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Biostimulant usage for preserving strawberries to climate damages

I. Bogunovic[1], B. Duralija[2], J. Gadze[2], I. Kisic[1]

[1]*Department of General Agronomy, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia*
[2]*Department of Pomology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia*

Abstract

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Climate changes affect horticultural production through the occurrence of late spring frosts. Therefore plant management is gaining more importance with the aim of improving plant condition. The research deals with the biostimulator containing amino-acids of animal origin (porcine blood) which can be obtained by chemical and/or enzymatic hydrolysis of an organic matrix. During vegetation period different cultivars of strawberries were included in the experiment: Asia, Alba and Clery. The effects of biostimulator on resistance to frost, yields and pomological characteristics were studied at three treatments (0.5 , 1.0 and 1.5 g/plant, and control 0 g/plant). The results showed that all investigated cultivars had a positive response to biostimulant application regarding the frost resistance. Natural frost resistance was the strongest for cv. Clery. Significantly lowest percentage of damage was recorded for cv. Alba at 1.5 g and cvs Clery and Alba at 0.5 g. In the case of late spring frosts and other climatic hazards for growing outdoors cvs Clery and Alba are recommended. All cultivars recorded decreased yields. The difference in yields between cultivars was a consequence of frost damages.

Keywords: *Fragaria* × *ananassa*; porcine blood; frost; yield

Climate change impacts are major threat for agricultural production. Two of the most important climatic factors, precipitation and air temperature, have undergone great changes during the last two decades in Central Croatia (Bogunovic, Kisic 2013). Despite an overall increase in mean daily temperatures, it is expected that there will be an increase in the number of devastating spring frosts due to the erratic weather patterns associated with global climate change (Gu et al. 2008). Due to several years of exploitation strawberries are often damaged by exposure and sensitivity to low temperatures. The most dangerous condition is the appearance of late spring frost, when strawberry plants emerge from dormancy. Because of the complex changes in physiology, metabolism, structure, and water content associated with cold acclimation, plants that are actively growing, flowering, or breaking dormancy typically have little to no frost tolerance (Sakai, Larcher 1987; Wisniewski et al. 2003). In the flowering stage open flowers freeze at –2°C and primary fruits at –1.5°C (Duralija 2004). Climatic adaptability is conditioned by properties with more complex hereditary basis which are very difficult to distinguish (Sherman, Beckman 2003). Also, mechanisms involved in frost damage and hardiness have turned out to be exceedingly complex and many aspects remain uncertain (Guy 1990; Thomashow 1999). However, the majority of the adverse effects are caused

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by inadequate condition of plants during the critical phenophases.

In the last decade, a great number of products known as biostimulants that are applied to crops appeared on the market (Boehme et al. 2004). Substances with biological origin have been used to avoid or counteract abiotic or biotic stress in horticulture. Many of these materials are natural products without chemicals or plant growth regulators (Russo, Berlyn 1991), and according to Crouch et al. (1992) can be classified into three major groups on the basis of their source and content: humic substances, hormone containing products and amino-acid containing product. The third group of biostimulants consists of mixtures of peptides and free amino acids which can be obtained by chemical and/or enzymatic hydrolysis of an organic matrix of plant or animal origin and their composition can be highly variable (Maini 2006). Biostimulants usage has become a common practice in sustainable agriculture, because their application reduces fertilizers and other chemical compound application in agriculture (Russo, Berlyn 1991). Some studies already investigated positive effects of biostimulants on plants. These studies mostly investigated biostimulant influence on plant growing, rooting, biomass of newly formed roots, early flowering and fruiting (Marfa et al. 2008) and thermal stress (Polo et al. 2006), but there is a small number of those that had experiments including ecological conditions of continental Croatia.

The main focus of this paper relies on the biostimulant containing amino-acids of animal origin (porcine blood). The effects of biostimulator on strawberry yields, pomological characteristics and resistance to frost in flowering stage were studied. This paper will try to answer which method of plant management is the most convenient for adaptation of horticultural practices to climate-induced damages. Accordingly, the optimal cultivar and biostimulant dose for maximizing strawberry resistance to frost, and ensuring stabile and high yields will be determined.

MATERIAL AND METHODS

Studies were conducted in the year 2011 at the commercial strawberry production fields located in Kupinecki Kraljevec near Zagreb (45°69'N, 15°87'E, 125 m a.s.l.). Cultivation followed good farming practices and the plants were watered through the fertirrigation each day. Experiment was established on the open field at two-rows with distance of 1.1 m on raised bed under white polyethylene mulch. The orientation of rows was in the N-S direction. The strawberry plant spacing was 0.3 m between rows and 0.3 m between plants in the row. Fertilization and plant protection included utilization of regional recommendations.

Strawberry plants cvs Asia, Alba and Clery were planted in 2010. Biostimulant used in the research is made from porcine blood and contains all amino acids (Table 1). On each cultivar 160 plants were followed, 480 plants in total. For each treatment and cultivar the experiment was established in a split-block design with 4 replications which consisted of 10 plants each. Treatment groups were composed of 10 plants for each cultivar with dif-

Table 1. Chemical composition of porcine blood biostimulant

Total organic matter	92.0%	Total amino acids	84.83%	Leucine	10.99%
Total nitrogen	13.4%	Free amino acids	16.52%	Lysine	7.19%
Organic nitrogen	12.0%	Alanine	6.90%	Methionine	0.71%
Ammonia nitrogen	1.0%	Arginine	5.22%	Phenylalanine	5.93%
Nitric nitrogen	0.4%	Aspartic acid	9.93%	Serine	3.88%
Potassium	3.32%	Cysteine	2.25%	Threonine	2.47%
Phosphorous	0.17%	Glutamic acid	7.25%	Tryptophan	inappreciable
Calcium	0.43%	Glycine	4.06%	Tyrosine	1.92%
Magnesium	195 ppm*	Histidine	6.34%	Valine	6.79%
Iron	4,061 ppm*	Izoleucine	0.15%	Proline	2.84%

*1 ppm = 10^{-6}

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Table 2. Flowering and maturity phenophases of investigated strawberries cultivars in 2011

Cultivar	Duration of bloom			Duration of maturity		
	beginning of bloom	end of bloom	duration (days)	beginning of maturity	end of maturity	duration (days)
Asia	April 24	May 21	29	May 18	June 18	30
Alba	April 21	May 20	29	May 21	June 23	32
Clery	April 19	May 17	28	May 19	June 28	39

ferent biostimulant formulation applied in water solution at the root zone. Treatments were: 0.5, 1.0, 1.5 g/plant and control treatment (0 g/plant). Biostimulant application was performed in four times during the vegetation period: April 16, April 23, May 9 and May 18, 2011.

Resistance to frost, flowering and ripening dynamics was monitored. Times of flowering and maturity were determined by visual observation and recorded by dates. Number of injured flowers was determined by counting. Meteorological data were registered and used with the automatic weather station (Model 450 WatchDog Data Logger; Spectrum Technologies, Inc., East Plainfield, USA) that was set at the studied field. Fruit weight was measured and compared to 0 g using digital scales (Velleman VTBAL22; Velleman, Gavere, Belgium). The fruits were harvested at the optimum time of harvest regardless of the size and appearance. Yield potential was determined by counting and calculation. One-way ANOVA was carried out to test whether the investigated properties varied significantly. In case of significant variation, the *post-hoc* Duncan multiple range test was applied to separate mean values at $P \leq 0.05$. All statistical analyses were carried out with the SAS Institute 9.1.3 (SAS Institute Inc., Cary, USA).

RESULTS AND DISCUSSION

Results of the flowering phenophases are presented in Table 2. Flowering of all varieties began within 5 days. The earliest beginning of flowering was recorded for cv. Clery on April 19 and the latest for Asia on April 24. The earliest end of flowering (May 17) was recorded for cv. Clery, while the latest was recorded for cv. Asia on May 21. Cvs Asia and Clery achieved the longest duration of flowering. Cv. Asia reached the earliest beginning of maturity (May 18), while the latest was for cv. Alba (May 21). The longest maturation time was determined for cv. Clery (39 days) and the shortest for cv. Asia (30 days).

Resistance to frost

Meteorological data (Fig. 1) showed that the critical temperatures for freezing injuries of strawberries were expressed in period May 4–6, and ranged up to a minimum of –3.11°C. The degree of resistance to frost depends on the content of dry matter, sugars, amino acids and proteins (Glišić et al. 2005). Study results on peach indicated that the concentration of protein, amino acids and sugars in floral buds varied

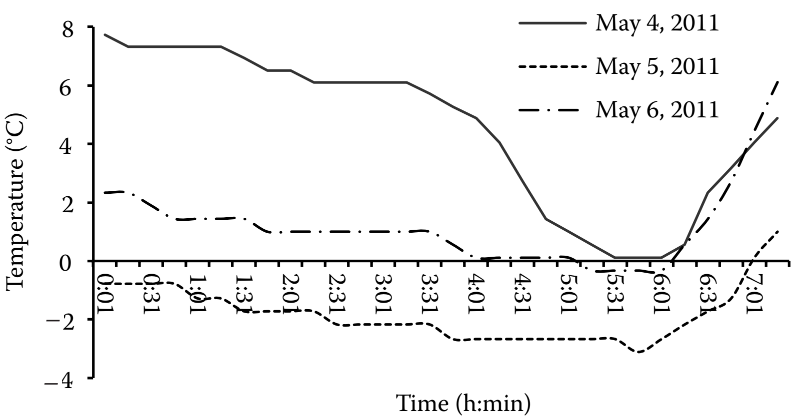

Fig. 1. Dynamics of night temperatures from weather station situated in field on the nights when frost was appeared

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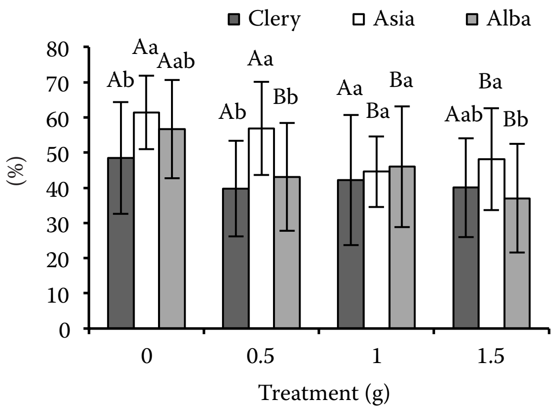

Fig. 2. Effect of root application of the biostimulant containing amino acids on percentage of frozen flowers
*different lowercase letters in the same column indicate significant differences in accordance with the Duncan's mean-separation test (P < 0.05). Different uppercase letters of the same cultivars between columns indicate significant differences in accordance with the Duncan's mean-separation test (P < 0.05). Hanging bars represent standard deviation

and were significantly greater in varieties resistant to frost (Lasheen, Chaplin 1971). Likewise, in apple study cold hardiness of flower buds was related to nutritive status in deblossomed apple trees, where the absence of fruit in the previous year increased cold hardiness of buds and their content of hydrophillic and acidic amino acids (Khanizadeh et al. 1992). Plants form amino acid with complex biochemical processes, which are necessary for the formation of proteins and vitamins. A large group of biostimulants whose proteins can be subjected to hydrolysis releases varying amounts of amino acids (Dimitrieva et al. 2003). By adding amino acids through biostimulators, this process accelerates because the plant adopts a free form, and the result is a faster metabolism of plants with increased resistance to stressful situations (Karlson 1993).

Results showed significant differences between treatments (F = 7.84, P < 0.0001), cultivars (F = 7.66, P = 0.0006) and the interaction between treatment and cultivar (F = 4.60, P < 0.0001) regarding frost resistance. *Post-hoc* test showed that all investigated cultivars had a positive response to biostimulant regarding frost resistance (Fig 2). Significant differences between treated plants and 0 g treatments were recorded on cvs Asia and Alba. All investigated cultivars (Asia, Alba and Clery) suffered from the highest percentage of damage in 0 g treatment with 61, 57 and 48%, respectively. Natural frost resistance was the strongest for cv. Clery (Fig 2). Significant difference in 0 g treatments was recorded between cvs Asia (as non-resistant with 61%) and Clery (the highest percentage of frost resistance with 48%). Significant differences in frost damage between cultivars were not recorded in 1 g treatment.

Treatments with 0.5 g and 1.5 g showed significant differences between cultivars (Fig. 2). Asia suffered from the highest damage at 0.5 g with 57% of frozen flowers and buds and 1.5 g with 48%. Significantly the smallest percentage of damage was found for cv. Alba at 1.5 g with 37% of frozen flowers and buds, and cvs Clery and Alba at 0.5 g (40% and 42%). When treatments were observed by varieties (Fig. 2), each variety recorded better results with lower percentage of frozen flowers when biostimulants were applied, but statistically justified usage of larger biostimulant doses was found only for Asia between 1 g and 1.5 g compared to 0.5 g and 0 g. According to Sagisaka and Araki (1983) arginine plays a role in protecting plants from freezing injuries. The arginine content of the biostimulant product used in this research was quite high (5.22%) and with a positive influence of proline on lessening the damage caused by abiotic stress (Filippini, Bonfiglioli 2005) can make a difference as the presented results showed in the previous chapter. Also, a study by Rajashekar et al. (1999) showed the possible involvement of glycine betaine on the cold tolerance in strawberry plants. All these studies showed some favorable effects of aminoacids and proteins on abiotic stress that can be held responsible for favorable effects on plants.

Yields

Crop yield is the most effective method of assessing economic benefits of production systems. The objective of planting any crop is to get the highest yield and the highest quality. In this experiment, strawberry harvesting stages lasted between 30–39 days, depending on cultivar. Basic statistical properties of the investigated cultivars are presented in Table 3. Mean fruit weight ranged from 14.86 g for cv. Clery to 22.06 g for cv. Asia. It is necessary to emphasize that all healthy fruits during harvest were collected regardless of their weight. Because of the great variation in size of sample population up to 846 fruits between cvs Clery and Asia it is necessary to compare coefficient of

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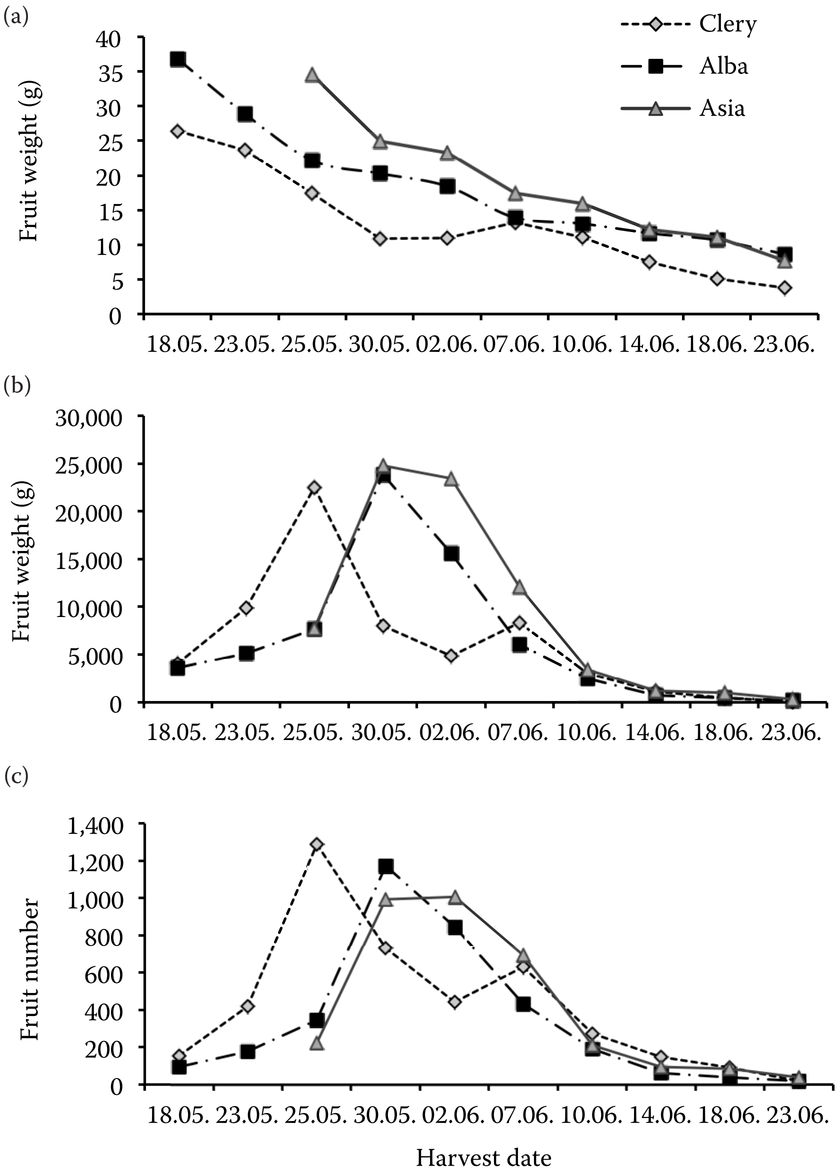

Fig. 3. Average fruit weight (a), yield dynamics (b) and fruit number per each harvest date (c)

variation (CV) instead of other parameters of variability. All variants showed relatively high CV (Table 3), but the highest variability was recorded for cv. Clery (56.69%), while the best homogeneity was recorded for cv. Asia (52.11%). Shape parameters, kurtosis and skewness, recorded values that indicated normal distribution (Table 3).

In addition to cultivar and planting date, harvest dynamics was directly affected by other factors such as mulch type, temperature and humidity at the time of ripening, irrigation, protection from disease and pests (Duralija et al. 2004), and this was the reason for considerable variations between different years (Stapleton et al. 2001). It is well known that the

Table 3. Basic statistical properties of fruit weight in investigated varieties (n = 160)

Cultivar	Mean	SD	Kurtosis	Skewness	Min.	Max.	Sum	Count	CL (95%)	CV
Clery	14.86	8.43	0.340	0.834	1	50	62,455	4,202	0.255	56.69
Alba	19.94	10.94	0.916	0.922	1	73	72,057	3,614	0.357	54.89
Asia	22.06	11.49	0.779	0.818	1	69	74,020	3,356	0.389	52.11

SD – standard deviation; CL – confidence level; CV – coefficient of variation; n – number of plants

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Table 4. Effect of root application of the biostimulant containing amino acids on yields, number of fruits and fruit weight (ANOVA)

Source	(*n*–1)	Yield (g/plant)	No. of fruits/plant	Fruit weight (g)
Treatment (T)	3	n.s.	n.s.	< 0.0001*
Cultivar (C)	2	0.0011*	< 0.0001*	< 0.0001*
T × C	6	n.s.	n.s.	< 0.0001*

*least significant difference, $P < 0.05$; n.s. – not significant; (*n*–1) – *n* minus 1 of degrees of freedom

first fruits have better quality and higher weight than the ones that mature later (Hortyński et al. 1991). In this study, similar situation can be observed in Fig. 3. All investigated varieties recorded a decrease of average fruit weight during the time scale, but the highest decrease of average fruit weight was recorded for cvs Clery and Asia from May 25 to May 30. The only deviation in fruit size was recorded for cv. Clery with an increase in average fruit weight between May 30 and June 10. By the end of May, air temperature was higher, plants grew faster, plant leaves, flowers and fruits increased more, and the strawberry yield also increased at the same time. From the beginning to the end of the harvest, the peak harvest period was at the end of May and beginning of June. Generally, each investigated variety had the highest yield potential at the third harvest date, approximately 7–12 days after the beginning of maturity. Only Asia achieved a shorter period from the first harvest to the max. recorded yield which was only five days after it began to decrease. Fruit number dynamics followed a pattern similar to yield dynamics.

Table 4 shows significant differences between cultivars in the yield (g/plant) and fruits number per plant. Also, fruits weight recorded a significant difference between treatments, cultivars and interaction treatment × cultivar (Table 4). The highest yields (Fig. 4a) were recorded for cvs Asia (462.6 g) and Alba (456.1 g) while significantly lower yield was recorded for cv. Clery (390.4 g). Cv. Asia achieved significantly the highest fruit weight with 22.1 g, while significantly the lightest fruit of 18.5 g was determined for cv. Clery (Fig. 4b). Strikić et al. (2011) recorded the fruit weight of cv. Clery of 15.4 g. Janković et al. (2010) in a three-year study recorded higher yields of cv. Clery (509.9 g). Fig. 4c shows that cv. Clery recorded significantly higher number of fruits per plant (26.2) in contrast to cvs Alba (22.9) and Asia (21.0). Kiprijanovski et al. (2010) in a two year research measured higher fruit numbers (39) and yield (539 g/plant) for cv. Alba, while fruits weight was similar to this research. Sylanaj and Shala (2008) measured similar fruit weight for cv. Alba (18.5 g), while the yields were 615.1 g per plant. Treatment with 0 g recorded significantly different fruit weight between all strawberry cultivars (Fig. 4d). The largest fruits were recorded for cv. Asia, and the smallest for cv. Clery. Also, fruit weights were considerably different in 0.5 g treatment, where cv. Asia had the largest fruits and cv. Clery had the smallest. Treatment with 1.5 g recorded indistinguishable fruit weight for cvs Clery and Asia, and 1 g treatment for cvs Clery and Alba.

Natural characteristics of cultivars and frost, together with different environmental conditions, were a key factor for large variations in yield, number of fruits per plant and average fruit weight. After a low-temperature period caused by late spring frost, plants ability to absorb minerals was impaired (Marfà et al. 2008) which could explain lower yields. According to Havis (1938), primary flowers are most susceptible to low temperatures. Apart from the probability that cv. Clery had naturally lower yields, it would probably be the reason for lower yield for cv. Clery. Although the results presented in Fig. 3 indicate that cv. Clery had the lowest percentage of damaged flowers and did not respond to the biostimulant, the problem was in the damage on the first flowers. Frost damage is highly dependent on the stage of development of flower buds (Simons, Doll 1976; Westwood 1993) and cv. Clery had the earliest flowering date and had the most flowers from all investigated cultivars exposed to frost. Thus, cv. Clery flowers should be at a more advanced stage of floral development with greater susceptibility to frost injury when exposed to frost than the other two investigated cultivars. Freezing injury in reproductive organs causes crop losses, since after blooming, no new flowers are produced during that season. As a consequence, fruit number and fruit weight are substantially reduced when primary

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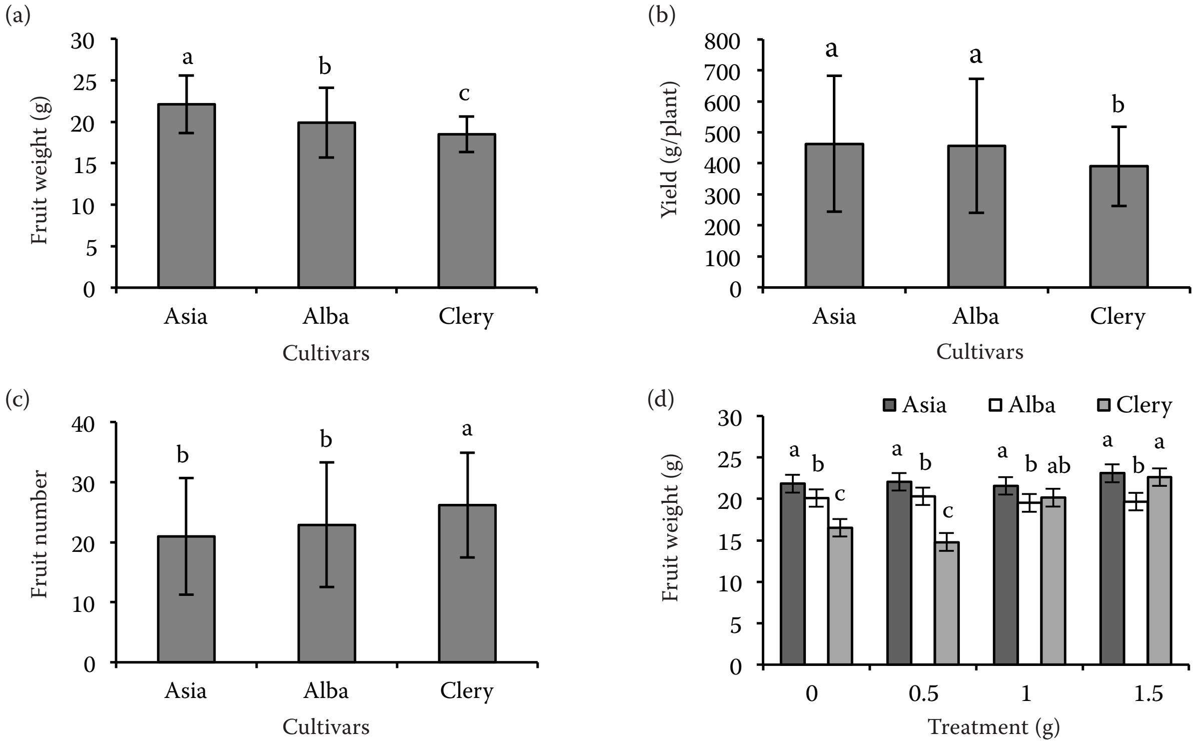

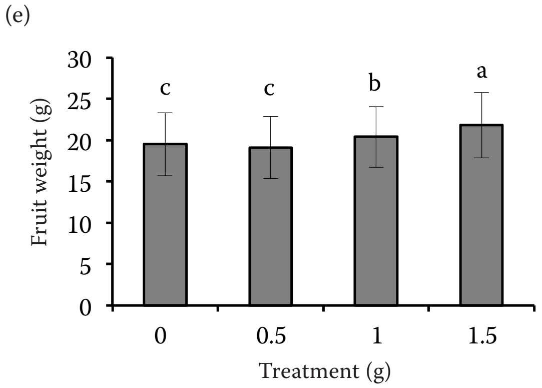

Fig. 4. Results from ANOVA and interaction test: (a) fruit weight per cultivar, (b) yield per cultivar, (c) fruit number per cultivar, (d) fruit weight at each cultivar per treatment, (e) fruit weight per treatment

different letters represent significant differences in accordance with the Duncan's mean-separation test ($P < 0.05$); hanging bars represent standard deviation

flowers are injured (Janick, Eggert 1968). Boyce and Strater (1984) also found that yield decreased by 30% when primary flowers of strawberry plants were removed during early bloom. In cv. Alba and especially cv. Asia cultivars inflorescences may escape early frost damage because they bloom later than cv. Clery. Biostimulant was certainly one of the major factors that improved resistance of strawberries to the negative temperatures. Also, biostimulant dose was evidently responsible for larger fruits, but it remains unclear how it affected yield of the studied cultivars.

CONCLUSION

Research showed that cv. Asia achieved the earliest beginning of maturity, while the latest was for Alba. The longest maturation time was determined for cv. Clery and the shortest for cv. Asia. All investigated cultivars had a positive response to biostimulant regarding frost resistance. The highest percentage of damage occurred in the 0 g treatment. Natural frost resistance was the strongest for cv. Clery. Treatments with 0.5 g and 1.5 g of biostimulant showed significant differences between cultivars and cv. Asia suffered from the highest damage percentage. Significantly the smallest percentage of damage was recorded for cv. Alba at 1.5 g and cvs Clery and Alba at 0.5 g treatment. Each cultivar recorded better results in treatments with biostimulant, but statistically justified usage of larger biostimulant doses was found only for cv. Asia between 1 g and 1.5 g, compared to 0.5 g and 0 g. In case of late spring frosts and other climatic hazards for growing outdoors in environmental conditions

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of continental Croatia, cvs Clery and Alba are recommended. Increasing biostimulant concentration is statistically justified for achieving heavier fruits. Asia variety is a cultivar with very large fruits. The lowest yield was recorded for cv. Clery, which had the highest number of fruits per plant. Apart from natural characteristics of each cultivar, the difference in yields between cultivars is a consequence of frost damages. Somewhat better results were recorded for cv. Alba and especially Asia cultivar, whose inflorescences may escape early frost damage because they bloom later than cv. Clery. Biostimulants increase resistance to frost and they are evidently responsible for larger fruits but in order to give a firmer conclusion for biostimulant influence on yields a longer period of investigation is necessary.

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Corresponding author:

Dr. Igor Bogunovic, University of Zagreb, Faculty of Agriculture, Department of General Agronomy, Svetosimunska 25, 10000 Zagreb, Croatia
phone: + 38 501 239 3815, fax: + 38 501 239 3981, e-mail: ibogunovic@agr.hr