

Full Length Research Paper

Effects of daminozide and day length on the flowering of some quality parameters and chlorophyll content of (*Chrysanthemum morifolium*) Ramat

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This study was conducted to determine the effects of different day lengths and daminozide on days to flower, some quality parameters and chlorophyll contents of spray chrysanthemum plants (*Chrysanthemum morifolium* cvs. 'Yellow Reagan' and 'White Reagan') grown under greenhouse conditions. Day length and daminozide significantly affected days to flower, stem length, stem diameter and flower numbers per stem. While stem fresh weight and chlorophyll *a* and *b* contents were affected by day length, daminozide had an insignificant effect on these parameters. Long day (LD) conditions delayed days to flower by 42 days as compared to short day (SD) conditions. SD conditions reduced stem length (84.47 cm) by almost 2- fold as compared to LD conditions (169.88 cm) . Under SD conditions, the stem lengths of plants treated (3000 mg L⁻¹) and untreated (control) with daminozide were 79.21 and 89.73 cm, respectively, whereas under LD conditions, they were 156.33 and 183.43 cm, respectively. The flower number increased per stem both under SD conditions and with daminozide application. The numbers of flowers per stem were 13.40 and 11.87 under SD and LD conditions, respectively, and 14.11 flowers were obtained from the plants treated with daminozide, while 11.17 flowers per stem were obtained from the plants untreated with daminozide. LD conditions increased both chlorophyll *a* (11.50 µg/mg) and chlorophyll *b* (5.93 µg/mg) contents as compared to SD conditions. In the study, compact and higher quality flowers with a desired stem length were obtained both under SD conditions and with daminozide application.

Key words: Chlorophyll, *Chrysanthemum*, daminozide, long day, short day, stem length.

INTRODUCTION

Chrysanthemum is one of the most important ornamental crops around the world, and it is produced as both cut flower and pot plant (van Der Ploeg and Heuvelink, 2006) . Photoperiod controls growth and flowering in many floriculture crops. During the production of photoperiodic crops in the greenhouse, photoperiod is artificially extended or shortened to maintain the vegetative growth of these crops or to induce flowering (Blanchard and Runkle, 2009). Such a manipulation of photoperiod provides growers with an efficient crop schedule.

Chrysanthemum is a short-day plant with a critical day length of approximately 13.5 h (Post, 1931; Furuta, 1954). When natural night lengths are long (>12 h), photoperiod is shortened by extending a blackout material over the crop in order to promote flowering in short day (SD) crops (Runkle and Fisher, 2004). Photoperiod is the shortest on December 21, whereas it increases until June 21 in the Northern Hemisphere, where Turkey is also located. It decreases thereafter. Thus, short-days must be supplied in this hemisphere for early flowering in the production of chrysanthemum during long-day times of the year (from March 21 to September 21). Flowering is uniform under photoperiods shorter than critical photoperiods, while under longer photoperiods, flowering is not uniform and often the buds do not develop normally (Furuta, 1954).

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Growth retardants have an inhibiting effect on cell division and enlargement of cell in plants. Therefore, they are widely used for height control in floriculture (Pasian, 1999). At the same time, growth retardants suppress the growth of vegetative shoots developing beneath the flower or may also increase the number of lateral shoots, resulting in a larger number of inflorescences (Whealy et al., 1988; Keever and Foster, 1989). Height control in plants has an important role in avoiding unacceptably tall plants that require more space, labor and higher transport costs, as well as in promoting yield and quality (Hayashi et al., 2001; Jaime and Silva, 2003; Karlovic et al., 2004).

Growth regulators, such as daminozide, chlormequat chloride and paclobutrazol, are successfully applied to obtain higher quality yield, to avoid lodging of plants and to control height, branching and flowering in chrysanthemum, like in many other plant species (Larson, 1985; Bailey and Whipker, 1998; Hayashi et al., 2001; Karlovic et al., 2004). Daminozide inhibited gibberellin biosynthesis or action and induced flowering (Halevy, 1986). It was reported that in chrysanthemum, different photoperiods had an important role in yield and quality (Korkut, 1990; Dutta et al., 1996; Hanke, 1996; ByungJoo et al., 2001) and daminozide had an important role in reducing plant height (Shawareb and Qrunfleh, 1988; Gregov et al., 1995; Saikia and Talukdar, 1997; Khobragade et al., 2002; JuHyoungh et al., 2004; Karlovic et al., 2004).

The aim of this study was to determine the effects of different day lengths photoperiods and daminozide on days to flower, some quality parameters and chlorophyll (chl) content of spray chrysanthemums grown under greenhouse conditions.

MATERIALS AND METHODS

The study was conducted in a plastic-covered greenhouse located at the Agricultural Research and Application Center of Agricultural Faculty at Suleyman Demirel University (latitude 37° 83' N, longitude 30°53' E, altitude 1,020 m) between June and November, 2006 in Isparta, Turkey. Rooted cuttings of spray chrysanthemum (*Chrysanthemum morifolium* Ramat. syn. *Dendranthema grandiflorum* [Ramat.] Kitam cvs. 'Yellow Reagan' and 'White Reagan') were planted on 06 June, 2006 in soil beds (3.0 m long and 1.0 m wide) at a density of 25 plants m⁻² (with five rows) in a randomized block design with 3 replications, and each replication contained 75 plants. Some characteristics of greenhouse soil (in 0 - 20 cm depth) in the study area were as follows: texture: clay loam, bulk density (g cm⁻³): 1.31, field capacity (Pw (%): 18.10, permanent wilting point (Pw (%): 7.43, EC (dSm⁻¹): 110, pH: 7.85, CaCO₃ (%): 30.12, organic matter (%): 3.45. Plants were fertilized at every irrigation application with 1 g L⁻¹ of a 21 N-4.8 P-17.5 K water soluble fertilizer (Poly-feed 21- 11-21+2MgO with micronutrients, Haifa Chemicals, Israel) during the experiment. The plants were pinched to seven nodes (Langton, 1987) 15 days after planting (DAP) and twenty six days later pruned to three stems per plant (Sugiura, 2004). Half of the plants were grown under the natural long day (LD) conditions, while darkening was applied to the other half of them in the period from July 08 to August 23. SD treatment was started 18 days after pinching and terminated when the flower buds showed color (August, 23). The SD conditions were achieved by closing the black polyethylene from 17:00 to 08:00 h.

Daminozide (Alar-85) was sprayed to half of the plants grown under both natural day length and SD conditions from foliar at a concentration of 3000 mg L⁻¹ at two growth stages. The first application was performed on the plants under both LD conditions when shoots reached about 30 - 35 cm height (July, 14), whereas the second application was performed during the budding period (under SD conditions: August 08, LD conditions: September, 19). When the buds showed color, the terminal flower bud was removed to provide more uniform and earlier flowering of other buds. Harvest was performed during the period when the central flower was completely open and the surrounding flowers were well developed (Laurie et al., 1969; Menguç, 1996). In this study were determined days to flower, stem length, stem diameter, number of flowers per stem, stem fresh weight, dry matter content and chl *a* and chl *b* contents.

Determination of chlorophyll *a* and *b* (µg/mg)

For chl analyses, leaf samples were obtained at the flowering stage and placed in 100 ml glass jars. Each jar was then filled with 99.5% ethanol, capped and stored in the dark at room temperature until the chl was extracted. Absorbances of the chl extract were measured by a spectrophotometer 'Perkin Elmer UV/VIS Lambda 20' 649 and 665 nm of the wavelength. Leaves were oven-dried at 70°C and the dry weight was determined. The chl contents were then calculated using the following equations (Wintermans and de Mots, 1965; Akcin, 1980):

$$\mu\text{g Chl } a = (13.70) \times (A_{665 \text{ nm}}) - (5.76) \times (A_{649 \text{ nm}})$$

ml solution

$$\mu\text{g Chl } b = (25.80) \times (A_{649 \text{ nm}}) - (7.60) \times (A_{665 \text{ nm}})$$

ml solution

The chl concentration, expressed as µg chl/mg dry weight, was then obtained. Data were subjected to analysis of variance (ANOVA) and means values were compared using Duncan's multiple range test at P = 0.05 level.

RESULTS

Days to flower

Days to flower were significantly affected by LD, daminozide, cultivar and the interaction between day length and cultivar (Table 1). Days to flower were significantly delayed under the natural LD conditions. The days to flower for plants under natural day length conditions were 136.6 days, whereas those under SD conditions flowered in 94.25 days. Daminozide application also slightly delayed flowering. When the cultivars were considered with respect to days to flower, 'Yellow Reagan' flowered in 117.08 days, while 'White Reagan' flowered in 113.83 days (Table 1). In the interaction between day length and cultivar, 'Yellow Reagan' and 'White Reagan' cultivars under SD conditions flowered in 95.17 and 93.33 days, respectively, whereas 'Yellow Reagan' and 'White Reagan' cultivars under natural LD conditions flowered in 139.0 and 134.33 days, respectively (Figure 1).

Table 1. Effects of different day lengths (natural day length and short day conditions) and daminozide on flowering period, stem length, stem diameter and number of flowers of two chrysanthemum cultivars.

	Flowering period (days)	Stem length (cm)	Stem diameter (mm)	Number of flowers (per stem)
Day length				
Natural condition	136.67a ^z	169.88a	6.85b	11.87b
Short day	94.25b	84.47b	6.99a	13.40a
Daminozide				
0 (Control)	115.17b	136.58	6.69b	11.17b
3000 mg L ⁻¹	115.75a	117.77	7.13a	14.11a
Cultivar				
'Yellow Reagan'	117.08	126.36	6.95	12.99a
'White Reagan'	113.83	127.94	6.87	12.28b
Significance				
Daylength (DL)	**	**	*	**
Daminozide (D)	*	**	**	**
DL x D	ns	**	ns	ns
Cultivar (C)	**	ns	ns	*
DL x C	**	ns	ns	**
D x C	ns	ns	ns	ns
DL x D x C	ns	ns	ns	ns

^zMeans within a column and main effect followed by different letters are significantly different at P<0.05, Duncan's multiple range test. ns: not significant; *, ** significant at P<0.05, P<0.01, respectively.

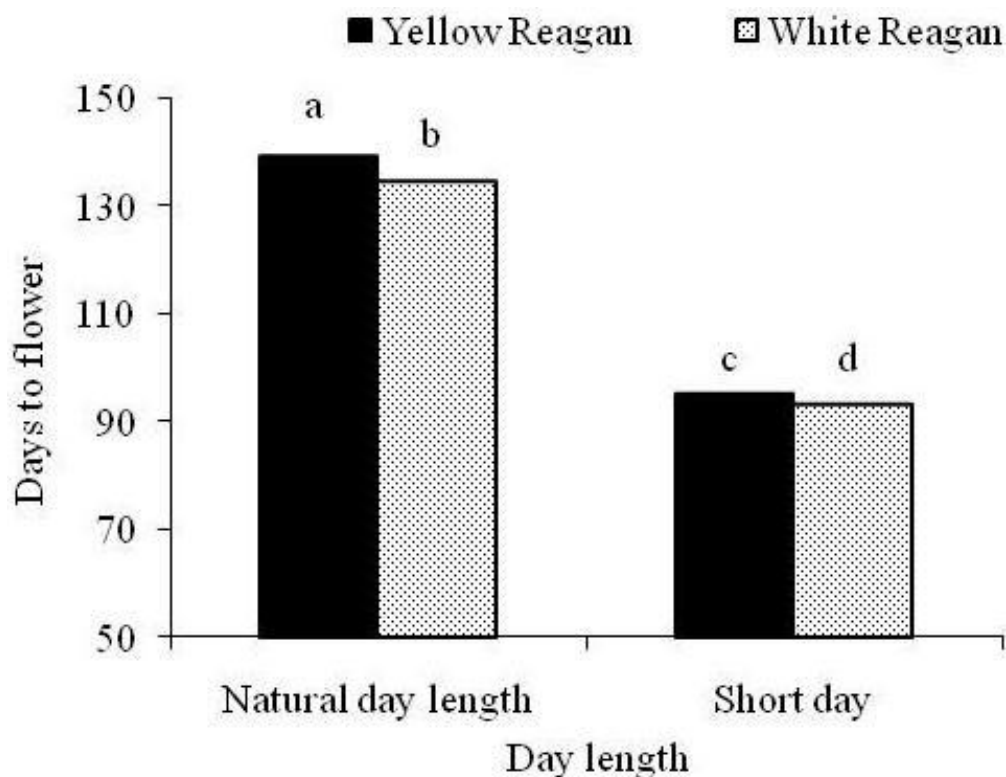


Figure 1. Effect of day length and cultivars on days to flower.

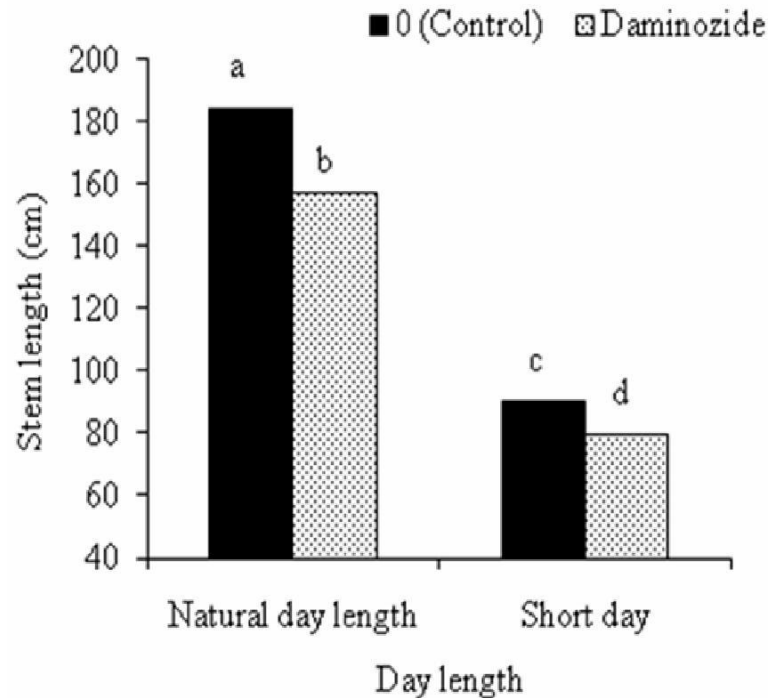


Figure 2. Effect of day length and daminozide on stem length.

Stem length

Day length and daminozide applications significantly affect stem length. The plants grown under the natural day length conditions had longer stems (169.88 cm) than those grown under SD conditions (84.47 cm). On the other hand, daminozide application reduced stem length (Table 1). The interaction between day length and daminozide also significantly affected stem length. In this interaction, the longest stem was obtained from plants treated with daminozide under natural day length conditions (183.43 cm), followed by the plants untreated with daminozide under the same conditions (156.33 cm) (Figure 2).

Stem diameter

SD and daminozide applications significantly increased stem diameter. The stem diameter was measured as 6.99 mm under SD conditions and as 6.85 mm under LD conditions. The stem diameter was 7.13 mm in the plants applied with daminozide and 6.69 mm in the plants untreated with daminozide. There was no significant difference in stem diameter between the cultivars (Table 1).

Number of flowers per stem

Number of flowers was strongly influenced by LD,

daminozide and cultivars. Plants under SD conditions (13.40 flowers per stem) had a higher number of flowers than the plants under LD conditions (11.87 flowers per stem) (Table 1). The flower number of the plants applied with daminozide was 14.11 flowers per stem, whereas it was recorded as 11.17 flowers per stem in the plants untreated with daminozide. When the cultivars were considered with respect to number of flowers per stem, a higher number of flowers were recorded in 'Yellow Reagan' cultivar (12.99 flowers per stem) than in 'White Reagan' cultivar (12.28 flowers per stem) (Table 1). The interaction between day length and cultivar also significantly affected the number of flowers. In this interaction, the highest number of flowers was determined as 14.19 flowers per stem and 12.63 flowers per stem in 'Yellow Reagan' and 'White Reagan' cultivars under SD conditions, respectively. Under natural day length conditions, 11.80 flowers per stem were recorded in 'Yellow Reagan' cultivar, while 11.94 flowers per stem were recorded in 'White Reagan' cultivar; however, there was no significant difference between the cultivars (Figure 3).

Stem fresh weight

The effect of LD and the interaction between LD and daminozide on stem fresh weight was found statistically significant; however, the effect of other applications was insignificant (Table 2). A higher stem fresh weight was

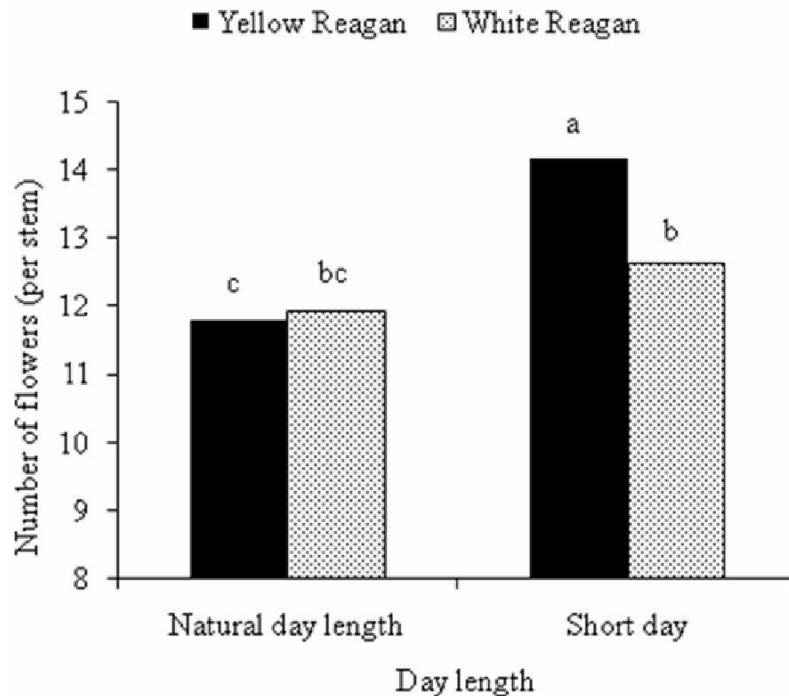


Figure 3. Effect of day length and cultivars on number of flowers.

recorded in the plants grown under natural day length conditions (130.08 g/stem) as compared to those under SD conditions (99.40 g/stem). Although, stem fresh weight was slightly high in the plants applied with daminozide, this difference was insignificant. A higher stem fresh weight was found in the plants both treated (128.12 g/stem) and untreated (132.03 g/stem) with daminozide and grown under natural LD conditions as compared to the plants treated (106.38 g/stem) and untreated (95.42 g/stem) with daminozide under SD conditions (Figure 4).

Dry matter content

In the study, the effect of all applications except LD on dry matter content was insignificant. The dry matter content of the plants under natural day length conditions (20.38%) was higher than those under SD conditions (18.79%). The dry matter content was 19.44% in the plants treated with daminozide and 19.73% in the plants untreated with daminozide. The dry matter contents ranged from 19.54 to 19.63% between the cultivars (Table 2).

Contents of chl *a* and *b*

Day length significantly affected the contents of chl *a* and *b*. Natural day length conditions increased the quantities

of chl *a* and *b* in the plants (Table 2). The contents of chl *a* and *b* were found 11.50 and 5.93 ($\mu\text{g}/\text{mg}$) in plants under natural conditions, respectively, whereas they were 9.51 ($\mu\text{g}/\text{mg}$) and 5.10 ($\mu\text{g}/\text{mg}$) under SD conditions, respectively. The contents of chl *a* and *b* were unaffected by daminozide application. When the cultivars were evaluated with respect to contents of chl *a* and *b*, 'White Reagan' cultivar had higher values in terms of the contents of both chl *a* (10.81 $\mu\text{g}/\text{mg}$) and chl *b* (5.79 $\mu\text{g}/\text{mg}$) as compared to 'Yellow Reagan' cultivar.

DISCUSSION

Since chrysanthemum is a short-day plant (Furuta, 1954), short days promote flowering in chrysanthemum (Runkle and Fisher, 2004). In the study, day length significantly affected days to flower. Plants under SD conditions flowered 42 days earlier than those under natural day length conditions. Similar results were reported by Korkut (1990), Hanke (1996) and Dutta and Ramadas (2000). Days to flower can be scheduled by manipulating the day length in photoperiodic crops like chrysanthemum, the year-round production of which can be achieved. This can provide growers with an opportunity to sell their crops at higher prices and achieve a higher profit. Days to flower were slightly delayed by daminozide application (0.58 days). It was reported that daminozide application delayed flowering by 1 to 3 days in chrysanthemum (Adriansen, 1985; Gregor et al., 1992). When the

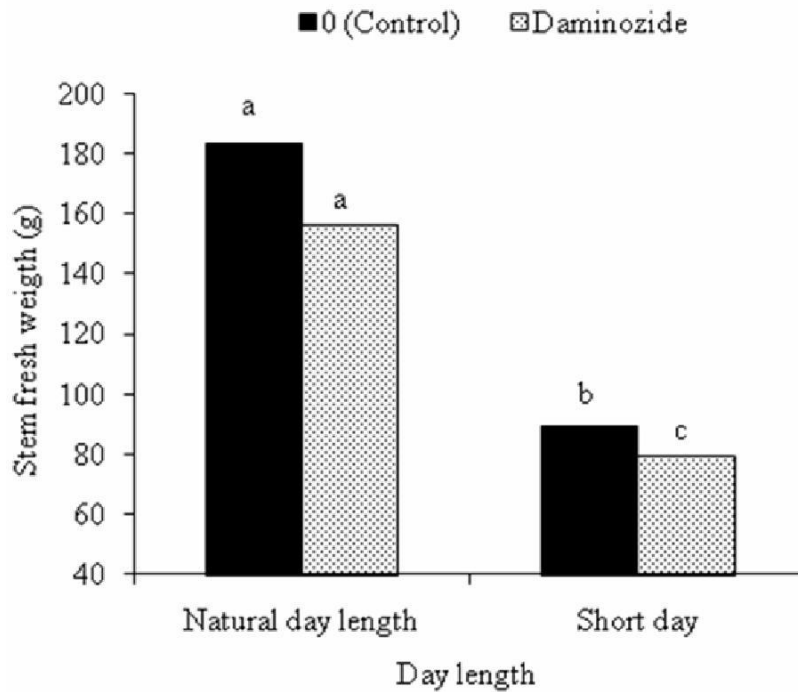


Figure 4. Effect of day length and daminozide on stem fresh weight.

Table 2. Effects of different daylengths (natural day length and short day conditions) and daminozide on stem fresh weight, dry matter, chlorophyll *a* and *b*.

	Stem fresh weight (g)	Dry matter (%)	chlorophyll <i>a</i> (µg/mg)	chlorophyll <i>b</i> (µg/mg)
Day length				
Natural condition	130.08a ^z	20.38a	11.50	5.93
Short day	99.40b	18.79b	9.51	5.10
Daminozide				
0 (Control)	112.23	19.73	10.18	5.32
3000 mg L ⁻¹	117.25	19.44	10.84	5.71
Cultivar				
'Yellow Reagan'	117.98	19.54	10.22	5.23
'White Reagan'	111.50	19.63	10.81	5.79
Significance				
Day length (DL)	**	**	**	**
Daminozide (D)	ns	ns	ns	ns
DL x D	*	ns	ns	ns
Cultivar (C)	ns	ns	ns	*
DL x C	ns	ns	ns	ns
D x C	ns	ns	ns	ns
DL x D x C	ns	ns	ns	ns

^zMeans within a column and main effect followed by different letters are significantly different at $P < 0.05$, Duncan's multiple range test. ns: not significant; *, ** at $P < 0.05$, $P < 0.01$, respectively.

cultivars were considered with respect to days to flower, 'White Reagan' cultivar flowered before (3 days) 'Yellow Reagan' cultivar. The interaction between day length and daminozide did not affect days to flower, whereas the

interaction between LD and cultivar significantly affected days to flower. LD conditions delayed the flowering of 'White Reagan' and 'Yellow Reagan' cultivars by about 41 and 44 days, respectively, as compared to SD conditions.

One of the most important indicators for the market value is the stem length in chrysanthemum, like in other cut flower species. Stem length was significantly affected by both day length and daminozide applications. In the study, the stem lengths under natural day length conditions were 2-fold longer than those under SD conditions. On the other hand, it was recorded that the daminozide application reduced stem length by approximately 19 cm. The stem lengths of plants treated and untreated with daminozide under day length conditions were found to be approximately 77 and 94 cm longer than those of the plants treated and untreated with daminozide under SD conditions, respectively. Our results are in agreement with the findings of Korkut (1990), Gregov et al., (1995), Velmurugan and Vadivel (2003), Karlovic et al. (2004) and El-Sheibany et al. (2007), who reported that SD and plant growth retardants reduced stem length in chrysanthemum. In the study, flowers with a quite long stem (>156 cm) were obtained under natural day length conditions. Nevertheless, although, varying by country, generally 70 - 80 cm long stems are preferred in the market. Both the risk of lodging in plants with very long stems and their requirement of more space, labor and higher transport costs (Larson, 1985; Bailey and Whipker, 1998; Hayashi et al., 2001; Jaime and Silva, 2003; Karlovic et al., 2004) increase the importance of plant height control in the production of chrysanthemum.

Stem diameter is an important criterion for determining stem strength. In the study, it was observed that SD conditions and daminozide application increased the stem diameter. Similar results were reported by Gregov et al., (1995), Mahalle et al. (2001), Khobragade et al. (2002), El-Sheibany et al. (2007) and (Kim et al. 2010). One of the important quality criteria for spray chrysanthemums is the number of flowers. The high number of flowers both affects the crop appearance and attracts the attention of consumers. In the study, SD conditions and daminozide application increased the number of flowers. There was a significant difference in the number of flowers between the cultivars. Under SD conditions, approximately 1.5 more flowers per stem were obtained as compared to LD conditions, whereas approximately 3 more flowers were obtained in daminozide application as compared to those untreated with daminozide (Table 1). Under SD conditions as compared to LD conditions, 'Yellow Reagan' cultivar had about 2.39 more flowers, while 'White Reagan' cultivar had 0.69 more flowers. The obtained results show similarity to the findings by Lazar et al. (1977), Gregov et al., (1995) and Velmurugan and Vadivel (2003), who reported that treatments of growth retardants increased the number of flowers in chrysanthemum. On the other hand, the obtained results regarding day length are inconsistent with the findings by Korkut (1990), who reported that the number of flowers decreased with the darkening.

Spray chrysanthemums are graded by stem length, stem fresh weight and maturity at the Dutch Flower Auction Association (VBN). In this grading, stem fresh

weights range from 25 to 105 g in spray chrysanthemums, depending on the grading codes (Anonymous, 2010). The stem fresh weights obtained in the study are in agreement with the above-mentioned standards. While stem fresh weight decreased under SD conditions, the daminozide application increased the stem fresh weight. The highest stem fresh weight was measured from the plants untreated with daminozide under LD conditions (132.03 g), whereas the lowest stem fresh weight was the plants untreated with daminozide under SD conditions (95.42 g) (Figure 4). It is known that dry matter content has a significant effect on the postharvest life of cut flowers. In the study, LD conditions increased the dry matter content. Similar results were reported by ByungJoo et al. (2004). Although, daminozide application slightly decreased the dry matter content, this decrease was statistically insignificant. Shawareb and Orunfleh (1988) and Mahalle et al. (2001) reported that treatments of growth retardants decreased the dry matter content in chrysanthemum.

Chl *a* is the principal pigment in plants. Levels of chl *a* and *b* (11.50 and 5.93 µg/mg, respectively) were found to be higher in the plants under LD conditions as compared to those under SD conditions (9.51 and 5.10 µg/mg, respectively). The low chl contents under SD conditions as compared to LD conditions can be explained by the scarcity of total period of lighting received by plants. Even though the levels of chl *a* (10.84 µg/mg) and *b* (5.71 µg/mg) in the plants treated with daminozide slightly increased as compared to the plants untreated with daminozide, this increase was statistically insignificant (Table 2). Shawareb and Qrunfleh (1988), Mahalle et al. (2001) and Kim et al. (2010) reported that treatments of growth retardants increased chl contents in chrysanthemum. While there was an insignificant difference in chl *a* between the cultivars, level of chl *b* (5.79 µg/mg) was higher in 'White Reagan' cultivar. Even though the increases or decreases in the contents of chl *a* and *b* are statistically insignificant, they cause some increase or decrease in the photosynthesis level in the plant, which indirectly affects plant development and, as a result, the crop (Tort and Dereboylu, 2003).

Conclusion

The effects of different day lengths (LD and SD) and daminozide on days to flower, some quality parameters and chlorophyll content in spray chrysanthemum cultivars ('White Reagan' and White Reagan) were tested in the study. SD conditions and daminozide application shortened the days to flower. SD conditions and daminozide reduced the stem length but increased the stem diameter and the number of flowers. LD conditions increased the stem fresh weight, the dry matter content and the contents of chl *a* and *b*. During the production of short-day plants like chrysanthemum under LD conditions, the plant height control through both short-day

application and treatments of plant growth retardants might also provide important advantages with respect to marketing, besides the increasing of yield and uniform quality in the commercial production of spray chrysanthemums.

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