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Yield and Fruit Quality of Pepper (*Capsicum annuum* L.) in Response to Bioregulators

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Abstract. **To test the effectiveness of different bioregulators in enhancing bell pepper (*Capsicum annuum* L.) yield and fruit quality, the commercial bioregulators CCC, NAA, GA_3, and Biozyme® were sprayed on plants at flower initiation, followed by two additional applications at 30-day intervals. Biozyme produced a significant increase in total yield but ≈40% of the fruit were not marketable. Treatment with NAA produced the highest yield of marketable fruit. Treatments did not affect fruit firmness compared to the control. Gibberellic acid increased fruit ascorbic acid and citric acid concentrations and Biozyme, GA_3, and CCC increased fruit soluble solids content. Biozyme treatment increased fruit fructose, sucrose, carotenoid, and lycopene concentration. Treatments had no effect on fruit calcium concentration or pH. Chemical names used: chlormequat chloride (CCC); naphthaleneacetic acid (NAA), gibberellic acid (GA_3); GA_3 + IAA (indole-3-acetic acid) + zeatine + micronutrients (Biozyme®).**

Bell pepper is a major crop in the southeast of Spain, and Almeria is the most important zone with the highest yields. Generally, growers apply P, K, and especially N to improve yields (Grattan and Grieve, 1993). However, heavy fertilizer application can result in the excessive accumulation of minerals in the soil, since the area already suffers from soil salinization from irrigation with saline water.

The use of bioregulators is a common horticultural practice to improve yields (Latimer, 1992). Bioregulators can affect rooting, flowering, fruiting and fruit growth, leaf or fruit abscission, senescence, regulation of some metabolic processes, and plant resistance to temperature or water stresses (Nickell, 1988).

Relatively few studies have reported on the effects of bioregulators on improving pepper yields. Nickell (1982) found that an antiauxin (toluiphtalamic acid) extended the spring and winter greenhouse production seasons of many plant species, including tomato (*Solanum esculentum*), potato (*Solanum tuberosum*) and pepper, by promoting fruit set and development. Berkowitz and Rabin (1988) reduced transplant shock and increased plant yield by applying abscisic acid to bell pepper seedlings immediately before transplanting. Csizinszky (1990) reported that the application of a mixture of growth regulators and nutrients increased pepper yield and nutrient availability in fruit. Hartz et al. (1995) treated pepper seeds and leaves with DCPTA [2-(3,4-dichlorophenoxy) triethylamine], but found that it was not effective in increasing vegetative growth or fresh fruit yield and quality. Elsayed (1995) applied several doses of Biozyme to several bell pepper cultivars and found that 2 $mL \cdot L^{-1}$ of Biozyme increased height and weight of plants of the cultivar Blemont.

The objective of our study was to determine the effects of four different commercial bioregulators on pepper production and fruit quality.

Materials and Methods

Experimental design. The experiment was conducted under controlled greenhouse conditions in the southeast of Spain. In Oct. 1992, bell pepper 'Lamuyo' was seeded in cell flats (cell size 3 × 3 × 10 cm) filled with peat-lite mixture, and the flats were placed on benches under the greenhouse conditions described below. Six weeks later, they were transplanted to a loamy soil with the following characteristics: sand (37.3%), silt (48.6%), and clay (14.1%), $CaCO_3$ equivalence (26.82 $g \cdot 100\ g^{-1}$), $CaCO_3$ active (14.35 $g \cdot 100\ g^{-1}$), P+K (14.57 $meq \cdot 100\ g^{-1}$), pH (H_2O) (8.45), pH (KCl) (8.01), electrical conductivity (4.63 $dS \cdot m^{-1}$). The relative humidity was 60% to 80% and the temperature 27 °C day ± 4 °C night. The experimental design was a randomized complete block with five treatments, including one control. Peppers were transplanted in two rows spaced 35 cm within rows and 50 cm between rows, and trickle-irrigated. Each treatment was replicated four times in four individual plots 4 m long × 2 m wide. Four bioregulators were applied to plants to runoff as aqueous foliar sprays containing the surfactant Tween 20 (0.5% v/v), using a stainless steel sprayer. Treatments were nontreated control, CCC (100 $mg \cdot L^{-1}$), NAA (27 $mg \cdot L^{-1}$), GA_3 (16 $mg \cdot L^{-1}$), and Biozyme [GA_3(32.2 $mg \cdot L^{-1}$) + IAA (indole-3-acetic acid) (32.2 $mg \cdot L^{-1}$) + zeatin (83.2 $mg \cdot L^{-1}$) + chelated micronutrients]. The first application was at flower initiation, followed by two sprays at intervals of 30 d. Plants received 150N–80P–288K ($mg \cdot L^{-1}$) weekly through the irrigation system. The fertilization was supplemented with the following micronutrient solution: $MnSO_4$ (2 $mg \cdot L^{-1}$), $ZnSO_4 \cdot 7H_2O$ (1 $mg \cdot L^{-1}$), $CuSO_4 \cdot 5H_2O$ (0.25 $mg \cdot L^{-1}$), H_3BO_3 (0.5 $mg \cdot L^{-1}$) and $Na_2Mo_4O \cdot 2H_2O$ (0.05 $mg \cdot L^{-1}$). Iron was supplied as Fe-EDDHA (5 $mg \cdot L^{-1}$). The final pH of the nutrient solution oscillated between 5 and 6.1.

Fruit sampling. Peppers were usually harvested weekly at mature red stage. Yield was determined by counting and weighting all fruit on each plant. The number of marketable and nonmarketable fruits were noted for each treatment. Marketable fruits were those of 140 to 170 g, with uniform size and brilliant red color. The nonmarketable fruits were physically damaged or those with unsuitable color or size.

Fruit analysis. Five representative pepper samples were selected randomly (picking marketable and/or nonmarketable fruits, depending on the treatment) from each treatment and used for fruit analysis. Firmness was determined on one side of fruits using a Magness-Taylor penetrometer. Soluble solids concentration (SSC) of a composite juice sample was measured with a hand-held refractometer. Ten grams of fresh fruit material was crushed in a mortar, and the extract was assayed for citric acid by titration with 0.1 N NaOH. Ascorbic acid concentration was analyzed according to the Association of Official Analytical Chemists (1984) colorimetric method. Chlorophyll was extracted by the procedure of Hiscox and Israelstam (1979). Disks of tissue (40 mg) were soaked in assay tubes with 10 mL of dimethylsulfoxide and placed into a water bath at 65 °C for 1.5 h. Chlorophyll was then measured by colorimetric spectrophotometry at several wavelengths (Bruinsma, 1963). Chlorophyll content was expressed as milligrams per gram fresh mass, according to the equations of Abadía and Abadía (1993). Carotenoids were measured as described by Jaspar (1965). Disks were incubated in acetone for 24 h and absorbance was read at a wavelength of 470 nm. The formula of Abadía and Abadía (1993) was then applied. Lycopene was measured according to Jaspar (1965).

For the determination of soluble carbohydrates and total Ca in fruit, five representative fruit were washed with a 1% soap solution, then rinsed three times with distilled water (Wolf, 1982). Samples were dried in a forced-air oven at 70 °C for 48 h, and then ground to pass a 20-mesh screen in a Wiley mill for analysis of soluble carbohydrates and total Ca.

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Soluble carbohydrates (glucose, fructose, and sucrose) were analyzed in dry fruit samples by spectrophotometry at 650 nm following the anthrone procedure (Irigoyen et al., 1992). For Ca determination, dry fruit material was digested with H_2SO_4 and H_2O_2 (Wolf, 1982). Total Ca concentration was measured by atomic absorption spectrophotometry. Interferences associated with Ca determination were eliminated by addition of 3% lanthanum in the diluted digest solution.

Statistical analysis. Data were subjected to analysis of variance in all experiments to determine significance of bioregulator treatments, and treatment means were separated by Duncan's multiple range test at $P < 0.05$ (Statgraphics, 1992). When appropriate, correlation analysis was used.

Results and Discussion

Number of fruits per hectare. Plants treated with NAA produced 33% more marketable fruits per hectare than did the control (Table 1). Yield of plants treated with CCC also increased slightly (18%), possibly because of an inhibition of the vegetative growth. Growth retardants applied to grape (*Vitis vinifera*) vines enhanced individual berry mass but decreased yield per hectare (Reynolds, 1988). Although GA_3 application generally induces parthenocarpic fruit set (Pharis and King, 1985), in our study this bioregulator did not increase yield. Biozyme increased the number of marketable fruit per hectare by 29% compared to control plants. The NAA treatment produced the smallest number of nonmarketable fruits and Biozyme treatment the largest. The total number of fruit was highest with Biozyme, principally due to the high number of nonmarketable fruits. The remaining bioregulators did not affect total yield significantly.

Fruit mass per hectare. When data were expressed as tons per hectare, treatment with NAA increased marketable yield 28% compared to control plants (Table 1). Our findings are consistent with those of Reynolds (1988), who reported that application of NAA to grape increased yield. Treatment with GA_3, CCC, and Biozyme did not affect marketable yield. Biozyme increased nonmarketable fruit yield by 39%. The highest total yield was obtained following NAA application.

Few studies have reported on the applicability of Biozyme in horticulture. Campos et al. (1994) demonstrated that the dry mass of bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) plants increased following Biozyme application. In our study, the increase in yield as kilograms per hectare or number of fruit per hectare was significant. Our results agree with those of Elsayed (1995), who demonstrated that Biozyme treatment increased yield of three bell pepper cultivars.

Fruit firmness was not affected by GA_3 or Biozyme (Table 2). Calcium concentration was greater with GA_3 than with Biozyme or CCC treatment, but no treatment differed from the control (Table 2). The greater fruit Ca concentration with GA_3 than with Biozyme treatment may account for the greater firmness with GA_3. That Ca plays a key role in the maintenance of fruit firmness and, therefore, quality is well-known. The requirements of Ca in fruit are related to cell wall stability and with membrane integrity (Shear, 1975). Our results could be supported by the high degree of correlation (at $P \leq 0.05$) obtained between these parameters ($r = 0.951$). This significant relationship indicates that fruit firmness was proportional to the level of Ca.

Citric acid and ascorbic acid concentration in fruit was increased by GA_3 treatment (Table 2). The increase in concentration of both organic acids appeared to affect fruit pH, as pH was lowest in fruits treated with GA_3. Our results are consistent with those of Akl et al. (1995), who reported that the application of GA_3 to orange trees produced an increase in the concentration of ascorbic acid and, therefore, increased fruit acidity. No significant differences were observed among the remaining treatments. Elsayed (1995) reported that Biozyme applied at 2 $mL \cdot L^{-1}$ increased the level of ascorbic acid in the pepper cultivar Atol. Our results may be due to the low amount of Biozyme applied or to the long intervals between application.

Biozyme, CCC, and GA_3 increased fruit SSC, Biozyme having the greatest effect, but only Biozyme increased the concentration of fructose and sucrose (Table 2). Auxin or gibberellins can cause accumulation of carbohydrates in several species and stimulation of the rate of export of assimilates from leaves (Archbold et al., 1982). The high level of soluble carbohydrates produced by the Biozyme treatment (Table 2) could be due to a high level of respiration caused by intensive fruit growth and high metabolic activity in response to Biozyme (Tazuke and Sakiyama, 1991).

Only GA_3 increased the chlorophyll *a* level (Table 3), and no treatment altered the concentration of chlorophyll *b*. The effectiveness of growth retardants, such as CCC, in increasing leaf chlorophyll concentration of many plant species is well-documented (Rademacher, 1991). The response of chlorophyll *a* and *b* observed with Biozyme may be accompanied by a change in fruit pigmentation. Biles et al. (1993) demonstrated that a loss of chlorophyll in pepper fruit accompanied the change of fruit pigmentation from orange to red. Biozyme treatment increased carotenoid concentration 36.4%. The highest concentration of lycopene was also obtained with Biozyme. The transformation of chloroplasts to chromoplasts in pepper fruits coincides with the accumulation of carotenoids, inducing fruit ripening (Bertrand et al. 1992). Therefore, the cumulative effects of carotenoid accumulation and lycopene deposition lead to a change in fruit color from orange to red (Hobson, 1993).

The number of marketable fruit harvested increased with CCC, NAA, and Biozyme treatment. Total yield increased greatly with Biozyme, but mainly because of an increase in yield of nonmarketable fruit. A recommendation is difficult with regard to Biozyme and CCC, but NAA produced good results in both yield and fruit quality. Modifications in Biozyme concentration or in application intervals may be beneficial in reducing nonmarketable yield. Possibly, higher doses of Biozyme (2 $mL \cdot L^{-1}$) sprayed at 15-d intervals would be more effective.

Table 1. Effects of bioregulator treatments on marketable and nonmarketable yield of bell pepper.

	Number of fruit/ha (thousands)			Yield ($t \cdot ha^{-1}$)		
Treatment	Marketable fruit	Nonmarketable fruit	Total	Marketable fruit	Nonmarketable fruit	Total
Control	200 c[z]	179 d	379 b	36 b	8 c	44 b
CCC[y]	236 b	213 c	449 ab	40 b	9 c	49 b
NAA	265 a	169 e	434 ab	46 a	9 c	55 a
GA_3	214 c	228 b	442 ab	37 b	10 b	47 b
Biozyme	258 ab	282 a	541 a	42 ab	11 a	53 a

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.
[y]CCC = chlormequat chloride; NAA = naphthaleneacetic acid; GA_3 = gibberellic acid; Biozyme = GA_3 + IAA (indole-3-acetic acid) + zeatine + micronutrients.

Table 2. Effects of bioregulator treatments on quality parameters of pepper fruits.

	Firmness	Total Ca	Ascorbic acid	Citric acid		SSC[z]	Glucose	Fructose	Sucrose
Treatment	(kg)	($mg \cdot g^{-1}$ dry mass)	($mg \cdot 100\ g^{-1}$ fresh mass)		pH	(%)	($mg \cdot g^{-1}$ dry mass)		
Control	0.60 ab[y]	2.20 ab	2.58 b	1.02 b	5.45 ab	0.805 b	27.61 ab	3.94 b	11.56 b
CCC[x]	0.57 ab	2.10 b	2.89 ab	1.10 b	5.48 a	1.000 a	28.63 ab	4.24 ab	12.45 ab
NAA	0.59 ab	2.14 ab	2.97 ab	1.26 ab	5.49 a	0.880 b	27.34 ab	4.04 ab	11.86 ab
GA_3	0.63 a	2.39 a	3.36 a	1.51 a	5.34 b	0.980 a	26.40 b	3.89 b	11.41 b
Biozyme	0.56 b	2.09 b	3.09 ab	1.38 ab	5.43 ab	1.020 a	30.29 a	4.48 a	13.14 a

[z]Soluble solids content.
[y]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.
[x]CCC = chlormequat chloride; NAA = naphthaleneacetic acid; GA_3 = gibberellic acid; Biozyme = GA_3 + IAA (indole-3-acetic acid) + zeatine + micronutrients.

Table 3. Effects of bioregulators on chlorophyll and assessory pigment concentrations in pepper fruits.

Treatment	Chlorophyll *a* (mg·g^{-1} fresh mass)	Chlorophyll *b* (mg·g^{-1} fresh mass)	Carotenoids (mg·g^{-1} fresh mass)	Lycopenes (g·100 g^{-1} fresh mass)
Control	0.058 b[z]	0.027 ab	0.022 b	25.0 b
CCC[y]	0.055 b	0.035 a	0.023 b	26.5 b
NAA	0.055 b	0.023 b	0.024 b	26.7 b
GA_3	0.073 a	0.023 b	0.027 ab	25.7 b
Biozyme	0.062 ab	0.021 b	0.030 a	29.5 a

[z]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.
[y]CCC = chlormequat chloride; NAA = naphthaleneacetic acid; GA_3 = gibberellic acid; Biozyme = GA_3 + IAA (indole-3-acetic acid) + zeatine + micronutrients.

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