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Article

The Effects of Growth Regulators and Apical Bud Removal on Growth, Flowering, and Corms Production of Two Gladiolus Varieties

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Abstract: Gladiolus is commonly propagated from corms. The multiplication rate of corms is low and to increase the propagation rate, we examined a combination of apical bud removal and the application of growth regulators. The experiments were conducted in two varieties, 'Rose Supreme' and 'White Prosperity', and over two seasons. The apical buds on the planting corms were either removed or left intact before the same corms were soaked in a suspension with either 100 ppm of benzyladenine (BA), 100 ppm of gibberellic acid (GA3), or pure water. The results showed that apical bud removal increased the number of corms and shoots. GA3 had limited the effect on corm and shoot production, but instead resulted in increased total leaf area and leaf weight per shoot. BA, on the other hand, increased the number of corms and shoots. Overall, the removal of the apical bud plus application of BA increased the number of corms and shoots but reduced the average corm diameter and leaf weight per shoot. This was clearer in 'Rose Supreme' than in 'White Prosperity'. To maximize flower production for the coming season, farmers need to produce a high number of planting corms, but they also need to balance this with a sufficient corm size and the production of flowers of good quality. The application of growth regulators in combination with apical bud removal should be fine-tuned to avoid a situation that leads to the production of too many small or too few large corms.

Keywords: apical dominance; benzyladenine; corm; flowering; gibberellic acid; growth regulator; propagation; quality

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1. Introduction

Gladiolus as a genus (family Iridaceae) contains species that are widely cultivated for their flowers and are popular as cut flowers, garden flowers, and pot plants. *Gladiolus grandifloras*, hereafter termed gladiolus, is the main species from where modern hybrids are bred. The species and its wild relatives are native to South Africa but are also found wild in the Mediterranean region [1,2]. Gladiolus is a global commodity and is in the eighth highest position in the world cut-flower trade [3]. It is cultivated all over the world, with major producing countries such as the USA, Holland, Italy, France, Poland, Bulgaria, Brazil, India, Australia, and Israel [4]. In addition, Japan, China, and India also have large production [5].

Commercially, gladiolus is propagated by the natural multiplication of corms and cormels [4,6]. However, the multiplication rate is low, which restricts the production. We have seen different strategies used to overcome this problem. Breeding new cultivars with a more rapid multiplication rate is one, and in vitro propagation is another. However, breeding takes many years and in vitro is costly [6,7]. Therefore, we suggest examining if current cultivation practices could be modified, e.g., by using growth regulators and apical bud removal to speed up the multiplication rates. From the literature, it is known that several practices could influence the results, such as the division of corms [8], removal of flower spikes [8,9], manual removal of apical buds [8], growth regulators application [10,11], and combined growth regulators and leaf regulation practices [12]. Memon et al. [6] suggested that dividing the corm is the best method to increase the number and size of daughter corms, while others recommended growth regulators [13–15]. We saw no studies on the combined effect of growth regulators and the removal of apical buds.

Therefore, the current study aims at examining the effects of apical bud removal coupled with the use of benzyladenine (BA) or gibberellic acid (GA3) as growth regulators. This was examined in two gladiolus cultivars, 'Rose Supreme' and 'White Prosperity'. As the trade-offs are important for farmers, in addition to corms and cormels, we studied the effects on various leaf and flower variables.

2. Materials and Methods

Field trials were conducted to examine the effects of apical bud removal combined with growth regulators on two gladiolus cultivars. Gladiolus has a typical geophytic nature, with corms as the main storage and propagation organ (Figure 1). On the nodes of the corm, several axillary buds are found. Due to apical dominance, only a few axillary buds will produce shoots and subsequently form one or two daughter (new mother) corms. In addition, several small corms (cormels) are produced in clusters between the mother and daughter corms, but these require about 3–4 seasons to produce mature corms depending on the size of corms or cormels, their variety, and the growing conditions [16,17].

Figure 1. The reproductive phase of 'White Prosperity' gladiolus treated with BA at 100 ppm + removal of the apical bud. From top to bottom: base of three shoots, new corms, cormels, single leaf cormel, contractile roots, and fibrous roots.

2.1. Treatments and Experimental Design

Three factors were examined: (1) apical bud removal (removed or not removed), (2) growth regulators (BA, GA3, or no growth regulator), and (3) variety ('White Prosperity' and 'Rose Supreme'). The two varieties were selected as they are well-known to growers. 'Rose Supreme' has pink flowers and 'White Prosperity' has white flowers with dark pink streaks on the petals [18]. For each variety, two groups of corms were prepared. In the first group, each corm was removed from the apical bud (R). Corms from the second group were left intact (non-removed apical bud, NR). The corms were then soaked either in a solution of benzyladenine (BA) or gibberellic acid (GA3), each at 100 ppm for 24 h (in early December). The control corms were dipped in distilled water at the same time. The concentration of growth regulators in the soaking solutions were selected based on previous results [11,19]. We used uniform-sized corms for both varieties and all treatments: all 1.9–2.5 cm in diameter (small corms, No.4) and imported from Agro Trading Company B.V., Holland, through a nursery in Egypt. The experiment was laid out as a randomized complete block design with three replicates per treatment. The same experiment was repeated over two seasons (2019/20 and 2020/21) under open field conditions.

2.2. Field Management

The research was carried out at the Experimental Station of the Horticulture Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, Egypt, (Latitude: 31°6.3792′ N, longitude: 30°56.5184′ E). The soil texture at the site was classified as a clay loam with 1.6% organic matter. The pH was 7.3–7.5 and the electrical conductivity was 1.4 dSm-1 during the first year and before planting. The total nitrogen content in the soil was 0.52%, the available phosphorus was 11.8 ppm, and the available potassium was 17.0 ppm. The climate of the region was typically Mediterranean, i.e., with mild and rainy winters and dry and hot summers. The maximum and minimum monthly mean temperature (recorded during the years of the experiment at the nearest meteorological station, Sakha) were 27.3 and 15.9 °C (November), 21.4 and 11.3 °C (December), 19.6 and 8.8 °C (January), 21.6 and 9.3 °C (February), 24.8 and 10.7 °C (March), 28.6 and 12.9 °C (April), 35.2 and 17.6 °C (May), and 40.0 and 20.6 °C (June), respectively. The light intensity at noon was averaged to 50 klx (measured with a light meter, ST-85 Auto-range illuminance meter, Beijing, China).

The field was prepared by well-decomposed farmyard manure that was applied before land preparation at the rate of 5 kg per plot and mixed well into the soil. Corms were planted at the depth of 7–8 cm at 15 cm intervals and with 4 rows on a ridge furrow with a width of 60 cm. One plot was 60 cm × 100 cm and contained 24 planted corms. Between each plot we placed a row of border plants as guards.

Irrigation was supplied at regular intervals—7–10 days—by a drip irrigation system to keep the soil moist but not flooded. Twelve grams per plot of additional fertilizer, Kristalon NPK 19-19-19 (Yara, Norway), was applied once monthly when the emerging was completed (in mid-December) until mid-April. Furthermore, other cultural practices, including hand weeding and pest and disease control, were taken up whenever necessary.

2.3. Plant Measurements and Sampling Methods

Three plants were sampled per plot and plant height was measured from the base of the plant to top of the upper floret (at basal florets that showed colors). The fresh leaves weight per shoot (g) and total leaf area per shoot (cm^2) were measured with a CI-202 laser area meter (CID Bio-Science Inc, Camas, DC, USA). The number of florets per spike were determined with only the main spike when more than one occurred on a plant. The corms were harvested during June–July at maturity, 65–70 days after spikes harvest. Following the North American Gladiolus Council [20], corms and cormels were classified into three broad groups based on their diameter, namely flowering stock corms (above 2.5 cm), planting stock corms (1.5–2.5 cm), and cormels (below 1.5 cm). Flowering stock corms are used for getting the flower spike of a standard size, while planting stock corms are used for the propagation of flowering size corms.

2.4. Statistical Analysis

Data analysis was carried out in R [21]. The data were surveyed using the R function, summary() and boxplot(). Outliers were identified but not removed. Using ANOVA, the main effects and all two-way interactions of the factors, season, variety, growth regulators, and apical bud removal, were analyzed. Several of the interactions were highly significant and we combined the interactions into new single factors for further analysis. Tukey's multiple comparisons of means at a 95% family-wise confidence level and Duncan's Multiple Range Tests were conducted on significant factors.

3. Results

A set of variables was recorded and an overview of the range of measured variables from our study is provided in Table 1. We saw a great span from the lowest to the highest value in most of the variables; for example, in the diameter of the flowering stock corms, which varied from 1.9–6.2 cm and the weight varied from 9.7–33.0 g. We first analyzed the results to find the effects of each of the three main factors: (1) variety, (2) growth regulators, and (3) apical bud removal. Illustrations of these results are presented as sets of boxplots. A boxplot is a visual illustration where the box is defined by a lower and an upper percentile in which 75% of the observations are found. Within the box, the marked line defines the median value, and outside the box, the whiskers and small circles show observations far from the median and outside the 25% and 75% percentiles.

Table 1. Variables with detailed descriptions and the observed ranges as detected in our study (minimum and maximum values).

Variables with Descriptions	Range
Flowering stock corms variables	
Number of flowering stock corms per plant	1–3
Diameter of flowering stock corm (cm)	1.9–6.2
Fresh weight of flowering stock corm (g)	9.7–33.0
Planting stock corm variables	
Number of planting stock corm per plant	7.0–26
Fresh weight of planting stock corm (g)	6.0-19.6
Cormels variables	
Number of cormels per plant	69–183
Vegetative and floral variables	
Total leaf area per shoot (cm)	278–630
Fresh weight of leaves per shoot (g)	20.1–62.7
Plant height (cm, at flowering)	57.3–126.7
Spike diameter (mm)	5.2–12.5
Number of spikes per plant	1–2

3.1. Variety

Two varieties, 'White Prosperity' and 'Rose Supreme', were included in the experiments. Across growth regulator and apical bud treatments, the two varieties differed in several of the examined variables. 'White Prosperity' was significantly taller than 'Rose Supreme' (19 cm taller on average, $p < 0.001$), and also had significantly more spikes and thicker spikes, but 'White Prosperity' had a lower leaf area per shoot (152 cm^2 lower on average, $p < 0.001$) than 'Rose Supreme' and a lower leaf weight per shoot (Figure 2). No clear differences were found between the two varieties for the other leaf and corm variables.

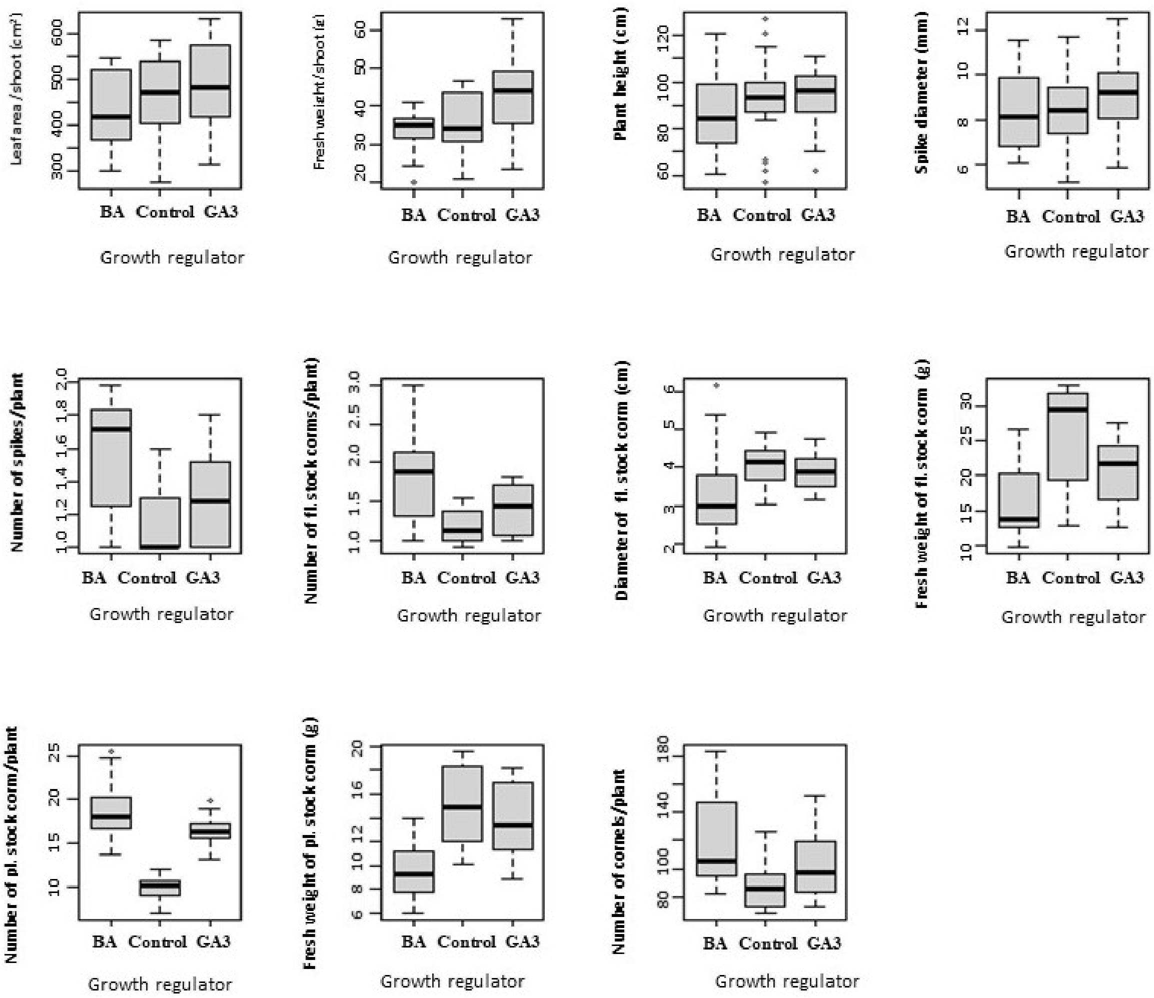

Figure 2. The effect of variety on the different corm and leaf variables illustrated as boxplots where a box defines a lower and an upper percentile, in which 75% of the observations are found, and the marked line defines the median value of a given variable.

3.2. Growth Regulators

Benzyladenine (BA) and gibberellic acid (GA3) were included in the experiment, as well as a control with pure water. Across the two varieties and the two apical bud treatments, corms soaked in 100 ppm of BA resulted in a reduced leaf area per shoot compared to the same concentration of GA3 (54 cm^2 less on average, $p < 0.001$), as well as a reduced leaf area compared to the control with no growth regulator (30.6 cm^2 less on average). Furthermore, BA showed a higher number of spikes per plant compared to GA3 and the control, but compared to GA3, the differences were significant in only one of the two seasons. On the other hand, the average diameter of each spike was lower with BA than with GA3 and the control. BA also showed a higher number of flowering stock corms per plant and a higher number of planting stock corms per plant than both GA3 and the control. On the other hand, the average corm weight was lowest with BA. (Figure 3). There was no clear effect from the growth regulators on plant height ($p = 0.06$) or the other variables not mentioned above (Tables S1–S3).

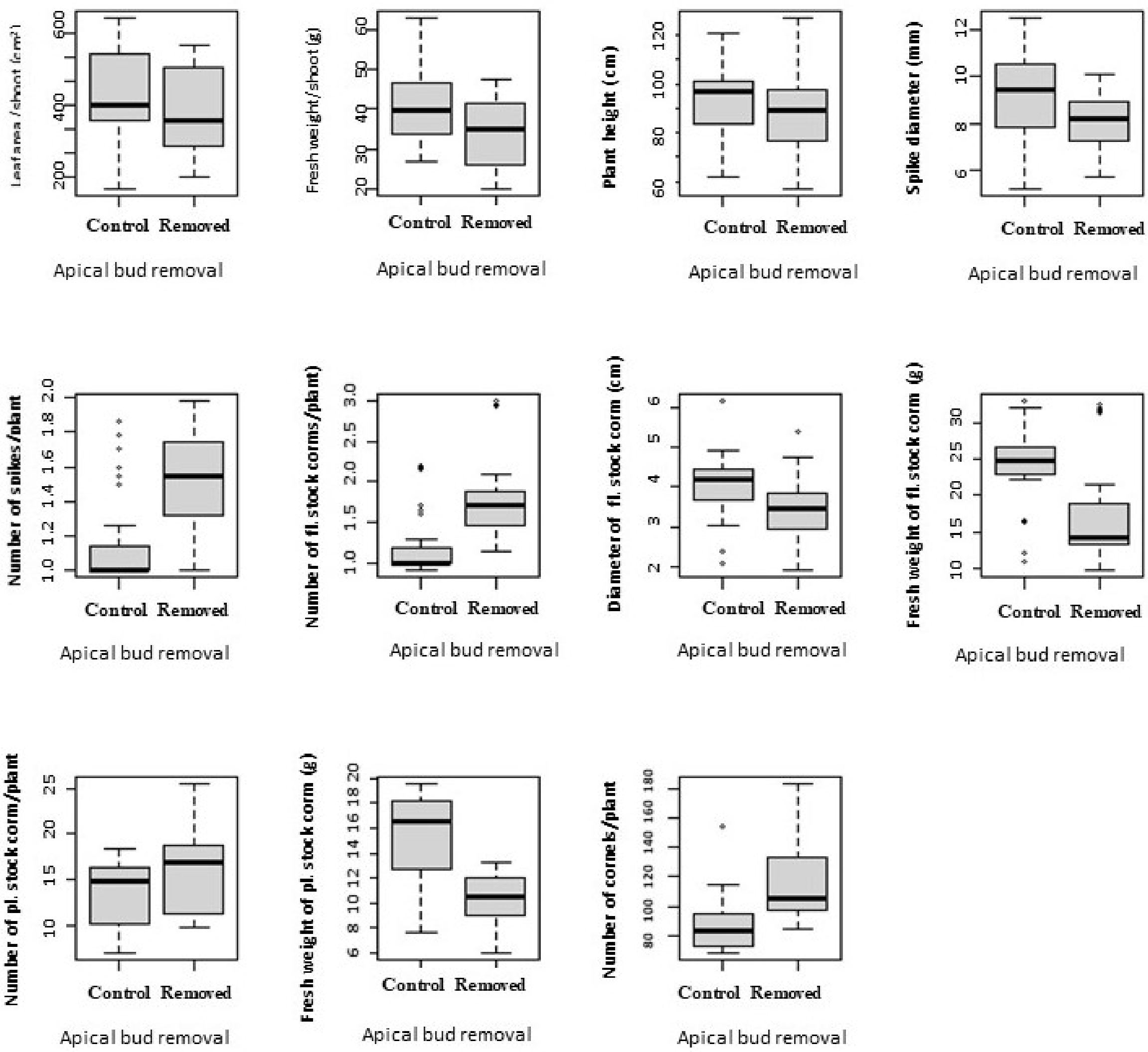

Figure 3. The effect of growth regulators on the different corm and leaf variables illustrated as boxplots where the boxes define a lower and an upper percentile, in which 75% of the observations are found, and the marked line defines the median value of a given variable.

3.3. Apical Bud Removal

Apical bud removal was compared to no apical removal (control). Across varieties and growth regulator treatments, the removal of apical buds increased the average number of spikes and the number of flowering stock corms, as well as the number of cormels per plant (Figure 4). Furthermore, removal resulted in a reduced leaf area per shoot (on average 47 cm^2 less than the control, Figure 4). Apical bud removal also influenced the average diameter of flowering stock corms, and plant height was to some extent affected, where apical bud removal resulted in slightly shorter plants (5.4 cm shorter on average, $p < 0.05$) compared to the control. For the examined variables not mentioned above, no clear differences were detected.

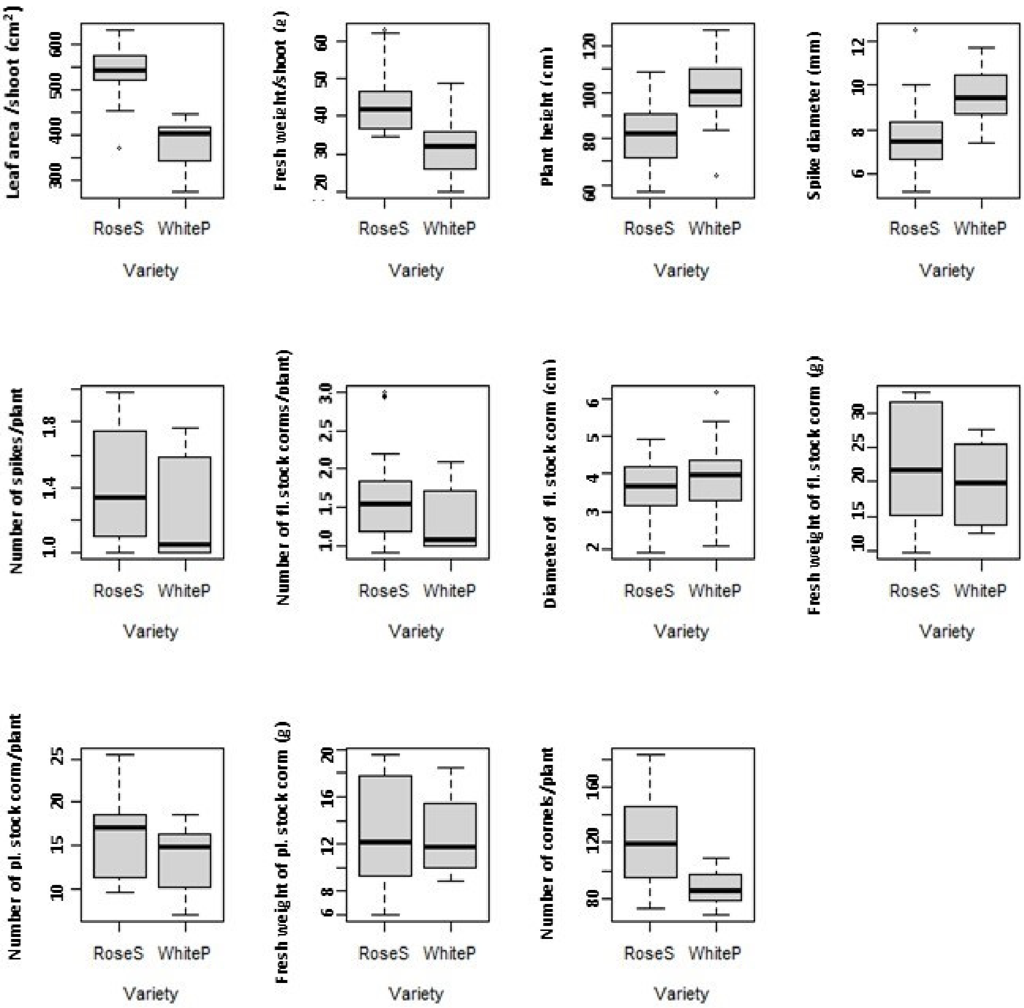

Figure 4. Effect of apical bud removal on the different corm and leaf variables illustrated as boxplots where the boxes define a lower and an upper percentile, in which 75% of the observations are found, and the marked line defines the median value of a given variable.

3.4. Interactions

The experiments were designed to also examine two- and three-way interactions. Our analysis showed such interactions. The number of flowering stock corms per plant was significantly influenced by the interaction of growth regulator × apical bud removal ($p < 0.001$). The highest number of flowering stock corm was produced by the combination of BA plus apical bud removal. For the variety, 'Rose Supreme', this combination resulted in 2.97 and 1.94 over the two seasons, respectively, while for "White Prosperity", it resulted in 2.00 and 1.83 flowering stock corms over the two seasons (Table S1).

The positive effect on the number of corms was balanced by a negative effect on the average fresh weight of the same corms. The interaction between growth regulator x apical bud removal × variety was highly significant ($p = 0.001$; Table 2). In 'Rose Supreme', growth regulators were of major importance, and in this variety, BA gave the lowest corm weight, GA3 had a medium weight, and the control with no use of growth regulators gave the highest corm weights. Here, apical bud removal was of limited importance. In the other variety, apical bud removal was of major importance. The lowest corm fresh weights were recorded where the apical buds were removed, regardless of growth regulators. Much of the same pattern as described for fresh weight was observed for the average diameter of the flowering stock corms with a significant effect from the interaction between variety x growth regulator ($p < 0.001$, Table S1).

Table 2. Sorted mean fresh weigh of flowering stock corm (from low to high) for the combination of growth regulators, apical bud removal, and varieties. Letters behind the numbers are results from the Duncan's Multiple Range Tests.

Combinations	**Mean Fresh Weight (g) of Flowering Stock Corm Per Plant**
BA + Removed apical bud + 'Rose Supreme'	11.7 d
No growth regulator + Removed apical bud + 'White Prosperity'	13.7 d
BA + Removed apical bud + 'White Prosperity'	13.8 d
BA + No removal of apical bud + 'Rose Supreme'	13.9 d
GA_3 + Removed apical bud + 'White Prosperity'	14.6 d
GA_3 + Removed apical bud + 'Rose Supreme'	19.1 c
GA_3 + No removal of apical bud + 'Rose Supreme'	23.6 b
BA + No removal of apical bud + 'White Prosperity'	25.2 b
No growth regulator + No removal of apical bud + 'White Prosperity'	25.3 b
GA_3 + No removal of apical bud + 'White Prosperity'	25.3 b
No growth regulator + Removed apical bud + 'Rose Supreme'	31.9 a
No growth regulator + No removal of apical bud + 'Rose Supreme'	32.0 a

The same pattern as described for flowering corms was observed for planting stock corms. Such corms are of importance for farmers as they represent the propagation material for next season's flower production. The number of planting stock corms was significantly affected by the interaction between variety x growth regulator x apical bud removal (p < 0.001). The combined treatment of BA and the removal of the apical bud gave the highest number of planting stock corms. This was the case in both varieties (Table 3). GA3 did not have the same effect, especially in 'White Prosperity', while no growth regulator and no apical bud removal resulted in the lowest numbers. The fresh weight of the same corms showed an effect on the interaction between growth regulator x apical bud removal (p < 0.001 for). Again, the lowest weights were found with the use of BA combined with apical bud removal and the highest weights occurred with no growth regulators combined with no apical bud removal.

Table 3. Sorted mean number of planting stock corms per plant (from low to high) as influenced by the combination of growth regulators, apical bud removal, and varieties. Letters behind the numbers are results from the Duncan's Multiple Range Tests.

Combinations	**Mean Number of Planting Stock Corms Per Plant**
No growth regulator + No removal of apical bud + 'White Prosperity'	7.66 g
No growth regulator + Removed apical bud + 'White Prosperity'	10.12 f
No growth regulator + No removal of apical bud + 'Rose Supreme'	10.15 f
No growth regulator + Removed apical bud + 'Rose Supreme'	11.37 f
GA3 + No removal of apical bud + 'White Prosperity'	14.46 e
BA + No removal of apical bud + 'White Prosperity'	15.03 de
GA_3 + Removed apical bud + 'White Prosperity'	16.31 cd
GA_3 + No removal of apical bud + 'Rose Supreme'	16.35 cd
BA + No removal of apical bud + 'Rose Supreme'	17.62 bc
BA + Removed apical bud + 'White Prosperity'	17.95 b
GA_3 + Removed apical bud + 'Rose Supreme'	18.46 b
BA + Removed apical bud + 'Rose Supreme'	23.66 a

For cormels, all treatments were composed of two grades (medium, >0.5 to $\leq$1.0, and small, <0.5 cm), of which medium-sized cormels were most frequent (generally 82–85%), while few small-sized cormels (14–19%) were produced. The mean number of cormels per plant was affected by season and the interaction between variety x growth regulators and the interaction between variety x apical bud removal ($p < 0.001$ for all). Much of the same pattern as described above for flowering and planting stock corms was observed for cormels. The combined treatment of BA and the removal of the apical bud gave the highest numbers of cormels, and this was found for both varieties (Table S2).

For vegetative and floral variables, a significant interaction was found for the fresh weight of leaves per shoot ($p < 0.001$). Compared to the controls, which yielded 36 g shoot^{-1} on average over the two seasons, corm soaked in 100 ppm of GA3 combined with no apical bud removal yielded 60 g shoot^{-1} in "Rose Supreme" and 43.8 g shoot^{-1} in 'White Prosperity', and this was the combination that gave the highest leaf yield (Table S3).

The number of spikes per plant was also affected by the same interaction ($p < 0.001$). Compared to the control, corm soaked in BA combined with apical bud removal increased the number of spikes the most, and the effect was clearest in the variety, 'Rose Supreme' (Table S1).

4. Discussion

For farmers, a high number of flowering stock corms means a high flower production, but farmers also need to balance the number with the size, and they need to produce a certain number of planting stock corms, as such corms function as the propagation material for next season's flower production. This study was designed to determine the combined effects of growth regulators and apical bud removal on such key variables in two common gladiolus varieties. Our results showed that both the number of flowering and planting stock corms, but also the number of small cormels, increased by using growth regulators combined with apical bud removal. At the applied concentration, benzyladenine (BA) had a stronger effect than gibberellic acid (GA3). The mechanism of the exogenous application of the given growth regulators is known to increase axillary bud induction and subsequent lateral shoot formation [11,14,19,22]. The effect is known to be modified by varieties as well as growth conditions [9,13]. The size of corm, number of axillary buds, and production practices can also influence lateral shoot formation and, subsequently, the total leaf area and fresh weight of leaves, and, in the end, the number of flowering stock corms per plant. Our data indicated that the two varieties showed varying degrees of response to BA and GA3. 'Rose Supreme' reached nearly a maximum response in the number of corms when apical buds were removed in combination with BA, while 'White Prosperity' did not produce the same high number. An alteration in the concentration could probably lead to a more satisfactory lateral shooting in 'White Prosperity'. We have no information that can explain the different responses in the two varieties.

The tradeoffs are, however, also present. The leaf area and leaf weight per shoot decreased when apical buds were removed, or when BA was applied. GA3, on the other hand, did not show the same negative effect, and when combined with no apical bud removal, GA3 resulted in the highest total leaf area and leaf weight production per shoot. This will increase the total shoot production per plant. Moreover, any treatment that advances the total leaf area and the photosynthetic capacity will also increase the subsequent planting stock corms production. Hence, their chances of reaching the critical corm diameter (or weight) that permits flower initiation and flower production in the following season increases. Too many small cormels will be contra-productive.

Removing the apical bud without any growth regulator decreased flowering stock corm diameter by 15%, while BA reduced it by 26% compared to no apical bud removal. This reduction, as well as the reduction caused by BA application, might be a result of the competition between the high number of corms and cormels that is created, and the available assimilates can't cope with what is needed for the corm tuberization. Such ideas have been raised after BA application [23,24] as well as GA3 application in gladiolus [25,26]. Apparently, the apical dominance that is reduced by manual or chemical increments also reduces the corm tuberization [11].

In the present study, a variation in cormel size was observed, with a large number of small cormels (<1.0 cm in diameter). The large-sized cormels, which produce an emergent leaf in their first season (Figure 1), may have been more frequent if the time or concentration had been different. Growth regulator (BA and GA3, but especially cytokinin) treatment of corm is known to enhance leaf cormel growth, an effect that was already noted in twin scales or bulbils of narcissus a long time ago [27]. However, any treatment that promotes leaf emergence cormels may enhance photosynthesis and increase the development and size. Although a single leaf from a cormel is very small, it allows growth, and hence the production of a bigger cormel. A small cormel needs more time to grow to a given flowering stock corm size than a big one. Therefore, large-sized cormels can be used as planting stock corm, as it is possible to attain a large flowering stock corm size in the following season under good growing conditions.

On average, the removal of apical buds without growth regulator application increased the number of flowering and planting stock corms per plant in both varieties. If BA was applied in combination with apical bud removal, the number of flowering stock corms increased by 145% and planting stock corms by 133% for "Rose Supreme" and increased by 92% and 134% in "White Prosperity" compared to non-removed apical buds dipped in water. Moreover, the treatment apparently forced a greater number of buds to shoot, which may enhance more carbohydrate assimilation. When the apical dominance is removed, the lateral buds have relatively high indole acetic acid concentrations, which is known to induce their elongation [28]. However, an increased number of corms and cormels may be attributed to the allocation of assimilates toward reproductive corms, which are considered to be more powerful sinks than other plant organs [29], or by an enhanced cytokinin production [30], which can be promoted by BA [31]. Moreover, the stolons might play an important role and help nutritious substance transportation from corm to cormels [32]. However, when corms are treated with BA or GA3 plus the removal of the apical bud, the apical dominance will decline sharply, and the lateral buds' induction will form multiple shoots and the branching of stolons, which again will form many new cormels.

5. Conclusions

On average, the number of flowering stocks corms increased from 1.2 to 1.8 per plant, and the number of planting stock corms increased from 9.8 to 18.6 per plant from the application of benzyladenine (BA) as a growth regulator. Gibberellic acid (GA3) also increased the numbers, but not at the same magnitude. Furthermore, apical bud removal increased the number of corms, but the increase became more obvious when the apical bud removal was coupled with growth regulators. BA combined with apical bud removal resulted in 2.2 flowering stock corms and 20.8 planting stock corms per plant, respectively. On the other hand, the average fresh weight as well as diameter of corms obviously decreased from the treatments. For example, BA combined with apical bud removal decreased the fresh weigh of flowering stock corms from 28.7 to 12.7 g and planting stock corms from 18.2 to 8.2 g per corm compared to the control with no treatment. This indicates that to maximize flower production, growers need to apply treatments in the year before to produce the optimal balance between number and size of planting stock corm for the flower production. The result was dependent on variety and we could also see some effect from season. Further research is needed to investigate the timing and concentration of the growth regulator treatment to avoid a situation that leads to a production of too many small or too few large corms.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8090789/s1, Table S1: Effects on spike diameter, number of spikes per plant, number of flowering stock corms per plant and diameter of flowering stock corm. Table S2: Effects on fresh weight of flowering stock corm, number of planting stock corms per plant, fresh weight of planting stock corm, and number of cormels per plant. Table S3: Effects on leaf area, leaf fresh weight and plant height.

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