

Anatomical changes in the epidermis of winter pea stipules and their area under usage of herbicide, stimulator of plant growth and microbial preparation

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Abstract. The use and search for new methods and ways to reduce negative herbicidal effect on crops is a key factor in increasing the level of yield and quality in modern agricultural conditions, including cultivation of crops such as winter peas. One of the factors that reflects the depth of the effect of herbicides on the plant organism may be the anatomical structure of the leaf, thus, the aim of the research was to study the characteristics of epidermis and size of stipules of winter peas with the complex use of stimulator of plant growth and microbial preparation in herbicide cultivation technology.

To determine the optimal combination of preparations and rates of their introduction, a field experiment was established in the Department of Biology of Uman National University of Horticulture (2018–2019), which included options: without herbicide, stimulator of plant growth and pre-sowing seed treatment with microbial preparation (control); treatment of plants with MaxiMox herbicide during the growing season in the rates of 0.8, 0.9, 1.0 and 1.1 L ha⁻¹ separately and in mixtures with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha⁻¹ without and against the background of pre-sowing treatment of seeds with microbial preparation Optimize Pulse in the rate of 3.28 L t⁻¹ (background). The experiment was repeated 3 times. Treatment of winter pea plants with preparations was carried out in the phase of 3–4 developed tendrils (BBCH 13–14). During the experiment it was found that treatment of winter pea plants with MaxiMox herbicide, especially with increasing level of the preparation to 1.1 L ha⁻¹ led to anatomical and morphological changes in plant stipules and affected the stipule size of winter pea crops. The number of epidermal cells on average decreased by 14–53 pcs (6–22%) at LSD_{05} 9.8 pcs, but their size increased by 28.42–394.52 μm^2 (2–35%) at LSD_{05} 71.7 μm^2 , while the size of the stipulate apparatus of crops increased on average by 2.7–4.6 thousand $\text{m}^2 \text{ha}^{-1}$ (13–22%) at LSD_{05} 1.3 thousand $\text{m}^2 \text{ha}^{-1}$. The complex application of the herbicide with stimulator of plant growth, especially against the background of pre-sowing treatment of seeds with the microbial preparation Optimize Pulse in the rate of 3.28 L t⁻¹, caused a decrease in the number of epidermal cells per unit of stipules surface on average by 50–84 pcs (21–35%) at LSD_{05} 9.8 pcs and with an increase in the stipule size by 9–12 thousand $\text{m}^2 \text{ha}^{-1}$ (44–59%) at LSD_{05} 1.3 thousand $\text{m}^2 \text{ha}^{-1}$, this may indicate the optimal effect of these mixtures of preparations on metabolic processes in plants against the background of reducing negative impact of the herbicide.

Key words: anatomy, epidermis, stipules, herbicide, winter pea (*Pisum sativum* L.), stimulator of plant growth, seed microbial preparation.

INTRODUCTION

The world area of pea crops is 8 million hectares with gross grain harvest - 16 million tons per year (FAOSTAT, 2019). Pea grain has a high content of protein, minerals, carbohydrates, fiber (Tosh et al., 2013) and is characterized by a complete absence of gluten proteins in beans, which is very important in meeting the needs of gluten-free diets for people suffering from celiac disease (Singh et al., 2017). Winter pea (*Pisum sativum* L.) has a number of advantages over spring forms: a longer and more effective period of symbiotic relationships with *Rhizobia leguminosarum* at the minimum expense of energy resources, low emissions of greenhouse gases into the atmosphere during growing season, and due to the formation of plants with high immunity - interrupting life cycle of many diseases and pests. Higher yields of winter peas compared to spring forms make it more cost-effective (McGee et al., 2017). Among negative factors that dramatically reduce the yield of peas is the presence of a weed component in crops that can reduce yields by 64.4%. The herbocritical period in peas is from 45 to 50 days, after which the crop can compete with weeds due to the growth of vegetative mass. A wide range of herbicides has now been developed to kill weeds at minimal cost compared to mechanical and other control methods (Shalini et al., 2017). However, their phytotoxic effects on pea plants are no exception, which is expressed in morphological, anatomical, physiological and biochemical changes that occur in plants. Herbicides affect the number of chloroplasts in assimilation tissues (Jung et al., 2008), disrupt the direction of biochemical and physiological processes (Warabi et al., 2001; Ha et al., 2003; Jung et al., 2004; Yang et al., 2006; Karpenko et al., 2019), causing primary anatomical and morphological changes (Guh & Kuk, 1997; Kamble, 2007a, 2007b), which generally cause growth inhibition or plant death. Thus, according to the data of Silva et al. (2017), in cassava plants (*Manihot esculenta* Crantz. cv. IAC-12) under the action of herbicides based on nicosulfuron (60 g a.i ha⁻¹), fluazifop (250 g a.i ha⁻¹), fomesafen (250 g a.i ha⁻¹), metribuzin (480 g a.i ha⁻¹), oxyfluorfen (720 g a.i ha⁻¹) and the mixture fluazifop + fomesafen (200 + 250 g a.i ha⁻¹) no visual signs of damage were observed, but there were changes in the anatomical structure of the leaf apparatus. In particular, in the treated cassava plants with a mixture of herbicides fluazifop + fomesafen (200 + 250 g a.i ha⁻¹), nicosulfuron (60 g a.i ha⁻¹), fluazifop (250 g a.i ha⁻¹) compared to the control, the thickness of the adaxial wall of the epidermis decreased by 75%, 60% and 50%. Treatment of pea plants with 0.2 mM aqueous solution of paraquate (Moskova et al., 2011) disrupted the cellular organization of the leaf - decreased intercellular space and cell size, including epidermal.

The results of the research by Cabral et al. (2017) showed that in plants *Inga marginata*, *Handroanthus serratifolius*, *Cedrela fissilis*, *Calophyllum brasiliense*, *Psidium myrsinoides*, *Tibouchina granulosa*, *Caesalpinia ferrea*, *Caesalpinia pluviosa*, *Terminalia argentea*, *Schizolobium parahyba* with two treatments on 60 and 80 days after planting with Gamit® 36CS (FMC, 360 g L clomazone) herbicide in the rate of 2 L ha⁻¹, there was a decrease in the thickness of the spongy parenchyma (SP) on average by 16.3% - after the first application and 17.9% - after the second application, while palisade parenchyma (PP), upper (adaxial, AET) and lower (abaxial, ABE) epidermis, decreased on average by 13.1, 8.22 and 7.73%, respectively, with double application of the herbicide. Pereira et al. (2017) reported that when the herbicide Sethoxydium® SPC (Nufarm, 2-[1-(ethoxyimino)butyl]-5-[ethythio)propyl]-3-hydroxy-2-cyclohexen-1-one

– 13.0%; other ingredients: 87.0%) in the rate of 184 g a. i. ha⁻¹ + Assist mineral oil was applied to *Urochloa decumbens* plants, restriction of epidermal cell growth, bulimorphic cells and total leaf thickness was observed in the leaves. Batista et al. (2018) note that in tomato plants, treated with different concentrations of herbicide Tordon® 22K (Corteva agriscience, 4-amino-3, 5, 6-trichloropicolinic acid, potassium salt – 24.4%; other ingredients – 75.6%) herbicide in the rates of 25% (1.0 L ha⁻¹), 50% (2.0 L ha⁻¹), 75% (3.0 L ha⁻¹) and 100% (4.0 L ha⁻¹), the thickness of the leaf blade increased with growing concentration of herbicide by 184%, however, there was a disorganization of leaf tissues: cells of irregular shape were formed.

Studies by Anastasov (2010a) showed a negative effect of the herbicide based on imazamox on certain anatomical parameters of sunflower leaves, which was accompanied by an increase in the size of the assimilation parenchyma, a decrease in the number of stomata and their atrophy, which caused their inefficient functioning. Inefficient functioning of stomata along with a significant increase in the size of the assimilation parenchyma and a decrease in the number of stomata per unit of leaf area, led to disruption of photosynthetic, transpiration and gas exchange processes, expressed by visible signs of phytotoxicity (deformation of leaves and tops of plants, inhibition of plant growth, chlorosis and subsequent formation of necrotic spots on sunflower leaves). According to Asharaf & Murtaza (2016), 0.04 M concentration of the herbicide 2,4-D caused a decrease in the number of stomata on the surface of a wheat leaf, as reported in other crops and other researchers (Anastasov, 2010b; Semerdjieva et al., 2015; Kamble, 2013).

Anatomical structure of the leaf may change due to the action of plant growth regulators. Thus, in the treated Heteroauxin bean plants (0.2 g L⁻¹), leaf thickness increased (due to the increase in assimilation tissue), the number of epidermal cells increased, the number of stomata and their area due to the effect of Heteroauxin on meristematic tissues at the stage of division, stretching of cells and formation of their sizes (Shevchuk et al., 2019). Studies of the anatomical structure of soybean leaves showed that complex application of the antihyperellin preparation Chlormequat Chloride at 0.5, 0.75 and 1.0% concentration with pre-sowing inoculation of seeds with the microbial preparation Optimize Pulse (2.8 L t⁻¹) increased the cell area of spongy parenchyma, columnar parenchyma cell volume and the number of chloroplasts (Chorna, 2016). According to Hrytsayenko & Ivasiuk (2014), the application of Fabian herbicide in the rate of 90 g ha⁻¹ in combination with the plant growth regulator Rehoplant in the rate of 50 mL t⁻¹ on the background of pre-sowing treatment of seeds with the microbial preparation Ryzobofit in the rate of 100 mL t⁻¹ with Rehoplant in the rate of 100 mL t⁻¹, contributed to the formation of mesomorphic features of soybean leaf apparatus: the size of epidermal cells and the total leaf area of crops increased.

The main indicator that determines potential productivity of crops is leaf surface area (Nichiporovich, 1971), the value of which can vary significantly depending on the varietal and climatic characteristics of the area, place of cultivation, as well as used preparations, including herbicides and plant growth regulators. According to Sivchev (1973), herbicides are able to inhibit the formation of leaves. Therefore, complex application of chemical and biological preparation can have a significant impact on the formation and size of photosynthetic apparatus of plants (Hoysyuk, 2003).

The results, obtained by Datsenko (2016), give reason to claim that application of the microbial preparation Diazobakterin in the rates of 150; 175 and 200 mL t⁻¹ in the mixture with plant growth regulator Radostim 250 mL t⁻¹ for pre-sowing treatment and treatment during growing season of buckwheat with the same plant growth regulator Radostim in the rate of 50 mL ha⁻¹ caused the activation of growth processes of individual tissues and organs, which was expressed in increase in leaf area by an average of 20–30%. Double spraying of pea plants during the growing season with plant growth regulators Gumaksid in the rate 0.6 L ha⁻¹ and AKM 0.5 L ha⁻¹, caused an increase in the area of stipules by 30–43% after the first treatment (phase 3–4 stipules) and by 15–18% after the second (budding phase) compared with untreated plants (Kalitka et al., 2015). The study by Pylypenko et al. (2016) showed that the application of suspension microbial preparation Rhizohumin in the rate of 150 mL t⁻¹ and complex application of N_{10–90}P_{10–90}K₆₀ under the main tillage, contributed to the formation of pea plants in the flowering phase of the largest assimilation surface of leaves (543.5–555.5 cm² per plant) and the content of pigments in them compared to the control version.

Unfortunately, studies on complex effects of herbicides, plant growth regulators and microbial preparations on anatomical changes in the stipulate apparatus of winter peas and their formation area in the scientific literature are hardly given, which determined the relevance and purpose of our studies.

MATERIALS AND METHODS

Field experiments were established at the experimental sites of the Department of Biology of Uman National University of Horticulture in 2018–2019. The soil of the experimental plots is low-humic heavy loam podzolized chernozem on the loess with a content of humus in the arable layer of humus 3.20–3.31%.

Weather conditions in the years of research on temperature and water regime were typical for this region, in particular the average temperature during the spring and summer vegetation (March-June) of the crop was + 13.0 °C, precipitation – 40.9 mm with their long-term average annual indicators of + 10.2 °C and 57.2 mm respectively.

The objects of the study were winter pea plants *Pisum sativum* L. (NS Moroz variety) - the originator NS Seme (Serbia) having aphid-type leaf (tendrils), herbicide MaxiMox s.c. (Bayer, active substance imazamox 40 g L⁻¹), stimulator of plant growth Agriflex Amino (Agrisol, a complex of 18 types of free L-amino acids (not less than 50%) of plant origin), microbial preparation Optimize Pulse (Monsanto BioAg, bacterial strain *Rhizobium leguminosarum*, minimum 2 × 10⁹ live cells mL⁻¹ + lipohitooligosaccharide in water solution).

The area of plots in the experiment was 100 m² with three repetitions. The sowing rate of winter pea seeds of NS Moroz winter variety was 1.1 million seeds per hectare. Treatment of winter pea seeds with the microbial preparation was carried out according to the rate, calculated on the weight of seeds on the day of sowing. Spraying of vegetative plants with herbicide and their tank mixture with stimulator of plant growth was carried out in the phase of 3–4 developed tendrils (BBCH 13-14) of the culture using a cordless sprayer Forte CL-18A, with a consumption rate of 300 L ha⁻¹.

A mixed type of weeding dominated in the experimental fields with a predominance of dicotyledonous weeds: *Fumaria officinalis* L., *Galium aparine* L., *Tripleurospermum*

inodorum L., *Viola arvensis* L., *Papaver rhoeas* L., *Thlaspi arvense* L., *Capsella bursa-pastoris* L., *Euphorbia agraria* L., *Descurainia Sophia* L., *Setaria glauca* L., *Setaria viridis* L.. The level of weediness of crops before the use of microbial preparations ranged from 118 to 182 pcs per m².

The scheme of the experiment included options: 1 – without the application of herbicide, stimulator of plant growth and pre-sowing treatment of seeds with the microbial preparation (control); 2, 3, 4, and 5 – treatment of plants with MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha⁻¹ (M0.8, 0.9, 1.0, 1.1); 6 – treatment of plants with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha⁻¹ (A1.0.); 7, 8, 9 and 10 – treatment of plants with MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha⁻¹ in a tank mixture with the stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha⁻¹ (M0.8, 0.9, 1.0, 1.1 + A1.0.); 11 – pre-sowing treatment of seeds with the microbial preparation Optimize Pulse in the rate of 3.28 L t⁻¹ (M0.8, 0.9, 1.0, 1.1 + O3.28); 12, 13, 14 and 15 – treatment of plants with herbicide MaxiMox in the rates of 0.8, 0.9, 1.0 and 1.1 L ha⁻¹ on the background of pre-sowing seed treatment with the microbial preparation Optimize Pulse in the rate of 3.28 L t⁻¹ (M0.8, 0.9, 1.0, 1.1 + O3.28); 16 – treatment of plants with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha⁻¹ on the background of pre-sowing seed treatment with the microbial preparation Optimize Pulse in the rate of 3.28 L t⁻¹ (A1.0 + O3.28); 17, 18, 19 and 20 – treatment of plants with MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha⁻¹ in a tank mixture with the stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha⁻¹ on the background of pre-sowing seed treatment with Optimize Pulse normal 3.28 L t⁻¹ (M0.8, 0.9, 1.0, 1.1 + A1.0 + O3.28).

Anatomical studies of the pea stipule epidermis were performed on a LEICA-295 system microscope using a MOV-1-15 eyepiece micrometer and a SHO-2 object micrometer scale. Sampling for anatomical studies was performed from the middle part of the stem, from 20 typical plants in each variant of the experiment in the budding-flowering phase (BBCH 51-60), according to the methodology proposed by Hrytsaenko et al. (2003): after that, cuts of the stipules were made with a cork drill No. 3.

Discoloration and clarification of the selected cuts were performed in javelin water (KOCI + KCl) for 3 days, then they were washed (with four changes) in distilled water, epidermis was removed and stained with a solution of crystal-violet stainer (mixture 1 mL⁻¹ 5% H₂SO₄ and 1 mL⁻¹ 1% of water stainer solution) for 10 minutes followed by washing in distilled water. The preparations prepared in this way were fixed under coverslips in glycerin (30 preparations were used). In particular, the number of epidermal cells by counting in the field of view of a microscope with the subsequent recalculation on 1 mm² of a stipule, the sizes - measurements of length and width by an eyepiece micrometer. The area of the stipules was determined by calculation using cuts (Hrytsaenko et al., 2003). The morphostructure coefficient (Km) was calculated according to the methodology of Karpenko et al. (2012), as the ratio of the number of epidermal cells per unit surface area of the stipule under the action of the preparation (or other factor) to the number of epidermal cells in the variant where the action of the preparation (factor) was excluded (control) (Karpenko, 2008; Karpenko et al., 2012). The accuracy of the experiment and the significance of the difference between the indicators (*LSD*) in the study were evaluated by the results of analysis of variance (Ehrmantraut et al., 2000) using Microsoft Excel. The sampling error did not exceed 5% of the mean values.

RESULTS AND DISCUSSION

During the experiment it was found that anatomical structure of epidermis of the stipulate plate of winter peas varied depending on the application of the herbicide alone and in combination with a stimulator of plant growth and a microbial preparation (Table 1). In particular, under the application of MaxiMox herbicide alone in crops in the rates of 0.8, 0.9, 1.0 and 1.1 L ha⁻¹ the number of epidermal cells per 1 mm² of pea stipules on average in 2018–2019 in the budding-flowering phase decreased relatively to control by 32, 53, 44 and 14 pieces that at *LSD*₀₅ 9.8 pcs. mm² was reliable, but there was an increase in their size (width and length) and, respectively, the area, which increased relatively to control by 200.12, 394.52, 132.12 and 28.42 μm² (18, 35, 12 and 2%), that at *LSD*₀₅ 71.7 μm² was essential for variants with MaxiMox 0.8, 0.9, 1.0 L ha⁻¹. Changes in the number and area of epidermal cells, on the one hand, may be a response of winter pea plants to reduced competition from segetal vegetation in crops (from 42 pcs m⁻² in the rate of 0.8 L ha⁻¹ to 13 pcs m⁻² - in the rate of 1.1 L ha⁻¹), and, as a consequence, improved lighting, moisture and mineral nutrition of cultivated plants. However, it is obvious that the maximum rate of herbicide (1.1 L ha⁻¹) showed some phytotoxic effect, which may indicate a decrease in cell area (compared to other rates) at the lowest level of weed infestation.

Treatment of plants with herbicide MaxiMox in the same rates in the mixture with the stimulator of plant growth Agriflex Amino 1 kg ha⁻¹ also caused a decrease in the number of cells both relatively to control and to options with self-application of the herbicide MaxiMox. However, in comparison with the variants with self-application of the herbicide, the area of epidermal cells increased by 52, 90, 273 and 188, respectively, that at *LSD*₀₅ 71.7 μm² was essential for the variants with MaxiMox 0.9, 1.0, 1.1 L ha⁻¹ + Agriflex Amino 1 kg ha⁻¹.

A similar tendency was observed in the variants with the application of herbicide MaxiMox in the rates of 0.8, 0.9, 1.0 and 1.1 L ha⁻¹ on the background of treatment of seeds with microbial preparation Optimize Pulse 3.28 L t⁻¹, where the area of epidermal cells relatively to the control variant increased by 312, 624, 421, 248 μm² and by 112, 129, 289 and 220 μm² relatively variants with self-application of the herbicide, that at *LSD*₀₅ 71.7 μm² was essential. Obviously, the reduction of herbocritical pressure on winter peas and the intensification of the bean-rhizobial apparatus contributed to the stabilization of the basic physiological and biochemical processes in plants, which reduced the phytotoxic effect of MaxiMox herbicide on plants and generally increased epidermal cell area.

Under the combined application of the studied preparations (MaxiMox 0.8–1.1 L ha⁻¹ + Agriflex Amino 1 kg ha⁻¹ + Optimize Pulse 3.28 L t⁻¹ (pre-sowing treatment seeds, background)) the number of cells per 1 mm² of stipulate epidermis decreased relatively to control by 50–84 pcs., by 25–36 pcs. - before variants with self-application of the herbicide MaxiMox and by 14–21 pcs. - before variants with MaxiMox + Agriflex Amino. Herewith, in these variants an increase in the area of cells relatively to control was observed by an average of 27–52%, that at *LSD*₀₅ 9.8 pcs. mm² was essential.

Table 1. Anatomical characteristics of epidermal cells of winter pea stipules under the effect of herbicide MaxiMox, stimulator of plant growth Agriflex Amino and microbial preparation Optimize Pulse (budding-flowering phase, average values for 2018–2019)

| Experimental variants | The number of cells per 1 mm ² , pcs. | Dimensions of one cell, μm | | Area of one cell, μm ² | K _M |
|---|--|----------------------------|-------|-----------------------------------|----------------|
| | | Length | Width | | |
| Control (without application of preparations) | 240 | 91.4 | 12.2 | 1,115.08 | 1 |
| M0.8 | 208 | 100.4 | 13.1 | 1,315.2 | 0.86 |
| M0.9 | 187 | 111.0 | 13.6 | 1,509.6 | 0.77 |
| M1.0 | 196 | 101.4 | 12.3 | 1,247.2 | 0.81 |
| M1.1 | 226 | 94.9 | 12.05 | 1,143.5 | 0.94 |
| A1.0 | 219 | 100.9 | 13.2 | 1,331.8 | 0.91 |
| M0.8 + A1.0 | 203 | 100.5 | 13.6 | 1,366.8 | 0.84 |
| M0.9 + A1.0 | 172 | 111.1 | 14.4 | 1,599.8 | 0.71 |
| M1.0 + A1.0 | 181 | 110.2 | 13.8 | 1,520.7 | 0.75 |
| M1.1 + A1.0 | 211 | 102.0 | 13.05 | 1,331.1 | 0.87 |
| O3.28 (pre-sowing treatment of seeds, background) | 217 | 101.1 | 13.7 | 1,385.07 | 0.90 |
| M0.8 + O3.28 | 196 | 101.2 | 14.1 | 1,426.9 | 0.81 |
| M0.9 + O3.28 | 166 | 111.5 | 14.7 | 1,639.05 | 0.69 |
| M1.0 + O3.28 | 181 | 110.5 | 13.9 | 1,535.9 | 0.75 |
| M1.1 + O3.28 | 201 | 102.5 | 13.3 | 1,363.2 | 0.83 |
| A1.0 + O3.28 | 206 | 101.8 | 13.9 | 1,415.02 | 0.85 |
| M0.8 + A1.0 + O3.28 | 183 | 101.6 | 14.6 | 1,483.3 | 0.76 |
| M0.9 + A1.0 + O3.28 | 156 | 112.4 | 15.1 | 1,697.2 | 0.65 |
| M1.0 + A1.0 + O3.28 | 167 | 109.9 | 14.4 | 1,582.5 | 0.69 |
| M1.1 + A1.0 + O3.28 | 190 | 103.5 | 13.7 | 1,417.9 | 0.79 |
| <i>LSD</i> ₀₅ | 9.8 | 5.2 | 0.68 | 71.7 | |

According to statistical analysis of quantitative variability, the studied indicators of the anatomical structure of the epidermis of pea stipules varied slightly and moderately. In particular, the indicators of the number of epidermal cells per 1 mm² and the area of cells μm² varied moderately - the coefficient of variation was $V = 11\%$, while the indicators of width and length of cells varied slightly ($V = 6\%$).

The obtained data give grounds to claim that the complex application of the studied preparations demonstrates a positive effect on growth processes in pea plants, due to the reduction of phytocenotic factor in the form of weed component (herbicide-destroying effect), stimulating effect of stimulator of plant growth (growth activation) and improved conditions of nitrogen nutrition (activation of symbiotic relations peas (*Pisum sativum* L.) - *Rhizobium leguminosarum* under the action of the microbial preparation), which is consistent with the studies of other scientists (Hrytsayenko & Ivasiuk, 2014; Karpenko et al., 2012; Viniukov et al., 2013). At the same time, the separate action of MaxiMox herbicide in our research, especially in the rate of 1.1 L ha⁻¹, showed a decrease in epidermal cells compared to other rates, despite the minimum weediness of crops, which is also consistent with the data of Moskova et al., 2011.

Studies have shown that the size of winter pea stipules varied depending on the combination of the studied preparations and correlated with the area of epidermal cells (generalized correlation coefficient was $r = 0.78$). Thus, in the budding-flowering phase (Fig. 1) in control (without application preparations) the total area of stipules was 20.5 thousand $m^2 ha^{-1}$. Application of MaxiMox in the rates of 0.8, 0.9, 1.0 and 1.1 $L ha^{-1}$ led to an increase in the size of stipules relatively to control by 3.9, 4.6, 4.2 and 2.7 thousand $m^2 ha^{-1}$ (19, 22, 20 and 13%) respectively, that at LSD_{05} 1.3 thousand $m^2 ha^{-1}$ was statistically reliable. During the treatment of winter pea plants with Agriflex Amino stimulator of plant growth in the rate of 1.0 $kg ha^{-1}$, stipules area increased relatively to control by 0.8 thousand $m^2 ha^{-1}$ (4%), that at LSD_{05} 1.3 thousand $m^2 ha^{-1}$ was not significant.

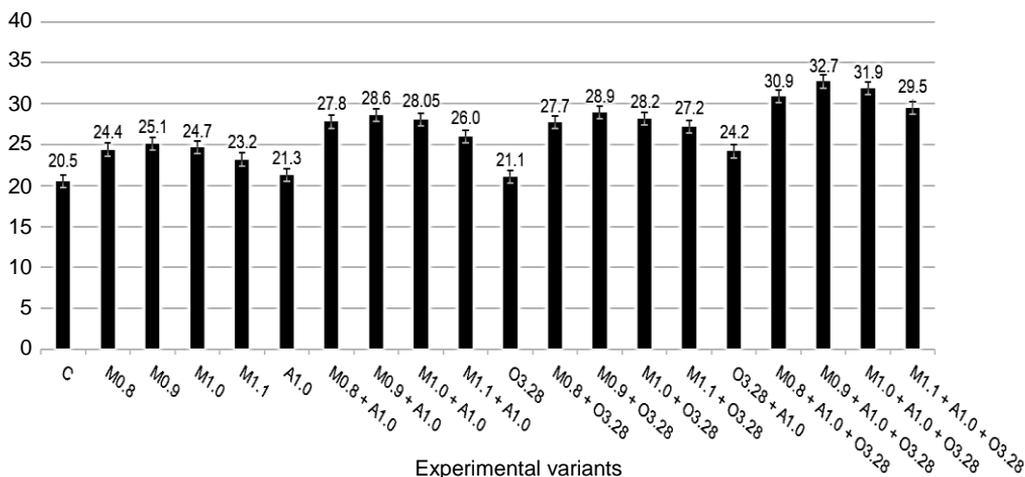


Figure 1. Area (thousand m^2 per ha) of winter pea stipules under the action of MaxiMox herbicide, stimulator of plant growth Agriflex Amino and Optimize Pulse microbial preparation (budding-flowering phase, average value for 2018–2019) (LSD_{05} : 1.3).

C – without application preparations (control); M0.8, M0.9, M1.0, M1.1 – MaxiMox 0.8, 0.9, 1.0, 1.1 $L ha^{-1}$; A1.0 – Agriflex Amino 1.0 $kg ha^{-1}$; M0.8, M0.9, M1.0, M1.1 + A1.0 – MaxiMox 0.8, 0.9, 1.0, 1.1 $L ha^{-1}$ + Agriflex Amino 1.0 $kg ha^{-1}$; O3.28 – Optimize Pulse 3.28 $L t^{-1}$ (pre-sowing treatment seeds, background); M0.9, M1.0, M1.1 + O3.28 – MaxiMox 0.8, 0.9, 1.0, 1.1 $L ha^{-1}$ + Optimize Pulse 3.28 $L t^{-1}$ (pre-sowing treatment seeds, background); O3.28 + A1.0 – Optimize Pulse 3.28 $L t^{-1}$ (pre-sowing treatment seeds, background) + Agriflex Amino 1.0 $kg ha^{-1}$; M0.8, M0.9, M1.0, M1.1 + A1.0 + O3.28 – MaxiMox 0.8, 0.9, 1.0, 1.1 $L ha^{-1}$ + Agriflex Amino 1.0 $kg ha^{-1}$ + Optimize Pulse 3.28 $L t^{-1}$ (pre-sowing treatment seeds, background).

The application of MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 $L ha^{-1}$ in tank mixtures with stimulator of plant growth Agriflex Amino 1.0 $kg ha^{-1}$ provided an increase in the area of stipules of winter peas by 7.3, 8.1, 7.55 and 5.5 thousand m^2 per ha (36, 39, 37 and 27%) to control, that at LSD_{05} 1.3 thousand m^2 per ha was statistically significant.

A similar dependence of the formation of the area of winter pea stipules was observed in variants using the herbicide MaxiMox in the rates of 0.8, 0.9, 1.0 and 1.1 $L ha^{-1}$ on the background of seed treatment with the microbial preparation Optimize Pulse in the rate of 3.28 $L t^{-1}$, that is consistent with the data obtained by Zabolotniy et

al., 2019). Under postemergence treatment with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha^{-1} on the background of seed treatment with the microbial preparation Optimize Pulse 3.28 L t^{-1} , the total stipulate area exceeded the control by 3.7 thousand m^2 per ha (18%) respectively, that at LSD_{05} 1.3 thousand m^2 per ha was essential.

Application of MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} in a tank mixture with stimulator of plant growth Agriflex Amino 1.0 kg ha^{-1} on the background of seed treatment with the microbial preparation Optimize Pulse 3.28 L t^{-1} provided the formation of stipules by 12.2–9 thousand m^2 per ha (59–44%) larger than control that at LSD_{05} 1.3 thousand m^2 per ha was statistically significant.

It is obvious that the complex application of a mixture of herbicide MaxiMox with the stimulator of plant growth Agriflex Amino on the background of seed treatment with the microbial preparation Optimize Pulse had a total positive effect on winter pea plants, which manifested in increased growth of stipules under several factors: phytocenotic (reduced effect of weeds on crops due to the herbicide) and physiological and biochemical (action of biologically active components of the plant growth regulator against the background of improving nitrogen nutrition due to active work of legume-rhizobial apparatus) as indicated in the studies by Ivasiuk (2017); Pidan (2017); Korobko (2019).

To find out the peculiarities of the formation of the stipular apparatus of peas, we calculated the coefficient of morphostructure (K_M), which characterizes the direction of passage of morpho-physiological processes in plants under the action of biologically active compounds: an increase of K_M to one or more indicates the formation of signs of xeromorphism in the leaf apparatus, and a significant decrease in K_M index indicates the formation of a mesomorphic type in the leaf apparatus of plants. Analyzing the obtained data (Table 1), the lowest K_M values were recorded in variants with the complex application of preparations (MaxiMox $0.8\text{--}1.1 \text{ L ha}^{-1}$ + Agriflex Amino 1 kg ha^{-1} + Optimize Pulse 3.28 L t^{-1}), where K_M fluctuated in the range of 0.69–0.79, while the highest K_M was observed in variants with independent application of the herbicide MaxiMox (close to 1.0).

It is safe to assume that the obtained K_M data indicate that the complex use of the studied preparations is one of the indirect factors in the formation of the stiff apparatus of the mesomorphic type in peas, which is typical for highly productive crops (Karpenko et al., 2018). Calculation of correlation dependence between generalized values ‘total area of stipules’ ↔ ‘area of cells’ demonstrates close correlation ($r = 0.78$), indicating a direct dependence of the formation of the area of the stipules on the peculiarities of the anatomical structure of cells of epidermis.

CONCLUSIONS

The obtained results give grounds to draw the following conclusions:

Application of MaxiMox herbicide $0.8\text{--}1.0 \text{ L ha}^{-1}$ leads to an increase in the area of epidermal cells additionally reducing their number, while increasing the rate of MaxiMox to 1.1 L ha^{-1} slightly reduced the size of epidermal cells and their area. The most optimal effect on winter pea stipules was the complex application of herbicide MaxiMox $0.8\text{--}1.1 \text{ L ha}^{-1}$ with the stimulator of plant growth Agriflex Amino 1.0 kg ha^{-1} on the background of pre-sowing bacterization of seeds with microbial preparation

Optimize Pulse 3.28 L t^{-1} , where a decrease in the number of epidermal cells per unit area of stipules with a simultaneous increase in their size was observed: the area increased by 27–52% with morphostructure coefficient 0.69–0.79, photosynthetic area of stipules increased by 44–59%.

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