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The Effect of Titanium Organic Complex on Pollination Process and Fruit Development of Apple cv. Topaz

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Abstract: Optimal weather conditions are necessary for the proper course of the flowering process and high activity of pollinators, which is the most important factor affecting pollination. Because weather conditions do not always favour effective pollination and because pollinators' activities are decreasing, the application of compound stimulating pollination and fertilization may be a good perspective for increasing yield. Titanium is considered a beneficial element for plants. Preliminary studies have indicated the positive effect of titanium organic complex on pollen adhesion to the stigma and pollen germination on the stigma of tomato and cucumber. Therefore, a 2-year experiment was designed to determine the effect of titanium organic complex application on the pollination process and fruit development of apple cv. Topaz (*Malus domestica* Borkh.) The experiment demonstrated the positive effect of titanium organic complex on fertilization of ovules because of effective pollination, seed setting, and fruit development of apple. Application of titanium organic complex improved pollen adhesion to the stigma and pollen germination on the stigma. In addition, titanium organic complex increased the number of pollen tubes growing through the pistil style, which resulted in fertilization that was more effective, as confirmed by the higher number of seeds set in fruits. Higher numbers of seeds set in fruits positively affected their weight and size. Therefore, fruit harvested from trees to which titanium organic complex was applied were characterized by greater weight, length, and diameter compared to fruits obtained from the trees to which titanium organic complex was not applied.

Keywords: self-pollination; incompatibility; pollen tubes; seed setting; *Malus domestica*; beneficial elements

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Citation: Bieniasz, M.; Konieczny, A. The Effect of Titanium Organic Complex on Pollination Process and Fruit Development of Apple cv. Topaz. *Agronomy* **2021**, *11*, 2591. https://doi.org/10.3390/agronomy11122591

Academic Editors: Youssef Rouphael and Giuseppe Colla

Received: 23 November 2021
Accepted: 17 December 2021
Published: 20 December 2021

1. Introduction

The effectiveness of pollination is one of the most important factors affecting yields of fruits and seeds. In biology, this parameter is measured by the number of seeds set in fruits. A higher number of seeds positively affects the weight and size of fruit [1,2]. Pollination is one of the most complicated processes in plants and results in a high number of well-developed fruits under optimal agrotechnical and weather conditions. Optimal weather conditions are necessary for the proper course of the flowering process and the high activity of pollinators, which is the most important factor affecting pollination. Low temperatures and rainfall limit pollinators' activity. Honey bees search for food at temperatures higher than 15 °C, and between 10 °C and 15 °C their activity is limited and below 10 °C no honey bees are at work. At 19 °C, bee flights reach a constantly high level. Air moisture and temperature determine the opening of anthers and pollen availability for pollinators. Optimal conditions are a temperature of 20 °C and an air moisture at 70% or lower. Low temperature and high air moisture decrease the activity of pollinators and slow down the release of pollen from anthers. Strong wind (i.e., more than 24 km/h) and a lack of light during cloudy weather limit the activity of pollinators and their number of flights per day [3,4]. High temperatures shorten the flowering period of many plant species, which increases the demand for high pollinator activity [5]. Because weather conditions are often not very favorable for effective pollination, the application of a compound that stimulates the effectiveness of pollination is very important and may be an effective way to improve pollination and consequently fertilization and fruit yield [1].

Apple trees belong to the to the group of monoecious species producing bisexual flowers [6] with a structure typical for the apple subfamily, *Maloideae*. A single apple blossom has the following parts arranged in four whorls: the pistil and the stamens, which are respectively female and male reproductive organs, as well as petals and sepals. The pistil is located in the innermost whorl. It is made up of five free pistil styles each with a stigma and ovary. An ovary, which contains ovules, is a rounded base of pistil [7]. In apple blossoms, typical wet stigmas are covered with a liquid secretion that is rich in proteins, polysaccharides [8,9], and lipids [10]. This sticky secretion increases adhesion of pollen grains to the stigma during pollination [1,11]. In a well-pollinated apple blossom, up to ten seeds may be developed; however, in some cultivars, the number of seeds may be even higher [7]. The central flower in the inflorescence is called a "king blossom" and is surrounded by 4–6 smaller flowers [12,13].

In the studies on flowering and fertilization, the term effective pollination period (EPP) was introduced. EPP determines a flower's fertility period. EPP is defined as the number of days during which pollination is effective and results in fertilization and fruit setting. It depends on flower quality, age of shoots, level of tree nourishment, and environmental conditions during flowering. EPP is always shorter than the flower's life is, and its length is determined by the longevity of the ovules minus the time needed for pollen tube growth and reaching the ovules. A flower's EPP can last from 2 to 10 days. Longer EPP increases the probability of flower pollination and fertilization [14–17]. A large number of pollen grains germinating on stigma stimulate the faster growth of pollen tubes through the pistil style. Delayed pollination reduces the number of pollen grains germinating on the stigma [18,19], which decreases the effectiveness of fertilization. Consequently, the reduced number of seeds in fruit may result in decreased fruit weight. A short effective pollination period is unfavorable, especially during low temperatures and the low activity of pollinators [20]. Reduction of the effectiveness of pollination and ovule fertilization in unfavorable environmental conditions has been observed by many authors [16,21]. Therefore, supporting the pollination and consequently the fertilization process may be very effective at obtaining a higher number of well-developed fruits.

Recently, the interest in using biostimulants in agriculture has grown significantly. A biostimulant is any substance or microorganism applied on plants to improve plant growth and development, enhance plant metabolism and improve yield [22–24]. Biostimulants can be divided into a few categories, one of which includes biostimulants based on beneficial elements [22]. Beneficial elements are not essential for plants, but applied at low concentrations, they positively affect plant growth and development as well as their tolerance to abiotic stress factors [25]. Titanium (Ti) is an element considered beneficial to plants and it mainly enhances photosynthesis by increasing iron ion activity, enhances pollen vigor, and increases nutrient uptake by plants [26]. Pais [27] discovered that titanium organic complex had beneficial effects on various plant species by promoting photosynthesis and plant growth. Wójcik [28] proved that spraying maiden apple trees in a nursery with titanium organic complex resulted in increased plant vigor and improved plant nutritional status. Dobromilska [29] demonstrated a higher total and marketable yield of tomato fruits after foliar application of titanium organic complex compared to nontreated plants. Similarly, Ochmian et al. [30] observed a significant increase of raspberry fruit yield after foliar application of titanium organic complex. Further studies demonstrated positive effects of foliar application of this compound on the yield of oilseed rape [31], winter wheat grains [32], and soybean seeds [33].

Titanium organic complex has been proven to enhance germination of pollen grains on stigma, which increased the yield of seeds and fruits [34]. Studies carried out by Dyki et al. [35] showed that foliar application of titanium organic complex resulted in the better adhesion of pollen grains to the stigma, better pollen germination on stigma, and a higher seed number in cucumber and tomato fruits. Janas [36] showed that in the cultivation of onion for seed production, treated plants demonstrated a higher seed yield and a better quality and health status after foliar spraying with that compound compared to nontreated plants. Similarly, Radkowski et al. [37] obtained a higher yield, thousand-grain weight, and germination capacity of timothy seeds due to foliar application of the titanium organic complex.

We hypothesized that foliar application of the titanium organic complex improves the adhesion of pollen grains to the stigma and pollen germination on the stigma, which results in better fruit development of apple cv. Topaz. Therefore, the aim of the study was to determine the effect of titanium organic complex application on the effectiveness of pollination and consequently fertilization and fruit development of apple cv. Topaz.

2. Materials and Methods

A 2-year experiment was conducted in an apple orchard belonging to the Experimental Station of the Faculty of Biotechnology and Horticulture, University of Agriculture in Kraków. Geographical coordinates of the station were as follows: h = 270 m, $\varphi = 50°09'$ N, $\lambda = 19°56'$ E.

2.1. Plant Cultivation

The 8-year old apple trees (*Malus domestica* Borkh.) cv. Topaz used in the experiment, were grafted on semi-dwarfing rootstock M26 planted at a spacing 2.5 × 4 m. The orientation of the rows was north to south. The pollinator of Topaz–cv. Šampion was planted in a row every sixth tree. Trees of uniform growth and health conditions were selected for the experiment. The crowns of the trees were formed into a spindles. During the study, all typical agrotechnical treatments were carried out according to the principles of good agricultural practice and the current principles of orchard protection.

2.2. Weather Conditions during the Flowering Period

In the first year of the experiment, the flowering period of apple trees started in the first decade of May and lasted very shortly. May was a warm and dry month, running from the perennial averages. The average temperature in May was 18.1 °C. Noteworthy are maximum daily temperatures of up to 29 °C. Such high temperatures shortened flowering period to a few days. The period from pink bud to full flowering lasted two days. It caused most of the flowers to be fully blooming at the same time (Figure 1a). In the second year of the experiment, May was cooler in terms of average air temperature (14.1 °C) than in the first year of experiment, and the maximum temperature reached 23.5 °C. The second year of the experiment in terms of temperature during flowering was close to the perennial average for that period (14.4 °C) (Figure 1b).

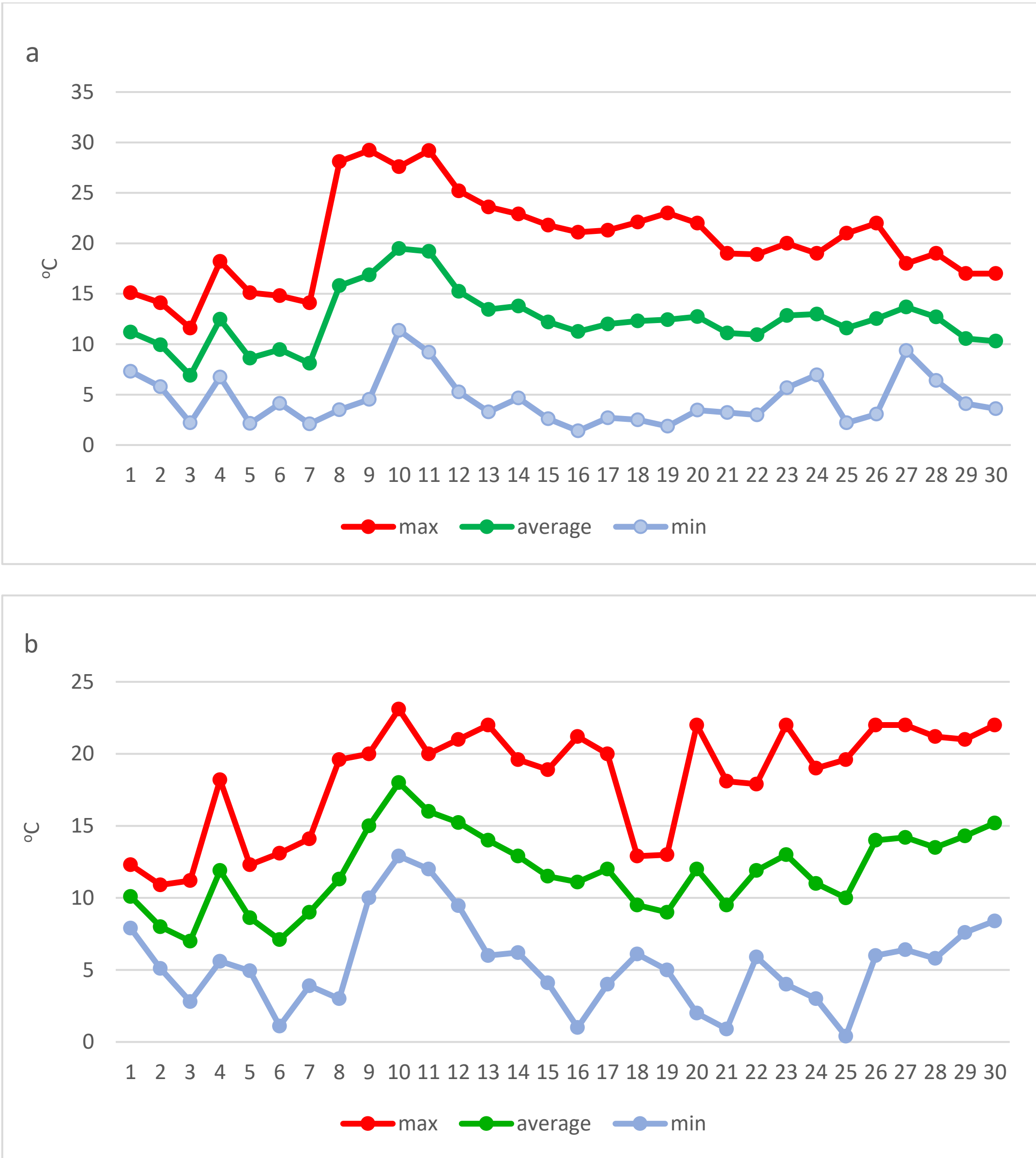

Figure 1. The course of maximum, average and minimum temperature in the periods of flowering. (**a**)—the first year of the experiment (2013 year); (**b**)—the second year of the experiment (2014 year).

2.3. Experiment Design and Treatments

The two-factor experiment was set up in May with the use of a randomized block design. The first experimental factor was the foliar application of titanium organic complex in a form of a commercial product TYTANIT (8.5 g of Ti/L; INTERMAG, Poland) [38], or a lack of it. The second factor of the experiment included the type of pollination: self-pollination or open-pollination. The experiment consisted of four treatments: self-pollination, self-pollination + titanium organic complex, open-pollination, and open-pollination + titanium organic complex. Treatments self-pollination and self-pollination + titanium organic complex were performed in order to observe flowers after pollination with its own pollen, which occurs in some years, especially under unfavorable weather conditions and low activity of pollinators. Each treatment consisted of 4 trees, and different trees for each treatment were selected in the orchard (total 16 trees were selected for the experiment). Each treatment consisted of two series (Series A and Series B) in four replications (each series: 50 flowers per each replication; in total 200 flowers per treatment): Series A was flowers selected for microscopic evaluation of pollination and Series B was flowers selected for observation of fruit development. The measurement within each series, i.e., for flowers selected for observation of pollination (Series A) and selected for observation of fruit development (Series B), were set up randomly on each tree. The results of the study were considered separately for self-pollination and open pollination by pollinators. Flowers in self-pollinated treatments were isolated by an agrotextile isolator at the stage of pink bud. After unfolding, the flowers were pollinated by hand, each flower three times at 2-day intervals. The presence of pollen grains on the stigma surface was verified with the use of a magnifying glass. Flowers in open-pollinated treatments were well marked for identification. Plants treated with titanium organic complex were sprayed twice with TYTANIT solution (foliar application) at a concentration of 0.05% at the beginning of flowering and during full flowering.

2.4. Observations and Measurements

2.4.1. Germination of Pollen Grains and Growth of Pollen Tubes

After petals had fallen, all pistils (50 pistils × 4 replications; in total 200 pistils per treatment) with ovaries selected for microscopic observations (Series A) were collected. Flowers from Series B were left on the trees to observe fruit development and ripening until harvest. Collected pistils and ovaries were fixed in chemical reagent FAA (formalin: ethanol 80%: glacial acetic acid; in a ratio 1:8:1) and then macerated in 30% NaOH (for 6 h). After maceration, the pistils were bleached in 6% H_2O_2 and finally stained in aniline blue [39]. Germinating pollen grains and growing pollen tubes were observed under a fluorescence microscope with UV light. The average number of pollen grains on the stigma of pistil; the average number of pollen tubes in a one-third section, a half section and at the end of pistil style (reaching the ovary); and the average number of fertilized ovules, were assessed. Observations were conducted in four replications, each of which included 50 pistils and ovaries. The assessments were made with the use of Light Microscope Discovery V12 Zeiss Stereo 3D and Fluorescence Microscope Imager M2 1022448558 1/1 Zeiss Axio (Carl-Zeiss-Strasse, Oberkochen, Germany).

2.4.2. Number of Seeds and Fruit Weight, Length, and Diameter

Fruits were harvested in September and their weight, diameter, and length were measured by a digital calliper. Measurements were conducted in four replications for each treatment. One replication included 30 randomly selected fruits (30 fruits × 4 replications; in total 120 fruits per treatment). In addition, the number of seeds in each selected fruit for measurement was estimated.

2.5. Statistical Analysis

The results were subjected to two-way analysis of variance using the ANOVA module of Statistica 10.PL (StatSoft Inc., Tulsa, OK, USA). Whenever some changes occurred, significant homogenous groups were determined by Tukey's Honest Significant Difference test. Results were considered significant at $p < 0.05$. The statistical analysis was performed separately for each year of the experiment.

3. Results

3.1. Germination of Pollen Grains and Growth of Pollen Tubes

In both years of the study, a higher average number of pollen grains germinating on stigma and a higher average number of pollen tubes growing in one-third, one-half, and at the end of the pistil style were observed in the open-pollination + titanium organic complex treatment compared to the open-pollination treatment. The same relation was observed for self-pollination + titanium organic complex treatment compared to self-pollination. All determined parameters (i.e., the average number of pollen grains germinating on pistil stigma, the average number of pollen tubes in one-third, one-half, and at the end of pistil style) were the highest in the open-pollination + titanium organic complex treatment (Table 1). In the one-third length of pistil style, the average number of pollen tubes was similar to the average number of germinating pollen grains on pistil stigma. However, in a one-half length of pistil style, we observed a strong reduction in the number of pollen tubes compared to the number of pollen tubes in the one-third length of pistil style. A small number of pollen tubes grew to the end of the pistil style (Table 1).

Table 1. The effect of titanium organic complex application on number of pollen grains germinating on pistil stigma and number of pollen tubes growing through pistil style (pieces).

Treatment	Average Number of Germinating Pollen Grains on Pistil Stigma (pcs)		Average Number of Pollen Tubes in One-Third Length of Pistil Style (pcs)		Average Number of Pollen Tubes in One-Half Length of Pistil Style (pcs)		Average Number of Pollen Tubes at the End of Pistil Style (pcs)	
	Year							
	1st Year	2nd Year	1st Year	2nd Year	1st Year	2nd Year	1st Year	2nd Year
Self-pollination	163.0 ± 0.68 a	155.1 ± 0.91 a	147.0 ± 0.89 a	132.2 ± 0.92 a	47.5 ± 0.68 a	31.1 ± 0.99 a	5.7 ± 0.69 a	4.0 ± 0.82 a
Self-pollination + titanium organic complex	430.5 ± 0.72 b	321.4 ± 0.74 b	421.0 ± 0.83 b	298.0 ± 0.89 b	310.2 ± 0.76 b	265.3 ± 0.61 b	12.5 ± 0.75 b	15.4 ± 0.93 b
Open-pollination	605.0 ± 0.81 c	548.7 ± 0.63 c	601.1 ± 0.76 c	421.3 ± 0.87 c	394.2 ± 0.74 b	199.5 ± 0.97 c	8.8 ± 0.88 b	4.2 ± 0.98 a
Open-pollination + titanium organic complex	729.0 ± 0.69 d	689.4 ± 0.77 d	719.3 ± 0.74 d	687.4 ± 0.69 d	438.7 ± 0.91 b	547.2 ± 0.66 d	24.4 ± 0.82 c	22.4 ± 0.91 c

Statistical analysis was performed separately for each year; significant homogenous groups were determined by Tukey's Honest Significant Difference test; a,b,c,d—means in columns followed by different letters differ at $p < 0.05$; ±—standard error; number of pistils evaluated for each treatment $n = 200$.

The model of pollen tube growth through pistil style was prepared on the basis of the result of the study. The percentage of pollen tubes growing to the base of the pistil style ranged from 1 to 3.8%. The first inhibition of growth was visible in 1/3 of the length of the pistil style and the second in the half of the length of pistil style. Regardless of the number of germinating pollen grains, about 50% of the pollen tubes are inhibited in the middle of the pistil style (Figure 2).

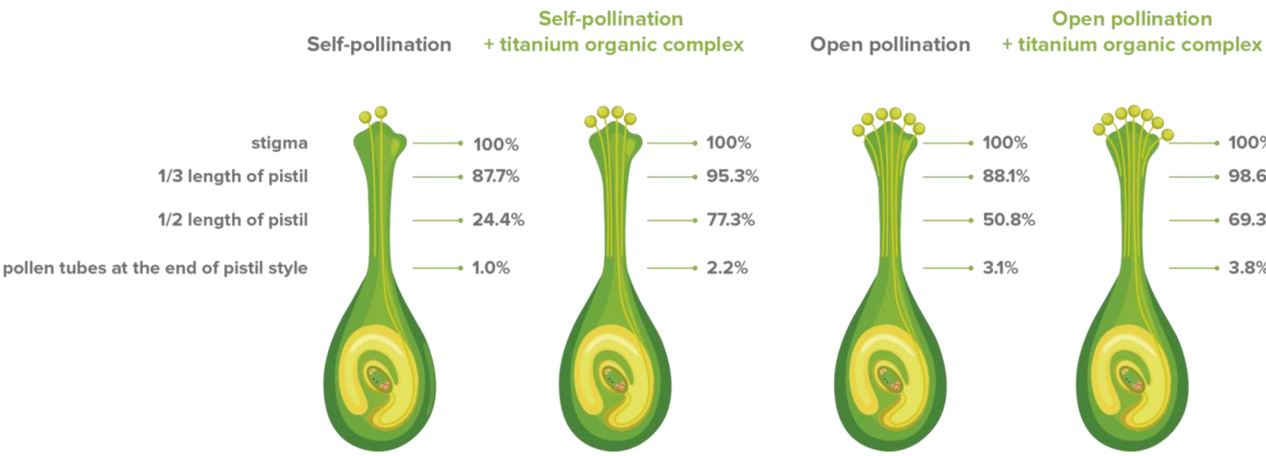

Figure 2. The model of pollen tube growth through pistil neck of apple cv. Topaz, based on the average of two year research.

Figure 3a,b show stigma of flowers pollinated by insects (open-pollination + titanium organic complex and open-pollination, respectively) with large numbers of pollen tubes growing through pistil styles. In the open-pollination + titanium organic complex treatment (Figure 3a), a higher number of pollen tubes was observed compared to open-pollination (i.e., without titanium organic complex treatment (Figure 3b). In the self-pollination treatment, a small number of pollen tubes were observed in the pistil styles of flowers not treated with titanium organic complex (Figure 3c). However, in the self-pollination + titanium organic complex treatment (Figure 3d), we observed a higher number of pollen tubes compared to self-pollination (Figure 3c).

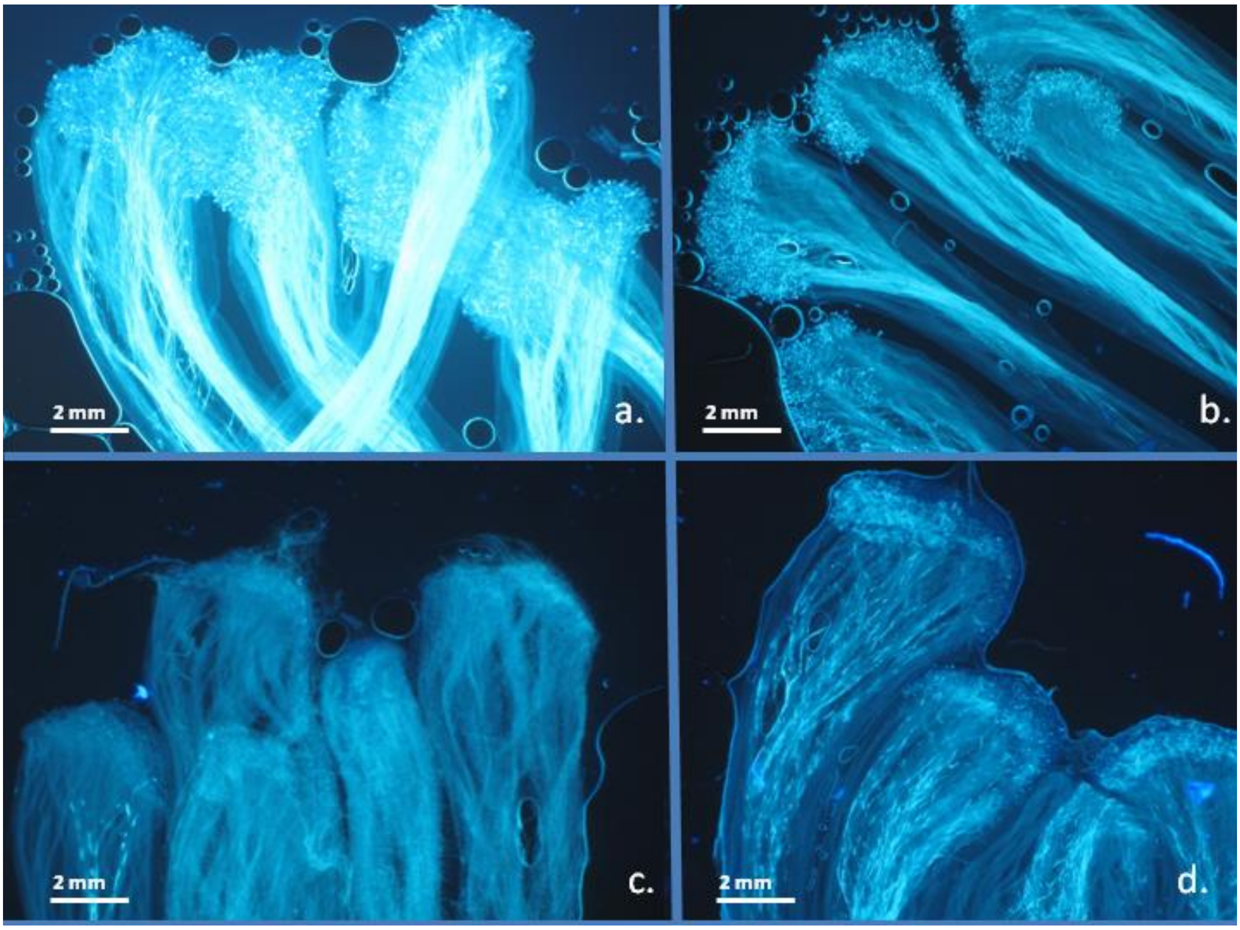

Figure 3. Pollen grains germinating on the stigmas. (**a**)—open-pollination + titanium organic complex, five pollinated stigmas, a large number of pollen tubes visible in lower part of pistil style; (**b**)—open-pollination, five pollinated stigmas; (**c**)—self-pollination, visible single pollen grains and single pollen tubes; (**d**)—self-pollination + titanium organic complex, visible pollen grains and numerous pollen tubes; the pictures were made with the use of Light Microscope Discovery V12 Zeiss Stereo 3D and Fluorescence Microscope Imager M2 1022448558 1/1 Zeiss Axio.

3.2. Number of Seeds in Fruit

The average number of seeds in apple fruit, in both years of the study, was the highest in the open-pollination + titanium organic complex treatment. Higher average numbers of seeds were observed in the open-pollination + titanium organic complex treatment compared to open-pollination. In the self-pollination + titanium organic complex and self-pollination treatments, the average number of seeds was lower compared to the open pollination + titanium organic complex and open pollination treatments, respectively. However, the average number of seeds in the self-pollination + titanium organic complex treatment was higher than it was in the self-pollination treatment (i.e., without application of this compound; Table 2).

Table 2. The effect of titanium organic complex application on the average number of seeds in apple fruit (pieces).

Treatment	Average Number of Seeds in Fruit (pcs)	
	Year	
	1st Year	2nd Year
Self-pollination	2.5 ± 0.89 a	1.5 ± 0.64 a
Self-pollination + titanium organic complex	4.5 ± 0.69 b	5.6 ± 0.62 b
Open-pollination	8.0 ± 0.97 c	7.8 ± 0.75 c
Open-pollination + titanium organic complex	9.5 ± 0.87 d	9.8 ± 0.59 d

Statistical analysis was performed separately for each year; significant homogenous groups were determined by Tukey's Honest Significant Difference test; a,b,c,d—means in columns followed by different letters differ at $p < 0.05$; ±—standard error; number of fruits evaluated for each treatment $n = 120$.

3.3. Fruit Weight, Length and Diameter

In the first year of the study, there were no significant differences in fruit length between self-pollination and self-pollination + titanium organic complex treatments. The highest fruit length was observed in the open pollination + titanium organic complex treatment. Similarly, there were no significant differences in fruit diameter between the self-pollination and self-pollination + titanium organic complex treatments. The highest fruit diameter was obtained in the open-pollination + titanium organic complex treatment (Table 3). In the second year of the study, the lowest fruit length was obtained in the self-pollination treatment, whereas the highest was in the open-pollination + titanium organic complex treatment. Fruits harvested from the trees in the open-pollination + titanium organic complex treatment were characterized by higher length than were fruits harvested in the open-pollination condition. A similar relation was observed for the self-pollination + titanium organic complex and self-pollination treatments. In the second year of the study, the highest fruit diameter was obtained in the open-pollination + titanium organic complex and open pollination treatments, and there were no significant differences between these two treatments. However, in self-pollinated trees, a higher fruit diameter was observed in the self-pollination + titanium organic complex treatment compared to self-pollination (Table 3).

Table 3. The effect of titanium organic complex application on length and diameter of apple fruit (mm).

Treatment	Fruit Length (mm)		Fruit Diameter (mm)	
	Year			
	1st Year	2nd Year	1st Year	2nd Year
Self-pollination	42.8 ± 0.98 a	38.3 ± 0.69 a	53.1 ± 0.77 a	55.2 ± 0.91 a
Self-pollination + titanium organic complex	41.2 ± 0.87 a	48.2 ± 0.72 b	56.6 ± 0.71 ab	60.2 ± 0.86 b
Open-pollination	54.7 ± 0.79 b	57.1 ± 0.79 c	69.0 ± 0.82 b	71.2 ± 0.79 c
Open-pollination + titanium organic complex	58.9 ± 0.95 c	60.1 ± 0.80 d	74.9 ± 0.84 c	72.8 ± 0.82 c

Statistical analysis was performed separately for each year, significant homogenous groups were determined by Tukey's Honest Significant Difference test; a–d—means in columns followed by different letters differ at $p < 0.05$; ±—standard error; number of fruits evaluated for each treatment $n = 120$.

Regardless of titanium organic complex application, in both years of the study fruits harvested from open-pollinated trees were characterized by higher weight than were fruits harvested from self-pollinated trees. In the open-pollination + titanium organic complex treatment, the weight of fruit was higher than it was in the open-pollination treatment. A similar relation was proven for the self-pollination + titanium organic complex and self-pollination treatments. In both years of the study, the highest fruit weight was obtained in the open pollination + titanium organic complex treatment (Figure 4).

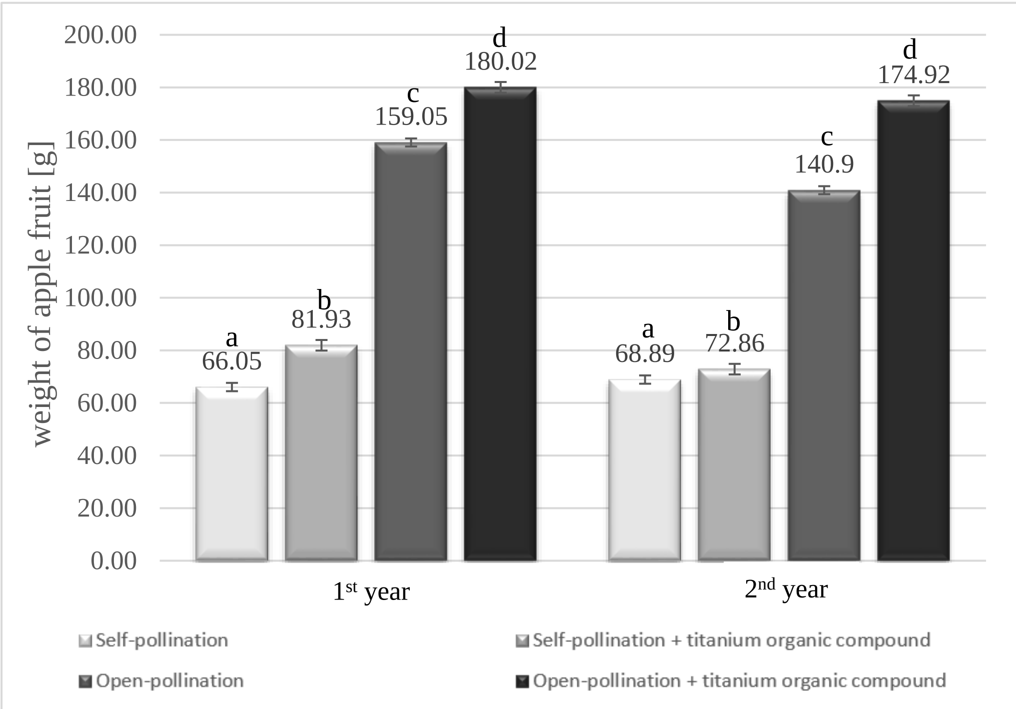

Figure 4. The effect of titanium organic complex application on the weight of apple fruit (g). Statistical analysis was performed separately for each year, significant homogenous groups were determined by Tukey's Honest Significant Difference test; a–d—means within each year followed by different letters differ at $p < 0.05$; number of fruits evaluated for each treatment $n = 120$.

4. Discussion

The average titanium content in surface soils is 0.33%. In soils, this element mainly occurs in minerals that are not water soluble and are resistant to weathering [40]; therefore, the availability of titanium from soil to plants is limited. Titanium is considered a beneficial element for plants [26,41]. Studies on titanium's effect on plants began at the beginning of 20th century, but the development of a titanium organic compound that was water-soluble, stable, nontoxic, and particularly effective in promoting plant growth and development was a milestone for considering titanium beneficial for plants [27]. Further studies have proven that titanium organic complex positively affects nutrient uptake by plants, content of chlorophyll, weight of phytomass, and yield quantity and quality [28,31–33].

Dyki et al. [35] were the first scientists to study the effect of titanium organic complex on the pollination process. They observed better adhesion of pollen grains to the stigma and their better germination on the stigma of tomato and cucumber after application of titanium organic complex. Moreover, they proved better seed setting in cucumber fruit after foliar spraying of plants with titanium organic complex. Besides these, few studies show the effect of titanium organic complex on the pollination process and consequently on seed setting and fruit development. Problems with the effectiveness of the pollination process in orchards, due to unfavourable environmental conditions and low activity of pollinators, occur with increasing frequency. Therefore, we designed an experiment aimed to test the beneficial effect of titanium organic compound on the effectiveness of pollination and, consequently, the fertilization process and fruit development of apple.

Our study confirmed that the application of titanium organic complex improved pollen adhesion to the stigma, pollen germination on stigma, ovule fertilization (with other compatible cultivars), and, consequently, fruit development of apple cv. Topaz. Apple trees are self-incompatible species; thus, to set good quality fruits, the trees need to be pollinated with pollen of other cultivars [42–44]. Fruit setting as a result of self-pollination in the genera *Pyrus* and *Malus* occurs in order to improve the biological preservation of the species. This process is not frequent and is irrelevant for the commercial production of fruits. Under unfavourable weather conditions, during low activity of pollinators and in the absence of compatible pollen, the incompatibility may be broken, which in turn leads to the fruit set. However, such fruits are of poor quality and do not present commercial value [45–47]. Despite the self-incompatibility of apple trees, we decided to test the effect of titanium organic complex on the pollination process separately for self and open pollination because we did not assess the replacement of open pollination through product application, but rather its supportive function. In our study, self-pollination should be considered as simulating the low activity of pollinators. Unfavorable environmental conditions increasingly have a negative effect on the activity of pollinators, which leads to the decreasing effectiveness of the pollination process [1,3]. Therefore, supporting the pollination process through application of titanium organic compounds may be favorable, especially under the condition of a low pollinator activity.

Pistils of apple flowers have five independent pistil styles that join half of the pistil style length. One hundred to two hundred pollen grains can be on each stigma of well-pollinated apple or pear flowers [48]. Thus, the competition of pollen tubes is very high, and only genetically compatible pollen tubes from well-formed pollen grains reach ovaries and lead to fertilization and the formation of the best quality seeds [49]. The results of our study proved that the application of titanium organic complex promoted pollen adhesion to the stigma and pollen germination on stigma. In both years of the study, both self-pollination and open-pollination treatments led to the average number of pollen grains germinating on stigma, which was higher after the application of titanium organic complex compared to nontreated plants. Similarly, Dyki et al. [35] observed a higher number of pollen grains germinating on stigmas of cucumber and tomato after application of titanium organic complex. In apple blossoms, stigmas are covered with a sticky liquid secretion [8,9], which increases the adhesion of pollen grains to the stigma during pollination [1,11,50]. We hypothesise that better adhesion of pollen grains to the stigma after application of titanium organic complex may result from the higher production of sticky secretion in the papilla of apple stigma, but it requires further studies for confirmation.

In both years of our study, the average number of pollen tubes in one-third, one-half, and at the end of a pistil style was higher after application of titanium organic complex compared to nontreated plants, in both self- and open pollinated flowers. According to Losada and Herrero [51], the kinetics of pollen tubes is related to the site in the pistil. The growth rate of pollen tubes is slower on the stigma, faster in the pistil style and again slower in the ovary, which results from chemical changes in the obturator secretory gland located near the micropyle. When the pollen tubes appear in the style of the pistil, the obturator secretes proteins, saccharides and glycoproteins, which are used by the growing pollen tubes [51]. The higher number of pollen tubes growing through the pistil style after application of titanium organic complex may be connected to the effect of titanium organic complex on the increasing secretion by the obturator, but this hypothesis also requires further study.

In our study, in a one-half length of pistil style, we observed the strong reduction in the number of pollen tubes compared to the number of pollen tubes in a one-third length of pistil style. Moreover, fewer pollen tubes grew to the end of the pistil style compared to the number of pollen tubes in one-third of the pistil style. In the reproduction of angiosperms, pollen tubes grow from stigma through the pistil style to the ovary, and their role is to deliver the male gametes for fertilization. The competition between growing pollen tubes is related to the fact that the ovaries are able to reach pollen tubes that "carry" high quality genetic material, which ensures the better development of seeds. The transmitting tissue of pollen tube is the place of possible pollen tube competition. The number of pollen grains germinating on flower stigmata often exceeds the number of ovules in the ovaries of pollinated flowers. Studies have shown that, to obtain well-developed seeds, the competition of many pollen tubes is necessary [52]. Hormaza et al. [49] studied the pollen tube dynamics in sweet cherry (*Prunus avium* L.) and observed constant reduction of the number of pollen tubes that grew through the pistil style.

Apple blossoms usually have two viable ovules in each ovary chamber. To ensure proper and symmetric development of apple fruit, each of the five pistil stigmas should be pollinated with compatible pollen. Effective pollination should lead to apple fruit containing around 10 seeds [53,54]. In our study, after application of titanium organic complex the number of pollen tubes reaching to the end of pistil style was always higher than 10. This relation was not observed for nontreated plants. Such a number of pollen tubes allows for fertilization of all ovules in an ovary, which may result in setting good-quality seeds. In both years of the study, the average number of seeds set in fruits developed from both self-pollinated and open-pollinated flowers was always higher after application of titanium organic complex, compared to nontreated plants. The favourable effect of titanium organic complex on seed setting results from pollination that is more effective, which was observed after application of this compound. Similarly, Dyki et al. [35] observed a higher number of seeds set in cucumber fruits after foliar application of titanium organic compound. In cultivation of onion for seed production, Janas [36] demonstrated a higher seed yield, as well as better quality and health status after foliar spraying with titanium organic complex. In addition, Radkowski et al. [37] proved that foliar application of titanium organic complex resulted in higher seed yield, thousand-grain weight, and germination capacity of timothy seeds.

According to Keulemans et al. [55], fruit weight is positively correlated with the number of well-developed seeds. In both years of our study, the average weight of individual apple fruit was the highest in the open-pollination + titanium organic compound treatment. In addition, the average number of seeds set in fruit was the highest in the open-pollination + titanium organic compound treatment. The weight of fruit is affected by the number of seeds in the fruit. An apple ovary has 10 ovules, so 10 seeds may form in fruit, due to effective pollination and fertilization of all ovules [56,57]. However, such favourable conditions for fertilization occur very rarely. In our study, using the open-pollination + titanium organic complex treatment, the average number of seeds in fruit was 9.5 and 9.8, depending on the year. Gibberellins and auxins present in seeds affect fruit development and fruit size; therefore, fruits with two to three seeds are characterized by a low weight and do not present commercial value [57]. The beneficial effect of titanium organic complex on fruit and seed yield was proven by several studies. Spraying tomato plants with titanium organic complex, Dobromilska [29] observed a higher total and marketable fruit yield after application, compared to nontreated plants. Titanium organic complex also had a beneficial effect on the fruit yield of eggplant, especially in years with unfavorable growing conditions [36]. Similarly, Ochmian et al. [30] observed a higher total yield and weight of 100 fruits of raspberry after application of titanium organic complex compared with nontreated plants.

5. Conclusions

Our study proved that titanium organic complex had a beneficial effect on pollination and fertilization processes by promoting adhesion of pollen grains to the stigma, growth of pollen tubes through pistil style, and consequently seed setting and fruit development. The presented study demonstrated that in combinations treated with a titanium organic complex, the number of pollen grains on pistil stigma was significantly higher than in nontreated combinations. With the reduction of pollen tubes above 90% at the end of pistil style, such a high number of pollen grains allowed more pollen tubes to grow into ovary and fertilize ovules more efficiently. The application of this compound may also be beneficial in the case of low frequency of pollinating insects and may support effective fertilization by self-pollination and pollen from pollinator cultivar, which will result in a higher weight of fruit. In case of open-pollination, the application results in better competition between pollen tubes growing through the pistil style, which leads to fertilization of ovules by pollen tubes “carrying” the most genetically valuable material. This effect of titanium organic complex may be relevant in cases of unfavorable weather and pollinator conditions during flowering (i.e., prolonged rainfall, extremely high or low temperatures, and low activity of pollinators). Various environmental anomalies appear cyclically, but have recently begun to appear more frequently. Therefore, the application of titanium organic complex prospectively supports the proper course of flowering, pollination and fertilization which may have an effect on yield quality and quantity.

Author Contributions: M.B. designed the experiment, methodology and carried out the experiment. A.K. worked up the results and prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Polish Ministry of Higher Education DS 3500.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: Monika Bieniasz declares no conflict of interest. Anna Konieczny is employee of INTERMAG Sp. z o.o., Olkusz, Poland, a company active in a sector of fertilizers and biostimulants.

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