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ORIGINAL ARTICLE

Effect of ethylene-releasing compound Esphon® on the anatomical structure, yield, and quality of Gooseberry (*Grossularia reclinata* (L.) *Mill.*)

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We were determined the effect of ethylene-releasing compound Esphon® on the morphogenesis and production process of gooseberry plants of the Mashenka variety. The Esphon treatment of gooseberry plants at the budding phase led to the modification of donor-acceptor relations in the plant, which was expressed through anatomical and morphological changes in vegetative organs, redistribution of assimilates towards the berries formation. The linear growth of shoots was inhibited, a more significant number of vessels in the xylem were formed under the drug's action compared to control, while the thickness of the cell walls of sclerenchyma fibers of cortex increased. The consequence of this restructuring was a more intensive accumulation of cellulose, hemicellulose, and lignin in annual gooseberry shoots as compared to control, as well as reserve forms of carbohydrates - sugars and starch that indicate a complete ripening of the shoot and is a prerequisite for high frost resistance of the crop. The leaf blade thickened due to the formation of a more powerful chlorenchyma under Esphon interaction. Retardant treatment caused an increase in the spongy's linear dimensions and columnar parenchyma volume - the primary assimilative leaf tissue. Optimization of the leaf's mesostructure organization enhanced the provision of morphogenetic processes with assimilates that caused an increase in the nonstructural carbohydrate content (sugars + starch) in the leaves compared to untreated plants at all stages of development. As a result of such regulation, a more powerful donor sphere increased the gooseberry crop yield, the content of sugars and ascorbic acid in the berries, the accumulation, and redistribution of assimilated flows from the vegetative organs to the fruits.

Keywords: gooseberry - *Grossularia reclinata* (L.) Mill, Esphon®, anatomical structure, mesostructure, yield, product quality.

Introduction

The current development of phytophysiology makes it possible to analyze the processes of accumulation and redistribution of photoassimilates between plant organs in the concept of donor-acceptor relationships ("source-sink"-system) (Yu et al., 2015; Bonelli et al., 2016; Kuryata et al., 2017). The processes of photosynthesis are considered as the donor, and the processes of growth, accumulation of reserve substances and zones of active metabolism during autotrophic nutrition (Zhang et al., 2013; Rogach et al., 2016; Kuryata and Polyvanyi, 2018), or the interaction between storage organs and growth processes at heterotrophic stages of seedling development are considered as the acceptor (Poprotska and Kuryata, 2017).

A hormonal system connects the donor and acceptor spheres of the plant, and other connections that ensure the mutual coordination of growth and photosynthesis (Wang et al., 2016; Kuryata et al., 2017; Rogach et al., 2018). Physiologically active substances are used to regulate these relationships. The application of exogenous phytoregulators makes it possible to change individual organs' growth activity, the intensity of photosynthesis, and the attracting activity of the fruit in the processes of carpogenesis (Matysiak and Kaczmarek 2013; Zhang et al., 2013; Kuryata and Golunova, 2018). The use of such compounds makes it possible to artificially simulate the various degrees of the tension of donor-acceptor relationships in a plant and determine what morphological, anatomical, and physiological changes cause the redistribution of assimilating flows between plant organs (Rogach et al., 2016). This effect can be achieved by mechanical methods, mainly by cutting off the shoots, removing fattening shoots. However, they require high physical costs; therefore, they are not economically feasible. A group of synthetic inhibitors of growth processes - retardants - is actively used among exogenous phytoregulators. The obtained research results confirmed the retardant application's high efficiency on crops' production process regulation (Koutroubas and Damalas, 2016; Panyapruek et al., 2016; Kuryata and Kravets, 2018). These compounds' physiological mechanisms are antihyberellins and block the synthesis of already synthesized gibberellins in the plant (Rademacher, 2016). Numerous studies have confirmed that the retardants treatment leads to artificial changes in morphogenesis, regulates the activity of growth processes, photosynthetic productivity per unit of leaf area and plants and cenosis in general, affects the processes of carpogenesis, a load of plants with fruits and seeds (Wang et al., 2016; Kuryata and Khodanitska, 2018). The retardant application often leads to a significant increase in crop productivity. Simultaneously, the effect of various types of retardants on berry crops has not been studied enough. In particular, there are no studies on the influence of various types of retardants on morphogenesis, features of the photosynthetic apparatus formation, the accumulation, redistribution of assimilates, and mineral nutrition elements between the organs of berry crops.

It is necessary to consider a berry growing crop's specific properties when choosing the type of retardant for solving practical problems of berry growing. The products of this crop ripen quickly, and the ripening period of berries begins immediately after the drug treatment. There are practically no works on the application of various types of retardants on gooseberry crops. Toxicological and environmental safety is an essential element of the retardant's application in the technology introduction. In this regard, it is advisable to use retardants from the ethylene-releasing compounds since they are rapidly degraded in the plant to the native metabolic product - ethylene gas. Simultaneously, physiological and biochemical changes in plants after ethylene producers treatment remain largely unexplored, limiting the practical use of these compounds. In this regard, the study's main task is to analyze the influence of ethylene-releasing compound Esphon on morphogenesis, redistribution of assimilates, and essential nutrients between plant organs connected with crop production.

Materials and methods

Field studies were carried out at a specialized farm FG "Dagor" (2015-2017) village Rakovo, Tomashpolsky district, Vinnytsia region. The area of the randomized block design is 30 m2, the repeatability is fivefold. The gooseberry treatment of the "Mashenka" varieties was applied via foliar spraying OP-2 with an aqueous solution of 0.1% Esphon (per active compound) once at the time of initiation of budding to complete wetting of leaves. Control plants were treated with water.
Esphon - 65% solution of (2-chloroethyl)phosphonic acid ($C_2H_6ClO_3P$). Manufacturer – LLC Agrosintez (Russia). It is a solid, white, hygroscopic, wax-like substance, readily soluble in water, ethyl and isopropyl alcohols, acetone, propylene glycol, and less soluble non-polar solvents - benzene, toluene. Molecular weight is 144,5 D, melting point - 74°C. It is a non-flammable compound and incompatible with alkaline salts in a solution. It has low toxicity for warm-blooded animals: LD_{50} for white rats orally 4220 mg/kg. The drug and its metabolites are excreted in the urine within seven days. It is not caused by embryotoxic, hepatogenic, and mutagenic effects, has no cumulative properties. Aqueous solutions with a pH value of 4,1-4,5 are stable, at higher pH values, which are characteristic of the plant cell sap, spontaneous non-enzymatic cleavage of Esphon begins with the free ethylene release with regulatory functions. Esphon belongs to the ethylene-releasing compounds according to the mechanism of action. The active substance quickly penetrates the plant and decomposes in its tissues with the formation of ethylene. The action of ethylene producers depends significantly on the air temperature. Recommended maximum dosage for application is at temperatures below 16°C. Wash-off resistance is acquired 4-5 hours after treatment (Kuryata and Poprotska, 2019).
The mesostructure organization of the leaf was determined at the end of the growing season on fixed material.
For preservation was used a mixture of equal parts of ethanol, glycerol, and water 1) with 1% formalin. Determination of individual cell size of chlorenchyma was carried out after the maceration of leaf tissues with a 5% solution of acetic acid in 2 mol/l hydrochloric acids.
Analysis of the leaf anatomy was made at the middle part of annual shoots at the end of the growing season. The study of the anatomical elements' size was performed by using a microscope "Mikmed-1" and ocular micrometer MOB-1-15x. Biochemical analysis was realized at the material fixed with liquid nitrogen, dried for 4 hours at 85°C in a drying-oven to an air-dry state.
The amount of sugars, reducing sugars, and starch in the vegetative organs and fruits was determined by the iodometric method. The content of cellulose, lignin, and hemicellulose was determined in the shoots. The product quality indicators - sugar content, ascorbic acid, and total acidity were established in ripe fruits (AOAC, 2010).
Statistical analysis of experimental data was performed by computer program «STATISTICA-6» StatSoft Inc. The reliability of obtained results between control and experiment variant was assessed using Student's t-test. Tables and figures show average values for the years of research and their standard errors.

Results and discussion

Many scientific studies have been devoted to the issues of plant morphogenesis under the action of retardants. In these studies, it was found that the application of quaternary ammonium salts, triazole derivative retardants changed the activity of the apical and lateral meristems of the stem and marginal meristems of the leaf (Kuryata and Poprotska, 2019). Simultaneously, it is not adequately explored the influence of ethylene-releasing compounds on the formation and functioning of the stem's anatomical structure and leaves of crops. However, this group of retardants is the most promising for reasons of environmental safety. In this regard, we investigated the anatomical, morphological, and mesostructural characteristics of gooseberry stems and leaves under conditions of different tension of donor-acceptor relations under the influence of Esphon.
It is known that gibberellins enhance and retardants inhibit linear shoot growth (Rademacher, 2016). In this case, there is a significant restructuring of the anatomical structure shoots. The treatment of chlormequat chloride on oil flax led to a thickening of the stem, an increase in the number of xylem vessels in a row, an increase in the thickness of cell walls of bast fibers, and improved resistance of flax plants to lodging (Kuryata and Khodanitska, 2018). Similar changes in oil poppy plants were found under the application of growth-regulating drugs (Kuryata and Polyvanyi, 2018). Analysis of the anatomical structure of annual gooseberry shoots at the end of the growing season indicates that the drug carried out a typical growth regulating effect: shoots were shorter for the action of Esphon. However, it was established no reliable growth in the thickness of the shoots (Table 1).
Changes in the thickness of the experimental variants' shoots were determined by the features of bark, wood, and core formation of annual shoots of retardant-treated plants, the bark thickness significantly increased, and the wood and core thickness decreased.
We registered significant number of vessels in wood were formed for the actions of Esphon; however, an increase in the number of vessels in the xylem row was accompanied by a decrease in their diameter. We found that the use of drugs contributed to the thickening of the sclerenchyma fibers of the cortex.
We were established among the group of structural biopolymers, a significant increase in the content of cellulose, lignin, a decrease in the content of pectins, and a tendency to increase the content of hemicellulose under the action of the drug. The lignin content is a test indicator of wood visibility, an essential condition for a plant's successful wintering.
Earlier, we revealed significant depot capacities of the vegetative organs of tomato plants - stems and roots - in the temporary accumulation of reserve carbohydrates with their subsequent use in physiological processes (Kuryata and Kravets, 2018).

There were no significant changes in the starch content in fully matured shoots of drug-treated plants, but the sugar content and their various forms - reducing sugars and sucrose significantly increased. An increase in the forms of reserve carbohydrates in wintering shoots is a prerequisite for increasing the frost resistance of woody plants.
It is known that the production process of plants depends on the ratio of the activities of donor and acceptor spheres of the plant (Yu et al., 2015; Bonelli et al., 2016). The donor sphere is primarily represented by the leaf apparatus and photosynthesis, which provide morphogenesis with assimilates. The mesostructure features of the leaf are essential for the donor sphere functioning of the plants. We found that the use of Esphon resulted in significant changes in the anatomical structure of gooseberry leaves (Table 3).

Table 1. Anatomical structure of annual shoots of Esphon-treated gooseberry cv. Mashenka at the end of vegetation.

Indicators	Control	Esphon
Shoot length, cm	27.7±0.8	18.16±0.4*
Stem thickness, mm	5.1±0.1	5.2±0.1
Bark thickness, μm	579.1±17.4	753.1±12.6*
Wood thickness, μm	822.4±24.6	693.0±20.8*
Diameter of core, μm	2371.8±72.2	2165.5±65.6
The number of xylem vessels in a wood layer, pcs.	16±0.2	20±0.4*
Diameter of a xylem vessel, μm	58.1±1.8	44.6±1.3*
Sclerenchyma cell wall thickness, μm	5.4±0.1	5.8±0.1*
The diameter of perimedular zone cells, μm	49.21±0.9	52.74±0.8*

Note.* - Difference is significant at $p<0.05$.

We recorded an increase in the perimedular zone cells of a core under Esphon treatment. We found that anatomical changes in fully formed shoots (October) were accompanied by changes in reserve carbohydrates and structural biopolymers (Table 2).

Table 2. Influence of Esphon on the chemical composition of gooseberry shoots cv. Mashenka at the end of the growing season (% per dry matter weight).

Substance	Control	Esphon
Cellulose	23.61±0.71	25.68±0.68*
Lignin	14.92±0.45	16.14±0.24*
Hemicellulose	17.28±0.52	18.09±0.54
Pectins	6.97±0.10	6.44±0.04*
Amount of carbohydrates (sugars + starch)	7.25±0.22	9.32±0.28*
Reducing Sugars	2.55±0.08	3.24±0.09*
Sucrose	2.66±0.05	4.02±0.08*
Sugar Content	5.35 ±0.16	7.47±0.29*
Starch	1.90±0.05	1.85±0.07

Note.* - Difference is significant at $p<0,05$.

Table 3. Influence of Esphon on the mesostructure organization of formed leaves of gooseberry cv. Mashenka.

Indicators	Control	Esphon
Thickness of leave, μm	244.0±8.1	293.7±10.1*
Thickness of chlorenchyma, μm	208.2±5.4	243.1±4.1*
Thickness of upper epidermis, μm	18.1±0.6	25.3±0.8*
Thickness of lower epidermis, μm	17.7±0.4	24.1±0.8*
Volume of palisade parenchyma, μm3	10305.5±355.2	11885.3±499.1*
Length of spongy cells, μm	28.1±0.5	31.0±0.9
Width of spongy cells, μm	23.6±0.5	23.5±0.3

Note.* - Difference is significant at $p<0.05$.

Significant thickening of the leaves of experimental variant plants indicates an increase in the concentration of structures providing photosynthesis per unit of leaf area. The thickening of the leaf occurred due to an increase in the thickness of the primary photosynthetic tissue - chlorenchyma, and due to a thickening of the upper and lower epidermis. Retardants treatment caused an increase in the spongy's linear dimensions and the volume of the primary assimilative leaf tissue - the palisade parenchyma.
Thus, the application of ethylene producer Esphon leads to the optimization of the leaf mesostructured that is an essential prerequisite for increasing their photosynthetic productivity. Our results indicate that the content of nonstructural carbohydrates (sugar + starch) in the leaves was consistently higher than in control due to the formation of a more powerful mesostructure under the drug's influence during the entire development (Table 4). In our opinion, this indicates a greater photosynthetic and donor activity of leaves for the action of this compound due to the formation of optimal mesostructure.

Table 4. Influence of Esphon on the sugars and starch content in leaves and stems gooseberry cv. Mashenka at various stages of plant development (% per dry matter weight).

Stages of development	Amount of carbohydrates (sugar+starch)		Sugar content		Starch content	
	Control	Esphon	Control	Esphon	Control	Esphon
Flowering stage	11.30±0.24	11.91±0.24	10.01±0.20	10.50±0.22	]1.31±0.02	1.40±0.03*
Fruit formation stage	10.30±0.21	11.70±0.24*	10.20±0.20	10.31±0.22	1.50±0.03	1.42±0.02
Fruit ripening stage	11.40±0.22	12.50±0.26*	10.30±0.21	10.21±0.21	1.10±0.02	2.30±0.05*

Note.* - Difference is significant at p<0,05.

In our opinion, an increase in the donor function of the leaves of Esphon treated plants is evidenced by the fact that in the fruit ripening phase due to the termination of processes of vegetative growth and the end of carpogenesis, the content of total carbohydrates in the gooseberry leaves was higher than in control.
The results of the study of the dynamics of the reducing sugars content in leaves in various phases of plant ontogenesis give a relatively clear idea of the characteristics of sugars accumulation and redistribution between plant organs (Table 4). In general, more reducing sugars accumulated during the flowering and fruit formation stages for drug action. The sugar content decreased in comparison with the control during the transition to the maturation phase, which, in our opinion, indicates a better provision of the carpogenesis process by carbohydrates. The higher starch content also evidences the more intensive accumulation of photoassimilants by the leaves of Esphon-treated gooseberry plants in them at the stage of fruit ripening. So, the accumulation and outflow of sugars from the leaves to the powerful acceptor zone - fruits formation and growth increased for the actions of ethylene producer Esphon. The symbiotic nitrogen fixation processes in soybeans intensified under the triazole derivative compound paclobutrazol (Kuryata & Golunova, 2018v). Treatment of gooseberry plants with tebuconazole and gibberellin significantly increased yield (Table 5).

Table 5. Influence of Esphon on yield and product quality of gooseberry cv. Mashenka.

Indicators	Control	Esphon
Yield per one bush, kg	2.90±0.05	3.20±0.04*
Titrated acidity, %	1.90±0.07	2.01±0.04
Content of ascorbic acid, mg/100 g	20.82±0.41	21.30±0.42
Content of sugar, % per fresh matter	7.40±0.16	8.31±0.16*

Note.* - Difference is significant at p<0.05.

The essential indicators of retardants application are the quality characteristics of products: ascorbic acid content, sugars, and total acidity. The results obtained indicate that the drug treatment increases the ascorbic acid and sugar content indicators in berries, which improves the product quality. The slight increase in berries' acidity is within the range of fluctuations that are typical for gooseberries under different climatic conditions. Thus, the data obtained indicate that the yield of gooseberry crop increased for the actions of Esphon due to the formation of a more powerful donor sphere, the accumulation and overload of assimilating' flows and essential nutrients from vegetative organs to fruits.

Conclusion

Application of retardant Esphon on gooseberry plants in the budding phase led to change donor-acceptor relations in the plant, expressed through anatomical and morphological changes in vegetative organs, redistribution of assimilates towards the formation of berries. The linear growth of shoots was inhibited, a more significant number of vessels were formed in the xylem of drug-treated plants compared to control, while the cell wall thickness of the sclerenchyma fibers of cortex increased. The consequence of this restructuring was the accumulation of cellulose, hemicellulose, and lignin in annual shoots of gooseberry compared to control and reserve forms of carbohydrates - sugars and starch, which is evidence of a complete ripening of the shoot and a prerequisite for higher frost resistance. The leaf blade thickened due to the formation of a more powerful chlorenchyma, an increase in the volume and linear dimensions of its cells for the actions of Esphon. Treatment of plants with retardants caused an increase in the spongy's linear dimensions and the volume of the primary assimilative leaf tissue - the palisade parenchyma. Optimization of the leaf mesostructure organization enhanced the provision of morphogenetic processes with assimilates, which turned out to be an increase in the content of nonstructural carbohydrates (amount of sugars + starch) in the leaves as compared to untreated plants at all stages of development. As a result of the more powerful donor sphere formation, the accumulation and redistribution of assimilating flow from vegetative organs to fruits, the yield of gooseberry crop, and sugars and ascorbic acid content in berries increased.

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