doi: 10.26114/pja.iung.429.2021.44.06

Polish Journal of Agronomy
2021, 44, 39–43

The effectiveness of using a preparation containing amino acids in the cultivation of strawberries under thermal stress conditions

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Abstract. The research was carried out in 2018 at the experimental station of the University of Life Sciences in Poznań. The plants of the strawberry variety Albion were exposed to sub-zero temperatures in the white bud phase and in full bloom. The possibility of mitigating the results of strawberries subjected to thermal stress was investigated using the Terra-Sorb complex biostimulator with a high content of amino acids. The intensity of flowering, the number, weight and area of strawberry leaves, the number of fruits on a bush and their quality parameters such as fruit weight, extract content and firmness were analysed. It was established that the impact of the sub-zero temperature on strawberry shrubs, regardless of the development phase of the plant, the number of inflorescences and healthy flowers was reduced significantly, the number of fruits, their average weight and firmness decreased. The use of the biostimulator on plants exposed to temperatures from -2 to -3 °C contributed to increasing the number of healthy strawberry flowers by several percentage points, the number of fruits per plant and increased their average weight significantly. The extract content in strawberries remained unchanged.

Key words: strawberry, negative temperatures, Terra-Sorb complex, strawberry yield and fruit quality

INTRODUCTION

Plants growing in their natural conditions are repeatedly exposed to the action of noxious stressors. The decrease in the yield of plants growing in such conditions may be as high as 50% (Atkinson, Urwin, 2012). The most common abiotic factors causing stress in plants include lack of water or nutrients, inadequate temperature or salinity. Thermal stress occurs as a result of the impact of temperature significantly different from an optimal one for a given species. Lowering the temperature below 0 ºC causes ice to form in plant tissues. That leads to damage to cell membranes and the outflow of metabolites, and finally to cell death.

The natural conditions of Poland allow for cultivating strawberries (*Fragaria ananassa*) practically throughout the country. The fruit of this species possesses nutritional and health-promoting properties. This stems from their high content of minerals, digestible sugars, a number of vitamins and antioxidants (Skupień, 2003; Rochalska et al., 2011). Strawberry, as a groundcover plant, is not particularly resistant to freezing temperatures. Most of new strawberry varieties with plentiful yield are not resistant to the conditions prevalent during the winter season. The risk of damage increases along with the intensification of climate conditions variability, especially in the absence of snow cover. Then, a rapid loss of water occurs, and the plants dry up quickly. In Poland, in conditions of repeated warm and snowless winters, spring frosts – periodic temperature drops below 0 ºC – are of more practical importance. During the period of flowering, spring frosts can cause significant damage to flowers, which in turn leads to a reduction in yield, sometimes by 50%. The sensitivity of strawberries to frost depends on its development phase. In the white bud stage, damage appears with a temperature drop, depending on the variety, from -2 to -3 ºC (Żurawicz, 2005). The more developed the flowers are, the more their sensitivity to low temperatures increases. When fully developed, they can be damaged when the temperature drops from -1.8 to 2.1 ºC (Bogunovic et al., 2015).

In practice, biostimulators are used in order to mitigate the effects of the impact of stressors on plants. These are substances containing mainly biologically active substances such as hormones, enzymes, microelements and amino acids. The purpose of their application is, among others, to stimulate plants by intensifying their metabolic processes to resist stress factors. It is possible thanks to the better development of the root mass of plants treated with biostimulants (Conselvan et al., 2017), which results in an easier nutrient uptake by them (Derkowska et al., 2015). This im-

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proves the vegetative growth of the aerial part of plants (Ennab, 2016) and also their general condition, which translates into an improvement of their tolerance to stress factors, including those related to low soil temperature and water deficiency (Filipczak et al., 2016). One example of such substances is the Terra-Sorb complex containing 20% of amino acids obtained by enzymatic hydrolysis of plant proteins. Moreover, it consists of N_{org} – 5.15%, organic matter – 25% and small amounts of micronutrients (Mg – 0.8%, Fe – 1%, Mn - 0.1%, Zn – 0.1%, Mo – 0.001%).

The aim of the study was to assess the effectiveness of the TerraSorb complex biostimulator containing amino acids in strawberry cultivation subjected to thermal stress.

MATERIALS AND METHODS

The study was carried out in 2018 at the Department of Dendrology, Orcharding and Nursery at the University of Life Sciences in Poznań. The subject of the study was one-year-old strawberry plants of Albion variety, planted in plastic containers with a capacity of 8 litres. The experiment covered 5 combinations: combination 1 – plants grown at optimal temperature (under control); combination 2 – plants in the white bud phase were exposed to a temperature of – 3 ºC; combination 3 – the same as in combination 2 with the addition of a biostimulator; combination 4 – plants in the full bloom phase were exposed to the temperature of – 2 ºC; combination 5 – the same as in combination 4 with the addition of a biostimulator. Each combination was represented by 6 replicates (containers with plants). The strawberry plants were exposed to the effects of low temperature in a controlled refrigerator-freezer in which the temperature drop proceeded at a rate of 5 º C per hour until the required level was reached. After a two-hour exposure at a given temperature, it was raised, retaining a growth rate of 5 º C per hour. Plants in the white bud phase were exposed to thermal stress in the first half of May, and in the case of those in the full bloom phase – after 10 days. When the process was completed, the containers with the plants were placed on an experimental plot.

In selected combinations, the Terra-Sorb complex biostimulator containing amino acids was applied. The day after the containers were taken out of the refrigerator, the substance was applied to the plants subjected to sub-zero temperature using a hand sprayer until the leaf blades were completely covered with the liquid. The next two sprayings were performed with an interval of 14 days. The total dose of the substance according to the manufacturer's instructions was 1.5 litre per 400 litres of water (after conversion). The protection was carried out in accordance with the recommendations of the fruit plant protection program and agrotechnical treatments were limited to periodic weeding and watering plants. No additional fertilization was applied.

During the experiment, the following measurements and observations were carried out: flowering intensity (the number of inflorescences and flowers per one plant), the strength of vegetative growth (number of leaves per plant, the average weight of a single leaf and its area), the number of fruits from a single plant and some of their quality parameters (average weight, firmness and extract content)

Flowering intensity was assessed 7 days after spraying the plants with the biostimulator. The total number of healthy inflorescences and flowers on the plant was counted for this purpose, excluding damaged flowers (with dark brown colour in the middle part).

The fruits were harvested three times during the growing season as they ripened. 5 fruits (a total of 30 from the combination) were collected from one replicate (container). The quality parameters of the fruit were assessed immediately after the first harvest that took place in mid-June. For this purpose, they were weighed with an accuracy of 0.1 g. Firmness was measured with a firmness gauge ("Fruit Pressure Tester mod. 302" by Facchini) mounted on a tripod, using a 1.5 mm diameter mandrel (the so-called Magness-Taylor test). Measurement values are expressed in (g mm^{-2}). The extract content was measured with an electronic refractometer DR 301-95 KRÜSS Optronic GmbH. Measurement values are expressed in% º Brix.

At the end of the growing season, the total number of (compound) leaves per plant was counted. Next, the leaves collected from each plant were weighed with the accuracy of 0.1 g and the average weight of one leaf was calculated. Thereafter, 5 leaves from the replicate (30 leaves from the combination) were collected and scanned. The leaf area was calculated with the DigiShape 1.9 computer program.

The results of measurements and observations were statistically processed using the one-way analysis of variance separately for each tested feature using Duncan's test at the significance level of $\alpha = 0.05$

RESULTS AND DISCUSSION

Two-hour exposure of strawberry plants to sub-zero temperature affected all the parameters being tested. Both the temperature level and the plant's development stage were significant. As a result of the sub-zero temperature, both the number of inflorescences and flowers on plants decreased. For example, the average number of healthy flowers on the plants exposed to the temperature of -2 °C in the full flowering stage (24.6 pcs.) was lower by over 26% than in the control combination (33.60 pcs.) (Table 1). At a lower temperature (-3 °C) in the white bud stage, the difference was smaller (by approx. 23%). Strawberries exposed to sub-zero temperature had worse growth. In the combination with the temperature - 3 °C, the average weight of the leaf was lower by about 25% and their area by about 22% compared to the control combination (Table 2). At the

Table 1. The effect of sub-zero temperature and biostimulant on the flowering intensity of strawberries.

No.	Combinations	Biostimulant	Number of inflorescences	Number of flowers
1	Control	-	6.25 b	33.60 c
2	-3 °C (white bud phase)	-	4.00 a	25.75 a
3		+	4.70 ab	28.80 b
4	-2 °C (full bloom phase)	-	4.50 ab	24.60 a
5		+	5.25 ab	28.70 b

Means marked with the same letters do not differ significantly at α = 0.05
+ with the use of a biostimulant; - without biostimulant

Table 2. The effect of sub-zero temperature and biostimulant on the strawberry leaf parameters.

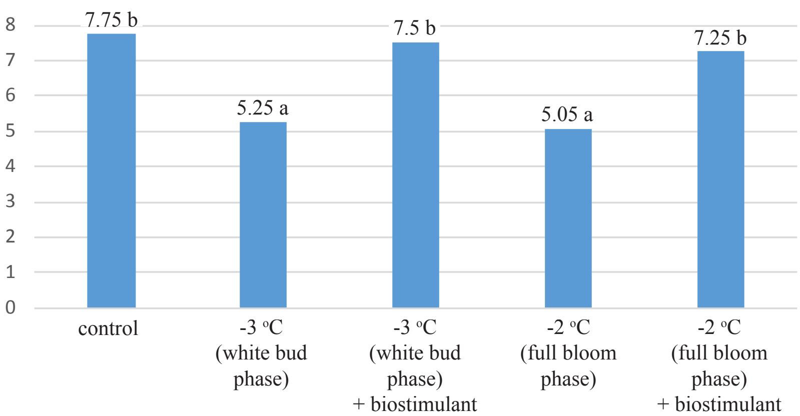
No.	Combinations	Biostimulant	The number of leaves per plant	The average weight of one leaf [g]	The surface of one leaf [cm^2]
1	Control	-	24.00 b	4.27 b	62.22 b
2	-3 °C (white bud phase)	-	20.25 a	3.18 a	48.44 a
3		+	23.95 b	3.57 ab	56.38 b
4	-2 °C (full bloom phase)	-	28.00 c	3.95 ab	58.57 b
5		+	31.75 d	3.84 ab	63.31 b

Means marked with the same letters do not differ significantly at α = 0.05

Table 3. The effect of sub-zero temperature and biostimulant on the quality parameters of strawberry fruit.

No.	Combinations	Biostimulant	Fruit weight [g]	Extract content [%° Brix]	Firmness [g mm^{-2}]
1	Control	-	12.51 c	8.04 a	175 c
2	-3 °C (white bud phase)	-	8.54 b	7.86 a	156 a
3		+	11.68 c	7.70 a	161 b
4	-2 °C (full bloom phase)	-	7.00 a	9.41 b	163 b
5		+	12.42 c	9.45 b	159 ab

Means marked with the same letters do not differ significantly at α = 0.05



Means marked with the same letters do not differ significantly at α = 0.05

Figure 1. The effect of sub-zero temperature on the number of strawberry fruit (per plant).

temperature of -2 °C, the differences in leaf weight and their area in these combinations were smaller (by 8% and 6%, respectively). Also, Klamkowski et al. (2008) report a decrease in the number, weight and area of strawberry leaves growing under stressful conditions.

The lower number of healthy flowers on the tested plants exposed to sub-zero temperatures (Table 1) resulted in a significantly smaller number of fruits as compared to control plants. For example, the average amount of fruit from plants exposed to the temperature of - 2 °C in the full bloom stage (5.05 pcs.) was lower by 34.8% than that of plants growing under optimal conditions (7.75 pcs.) (Fig. 1). According to Klamkowski et al. (2013), the yield of strawberries growing under stressful conditions may be even 45% lower than that of shrubs growing under optimal conditions. Starck (2002) explains such a decrease in yield by the limitation of the plant growth process.

To achieve profitability of production, not only the amount of produced fruit is vital, but also its appropriate quality. The features determining the quality of strawberries are usually their weight, firmness and extract content. From among the listed qualitative characteristics, the thermal stress in the experiment affected negatively the firmness and average fruit weight. In this case, the development stage of the plant was relevant as well. Under the influence of the temperature of -2 °C in the full bloom stage, the average fruit weight (7.00 g) was lower by over 40% than in the control combination (12.51 g) (Table 3). As a result of applying the temperature of -3 °C in the white bud stage, the difference with regard to control combinations did not exceed 32%.

An important feature which determines the quality of strawberries is their firmness (hardness). They are particularly sensitive to mechanical damage due to the specific structure of their tissues and high water content (Garcia et al. 2001). Fruits with high firmness endure transport better, remain shelf-stable in trade for a longer time, and withstand storage better. In the experiment, fruit firmness, depending on the combination, fluctuated from 156 g mm^{-2} to a maximum of 175 g mm^{-2} (control combination) (Table 3). These values were slightly lower than those obtained in the experiment by Chełpiński et al. (2010) which ranged from 160 to 180 g mm^{-2}.

The content of extract in the fruit from strawberry plants growing at an optimal temperature (8.04 %º Brix) was significantly lower than in the fruit from strawberries kept at -2 °C in the full bloom stage (9.41%º Brix) (Table 3). This could result from the different size of the fruits, which, apart from the climatic conditions and the variety, may modify the quality parameters of strawberries. The fruits with the highest weight were obtained from the control combination (Table 3, which, due to the dilution effect, could have resulted in a lower extract content (Table 3). The research by De Salvador et al. (2006) demonstrated a strong positive correlation between fruit size and its weight, and negative correlation between fruit weight and extract content as well as firmness. Similarly, in the experiment by Zydlik and Zydlik (2015), the content of refractometric extract in large fruits was lower than in small fruits.

The analysis of the obtained results shows that spraying strawberry plants exposed to sub-zero temperatures with the biostimulator had a positive effect on the flowering and the number of leaves and fruit per plant. In the combinations with the temperature of -2 and -3 °C, plants treated with Terra-Sorb complex had between ten and twenty percentage points more healthy flowers than in combinations without this treatment (Table 1).

The large assimilation surface of the leaves, apart from ensuring an optimal course of the photosynthesis process, facilitates the wintering of plants. It protects flower buds from freezing and allows plants to accumulate an appropriate amount of reserve substances. The effect of the biostimulator on the growth of strawberry plants was relatively small. In the plants exposed to sub-zero temperatures and sprayed with Terra-Sorb complex, only the number of leaves per plant was higher by 13% (-2 °C) and 18% (-3 °C) compared to the combinations without spraying (Table 2). In combination with the temperature of -3 °C, the area of one leaf also increased significantly. Similar significant differences were not found in the case of the average weight of one leaf and its area.

The treatment of strawberry plants exposed to sub-zero temperatures with a biostimulator, with more healthy flowers in these combinations, resulted in a greater quantity of fruit per plant - an average increase from 5 to 7 pieces (Figure 1). According to the authors, it was possible due to the beneficial effects of biologically active amino acids in the Terra-Sorb complex preparation. Applied in the form of foliar spraying, especially under stressful conditions, they are easily absorbed by plants that do not have to expend energy for their synthesis. In addition, the preparation supplies the soil with a number of micro-elements, which contributes to the improvement of plant growth and development and has a positive effect on plant yield.

The experiment proved that as a result of using the biostimulator, the weight of a single fruit increased significantly, which may contribute to a higher yield per area unit. In the combinations with the temperature of -2 °C in the fully flowering phase, with the application of Terra-Sorb complex preparation, the average fruit weight (12.24 g) was significantly higher than in the plants not treated with this complex (7.00 g) and comparable to the average fruit weight in the control combinations (12.51 g) (Table 3). The results of previous experiments confirm the positive effect of Terra-Sorb complex on the increase in the average weight of strawberry fruits (Bogunovic et al., 2015; Zydlik, Zydlik, 2015, 2016).

Among the fruit quality parameters tested in the experiment, preparation Terra-Sorb complex modified the average fruit weight to the greatest extent. The fruit from the plants exposed to sub-zero temperatures, both those treated with the biostimulator and the ones without this treatment, had comparable extract content (Table 3). A similar conclusion was reached in the previous experience by the authors of the study (Zydlik, Zydlik, 2016), in which the biopreparations used in strawberry cultivation did not significantly change the content of the extract in the fruit. EI-Boray et al. (2015) are of a different opinion. In their experiment, some fruit quality parameters were improved as a result of the foliar treatment of plants with preparations containing natural ingredients.

CONCLUSIONS

1. As a result of the impact of the sub-zero temperature on strawberry shrubs, regardless of the development phase of the plant, the number of inflorescences and healthy flowers was reduced significantly, the number of fruits, their average weight and firmness decreased.
2. The number of healthy flowers on strawberry shrubs exposed to sub-zero temperatures in treatments with Terra--Sorb complex biostimulator was several percent higher than on the shrubs without this treatment.
3. In plants exposed to a sub-zero temperature and sprayed with Terra-Sorb Complex, in each development phase, the number of leaves increased significantly.
4. The use of the biostimulator on plants exposed to sub-zero temperatures increased the number of fruits per plant and significantly increased their weight.
5. No significant influence of Terra-Sorb complex was found with regard to such quality parameters of fruit like extract content.

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received – 7 July 2020
revised – 11, December 2020
accepted – 28 January 2021