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Effect of Ecotol on Cold Resistance Indices of *Begonia grandis* Dryander

E. A. KARPOVA, T. D. FERSHALOVA, A. A. PETRUK

Central Siberian Botanical Garden, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

E-mail: karpova@csbg.nsc.ru

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Abstract

Begonia grandis is a unique representative of the genus *Begonia* inhabiting the temperate zone. The mechanisms of adaptation of this plant to low temperatures, in particular under the influence of physiologically active substances, are of great interest. The effects of the product of straw processing, ecotol, on the dynamics of the parameters of physiological state of *Begonia grandis* plants introduced in West Siberia (Novosibirsk), in a greenhouse and at an open ground were studied during air temperature drops at the end of the growing season. The concentrations of chlorophylls *a* and *b*, carotenoids, anthocyanins, flavones (luteolin, orientin) and flavonols (quercetin, kaempferol, hyperoside, isoquercitrin, and astragalin) were determined, and the ratios chlorophyll a/b, chlorophyll $(a + b)$/carotenoids, chlorophyll $(a + b)$/anthocyanins) in the leaves were calculated. With a decrease in the diurnal temperature to 10 °C and a short-term drop to negative temperatures, the open-ground plants treated with ecotol retained a higher proportion of the leaves (59.9 % of their initial number) compared to the control ones (5.8 %), and the concentrations of chlorophyll and antioxidants (carotenoids, anthocyanins, isoquercitrin) in them was more significant (up to 3.1, 1.2, 12.8 and 11.2 mg/g, respectively) than in the leaves of control plants (up to 1.4; 0.5; 1.8 and 7.0 mg/g). The properties of ecotol as a complex stimulator of plant resistance were confirmed. Under low-temperature stress, it provided a higher physiological state of *B. grandis* plants during their transition to dormancy.

Keywords: low-temperature stress, chlorophyll $(a + b)$, carotenoids, anthocyanins, isoquercitrin

INTRODUCTION

Adaptation of plants to the low-temperature stress plays the most important part under the conditions of moderate climate, which is characterized by substantial changes of air temperature during spring and autumn. A unique representative of *Begonia* genus, *Begonia grandis* Dryander occurs in the zone of moderate climate [1]. In the sites of natural growth (the south-western part of China) the plants are protected from frost-killing under the conditions of low temperature by passing into the dormant state, which is accompanied by a complete loss (abscission) of shoots. During autumn and winter, with temperature drop to 5 °C, the plant forms a bulbus under the ground at a depth of 3–7 cm. This bulbus conserves vitality at a temperature down to –20 °C [2]. Investigation of the dynamics of parameters related to the physiological state of *B. grandis* plants with a decrease in air temperature is important to understand the adaptation of this rare taxon and to enhance the efficiency of begonia introduction indoors and outdoors. The most informative parameters of the physiological state of plants characterizing the stability under stress conditions are the concentrations of chlorophylls, various antioxidants (carotenoids, anthocyanins, flavons and flavonols) and some specific relations between the concentrations of pigments [3–7].

Photosynthetic pigments (chlorophylls and carotenoids) are parts of the system providing the transformation of solar energy into the energy of plant growth and development. Their content characterizes the intensity of assimilation processes [8]. Carotenoids, along with anthocyanins, serve as the protective components of the photosynthetic apparatus [9]. The content of these compounds increases during any deviations from optimal conditions, including a decrease in air temperature [10–13]. A similar accumulation of flavons and flavonoids was also revealed [4, 14].

In the leaves of shade-enduring plants, including *B. grandis*, anthocyanins provide scattering of excessive solar radiation [4]. Though the participation of anthocyanins in adaptation to low-temperature stress was also established for some plants, the results of the majority of experiments do not confirm their decisive role in adaptive reactions [15].

We studied the dynamics of photosynthesizing pigments, anthocyanins and other flavonoids in the leaves of *B. grandis* during cultivation outdoors and indoors in the Novosibirsk Region previously [16, 17]. The periods of adaptation to the low-temperature stress were characterized by the lower content of the sum of chlorophylls ($a + b$), the ratio of the concentrations of chlorophylls ($a + b$)/carotenoids and chlorophylls ($a + b$)/anthocyanins in comparison with favourable periods, and higher content of flavons and flavonols, the major of which are C-glycosyl flavon (orientin) and O-glycosides of quercetin [16, 18–20]. With a decrease in air temperature, the composition of O-glycosides and free aglycons changes. Four O-glycosides including isoquercitrin were revealed only in the leaves of the plants growing outdoors [16].

At the next stage of the investigation, it appears interesting to study the changes in the adaptive response of photosynthesizing pigments and flavonoids to low-temperature stress of different intensities in the presence of ecotol. Ecotol (a natural complex of compounds) is a product of aerobic treatment of plant raw material (straw, leaves) by microorganisms and fungi; it contains lignins, the derivatives of benzene and furan, quinones, indoles, biogenic amines, melanins [21–23]. A positive effect of this multicomponent preparation was demonstrated for the growth of young tree plants of *Acer platanoides* L. at increased temperatures, as well as for the parameters of physiological state (chlorophyll fluorescence in the bark of young sprouts, protein content in the leaves) of *Fraxinus pennsylvanica* and *Sorbus aucuparia* in the case of lead excess in soil [22]. In addition, an increase in the stability of spring wheat to drought was detected, which was expressed in a substantial increase in the crop productivity [23].

The goal of the study was to investigate the effect of ecotol on the dynamics of the content of pigments and phenolic compounds, as well as the ratios of the concentrations of chlorofylls a/b, chlorophylls ($a + b$)/carotenoids and chlorophylls ($a + b$)/anthocyanins in the leaves of *B. grandis* plants the indoor and outdoor conditions under air temperature decrease at the end of vegetation period.

EXPERIMENTAL

Materials and methods

The samples were the leaves of *B. grandis* Dryander subsp. *grandis* (syn. *Begonia discolor* R. Brown, *Begonia evansiana* Andrews, *B. grandis* subsp. *evansiana* (Andrews) Irmsch.) plants introduced under greenhouse conditions and outdoors at the Central Siberian Botanical Garden SB RAS (CSBG SB RAS) growing during the vegetation period of the year 2018. Table 1 shows the dynamics of air temperature and visual evaluation of the state of plants. The samples for the determination of biochemical parameters were the fragments of the central part (between large veins) of mature leaves of the medium floor, without any signs of damage. To determine pigments, the fragments of leaves collected from every five of 25 plants were brought together. The resulting 5 average samples were homogenized with the help of a mortar and pestle, and used for analysis immediately. To study phenolic compounds, the samples were prepared similarly from the leaves dried in the air and homogenized with the help of electric coffee grinder Bosch MKM 6004 (Robert Bosch GmbH, Slovenia).

Ecotol was manufactured under laboratory conditions. The straw of Khakasskaya wheat dried to the air-dry state was ground, placed into a bioreactor 50 L in volume, poured with water at a ratio of 1 : 1, and switched to the aerator with air flow rate of 35 L/min [23]. The process was run till the completion of aerobic fermentation. The concentrations of extractives in the preparation during treatment was 0.5 mg/mL. The treatment of plants with ecotol was performed since early September under the conditions of night temperature higher than 7 °C (see Table 1). The treatment with ecotol by means of wetting

TABLE 1

Air temperature and visual evaluation of the state of *Begonia grandis* plants during the study (2018)

Date	Greenhouse			Outdoors		
	Temperature, °C	State of plants; development phase (leaves, % of their initial number)		Temperature, °C*	State of plants; development phase (leaves, % of their initial number)	
		E− (reference)	E+		E− (reference)	E+
03.09	14	Healthy plants without any signs of necrosis; vegetation (100.0±0.0)	Healthy plants without any signs of necrosis; vegetation (100.0±0.0)	10 (7)	Healthy plants without any signs of necrosis; vegetation (100.0±0.0)	Healthy plants without any signs of necrosis; vegetation (100.0±0.0)
13.09	13	The same; budding (100.0±0.0)	30 % of the leaves became yellowish; blossoming (95.2±4.0)	7 (1)	30 % of the leaves became yellowish; blossoming (85.6±4.1)	The majority of leaves are yellow, 20 % of leaves are with necrosis along the edges; blossoming (89.4±7.6)
18.09	10	The same; blossoming (96.2±3.8)	10 % of the leaves became yellowish; blossoming, fruiting (93.6±5.8)	7 (−1)	30 % of the leaves are yellowing; blossoming, fruiting (52.3±2.4)	All leaves became brown; blossoming, fruiting (77.1±4.5)
18.10	18	The same; fruiting (90.0±4.3)	20 % of the leaves became yellowing; fruiting (91.0±3.5)	2 (−3)	Stems with sole leaves; aboveground parts perish (5.8±0.6)	Leaves are brown, about 5 % of the leaves are with necrosis along edges; aboveground parts perish (59.9±5.8)

Note. E−, E+ − without treatment and after treatment with ecotol, respectively.

* Diurnal air temperature; temperature at the night preceding the observation is shown in parentheses.

(200 mL per one plant) at the root was carried out with 25 plants at the stage of active growth in the greenhouse and 25 plants growing outdoors. Wetting was carried out three times at an interval of 5 days. Untreated plants cultivated under the corresponding conditions served as reference.

Novosibirsk is situated in the forest-steppe zone with continental climate. Frost-free period 142 days long on average lasts from May to August [24]. The period of *B. grandis* vegetation starts from the moment of tuber sprouting in the greenhouse in the first decade of February, continues through blossoming (August−September) and then perishing of aboveground parts. The cache-pots with plants are brought outdoors during the third decade of May, and the transition to dormancy occurs in the third decade of September − October. In the greenhouse, dormancy starts at the end of November.

For the experiment, all the plants were planted in identical cache-pots 3 L in volume. The soil mixture was composed of leafy and garden soil with the addition of peat and sand. One group of plants was arranged in greenhouses (gable-roofed reinforced concrete structures closed with polycarbonate) on shelves 1 m high. Another part of plants was arranged outdoors at the experimental plot on identical shelves. Under natural conditions, *B. grandis* grows under the forest canopy, so in both cases, the curtains made of waterproof cloths were stretched above the plants to prevent them from bright sunrays and rainwater. Illumination under the artificial curtain in both versions of the experiment varied from 500 to 2000 lux. So, the differences between indoor and outdoor plants in all the major ecological parameters except temperature mode were excluded.

The smallest difference between day and night temperatures is observed outdoors in July (up to 10 °C). Since August, diurnal temperature variations become more substantial, and they may reach 20 °C in September. Air temperature in greenhouses during the operation of stationary warming system (October−April) is 20 °C on average. Since May till September, air temperature is substantially dependent on weather conditions. Temperature variations in the greenhouse are 5−10 °C [25].

During the time of the investigation, diurnal air temperature in the greenhouse decreased since 31.08 till 18.09, and then increased by 18.10 (see Table 1). Outdoors, the conditions at the end of August were similar to indoor conditions, but in September a gradual decrease in the bight and average diurnal temperature occurred. A drop of air temperature at night to 1 °C was observed on 13.09, to −1 °C on 18.09, and to −3 °C on 18.10.

Evaluation of the physiological state of plants was carried out over the following criteria: visual estimation of the number and state of leaves; the sequence of development phases (blossoming, fruiting, the transition to dormancy); the content of the sum of chlorophylls a and b (chlorophylls $(a + b)$). The criteria of stress and adaptation that were determined in experiments included the content of carotenoids and anthocyanins; the ratios of chlorophylls $(a + b)$/carotenoids and chlorophylls $(a + b)$/anthocyanins; the sum of phenolic compounds and the basic flavonoid component (orientin), as well as the content of the sum of O-glycosides (hyperoside and isoquercitrin).

To determine the content of chlorophylls and carotenoids, the homogenate of fresh leaves was weighted and extracted with 96 % ethanol. The optical density of the extract was measured at the wavelength of 470, 649 and 664 nm with the help of UV-Vis spectrophotometer Agilent 8453 (Agilent Technologies, USA). The calculation was carried out using equations

$C_a = (13.36A_{664} - 5.19A_{649})$

$C_b = (27.43A_{649} - 8.12A_{664})$

$C_c = (1000A_{470} - 2.13C_a - 97.64C_b)/209$

where C_a, C_b and C_c are concentrations of chlorophyll a, chlorophyll b and carotenoids, respectively, mg/mL of the extract; A_{664}, A_{649} and A_{470} are optical densities of the extract measured at 664, 649 and 470 nm, respectively [26].

For the quantitative estimation of anthocyanins, the weighted portion of the homogenate was extracted with a 1 % aqueous solution of HCl until the extractant was colourless. The optical density of the extract was measured at the wavelength of 529 and 650 nm. The content of the sum of anthocyanins was calculated using the molar extinction coefficient of cyanidine-3-rutinoside in a 1 % aqueous solution of HCl, which is equal to 28 840 [27]. A correction for the content of chlorophylls and the products of their degradation was taken into account using the equation

$A = A_{529} - 0.288A_{650}$

where A_{529} and A_{650} are the optical densities of the extract measured at 529 and 650 nm, respectively [28].

The composition and content of phenolic compounds in the leaves were studied by means of high-performance liquid chromatography (HPLC). An exact weighted portion of the plant material (0.1 g) was thoroughly extracted with 70 % ethanol on a water bath at a temperature of 60−70 °C. The analysis was carried out with an Agilent 1200 liquid chromatograph (Agilent Technologies, USA), equipped with a Zorbax SB-C18 column (4.6 mm × 150 mm × 5 μm; Agilent Technologies), a detector with the diode matrix and ChemStation system for chromatographic data processing. The eluents were 0.1 % aqueous solution of H_3PO_4 (eluent A) and methanol (eluent B). Chromatographic separation was carried out in the gradient mode under the following conditions: 0−27 min 32−33 % B, 28−38 min 33−46 % B, 39−50 min 46−56 % B, 51−54 min 56−100 % B, 55−56 min 100−32 % B. The volume of the sample introduced into the chromatograph was 10 μL; column temperature 25 °C; the flow rate of the solvent 1 mL/min. Detection was carried out at 255, 270, 290, 325, 340, 350, 360 and 370 nm. Identification of the known compounds was carried out by comparing retention times and UV spectra of the analyzed peaks with the peaks of standard samples of quercetin, kaempferol, orientin, luteolin (Sigma-Aldrich, USA), hyperoside, isoquercitrin, astragalin (Fluka, Sigma-Aldrich Chemie GmbH, Germany).

Calculation of the content of non-identified components was carried out relying on the standard areas of the peaks of gallic acid (for phenolcarboxylic acids) and hyperoside (for flavonoids). The class of compounds was determined according to the spectral characteristics of the peaks (the number of absorption peaks, wavelengths of maxima, the ratio of their intensities).

The content of pigments and phenolic compounds was measured three times in each of 5 extracts, the arithmetic mean and standard error were calculated over 15 parameters of biological and analytical repetitions [29]. The content of the components was calculated in 1 g of absolutely dry mass. Statistical treatment was performed using the Statistica 7.0 software (Statsoft Inc., USA).

RESULTS AND DISCUSSION

Within 10 days after the treatment with ecotol, 10 to 30 % of the leaves got yellow. A definite part of leaves was observed to keep the yellow colour in the treated greenhouse plants (E+) till the end of the experiment. Untreated plants (E−) looked healthy during the entire period. The fractions of fallen leaves with respect to their initial number on the treated and reference plants in the greenhouse did not exhibit any significant difference from each other and did not change significantly during the whole time of the investigation. Chlorophyll ($a + b$) content in the leaves of greenhouse plants decreased from the start to the finish of the experiment, and it was reliably higher (3.9 mg/g) in the leaves of the plants treated with ecotol in comparison with the leaves of reference samples (2.8 mg/g) (Fig. 1, *a*).

With a decrease in temperature to positive values (from 10 to 0 °C) after 13.09 we also observed yellow colouring of the leaves of plants growing outdoors, and the regions with necrosis appeared. Under the action of negative temperatures, the majority of leaves became brown. However, the plants treated with ecotol kept a substantially higher percentage of leaves during the whole time of the experiment in comparison with the reference plants. By the end of the observation period, the percentage of leaves with respect to their initial number was much higher in the treated plants (59.9 %) than in reference samples (5.8 %) (see Table 1). The general view of the plants growing outdoors, both treated with ecotol and untreated ones, is shown in Fig. 2 at the beginning (*a* and *b*, respectively) and at the end of the experiment (*c* and *d*, respectively). With a decrease in temperature, chlorophyll content in the leaves of outdoor plants decreased in both versions of the experiment (see Fig. 1, *b*). During almost the whole period of investigation (excluding the last chill on 18.10), chlorophyll content in the leaves of untreated plants was higher in comparison with the plants treated with ecotol. However, the plants treated with ecotol survived the last chill, which was critical for plant vitality outdoors, better than the reference plants: chlorophyll content in the leaves of the treated plants (3.1 mg/g), similarly to the greenhouse conditions, was higher than in the leaves of untreated samples (1.4 mg/g).

The ratio of chlorophylls a/b in the leaves of greenhouse and outdoor plants varied insignificantly (Fig. 3, *a*, *b*), with the exception of the point of observation on 13.09 for the leaves of untreated plants in the greenhouse. The content of the sum of chlorophylls at this observation point in the leaves of reference plants increased substantially, mainly due to chlorophyll *b*.

Carotenoid content in the leaves of greenhouse plants increased with a decrease in temperature, and then, after having achieved the optimal conditions at the end of September – October, it again decreased to the values characteristic of early September. At all observation points except 18.09, the leaves of untreated plants contained a substantially lower amount of carotenoids, and on that day the values under investigation differed from each other insignificantly in the two versions of the experiment (see Fig. 1, *c*). The dynamics of carotenoid content were similar in the plants growing outdoors: the amount of carotenoids decreased by the end of the experiment, and the decrease was especially significant in the leaves of reference samples. The leaves of the plants treated with ecotol were characterized by more smoothed dynamics of carotenoid content (see Fig. 1, *d*).

The content of anthocyanins in the leaves of greenhouse plants treated with ecotol was higher than their content in the leaves of reference samples during the entire experiment (see Fig. 1, *e*). Outdoors, till temperature drop on 18.09, anthocyanin content in the leaves of plants treated with ecotol was lower than in the leaves of reference samples. However, during the chill period, the content of anthocyanins in the leaves of reference plants increased critically (to 1.8 mg/g), while in the leaves of treated plants it decreased substantially (up to 12.8 mg/g) (see Fig. 1, *f*).

The ratios of chlorophylls ($a + b$)/carotenoids (see Fig. 3, *c*, *d*) and chlorophylls ($a + b$)/anthocyanins (see Fig. 3, *e*, *f*) were decreasing from the start till the end of the experiment, excluding the observation point on 13.09 in the leaves of untreated greenhouse plants. The dynamics of both ratios were similar to each other. However, by the end of the period of observations, the differences between ecotol-treated and reference plants outdoors were higher than those with respect to the ratio of chrolophylls/carotenoids.

The content of the sum of phenolic compounds (phenolic acids, O-glycosides of flavonols and C-glycosylflavones) increased, with a decrease in air temperature, in the leaves of outdoor plants, both treated with ecotol and untreated (see Fig. 1, *h*). In the leaves of untreated plants, an increase in the content of phenolic compounds proceeded to a higher extent due to orientin (Fig. 4, *a*), while in the case of treatment with ecotol it was mainly due to O-glycosides (see Fig. 4, *b*). It did not change substantially in the leaves of greenhouse plants (see Fig. 1, *g*).

Isoquercitrin content in the leaves of greenhouse plants was insignificant at all observation points (and much higher in the leaves of outdoor plants) and increased multiply with a decrease in temperature; this increase was most significant in the leaves of the plants treated with ecotol (see Fig. 1, *i*, *j*).

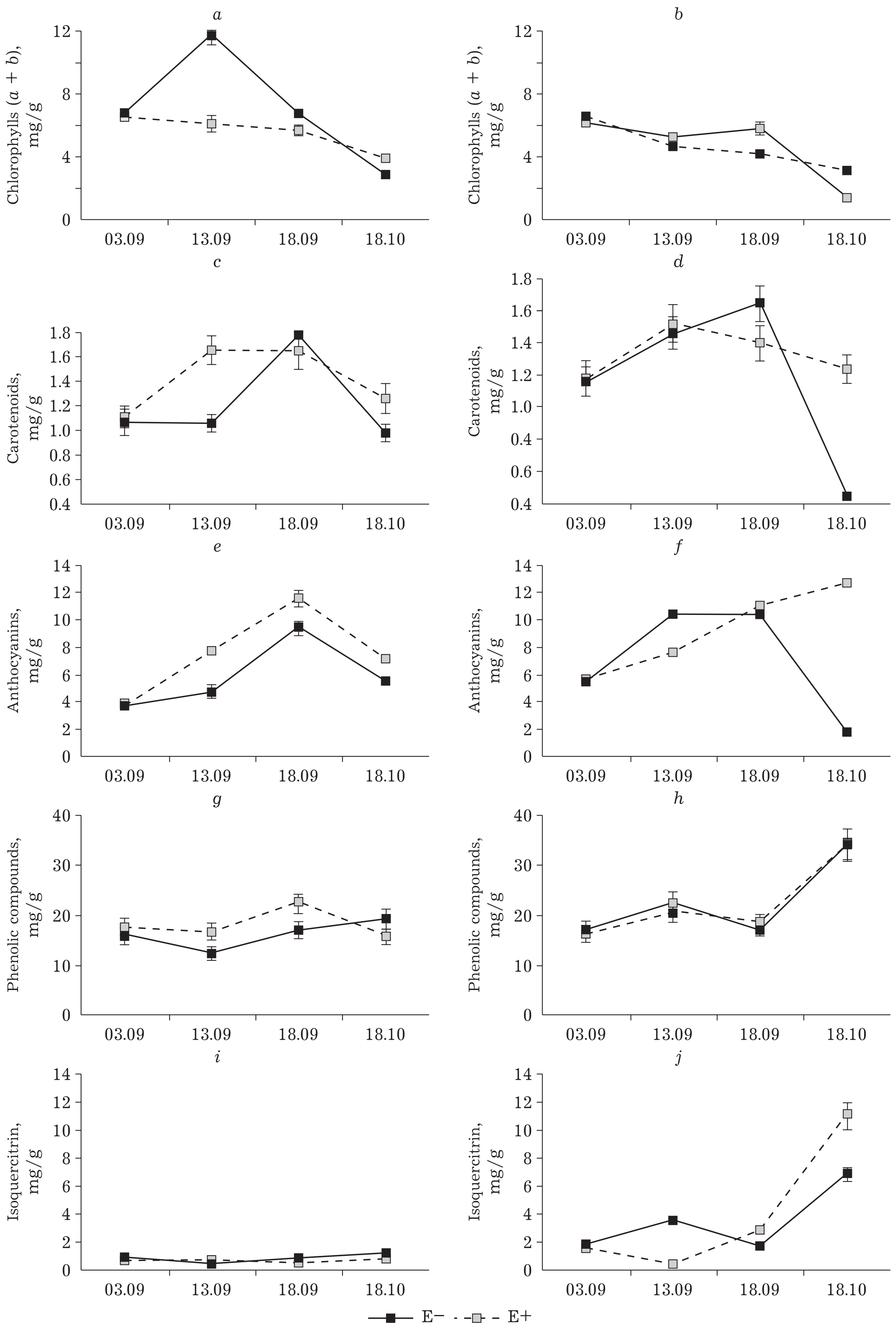

Fig. 1. Content of the sum of chlorophylls (*a* + *b*) (*a*, *b*), carotenoids (*c*, *d*), anthocyanins (*e*, *f*), the sum of phenolic compounds (*g*, *h*) and isoquercetrin (*i*, *j*) in the leaves of *Begonia grandis* plants growing in the greenhouse (*a*, *c*, *e*, *g*, *i*) and outdoors (*b*, *d*, *f*, *h*, *j*) treated with ecotol (E+) and nontreated with ecotol (E−), during the period of air temperature decrease and chill (2018).

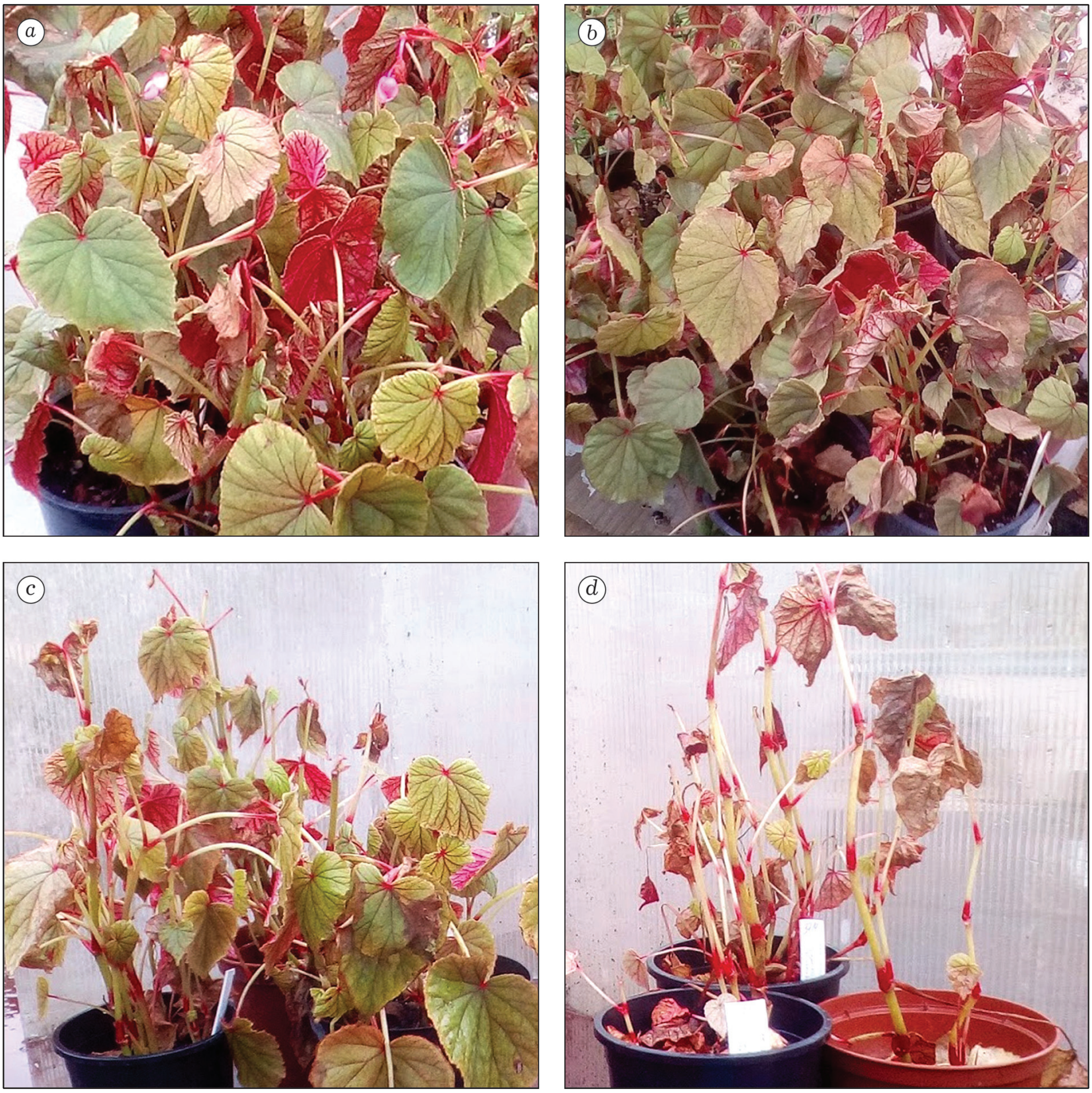

Fig. 2. General view of ecotol-treated and reference *Begonia grandis* plants growing outdoors, immediately after the treatment with ecotol 03.09 (*a* and *b*, respectively) and at the end of the experiment after the night chill on 18.10 (*c* and *d*, respectively) (2018).

A decrease in temperature under the natural growing conditions serves as a factor regulating the cross adaptation and the vital activity of the plant itself and its progeny [30]. The versions of the experiment demonstrate the effect of ecotol on *B. grandis* plants during two periods of hardening, which precede the period of dormancy under natural conditions, when negative temperatures follow low positive ones. Preliminary investigation of the dynamics of the parameters of physiological state of *B. grandis* for several years allowed us to estimate the state of plants treated with ecotol and to reveal the signs of stress and adaptation in them. The state of *B. grandis* plants under optimal conditions is characterized by the following ranges of pigment concentrations: chlorophylls 4–8 mg/g, carotenoids 1–1.5 mg/g, and anthocyanins 2–4 mg/g. The ratios of chlorophylls $(a + b)$/carotenoids and chlorophylls $(a + b)$/anthocyanins are 4–6 and 2.5–4, respectively. In the state of stress and adaptation, the content of carotenoids and anthocyanins in the leaves increases, and the values of the indicated ratios become lower than 4 and 2.5, respectively [17].

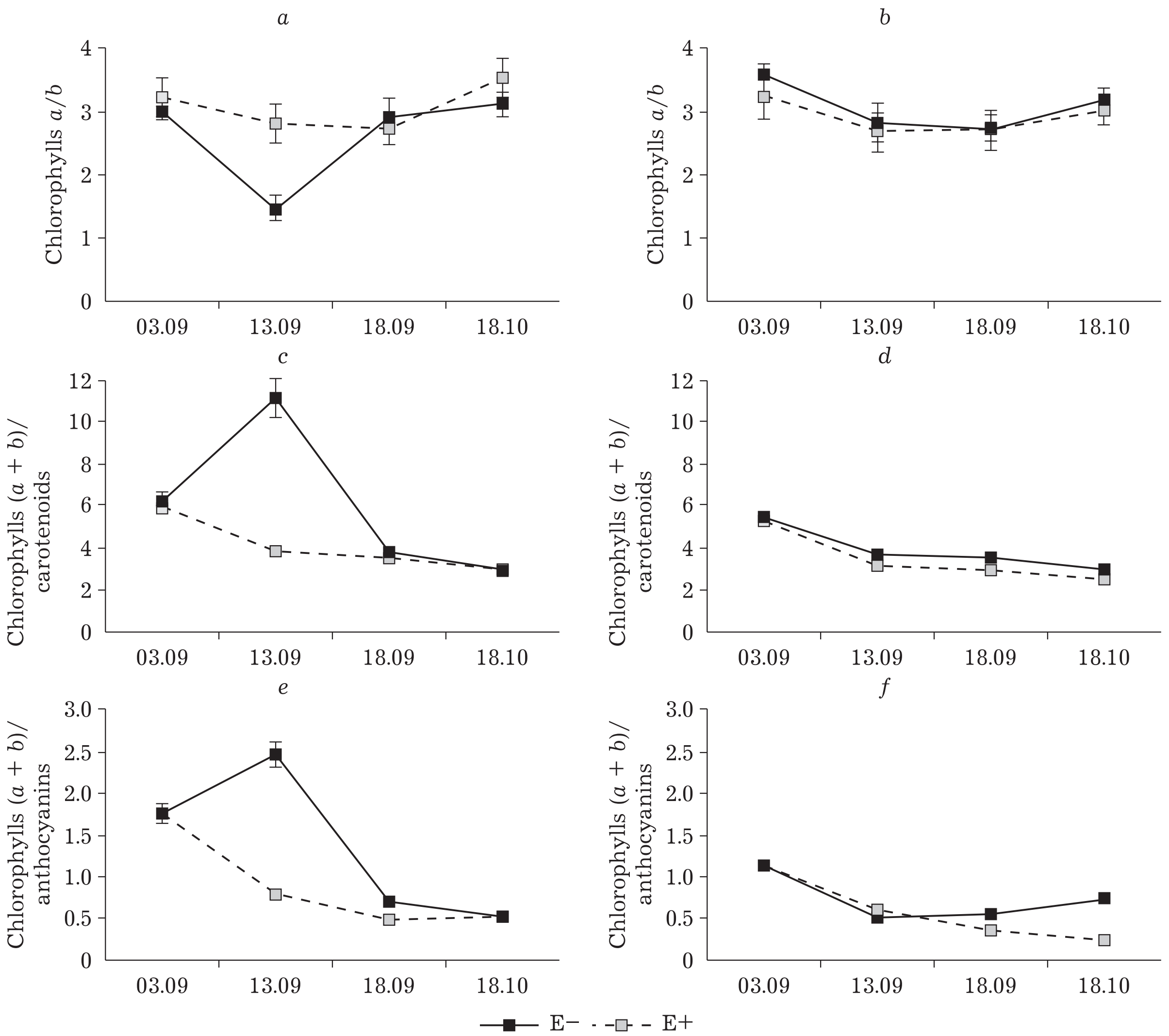

Fig. 3. The ratios of chlorophylls *a*/*b* (*a*, *b*), chlorophylls (*a* + *b*)/carotenoids (*c*, *d*) and chlorophylls (*a* + *b*)/anthocyanins (*e*, *f*) in the leaves of *Begonia grandis* plants growing in the greenhouse (*a*, *c*, *e*) and outdoors (*b*, *d*, *f*) treated with ecotol (E+) and non-treated with ecotol (E−) during the period of air temperature decrease and chill (2018).

An increase in the content of carotenoids and anthocyanins is considered as the major indices of the stress and adaptation states [3, 31, 32]. An increase in the content of carotenoids and anthocyanins accompanying a decrease in the sum of chlorophylls was revealed in the leaves of greenhouse plants after the treatment with ecotol even by 13.09. This is the evidence of the formation of the adaptation state in the treated greenhouse plants. A similar state in the reference samples was recorded only on 18.09 (see Fig. 1, *a*, *c*, *e*). Under the conditions of permanently decreasing air temperature outdoors, a decrease in the content of the sum of chlorophylls and an increase in the content of carotenoids and anthocyanins in the leaves of ecotol-treated and untreated plants were almost synchronous. This suggests the absence of the signs of stress caused by the treatment with ecotol in outdoor plants.

A decrease in carotenoid and anthocyanin content in the damaged leaves of reference plants (down to 0.5 and 1.8 mg/g, respectively) as a result of their destruction under the effect of the chill on 18.10 deserves attention too. Their concentrations were substantial at the beginning of observation: 1.2 and 12.8 mg/g, respectively (see Fig. 1, *d*, *f*). It was demonstrated previously that the state of adaptation in *B. grandis* plants is provided by an increase in anthocyanin content only within the limit of insignificant temperature difference, while a more substantial temperature drop causes a decrease in their concentration with an increase in the concentration of flavonols [12]. A decrease in the content of anthocyanins after the

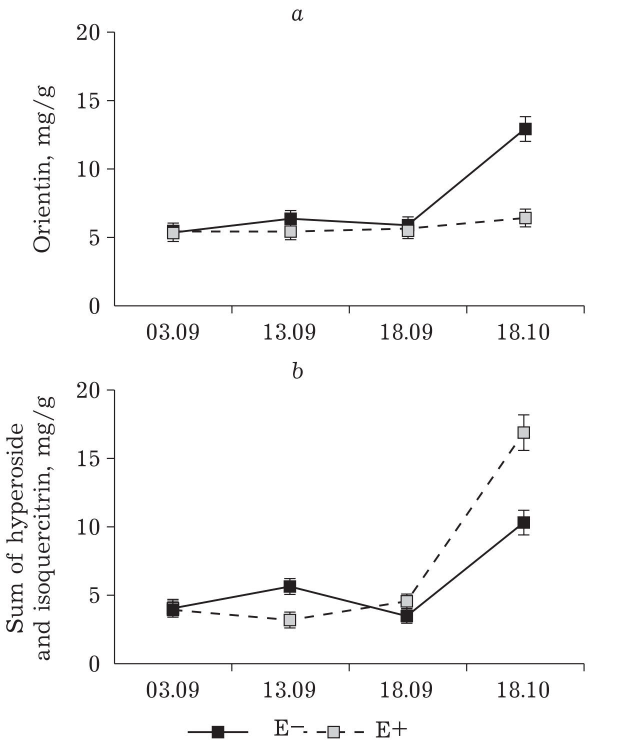

Fig. 4. Content of orientin (*a*) and a sum of hyperoside and isoquercitrin (*b*) in the leaves of *Begonia grandis* plants growing outdoors, treated with ecotol (E+) and non-treated with ecotol (E−) during the period of air temperature decrease and chill (2018).

chill on 18.10 in the leaves of reference plants confirms these results. It was detected that the content of anthocyanins increased substantially in the leaves of the samples treated with ecotol (see Fig. 1, *f*). Therefore, ecotol caused a modification of the adaptive response of plants through the accumulation of anthocyanins in the leaves under the conditions of a substantial decrease in air temperature. Similar differences were revealed also in the dynamics of carotenoid content in the leaves of outdoor plants (see Fig. 1, *d*). In this situation, unlike for anthocyanins, the content of carotenoids in the leaves of ecotol-treated plants decreased after 18.09, however, this decrease was not so substantial as that in the leaves of reference samples. So, the effect of ecotol with respect to carotenoids may be called protective.

It is known that the action of chill causes also changes in the ratio of chlorophylls *a*/*b*. However, the role of different groups of chlorophylls in the stability against chill has not been fully revealed yet. An opinion exists that chlorophyll *a* participates in adaptation to low temperature. However, the response of plants to cooling not always involves a more intense decrease in the content of chlorophyll *a* in comparison with chlorophyll *b* and a decrease in the ratio of chlorophylls *a*/*b*. In some cases cooling causes an increase in the content of chlorophyll a and the ratio of chlorophylls *a*/*b* [33]. A decrease in temperature in both versions of the experiment promoted a more substantial decrease in the content of chlorophyll *a* in comparison with chlorophyll b and a decrease in their ratio (see Fig. 3, *a*, *b*). This corresponds to the data reported in [34] concerning a more substantial contribution from chlorophyll a into a decrease in the concentration of the sum of chlorophylls at low temperature, which usually affects both its forms [35]. The values of this ratio were at the boundary or above the level of optimal values for shade-resistant plants (2.1−2.6) during the whole period of investigation in all versions of the experiment [17]. However, a decrease in the ratio of chlorophylls *a*/*b* was not significant even in outdoor plants, and the treatment with ecotol did not have a substantial effect on the change of this value.

Not very high values of the ratio of chlorophylls ($a + b$)/carotenoids (<4) provide evidence of the formation of the state of stress and adaptation in all plants after 13.09 or 18.09, and the values of the ratio of chlorophylls ($a + b$)/anthocyanins (<2.5) confirm the presence of stress in plants since the start of observation on 03.09 (excluding the reference plants in the greenhouse on 13.09). An increase in the ratio of chlorophylls ($a + b$)/carotenoids and chlorophylls ($a + b$)/anthocyanins in the leaves of reference plants in the greenhouse by 13.09 was likely to be caused by the preparation for blossoming (all other plants had been already blossoming by that moment). A decrease in air temperature could serve as one of the stimuli for blossoming for outdoor plants. Ecotol might have manifested a similar effect on the plants in the greenhouse [36].

The content of the sum of phenolic compounds (phenolic acids, O-glycosides of flavonols and C-glycosylflavones), similarly to our previous investigation [16], increased substantially with a decrease in air temperature. The most substantial increase in their sum was observed in outdoor plants, both treated with ecotol and reference ones (see Fig. 1, *h*). This points to a substantial role of phenolic compounds in the formation of the state of plant adaptation to decreased temperature. Ecotol modified the response of plants through an additional increase in the content of O-glycosides of flavonols including isoquercitrin (see Fig. 1, *j*).

In the investigation of the dynamics of the chemical composition of leaves during the years 2013−2015, isoquercitrin was not detected in the leaves of greenhouse plants [16]. However, in the current experiment, it was determined in a low concentration in the leaves of greenhouse plants during the whole period of observations (0.6–1.3 mg/g) (see Fig. 1, *i*).

So, the results of the investigation provide evidence that a definite stress effect of ecotol on the plants manifests itself only under temperature conditions that are optimal for vegetation. It is similar to a decrease in temperature in its consequences; most probably, it is necessary for passing to blossoming. This demonstrates the possibilities of blossoming regulation both by decreasing air temperature and by the treatment with ecotol. The treatment with ecotol is especially important in greenhouses where the plants from different ecological groups are grown simultaneously together, and a decrease in air temperature for the plants tolerant to cooling may kill the species that are more heat-loving species.

Under the conditions of low-temperature stress, the treatment with ecotol does not lead to additional stress response, but adaptation response is modified providing better physiological state of the plants when passing to dormancy, namely the conservation of the larger percentage of leaves, prevention of the destruction of carotenoids in them, and accumulation of anthocyanins, flavones and flavonols.

Most probably, this action was the reason for an increase in the vitality of bulbs of treated plants and promoted their sprouting, which started at the next vegetation period of 2019 two weeks earlier than in untreated plants. The plants from these bulbs passed to blossoming in the second decade of July, which was much earlier than the terms averaged over many years (August). Investigation of the effect of ecotol on the generative organs of *B. grandis* is the future task.

The positive effect of ecotol on the physiological state of *B. grandis* under the conditions of low-temperature stress agrees with its similar effect on woody plants at increased temperatures and increased content of lead in soil [22], as well as on the spring wheat plants under the conditions of drought [23]. This is the evidence that the production of ecotol is promising as a complex of physiologically active components activating the adaptive possibilities of plants with the help of ecologically safe technology of processing the wastes from agriculture and municipal facilities (straw and leaves).

CONCLUSION

We investigated the effect of the product of straw processing (ecotol) on the dynamics of the parameters of the physiological state of *B. grandis* plants, namely the content of the sum of chlorophylls ($a + b$), carotenoids, anthocyanins, flavones and flavonols, as well as the ratios of chlorophylls a/b, chlorophylls ($a + b$)/carotenoids and chlorophylls ($a + b$)/anthocyanins in the leaves of *B. grandis* subsp. grandis plants introduced in West Siberia (Novosibirsk), with a decrease in air temperature at the end of vegetation period under the conditions of greenhouse and outdoors.

After the treatment with ecotol, the adaptive response of the plants to the low-temperature action was modified, which provided a higher physiological status of plants during the transition to dormancy in comparison with untreated plants. The treated plants conserved more leaves with a higher content of the sum of chlorophylls ($a + b$), carotenoids, anthocyanins and O-glycosides of flavonols, in particular isoquercitrin. These properties, along with the previously described positive effects on the physical state of plants during adaptation to high-temperature stress and technogenic pollution, characterize ecotol as a complex preparation that causes an increase in the adaptivity potential of plants.

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REFERENCES

1 Gu C., Peng C. I., Turland N. J., Begoniaceae, in: Flora of China, Vol. 13 (Clusiaceae through Araliaceae), Z. Y. Wu, P. H. Raven, D. Y. Hong (Eds), Beijing: Science Press, and St. Louis: Missouri Botanical Garden Press, 2007. P. 153–207.

2 Li X., Tian D., Li C., Liu K., Li X., Nakata M., The history, culture, utilization, germplasm diversity and research advances of *Begonia grandis* Dry, *Botanical Research*, 2014, Vol. 3, P. 117–139 (In Chinese).

3 Chalker-Scott L., Enviromental significance of anthocyanins in plant stress responses, *J. Photochem. Photobiol. B*, 1999, Vol. 70, No. 1, P. 1–9.

4 Merzlyak M. N., Chivkunova O. B., Solovchenko A. E., Naqvi K. R., Light absorption by anthocyanins in juvenile, stressed, and senescing leaves, *J. Exp. Bot.*, 2008, Vol. 59, No. 14, P. 3903–3911.

5 Chudinova L. A., Orlova N. V., Physiology of Plant Resistance. A textbook to the special course [in Russian], Perm: the Perm State University, 2006. 124 p. [Electronic resource]. URL: http://www.psu.ru/files/docs/fakultety/bio/fiziologiya-ustojchivosti-rastenij.pdf (Accessed 01.06.2020).

6 Nenko N. I., Kiseleva G. K., Ulyanovskaya E. V., Karavaeva A. V., Physiological-biochemical evaluation of the resistance of apple trees to the stressors of winter and summer periods [in Russian], *Izv. Vuzov. Prikladnaya Khimiya i Biotekhnologiya*, 2016, Vol. 6, No. 3, P. 65–71.

7 Kancheva R., Borisova D., Georgiev G., Chlorophyll assessment and stress detection from vegetation optical properties, *Ecological Engineering and Environment Protection*, 2014, Vol. 1, P. 34–43.

8 Liu C., Liu Y., Lu Y., Liao Y., Nie J., Yuan X., Chen F., Use of a leaf chlorophyll content index to improve the prediction of above-ground biomass and productivity, *Peer J.*, 2019, No. 1, e6240. URL: https://doi.org/10.7717/peerj.6240 (Accessed 25.11.2019).

9 Domonkos I., Kis M., Gombos Z., Ughy B., Carotenoids, versatile components of oxygenic photosynthesis, *Prog. Lipid Res.*, 2013, Vol. 52, No. 4, P. 539–561.

10 Haldimann P., Low growth temperature-induced changes to pigment composition and photosynthesis in Zea mays genotypes differing in chilling sensitivity, *Plant Cell Environ.*, 1998, Vol. 21, P. 200–208.

11 Solecka D., Kacperska A., Boudet A. M., Phenylpropanoid and anthocyanin changes in low-temperature treated winter oilseed rape leaves, *Plant Physiol. Biochem.*, 1999, Vol. 37, No. 6, P. 491–496.

12 Tian J., Han Z., Zhang L., Song T., Zhang J., Li J., Yao Y., Induction of anthocyanin accumulation in crabapple (*Malus* cv.) leaves by low temperatures, *Hort Science*, 2015, Vol. 50, No. 5, P. 640–649.

13 Gould K. S., Lister C., Flavonoid Functions in Plants, in: Flavonoids: Chemistry, Biochemistry and Applications, Ш. M. Andersen, K. R. Markham (Eds.), New-York, *etc.*: Taylor & Francis Group, 2006. P. 397–443.

14 Di Ferdinando M., Brunetti C., Fini A., Tattini M., Flavonoids as Antioxidants in Plants under Abiotic Stresses, in: Abiotic Stress Responses in Plants. Metabolism, Productivity and Sustainability, P. Ahmad, M. N. V. Prasad (Eds.), New York, *etc.*: Springer, 2012. P. 159–180.

15 Chupakhina G. N., Maslennikov P. V., Skrypnik L. N., Natural Antioxidants (Ecological Aspect) [in Russian], Kaliningrad: Publishing House of I. Kant Baltic Federal University, 2011. 111 p.

16 Karpova E. A., Fershalova T. D., Petruk A. A., Flavonoids in adaptation of Begonia grandis Dryander subsp. grandis introduced in West Siberia (Novosibirsk), *Journal of Stress Physiology & Biochemistry*, 2016, Vol. 12, No. 3, P. 44–56.

17 Karpova E. A., Fershalova T. D., Dynamics of the content of pigments in the leaves of *Begonia grandis* Dryander subsp. grandis during introduction in West Siberia (Novosibirsk) [in Russian], *Vesnt. Tom. Gos. Un-ta. Biologiya*, 2016, No. 1 (33), P. 140–158.

18 Zhang J., Chen Y., Li B., Wang M. Chemical constituents of *Begonia evansiana* Andr., *Zhongguo Zhong Yao Za Zhi*, 1997. Vol. 22, No. 5. P. 295–296, 320 (in Chinese).

19 Iwashina T., Saito Y., Peng C.-I., Yokota M., Kokobugata G., Foliar flavonoids from two *Begonia* species in Japan, *Bull. Natl. Mus. Nat. Sci., Ser. B.*, 2008, Vol. 34, No. 4.,P. 175–181.

20 Joshi K. R., Devkota H. P., Nakamura T., Watanabe T., Yahara S., Chemical constituents and their DPPH radical scavenging activity of Nepalese crude drug *Begonia picta*, *Rec. Nat. Prod.*, 2015, Vol. 9, No. 3, P. 446–450.

21 Lebedev G. V., Sabinina E. D., Lebedeva N. G., Bubenchikova Z. I., Abramenkova N. A., Zhiznevskaya G. Ya., Prokhorov S. F., Vostrov I. S., Pleshkov D. A., Leonova S. S., Demidov A. S., Ecotols. Production and Application [in Russian], Moscow: FGUP VIMI, 2004. 116 p.

22 Fitiskina N. V., Polyfunctionality of the Action of Ecotol on Tree Young Plants under Unfavourable Environmental Conditions [in Russian], Candidate's Thesis in Biology, Moscow, 2017. 159 p.

23 Fitiskina N. V., Kartashova E. R., Yurina T. P., Oleskin A. V., Kurchenko V. P., Kalabin G. A., Kudoyarova G. R., Arkhipova T. N., Physiologically active compounds in the products of microbiological decomposition of the straw of cereals and their effect on the growth and crop productivity of plants under unfavourable conditions [in Russian], *Ekobiotekh.*, 2018, Vol. 1, No. 3, P. 161–176.

24 Luchitskaya I. O., Belaya N. I., Arbuzov S. A., Climate of Novosibirsk and Its Changes [in Russian], Novosibirsk: Publishing House of SB RAS, 2014. 224 p.

25 Fershalova T. D., Baykova E. V., Introduction of Begonias in Greenhouses and Indoors, Novosibirsk: Geo, 2013. 157 p. (in Russ.).

26 Lichtenthaler H. K., Buschmann C., Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy, in: Current Protocols in Food Analytical Chemistry [Electronic resource]. F4.3.1-F4.3.8. New York: John Wiley & Sons, 2001. URL: http://www.thyssen-web.de/assets/files/fd_documents/sp_buche/uv_vis_pigmente.pdf (Accessed 25.11.2019).

27 Horbowicz M., Mioduszewska H., Koczkodaj D., Saniewski M., The effect of methyl jasmonate and phenolic acids on growth of seedlings and accumulation of anthocyanins in common buckwheat (*Fagopyrum esculentum* Moench), *Acta Agrobot.*, 2009, Vol. 62, No. 1, P. 49–56.

28 Sims D. A., Gamon J. A., Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages, *Remote Sens. Environ.*, 2002, Vol. 81, P. 337–354.

29 Zaytsev G. N., Mathematical Analysis of Biological Data [in Russian], Moscow: Nauka, 1991. 184 p.

30 Wojciechowska R., Kalisz A., Sękara A., Nosek M., Cebula S., Miszalski Z., Kunicki E., Grabowska A., Alterations in chlorophyll a fluorescence and pigments concentration in the leaves of cauliflower and broccoli transplants subjected to chilling, *Not. Bot. Horti Agrobot. Cluj-Napoca*, 2016, Vol. 44, No. 1, P. 17–24.

31 Othman R., Mohd Zaifuddin F. A., Hassan N. M., Carotenoid biosynthesis regulatory mechanisms in plants, *J. Oleo. Sci.*, 2014, Vol. 63, No. 8, P. 753–760.

32 Uarrota V. G., Stefen D. L. V., Leolato L. S., Gindri D. M., Nerling D., Revisiting Carotenoids and Their Role in Plant Stress Responses: From Biosynthesis to Plant Signaling Mechanisms During Stress, in: Antioxidants and Antioxidant Enzymes in Higher Plants, D. Gupta, J. Palma, F. Corpas (Eds.), Cham: Springer, 2018, P. 207–232.

33 Babenko L. M., Kosakivska I. V., Akimov Yu. A., Klymchuk D. O., Skaternya T. D., Effect of temperature stresses on pigment content, lipoxygenase activity and cell ultrastructure of winter wheat seedlings, *Genet. Plant Physiol.*, 2014, Vol. 4, No. 1–2, P. 117–125.

34 Kalisz A., Jezdinskə A., Pokluda R., Sękara A., Grabowska A., Gil J., Impacts of chilling on photosynthesis and chlorophyll pigment content in juvenile basil cultivars, *Hortic. Environ. Biotechnol.*, 2016, Vol. 57, No. 4, P. 330–339.

35 Oksanen E., Freiwald V., Prozherina N., Rousi M., Photosynthesis of birch (*Betula pendula* Roth) is sensitive to spring-time frost and ozone, *Can. J. For. Res.*, 2011, Vol. 35, P. 703–712.

36 Pyatygin S. S., Stress in plants: a physiological approach [in Russian], *Zhurn. Obshchey Biologii*, 2008, Vol. 69, No. 4,