

# Effect of cultar and triadimefon on tomato (*Lycopersicon esculentum* Mill.)

K. N. ASARE-BOAMAH, E. ANLAAKU & A. K. I. GALYUON

Department of Botany, University of Cape Coast, Cape Coast, Ghana

## SUMMARY

Both vegetative and preproductive growth of tomato (*Lycopersicon esculentum* L. var. *Wosowoso*) were influenced by the application of cultar (100 ppm) and triadimefon (200 ppm). The fresh and dry weights of leaves, stems and roots were significantly ( $P \leq 0.05$ ) decreased by the chemicals and the elongation of the stem was significantly ( $P \leq 0.05$ ) retarded. The expansion of the epidermal and palisade cells and leaf area were significantly ( $P \leq 0.05$ ) reduced. Although, cultar and triadimefon had no effect on the number of stomates at the adaxial surface of the leaves, the size of the stomatal aperture was decreased significantly ( $P \leq 0.05$ ). The chemicals increased chlorophyll and carotenoid levels in the leaves compared to the controls. The levels of total soluble carbohydrates and relative water content in the leaves also were increased significantly ( $P \leq 0.05$ ). Although cultar and triadimefon delayed the onset of flowering, the number of flowers per plant in the chemically-treated plants was significantly ( $P \leq 0.05$ ) higher than in the control plants. Yield in tomato was also significantly ( $P \leq 0.05$ ) increased by cultar.

## RÉSUMÉ

ASARE-BOAMAH, K. N., ANLAAKU, E. & GALYUON, A. K. I.: *Effet de cultar et de Triadimefon sur de tomate*. (*Lycopersicon esculentum* Mill.). La croissance végétative et reproductrice de tomate (*Lycopersicon esculentum* L. var. *Wosowoso*) étaient à la fois influencées par l'application de cultar (100 ppm) et triadimefon (200 ppm). Les poids des feuilles, des tiges, et des racines fraîches et sèches étaient considérablement ( $P \leq 0.05$ ) réduites par les produits chimiques et l'élongation de la tige était considérablement ( $P \leq 0.05$ ) retardée. L'expansion des cellules épidermiques et palissades et la superficie de la feuille étaient considérablement réduites. Bien que cultar et triadimefon n'avaient pas d'effet sur le nombre de stomates à la surface adaxiale des feuilles, la taille de l'orifice stomatal était réduit considérablement ( $P \leq 0.05$ ). Les produits chimiques ont augmenté les niveaux de chlorophylle et de caroténoïde dans les feuilles comparé avec les contrôles. Les niveaux de féculents soluble totale et le contenu d'eau relative dans les feuilles étaient aussi augmentés considérablement ( $P \leq 0.05$ ). Bien que cultar et triadimefon ont tardé le début de la floraison, le nombre des fleurs par plante dans les plantes traitées avec des produits chimiques était considérablement ( $P \leq 0.05$ ) plus élevé que dans les plantes de contrôles. Le rendements dans la tomate était aussi augmenté considérablement ( $P \leq 0.05$ ) par cultar.

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## Introduction

Cultar (Paclobutrazol or PP333) [(2R,3R,+2S,3S)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol]] and triadimefon [(1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanone)] are triazole derivative compounds with both plant growth regulating and fungicidal properties although cultar is manufactured as a plant growth regulator and triadimefon as a fungicide (Fletcher, 1985). It has been reported that cultar retards vegetative growth and increases

yield in several plant species (Couture, 1982; Quinlan & Richardson, 1984; Addo-Quaye, Daniels & Searsbrick, 1985; Sankhla *et al.*, 1985; Martin & Dabek, 1988). It shifts the partitioning of photo-assimilates from leaves to floral branches and increases carbohydrate and chlorophyll levels (Addo-Quaye, Daniels & Searisbrick, 1985; Steffens, Bryun & Wang 1985; Steffens *et al.*, 1985; Wang, Bryun & Steffens, 1985). Triadimefon is also known to cause growth retardation, reduce leaf area, increase leaf diffusive resistance in beans

and tomato (Fletcher, 1985; Asare-Boamah *et al.*, 1986), retard vegetative growth in barley, tomato and cotton (Buchenaer & Rhoner, 1981) and increase yield in some species (Buchenaer & Rhoner, 1981; Hedden & Graebe, 1985), and their growth regulating properties are attributed to their anti-gibberellin activities (Hedden & Graebe, 1985).

The above findings suggest, therefore, that by manipulating the vegetative growth of crops, using these chemicals, more plants could be grown per unit land area to enhance yields and increase land use efficiency. In the present report, additional information is provided on the effects of cultar and triadimefon on the height of the stem, expansion of the epidermal and palisade cells, the size of the stomatal aperture, number of stomates, the levels of soluble carbohydrates, relative water content, the opening of flowers, number of flowers and yield in tomato plants.

### Materials and methods

#### *Plant growth and treatment*

Seeds of tomato (*Lycopersicon esculentum* L. var. Wosowoso) were obtained from the University of Cape Coast Teaching and Research Farm, Cape Coast, Ghana. The seeds were nursed in a rectangular box (30 cm × 20 cm). When the seedlings were 12 days old (approximately 10 cm tall), one set of 30 seedlings were transplanted into black plastic buckets (11.5l) for yield studies and the other of 90 seedlings in black polythene bags (40 cm × 25 cm) for physiological and anatomical studies. The buckets and plastic bags were filled with sandy loam soil and each had a seedling. The seedlings were kept in the open at the Botanic Garden, University of Cape Coast. When the seedlings were 17 days old (approximately 13 cm tall), groups of 100 plants were soil-drenched with 200 ml of one of the following; water (control), 100 ppm cultar (23.5 per cent WP) and 200 ppm triadimefon (25 per cent WP). The concentration of cultar and triadimefon chose in this study were based on previous experience with the chemicals (Asare-Boamah, 1985; Asare-Boamah & Boateng, 1989).

#### *Growth measurement*

Ten uniform seedlings per treatment were used for fresh weight and dry weight studies 28 days after treatment (DAT). The fresh weights of shoots and roots were determined by weighing the fresh materials and the dry weights after drying the materials in an oven at 80 °C for 48 h. The surface area of the 4th leaf was determined by the graph method (Edje, 1987) at 21 DAT using 10 plants per treatment. Stem height was also measured at 21 DAT using a meter rule in 10 plants per treatment.

#### *Anatomical studies*

The number of stomates and sizes of stomatal aperture (at 0900h GMT) at the adaxial surface of the leaves were studied using bexol glue at 21 DAT. The number of stomates per field of view under high power was counted. Twenty fields of view were used in each treatment. Permanent slides were prepared for transverse sections of the 4th leaf of the seedlings 35 DAT. The 4th true leaf was chosen because at the time of the application of the chemical, the 4th leaf had just been initiated and its growth and development was expected to be influenced by the chemical treatment. The length and width of the epidermal and palisade cells were measured using the eye piece graticule and stage micrometer. Five plants per treatment were used in each case.

#### *Relative water content (RWC)*

The RWC was determined using the method described by Weatherly & Slatyer (1957). Twenty leaf discs (0.7 cm diameter) from the 4th leaf were obtained from the leaves of 5 plants per treatment. The fresh weight (FW) was immediately taken. They were then floated in distilled water for 3 h, dried with filter paper and the saturated weight (SW) determined. The leaf discs were then oven-dried at 80 °C for 24 h and their dry weights (DW) determined. The RWC was calculated according to the equation by Weatherly & Slatyer (1957), viz:

$$RWC = \frac{FW - DW}{SW - DW} \times 100$$

New set of plants were used at each reading.

#### Pigment determination

Total chlorophyll and carotenoid contents were determined using 10 leaf discs (0.7 cm diameter) taken from portions of the 3rd and 4th leaves between the main veins using 5 plants per treatment. The pigments were extracted in 80 per cent acetone, centrifuged for 10 min and the supernatant decanted. The absorbance of the supernatant at 663, 645 and 480 nm were recorded with Spectronic 20 spectrophotometer. Total chlorophyll was calculated using the formula, viz: Mg total chlorophyll/g tissue

$$= [20.2(A_{645}) - 8.02(A_{663})] \times (V/1000W),$$

where A is the absorbance at the specific wavelength; V the final volume of the 80 per cent acetone-chlorophyll extract and W the fresh weight in grams of the tissue extracted. The carotenoid levels were calculated using the formula given by Kirk & Allen (1965), viz:

$$\Delta A \frac{CAR}{480} = \Delta A_{480} - 0.114 \Delta A_{663} - 0.638 \Delta A_{645}$$

#### Total soluble carbohydrates

The total soluble carbohydrates in one gram of leaves were estimated using the anthrone reagent method as described by Whitham, Blaydes & Devlin (1971). Readings were taken at weekly intervals from the day of chemical application to the 28th day. One gram of the leaves was weighed and homogenized three times with 80 per cent ethanol. Leaves were taken from five plants per treatment and bulked and three replicates of one gram leaf from each treatment used. The volume was made up to 100 ml and the slurry filtered by suction. The filtrate was centrifuged for 30 minutes. An aliquot (0.4 ml) of supernatant was

pipetted into a test tube and the ethanol evaporated. Distilled water (3 ml) was then added. Anthrone (6 ml) was added to the test tube and the mixture heated for 3 min. The absorbance of the cooled mixture was measured at 600 nm and the total soluble carbohydrates estimated from the standard curve.

#### Reproductive growth

Ten plants per treatment were used in this study. The number of days to first flower(s) appearance after treatment was noted and the number of flowers per plant 38 days after treatment were counted. The number, fresh weight and size (diameter) of fruits were determined 42 DAT.

#### Experimental design

The completely randomized design was used for the experiment and the results were analysed statistically using ANOVA. The differences between the means were separated by the least significant difference (LSD) and the Duncan's multiple range test (DMR).

### Results

The fresh and dry weights of leaf, stem and root as well as stem height were significantly ( $P \leq 0.05$ )

TABLE 1

*Effects of Cultar and Triadimefon on Vegetative Growth of Tomato*

Treatment (ppm)	Stem height (cm)	Mean fresh weight (g)			Mean dry weight (g)		
		Leaf	Stem	Root	Leaf	Stem	Root
Control (0)	22.7b	17.6c	28.8b	15.2b	5.8b	4.2b	4.9b
Cultar (100)	14.2a	12.9a	19.1a	5.9a	2.5a	2.3a	2.5a
Triadimefon (200)	15.1a	15.9b	21.3a	6.1a	2.7a	2.6a	2.9a

Means in the same column followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range Test.

reduced by cultar and triadimefon as compared to the control (Table 1). With the exception of leaf fresh weight, there was no significant difference between cultar-treated and triadimefon-treated plants although cultar tended to be more effica-

cious than triadimefon.

The expansion of the epidermal and palisade cells were significantly ( $P \leq 0.05$ ) retarded by the chemical treatments (Table 2). Cultar significantly ( $P \leq 0.05$ ) retarded the expansion of these cells more

TABLE 2

*Effects of Cultar and Triadimefon on Epidermal and Palisade Cells and Leaf Surface Area of Tomato Plant*

Treatment (ppm)	Mean length ( $\mu\text{m}$ ) of cell epidermis	Mean width ( $\mu\text{m}$ ) of cell palisade	Mean leaf area ( $\text{cm}^2$ )
Control (0)	4.8c	6.3c	29.6c
Cultar (100)	1.8a	3.7a	21.4a
Triadimefon (200)	2.6b	4.6b	25.4b

Means in the same column followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range Test.

than triadimefon. The 4th leaf showed corresponding reduction in leaf area in the chemically-treated plants as compared to that of the control. Cultar was the most effective in leaf area reduction compared to triadimefon-treated and control plants. Cultar reduced leaf surface area by about 28 per cent, while with triadimefon the reduction was only 14 per cent as compared to the control.

Although there was no significant ( $P \leq 0.05$ ) difference in the distribution of stomates at the adaxial surface of the leaves, the stomatal aperture of both

TABLE 3

*Effects of Cultar and Triadimefon on the Number of Stomates and Size of Stomatal Aperture on the Adaxial Surface of the 4th Leaf of Tomato*

Treatment (ppm)	Mean number of stomates	Mean size (pm) of stomatal aperture
Control (0)	5a	0.36c
Cultar (100)	4a	0.22a
Triadimefon (200)	5a	0.28b

Means in the same column followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range Test.

those treated with cultar and triadimefon were significantly ( $P \leq 0.05$ ) reduced as compared to the control (Table 3). It was observed that leaves of cultar-treated plants had more trichomes at the adaxial surface than those of triadimefon-treated plants. The control plants had the least trichomes.

Leaves of cultar-treated plants maintained higher relative water content (RWC) than those of

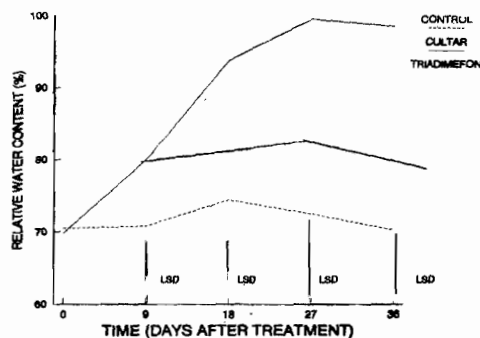


Fig. 1. Effects of water (---), cultar (—) and triadimefon (···) on relative water content of tomato leaves. triadimefon-treated and control plants (Fig. 1). However, the RWC in leaves of triadimefon-treated plants was higher than that of controls.

The chemically-treated plants looked greener compared to the controls. Fourteen days after treatment, both chemicals significantly increased chlorophyll levels in the 3rd and 4th leaves as compared to the control (Fig. 2A). Initially, the leaves of both the chemically-treated and control plants showed increasing levels of chlorophyll with age up to 21 days (in control leaves) and 28 days after treatment (in the chemically-treated leaves). Thereafter, the chlorophyll levels in leaves of the control and triadimefon-treated plants began to decline. It was observed that the period of decline in chlorophyll levels in leaves of both control and chemically-treated plants coincided with flower opening. Like chlorophyll levels, the leaves of treated plants had higher levels of carotenoids as compared to the controls (Fig. 2B).

Cultar and triadimefon increased significantly ( $P \leq 0.05$ ) the amount of total soluble carbohydrates in the 3rd and 4th leaves of tomato 21 and 28 DAT

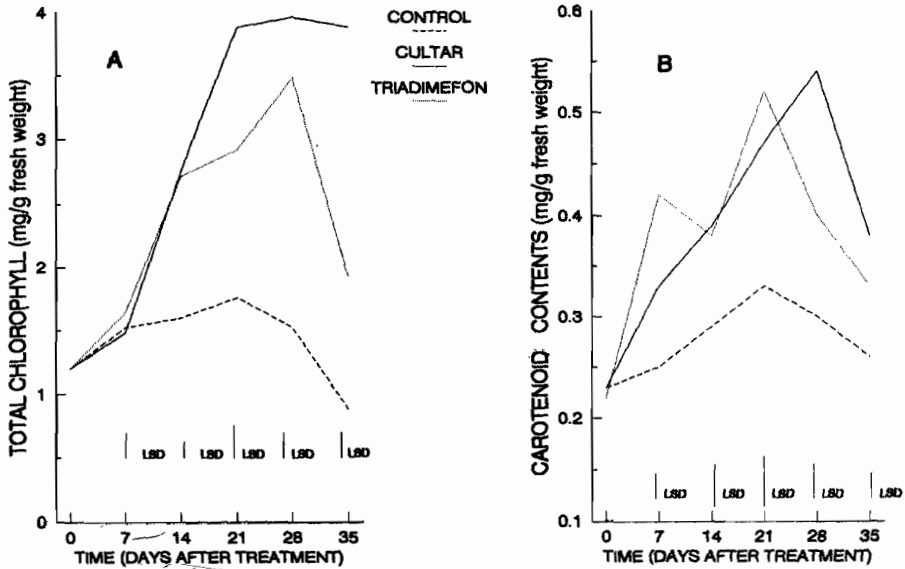


Fig. 2. Effects of water (-----), cultar (\_\_\_\_) and triadimefon ( . . . ) on total chlorophyll (A) and carotenoid (B) contents.

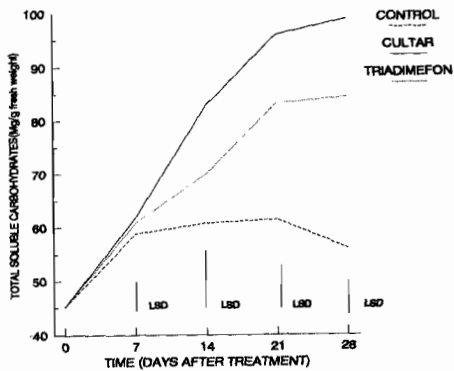


Fig. 3. Effects of water (-----), cultar (\_\_\_\_) and triadimefon ( . . . ) on total soluble carbohydrates of tomato leaves.

as compared to the control (Fig. 3). The leaves of the chemically-treated plants showed increasing levels of total soluble carbohydrates with age up to 21 DAT and, thereafter, there was a decline in the leaves of control plants. The decline in total soluble carbohydrates also coincided with the onset of flowering. Throughout the period of study, cultar-treated plants maintained higher levels of total soluble carbohydrates than those of

triadimefon but the difference was not significant. Cultar and triadimefon delayed flower opening as compared to the control plants. Cultar delayed flowering by 8 days whereas triadimefon delayed it by 6 days compared to the controls. Although flower opening was delayed in the chemically-treated plants, these had a higher number of flowers than the control plants 38 DAT (Table 4).

TABLE 4

*Effects of Cultar and Triadimefon on the Number of Days to First Flower Appearance and Number of Flowers*

Treatment (ppm)	Days to first flower appearance	Mean number of flowers per plant 38 days after treatment
Control (0)	20	27a
Cultar (100)	28	40c
Triadimefon (200)	26	33b

Means in the same column followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range Test.

Cultar-treated plants had significantly ( $P \leq 0.05$ ) more flowers than those treated with triadimefon. Treatment with cultar significantly ( $P \leq 0.05$ ) increased yield factors as compared to the controls and triadimefon treatment (Table 5). Cultar increased the number and fresh weight of the fruits. The size of cultar-treated tomato fruits as measured by the diameter of the fruit was significantly larger than those of the triadimefon-treated plants.

TABLE 5

*Effects of Cultar and Triadimefon on Yield Factors of Tomato*

<i>Treatment (ppm)</i>	<i>Mean total number of fruits/plant</i>	<i>Mean fresh weight of fruits/plant (g)</i>	<i>Mean fruit diameter (cm)</i>
Control (0)	19a	37.2a	3.2ab
Cultar (100)	30b	39.8b	3.3b
Triadimefon (200)	17a	36.6a	3.0a

Means in the same column followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range Test.

Triadimefon tended to reduce the number of fresh weight and size of the fruits, but not significantly ( $P \leq 0.05$ ) as compared to the control.

### Discussion and conclusion

The triazole compounds significantly reduced the vegetative growth of tomato (Table 1). The data supports earlier reports in snapbean (Buchenauer & Rhoner, 1981), soybean (Lee, Bryun & Steffers, 1985; Sankhla *et al.*, 1985), beans (Asare-Boamah *et al.*, 1986) and clove (Martin & Dabek, 1988). The retardation of vegetative growth has been attributed to the inhibition of GA biosynthesis (Buchenauer & Rhoner, 1981; Hedden & Graebe, 1985). In contrast to the retardation of growth, Fletcher & Nath (1984) reported stimulation of growth in radish, pea and soybean under water stress conditions. It appears, therefore, that the type of effect induced by triazoles may be dependent on species and/or environmental conditions.

The leaf surface area of cultar-treated plants was reduced by about 28 per cent as compared to the controls. The leaves of triadimefon-treated plants

were significantly broader than those of cultar-treated plants. The reduction in leaf surface area by cultar and triadimefon has been reported earlier by Sankhla *et al.* (1985) in soybean, and Asare-Boamah *et al.* (1986) in beans. The expansion of the epidermal and palisade cells were severely retarded and the reduction in the sizes of these cells might partially account for the reduction in leaf surface area associated with triazoles treatment. Similar results were reported in wheat leaves when triadimefon and S-3307 (both triazoles) were applied as seed treatment (Gao, Hofstra & Fletcher 1988). Cultar and triadimefon had no significant influence on the number of stomates at the adaxial surface of tomato leaves. In wheat, however, triadimefon and S-3307 (both triazoles) at concentrations of 1.0 g and 0.10 g active ingredient/kg of seed, respectively, increased the number of stomates per unit leaf area (Gao, Hofstra & Fletcher, 1988). Both cultar and triadimefon significantly reduced the opening of the stomatal apertures compared to the control. Triadimefon (Asare-Boamah *et al.*, 1986) and cultar (Wample & Culver, 1983) reduced transpiration by decreasing the leaf area, and increasing leaf diffusive resistance in beans and soybeans, respectively. It suggests, therefore, that the increase in leaf diffusive resistance in triazole-treated plants may be partially accounted for by the reduction in stomatal aperture as observed in this study. The reduction in stomatal aperture also indicates that these chemicals can be used in plants growing in drought environment to reduce water loss and thereby improve water use efficiency.

It appears that the triazoles employ several mechanisms including reduction in stomatal aperture and leaf area to minimize transpirational loss and hence confer drought tolerance to plants (Asare-Boamah *et al.*, 1986). In this study, it was observed that there were more trichomes in leaves of the cultar-treated plants than in those of triadimefon-treated and control plants although this observation was not quantified. This observation is under investigation in the laboratory at the moment. The increased number of trichomes in leaves of the treated plants may enhance drought

tolerance in treated plants. In wheat, triadimefon and S-3307 reduced the length of trichomes (Gao, Hofstra & Fletcher, 1988); however, no such measurements were taken in the present study. The triazole derivatives increased significantly the RWC of the leaves as compared to the controls. Uniconazole, another triazole derivative, was reported to have increased the RWC in wheat (Fletcher, Santakumari & Murr, 1988), which has been confirmed by the results of the present study. The high RWC of the triazole-treated plants (Fig. 1) may help the plants to tolerate water stress as reported by Sankhla *et al.* (1985) and Asare-Boamah *et al.* (1986).

There was an increase in the levels of photosynthetic pigments in the treated plants as compared to the controls. This may contribute to increase in yield in the treated plants. Similar results have been reported in sunflower (Wample & Culver, 1983). The mechanism of delay in flower opening by the triazoles in tomato is not clearly understood. However, according to Hedden & Graebe (1985), the triazoles inhibit GA biosynthesis, and since GA is important in the flowering process, it is likely that the delay in flower opening by the triazoles observed in this study may be due to the decrease in GA levels in the treated plants. It was found that if triadimefon or cultar is applied at certain age range (28-35 days old seedlings) in tomato and bean the chemicals cause flower abortion and fruit failure (Asare-Boamah, Galyuon & Boateng, 1991). This indicates that the age at which the chemicals are applied may influence flower production and fruit set. The triazole-treated plants had more flowers 38 DAT despite the delay in flower opening. The increase in number of flowers may partially account for the increase in yield in cultar-treated plants, since increase in yield by triazole derivatives has been reported in cloves by Martin & Dabek (1988), radish and soybean under water stress by Fletcher & Nath (1984) and in apples by Kolbe (1981).

The application of 100 ppm cultar on tomato reduced vegetative growth, maintained higher water levels in the leaves and increased yield as com-

pared to the plants treated with triadimefon. It is, therefore, concluded that cultar is more efficacious as a plant growth regulator than triadimefon. If the results could be reproduced in the field, then cultar will be more beneficial to tomato growers than triadimefon.

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