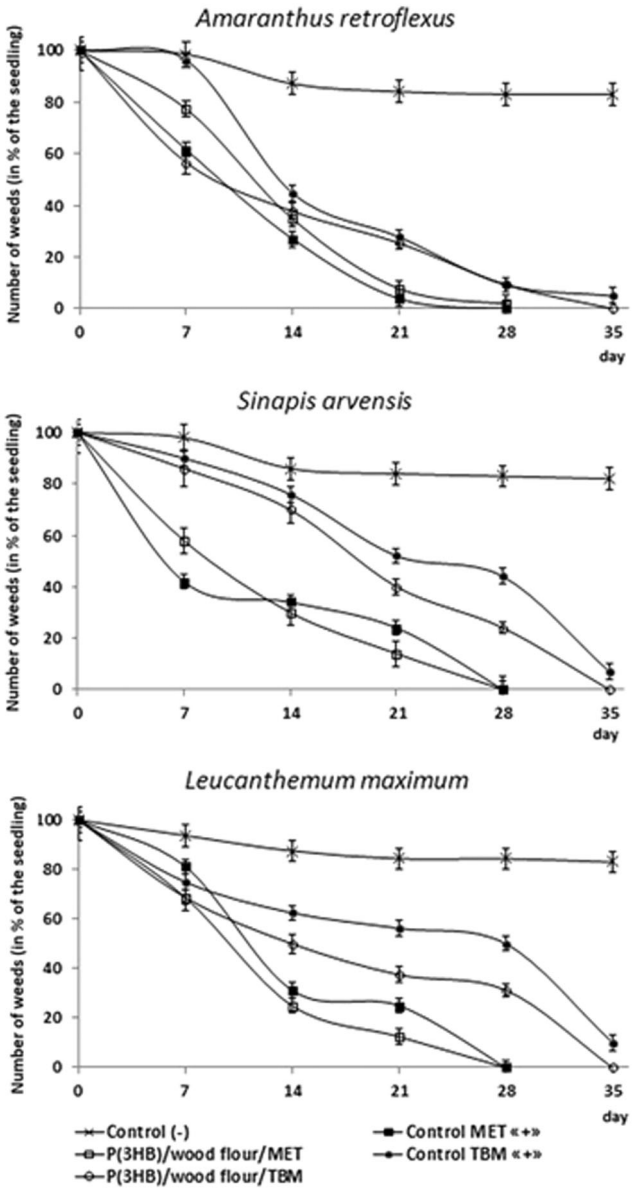

Figure 1. Weed mortality dynamics (weeds as % of seedlings): *Amaranthus retroflexus*, *Sinapis arvensis*, *Leucanthemum maximum*. Negative control (intact plants without herbicide treatment); positive control (free metribuzin and tribenuron-methyl); embedded herbicides: P(3HB)/wood flour/MET and P(3HB)/wood flour/TBM.

a_k – the number of weed plants per m^2 to determine the initial infestation rate in the control;
b_k – the same in the second and following counts.

Study of the effect of herbicides on plant photosynthesis parameters

The effect of herbicides on the photosynthetic activity of plants was studied using leaves of the weeds: rapid chlorophyll fluorescence was measured employing pulse amplitude modulated fluorometers (PAM) (Walz, Effeltrich, Germany). Before measurements, the leaves were dark adapted for 30 min. Actinic light intensity was increased stepwise (0, 66, 90, 125, 190, 285, 420, 625, and 820×10^{-6} M photons·m^{-2}·s^{-1}) at 10 s intervals. Fluorescence parameters were measured at ambient temperature and humidity. The following fluorescence parameters were measured: $Y(II)_{max}$ – maximum quantum yield of Photosystem-II (PSII); $Y(NPQ)_{max}$ – maximum quantum yield of non-photochemical quenching; ETR_{max} – maximum rate of non-cyclic electron transport.

Quantification of chlorophyll-protein complexes

To extract pigments of photosynthesis from fresh plant material, a 40–60 mg leaf sample (without midrib) was placed into a 15-ml test tube. Five ml 95% ethyl alcohol and 10 mg $CaCO_3$ powder were added, and the test tube was plugged with a PE foam stopper. Pigments were extracted in water bath at a temperature of 60 °C until the leaf was completely bleached (usually for 20–30 min). Then, the test tubes were placed into a refrigerator for 12 h (+4 °C) and left in the dark until pigment extraction was completed and solution clarified. Pigments were quantified by spectrophotometry. Optical density was measured using a Specol-1300 spectrophotometer (Germany). Concentrations of C_a and C_b chlorophylls and total xanthophylls and carotenes C_{x+c} (μg/ml) were calculated using conventional formulas.[13] Pigment contents were expressed as mg × g^{-1} fresh mass.

Statistical analysis

Results were expressed as the average from three parallel experiments performed with triplicates and presented as the mean ± SE. Statistical comparisons of the means between each treatment and the control were performed using analysis of variance. Statistical probability (P) was set at 0.05.

Results and discussion

The effect of herbicides on weed mortality dynamics

Experimental formulations of both MET and TBM showed herbicidal activity toward the weeds comparable or even superior to the herbicidal activity of free MET and TBM (Fig. 1).

The experiment with red-root amaranth, field mustard, and max chrysanthemum showed that both herbicides inhibited the growth of the three weeds. The herbicidal activity was detected at day 7, and metribuzin was more effective than tribenuron-methyl. The highest mortality rate of the weeds was achieved earlier in the MET treatment than in the TBM treatment (at day 14), when the abundance of plants treated with MET was no more than 25%–30% of the initial abundance; at day 28, all plants died.

A search of the literature on the efficacy of metribuzin revealed that research mainly focused on biological effects of different concentrations of this herbicide on various weed species, but no comparison was made between the efficacy and properties of the free and embedded herbicide. Metribuzin is effective against various broadleaf weeds and grasses, and traditional pre-emergence soil applications of this herbicide in potato fields proved that it effectively

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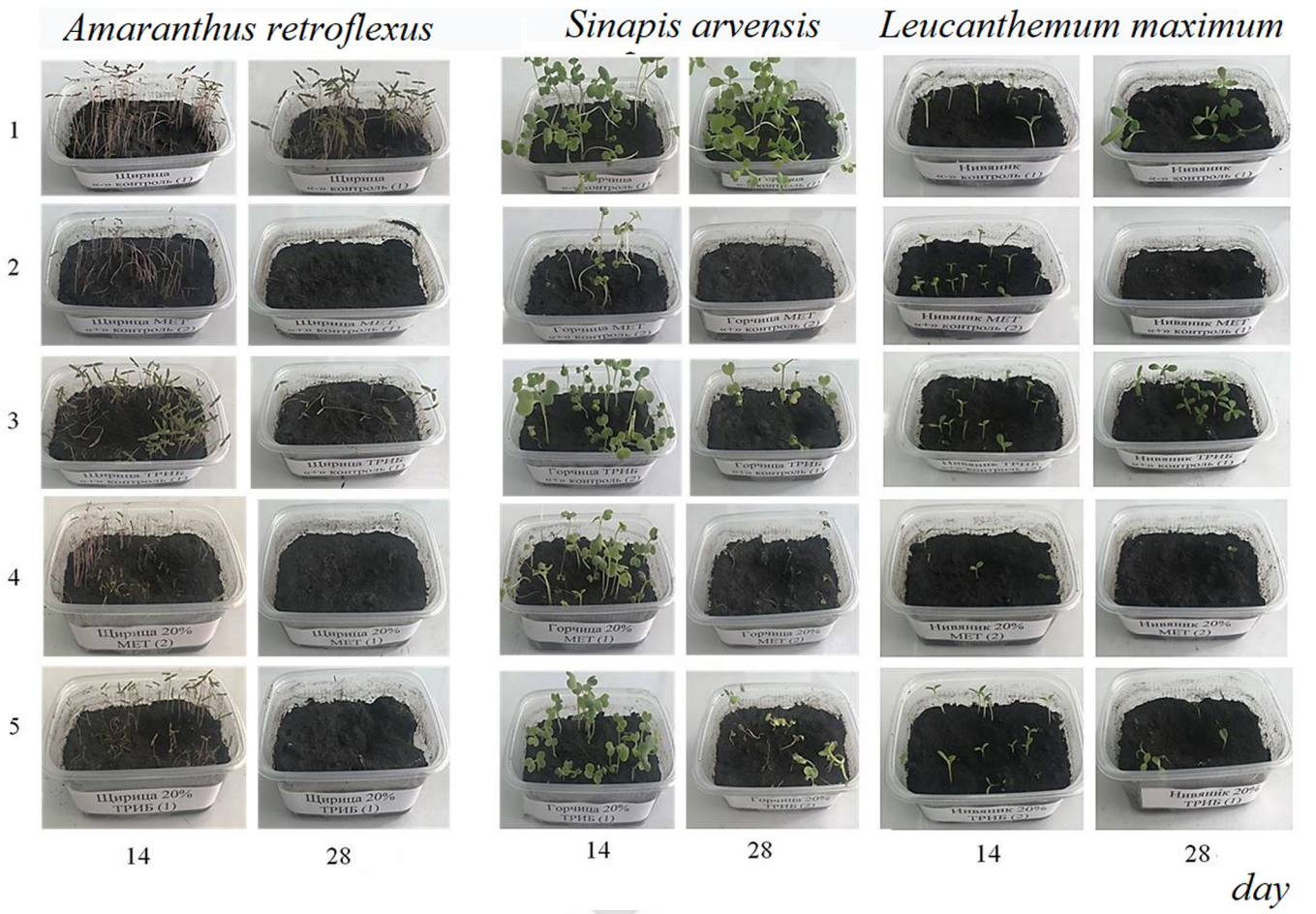

Figure 2. Photographs of laboratory ecosystems with different forms of metribuzin and tribenuron-methyl: 1 – negative control (intact plants, with no herbicides); free herbicides (positive control): 2 – metribuzin and 3 – tribenuron-methyl; experimental formulations: 4 – P(3HB)/wood flour/MET and 5 – P(3HB)/wood flour/TBM.

controlled *Amaranthus retroflexus* and *Chenopodium album* without reducing potato yield.[14] The effects of different metribuzin doses (105 to 525 g/ha) on red-root amaranth (*Amaranthus retroflexus* L.) and field mustard (*Sinapis arvensis* L.) were investigated in this study, showing that the biomass of red-root amaranth was reduced by 90% when MET was applied at the BBCH 12–15 and BBCH 16–19 growth stages, respectively, at a concentration of 525 g/ha. The decrease in the biomass of *S. arvensis* in the same growth stages, BBCH 12–15 and BBCH 16–19, occurred at lower metribuzin concentrations (263 g/ha). Metribuzin was used in maize fields to test its efficacy against the *Portulaca oleracea*, *Amaranthus retroflexus*, and *Echinochloa colonum* weeds, and the biomass of the weeds was reduced by 97.7%, 96.9%, and 97.2%, respectively.[15] These results were consistent with the data reported by Medd et al.,[16] suggesting that herbicide efficacy varied depending on the weed species. In addition, weeds at the BBCH 12–15 growth stage were found to be more sensitive to the effects of herbicides. This finding is consistent with the conclusion made by Riethmuller-Haage et al.,[17] suggesting that the metribuzin dose necessary to control weeds varied depending on the leaf area and number of leaves and that the effect of herbicides became weaker as the plants were growing. A few studies addressed the relationship between the herbicidal activity and the form of herbicide delivery. Experiments with polycaprolactone nanocapsules with the atrazine herbicide, whose mode of action is similar to that of metribuzin – inhibition of plant photosynthesis, showed higher efficacy of encapsulated atrazine compared to the free herbicide used for post-emergence treatment of *Amaranthus viridis* and *Bidens pilosa*.[18]

Experiments with tribenuron-methyl showed that, regardless of its form, its herbicidal action toward field mustard and max chrysanthemum was weaker than the effect of MET (Fig. 1). Only the mortality rate of red-root amaranth reached its peak at the same dates as in the MET experiment. The highest mortality rates of the other weed species (field mustard and max chrysanthemum) were achieved later. At day 21, the abundances of both weed species were reduced to 50%–60%; a week later (day 28), weed mortality did not reach 100%, in contrast to the MET treatments. The abundance of the remaining weed plants was rather high, between 25%–35% and 40%–50%. A significant difference was noted between the herbicidal activities of the embedded TBM and the unembedded herbicide. The embedded TBM was more effective, killing all weed plants by day 35. At the same time point, in the ecosystems with the free TBM, 5%–10% field mustard and max chrysanthemum plants still remained alive.

Table 2. Biological efficacy (C_{cor}) of the P(3HB)/wood flour/MET and P(3HB)/wood flour/TBM experimental formulations compared to the efficacy of free metribuzin and tribenuron-methyl.

	Biological efficacy C_{cor} (%)		
Treatment/control	14 day	21 day	28 day
Amaranthus retroflexus			
Control MET (400 g/ha) «+»	66.7 ± 5.2	95.0 ± 4.2	100.0
Control TBM (20 g/ha) «+»	44.8 ± 3.6	64.0 ± 4.0	88.0 ± 4.2
P(3HB)/wood flour/MET	57.1 ± 4.3	90.0 ± 3.7	97.5 ± 2.1
P(3HB)/wood flour/TBM	53.3 ± 4.7	67.0 ± 5.4	92.0 ± 3.4
Sinapis arvensis			
Control MET (400 g/ha) «+»	60.5 ± 5.0	71.4 ± 3.3	100.0
Control TBM (20 g/ha)«+»	11.6 ± 1.2	38.1 ± 2.6	45.0 ± 2.1
P(3HB)/wood flour/MET	65.1 ± 5.6	83.3 ± 4.0	100.0
P(3HB)/wood flour/TBM	18.6 ± 1.8	52.4 ± 3.7	70.0 ± 3.4
Leucanthemum maximum			
Control MET (400 g/ha) «+»	64.3 ± 5.3	70.4 ± 3.8	100.0
Control TBM (20 g/ha) «+»	28.6 ± 1.7	33.3 ± 2.2	40.7 ± 2.5
P(3HB)/wood flour/MET	71.4 ± 5.9	85.2 ± 3.0	100.0
P(3HB)/wood flour/TBM	42.9 ± 3.8	55.6 ± 3.3	63.0 ± 3.9

The latest literature data suggest that the effect of tribenuron-methyl, like the effect of metribuzin, varies depending on the target weed species and the time and rate of herbicide application. Gherekhloo et al.[19] described the dose-dependent effect of TBM on different generations of *Sinapis arvensis* and showed that as plants became more resistant, the standard dose of TBM (15 g/ha) needed to be increased by 2.2–16.8 times. Similar results were obtained in another study of TBM effect on *Sinapis arvensis*.[20] TBM was found to be less effective in weed control than triazine and chlorotriazine herbicides.[21] However, the ability of embedded or free sulfonylurea herbicides to inhibit growth and development of various weeds was confirmed in a number of studies,[22] including studies performed with such weeds as *Amaranthus tuberculatus*, *Amaranthus palmeri*, and *Amaranthus* spp.[23]

Biological efficacy of embedded herbicides

The herbicidal efficacy of different forms of herbicides and the dissimilarities between the effects of MET and TBM and their embedded and unembedded forms are clearly seen in the photographs of the weed stands taken at different time points of the experiment (Fig. 2). In the MET treatments, no weeds can be seen at the end of the experiment while in the TBM treatments, some of the field mustard and max chrysanthemum plants remained alive, and their abundances were different in the ecosystems with the free and embedded TBM.

Results are summarized in Table 2, which shows the dynamics of biological efficacy determined for the experimental formulations of metribuzin [P(3HB)/wood flour/MET] and tribenuron-methyl [P(3HB)/wood flour/TBM] compared to the effects of the free herbicides and relative to the abundance of weed plants in the negative control. At day 28, the biological efficacy of the embedded MET was 100% in the red-root amaranth, field mustard, and max chrysanthemum stands. The biological efficacy of the embedded TBM was generally somewhat lower than the efficacy of the embedded MET: 97.1% for red-root amaranth, 70% for field mustard, and 63% for max chrysanthemum at day 28. The herbicidal activity of free TBM was inferior to that of the experimental formulations, and at day 28, the biological efficacy of free TBM was 88.0% for red-root amaranth and slightly above 40% for field mustard and max chrysanthemum. The somewhat lower herbicidal activity of TBM may be associated with the well-known ability of this herbicide to be metabolized in higher plant tissues to yield compounds that are not toxic to plants. Thus, it is very important to be able to prolong and enhance its effects by loading it into a degradable matrix.

Experiments showed that embedding of the herbicides enhanced their activity toward the weeds rather than decreased it.

Functional activity of the photosynthetic apparatus of weeds affected by herbicides

The study of the effect of the free and embedded metribuzin on chlorophyll fluorescence parameters in different weed species showed that both MET forms considerably inhibited the photosynthetic apparatus of the three weed species compared to the negative control (Fig. 3). At day 14, the values of the fluorescence parameters ($Y(II)_{max}$ and ETR_{max}) in different species dropped by a factor of 2 to 8 relative to the negative control. Then, both parameters continued declining, and at day 21, they reached almost zero level. The reason for such dramatic inhibition of plant photosynthetic activity by metribuzin is the well-known negative effect of this herbicide on photosynthesis reactions.[10, 24]

The decrease in the photosynthesis parameters, $Y(II)_{max}$ and ETR_{max}, of the weeds affected by MET may be directly related to the increase in concentration of hydrogen peroxide, which is a strong inhibitor of photosynthesis: even low H_2O_2 concentrations inhibit CO_2 fixation by oxidizing thiol groups of some enzymes in the Calvin cycle.[25] The decrease in ETR may be associated with the decrease in the ascorbate pool, which in turn impairs antioxidant protection of cells.[10]

The maximum quantum yield of non-photochemical quenching, $Y(NPQ)_{max}$, in the plants decreased insignificantly at day 14, approaching zero at day 21, similar to parameters $Y(II)_{max}$ and ETR_{max} (Fig. 3). NPQ is directly or indirectly related to light harvesting by photosynthetic antenna complexes, their structure, transfer of the captured energy to reaction centers, electron transport, proton transport across the membrane, ATPase activity, and carbon assimilation.[26–28] Thus, metribuzin, regardless of its delivery form, interrupted the key processes of photochemistry in the weeds, as evidenced by the dynamics of parameters of photosynthetic activity – $Y(II)_{max}$, ETR_{max}, and $Y(NPQ)_{max}$. A similar decrease in the photosynthesis variables was observed in the experiments with MET-treated wheat plants.[8] Qien et al.[29] reported the effects of sublethal doses of atrazine (Atr) and methyl viologen (MV) on the photosynthetic electron transport in *Arabidopsis thaliana*. Four herbicides (paraquat, norflurazon, flazasulfuron, and atrazine) used in experiment with *Lemna minor* inhibited

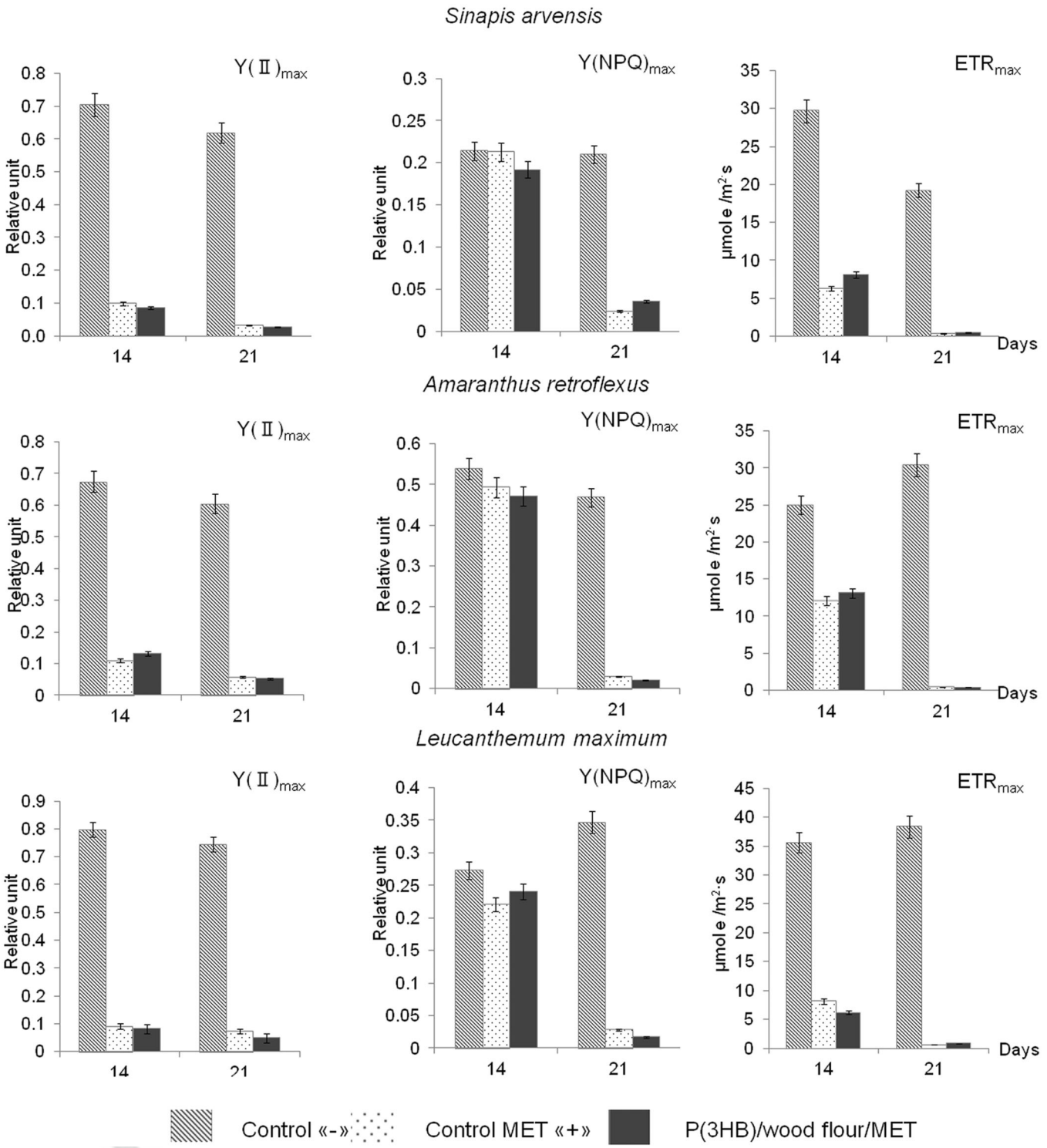

Figure 3. Changes in chlorophyll fluorescence parameters of weeds affected by different forms of metribuzin: Control (+) – positive control (free MET); MET (Granules) – experimental herbicide formulation, P(3HB)/wood flour/MET; Control (–) – negative control (intact plants).

carotenoids, and interrupted protein biosynthesis, blocking electron transport of PSII.[30] Maximum quantum yield decreased when the *B. pilosa* weed and soybean (*Glycine max*) were treated with a commercial formulation of atrazine and the herbicide encapsulated in nanocapsules of poly(ε-caprolactone).[31] An ETR decrease was detected after atrazine treatment of maize, weeds, and *Calophyllum brasiliense*.[32] Application of nanocapsules with atrazine decreased Y(II) and ETR in the *Amaranthus viridis* and *Bidens pilosa* weeds.[33]

The effect of tribenuron-methyl on photosynthetic activity of weeds is more complex and controversial. Herbicides based on sulfonylurea, containing TBM as the active ingredient, do not directly affect the photosynthesis system but rather impact the function of acetolactate synthase (ALS). The adverse effects of sulfonylureas, inhibiting ALS, such as

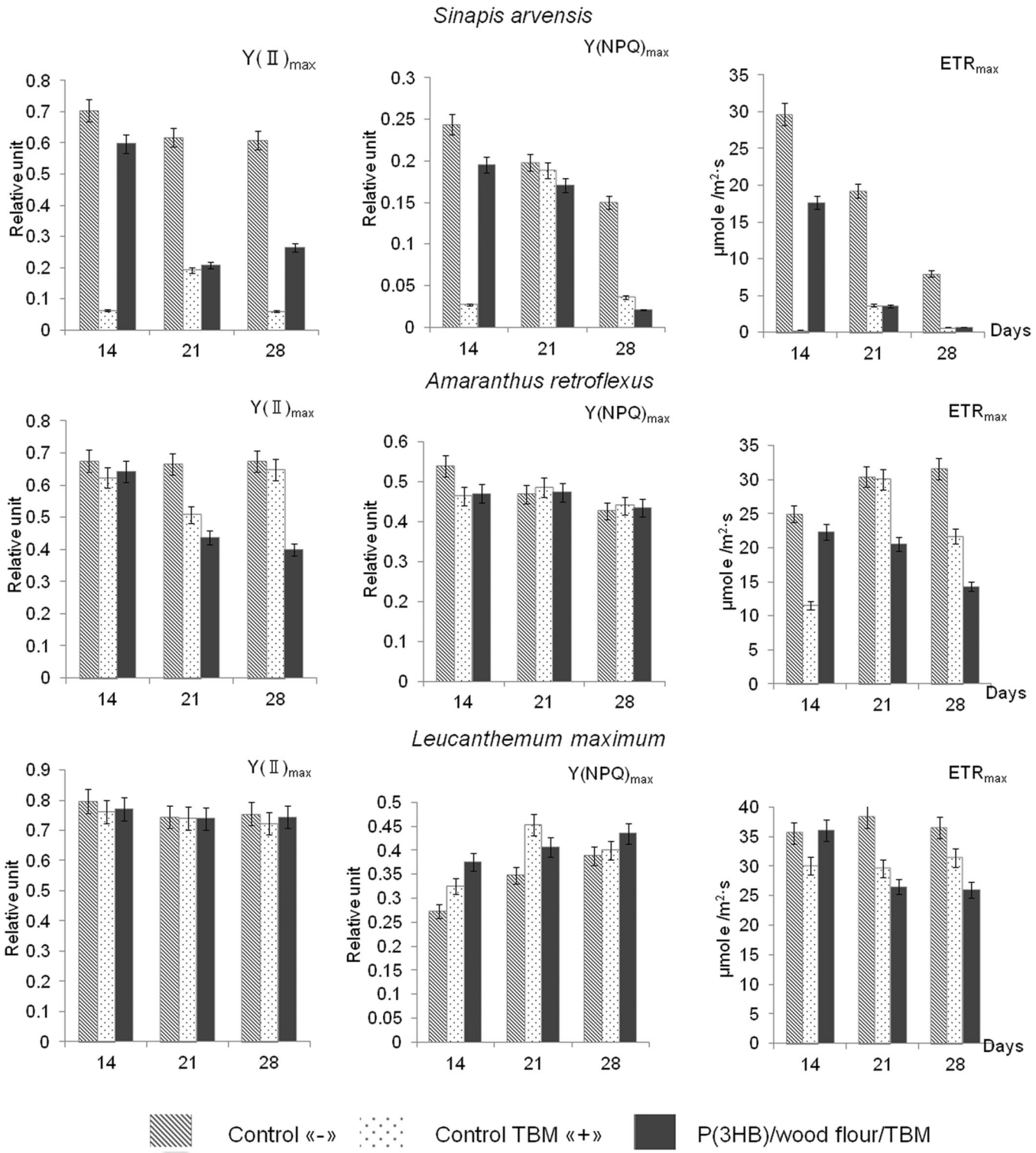

Figure 4. Changes in chlorophyll fluorescence parameters of weeds affected by different forms of tribenuron-methyl: Control (+) – positive control (free TBM); TBM (Granules) – experimental herbicide formulation, P(3HB)/wood flour/TBM; Control (−) – negative control (intact plants).

chlorosis, necrosis, and inhibition of growth of plants were described by Agostinetto et al.[8] Relatively recent research showed that many of the effects produced by ALS inhibiting herbicides were associated with biogenesis of ribosomes, secondary metabolism, cell wall modification, and cell growth.[34,35] For instance, the glyphosate can affect other physiological processes in the plant.[34] Numerous studies demonstrated decreases in the photosynthetic rate of plants following treatment with glyphosate.[36,37] Moreover, glyphosate can indirectly affect photosynthesis by inhibiting chlorophyll biosynthesis or inducing chlorophyll degradation,[38] decreasing stomatal conductance,[39] and provoking nutritional disturbances.[40] Measurements of chlorophyll fluorescence parameters in weeds are shown in Figure 4. Experiments with field mustard and red-root amaranth demonstrated a considerable inhibitory effect of tribenuron-

Table 3. Changes in photosynthesis pigment contents of weeds treated with the free and embedded metribuzin and tribenuron-methyl.

	Chlorophyll *a*, mg/g			Chlorophyll *b*, mg/g			Carotenoids, mg/g		
	14 day	21 day	28 day	14 day	21 day	28 day	14 day	21 day	28 day
	Amaranthus retroflexus								
Control «−» (no herbicide applied)	0.34 ± 0.05	0.79 ± 0.05	1.56 ± 0.03	0.13 ± 0.02	0.31 ± 0.04	0.52 ± 0.03	0.08 ± 0.03	0.20 ± 0.02	0.43 ± 0.03
Control TBM «+»	0.23 ± 0.02	0.75 ± 0.05	1.40 ± 0.03	0.08 ± 0.02	0.26 ± 0.03	0.47 ± 0.03	0.06 ± 0.02	0.22 ± 0.02	0.38 ± 0.03
P(3HB)/wood flour/TBM	0.22 ± 0.02	0.56 ± 0.04	1.30 ± 0.03	0.08 ± 0.02	0.23 ± 0.03	0.44 ± 0.03	0.06 ± .0.01	0.13 ± 0.01	0.34 ± 0.02
Control MET «+»	0.33 ± 0.05	0.75 ± 0.05	—	0.12 ± 0.03	0.26 ± 0.03	—	0.08 ± 0.02	0.22 ± 0.02	—
P(3HB)/wood flour/MET	0.30 ± 0.04	0.51 ± 0.04	—	0.11 ± 0.02	0.21 ± 0.02	—	0.07 ± 0.02	0.19 ± 0.02	—
	Sinapis arvensis								
Control «−» (no herbicide applied)	0.76 ± 0.04	1.40 ± 0.06	1.65 ± 0.05	0.26 ± 0.03	0.57 ± 0.04	0.67 ± 0.04	0.16 ± 0.02	0.38 ± 0.04	0.43 ± 0.03
Control TBM «+»	0.47 ± 0.03	1.32 ± 0.03	1.52 ± 0.03	0.18 ± 0.02	0.53 ± 0.03	0.62 ± 0.03	0.16 ± 0.02	0.34 ± 0.02	0.38 ± 0.02
P(3HB)/wood flour/TBM	0.44 ± 0.04	1.27 ± 0.04	1.48 ± 0.03	0.16 ± 0.02	0.50 ± 0.03	0.58 ± 0.03	0.13 ± 0.02	0.33 ± 0.03	0.36 ± 0.03
Control MET «+»	0.36 ± 0.03	1.24 ± 0.03	—	0.14 ± 0.02	0.50 ± 0.02	—	0.11 ± 0.01	0.34 ± 0.03	—
P(3HB)/wood flour/MET	0.43 ± 0.03	0.94 ± 0.03	—	0.18 ± 0.02	0.44 ± 0.03	—	0.13 ± 0.02	0.28 ± 0.02	—
	Leucanthemum maximum								
Control «−» (no herbicide applied)	0.63 ± 0.05		1.03 ± 0.06	1.16 ± 0.07	0.23 ± 0.03	0.39 ± 0.03	0.42 ± 0.03	0.13 ± 0.02	0.24 ± 0.03	0.27 ± 0.03
Control TBM «+»	0.42 ± 0.04		0.95 ± 0.05	1.02 ± 0.03	0.18 ± 0.02	0.36 ± 0.02	0.40 ± 0.03	0.12 ± 0.02	0.21 ± 0.01	0.25 ± 0.03
P(3HB)/wood flour/TBM	0.41 ± 0.04		0.92 ± 0.05	0.95 ± 0.02	0.17 ± 0.03	0.35 ± 0.02	0.35 ± 0.02	0.11 ± 0.01	0.20 ± 0.02	0.23 ± 0.02
Control MET «+»	0.31 ± 0.03		0.84 ± 0.03	—	0.15 ± 0.02	0.34 ± 0.03	—	0.09 ± 0.01	0.22 ± 0.02	—
P(3HB)/wood flour/MET	0.36 ± 0.03		0.82 ± 0.03	—	0.17 ± 0.01	0.33 ± 0.02	—	0.11 ± 0.01	0.20 ± 0.01	—

methyl on photosynthesis and a decrease in $Y(II)_{max}$ and ETR_{max}. The photoactivity of the field mustard plants was the least stable when affected by tribenuron-methyl: the $Y(II)_{max}$ and $Y(NPQ)_{max}$ of this plant considerably decreased, in contrast to ETR_{max}, at day 14 in the positive control (with free TBM). At the same time, $Y(II)_{max}$, $Y(NPQ)_{max}$, and ETR_{max} in experiments with red-root amaranth and max chrysanthemum did not change significantly during the experiment. The reduction in $Y(II)_{max}$ suggested substantial damage to the photosynthetic apparatus of red-root amaranth and, especially, field mustard. There are literature data suggesting that in some plants, e.g., in *Radix isatidis*, TBM causes a decrease in Y(II) and ETR in PSII.[25] Trace concentrations of imazethapyr (ALS inhibitor) caused a dramatic increase in reactive oxygen species (ROS) in *A. thaliana*.[29]

In experiments with max chrysanthemum, TBM considerably decreased ETR_{max} and somewhat increased $Y(NPQ)_{max}$ relative to the intact plants. TBM did not affect the $Y(II)_{max}$ of max chrysanthemum, and it was similar to the control level during the experiment. Similar results (except a decrease in Y(NPQ)) were obtained after treating cornflower with tribenuron-methyl: no significant changes were observed in Y(II) but ETR and Y(NPQ) decreased.[34]

Thus, differences in changes of parameters $Y(II)_{max}$, $Y(NPQ)_{max}$, and ETR suggest that application of the free TBM causes a pronounced response in most plants in the early phase of the experiment but it usually becomes less intense later. By contrast, the embedded TBM produces a somewhat delayed effect, which becomes stronger by the middle of the experiment and reaches its peak over time.

The effect of herbicides on the contents of photosynthesis pigments

Another important indicator of plant photosynthetic activity, along with chlorophyll fluorescence parameters, is the state of chlorophyll-protein complexes.

The study of photosynthetic activity of weeds treated with the free and embedded herbicides included analysis of changes in chlorophyll a, b and carotenoid contents in chlorophyll-protein complexes. The pigment contents of different plant species treated with free and embedded herbicides had similar patterns of change. Regardless of the herbicide used, its form, and plant species treated, chlorophyll *a* and *b* increased as the plants were growing and developing, but the pattern of change of carotenoids was more intricate. However, concentrations of the pigments varied significantly depending on the plant species. Intact and herbicide-treated plants of two species (*Sinapis arvensis* and *Leucanthemum maximum*) contained almost twice as high concentrations of the green pigments and carotenoids compared with *Amaranthus retroflexus* (Table 3). Changes in the pigment concentrations were compared in the experiments with herbicides that had different modes of action (metribuzin and tribenuron-methyl) and with different forms of the herbicides (free and embedded ones) (Table 3). Both herbicides caused a decrease in chlorophyll *a* and *b* relative to the intact plants, regardless of the herbicide form, and the patterns of change were similar while quantitative changes differed somewhat. The greatest decrease in chlorophyll *a* was 53% and chlorophyll *b* 46% in *Sinapis arvensis* at day 14 in the experiment with the free MET.

In contrast to the green pigments, carotenoid contents of all weed species were quantitatively similar in the intact and herbicide-treated plants. As plants developed, carotenoid contents increased in all species in the experiments with both free and embedded MET and TBM (Table 3).

Thus, both free and embedded MET and TBM caused a quantitatively similar decrease in chlorophyll *a* and *b* in various weed species compared to intact plants. The decrease in concentrations of photosynthesis pigments in plants treated with herbicides is usually attributed to the damage to the chlorophyll synthesis system and increased degradation of pigments, which is also caused by natural aging of plants.[38] This is consistent with the available literature data on the effects of some herbicides on plants. For

example, a study by Qian et al.[29] showed a strong effect of trace concentrations of imazethapyr (an ALS inhibitor) on *A. thaliana* plants and a decrease in chlorophyll synthesis. Treatment of rape with amidosulfuron (an ALS inhibitor) also resulted in a considerable chlorophyll decrease.[35] Treatment of willow with the glyphosate-based herbicide caused a decrease in chlorophyll concentrations and an increase in carotenoid concentrations at day 7, followed by a decrease.[41] An increase in carotenoids was observed in *Centaurea cyanus* L. biotopes treated with tribenuron-methyl.[34] Carotenoids usually take part in protection from oxidative damage by detoxifying oxygen singlets produced by photosynthesis or enzymatic transformation of other ROS into oxygen singlets.[42] The present study suggests that findings obtained in research of photosynthetic activity of plants using chlorophyll fluorescence parameters have high information value.

Conclusion

The study of the efficacy of the free and embedded forms of metribuzin and tribenuron-methyl in controlling various weeds showed that embedding of the herbicides in degradable matrix did not decrease their activity but prolonged their action and, in some cases, even enhanced their efficacy. Embedded MET, which caused 100% mortality of the weeds, was found to be the more effective herbicide. The herbicidal activity of the embedded TBM was superior to its activity in the free form: the embedding of this herbicide, which is quickly inactivated and metabolized in plant tissues, enhanced and prolonged its action. The study showed that the herbicides decreased the main parameters of fluorescence [$Y(II)_{max}$, $Y(NPQ)_{max}$, and ETR] and concentrations of photopigments. Comparison of the free and embedded MET did not reveal any differences between qualitative and quantitative changes in fluorescence parameters of the various plants affected by the two forms. The effect of the embedded TBM was somewhat delayed in the early phase of the experiment but lasted longer than the effect of the free TBM and increased over time.

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