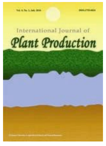

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Sunflower morphology and yield as affected by foliar applications of plant growth regulators

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Abstract

Plant height of sunflower is a major agronomic trait affecting crop performance in the field and its adaptation to mechanical harvest. Field experiments were conducted to study the effect of foliar application of paclobutrazol (PBZ) at 12.5 g ai/ha, mepiquat chloride (MPC) at 25.0 g ai/ha and chlormequat chloride (CCC) at 1,500 g ai/ha in single or double applications on sunflower plant morphology, growth and achene yield and oil content. Single applications of PBZ or MPC reduced sunflower plant height at maturity by 11.1% and 11.7%, respectively. Single applications of PBZ, MPC or CCC did not reduce the above ground dry weight of the plants, provided similar achene yield per plant and 100-achenes weight compared with those of the non-treated control, whereas increased the total number of achenes as well as the number of filled achenes per capitulum. Double applications of PBZ, MPC or CCC reduced plant height by 13.9%, 15.6% and 13.4%, respectively, but proved in certain cases to have a phytotoxic effect on achene and oil production. Double application of PBZ resulted in lower achene and oil yield than the non-treated control, whereas double application of MPC resulted in lower above ground dry weight at maturity, achene yield and 100-achenes weight. Double application of CCC resulted in reduced 100-achenes weight. Overall, PBZ and MPC under the single dose scheme applied can reduce plant height in sunflower without adverse effects on achene and oil yields, thus providing a basis for reducing the risk of plant lodging.

Keywords: Height; Lodging; Chlormequat chloride; Mepiquat chloride; Paclobutrazol.

Introduction

Sunflower (*Helianthus annuus* L.) is gaining popularity as an oilseed and a feedstock crop, because it shares several positive agronomic features with other common oil crops such as canola and soybean, yields well in a variety of conditions and can be grown easily and profitably at both small farms and large field scale. In addition, seeds from non-oilseed sunflower types are popular as a snack and bird food. In Greece, sunflower is principally grown in the northern part of the country (70% of the total sunflower area), mostly as a rainfed rotation partner to winter cereals (Kallivroussis et al., 2002). The crop, having suffered a significant acreage decrease till 2004, is gradually recovering over the last few years (FAOSTAT, 2012) mainly because of the increased interest for biodiesel production combined with the low prices of winter cereals, factors which have led farmers to turn to more profitable crops.

Sunflower is disadvantaged by its tall growth coupled with its relatively limited root system (Weiss, 2000) and this disadvantage is often manifested in its tendency towards lodging (uprooting). Lodging, the permanent displacement of the stem from the upright position, can be a major problem in sunflower cultivation, particularly under irrigation (Hall et al., 2010; Sposaro et al., 2010). When sunflower plants start to mature, the increased weight of the heads on the tall stems makes the plants particularly vulnerable to wind damage. Heavy rainfalls or strong winds during that stage further increase the possibility of plant lodging due to insufficient support by the wet soil. Susceptibility to lodging often depends on complex interactions between the mechanical properties of the stems and the soil-root system, the shape of the upper sections of the plant that capture wind gusts and the amount of rainfall (Sposaro et al., 2010). Short sunflower plants with increased resistance to lodging may have advantages under adverse growth conditions and are often preferred by farmers for reasons of ease of harvest (Fick and Miller, 1997).

Plant growth regulators (PGRs) include a wide variety of chemicals that are often used to regulate plant growth at various crop stages. Growth retardants, a group of PGRs, have found wide use in the agricultural practice with main focus on the improvement of resistance to lodging and canopy structure of crop plants (Arteca, 1995; Grossmann, 1990; Rademacher, 2000). Various PGRs have been tested in several field crops in an effort to reduce stem length, increase resistance to lodging and improve yield. Previous reports regarding these aspects include mainly cereals (Cox and Otis, 1989; Naylor, 1989; Rajala and Peltonen-Sainio, 2001; Sanvicente et al., 1999; Street et al., 1986; Toyota et al., 2010), as well as linseed (Leitch and Curt, 1999), cotton (Kerby, 1985; Lamas et al., 2000; Reddy et al., 1992; Zhao and Oosterhuis, 2000) and peas (Elkoca and Kantar, 2006). However, the effects of PGRs on crop plants are often highly variable with distinct cases of negative impact on yield.

The application of PGRs aiming to reduce stem length in sunflower provides a potential means of overcoming problems of lodging in this crop. However, there is very little information on possibilities for using growth regulators in sunflower (Spitzer et al., 2011). Previous research has shown that paclobutrazol affected the growth and production of sunflower grown in hydroponic cultivation by reducing the final plant height and the head diameter (Wanderley et al., 2007). Also, double application of chlormequat chloride plus ethephon reduced sunflower height by a maximum of 63 cm, whereas single application of ethephon reduced sunflower height by a maximum of 35 cm (Spitzer et al., 2011). However, the inconsistent performance of many PGRs in the field and the diverse effects on plant growth related to the timing of application (i.e. the growth stage of crop), the application dose of the PGR, as well as the variable effects of the environmental conditions are considered major limitations in the use of PGRs (Elkoca and Kantar, 2006; Leitch and Kurt, 1999; Scarisbrick et al., 1985).

The purpose of this study was to investigate the effects of foliar application of three PGRs, i.e. paclobutrazol (PBZ), mepiquat chloride (MPC) and chlormequat chloride (CCC) on non-oilseed sunflower morphology, growth and achene yield and oil content under field conditions.

Materials and Methods

Experimental design and cultural practices

A field experiment with a local population (i.e. traditional landrace) of non-oilseed sunflower was carried out during 2003 and 2004 in Orestiada (41^o 33′ N latitude, 26^o 31′ E longitude, 33 m a.s.l) in northern Greece. The experiment was established on a clay loam soil (28.7% clay, 46.1% silt and 25.2% sand) with pH 6.6 (1:1 with H_2O), organic matter content 2.4%, CEC 27.7 milliequivalents/100 g, N-NH_4 9.3 ppm, P (Olsen) 46.9 ppm and K 457.6 ppm (0 to 30 cm depth). The previous crop in the experimental field was sugar beet (*Beta vulgaris* L.). Meteorological data during the two growing seasons of the experimentation are given in Figure 1.

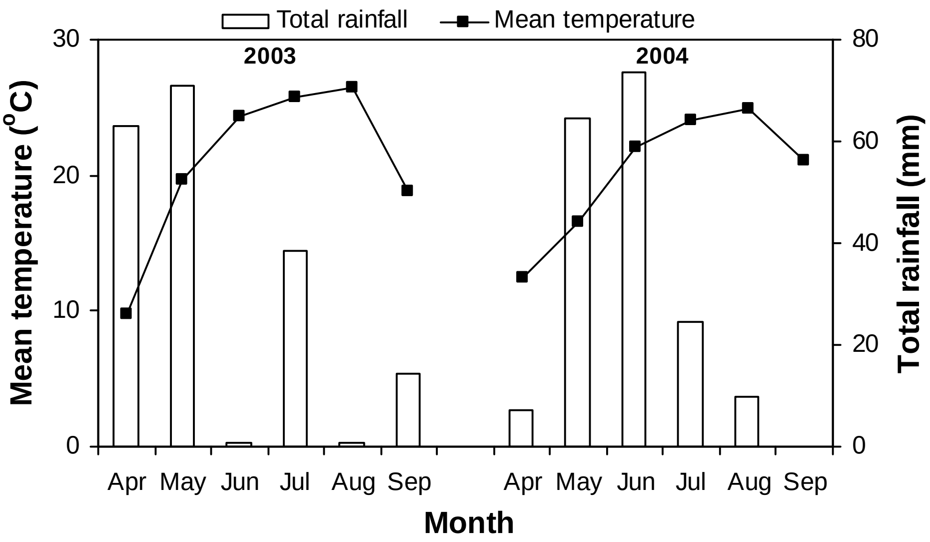

Figure 1. Mean monthly temperature and total monthly rainfall during the experimentation.

Soil preparation was done in mid spring of each year and consisted of conventional tillage, including chisel ploughing followed by harrowing twice. Sunflower was sown on May 3 2003 and April 25 2004 at a seeding rate of 7 kg/ha. Row to row and plant to plant (on the row) distances were 75 cm and 30 cm, respectively, yielding the usually practiced crop density of 44,400 plants/ha. Plots consisted of 4 rows of the crop, 8-m long each. The field was fertilized with 50 kg N/ha as ammonium sulfate, 50 kg P_2O_5/ha as superphosphate and 50 kg K_2O/ha as potassium sulfate, which were broadcast applied and incorporated into the soil before sowing. Two irrigations were applied at the early growth stages of the crop and one before flowering. Weeds were controlled manually during the early crop growth.

In 2003, single PGR treatments consisting of paclobutrazol (PBZ) at 12.5 g ai/ha, mepiquat chloride (MPC) at 25.0 g ai/ha and chlormequat chloride (CCC) at 1,500 g ai/ha were applied 33 days after sowing, when plants had five to six true leaves (BBCH 15-16) (Meier, 2001). A non-treated control was included for comparison. In 2004, an extra application was made for all PGR treatments in order to better clarify the proper application scheme. Thus, the treatments in 2004 were: PBZ at

12.5 + 12.5 g ai/ha, MPC at 25.0 + 25.0 g ai/ha and CCC at 1,500 + 1,500 g ai/ha. In this case, the first application of PGR treatments took place 26 days after sowing, at the four to five true leaves (BBCH 14-15) of sunflower plants and the second application took place two weeks after the first one, at the five to six true leaves (BBCH 15-16) (Meier, 2001). The treatments were arranged in a randomized complete block design with four replications. All PGRs were applied with a portable hand-held field plot sprayer at 250 kPa pressure using a water carrier volume of 500 L/ha.

Data collection

Visual rating of sunflower injury was based on a scale of 0 (no injury) to 100% (plant death) 7 days after treatment. Plant height, number of stem nodes and stem diameter were measured at maturity using 10 plants from each plot labeled 2 days prior to the PGR treatments. Plant height was measured from the soil surface to the top of the uppermost plant organ. Stem diameter measurements were taken from the same (second) internode of the plants throughout the trial. Plant samples, composed of three plants by each plot, were taken at flowering and maturity. The plants were cut at the ground level and separated into leafs, stems and capitula. At maturity, capitula were further separated into vegetative components and achenes. Filled and unfilled achenes were separated by hand and the number of achenes for each group was determined. All plant samples were oven-dried at 70 ^{o}C to constant weight and weighted. Achene yield (expressed as g/plant) was determined on the basis of the filled achenes. Achene oil content was determined with the Soxhlet extraction method according to the official methods of the American Oil Chemists’ Society (AOCS, 1983).

Data analysis

All measured and derived data were subjected to analysis of variance (ANOVA) using one-way ANOVA with four treatments (three chemical treatments plus the non-treated control) and four replications according to Steel and Torrie (1980). Because there was an extra application for all PGRs in the second year (2004), the data were analyzed separately for each year. Least significant difference (LSD) values at 5% level of significance were calculated for each variable and used for mean separation.

Results

Weather conditions prevailed during the experimentation were similar the two growing seasons. Total seasonal rainfall was 188.3 mm in 2003 and 180.1 mm in 2004. However, differences in terms of rainfall distribution between growing seasons were recorded, with June 2004 being much rainier (73.5 mm) in comparison to June 2003 (0.6 mm) (Figure 1). Mean temperature during the whole growing season was 20.8 ^{o}C in 2003 and 20.2 ^{o}C in 2004.

Phenological stages and crop injury

All PGR treatments, both in 2003 as well as in 2004, resulted in no shift of the various phenological stages compared with the non-treated control. Averaged across years and treatments flowering was recorded 86 days after sowing and maturity was recorded 144 days after sowing (data not shown). No injury at any treatment in both years was observed.

Plant height, number of stem nodes and stem diameter

In 2003, significant reduction of plant height was observed with PBZ (by 29.1 cm or 11.1%) and with MPC (by 30.8 cm or 11.7%) compared with the non-treated control (Table 1). On the other hand, plant height was not affected by CCC application. In 2004, applications of PBZ, MPC or CCC reduced plant height by 13.9%, 15.6% or 13.4%, respectively, compared with the non-treated control. In both years, the reduction in plant height was not accompanied by a reduction in the number of stem nodes per plant or by a significant effect on stem diameter compared with the non-treated control, with the exception of MPC in 2003, which resulted in thinner stems than that of the non-treated control (Table 1).

Table 1. Plant height, number of nodes and stem diameter of sunflower plants at maturity as influenced by the foliar treatments of PGRs.

Treatment	Rate (g ai/ha)	Plant height (cm)		No. nodes per plant		Stem diameter (mm)	
		Growing season 2003					
Paclobutrazol (PBZ)	12.5	233.4	b	30.8	a	37.0	ab
Mepiquat chloride (MPC)	25.0	231.7	b	30.2	a	35.5	b
Chlormequat chloride (CCC)	1,500	256.7	a	34.8	a	39.2	a
Non-treated control	---	262.5	a	34.5	a	39.5	a
		Growing season 2004					
Paclobutrazol (PBZ)	12.5+12.5	294.2	b	30.3	a	37.0	b
Mepiquat chloride (MPC)	25.0+25.0	288.3	b	30.0	a	35.5	b
Chlormequat chloride (CCC)	1,500+1,500	295.8	b	30.7	a	42.2	a
Non-treated control	---	341.7	a	32.7	a	37.5	ab

Different letters within each column in each growing season indicate statistically significant differences at P=0.05.

Above ground dry weight

Differences among treatments in above ground dry weight were observed, but these differences were not consistent across years or growth stages (Table 2). In both years, a significant increase of above ground dry weight at flowering was observed with PBZ and CCC compared with the non-treated control. On the contrary, above ground dry weight at flowering obtained with MPC was similar to that of the non-treated control.

PGR treatments resulted in above ground dry weight at maturity similar to that of the non-treated control in both years, except from CCC in 2003 and MPC in 2004 (Table 2). CCC application in 2003 increased above ground dry weight at maturity, whereas the double application of MPC in 2004 reduced the above ground dry weight at maturity compared with the non-treated control.

Table 2. Above ground dry weight of sunflower plants at flowering and maturity as influenced by the foliar treatments of PGRs.

Treatment	Rate (g ai/ha)	Above ground dry weight (g/plant)			
		Flowering		Maturity	
		Growing season 2003			
Paclobutrazol (PBZ)	12.5	470.8	a	745.0	b
Mepiquat chloride (MPC)	25.0	368.1	b	776.0	ab
Chlormequat chloride (CCC)	1,500	476.5	a	867.9	a
Non-treated control	---	369.0	b	757.0	b
		Growing season 2004			
Paclobutrazol (PBZ)	12.5+12.5	545.1	a	694.5	ab
Mepiquat chloride (MPC)	25.0+25.0	424.8	b	625.0	b
Chlormequat chloride (CCC)	1,500+1,500	590.6	a	848.1	a
Non-treated control	---	502.5	b	809.9	a

Different letters within each column in each growing season indicate statistically significant differences at P=0.05.

Achene yield, yield components, achene oil content and oil yield

In 2003, chemical treatments resulted generally in increased total number of achenes per capitulum and number of filled achenes per capitulum than the non-treated control, with the greatest increase in these variables observed with CCC (Table 3). However, no significant differences among treatments in 100-achenes weight and achene yield were observed. In 2004, all chemical treatments yielded similar or significantly higher total number of achenes per capitulum and similar number of filled achenes per capitulum compared with those of the non-treated control (Table 3). In addition, all chemical treatments reduced 100-achenes weight compared with the non-treated control, significantly only for MPC (24%) and CCC (21%). The double application of PBZ or MPC resulted in significantly lower achene yield by 21.7 and 21.2%, respectively than the non-treated control. The double application of CCC reduced achene yield by 10% compared to the non-treated control, but the difference was not significant.

In both years, none of the PGR treatments affected achene oil content (Table 3). In 2003, CCC or PBZ application increased oil yield per plant, whereas MPC application resulted in similar oil yield per plant compared with that of the non-treated control. In 2004, the double application of MPC or CCC did not affect oil yield per plant, whereas the double application of PBZ resulted in considerably lower oil yield per plant compared with that of the non-treated control (Table 3).

Table 3. Achene yield, 100-achenes weight (filled), number of total achenes and filled achenes per capitulum, achene oil content and oil yield of sunflower plants as influenced by the foliar treatments of PGRs.

Treatment	Rate (g ai/ha)	Achene yield (g/plant)		100-achenes weight (g)		No. total achenes per capitulum		No. filled achenes per capitulum		Achene oil content (%)		Oil yield (g/plant)	
		Growing season 2003											
Paclobutrazol (PBZ)	12.5	203.0	a	16.6	a	1788	b	1255	bc	29.5	a	59.8	ab
Mepiquat chloride (MPC)	25.0	205.0	a	16.8	a	1733	b	1276	b	24.9	a	51.1	bc
Chlormequat chloride (CCC)	1,500	224.5	a	16.3	a	1933	a	1392	a	27.7	a	61.5	a
Non-treated control	---	197.5	a	16.6	a	1564	c	1178	c	24.9	a	49.3	c
		Growing season 2004											
Paclobutrazol (PBZ)	12.5+12.5	151.7	b	18.5	ab	1386	b	828	b	18.2	b	27.9	b
Mepiquat chloride (MPC)	25.0+25.0	152.7	b	14.9	b	1667	ab	1020	a	23.8	a	35.8	ab
Chlormequat chloride (CCC)	1,500+1,500	174.6	ab	15.5	b	1942	a	1124	a	20.2	ab	34.8	ab
Non-treated control	---	193.9	a	19.6	a	1575	b	988	ab	20.3	ab	38.9	a

Different letters within each column in each growing season indicate statistically significant differences at P=0.05.

Discussion

Data of this study showed that the height of sunflower plants can be reduced by foliar application of PBZ, MPC and CCC, but in certain cases a detrimental effect occurred in other plant parameters, particularly with the double applications of those PGRs. Single applications of PBZ or MPC reduced plant height at maturity by 11.1% and 11.7%, respectively, compared with the non-treated control. These treatments did not show any detrimental effect on other growth and yield parameters and thus they could be considered for the control of plant height in sunflower. Significant reduction in plant height was also observed with the double applications of all PGRs. However, these treatments resulted in lower achene yield than the non-treated control, which indicates a certain phytotoxic effect on sunflower plants in terms of seed and oil production. The reduction of achene yield observed with double application of PGRs was due to decrease of individual seed weight, because total number of achenes per capitulum or filled achenes per capitulum was at least similar to that of the non-treated control. Seed weight in sunflower has been reported to have the highest direct positive effect on seed yield per plant (Marinkovic, 1992) along with the number of seeds per head and the head diameter (Yasin and Singh, 2010). In addition, seed weight is of paramount importance in non-oilseed sunflower because it is associated with seed size, a trait that is often used in grading seeds, with the largest ones being preferred by customers. Oil yield is a function of achene yield (grain number and weight) and achene oil content (oil concentration) (De la Veja and Hall, 2002). In the present study, various combinations of these components resulted in oil yield per plant higher or at least similar to that of the non-treated control with single applications of PGRs (2003) and in similar or lower oil yield per plant with double applications of PGRs (2004).

The results of this study generally agree with those previously reported in the literature for sunflower or for other field crops regarding plant height, but with different PGRs or different combinations of PGRs. Lovett and Campbell (1973) reported that the height of sunflower plants treated with CCC represented 60% of that of the untreated plants. A recent study (Spitzer et al., 2011) found that sunflower plant height was reduced by as much as 63 cm with double application of CCC plus ethephon and by as much as 35 cm with ethephon alone. In cotton (*Gossypium hirsutum*), several studies have reported reduction of plant height mainly because of reduction in the internode length due to MPC treatment (Kerby, 1985; Lamas et al., 2000; Reddy et al., 1992; Zhao and Oosterhuis, 2000). Spring applications of PBZ in oilseed rape (*Brassica napus*) prior to a period of rapid crop development reduced plant height, but also they had an adverse effect on the yield components and they offered no practical advantage for the commercial production of oilseed rape (Scarisbrick et al., 1985).

The shortening effect of PGRs on sunflower plants observed in the present study was due to the reduction of the internode length, because the number of stem nodes was not affected by the chemical treatments (Table 1). These results are in agreement with those of Lovett and Campbell (1973), who reported a reduction in the internode length of sunflower plants after application of CCC. Scarisbrick et al. (1985) found that PBZ significantly reduced the lengths of the early-formed internodes of oilseed rape at the base of the plant, whereas the middle and the upper internodes later became longer than those of the control. Weiss (2000) also reported that certain growth regulators generally reduced height of sunflower at maturity by affecting the internode length, although there may be differing cultivar reaction to the chemical used or to the dose applied. Regarding the stem diameter of sunflower plants, results of the present study indicated that it was not affected by PGRs application. Similar results have been reported by Sanvicente et al. (1999), who found no changes in culm diameter of barley after PGR application. On the contrary, an increase in stem width of sunflower plants treated by CCC was reported (Lovett and Campbell, 1973). No effect of PGRs on the time of appearance of sunflower phenological stages was observed. On the other hand, application of PBZ and CCC was found to increase above ground dry weight of plants at flowering compared with the non-treated control. With reference to phenological stages duration and growth, variable effects have been reported in the literature, particularly with PBZ. The application of PBZ markedly delayed floral initiation in sunflower and this effect was dependent on plant age (Almeida and Pereira 1996). On the contrary, PBZ foliar applications (up to 80 mg/L) for the control of height in potted sunflower had little effect on growth and flowering responses of the plants (Whipker and Dasoju, 1998). Davis et al. (1988) reported that PBZ can cause several physiological alterations that are generally correlated with optimized yield formation in various crops such as enhanced carbohydrate synthesis, promoted flowering and fruit set and stimulated translocation of photoassimilates towards seeds. The increased above ground dry matter accumulation of sunflower plants at flowering obtained by PBZ and CCC in the present study suggests a positive effect of these PGRs on the amount of photoassimilates that were available for translocation from the vegetative organs to the achenes. In several crops it has been found that increased dry matter translocation to the seeds during the filling period was associated with increased above ground dry matter at anthesis (Papakosta and Gagianas, 1991; Koutroubas et al., 2004). In Ethiopian mustard (*Brassica carinata*), PBZ increased total dry matter of plants and partitioning coefficients, but the seed yield per plant increased mainly due to increase in the number of siliquae (i.e. pods) per plant (Setia et al., 1995). In potato, although PBZ decreased the total biomass production, it improved yield by partitioning more assimilates to the tubers (Tekalign and Hammes, 2004; Tekalign and Hammes, 2005). A previous study in sunflower with different PGRs (Beltrano et al., 1994) found that foliarly applied gibberellic acid and benzyladenine increased achene number and achene weight, while reduced the percentage of empty achenes in the inner and middle portion of the capitulum of the plants due to a preferential distribution of photoassimilates from the outer to the inner portion of capitulum. This modified pattern of photoassimilate distribution also might be the case for the increased number of filled achenes that were observed with most PGR treatments tested in the present study.

No foliar injury was observed on sunflower leaves by any PGR treatment in the present study. Spitzer et al. (2011) observed some extent of foliar injury with chlormequat chloride on sunflower. Indeed, foliar sprays of CCC can cause yellow spotting, haloing, or discoloration of newly expanding leaves in ornamental plants, usually visible within 3 to 5 days after spray application as a result of damage to the chloroplasts (Lopez and Currey, 2010). Problems usually occur when the concentration of CCC sprays exceeds 1,500 ppm, but leaves usually 'green up' within a few weeks, depending on the sensitivity of cultivars and species (Lopez and Currey, 2010). Additionally, damage such as flower deformation was not observed in the present study. Wanderley et al. (2007) reported that high rates of PBZ caused damage (i.e. flower deformation) to sunflower grown in a hydroponic solution. Moreover, application of PBZ on a spring variety of oilseed rape has been reported to reduce the size of petals and sepals on the first-formed flowers (Scarisbrick et al., 1985).

Overall, results of this study showed that PBZ and MPC under the single dose scheme applied can reduce plant height in sunflower without adverse effects on achene and oil yields, thus providing a basis for reducing the risk of plant lodging. CCC produced a similar effect only by means of a split double dose application. However, the split double dose application scheme tested in this study proved in certain cases to have adverse effects on achene and oil yields. In terms of lodging risk, the practical importance of the plant height reduction obtained by the foliar application of the PGRs should be further investigated, including factors like cultivar response, split application of PGRs at lower doses or at different crop stages and different moisture regimes (i.e. irrigated or rainfed crop).

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