




## Article

# Impact of Nitrogen Fertilization on Fruit Parameters of Four *Cucurbita maxima* Cultivars Grown in Poland

Karolina Kaźmińska <sup>1</sup>, Aleksandra Korzeniewska <sup>1</sup>, Renata Słomnicka <sup>1</sup> , Agnieszka Gniazdowska <sup>2</sup>   
and Grzegorz Bartoszewski <sup>1,\*</sup> 

<sup>1</sup> Department of Plant Genetics Breeding and Biotechnology, Institute of Biology, Warsaw University of Life Sciences, ul. Nowoursynowska 159, 02-776 Warsaw, Poland; karolina\_kazminska@sggw.edu.pl (K.K.)

<sup>2</sup> Department of Plant Physiology, Institute of Biology, Warsaw University of Life Sciences, ul. Nowoursynowska 159, 02-776 Warsaw, Poland; agnieszka\_gniazdowska@sggw.edu.pl

\* Correspondence: grzegorz\_bartoszewski@sggw.edu.pl

**Abstract:** *Cucurbita maxima* Duchesne is a cucurbit species cultivated worldwide due to its economic and nutritional value. *C. maxima* winter squash fruits are rich in carotenoids and dietary fiber, making them valuable raw materials for food products, especially for infants and children as ingredients in pomaces, mousses, and juices. Therefore, both the yield and quality of fruits are economically important traits. These traits depend mainly on factors such as cultivar and plant growing conditions, including nitrogen fertilization. This study aimed to evaluate the nitrate content in the fruit as well as the yield and fruit parameters of four winter squash cultivars, Bambino, Justynka F<sub>1</sub>, Otylia F<sub>1</sub>, and Mammoth Gold, under different nitrogen fertilization regimes of 100%, 50%, and 30% of the standard nitrogen dose. Two field experiments were conducted in 2021 and 2022. This study revealed that the nitrogen dose had a significant effect on the nitrate content in the fruit flesh. A lower nitrogen dose resulted in a lower nitrate content in the fruit. In addition, a decrease in fruit yield occurred when the plants were fertilized with 30% of the standard nitrogen dose, whereas when the plants were fertilized with 50% of the standard nitrogen dose, the fruit yield remained relatively unaffected. The cultivars used in the experiment differed significantly in terms of the traits studied. The lowest nitrate content in fruit flesh was found for Justynka F<sub>1</sub>; however, the most favorable fruit parameters were obtained for Otylia F<sub>1</sub>, for which, in both years of the experiment, high yield and fruit weight, dry matter content, and the thickest flesh among the tested cultivars were achieved. This study contributes to the understanding of fruit quality and cultivation techniques for winter squash under the climatic conditions of Poland.

**Keywords:** cucurbits; fruit yield and quality; nitrate content; sustainable agriculture



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## 1. Introduction

*Cucurbita maxima*, commonly known as winter squash, originated in the New World and was introduced to Europe after the discovery of the American continent [1–4]. It has a long tradition of cultivation in Mediterranean countries, including Italy and France, where many valuable cultivars have been developed [5,6]. Winter squash cultivation has spread to Central and Eastern European countries, including Germany, Poland, Lithuania, Ukraine, and Russia [1,2]. This spread was possible because, in various countries, different germplasms have been selected and bred for acclimatization to the local climate, better agronomic characteristics, various fruit shapes, higher fruit yields, and higher carotenoid contents [4,6]. Currently, in Poland, winter squash, together with cucumber, zucchini, and

Styrian pumpkins, is a commonly cultivated cucurbit crop [7]. According to the latest statistics, since 2020, Poland has been a leading producer of pumpkins in the EU. With more than 400 thousand tons produced in 2022, Poland's share of the total pumpkin harvest in the EU was nearly 40% [8,9].

*C. maxima* fruits have positive effects on human health. Winter squash fruits are rich in nutrients such as carotenoids, sugars, vitamins C and E, minerals, and fiber [10,11]. Therefore, winter squash fruits are valuable raw materials for the production of new health-promoting foods [12–14]. Fruit preserves made from *C. maxima*, such as juices, pomaces, and mousses, are valuable for infant and young child nutrition. For this reason, the fruits of winter squash must meet strict quality standards, such as the nitrate content of the raw materials [15]. Human exposure to nitrate reaction products and metabolites, including nitrates, may cause direct risks to human health. Nitrate reduction can occur directly in the plant material or after consumption of the product, so the use of low-nitrate vegetables in the food industry is recommended, especially in products for children [16]. Like cucumber, pumpkin is a vegetable with a low capacity for nitrate accumulation (200–500 mg/kg) [17]. Unfortunately, *C. maxima* fruits can accumulate relatively high amounts of nitrates due to several factors. Nitrogen fertilization, genotype, and climatic conditions strongly influence the variability of nitrate content in plants [16,18,19]. In the European Union (EU), the Nitrate Directive [20,21] requires farmers to adopt improved nitrogen management practices. Nitrogen positively affects plant growth and development. However, an excessive nitrogen supply leads to increased vegetative growth and has a limiting effect on root and fruit development. Therefore, the effective use of nitrogen influences both environmental safety and crop productivity [22].

Efforts are currently underway to identify *C. maxima* cultivars with low nitrate accumulation capacity to meet standards for fruit intended for baby food processing. These standards also consider fruit size, flesh thickness, and dry matter content. Therefore, there is a need to identify winter squash cultivars and develop a cultivation system to reduce nitrate accumulation in fruits and meet the expectations of the processing industry.

Several studies have indicated that fluctuations in the winter squash fruit parameters are affected by cultivar and environmental conditions. To date, only a few studies have investigated the composition and yield of winter squash produced from temperate climate zones, such as those in Poland [18,19,23]. There is also insufficient knowledge on nitrate accumulation by *C. maxima* cultivars under changing nitrogen fertilization conditions.

This study aimed to investigate changes in the winter squash fruit parameters, including nitrate content, as well as yield and fruit size components based on different cultivars and three nitrogen fertilization regimes, under the climatic conditions of central Poland.

## 2. Materials and Methods

### 2.1. Plant Material

Four cultivars of the winter squash *Cucurbita maxima* Duchesne were used in the experiments (Figure 1). Three of them represented Eastern European cultivars similar to the old cultivar Jaune Gros de Paris, whereas one represented the Hubbard-type cultivar. The first group included the traditional Polish winter squash cultivar Bambino, the large-fruited Mammoth Gold, and the Polish hybrid cultivar Otylia F<sub>1</sub>. The Hubbard-type cultivar was the Polish hybrid Justynka F<sub>1</sub> (Figure 1). The cultivars Bambino and Mammoth Gold had a vine growth habit, and Otylia F<sub>1</sub> and Justynka F<sub>1</sub> had a semi-bush growth habit. Seeds of all cultivars used in the experiments were purchased from W. Legutko Seed Company (W. Legutko Przedsiębiorstwo Hodowlano-Nasienne, Jutrosin, Poland).



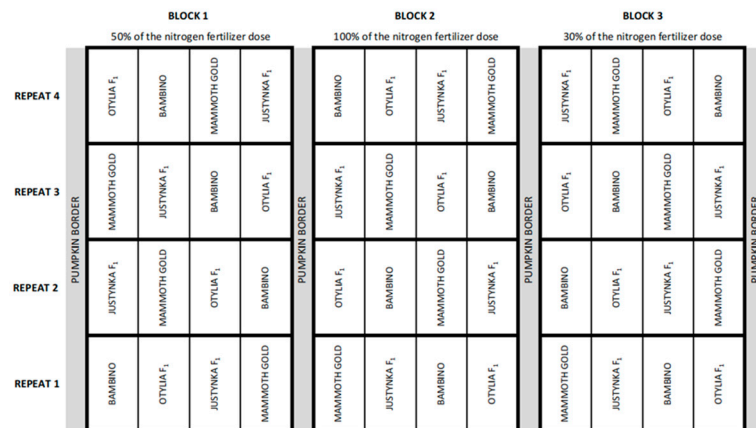
**Figure 1.** Photos of whole fruits and fruit cross-sections of the four cultivars tested: (A,B)—Bambino; (C,D)—Otylia F<sub>1</sub>; (E,F)—Justynka F<sub>1</sub>; (G,H)—Mammoth Gold. (I,J) View of the experiment (2022): (I)—plants approximately four weeks after planting; (J)—ripe fruit collected and arranged for fruit assessment and sample collection. Scale bars: 10 cm.

## 2.2. Field Experiments

Two open-field experiments were conducted at the Experimental Station ‘Wolica’ of the Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences, Poland (52.14582336609585, 21.06815958259584). Field trials were conducted in 2021 and 2022 on Class IVb soil with a granulometric composition of sandy loam, a pH of 6.2, and a humus content of 1.3%.

The experiments were set up using a split-plot design with three blocks and four replicates of 24 plants each. In total, 48 plots, each approximately 50 m<sup>2</sup>, were assessed each year. A border of a different pumpkin cultivar separated the experimental blocks. The detailed scheme of the experiment is shown in Figure 2. The factors in the experiments were three nitrogen fertilization rates (factor A) and four cultivars (factor B). Nitrogen dose served as the whole-plot factor, whereas cultivar served as the split-plot factor.

Seeds of all the tested cultivars were sown in multiple pots containing peat substrate in mid-May. The seedlings were planted on 30 May, according to the scheme shown in Figure 2. Approximately three weeks after planting, the copper-based fungicide Nordox 75 WG (Nordox, Oslo, Norway) was applied at 1 kg/ha for plant protection. During cultivation, hand weeding was performed as needed. The plants were pollinated by insects. In September, approximately 110 days after sowing, fruits were harvested and used for phenotypic evaluation.



**Figure 2.** Scheme of the experiments conducted in 2021 and 2022 at the Experimental Station ‘Wolica’ of the Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences, Poland. One plot (cultivars: Bambino, Otylia F<sub>1</sub>, Justynka F<sub>1</sub>, and Mammoth Gold) included 24 plants (three rows of eight plants each), with a spacing of 1.6 × 1.5 m (2 m<sup>2</sup> for each plant) and an area of 50 m<sup>2</sup> (4.8 × 10.4 m). One block contained 288 plants, and its area was 921.6 m<sup>2</sup> (19.2 × 48 m). The borders were 1.6 m wide.

### 2.3. Weather Conditions

The weather conditions for the field experiment period, from June to September 2021 and 2022, are compared with the multiyear (30-year) average in Table S1. The individual study years differed from each other in terms of temperature and precipitation. The year 2021 was characterized by higher average temperatures in July and September as well as greater rainfall in July and August, whereas in 2022, lower rainfall in June and August was recorded. Rainfall was half of the multiyear average. Low precipitation in August (34.4 mm compared with the multiyear average of 65.2 mm) and high temperatures (2.6 °C higher than the multiyear average) resulted in drought at the end of the season.

### 2.4. Fertilization Regimes

To determine the fertilizer requirement (NPKMg), a soil nutrient content analysis was conducted before the experiments at an agrochemical service station (OSChR, Warsaw, Poland). Fertilizer amounts were then calculated on the basis of the recommended nutrient doses for winter squash cultivation: (N) 150 kg/ha, phosphorus (P) 120 kg/ha, potassium (K) 350 kg/ha, and magnesium (Mg) 130 kg/ha [24]. The fertilizers (NPKMg) were applied separately for each experimental block. The following single-component fertilizers were used: ammonium nitrate (34% N), triple superphosphate (46% P), potassium sulfate (50% K), and magnesium sulfate (15% Mg).

Three nitrogen doses were tested: 30%, 50%, and 100% of the optimal nitrogen level recommended for winter squash production. Each nitrogen dose represented a different experimental block: block 1—50% N, block 2—100% N, and block 3—30% N. For the 30% N treatment, the entire nitrogen fertilizer dose was applied two weeks before planting. However, for the 50% N and 100% N treatments, 2/3 of the nitrogen fertilizer was applied two weeks before planting, and 1/3 was applied three weeks after planting.

### 2.5. Phenotypic Evaluation of the Fruit and Yield

Fruit characteristics included average fruit weight (kg), number of fruits per plant, marketable yield (kg/100 m<sup>2</sup>, kg/one are), fruit diameter and length (cm), and flesh thickness (cm). The ripe, fully grown fruits from each plot/replicate were collected, weighed, and counted. The marketable fruit yield was estimated using the following formula:  $\left( \frac{\text{fruit number} \times \text{fruit weight}}{\text{number of plants per plot per replicate}} \right)$  and is calculated as kg per 100 m<sup>2</sup> (one are). For fruit

measurements (length, diameter, and flesh thickness), a minimum of six fruits from a plot/replicate were taken. The data were collected and organized using Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA).

### 2.6. Nitrates and Dry Matter Content in the Fruits

The samples of fruit flesh were collected from ripe, full-grown fruits. Six fruits were selected from each plot/replicate for nitrate assessment, sampled for flesh (approximately 100 g), and used for measurements. The nitrate content (mg/kg) was determined by the spectrophotometric method (flow injection analysis—FIA, [25]). To determine dry matter content, fruit fragments taken from a given cultivar were combined in a block of experiments. A total of twelve samples were prepared, with four samples for each block. The dry matter content (g/100 g FW) was determined by drying the samples at 102 °C. All the measurements were carried out in an accredited laboratory of NQAC Rzeszów (Nestlé Quality Assurance Center, Rzeszów, Poland).

### 2.7. Statistical Analysis

The results of the analyzed characteristics are presented as the mean values with standard deviations quantified for both years of cultivation. A mixed-design analysis of variance model (split-plot ANOVA) was used to evaluate the effects of nitrogen fertilization rates (factor A) and cultivar (factor B) on the nitrate content of fruit flesh, fruit yield, fruit weight, fruit number, fruit length, fruit diameter, and flesh thickness. The results were analyzed using Statistica 13.1 PL software (TIBCO Software, Inc., Palo Alto, CA, USA) at a significance level of  $p < 0.05$ . Graphs were prepared using GraphPad Prism v7 (GraphPad Software Inc., San Diego, CA, USA).

## 3. Results

Variance analysis indicated that the nitrogen fertilization dose (factor A) had a considerable effect on the nitrate content and fruit yield in both years of the experiment (Table 1). Additionally, a significant effect of cultivar (factor B) was observed for all the examined traits. The interaction effect of the two factors ( $A \times B$ ) was significant for fruit weight in both years and for nitrate content in 2022.

**Table 1.** Variability of examined traits of the four *C. maxima* cultivars under the three nitrogen fertilization treatments. Experimental Station ‘Wolica’, 2021 and 2022. A mixed-design analysis of variance model (split-plot ANOVA) was used to evaluate the effects of nitrogen fertilization rates (factor A) and cultivars (factor B) on fruit traits. Asterisks indicate significance levels: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

| Source of Variation | Experiment/Year | Trait                   |     |                                      |     |                   |     |                  |     |                   |     |                     |     |                            |     |
|---------------------|-----------------|-------------------------|-----|--------------------------------------|-----|-------------------|-----|------------------|-----|-------------------|-----|---------------------|-----|----------------------------|-----|
|                     |                 | Nitrate content (mg/kg) |     | Fruit Yield (kg/100 m <sup>2</sup> ) |     | Fruit Weight (kg) |     | Fruit Number (n) |     | Fruit Length (cm) |     | Fruit Diameter (cm) |     | Fruit Flesh Thickness (cm) |     |
| Blocks              | I/2021          | 2.30                    |     | 0.40                                 | 0   | 0.34              |     | 0.44             |     | 5.45              |     | 0.27                |     | 0.45                       |     |
|                     | II/2022         | 1.19                    |     | 0.53                                 |     | 4.46              |     | 10.36            | *   | 2.20              |     | 1.12                |     | 1.44                       |     |
| Factor A            | I/2021          | 16.69                   | *   | 5.19                                 | *   | 2.99              |     | 16.64            | **  | 13.31             | **  | 2.09                |     | 8.56                       | *   |
|                     | II/2022         | 35.26                   | *** | 13.89                                | **  | 56.16             | *** | 9.48             | *   | 1.48              |     | 18.83               | **  | 3.12                       |     |
| Factor B            | I/2021          | 15.74                   | **  | 65.37                                | *** | 151.74            | *** | 95.92            | *** | 169.76            | *** | 129.90              | *** | 96.64                      | *** |
|                     | II/2022         | 9.12                    | *** | 307.38                               | *** | 851.87            | *** | 710.92           | *** | 197.60            | *** | 269.34              | *** | 139.63                     | *** |
| Interaction A × B   | I/2021          | 1.75                    |     | 1.58                                 |     | 3.20              | *   | 1.51             |     | 2.58              | *   | 0.23                |     | 1.39                       |     |
|                     | II/2022         | 6.66                    | *** | 1.81                                 |     | 4.94              | **  | 1.85             |     | 0.57              |     | 1.49                |     | 2.08                       |     |

### 3.1. Effects of Nitrogen Fertilization Dose on Fruit Traits

Reducing the nitrogen fertilization doses to 50% and 30% resulted in a significant reduction in the average nitrate content in the fruits (Table 2, Supplementary Tables S2 and S3). When the nitrogen dose was reduced to 30%, the average nitrate content in the fruit

flesh did not exceed 200 mg/kg in either year of the experiment (143 mg/kg in 2021 and 129 mg/kg in 2022). However, when the nitrogen dose was reduced to 50%, variable results were obtained: the average nitrate content was 89 mg/kg in 2021 and 260 mg/kg in 2022. When the optimal nitrogen dose (100%) was applied, in both years, the nitrate content exceeded 300 mg/kg (305 mg/kg in 2021 and 492 mg/kg in 2022). A comparison of the two years revealed that the average nitrate content in fruits in 2022—a year with drought in August—was significantly higher (129 to 493 mg/kg) than that in 2021 (89 to 305 mg/kg).

**Table 2.** Effect of nitrogen fertilization dose on nitrate content in fruit flesh and average values of yield-related traits and fruit dimensions in two years of experiments, 2021 and 2022. Letters indicate homogenous groups;  $p < 0.05$ .

| Nitrogen Dose (Factor A) | Experiment/Year | Trait                   |   |                                      |   |                   |   |                  |   |                   |   |                     |   |                            |   |
|--------------------------|-----------------|-------------------------|---|--------------------------------------|---|-------------------|---|------------------|---|-------------------|---|---------------------|---|----------------------------|---|
|                          |                 | Nitrate content (mg/kg) |   | Fruit Yield (kg/100 m <sup>2</sup> ) |   | Fruit Weight (kg) |   | Fruit Number (n) |   | Fruit Length (cm) |   | Fruit Diameter (cm) |   | Fruit Flesh Thickness (cm) |   |
| 100%                     | I/2021          | 305.0                   | c | 362.1                                | b | 5.3               | a | 1.7              | b | 21.2              | a | 25.1                | a | 4.2                        | a |
|                          | II/2022         | 492.6                   | b | 266.8                                | b | 5.4               | b | 1.3              | a | 20.6              | a | 25.5                | b | 4.3                        | a |
| 50%                      | I/2021          | 89.0                    | a | 334.9                                | b | 5.9               | b | 1.4              | a | 22.8              | b | 26.8                | b | 4.8                        | b |
|                          | II/2022         | 260.2                   | a | 275.5                                | b | 5.6               | b | 1.3              | a | 21.7              | a | 26.4                | b | 4.4                        | a |
| 30%                      | I/2021          | 143.0                   | b | 296.4                                | a | 4.9               | a | 1.4              | a | 20.6              | a | 24.9                | a | 4.4                        | a |
|                          | II/2022         | 129.3                   | a | 235.1                                | a | 4.7               | a | 1.3              | a | 20.6              | a | 24.2                | a | 4.2                        | a |

The nitrogen dose significantly affected fruit yield in both years of the experiment (Table 2, Supplementary Tables S2 and S3). The average fruit yields at the 100% and 50% nitrogen doses did not differ significantly between the years and were 362 and 335 kg/100 m<sup>2</sup> in 2021 and 267 and 276 kg/100 m<sup>2</sup> in 2022, respectively. A significantly lower fruit yield (296 kg/100 m<sup>2</sup> in 2021 and 235 kg/100 m<sup>2</sup> in 2022) was obtained only at the 30% nitrogen dose, which was 12% (2022) to 20% (2021) lower than the standard dose.

The effect of nitrogen fertilization dose on fruit weight was evaluated. In 2022, the overall average weight of single fruits was significantly lower when the fertilization rate was reduced to 30% (4.7 kg compared with 5.6 kg at 50% and 5.4 kg at 100%). Different results were obtained in 2021, when the highest fruit weight was recorded at the 50% nitrogen dose (5.9 kg), while no significant difference was observed between the 100% and 30% nitrogen doses (Table 2, Supplementary Tables S2 and S3).

The average number of fruits per plant in 2022 did not differ regardless of the nitrogen fertilization rate (1.3 fruits per plant), whereas in 2021, the highest number of fruits was observed with standard fertilization (1.7 compared with 1.4 at 50% and 30%) (Table 2, Supplementary Tables S2 and S3).

Various effects of nitrogen fertilization doses on fruit dimensions (length, diameter, and flesh thickness) were observed among the tested varieties. In 2021, the largest fruit dimensions were observed at a fertilization rate of 50% (Table 2). In the second year of the experiment (2022), a significant difference was noted only for fruit diameter, which was lowest at the 30% nitrogen dose (Table 2, Supplementary Tables S2 and S3). A relatively high fruit yield was obtained in 2021, ranging from 296 to 362 kg/100 m<sup>2</sup>, whereas in 2022, it ranged from 235 to 276 kg/100 m<sup>2</sup>. The number of fruits was also greater in 2021, ranging from 1.4 to 1.7 per plant, than it was 1.3 in 2022. The average fruit weight and dimensions (length, diameter, and flesh thickness) were similar in both years of the experiments (Table 2, Supplementary Tables S2 and S3).

### 3.2. Cultivar Performance

The cultivars chosen for the experiment differed from each other in the traits taken into consideration (Table 3, Supplementary Tables S2 and S3). Evaluating the results obtained

for individual cultivars in both years of the experiment, the lowest nitrate content in the fruit flesh was found for Justynka F<sub>1</sub>, with averages of 27 mg/kg in 2021 and 183.9 mg/kg in 2022. The average nitrate content of the Otylia F<sub>1</sub> and Mammoth Gold cultivars was similar in both years: 293.0 and 261 mg/kg in 2021 and 389.0 and 293.7 in 2022, respectively. For the Bambino cultivar, the average nitrate content differed from that of the other cultivars in 2021, being at an intermediate level (135 mg/kg), whereas in 2022, it was similar to that of the Otylia F<sub>1</sub> and Mammoth Gold cultivars (Table 3, Supplementary Tables S2 and S3). In 2021, the nitrate content for Justynka F<sub>1</sub> cultivar was very low at all nitrogen fertilization doses and did not exceed 100 mg/kg. In 2022, the average nitrate content also did not exceed 100 mg/kg for this cultivar, but only for 50% and 30% nitrogen fertilization doses. The nitrate content in the Bambino and Otylia F<sub>1</sub> fruits was significantly lower in both years at 50% and 30% of nitrogen fertilization, but the lowest nitrate concentration at 50% N fertilization was observed in Justynka F<sub>1</sub>. The results for Mammoth Gold were similar to those for these two cultivars in the first year of the study, and no significant differences in nitrate content in the fruit flesh depending on the nitrogen doses were found in the second year (Figure 3, Supplementary Tables S2 and S3).

**Table 3.** Effect of four *Cucurbita maxima* cultivars treated with three different nitrogen fertilization doses on nitrate contents in fruit flesh, average values of yield-related traits, and fruit dimensions across two years of experiments, 2021 and 2022. A minimum of six fruits from a plot/replicate were taken for fruit length, diameter, and flesh thickness measurements. Letters indicate homogenous groups;  $p < 0.05$ .

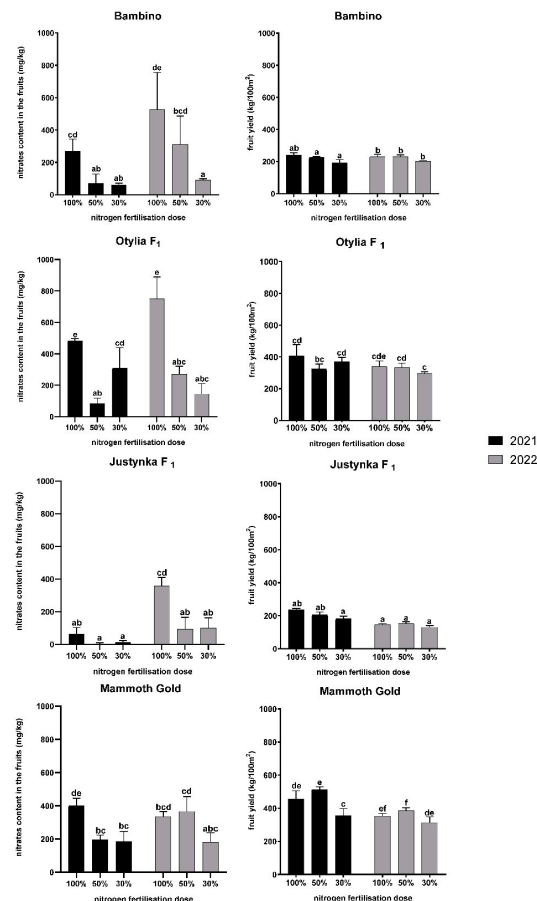
| Cultivar (Factor B)     | Experiment/Year | Trait                   |   |                                      |   |                   |   |                  |    |                   |   |                     |   |                            |   |
|-------------------------|-----------------|-------------------------|---|--------------------------------------|---|-------------------|---|------------------|----|-------------------|---|---------------------|---|----------------------------|---|
|                         |                 | Nitrate Content (mg/kg) |   | Fruit Yield (kg/100 m <sup>2</sup> ) |   | Fruit Weight (kg) |   | Fruit Number (n) |    | Fruit Length (cm) |   | Fruit Diameter (cm) |   | Fruit Flesh Thickness (cm) |   |
| Bambino                 | I/2021          | 135.0                   | b | 230.8                                | a | 4.5               | b | 1.1              | a  | 19.8              | b | 26.0                | b | 4.4                        | b |
|                         | II/2022         | 309.5                   | b | 220.7                                | b | 4.8               | b | 1.1              | a  | 20.4              | b | 25.7                | b | 3.8                        | b |
| Otylia F <sub>1</sub>   | I/2021          | 293.0                   | c | 394.6                                | b | 6.9               | c | 1.2              | ab | 22.7              | c | 29.0                | c | 5.4                        | c |
|                         | II/2022         | 389.0                   | b | 322.0                                | c | 7.2               | c | 1.0              | a  | 23.5              | c | 29.0                | c | 6.1                        | c |
| Justynka F <sub>1</sub> | I/2021          | 27.0                    | a | 230.3                                | a | 2.0               | a | 2.4              | c  | 14.8              | a | 18.4                | a | 3.4                        | a |
|                         | II/2022         | 183.9                   | a | 142.9                                | a | 1.5               | a | 2.1              | b  | 12.8              | a | 17.3                | a | 3.2                        | a |
| Mammoth Gold            | I/2021          | 261.0                   | c | 468.7                                | c | 7.4               | c | 1.3              | b  | 28.9              | d | 29.0                | c | 4.7                        | b |
|                         | II/2022         | 293.7                   | b | 350.9                                | d | 7.4               | c | 1.1              | a  | 27.1              | d | 29.6                | c | 4.1                        | b |

The best-yielding varieties were Mammoth Gold (468.7 kg/100 m<sup>2</sup> in 2021 and 350.9 kg/100 m<sup>2</sup> in 2022) and Otylia F<sub>1</sub> (394.6 kg/100 m<sup>2</sup> in 2021 and 322 kg/100 m<sup>2</sup> in 2022), whereas the lowest yields were obtained for Justynka F<sub>1</sub> (230.3 kg/100 m<sup>2</sup> in 2021 and 142.9 kg/100 m<sup>2</sup> in 2022). For Bambino, in both years, very similar yields were obtained that ranked at the average level (230.8 kg/100 m<sup>2</sup> in 2021 and 220.7 kg/100 m<sup>2</sup> in 2022) (Table 3, Supplementary Tables S2 and S3). For Mammoth Gold, the yield was significantly lower at a fertilization rate of 30%, suggesting that this cultivar is most sensitive to low nitrogen levels (Figure 3, Supplementary Tables S2 and S3).

In Justynka F<sub>1</sub>, fruits with significantly lower weights (2.0 kg in 2021 and 1.5 kg in 2022) were observed. In contrast, the highest fruit weight in both years of the experiment was found for the Mammoth Gold (7.4 kg) and Otylia F<sub>1</sub> (6.9 kg in 2021 and 7.2 kg in 2022) cultivars. The Bambino cultivar had slightly lower average fruit weights of 4.5 kg in 2021 and 4.8 kg in 2022 (Table 3, Supplementary Tables S2 and S3).

In both years of the experiment, the largest fruits were observed in the Mammoth Gold cultivar (fruit diameter: 29.0 cm in 2021 and 29.6 cm in 2022; fruit length: 28.9 cm in 2021 and 27.1 cm in 2022) and Otylia F<sub>1</sub> (fruit diameter: 29.0 cm in both years; fruit length: 22.7 cm in 2021 and 23.5 cm in 2022). Bambino had slightly smaller fruits with a 26 cm

(2021) and 25.7 cm (2022) in diameter and 19.8 cm (2021) and 20.4 cm (2022) in length. The cultivar Justynka F<sub>1</sub> produced the smallest fruits, with diameters and lengths of 18.4 cm (2021) and 17.3 cm (2022) and 14.8 cm (2021) and 12.8 cm (2022), respectively (Table 3, Supplementary Tables S2 and S3). The highest average flesh thickness was observed for Otylia F<sub>1</sub>, ranging from 5.4 cm in 2021 to 6.1 cm in 2022. In contrast, the thinnest flesh was characterized by the variety Justynka F<sub>1</sub> (3.4 cm in 2021 and 3.2 cm in 2022). The Mammoth Gold and Bambino cultivars were characterized by similar flesh thicknesses, which ranged, respectively, from 4.7 to 4.4 cm in 2021 and 4.1 to 3.8 cm in 2022 (Table 3, Supplementary Tables S2 and S3).



**Figure 3.** Average nitrate content in fruit flesh and fruit yield of four winter squash cultivars cultivated with three different nitrogen fertilization doses across two years of experiments, 2021 and 2022. The error bars represent the standard deviations (SDs), and the letters indicate homogenous groups;  $p < 0.05$ .

#### 4. Discussion

Nitrogen fertilization management has become increasingly important in agricultural and horticultural production systems. The overuse of synthetic nitrogen fertilizers negatively impacts terrestrial and marine ecosystems and contributes to environmental harm by increasing N<sub>2</sub>O emissions from agricultural soils, potentially threatening the Paris Agreement's climate goal of limiting global warming to 1.5 °C [26]. In addition, excessive use of nitrogen fertilizers has negative effects on plants and the raw material extracted from them. However, nitrogen deficiency limits crop growth and significantly reduces yields [16]. Therefore, agronomic research is increasingly focusing on finding nitrogen fertilization practices that maximize crop production and improve the quality of raw materials while minimizing environmental impacts.



In the present study, the effects of varying nitrogen fertilization doses (100%, 50%, and 30% of the standard dose recommended by Sady [24]) on fruit yield, fruit size, and the nitrate content of fruit flesh were studied over two consecutive years (2021 and 2022). In a two-year experiment, four *C. maxima* winter squash cultivars—Bambino, Otylia F<sub>1</sub>, Justynka F<sub>1</sub>, and Mammoth Gold—bred or adapted for cultivation under Poland's climatic conditions were used.

We noted that decreasing the nitrogen fertilization rate had a significant effect on the overall nitrate content in the fruit flesh in both years of the experiment. We found that reducing nitrogen fertilization to 50% and 30% significantly reduced the average nitrate content of the fruits of the cultivars tested. Moreover, at 30% nitrogen dose the nitrate content in the flesh remained below the European Union standard (200 mg/kg) in both years of the experiments [15]. A similar relationship has been noted for other crops, such as potato and radish, in which the nitrate content increased when relatively high doses of nitrogen fertilizer were applied [27,28].

We observed differences among the cultivars in terms of nitrate accumulation in the fruit flesh. The lowest nitrate contents were detected in the fruit flesh of small-fruited Justynka F<sub>1</sub>, which represents the horticultural group of *C. maxima* named Hubbard-type. The nitrogen content determined in this study was similar to that reported in previous studies for cultivars similar to Justynka F<sub>1</sub> [18]. The average nitrate concentrations before storage obtained by Biesiada et al. (2009) [18] for the Amazonka and Karowita cultivars (Justynka F<sub>1</sub> cultivar type) were 279 mg/kg and 245 mg/kg, respectively. However, the same authors in other publication reported significantly higher total nitrate contents, varying from 390 to 690 mg/kg, depending on the *C. maxima* cultivar. The nitrate contents of the small-fruited cultivars (Ushiki Kuri, Amazonka, Karowita, and Ambar) ranged from 390 to 650 mg/kg, whereas those of the large-fruited cultivars Bambino and Melonowa Żółta ranged from 570 to 690 mg/kg [19]. Studies in tomatoes have also shown different effects of nitrogen fertilization on fruit parameters in small-fruited and large-fruited cultivars [29].

In our study, the high nitrate content in fruit in 2022 may have been caused by unfavorable weather conditions, including drought during intensive fruit growth. Limited precipitation in June, after the seedlings were planted, hindered nutrient uptake, proper rooting, and vegetative growth of the plants. Low precipitation in August (34.4 mm compared with a multiyear average of 65.2 mm) and high temperatures (2.6 °C above the multiyear average) caused drought, affecting fruit development and growth. Under conditions of insufficient sunlight and/or a negative water balance, photosynthesis may be reduced, and the nitrate content in plant tissues may increase. Thus, periods of drought can cause nitrate (V) accumulation in plants due to a reduction in the rate of conversion to nitrate (III) (nitrite) [30]. Drought occurred in August 2022, which may explain the relatively high nitrate content in the fruit.

The applied nitrogen dose significantly affected fruit yield in both years of the experiment. We found that the fruit yield did not differ significantly between the 100% and 50% nitrogen treatments in either year of the experiment. However, when nitrogen fertilization was reduced to 30%, a significantly lower fruit yield was obtained, ranging from 12% (2022) to 20% (2021) below the standard fertilization level. Similar results were noted for corn, where a reduction in nitrogen fertilization to 30% resulted in a 10% yield loss [31].

We also observed that the cultivars differed in terms of fruit yield. The highest fruit yield in both years of the experiment was obtained for the large-fruited varieties Mammoth Gold and Otylia F<sub>1</sub>, whereas the lowest was obtained for the small-fruited variety Justynka F<sub>1</sub>. These results are much lower than those reported in other studies, where yields for small-fruit cultivars ranged from 30 to 50 t/ha, and those for large-fruit cultivars ranged from 50 to 100 t/ha [23,32–34]. However, this may be caused by inferior soil quality and/or

lower levels of mineral fertilizer application and a lack of organic fertilization. In contrast to the nitrate content, a lower fruit yield was obtained in 2022. This may also be due to adverse weather conditions this year.

Little effect of varying nitrogen fertilization rates on the fruit dimensions (length, diameter and flesh thickness) of the tested varieties was observed. The values were similar in both years of the experiments. However, the largest fruits were obtained from the Mammoth Gold and Otylia F<sub>1</sub> cultivars, whereas Justynka F<sub>1</sub> produced the smallest fruits. Winter squash fruits intended for processing have to meet a size standard due to the requirements of a specific processing factory, which are generally 25–50 cm. Additionally, the minimum flesh thickness of pumpkins cultivated for processing is 3 cm [35]. Justynka F<sub>1</sub> was the only cultivar tested that did not meet the size standard for fruit intended for processing. Nevertheless, Justynka F<sub>1</sub>, due to its fruit size, can be recommended for consumption by individual consumers. All the cultivars tested achieved a flesh thickness of more than 3 cm.

In the 2022 experiment, higher dry matter content in fruit flesh was observed for Otylia F<sub>1</sub> and Justynka F<sub>1</sub>, while similar values for this trait were recorded for Bambino and Mammoth Gold in both experiments (Supplementary Table S4). Other authors also obtained different dry matter content results depending on the year of the study [23]. All the cultivars tested exceeded the minimum dry matter content for fruit processing, which was 5.5% (Supplementary Table S4). The highest dry matter content in both years was recorded for the small-fruited Justynka F<sub>1</sub> cultivar, whereas the lowest dry matter content was recorded for Mammoth Gold.

## 5. Conclusions

The results of the present study revealed differences in the fruit parameters of winter squash, depending on the cultivar/genotype and dose of nitrogen fertilization as well as the year of cultivation (climatic conditions). We noted that decreasing the nitrogen fertilization rate significantly affected the nitrate content of the fruit flesh and fruit yield in both years of the experiment. Reducing nitrogen fertilization to 50% and 30% significantly reduced the average nitrate content of the fruits of the *C. maxima* cultivars tested. However, the fruit yield did not differ significantly between the 100% and 50% nitrogen treatments in either year of the experiment. Therefore, decreasing the nitrogen dose up to 50% of the recommended dose seems reasonable. The tested varieties differed in terms of nitrate accumulation in the fruit, and the lowest values for this trait were recorded for the Justynka F<sub>1</sub> variety, for which the nitrate value did not exceed 200 mg/kg at both the 30% and 50% reduced doses. However, this variety was the only one that did not meet the fruit size standard for processing. Thus, the most promising of the tested cultivars is Otylia F<sub>1</sub>. This cultivar achieved high yield and fruit weight in both years of the experiment, with dry matter at 8 to 10 g/100 g, and was characterized by the thickest flesh among the tested varieties. Although the nitrate content of the Otylia F<sub>1</sub> fruit varied considerably depending on the experimental year and nitrogen fertilization dose, it remained within the standard range at the 50% nitrogen dose. The presented research is part of the strategy for sustainable agricultural development. This work provides new knowledge on the performance of *C. maxima* cultivars suitable for processing and strategies for using limited nitrogen fertilization in winter squash production.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture15010042/s1>, Table S1: Mean temperature and precipitation during field experiments (WULS meteorological Station, Warsaw, Poland, 52°09'37.37" N 21°03'11.92" E); Table S2: Average nitrate contents in fruit flesh, average values of yield-related traits and fruit dimensions for four cultivars of winter squash (*Cucurbita maxima*

Duchesne) in relation to three doses of nitrogen fertilization for field experiment conducted in 2021; Table S3: Average nitrate contents in fruit flesh, average values of yield-related traits, and fruit dimensions for four cultivars of winter squash (*Cucurbita maxima* Duchesne) in relation to three doses of nitrogen fertilization for field experiment conducted in 2021; Table S4: The average dry matter contents in fruit flesh for four cultivars of winter squash (*Cucurbita maxima* Duchesne) for field experiments conducted in 2021 and 2022.

**Author Contributions:** K.K. designed the study, performed the research and data analysis, and wrote a draft of the manuscript; A.K. participated in designing the study and fruit phenotyping; R.S. participated in the data analysis; A.G. participated in the data analysis; G.B. supervised the study, participated in the data analysis, and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare that they have no conflicts of interest.

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