Changes in the content of chlorophyll in leaves when using pesticides and microfertilizers

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Abstract. One of the ways to reduce the phytotoxicity of herbicides on cultivated plants is the use of trace element fertilizers. The article presents the results of research on the influence of liquid trace element fertilizers on the content of chlorophyll in leaves and the productivity of sugar beet. It was found that three days after the treatment of crops with herbicides together with microfertilizers, the amount of chlorophyll a and b pigments was 4% higher than when applying herbicides without microfertilizers. In the control version, where pesticides and microfertilizers were not applied, the content of chlorophyll in the leaves was 0.350% by weight. As the observations showed, the photosynthetic activity of sugar beet plants was restored 4-5 days after the treatment by herbicides. Herbicides, with the simultaneous introduction of micro-fertilizers, had a less negative effect on the photosynthesis of sugar beet than herbicides alone. The biological yield of root crops in the variant with the combined use of pesticides and microfertilizers was 56.36 t/ha. The polarization of sugar beet root crops with the combined use of pesticides and microfertilizers was 0.64% higher than in the variant without microfertilizers.

1. Introduction

Obtaining stable and high yields of sugar beet with good technological properties of the root crop is often limited by the influence of environmental factors, including the infestation of its crops. Every year, agricultural producers do not receive from 10 to 30% of the crop from weeds, and they spend significant funds on clearing crops from weeds. In recent years, there has been a tendency to increase the soreness of crops of all varieties, including sugar beet, which was the result of an increase in fallow lands, violations of agricultural techniques of cultivation, primarily, non-compliance with crop rotations and the system of tillage. The species composition of weeds has also changed for the worse. There was a significant increase in the number of perennial and hard-to-kill annual plants.

An important component of the modern intensive technology of sugar beet cultivation without manual labor, which allows the most complete use of the potential of the beet plant, is the effective and reliable protection of the crop from weeds [1]. Increasing the efficiency of sugar beet production on an intensive basis requires continuous improvement of certain elements of the technology, including the use of existing chemicals with greater efficiency, which occupy 30% of the cost structure by volume [2, 3].

Among the complex of harmful organisms, they cause the greatest damage to the sugar beet crop, losses without protective measures can exceed 80%. Weeds pose the greatest danger in the first 68 weeks, since sugar beet, due to its biological characteristics, primarily slow growth in the first half of
the growing season, is completely deprived of the opportunity to compete with exclusively plastic, fast-growing weeds, the which seedlings number sometimes exceeds the number of beet plants per unit area by ten times. The correct choice, effective various action spectrum herbicides complex timely and high-quality application, adequate to the nature of infestation, allows to successfully solve the problem of protecting sugar beet from weeds during the growing season [4].

Herbicides that selectively act on weeds can cause the appearance of damage symptoms in cultivated plants. The risk of damage to agricultural crops increases when using large amounts of pesticides or when the processing time coincides with the passage of the of plant development critical stage. The nature of the damage depends on the type of herbicide. This can be chlorosis, falling or yellowing of leaves, wilting.

Most herbicides are physiologically active substances. The basis for the widespread use of chemical agents in the control of weeds is their selective toxic effect on weeds [5].

To protect vegetative cultivated plants from the negative effects of post-emergence chemical weeding, synthetic organic antidotes are used, they increase the resistance of plants to herbicides without reducing the effectiveness of their action on weeds. These are substances that stimulate the adaptive capabilities of cultivated plants to herbicides, with the properties of immunomodulators and adaptogens. On the territory of the Russian Federation, a limited number of compounds with antidote activity included in some herbicides are of practical importance: mefenpyr-diethyl, cloquintoset-mexyl, naphthalene anhydride, cyprosulfamide, isoxadiphene-ethyl.

An integral part of the modern technology of growing sugar beet has become the use of microfertilizers, which help to accelerate the processes of internal metabolism, the dynamics of plant development, and as the result, to obtain high yields with good technological qualities of root crops. Thanks to the production of chelated forms of microfertilizers, which are characterized by a high digestibility coefficient, they have become actively used for foliar top dressing of most agricultural crops. This provided a significant increase in crop productivity. Non-root top dressing allows to evenly distribute usually small doses of microfertilizers, which in the case of even a minimal overdose can have a negative effect; spraying with fertilizers dissolved in water of aboveground sugar beet orchards eliminates the possibility of binding the nutrients to the soil-absorbing complex, which significantly increases the coefficient of their use by plants. Non-root processing allows to balance nutrient imbalances quickly and purposefully during the most maximum need. Leaf fertilizing is convenient to combine with pesticide treatments [6].

During the growth and development, cultivated plants are often subjected to periodically repeated physical, light, temperature, and chemical stresses, which can lead to significant losses in yield and product quality. To overcome stress, the plant requires additional effort, which is spent at the expense of the main process of metabolism. Therefore, the question of protecting vegetative plants when they are damaged by herbicides remains open, especially for economically important crops, including sugar beet [7, 8].

2. Materials and methods
The research was carried out on chestnut soils. The experience was laid down according to the scheme: 1. Without pesticides and microfertilizers; 2. Pesticides without fertilizers; 3. Pesticides with microfertilizers: POLYDON Amino Zinc (L-amino acids and oligopeptides-250 g/l; N total – 100 g/l; Zn – 80 g/l) – 0.6 l/ha; POLYDON Amino Boron – Molybdenum (L-amino acids and oligopeptides-200 g/l; B – 75 g/l; N total – 50 g/l; Mo – 25 g/l) – 0.6 l/ha; POLYDON Amino Manganese (L – amino acids and oligopeptides-250 g/l; N total - 100 g / l; Mn-80 g / l) - 0.6 l / ha; POLYDON Boron (B-150 g / l; N total. - 50 g / l; Mo-l g / l) - 1.0 l / ha. Term of microfertilizers application is: 1-3-4 pairs of real leaves; 2-10 days after the first application. Trace elements in the studied fertilizers are in chelated form. Soil is a leached chestnut medium-clay. The precursor of sugar beet is pure steam. The tank mixture of herbicides was prepared taking into account the number of weeds, their phase of development, as well as the species composition. Agro-techniques included: diskling to the depth of 10-12 cm (Horsch Joker + John Deere 9RT), plowing – 30 cm (Gregoire Besson SPSF 9RT + John
Deere 9), disking (Horsch Joker + John Deere 9RT) – 10-12 cm, early spring harrowing (VELES AGS-22-2U + John Deere 9RT), pre-sowing cultivation (KBM-14.4 PS + John Deere 8). Seeding is Gaspardo Maestra 12 + John Deere 9RT. The seeding rate is 1.25 seeding units. The depth of seeding is 3-4 cm. The flow rate of the working fluid during non-root processing is 200 l/ha. The plot area is 14 hectares.

In general, during the entire growing season of sugar beet, the amount of precipitation varied from 104 to 193 mm, but they fell unevenly; the sum of active temperatures is from 2550 °C to 2753 °C, and the hydrothermal coefficient is 0.38-0.76, which characterizes the prevailing weather conditions with insufficient moisture.

3. Results and discussion
The phase of full shoots was marked on May, 6...11. At this time, the sugar beet crops were treated by herbicides. The results of the survey of the experimental site before processing the crops showed that the species composition of the weed flora was represented by the following species: chicken millet, blue bristle, common pickle, white marsh, pinched pike, field bindweed. Among the biological subtypes, the subtype of annual weeds is most represented – 5 species or 80% of the total species composition. At the same time, the largest number of specimens was the tilted-back schiritsa – 9.1 pcs/m².

The assessment of species composition, population density and population dynamics of weeds is the first step in the prediction of agricultural land contamination to allow engaged into informed choice of methods and means of struggle, safe to culture and the most effective against targets. As shown by the survey of crops, the first herbicidal treatment of sugar beet crops led mainly to a halt in the growth and development of weeds, which contributes to the normal development of cultivated plants in their growth initial phases. Herbicides used in the farm, after the first treatment, showed the efficiency (in quantity) equal to 10.45%.

The second treatment of sugar beet crops by herbicides was carried out when 1-2 pairs of real leaves were formed in the cultivated plant. Before processing, the weediness of the crops was low and was represented by the following species: white marsh - 1.8 pcs/m², common pickle - 3.9 pcs/m², bindweed - 5.3 pcs/m², tilted pike – 1.1 pcs/m², blue bristle – 0.8 pcs/m². On the fifth day after the treatment, chlorosis of the growth point and drying of the leaf edge were observed in all treated areas in aspens, in cereals and dicotyledonous annuals-growth arrest, leaf necrosis.

The third treatment by herbicides with simultaneous application of liquid microelement fertilizers was carried out with the formation of 3-4 pairs of real leaves. Before processing, weeds were noted in sugar beet crops: sedge - 1.10 pcs/m², common pickle - 2.4 pcs/m², mountain bindweed - 2.1 pcs/m², white marsh – 1.3 pcs/m². New plants amaranth thrown back, and annual grass weeds were not detected.

The use of modern herbicides, regardless of the manufacturer, with mandatory compliance with the regulations for their use, will contribute to stopping the weed plants development and growth, and, accordingly, to their death. Herbicides, used on sugar beet crops against annual dicotyledonous weeds, contain active substances of phenmedipham, desmedipham (derivatives bicarbonate). Desmedifam has the characteristic properties of a photosynthesis inhibitor, such as increasing phytotoxicity at high light intensity and dependence of phytotoxicity on climatic conditions. The activation of proton movement, equivalent to the increase in cyclic photophosphorylation, becomes possible due to the fact that non-cyclic electron transport is interrupted by these herbicides, and thus the possibility of interference of photosystem II in the redox process is excluded. The inhibition of photosynthetic electron transport by fenmedifam is well proven. Its selectivity is due to the rapid detoxification in resistant plants and the difference in the rate of absorption and movement.

More specific is the use of herbicides for the growing season of sugar beet. For postemergence applications are recommended, as a rule, drugs are so-called betalal groups (based on active ingredients of phenmedipham and desmedipham). The disadvantage of these herbicides is the temperature restrictions: the optimal temperature for their use is 15-25 °C. Failure to comply with
these requirements can lead to serious negative consequences: leaf burns, oppression, and even the death of beets. Slowing down the root crop weight gain and leaves of the crop under the influence of herbicides in warm weather conditions can lead to a significant shortage of the crop. Therefore, when conducting the experiment, more attention was paid not to the biological effectiveness of herbicides, but to the state, further growth and development of sugar beet plants after exposure to pesticides. The assessment of the beet plants state showed that in the variant with the use of herbicides, 95% of the number of examined plants had the 3rd pair of real leaves. In the area where no pesticide treatment was carried out, sugar beet plants were in competition for life factors with weeds, but 98% of the plants had the 3rd pair of real leaves. The development of plants can be described by the intensity of leaf color, which is directly related to the content of chlorophyll in the green parts of plants and the intensity of photosynthesis. Determination of the sugar beet leaves color intensity showed that the leaves that were not exposed to pesticides, but had small restrictions on the use of sunlight, it was 46.28 units (indications of the portable chlorophyll level meter in the leaves of atLEAF+), on the variant with the use of herbicides – 48.01 units.

One of the indicators of the photosynthesis intensity is the content of chlorophyll. The intensity of photosynthesis increases with the increase in the content of chlorophyll. Chlorophyll is the most important component of the photosynthetic apparatus of leaves. Its content is determined by the genetic nature of the crop, as the result of which it can be used as a physiological indicator that characterizes the ontogenetic, age-related and genetic characteristics of the plant. The amount of pigments reflects the reaction of the plant organism to the growing conditions.

The determination of the chlorophyll content before the third treatment showed that in sugar beet leaves that were not exposed to pesticide loads, the chlorophyll content was 0.319% by weight of raw leaves, and the content of chlorophyll pigments a and b was 2.06% and 0.96% by weight of raw leaves. The analysis of the sugar beet assimilation surface in the plant protection method showed that the chlorophyll content in the leaves was higher than in the control version and amounted to 0.336% of the raw mass. At the same time, the content of chlorophyll a and b pigments was 2.187% and 1.021%, respectively, and their sum was 5.8% higher than in the control variant.

Three days after the third treatment, the chlorophyll content was determined to assess the condition of sugar beet plants after exposure to herbicides. As the result, it was found that the herbicides used had the effect on the sugar beet plants immune system, and, accordingly, on the content of photosynthetic pigments of the leaves. When using herbicides together with microfertilizers, the amount of chlorophyll a and b pigments was 3.157% of the leaf weight, which is 4% more than when applying herbicides without microfertilizers (3.029%). In the control variant, where pesticides and microfertilizers were not applied, the chlorophyll content in the leaves was 0.350% by weight.

The results of determining the plants roots and the green part mass showed that the mass of one plant in the control variant was less than in the variants with herbicide treatments. The weight of one root crop when treated with pesticides together with microfertilizers was 1.54 g, which is 37.5% more than the weight of the root crop when using only pesticides and 0.58 g more than in the control one. A similar pattern was found when determining the mass of the one plant’s leaves.

Visual assessment of the sugar beet plants condition only confirms that the development of plants after three herbicide treatments without joint application of microfertilizers and in the control variant lags behind the plants where there was a joint application of herbicides and microfertilizers. This can be explained by the fact that in the control variant, sugar beet plants lagged behind in growth and development due to the lack of basic life factors due to high contamination of crops, and in the variant using only herbicides due to a longer period of stress from the effects of herbicides

After 10 days, the fourth treatment of sugar beet crops by herbicides was carried out. Before processing, the analysis was conducted on a total leaf chlorophyll content and pigments of chlorophyll a and b: on the control option, the total chlorophyll content was 0.348% wet weight of leaves, and the contents of photosynthetic pigments – 1.064% (chlorophyll b) and 2.280% (chlorophyll a). Sugar beet leaves subjected to herbicide treatments without microfertilizers contained 0.336% of the raw mass of chlorophyll, and 0.344% of the raw mass with microfertilizers. Two days after the crops treatment, the
second analysis of sugar beet leaves was carried out, which showed the decrease in the intensity of photo-synthesis and the content of chlorophyll under the influence of pesticides. When treating crops by herbicides together with microfertilizers, the total content of chlorophyll was 88.6% in relation to the control one, when treating only by herbicides, the inhibition of photochemical reactions in the leaves was more significant – 85.2% in relation to the control one. The inhibition of photochemical reactions leads to the sharp decrease in the assimilation of carbon dioxide by leaves, and, accordingly, to the decrease in the productivity of photosynthesis and the formation of organic matter. The leaves of the plants acquire a light green color.

As shown by observations of crops and laboratory studies, 4-5 days after the treatment by herbicides, the activity of photosynthesis in sugar beet plants was restored. Herbicides, with the simultaneous introduction of microfertilizers, had a less negative effect on the photosynthesis of sugar beet than herbicides alone. The phytosanitary condition of the crops was characterized as a good one. The use of herbicides, both with and without simultaneous application of liquid microfertilizers, contributed to the purification of sugar crops from weeds.

30 days after the last herbicide treatment, a laboratory analysis of sugar beet plants was carried out according to the following indicators: the content of chlorophyll, the content of the main macronutrients in root crops (Table 1).

### Table 1. Biometric and qualitative indicators of sugar beet plants.

| Indicator                           | Control | Herbicides + micronutrient fertilizers | Herbicides |
|-------------------------------------|---------|---------------------------------------|------------|
| Leaf weight, g                      | 93.48   | 118.64                                | 101.76     |
| Root crop weight, g                 | 75.60   | 132.67                                | 113.74     |
| Total chlorophyll content, % of leaf weight | 0.348 | 0.356                                | 0.352     |
| Chlorophyll content a               | 2.278   | 2.327                                 | 2.296     |
| Chlorophyll content b               | 1.064   | 1.087                                 | 1.072     |
| Chlorophyll content a + b           | 3.342   | 3.413                                 | 3.368     |
| Content in root vegetables, %       |         |                                       |           |
| N                                   | 0.84    | 0.77                                  | 0.70      |
| P                                   | 0.12    | 0.15                                  | 0.14      |
| K                                   | 1.22    | 1.06                                  | 1.10      |

The intensity of photosynthesis is the indicator of the work of carbon assimilation photochemical reactions at the plastid and cellular levels, while in the whole plant photosynthesis is controlled by higher-order regulatory systems. The relationship between growth and photosynthetic support of the growth process is constantly changing in ontogenesis and depends on environmental factors. The assimilation of carbon by the whole plant, as the indicator of the entire leaf apparatus photosynthesis efficiency, decreased under the influence of the herbicide. In dynamics, this process characterizes the time of the plant adaptation to the herbicide and the environment and is most often aimed at activating the growth point and regulating the energy of new leaves regrowth. Adaptation processes are faster in conditions of sufficient moisture and heat. This explains the difference in plant weight: under conditions of sufficient moisture and optimal temperature regimes, sugar beet plants quickly adapted to the phytotoxic action of herbicides when they were applied together with liquid microfertilizers. The total content of chlorophyll in sugar beet leaves during the combined treatment by herbicides and microfertilizers was 0.356%, which is 2.3% more than in the control variant, where beet plants were not negatively affected by herbicides, but in the process of ontogenesis constantly competed for the main factors of life. The use of liquid fertilizers together with herbicides contributed to a faster recovery of photosynthesis intensity and adaptation to the herbicides phytotoxic effects in the shortest possible time. In this variant, the weight of the root crop was 132.67 g, which is 18.93 g more than in the economic variant.
In the second decade of July, preventive treatment of sugar beet crops by fungicides against diseases of the leaf apparatus was carried out. Three days after the processing, the total chlorophyll content in the leaves was determined. The results of the laboratory analysis showed that the fungicides in the recommended doses did not have a negative effect on sugar beet plants. The total content of chlorophyll in the variant where previously only pesticides were used was 0.236%, which was 101.7% compared to the control variant. In the beet leaves in the variant where there was a joint application of pesticides and liquid trace element fertilizers, the total content of chlorophyll was 127.6% compared to the control, and the sum of chlorophyll a and b was 2.602% by weight of raw leaves, which is 15% more than in the variant where the crops were treated only by pesticides. The determination of the root crop mass confirmed the earlier conclusions about the effectiveness of local application of pesticides and microfertilizers-the mass of the root crop was 256 g.

Sugar beet harvesting was carried out on September, 05...07. The phytosanitary condition of the crops was noted as a good one (only the variants where the chemical protection of the crops was carried out were evaluated); signs of foliage diseases were not detected, weeds were recorded singly (sow-thistle, pigweed white).

The leaves raw mass laboratory analysis showed that beet plants from the variant where only pesticides were used had a high content of total chlorophyll - 0.282% of the raw mass, the amount of chlorophyll a and b was 2.663%, which was 158% and 164%, respectively, to the control level. In the variant where pesticides and liquid trace element fertilizers were used together, the total content of chlorophyll was 0.222%, and the sum of chlorophyll a and b was 2.148%. This can be explained by the fact that sugar beet plants, which were exposed to phytotoxic effects of herbicides during the growing season, quickly adapted and restored the intensity of photosynthesis and the formation of organic matter during the joint treatment by pesticides and microfertilizers. When sugar beet crops were treated by pesticides only, the period of adaptation and restoration of photosynthesis intensity was prolonged. Therefore, the growing season of these plants was prolonged, and the intensity of photosynthesis was slightly higher during the harvesting. According to the literature data, it is known that for the formation of a root crops high yield, sugar beet plants need a sum of active temperatures equal to 2200-2300 °C. On the harvesting date, the amount was already 2382 °C. Therefore, the beginning of the harvesting was set correctly, and further delay with the beginning of the harvesting would be impractical.

The biological yield of root crops in the variant with the combined use of pesticides and microfertilizers was 56.36 t/ha, in the variant with the use of pesticides only – 51.58 t/ha. To date, high yields of sugar beet root crops are achieved by almost all economic entities, but not all pay close attention to the production of root crops with high technological qualities. And this is not only the sugar content of root crops, but also molasses-like substances, on the content of which the pure sugar yield depends (Table 2).

| Variant                  | Content, mmol/100 g | The content of the purified sugar, % |
|-------------------------|---------------------|-------------------------------------|
|                         | Na      | K       | N       |                     |
| Pesticides + micronutrient fertilizers | 1.31    | 5.28    | 0.80    | 16.37               |
| Average                 | 1.44    | 4.14    | 0.67    | 16.38               |
| Pesticides              | 1.38    | 4.71    | 0.74    | 16.38               |
| Average                 | 1.26    | 3.95    | 1.00    | 15.61               |
| Average                 | 1.25    | 3.42    | 1.22    | 15.94               |
|                         | 1.26    | 3.69    | 1.11    | 15.78               |

The analysis of root crops showed that the polarization of sugar beet root crops with the combined use of pesticides and microfertilizers was 19.46%, which is 0.64% more than in the variant without
microfertilizers. The content of refined sugar, taking into account losses in the formation of molasses, was also the highest in the variant with the combined use of liquid trace element fertilizers and pesticides – 16.38%.

4. Conclusion
Thus, the conducted studies showed that 4-5 days after the treatment by herbicides, the photosynthetic activity of sugar beet plants was restored. Herbicides, with the simultaneous introduction of microfertilizers, had a less negative effect on the photosynthesis of sugar beet than herbicides alone. The combined use of microfertilizers and chemicals to protect sugar beet crops from weeds and diseases is more effective, contributes to higher yields with high root crops technological quality.

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