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Opportunities to Improve Effectiveness of Pollination of Blueberry CV. 'Bluecrop'

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Abstract: In the production of blueberries, pollination is a key factor determining high quality yield. Problems with the process of pollination in blueberries occurs frequently. Lack of the effective pollination results in a lower yield and production of small fruits with low commercial value. The experiment described in this paper was carried out to test the effect of titanium organic complex (TOC) on the pollination and fertilization processes, seed formation, fruit setting and fruit development of blueberry in two consecutive years. The experiment consisted of two experimental factors: pollination type (self or open pollination) and application of TOC or lack of it. The presented study proves that TOC stimulates pollen adhesion and germination on the stigma, fruit setting, seed setting and fruit development. In self-pollinated flowers, the use of TOC increased the mass of each berry by 28% (in the first year) and 26% (in the second year), compared to non-treated plants. In open pollinated flowers, the application of TOC increased fruit mass by 38% (in the first year) and 29% (in the second year), compared to non-treated plants. Therefore, TOC may be treated as a substance stimulating the total fruit yield and mass of individual fruit.

Keywords: titanium organic complex; fruit set; number of seeds; fruit mass; fruit size; fertilization; self-compatibility; self-incompatibility; biostimulant

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1. Introduction

Annual worldwide production of berries continuously increases. The most important cultivated berry species are strawberry, blueberry, raspberry, Kamchatka berry and kiwi. In the modern cultivation of berries, not only the quantity, but also the quality of yield are important—the size and mass of the fruit, its chemical composition and storage capacity [1–6].

The genus *Vaccinium* includes 300–400 species growing in the Northern Hemisphere, from the tropics to the polar zone. Highbush blueberry (*Vaccinium corymbosum* L.) includes cultivars derived from several species of the genus *Vaccinium*. The blueberry cultivars that are currently grown were created thanks to the interspecies crosses of American highbush blueberries. The most important role in the creation of cultivars was played by four species of highbush blueberry: *Vaccinium corymbosum* L., *Vaccinium australe* Small., *Vaccinium arboreum* L. and *Vaccinium angustifolium* Ait. Among the cultivated species and hybrids of the genus Vaccinium, the highbush blueberry is the most noteworthy because of the highest yield, compared to the other species of this genus [7].

The first studies on the self-fertility of highbush blueberry started shortly after Coville introduced it to cultivation in 1921 [8]. The flower structure of the plants from the genus *Vaccinium*, e.g., the colour of the perianth, heterostyle, the distance between the holes in anthers and the shape of the pistil stigma, show that the species is adapted to cross pollination. The absence of pollinating insects or their low activity results in small number of pollen grains on stigma. During the pollination of *Vaccinium corymbosum* with its own pollen, incompatibility was observed, consisting of a very slow growth of the pollen tube from its own pollen. However, if cross pollination did not occur during the flowering, then at the end of the flowering, the ovule is fertilized with its own pollen [9,10]. Cross pollinated blueberries demonstrated accelerated fruit ripening and improved quality of yield because of bigger and better shaped fruits [10,11]. In cross pollinated plants, the share of early ripening fruits was higher by about 43%, the share of large fruits by 13%, and the share of small fruit was reduced by 66% in total yield. The higher share of bigger and better-formed fruits in the total yield results in a significant increase in the income of fruit producers [12]. Bieniasz [10] and Ehlenfeldt and Martin [13] reported that cross pollination had an effect on the increase in individual blueberry fruit mass by about 30% and acceleration of fruit maturation by 7–14 days. The most effective pollination of flowers occurred when there were several cultivars in the quarters. It was advantageous to plant the cultivars alternately along rows in accordance with the flight of the bees [14]. Lang and Danka [15] compared the self-pollination of blueberry cv. 'Sharpblue' and open pollination of this cultivar with the pollen of cv. 'Gulfcoast'. The authors showed that open pollination did not increase the percentage of fruit set, whereas fruit mass was higher (on average by 1 g) due to the greater number of seeds in the berry. In the case of self-pollination, fruit mass was 0.88 g. Fruits from open-pollinated plants ripened 10–14 days earlier than fruits from self-pollinated plants.

Plants need macro and micronutrients for proper growth and development. These elements are essential for plants, and their primary function is plant nutrition. Besides the essential elements, there are also elements defined as beneficial for plants. Beneficial elements are not essential for completing the life cycle of all plant species, but if applied at low concentrations, they have positive effects on plant growth, development and their tolerance to stress factors [16–18]. In plant nutrition, titanium is classified as a beneficial element. Research on its effect on plants started at the beginning of the twentieth century, and various titanium compounds were used in the studies [18,19]. Pais [20] discovered that titanium organic complex (TOC) was water soluble, stable, nontoxic and very effective in stimulating plant growth, development and consequently yield. Further research proved that TOC increased nutrient uptake, content of chlorophyll, growth of the phytomass, seed yield and improved the quantity and quality of fruits [21–24].

It has been proven that the TOC stimulates the processes of pollination and fertilization and as a consequence, seed setting and fruit development [25–27]. Dyki et al. [25] demonstrated that TOC enhanced pollen adhesion and germination on the stigma, seed setting and fruit development of tomato and cucumber. Bieniasz and Konieczny [27] proved that TOC stimulated pollination and ovule fertilization, formation of seeds and development of the apple fruits cv. 'Topaz'. Janas [26] and Radkowski et al. [28] in solanaceous plants and timothy obtained the higher number of seeds and their better quality after the use of TOC.

The hypothesis of our experiment assumed that foliar spraying of blueberry plants cv. 'Bluecrop' with TOC had a beneficial effect on pollen adhesion to the stigma and pollen germination on the stigma, thus improving seed setting and fruit development. The objective of our experiment was to test the effect of TOC on the pollination and fertilization processes, seed and fruit setting as well as diameter, length and mass of the blueberry fruit cv. 'Bluecrop'.

2. Materials and Methods

2.1. Plant Cultivation

A two-year experiment was conducted in the Experimental Station (geographical coordinates: h = 270 m, φ = 50°09′ N, λ = 19°56′ E) belonging to the Faculty of Biotechnology and Horticulture, University of Agriculture in Kraków, Poland.

Blueberry plants (*Vaccinium corymbosum* L.) cv. 'Bluecrop' of uniform growth and health status were chosen for the experiment. The specimens were planted in open-field (without cover), in a space with 1.2 m between plants and 3 m between rows on a gentle, southern slope. The plants grew in clay soil having a pH of 4.5–5.0. Every year, a layer of sawdust made of coniferous was poured on the soil. An irrigation system was placed on the plots where the plants grew. The irrigation was used as needed, and the frequency of irrigation was adjusted to the weather conditions. The annual rainfall in the region where the experiment was carried out (Małopolska, Poland) is 700–720 mm. During the experiment, all typical agrotechnical treatments were performed in accordance with the principles of good agricultural practice and current principles of blueberry protection. The hives with bumblebees were located 100 m from the blueberry plants.

2.2. Weather Conditions during the Flowering Period

The weather conditions during the flowering period of blueberry (May) were different in each year of the experiment. In the 1st year of the experiment the average daily temperature in May was 18.1 °C. The maximum daily temperature in this month reached 29 °C (Figure 1a). In the 2nd year of the experiment, the average daily temperature in May was 14.1 °C, and the maximum daily temperature reached 23.5 °C (Figure 1b). In the years with weather conditions typical for a moderate climate, the flowering period of blueberry lasts 10–14 days. In the 1st year of the experiment, due to the high temperatures during the flowering period, the flowering period of blueberry was shorter and lasted 8 days (from 6th to 13th May). In the 2nd year of the experiment, the flowering period of blueberry was typical for a moderate climate and lasted 15 days (from 4th to 18th May).

2.3. Experiment Design

The 2 × 2 factorial experiment was set up in May, using the randomized block design. The first factor of the experiment included application of titanium organic complex (TOC) in the form of the commercial product TYTANIT (8.5 g of Ti/L, INTERMAG, Poland) [29] or the lack of it. The second experimental factor was the type of pollination: self- or open pollination. The experiment consisted of 4 treatments (self-pollination, self-pollination + TOC, open pollination, open pollination + TOC).

In order to evaluate the efficacy of pollination and fertilization as well as fruit growth and development, measurement points were set up for all treatments. In each treatment, the plants were divided into two series: Series A and B. Series A included flowers chosen for the microscopic assessment of pollination and fertilization, while Series B included flowers chosen for the observation of fruit setting and fruit development. Series A was set up in four replications and one replication consisted of 50 flowers (4 × 50 = 200 flowers per treatment). Series B was also set up in four replications and one replication consisted of 100 flowers (4 × 100 = 400 flowers per treatment) (Figure 2). Flowers from the Series B were counted in the stage of budding, and then, after the fruit setting, all ripe fruits were counted. Based on this, the percentage of fruit set in each treatment was calculated. The results of the experiment were considered individually for self- and open pollination.

Flowers in self-pollinated treatments were set apart using an isolator made of agrotextile at the development stage when flowers at the base of the cluster were in a balloon stage. After unfolding, the flowers were hand pollinated. Each flower was pollinated 3 times at two-day intervals and the presence of pollen on stigma was checked using a magnifying glass. Flowers in open pollinated treatments were marked only for identification. In the vicinity of the experimental plot with cv. 'Bluecrop', the following cultivars were grown: 'Heerma', 'Croatan', and 'Darrow', which yielded pollen that was favourable to the open pollination of 'Bluecrop'.

Blueberry plants in the treatments with self-pollination + TOC and open pollination + TOC were sprayed two times with a solution of TYTANIT (foliar application) at a concentration of 0.05% (200 mL of TYTANIT/ha): at the beginning of flowering and during full flowering.

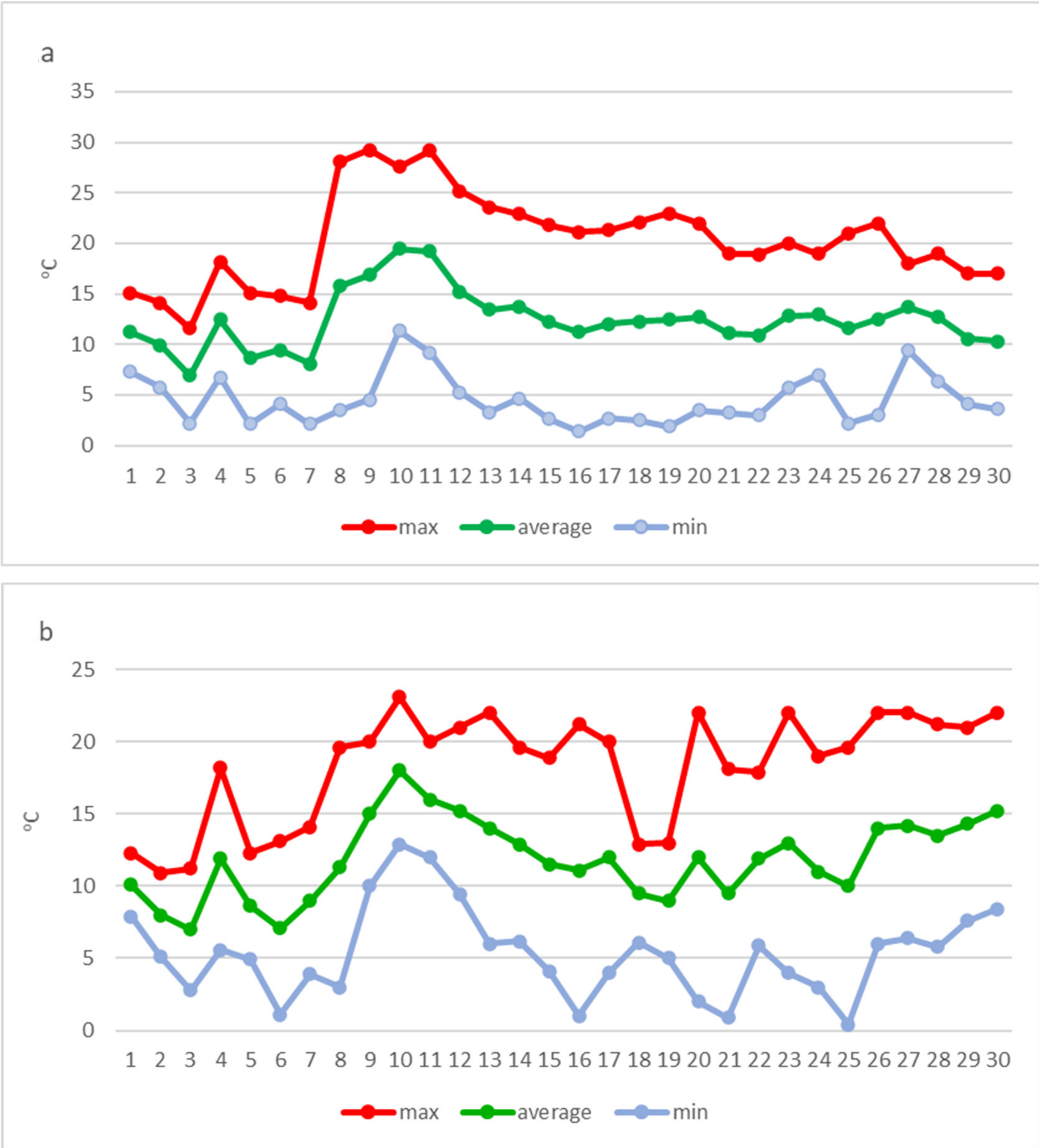

Figure 1. The maximum, average and minimum temperatures during the flowering. (**a**) The 1st year of the experiment; (**b**) the 2nd year of the experiment.

2.4. Observations and Measurements

2.4.1. Germination of Pollen Grains and the Growth of Pollen Tubes

After the petals had fallen, all pistils with the ovaries of flowers from Series A were collected for microscopic observation of the germination of pollen grains and the growth of pollen tubes. In total, 200 pistils (50 pistils × 4 replications) were collected per treatment. Collected pistils and ovaries were fixed in the chemical reagent FAA (formalin: ethanol 80%: glacial acetic acid; in a ratio 1:8:1). Then the pistils were macerated in 30% NaOH for 6 h. After maceration, the pistils were bleached in 6% H_2O_2 and then stained in aniline blue [30,31]. Germination of pollen grains and the growth of pollen tubes were assessed with the use of fluorescence microscope with UV light. Pollen tubes whose length exceeded the size of the pollen grains by two times were considered germinated. The average number of germinating pollen grains on the stigma was estimated. Assessment was performed in 4 replications for each treatment, with each replication including 50 pistils with ovaries. The observations were made using Light Microscope Discovery V12 Zeiss Stereo 3D and Fluorescence Microscope Imager M2 1022448558 1/1 Zeiss Axio (Carl-Zeiss-Strasse, Oberkochen, Germany).

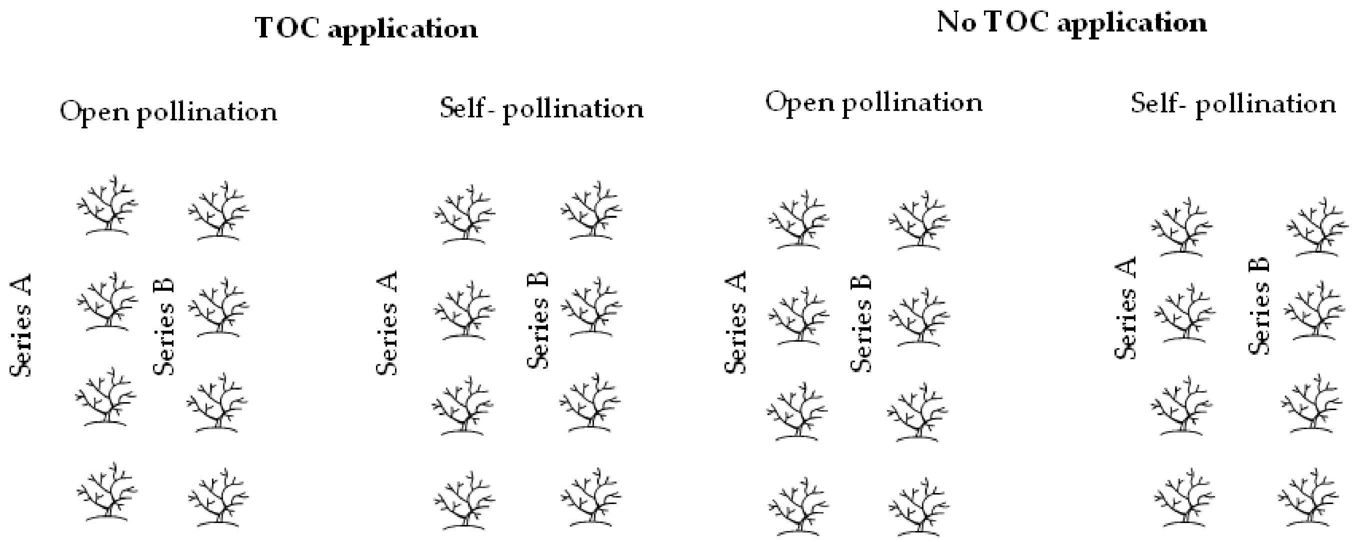

Figure 2. The scheme of the experiment. TOC application—application of the titanium organic complex; no TOC application—no application of the titanium organic complex; Series A—plants, for which flowers were selected for microscopic observation of pollination and fertilization; Series B—plants, for which flowers were selected for measurements of fruit set and development. TOC—titanium organic complex.

2.4.2. Number of Seeds, Percent of Fruit Set, Fruit Mass, Length and Diameter

The flowers of Series B were left on the trees to evaluate fruit setting and development of the fruit. The percentage of fruit set for each blueberry plant was assessed before the harvest. Fully mature fruits were harvested after counting, in a full summer. Fruits were weighted and then their diameter and length were measured by a digital caliper. Measurements were performed in 4 replications for each treatment. A total of 30 fruits per replication were chosen. In total, 120 fruits were randomly selected for each treatment (30 $\times$ 4 replications). Furthermore, the number of seeds in fruits chosen for the measurements was evaluated. The mass, length and diameter were calculated as a mean value of all fruits set in the treatment.

2.5. Statistical Analysis

The results were subjected to a two-way analysis of variance using the ANOVA module of SATISTICA 10.PL (StatSoft Inc., Tulsa, OK, USA). We used Tukey's honest significant difference test to determine the significance between means. Results were considered significant at a probability level below 0.05 ($p < 0.05$). For each year of the experiment, the statistical analysis was carried out separately.

3. Results

3.1. Germination of Pollen Grains

In the first year of the study, the highest average number of pollen grains germinating on the stigma was obtained for the treatments: open pollination and open pollination + TOC. The average number of germinating pollen grains in self-pollination + TOC was higher than in self-pollination (Table 1). In the second year of the study, the highest average number of pollen grains germinating on the open pollination stigma was observed in the treatment + TOC. A higher average number of germinating pollen grains was obtained in open pollination + TOC than in open pollination. The same relation was observed for self-pollination + TOC and self-pollination (Table 1).

Table 1. The effect of experimental factors: type of pollination and application of titanium organic complex (TOC) on the germination of pollen grains on the stigma.

Treatment	Average Number of Germinating Pollen Grains on Stigma [pcs.]	
	Year	
	First Year	Second Year
Self-pollination	52.1 ± 0.74 a	38.1 ± 0.79 a
Self-pollination + TOC	78.1 ± 0.82 b	111.3 ± 0.88 b
Open pollination	236.1 ± 0.79 c	268.2 ± 0.75 c
Open pollination + TOC	253.2 ± 0.84 c	298.3 ± 0.71 d

Statistical analysis was performed separately for each year; TOC—titanium organic complex; a, b, c, d—means in columns followed by different letters differ at $p < 0.05$; ±—standard error.

Microscopic observations of stigmas and styles of the pistils in the UV light showed that in the case of open pollination, numerous pollen grains germinated on the stigma and numerous pollen tubes in the pistil style were visible (Figure 3A). In open pollination treatment + TOC a higher number of pollen grains was observed on the stigma than in the open pollination treatment. A very bright glow of callose in the UV light in the pollen tubes confirms the high vigor of the pollen tubes (Figure 3B). Figure 3C shows some pollen grains visible on the stigma in self-pollinated plants. Additionally, germinating pollen tubes did not show chemotropism towards the ovary (Figure 3C). A higher number of pollen grains were visible in the + TOC than in the self-pollination. Additionally, more pollen tubes were detected in the pistil (Figure 3D).

3.2. Fruit Set

In the first year of the study, the highest average percentage of fruits set by plants was obtained in open pollination + TOC. The percentage of fruits set between treatments: open pollination and self-pollination + TOC did not differ significantly. The average percentage of fruits set by plants in self-pollination was lower than in self-pollination + TOC (Table 2). In the second year of the study, the average percentage of fruits established by plants was the highest in open pollination and open pollination + TOC. The average percentage of fruits set by plants in the treatment self-pollination + TOC was higher than in self-pollination (Table 2).

3.3. Average Number of Seeds in Fruit

In the first year of the experiment, the average number of seeds in fruit was the highest in open pollination + TOC. The average number of seeds in fruit in self-pollination + TOC was higher than in self-pollination (Table 3). In the second year of the study, the highest average number of seeds in fruit was obtained in the open pollination and open pollination + TOC treatments. The average number of seeds in fruit in self-pollination + TOC was higher than in self-pollination (Table 3).

3.4. Fruit Length and Diameter

In the first year of the experiment, no significant differences in fruit diameter between open pollination + TOC, open pollination and self-pollination + TOC were observed. However, there was a tendency towards higher fruit diameter in open pollination + TOC. Fruit length was the highest in open pollination + TOC, whereas it was the lowest in self-pollination (Table 4). In the second year of the study, fruit diameter was higher in open pollination + TOC than in open pollination. The same relation was observed for self-pollination + TOC and self-pollination. The lowest fruit length was obtained in self-pollination. In self-pollination + TOC, fruit length was higher than in self-pollination (Table 4).

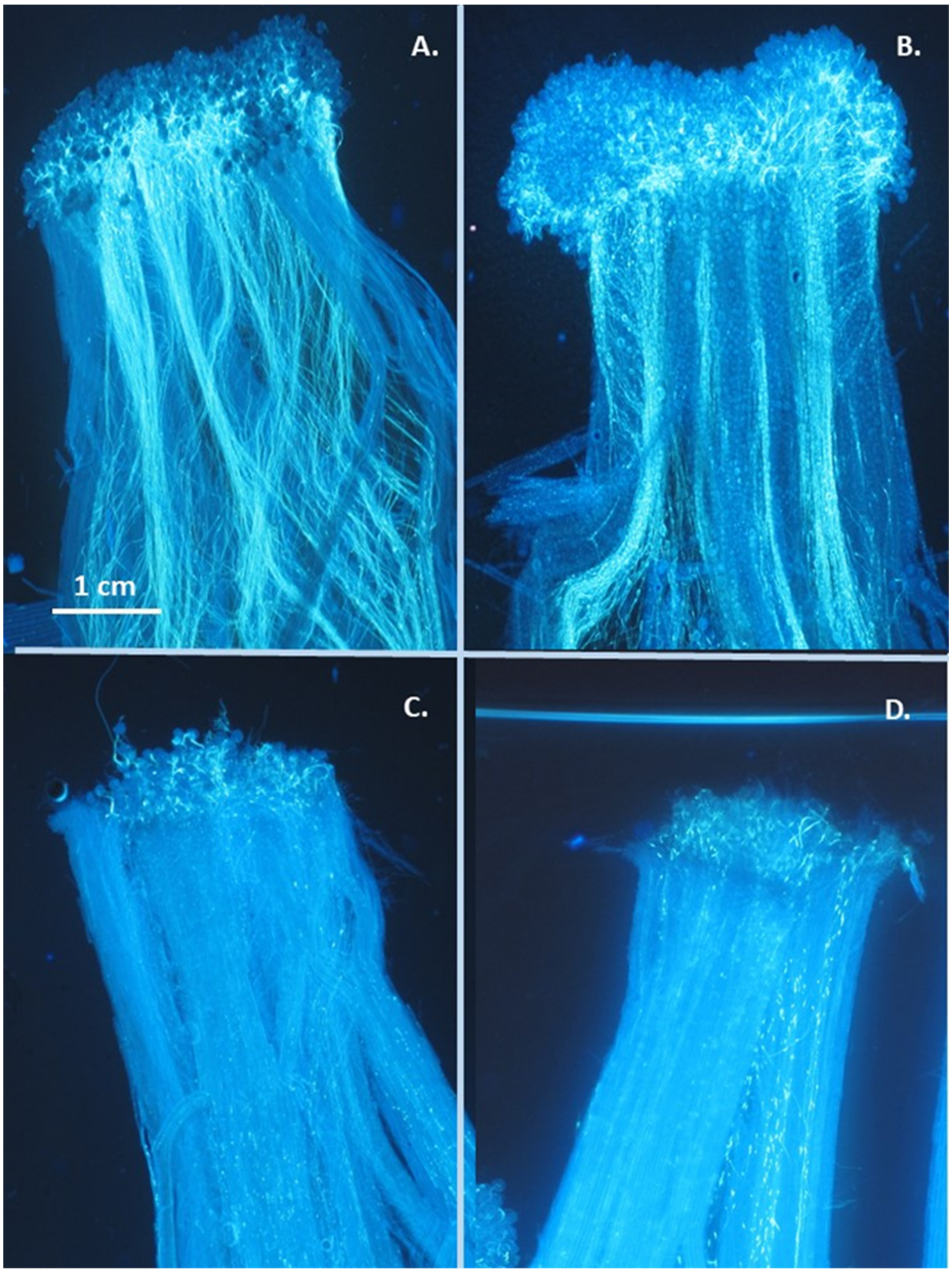

Figure 3. Stigmas of blueberry after different types of pollination (self- or open pollination) and application of TOC or lack of it. (**A**) Open pollination; (**B**) open pollination + TOC; (**C**) self-pollination; (**D**) self-pollination + TOC. TOC—titanium organic complex.

Table 2. The effect of experimental factors: type of pollination and application of titanium organic complex (TOC) on average percent of fruits set by blueberry plants (%).

Treatment	Average Percent of Fruits Set by Plants (%)	
	Year	
	First Year	Second Year
Self-pollination	38.9 ± 0.89 a	31.2 ± 0.64 a
Self-pollination + TOC	49.7 ± 0.87 b	43.4 ± 0.62 b
Open pollination	46.7 ± 0.97 ab	62.1 ± 0.75 c
Open pollination + TOC	55.0 ± 0.69 c	63.3 ± 0.59 c

Statistical analysis was performed separately for each year; TOC—titanium organic complex; a, b, c—means in columns followed by different letters differ at $p < 0.05$; ±—standard error.

Table 3. The effect of experimental factors: type of pollination and application of titanium organic complex (TOC) on the average number of seeds in individual blueberry fruit (pieces).

Treatment	Average Number of Seeds in One Fruit (pcs.)	
	Year	
	First Year	Second Year
Self-pollination	56.1 ± 0.89 a	48.2 ± 0.64 a
Self-pollination + TOC	74.8 ± 0.69 b	58.0 ± 0.62 b
Open pollination	67.6 ± 0.97 ab	74.1 ± 0.75 c
Open pollination + TOC	88.2 ± 0.87 c	77.6 ± 0.59 c

Statistical analysis was performed separately for each year; TOC—titanium organic complex; a, b, c—means in columns followed by different letters differ at $p < 0.05$; ±—standard error.

Table 4. The effect of experimental factors: type of pollination and application of titanium organic complex (TOC) on the diameter and length of individual blueberry fruit (mm).

Treatment	Fruit Diameter (mm)		Fruit Length (mm)	
	Year			
	First Year	Second Year	First Year	Second Year
Self-pollination	11.6 ± 0.71 a	11.1 ± 0.89 a	8.9 ± 0.73 a	8.2 ± 0.84 a
Self-pollination + TOC	12.9 ± 0.79 b	13.3 ± 0.77 c	9.8 ± 0.69 b	9.9 ± 0.00 b
Open pollination	12.6 ± 0.72 b	12.4 ± 0.83 b	9.3 ± 0.78 b	9.0 ± 0.79 b
Open pollination + TOC	13.8 ± 0.84 bc	13.5 ± 0.81 c	10.1 ± 0.76 c	9.8 ± 0.82 b

Statistical analysis was performed separately for each year; TOC—titanium organic complex; a, b, c—means in columns followed by different letters differ at $p < 0.05$; ±—standard error.

3.5. Fruit Mass

In both experimental years, the average fruit mass was the highest in open pollination + TOC. The average fruit mass in open pollination + TOC was always higher than in open pollination. The same relation was observed for self-pollination TOC and self-pollination (Figure 4).

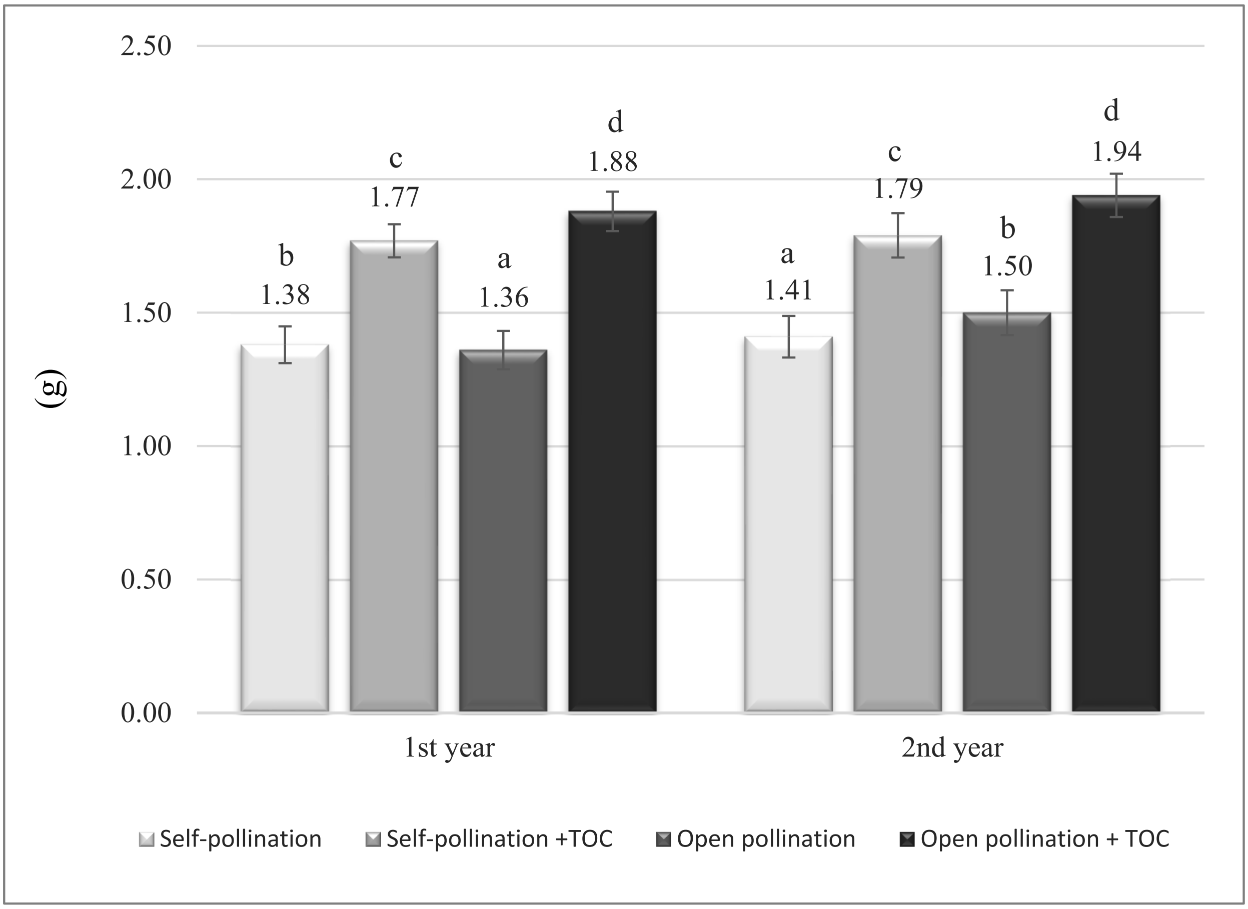

Figure 4. The effect of experimental factors: type of pollination and application of titanium organic complex (TOC) on the mass of blueberry fruit (g). Statistical analysis was performed separately for each year; TOC—titanium organic complex; a, b, c, d—means within each year followed by different letters differ at $p < 0.05$.

4. Discussion

Among the cultivars of the genus *Vaccinium*, highbush blueberry is the most noteworthy because, compared to other species of blueberries, its yield is the highest and ranges from 5 to 17 t/ha [7,32]. Because of their high content of phenolic acids and flavonoids, blueberries are considered a health-promoting fruit [33,34]. Therefore, in the cultivation of blueberries, attention is paid not only to the quantity of yield but also its quality, especially the size and mass of the fruit, the content of beneficial compounds and storage properties [10].

In plant nutrition, titanium is classified as a beneficial element [18,19]. Pais [20] carried out many studies on various plant species and proved that TOC stimulated plant growth and yield. Dyki et al. [25] performed the first studies, which showed that TOC positively affected pollination and fertilization processes, seed formation and fruit development of tomato and cucumber. Research carried out by these authors was followed by Bieniasz and Konieczny [27] and Bieniasz et al. [35], who also demonstrated that TOC positively affected the pollination process, seed setting and mass of individual fruit of apples and strawberries, respectively. Janas [26] and Radkowski et al. [28] in solanaceous crops and timothy demonstrated a higher number of seeds and their better quality as a result of the use of TOC, but did not conduct analysis of pollen adhesion to the stigma and growth of the pollen tube. Similarly to the above mentioned authors, the results of our study confirmed the positive effect of TOC on the process of pollination and fruit development.

According to Chaves and Lirene, most of the species of the genus *Vaccinium* are cross pollinated, which increases the fruit set and fruit yield from 20 to 100% [36]. Therefore, at least two cultivars with a similar flowering period should be planted in commercial plantations [37]. However, in cultivation on large areas, self-fertile cultivars are very valuable due to the convenience of agrotechnical work. Large plots with only one cultivar are planted more and more often. In some plantations, the distance between cultivars is very huge, which makes open pollination difficult, because pollinating insects are not able to transport a sufficient amount of pollen between cultivars. In some years during flowering, weather conditions limit the frequency of pollinating insects, which decreases the number of pollen grains on the stigma, thus limiting the effectiveness of pollination [38,39]. In these cases, the use of TOC may contribute to the improvement of fruit quality by stimulating the pollination process and consequently fruit setting and development. Therefore, we evaluated the impact of TOC for both types of pollination: self and open. There are few self-fertile cultivars among highbush blueberry cultivars. Among the popular self-fertile cultivars, cv. 'Duke' is characterized by full self-fertility and cv. 'Bluecrop' by a partial self-fertility [40]. The average lifetime of individual flowers lasts from 4 to 7 days [41]. In the case of pollination of *Vaccinium corymbosum* flowers with their own pollen, an incompatibility was observed, manifested by a very slow growth of the pollen tube, total inhibition of pollen tube growth and looping of the pollen tubes [9,10]. Compatible pollen grains quickly adhere to the stigma due to physical and chemical changes in the compounds responsible for adhesion of pollen to the stigma [42]. Parrie and Lang [43] and Dogterom et al. [44] reported that the most effective covering of the stigma with pollen was observed when the number of pollen grains on the stigma was between 200 and 300. In our study the amount of pollen grains on the stigma was always higher in case of open-pollinated plants, compared to self-pollinated plants. In open-pollinated plants, the average number of pollen grains was between 236.1 and 298.3, whereas in self-pollinated plants, the number ranged between 38.1 and 111.3.

Our experiment confirmed the positive effect of TOC on the amount of pollen grains on the stigma. Generally, for self-pollination and open pollination treatments, more pollen grains germinated on the stigma of flowers sprayed with TOC, compared to plants where this compound was not applied. Only in the first year of the experiment were there no statistically significant differences between the treatments open pollination and open pollination + TOC. However, there was a tendency to a higher number of germinating pollen grains on the stigma of plants sprayed with TOC. Bieniasz and Konieczny [27] obtained similar results in the cultivation of apple. In the two following growing seasons, these authors sprayed an apple tree with TOC and observed more pollen grains on stigma and more pollen tubes reaching the ovary. The authors hypothesized that TOC may stimulate production of sticky secretion in the papilla of apple stigma, which increases the adhesion of pollen grains to the stigma. Bieniasz et al. [35] conducted a three-year experiment, on three cultivars of strawberry, and demonstrated a higher pollen viability, pollen germination capacity and more pollen grains germinating on the surface of the stigma after application of TOC. The growth rate of pollen tube is affected by the concentration of calcium ions (Ca2+) in the tissues of the stigma [45]. The papilla are a source of exogenous Ca for pollen grains; thus, they participate in the process of pollen tube growth [46]. Borkowski and Dyki [47] suggested that Ti ions have direct and indirect effect on the regulation of intracellular Ca level, improving the adhesion of pollen grains to the stigma and stimulating the germination and growth of the pollen tubes.

Krebs and Hancock [48] proved that the open pollination of *Vaccinium corymbosum* contributed to an increase in fruit set. Bieniasz [10] carried out a two-year experiment aimed at comparing the fruit set of cv. 'Bluecrop' in the case of self- and open pollination and demonstrated variable results. In the first year of the study, the author did not observe significant differences in fruit set between the self and open pollinated flowers, whereas in the second year of the experiment, open pollination increased the percentage of the fruit set. 'Bluecrop' is a partially self-fertile cultivar, but open pollination is a beneficial factor affecting the fruit set. In our experiment, the percentage of fruit set was generally higher in the open pollination treatments compared to self-pollination. Spraying blueberry with TOC increased the percentage of fruit set by plants. In both years of the experiment, in self-pollinated flowers, the use of TOC increased fruit setting, in comparison to non-treated plants. In open-pollinated plants, the application of TOC increased fruit setting only in the first year of the experiment. The first year of the experiment was characterized by higher maximum daily temperatures compared to the second year of the experiment. In such unfavourable conditions, the positive impact of TOC on fruit setting was more visible. There are about 100–110 ovules in the ovary of highbush blueberry. Fruits of high dessert quality usually have 60–80 seeds set. Not all seeds are always properly developed, yet they also increase the mass of the fruit [49]. In the first year of our experiment, the average number of seeds in blueberry fruit was high, which affected the mass of the fruits. The average number of seeds in fruits harvested from open-pollinated plants and sprayed with TOC reached 88.2, which consequently led to the increase in the fruit mass by 38% in relation to the non-treated plants. In the second year of the experiment, we did not see the difference in the number of seeds between open pollination and open pollination + TOC treatments. However, there was a significant difference in the fruit mass. Fruits obtained from open-pollinated plants and sprayed with TOC were characterized by a 29% higher mass, compared to open-pollinated plants. The same relationship was observed in each year of this study in self-pollinated plants. Both the number of seeds in individual fruit and fruit mass were higher in the self-pollination + TOC treatment, in comparison to the self-pollination treatment. Bieniasz et al. [35] demonstrated a higher number of seeds and higher fruit mass of three strawberry cultivars—'Albion', 'Murano' and 'St Andreas' as a result of the application of TOC. Many authors have demonstrated that blueberry cultivars differ among each other in terms of mass and number of seeds set [49]. It is believed that the growth of blueberry fruit is stimulated by fertilized ovules and in consequence by developing seeds. Seeds set in fruits, during their development, produce hormones—auxins and gibberellins—which affect the mass of fruits [13].

Several studies have proven the beneficial effects of TOC on total yield and/or mass of the individual fruit of eggplant [26], tomato [22], apple [27], raspberry [23] and strawberry [35]. Therefore, TOC may be treated as a substance stimulating total fruit yield and mass of individual fruit, which may contribute to the higher share of extra class fruit in total yield.

5. Conclusions

Our research is the first to demonstrate that TOC positively affects the pollination process, seed settings and fruit development of blueberry. Blueberry is one of the most important berries cultivated all over the world. Effective pollination of the blueberry flower is one of the most important factors, which has an effect on fruit setting, yield quantity and quality. In the commercial production of blueberries on large areas, pollinating insects are not always able to transfer pollen from different cultivars. Additionally, in some years, during flowering, weather conditions limit the frequency of pollinating insects, which decreases the number of pollen grains on the stigma, thus limiting the effectiveness of pollination. Therefore, as a result of non-effective pollination, smaller fruits with a lower number of seeds are formed. The presented study proves that application of TOC during flowering stimulates pollen adhesion to the stigma, pollen germination on the stigma, fruit setting, seed setting and fruit development, both in case of self- and open-pollinated flowers. Therefore, TOC may be treated as a substance stimulating total fruit yield and the mass of individual fruit, which may contribute to the higher share of extra- class fruit in total yield. Titanium is classified as a beneficial element. Beneficial elements positively affect plant growth and development when applied at low concentrations. Therefore, the single dose of the formulation tested in this study was very low (200 mL/ha). The formulation has been implemented to the agricultural practice in many countries. Based on the available data, the average cost of application of the formulation is about a dozen dollars per hectare. High effectiveness and low application cost per hectare make the formulation very profitable for the growers.

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