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Titanium Organic Complex Improves Pollination and Fruit Development of Remontant Strawberry Cultivars under High-Temperature Conditions

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Abstract: Heat stress negatively affects pollination, fertilization, and consequently, the development of strawberry fruits. It was proved that foliar application of titanium organic complex improved pollination and fertilization processes of some plant species, which resulted in better fruit development. Therefore, a three-year experiment was designed to determine the effect of the titanium organic complex on pollination and fertilization processes and consequently on fruit development of three remontant strawberry cultivars grown under canopies, under conditions of high temperature. The experiment demonstrated that high temperature disrupted the flowering physiology of strawberry. Under such conditions, the viability and germination of pollen as well as the receptivity of stigma were significantly reduced. The application of titanium organic complex during flowering had a beneficial effect on the pollination and fertilization processes of strawberries. Plants treated with the titanium organic complex were characterized by higher pollen viability, better pollen germination, a higher number of achenes in fruit, and a higher weight of individual fruit, compared to the not treated plants. The obtained results suggest that application of titanium organic complex during flowering may alleviate the stress caused by high temperature and contribute to the improvement of the quantity and quality of a crop.

Keywords: beneficial elements; biostimulation; everbearing strawberry; flowering; achenes number; fruit quality

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1. Introduction

Strawberries are among the most consumed dessert fruits in the world after raspberries, blueberries, dogwood, Kamchatka berries, and kiwi [1–5]. They are cultivated in many regions of the globe, from the Arctic to the tropics, in the field, and increasingly using soilless techniques. In the recent years, research on fruit quality has focused on issues important for cultivation, such as agrotechnology and selection of varieties [6]. Many authors report that the quality of dessert fruit depends on many factors that affect the attractive appearance, health, and post-harvest durability, as well as the high content of bioactive compounds. Therefore, the research work covers a wide range of topics, from the selection of the variety and substrate type to the biological protection and balanced fertilization with calcium supplementation to the durability during transport and storage [7–17].

The Fragaria genus includes 21 species distributed in the northern temperate and Holarctic zones [18–21]. The cultivars of strawberry (*Fragaria* × *ananassa*) are hybrids of two American species: *F. chiloensis* and *F. virginiana*. Long-day cultivars, blooming when the day is longer than night, are a large group. These cultivars do not repeat flowering (no remontant). An example of a cultivar from this group is the commonly cultivated 'Honeoye'. Recently, strawberry growers have focused on cultivars that are neutral to the duration of the day (remontant). This type of cultivar is valuable because the plants bloom and bear fruit throughout the entire growing season [22,23]. Temperature is an important factor affecting the quality of strawberry flowers. The inception of flowering in short-day cultivars can occur in any photoperiod if the temperature is low enough, generally <15 °C [24]. Sønsteby and Heide [25] reported that, in strawberries, flower induction was closely related to photoperiod and temperature. The flowering of long-day cultivars is inversely proportional to the inhibitory effect of temperature on long days. This relationship is stronger in the case of strawberries that are sensitive to the length of the day (non-remontant). In the case of strawberries that are neutral to the length of the day, the temperature is the main factor determining the proper initiation and differentiation of flower buds. Heat stress negatively affects the flowering stage and the development of the strawberry. The effect of one-time, severe heat stress at different stages of flower development is poorly understood. Ledesma and Kawabata [26] confirmed that the effect of heat stress was different for various cultivars. Plants subjected to 4 h of heat stress (above 40 °C) before the opening of the flowers and during anthesis reacted with a reduction in the weight of their fruit. Bradford et al. [23] proved that the number of flowers and inflorescences depended on the temperature, and in the 'Honeoye' were the highest between 14 and 17 °C and decreased with increasing temperature to 20 °C. At a temperature of 26 and 29 °C, there was a complete inhibition of flowering.

A dessert strawberry should be an even, symmetrically shaped, large fruit [9]. An important agrotechnical problem in the cultivation of dessert strawberries is fruit deformation. Strawberry is a collective fruit with achenes on the surface [27]. The number and distribution of achenes on the surface of the fruit determines its quality. In the creative breeding of strawberry cultivars, instead of assessing the quality and number of stamens, attention is paid to the number of functional pistils, as it determines the size and shape of the set fruit. Cultivars demonstrating problems with pollination occur on the market. One example is the cultivar 'Redgauntlet' [28], which is very prone to pollination problems. It is very important that the cultivars launched on the market are well assessed in terms of flower structure and functionality, as the effectiveness of pollination depends on them. The flower structure of the *Fragaria* species can be stamen (male), pistil (female), or bisexual. In cultivation, bisexual flowers occur the most frequently and such clones and cultivars are preferred [29].

In the study conducted by Ledesma and Kawabata [26], high temperatures reduced the size and weight of strawberries, but cultivars did not respond to this stress in the same way. The cultivars 'Nyoho' and 'Toyonoka' responded to high temperature (day/night 30/25 °C) with a reduced number of inflorescences and a lower fruit set, compared to the plants grown under the temperature of 23 °C during day and 18 °C at night. However, the differences between the cultivars were not statistically significant. Cui et al. [30] proved that treating flowers with high temperature during anthesis resulted in damage to the stigmas in the apical part of the flower, which contributed to fruit deformation. Therefore, strawberry breeding programs should be based on the selection of heat-resistant clones [31].

Titanium (Ti) is considered to be a beneficial element for plants [32,33]. The results show that the application of titanium enhanced photosynthesis improves the vigor and nutritional status of plants [34,35]. Pais [32] proved that titanium organic complex positively affected the growth and development of various plant species. Since these results, many studies have demonstrated the beneficial effect of the titanium organic complex on the yield quantity and quality of various crops, including raspberry [36], tomato [37], winter wheat [38], oilseed rape [35], and soybean [39]. Dyki et al. [40] were the first to observe better adhesion of pollen to the stigma, enhanced germination of pollen grains on the stigma, and a higher seed number in tomatoes and cucumber fruits after the application of the titanium organic complex. Similarly, Bieniasz and Konieczny [41] demonstrated the positive effect of the organic titanium complex on the pollination and fertilization processes of apples. The authors found that plants treated with the titanium organic complex were characterized by better adhesion of pollen grains to the stigma, better growth of pollen tubes through the pistil style, and consequently, better seed setting and fruit development, compared to non-treated plants. Janas et al. [42] also obtained a higher number of seeds and better seed quality after application of the titanium organic complex in the cultivation of solanaceous vegetables. Radkowski et al. [43] demonstrated the same effect in the cultivation of timothy grass.

We hypothesize that foliar application of titanium organic complex improves the adhesion of pollen grains to the stigma and pollen germination on the stigma, which results in better fertilization, seed setting, and fruit development of remontant strawberries grown under canopies, under high summer temperature conditions. Our objective in this study was to assess the effectiveness of the pollination of strawberries grown under canopies, depending on the temperature during the flowering period. Our goal was to test the possibility of the biostimulation of strawberry flowers with titanium organic complex in order to obtain high value fruits under conditions of high-temperature stress.

2. Materials and Methods

A 3-year experiment was carried out to monitor the flowering, flower quality, and effectiveness of pollination and fertilization of strawberries in southeastern Poland. The experimental field was located close to Kraków. Geographical coordinates were as follows: h = 210 m, φ = 50°02′11″ N, and λ = 19°81′19″ E.

2.1. Plant Material

The plant materials were remontant (everbearing) strawberries (*Fragaria* × *ananassa*) of three economically important cultivars: 'Murano', 'St Andreas', and 'Albion'. Plants were grown in gutters, under canopies, and in mats with coconut substrate in white foil (mat dimensions: 200 mm × 100 mm × 1000 mm). During the study, all typical agrotechnical treatments were carried out according to the principles of good agricultural practice and the current principles of strawberry protection.

2.2. Weather Conditions during the Flowering Period

In southeastern Poland, the climate is moderate. The long-term (20-year period) average daily temperature in July is 14.3 °C. Climate conditions are favorable for the cultivation of plants under cover. However, recent years have seen heat waves. During the experiment, heat waves lasting between 10 and 14 days occurred in July and August. In July of each year of the experiment, the average maximum monthly temperatures in the green mass of plants were about 35 °C, while in August, the average maximum monthly temperatures were close to 45 °C. Such high temperatures lasted for several hours during the day. Average and maximum monthly temperatures during the day, measured in the green mass of plant, are shown in the Figure 1.

2.3. Experiment Design

The one-factor experiment with the use of randomized block design was set up. The experimental factor was foliar application of titanium organic complex in the form of commercial product TYTANIT (8.5 g Ti/L, INTERMAG, Olkusz, Poland) [44].

In mid-May of the three experimental years, the A+ class strawberry plants of 3 cultivars were planted in mats with coconut substrate. There were 8 plants per mat. The experiment was set up in 2 treatments for each cultivar. The first treatment (control) was for plants not treated with the titanium organic complex. The second treatment was for plants treated with titanium organic complex. Each treatment in the experiment consisted of 4 replications. There were 32 plants per replication.

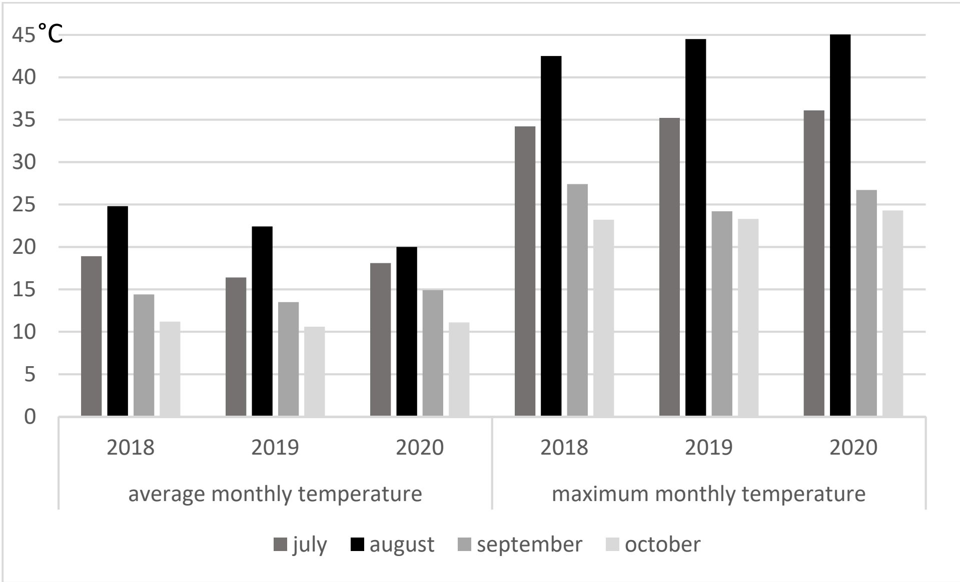

Figure 1. Average and maximum monthly temperatures during the day measured in the green mass of strawberry plants (°C).

Titanium organic complex was applied via foliar spraying, at a concentration of 0.05%, twice in each flowering cycle, every 7 days (at the beginning of flowering and during full flowering). There were 4 flowering cycles in each year of the experiment (June, July, August, and September). A total of eight sprayings of the titanium organic complex were made in each year of the experiment.

The first fruits were harvested in July and the last in mid-October. During the flowering period, hives with bumblebees were placed in the area of cultivation. The effectiveness of flowering was assessed separately for each year and each cultivar in 4 evaluation periods: July, August, September, and October. The following parameters were assessed: pollen viability and germination, number of pollen grains on the stigma, the number of set achenes (seeds), weight of the fruits, and correlation between fruit weight and the number of achenes.

2.4. Observation and Measurements

2.4.1. Pollen Quality

To assess the viability and germination of pollen, 50 flowers with closed anthers from each treatment were collected. Then, the anthers were cut off and were put into dry Petri dishes. After storage for 24 h at room temperature, the anthers opened, and the tests were performed to assess pollen viability and pollen germination. The pollen viability was assessed according to the color method by Alexander [45]. The pollen was collected from the freshly opened flowers and placed on glass slides, and then Alexander's dye was applied. The dye stained viable pollen grains in a fuchsia color, while non-viable pollen grains remained green. The pollen viability was expressed as a percentage of viable pollen grains. The pollen germination was determined on the basis of germination in vitro on an agar medium (0.6% agar) supplemented with 10% sucrose. The pollen from freshly opened flowers was collected and placed on Petri dishes containing the medium mentioned above. The dishes were closed and incubated at 24 °C for 24 h. Then, the number of germinating pollen grains was counted, and the germination capacity was expressed as a percentage of germinating grains.

2.4.2. Effectiveness of Pollination

For both treatments, at the end of each evaluation period, the number of pollen grains on the stigma was assessed through microscopic observations. In total, 50 flowers were collected from each treatment, and then the pistils were isolated. The isolated pistils were fixed in the chemical FAA reagent (formalin: ethanol 80%: glacial acetic acid; in a ratio 1:8:1) for 10–12 h. Then, the pistils were macerated in 30% NaOH for 2–3 h. After maceration, the pistils were bleached in 6% H_2O_2 and stained with aniline blue [46]. Pollen grains on the stigma were observed using a Zeiss Axio Imager M2 fluorescence microscope (Carl-Zeiss-Strasse, Oberkochen, Germany) and ultraviolet light with a wavelength of about 356 nm. Under these conditions, the callose fluoresced with a bright yellow-green color. The observations were performed in 4 replications for each treatment. One replication consisted of 50 pistils.

2.4.3. Fruit Quality

In each year of the study, the fruits were harvested in 4 cycles: July, August, September, and October. For each treatment, the average weight in one harvest cycle was calculated based on the data from 4 replications. One replication consisted of 200 fruits. In addition, the number of achenes in the fruit selected for weight measurements was estimated.

Moreover, a dessert quality fruit model for the harvested fruit of each cultivar was created. The number of set achenes, which is necessary to obtain a dessert quality fruit was estimated. In order to estimate the correlation between the number of achenes and fruit weight, 300 fruits of different sizes were randomly selected from each cultivar. Each fruit was weighted separately, and the number of achenes set in the fruit was calculated.

2.4.4. Statistical Analysis

The results were subjected to a one-way analysis of variance using the Statistica 10.PL ANOVA module (StatSoft, Inc., Tulsa, OK, USA). Whenever differences occurred, significant homogenous groups were determined with the use of Tukey's test. Results were considered as significant at $p < 0.05$. The statistical analysis was performed for each cultivar and each year separately.

3. Results and Discussion

3.1. Weather Conditions

In southeast Poland, the climate is moderate. However, heat waves have been observed in recent years. During the experiment, heat waves that lasted for 10–14 days occurred in July and August. The average monthly temperatures from July to October were within the range of values well tolerated by strawberry plants, while the maximum monthly temperatures in July and August were problematic. Maximum temperatures lasted for several hours a day between 12 and 3 pm. In each year of the experiment, in August, the maximum monthly temperature in the plant green mass reached 45 °C. Despite the systematic supply of nutrient solution, plant thermoregulation was insufficient. At such high temperatures the physiology of flowering was disturbed, and the activity of pollinating insects decreased. In strawberry plantations, the activity of bees was recorded at temperatures ranging from 16.49 to 38.91 °C [47]. Cooper et al. [48] studied bee feeding patterns and showed that bees stop feeding at air temperatures above 35 °C. There is a study on the population of Saudi bees, representing a breed distinct from *Apis mellifera jemenitica*, with increased tolerance to high temperatures during flowering [49]. Bumblebees are very popular pollinators in protected agriculture. They start feeding at 12 °C and are the most active at temperatures of 25 °C, and their activity decreases above this temperature. The bumblebee rarely feeds at temperatures above 27 °C. The activity of the bumblebee is related to its body weight. The larger the individual, the greater its activity is, even at lower temperatures [50]. Bumblebees are very useful for pollinating strawberries in greenhouses. The yield of bumblebee pollinated plants was over 30% higher than control plants [51]. Zaitoun et al. [52] proved that bumblebees increased the fruit set of strawberry by 60% compared to the control. Additionally, strawberries pollinated by bumblebees produced no defective berries and were characterized by larger fruits compared to the strawberries cultivated with other pollination techniques.

3.2. Pollen Quality

In our study, the viability of pollen grains tended to change along with changes in temperature in individual evaluation periods. In July 2018 and 2020, the pollen viability of all three tested cultivars was very high and did not differ between treatments, which proved that tested cultivars had genetically viable pollen (Table 1).

Table 1. The effect of titanium organic complex application on the viability of pollen grains of three strawberry cultivars (%).

	Pollen Viability (%)											
	2018				2019				2020			
Cultivar	VII	VIII	IX	X	VII	VIII	IX	X	VII	VIII	IX	X
'Murano'	99.1 a	70.3 a	89.1a	79.6 a	90.3 a	89.9 a	89.4 a	90.0 a	98.9 a	81.1 a	79.5 a	84.5 a
'Murano' + T	100.0 a	83,2 b	90.2 b	93.1 b	98.2 b	93.2 b	97.2 b	92.2 a	99.2 a	93.2 b	98.0 b	91.3 b
'St Andreas'	99.3 a	79.1 a	92.3 a	89.1 a	95.4 a	79.0 a	90.1 a	95.2 a	91.3 a	86.1 a	92.1 a	89.4 a
'St Andreas' + T	99.8 a	89.7b	94.5 a	92.9 b	95.1 a	87.2 b	98.4 b	98.4 a	93.4 a	85.2 a	97.4 a	94.0 b
'Albion'	96,3 a	77.9 a	92.3 a	83.4 a	91.0 a	83.4 a	73.8 a	93.6 a	90.9 a	80.6 a	87.3 a	86.3 a
'Albion' + T	99.3 a	87.4b	99.5b	97.1 b	92. 3a	89.6 b	89.2 b	98.2 a	97.7 a	90.2 b	91.3 b	95.0 b

Statistical analysis was performed separately for each cultivar in each year. a,b—means that columns followed by different letters differ at $p < 0.05$; 'Murano', 'St Andreas', 'Albion'—control, no application of titanium organic complex; 'Murano' + T, 'St Andreas' + T, 'Albion' + T—application of titanium organic complex; VII, VIII, IX, and X—July, August, September, and October, respectively.

The viability of strawberry pollen is variable and depends on the cultivar and on the weather conditions. Weather conditions significantly affect the quality of pollen. In a study carried out by Kaczmarska et al. [53] on pollen viability under moderate climate conditions, on non-remontant strawberry cultivars, the percentage of viable pollen grains ranged from 33.8 to 70.8%. The material was collected on four dates and for only one variety on all dates pollen viability was invariably the same.

Our study is the first to prove that the application of titanium organic complex significantly improves pollen viability of strawberry. Plants treated with the titanium organic complex were characterized by a significantly higher percentage of viable pollen compared to those of the control, that is, those without treated plants. This relationship was repeated in all years of the study. For all three cultivars, it was not observed only in July 2018, July 2019, and October 2019 (Table 1).

The stress of high temperatures during flowering or just before anthesis damages the pollen of many crops: grains [54], canola [55], pepper [56], and bean [57]. In the case of strawberry cultivars, Cui et al. [30] as well as Ledesma and Sugiyama [58] reported a reduction in pollen viability under conditions of high temperature during the anthesis or just before the opening of flowers. The decrease in the percentage of viable strawberry pollen due to high temperature was noticeable, but different for different cultivars. Cui et al. [30] proved that the negative effect of high temperature was greater for the cultivar 'Maehyang' than for 'Seolhyang'.

Pollen viability is the first test of a plant's ability to set fruit. However, the most important test is the capacity for pollen germination. In the three years of our study, a significantly higher percentage of germinating pollen grains was observed in the combinations treated with titanium organic complex compared to non-treated. Only for cv. 'Albion' in June and September 2018, for cv. 'St Andreas' in July 2019, and for all three cultivars in July 2020, we found that there was no effect of titanium organic complex on pollen germination (Table 2). Other researchers have proven the beneficial effect of titanium organic complex on the pollination process in cultivation of tomato, cucumber, and apple [40,41]. However, these authors did not test pollen viability or germination capacity.

Table 2. The effect of titanium organic complex on the germination of pollen grains of three strawberry cultivars (%).

	Pollen Germination (%)											
	2018				2019				2020			
Cultivar	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**
'Murano'	89.3 a	45.2 a	88.3 a	90.1 a	81.1 a	41.0 a	71.7 a	80.0 a	87.6 a	40.8 a	77.0 a	73.2 a
'Murano' + T	94.1 b	52.9 b	92.7 b	98.6 b	89.6 b	48.9 b	80.1 b	86.3 b	88.2 a	57.2 b	83.6 b	89.1 b
'St Andreas'	85.1 a	41.2 a	90.4 a	91.4 a	86.2 a	40.0 a	74.0 a	83.5 a	86.2 a	41.4 a	74.2 a	88.7 a
'St Andreas' + T	94.8 b	48.2 b	97.4 b	98.2 b	88.8 a	51.1 b	88.6 b	90.1 b	89.6 a	49.2 b	87.2 b	98.2 b
'Albion'	89.2 a	48.4 a	87.4 a	90.0 a	85.6 a	43.8 a	75.3 a	43.8 a	83.8 a	42.2 a	72.7 a	87.6 a
'Albion' + T	89.8 a	56.2 b	89.9 a	96.8 b	89.9 b	49.2 b	85.2 b	49.9 b	84.6 a	54.9 b	84.8 b	94.7 b

Statistical analysis was performed separately for each cultivar in each year. a,b—means that columns followed by different letters differ at $p < 0.05$; 'Murano', 'St Andreas', 'Albion'—control, no application of titanium organic complex; 'Murano' + T, 'St Andreas' + T, 'Albion' + T—application of titanium organic complex; VII, VIII, IX, and X—July, August, September, and October, respectively.

Poor pollen germination in strawberries can result in the poor fertilization and deformation [30]. Karapatzak et al. [59] observed a short-term reduction in remontant strawberry yield due to high temperature (thermodormance). In a two-year experiment with 'Everest' and 'Diamante', researchers observed that the pollen of these varieties lost its germination capacity after exposure to a period of high temperature (30/20°Cday/night). The same relationship was observed in our study.

Our study is the first to demonstrate that foliar application of the titanium organic complex during the flowering period significantly improves the pollen quality of strawberries, that is, the viability of pollen and pollen germination capacity. Therefore, this compound may be helpful in reducing the destructive effect of high temperature on strawberry pollen.

3.3. Effectiveness of Pollination

Cui et al. [30] proved that high temperature reduced pollen viability, germination capacity, and pollen tube growth in strawberries, especially for the cultivar 'Toyonoka'. The authors concluded that successful pollination is very important in the case of strawberries, as only fertile ovaries can lead to the expansion of the primordium and the formation of a valuable fruit. When most of the ovules are fertilized, a well-shaped, marketable fruit is formed. It was also confirmed that the pollen of different strawberry cultivars had different tolerances to high-temperature stress [30]. If pollen grains matched the stigma genetically, the chemical and physical interaction between grains and the receptive stigma was so strong that even heavy rainfall during pollination did not significantly reduce the number of grains on the stigma [60]. Maji et al. [61] demonstrated that the heat damage of the stigma reduced seed setting in chickpeas.

In our experiment, the average number of pollen grains present and germinating on the stigma was monitored in each year, in the four evaluation periods. In the months when high temperatures did not disturb flowering physiology (July, September, and October), a dozen germinating pollen grains were observed on the stigma, while in August, during extremely high temperatures, the number of pollen grains decreased to a single grain (Table 3). In July, September, and October, the average maximum monthly temperatures were about 35 °C, 25 °C, and 20–25 °C, respectively. On the other hand, in August, the average maximum monthly temperatures reached 45 °C.

A statistically significant difference in the number of germinating pollen grains on the stigma was observed between the combinations: application of the organic titanium complex and lack of it. In each year of the study, each evaluation period and each cultivar plant treated with titanium organic complex were characterized by a higher number of pollen grains germinating on the stigma, compared to non-treated plants. The only exception was cv. 'Murano' in September 2019, where we did not observe a significant effect of titanium organic complex on pollen germination (Table 3). Such results may suggest that the organic titanium complex improves stigma receptivity by decreasing physiological stress (Table 3, Figure 2), which may be important in terms of potential yield.

Table 3. The effect of titanium organic complex on average number of pollen grains on the stigma of three strawberry cultivars (pcs.).

	Average Number of Pollen Grains Germinating on a Stigma (pcs.)											
	2018				2019				2020			
Culivar	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**
'Murano'	8.1 a	1.3 a	10.9 a	10.2 a	11.2 a	1.3 a	10.1 a	9.2 a	10.4 a	1.2 a	8.9 a	7.2 a
'Murano' + T	18.2 b	9.8 b	18.7 b	17.8 b	16.5 b	7.8 b	15.6 b	13.7 b	15.7 b	6.9 b	9.9 a	10.6 b
'St Andreas'	13.5 a	1.5 a	11.5 a	13.2 a	12.1 a	2.4 a	9.7 a	8.8 a	10.2 a	2.2 a	8.4 a	9.8 a
'St Andreas' + T	16.6 b	8.6 b	18.6 b	18.5 b	18.5 b	9.9 b	16.6 b	15.1 b	17.2 b	8.9 b	14.7 b	15.1 b
'Albion'	11.1 a	1.8 a	15.3 a	11.0 a	12.8 a	3.2 a	7.8 a	7.9 a	9.8 a	3.0 a	10.7 a	11.0 a
'Albion' + T	18.5 b	9.8 b	19.3 b	16.8 b	19.1 b	8.5 b	17.2 b	15.4 b	13.8 b	9.4 b	14.9 b	16.6 b

Statistical analysis was performed separately for each cultivar in each year. a,b—means that columns followed by different letters differ at $p < 0.05$; 'Murano', 'St Andreas', 'Albion'—control, no application of titanium organic complex; 'Murano' + T, 'St Andreas' + T, 'Albion' + T—application of titanium organic complex; VII, VIII, IX, and X—July, August, September, and October, respectively.

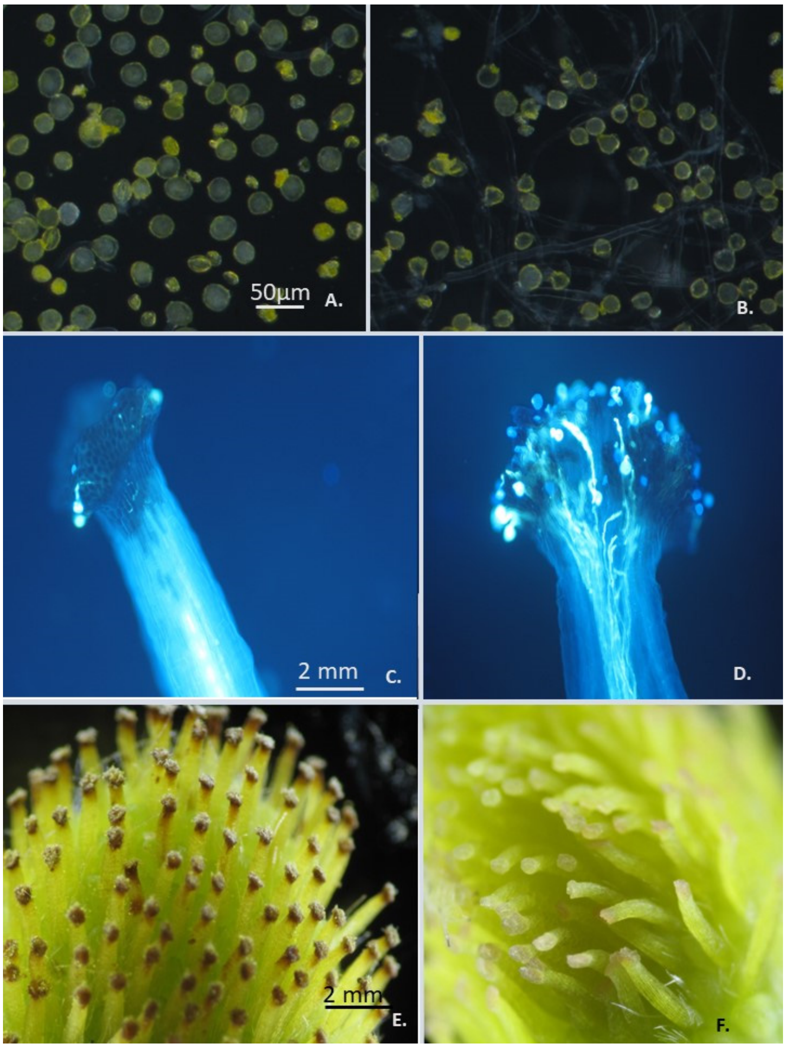

Figure 2. Pollen and stigmas of strawberry flowers collected 48 h after occurring the maximum monthly temperatures of 30 °C and 45 °C. (**A**) Non-germinating strawberry pollen (at maximum

monthly temperatures above 45 °C); (**B**) germinating strawberry pollen (at maximum monthly temperatures below 30 °C); (**C**) single pollen grains on stigma (at maximum monthly temperatures above 45 °C); (**D**) numerous pollen grains on stigma (at maximum monthly temperatures below 30 °C); (**E**) dried stigmas in freshly opened flowers (at maximum monthly temperatures above 45 °C); (**F**) receptive stigmas in freshly opened flowers (at maximum monthly temperatures below 30 °C).

Dyki et al. [40] obtained similar results; they observed better pollen adhesion and better pollen germination on the stigma of cucumber and tomato after application of titanium organic complex. Similarly, Bieniasz and Konieczny [41] proved that the foliar application of the titanium organic complex during the flowering period improved pollen adhesion to the stigma, pollen germination on the stigma, and the growth of the pollen tube through the pistil style of apples.

3.4. Fruit Quality

The uniform shape and size of the fruits of many species is affected by the number and quality of the set seeds. This relationship also applies to most *Fragaria* species and, above all, cultivars [53]. In each year and in each harvest cycle (July, August, September, and October) of our experiment, all tested cultivars treated with titanium organic complex were characterized by a significantly higher number of achenes, compared to untreated plants (Table 4).

Table 4. The effect of titanium organic complex on average number of achenes on the fruit of three strawberry cultivars (pcs.).

	Average Number of Achens (pcs.)											
	2018				2019				2020			
Cultivar	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**
‘Murano’	250 a	120 a	255 a	239 a	234 a	110 a	243 a	311 a	231 a	111 a	256 a	341 a
‘Murano’ + T	311 b	199 b	311 b	321 b	342 b	166 b	289 b	421 b	329 b	178 b	327 b	402 b
‘St. Andreas’	201 a	111 a	321 a	276 a	298 a	136 a	266 a	321 a	311 a	186 a	301 a	347 a
‘St Andreas’ + T	411 b	145 b	424 b	374 b	431 b	198 b	379 b	417 b	445 b	201 b	431 b	426 b
‘Albion’	321 a	116 a	312 a	298 a	321 a	116 a	231 a	235 a	298 a	178 a	299 a	296 a
‘Albion’ + T	430 b	167 b	465 b	399 b	389 b	279 b	412 b	301 b	389 b	230 b	411 b	389 b

Statistical analysis was performed separately for each cultivar in each year. a,b—means that columns followed by different letters differ at $p < 0.05$; ‘Murano’, ‘St Andreas’, ‘Albion’—control, no application of titanium organic complex; ‘Murano’ + T, ‘St Andreas’ + T, ‘Albion’ + T—application of titanium organic complex; VII, VIII, IX, and X—July, August, September, and October, respectively.

Similarly, Dyki et al. [40] observed better seed setting in cucumber fruit after application of titanium organic complex. Janas et al. [42] demonstrated a higher seed yield and better quality after foliar spraying of eggplant and tomato with the titanium organic complex. Radkowski et al. [43] also proved the beneficial effect of the titanium organic complex on the yield of timothy seeds and their germination capacity. The latest studies carried out by Bieniasz and Konieczny [41] on apples showed that the titanium organic complex improved the seed setting in apples. The authors demonstrated that apples from plants sprayed with a titanium organic complex were characterized by 9 or 10 seeds in a chamber, which confirmed very effective fertilization resulting from effective pollination. In August of each year of the experiment, when the daily maximum temperatures were extremely high, there was a tendency toward a lower number of achenes compared to other months (Table 4). Achenes are directly related to the development of the fruit. They secrete auxins to adjacent cells, which promotes the thickening of the flower bottom by increasing the number and size of cells [62]. The growth and shape of the fruit is strongly determined by the number and location of achenes on the flower bottom. Uneven distribution or an insufficient number of fertilized ovules lead to the formation of small, misshapen fruits. Many factors contribute to the deformation of the fruit, but the most important seems to be the genotype, the position of the flower in the inflorescence, and the viability of the stamen and ovules. However, environmental factors, pollination, and plant nutrition must also be taken into account [27].

The main quality parameter of strawberries is their weight. In our study, fruits were harvested from the beginning of July to the end of October (Table 5).

Table 5. The effect of titanium organic complex on average weight of fruit of three strawberry cultivars (g).

	Average Fruit Weight (g)											
	2018				**2019**				**2020**			
Cultivar	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**	**VII**	**VIII**	**IX**	**X**
'Murano'	17.2 a	12.1 a	18.2 a	17.2 a	16.3 a	11.8 a	12.7 a	15.5 a	13.8 a	14.5 a	15.3 a	14.7 a
'Murano' + T	20.5 b	14.5 b	21.5 b	20.5 b	19.2 b	14.6 b	16.0 b	17.4 b	15.7 b	16.2 b	17.7 b	17.8 b
'St Andreas'	16.8 a	12.1 a	16.8 a	16.8 a	17.1 a	15.4 a	15.8 a	14.7 a	14.2 a	17.2 a	14.5 a	17.9 a
'St Andreas' + T	19.3 b	14.9 b	22.3 b	19.3 b	21.5 b	17.8 b	18.2 b	17.1 b	17.1 b	25.1 b	18.0 b	22.3 b
'Albion'	16.8 a	12.3 a	15.8 a	16.8 a	17.2 a	13.2 a	15.5 a	14.5 a	14.1 a	16.8 a	17.6 a	16.8 a
'Albion'+T	18.6 b	17.1 b	21.6 b	18.6 b	24.2 b	17.7 b	19.2 b	17.8 b	15.9 ab	23.3 b	20.1 b	24.3 b

Statistical analysis was performed separately for each cultivar in each year. a,b—means that columns followed by different letters differ at $p < 0.05$; 'Murano', 'St Andreas', 'Albion'—control, no application of titanium organic complex; 'Murano' + T, 'St Andreas' + T, 'Albion' + T—application of titanium organic complex; VII, VIII, IX, and X—July, August, September, and October, respectively.

In another experiment, strawberries of the same cultivars, 'St Andreas' and 'Albion', bore fruit in a very similar period, i.e., from late June to early November [12]. In our experiment, the average fruit weight was subjected to changes throughout the growing season. The lowest fruit weight was recorded in August, which was a consequence of disturbances in the biology of flowering and a smaller number of established achenes. Statistical analysis showed that in each year of the experiment, the plants of all assessed cultivars treated with titanium organic complex were characterized by a significantly higher fruit weight compared to non-treated ones (Table 5). Bieniasz and Konieczny [41] also proved the beneficial effect of titanium organic complex on fruit weight as a result of effective pollination and consequently fertilization in the cultivation of apple. Ochmian et al. [36] observed a higher total yield and a higher weight of raspberry fruit after application of titanium organic complex, compared with non-treated plants. Similarly, Dobromilska [37] showed a higher yield of tomato after treating plants with titanium organic complex.

Kaczmarska et al. [53] studied the relationship between pollen fertility, the number of achenes, and the weight of the achenes and fruit weight, and proved that both the number of achenes in the individual fruit and their weight are positively correlated. This relationship was especially visible in the group of large fruit. However, the weight of achenes in individual fruit significantly affected the weight of small and large fruits. The authors concluded that pollen viability had a positive effect on the weight of achenes in large fruit, while no direct relationship was found between the fertility of pollen and weight of the strawberries [53]. Fruit deformations can also be associated with low temperatures during flowering. Low temperatures contribute to fertilization disorders, deformation, or reduction in weight. Low temperatures not only reduced the ability of pollen to fertilize, but also caused the inability of the ovule to accept sperm [63]. These results suggest that any thermal disturbance during flowering causes the distortion of fruit and the reduction of weight. Therefore, the application of titanium organic complex, which supports the processes of pollination and fertilization, may be beneficial for obtaining the proper quantity and quality of yield, especially in harsh environmental conditions.

Regardless of the combination, based on the weight of the fruit harvested in different cycles and the number of achenes set in the fruit, it was noted that the most valuable fruit, which weighed more than 18 g, had over 250 achenes set (Figure 3).

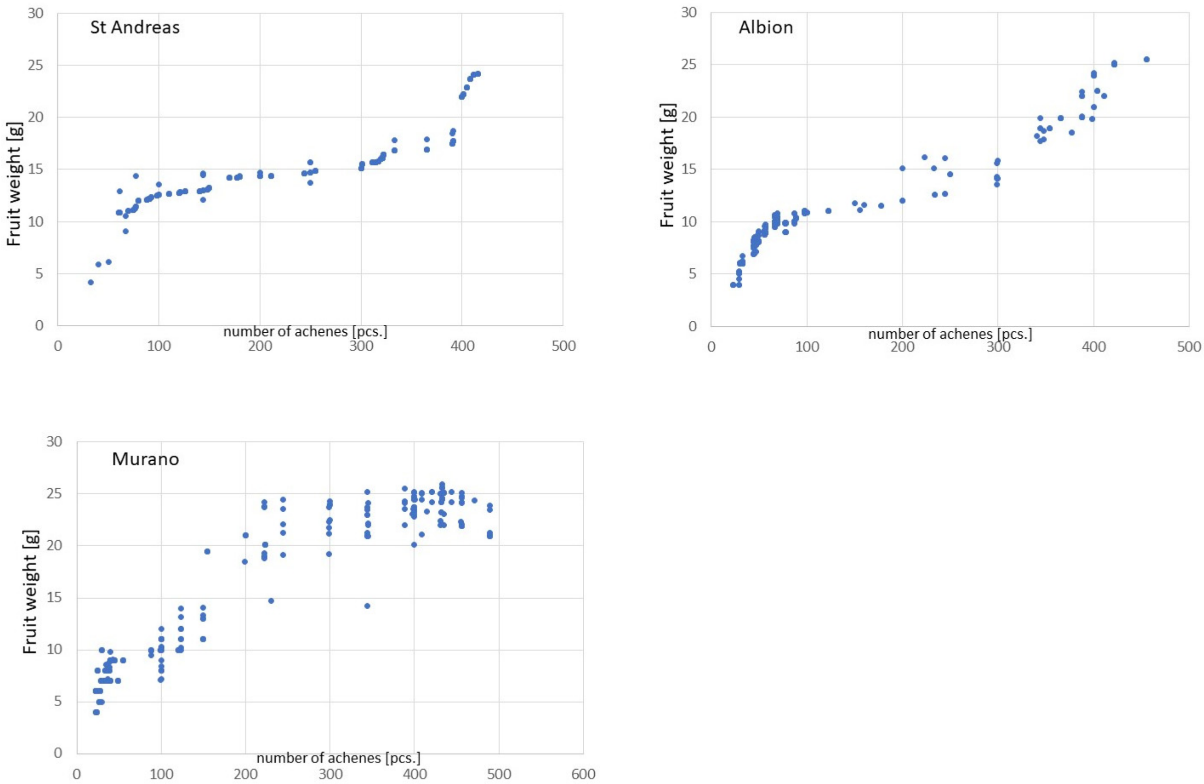

Figure 3. Relationship between the number of achenes and the weight of strawberry fruit.

Webb et al. [64] proved a similar relationship. The authors determined the number of achenes in a different way, expressing it as the number of achenes per 1 cm^2 of fruit. It was found that there was a limit to the number of achenes above which the weight of the fruit did not increase. Kaczmarska et al. [53] proved that the weight of the fruit no longer increased from 200 to 370 achenes. In our study, fruit having over 200 achenes weighed more than 13 g.

4. Conclusions

The maximum monthly temperatures reaching 45 °C in plant biomass, lasting several hours a day, disrupted the flowering physiology of strawberries. Under such conditions, the viability and germination of pollen as well as the receptivity of the stigma were significantly reduced. The surface of stigma in newly opened flowers was disturbed, which resulted in lower adhesion of pollen grains to the stigma. Fruits derived from flowers blooming under conditions of high temperature were characterized by a lower weight and lower number of set achenes.

The application of titanium organic complex during flowering had a beneficial effect on the pollination and fertilization processes of strawberries. Plants treated with the titanium organic complex were characterized by higher pollen viability, better pollen germination, a higher number of achenes in fruit, and a higher weight of individual fruit. Titanium organic complex helped to improve the effectiveness of strawberry pollination and fertilization under conditions of high temperatures. The obtained results suggest that application of titanium organic complex during flowering may alleviate the stress caused by high temperatures and contribute to the improvement of the quantity and quality of a crop. This is especially important in view of global warming.

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