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Smoke-water and a Smoke-isolated Butenolide Improve Growth and Yield of Tomatoes under Greenhouse Conditions

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SUMMARY. Smoke shows promising results in stimulating germination and vigor. The biologically active butenolide compound isolated from smoke has potential to become a valuable tool in horticulture. 'Heinz-1370' tomato (*Solanum lycopersicum*) seedlings showed a positive response to smoke and were therefore tested with smoke-water and butenolide for growth, yield, and nutritional composition. Smoke-water (1:500, by volume) treatment showed the maximum height, number of leaves, and stem thickness from 57 to 78 days after sowing. The percentage of plants with fruit from 85 to 95 days after sowing was much higher with the application of smoke-water and butenolide solution than in the control. The total number of marketable fruit was significantly greater ($P \leq 0.05$) for smoke-water–treated (1:500, by volume) tomato plants (168) than for the control (124). Butenolide and the lower concentration of smoke-water (1:2000, by volume) yielded more fruit, but was not significantly ($P \geq 0.05$) different from the control. In spite of achieving a greater number of fruit, smoke treatments did not significantly ($P \leq 0.05$) change the size, weight, and nutritional composition (ascorbic acid, β-carotene, lycopene, and total soluble solids) of fruit. The harvest indices of smoke-water– and butenolide-treated plants significantly improved ($P \leq 0.05$), suggesting the possible use of smoke technology for tomato cultivation.

Fire is an integral element of rural agronomy. In traditional farming communities, the burning of fields is an inexpensive and efficient method of eliminating the residue of previous crops. In addition, it protects fields from weeds, pests, diseases, and it increases the fertility of the soil (Altieri, 1993; Meland and Boubel, 1966). Consequently, the burnt fields show an improvement in crop yield. However, there are several unclear explanations about the interaction of burnt soil and the growth of a crop (Lal and Ghuman, 1989).

The use of fire, smoke, and ash for drying and storing grains is traditionally a common practice (Haile, 2006; Pierrot, 1988). This indigenous custom of treating grains with smoke improves germination and seedling vigor of a crop (Modi, 2002, 2004; Paasonen et al., 2003). Smoke is currently being extensively investigated because of its stimulatory properties. Sparg et al. (2006) showed that not only the percentage of germination, but also the seedling vigor of a commercial maize (*Zea mays*) variety could be enhanced using different combinations of aerosol smoke and smoke-water soaking. Therefore, smoke treatment does not only enhance germination, but can also stimulate seedling vigor (Brown et al., 2003; Sparg et al., 2005). This indicates that smoke has some chemical properties that interact effectively with plant metabolism.

The aqueous smoke extracts prepared from dry plant material of gymnosperm (leaves) and a number of angiosperms showed a positive response to the germination of light-sensitive lettuce seeds (Jäger et al., 1996). This indicates that a range of plant material can be used in preparing a smoke stock solution.

Recently, the biologically active compound, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, known as butenolide, was isolated from burnt cellulose (Flematti et al., 2004) and plant-derived smoke (Van Staden et al., 2004). Although this compound is now recognized as a germination cue for many smoke-exposed and smoke-nonexposed naturally occurring species, it also shows a good potential for agricultural and horticultural crops. Butenolide-treated maize kernels

Units

To convert U.S. to SI, multiply by	U.S, unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.4536	lb	kg	2.2046
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274×10^{-5}
0.1	ppm	mg/100 g	10
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

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showed a better percentage of germination with higher seedling vigor indices than untreated kernels. Seedlings of bean (*Phaseolus vulgaris*), okra (*Abelmoschus esculentus*), and tomato showed a similar improved response (Van Staden et al., 2006). Jain and Van Staden (2006) indicated that butenolide may be instrumental in mobilizing seed reserves in developing tomato seedlings. In another study, foliar treatment of tomato seedlings with butenolide showed better growth performance than untreated seedlings (Kulkarni et al., 2007).

Tomato is an extensively cultivated vegetable crop around the world and is popular for greenhouse production. Seedlings of tomato displayed a positive response to smoke (Kulkarni et al., 2007), and its application can potentially be a cost-effective technique for mass-scale production. However, the influence of smoke treatments on growth, fruit yield, and nutritional composition of tomato has not yet been investigated. Therefore, this study was conducted to evaluate the effects of smoke-water and smoke-isolated butenolide treatments on yield and quality of tomatoes.

Materials and methods

SEED SOURCE. Experiments were conducted on commercial seeds of 'Heinz-1370' tomato purchased from McDonalds Seeds (Pietermaritzburg, South Africa).

SMOKE-WATER PREPARATION. Dry red grass (*Themeda triandra*) leaf material (5 kg) was ignited in a 20-L metal drum, and using compressed air, the smoke was continuously bubbled through a glass column containing 500 mL of tap water for 45 min (Baxter et al., 1994). This smoke extract (500 mL) was filtered through filter paper (No. 1; Whatman, Clifton, NJ) and was used as stock solution. Two concentrations of smoke-water were tested. These concentrations were prepared by diluting 1 mL of the above stock solution with 500 or 2000 mL (smoke-extract:water ratio 1:500 and 1:2000, by volume) of tap water for soil drenching. A similar method of preparing aqueous smoke extract with different plant material has been described in many studies (Boucher and Meets, 2004; Brown, 1993; De Lange and Boucher, 1990; Jäger et al., 1996). The apparatus for producing smoke-saturated water has been illustrated by De Lange and Boucher (1990) and Van Staden et al. (2004).

ISOLATION OF BUTENOLIDE. The butenolide used in this trial was isolated from plant-derived smoke-water using bioactivity-guided fractionation, isolation, and identification as described by Van Staden et al. (2004). The concentration of pure butenolide solution tested in this trial was 1 nM.

EXPERIMENTAL CONDITIONS AND DESIGN. The study was initiated on 6 Aug. 2006 in a greenhouse of the University of KwaZulu-Natal Botanical Garden at Pietermaritzburg, South Africa (lat. 29°37′S, long. 30°16′E). Three seeds were sown at a depth of 1.5 cm in a 4-L plastic pot (204 mm wide by 175 mm high) containing a soil mixture (1 compost:1 garden soil, by volume). The control pots were water-irrigated three times weekly. The treatment pots were irrigated twice weekly with smoke-water concentrations or butenolide solution and once with water until they reached field capacity. The experimental design was a randomized complete block with 15 replicates of each treatment. The spacing between the pots was 50 cm. The temperature in the greenhouse during the experiment was 22 ± 2.5 °C with a photosynthetic photon flux density of daylight hours averaging 435 ± 85 $\mu mol \cdot m^{-2} \cdot s^{-1}$. On 26 Aug. 2006, the seedlings were thinned so that only the best seedling was retained in each pot. Using thin plastic insulated wire, the stem of each young tomato plant (45 d old) was tied to a bamboo stick (1.5 cm thick by 120 cm long) that was deeply fitted in the soil. On 20 Oct. and 20 Nov. 2006, the plants were supplied with a liquid dose (500 mL) of commercial fertilizer Kompel [Chemicult Products (Pty) Ltd., Camps Bay, South Africa; 10 g of powder dissolved in 5 L of tap water] containing 6.5% nitrogen, 2.7% phosphorus, 13% potassium, 7% calcium, 7.5% sulfur, 0.15% iron, 0.024% manganese, 0.024% boron, 0.005% zinc, 0.002% copper, and 0.001% molybdenum.

GROWTH, YIELD, AND NUTRITIONAL COMPOSITION. At different time intervals, the stem thickness of the plants (1 cm above soil surface) was measured using vernier callipers. Simultaneously, the number of leaves and height of plants were recorded up to the first fruiting stage. Harvesting of tomatoes commenced on 29 Nov. 2006 and continued until 8 Jan. 2007. At each harvesting, the percentage of plants with fruit was recorded. Light red fruit were hand-harvested twice weekly and the weight and size of the fruit were recorded. The harvested tomatoes were stored at normal room temperature to fully ripen for nutritional composition analysis. In this study, only marketable fruit (>50 mm) were considered for all the analyses. At the end of the experiment, the harvest index (HI) was calculated as the ratio of fruit yield to total aboveground biomass yield on a fresh weight basis (Henderson et al., 2000; Prasad et al., 2006). Five fully ripe (red color) tomatoes were blended, centrifuged, and total soluble solids (%) and acidity were determined on the clear supernatant using a refractometer (PR-101; Atago Co., Ltd., Tokyo) and pH meter [HI 223; Hanna Instruments (Pty) Ltd., Durban, South Africa], respectively (Moretti et al., 1998; Stirzaker et al., 1992). This procedure was repeated for five different harvests and the values were averaged. The ascorbic acid content was determined by the method of Teklemariam and Sparks (2004), and β-carotene and lycopene content were estimated by the procedure of Navarro et al. (2006) and calculated according to the equations of Nagata and Yamashita (1992). Before estimating, fully ripe tomato samples (outer thick layer) of five different harvests were freeze-dried and stored at –70 °C. The average ascorbic acid, β-carotene, and lycopene content are expressed on a fresh weight basis.

STATISTICAL ANALYSIS. Statistical analysis was conducted using GenStat (version 9.1; Rothamsted Research, Harpenden, UK). Average CV was calculated for growth data (height, number of leaves, and stem thickness) and plants with fruit (%). Yield and nutritional analysis results of marketable tomato fruit were analyzed using one-way analysis of variance and tested at 5% level of least significant difference (LSD).

Results and discussion

EFFECT ON PLANT GROWTH. Soil application of two concentrations of smoke-water or a butenolide solution increased the height of tomato plants compared with the control (Fig. 1A). Smoke-water–treated (1:500) plants achieved the greatest height. At the first fruiting stage [78 d after sowing (DAS)], the smoke-water–treated (1:500) plants were taller by 14.2 ± 0.4 cm than untreated (control) tomato plants. In all smoke treatments, the number of leaves from 57 to 78 DAS was greater than the control (Fig. 1B). Smoke-water (1:500) showed an increment of about three leaves over the control. In a similar study, maize plants that were drenched with smoke-water reached a leaf stage of more than six leaves 30 DAS, whereas control plants only had more than four leaves (Van Staden et al., 2006). These results indicate that smoke-water has the ability to improve the aboveground biomass. Stem thickness was greater for smoke-water— and butenolide-treated tomato plants compared with control plants (Fig. 1C). The smoke-water (1:500) application resulted in thicker stems than any other treatment evaluated. In a recent and similar study on tomato, foliar treatments of smoke-water and butenolide on young seedlings showed a significant improvement in growth (Kulkarni et al., 2007). However, this study was limited to seedlings that were grown for later transplanting to the field. In the present trial, the smoke-water—and butenolide-treated plants sustained increased growth until the fruiting stage. This finding clearly indicates that tomato plants react positively to these treatments.

Effect on fruiting. A few plants started fruiting at 79 DAS. There was a sharp rise in the percentage of smoke-treated plants with fruit from 85 to 95 DAS (Fig. 2). On 85 and 95 DAS, nearly 58% and 42%, respectively, more plants were fruiting when treated with smoke-water (1:500) than the control. At the same sample dates, butenolide treatment also showed an increase of 42% and 37%, respectively, over the control. All the control plants were only fruiting by 113 DAS. This shows that the plants subjected to smoke-water and butenolide treatments fruited earlier. This will be an advantage to growers or farmers to sell their fruit earlier in the market at a higher price.

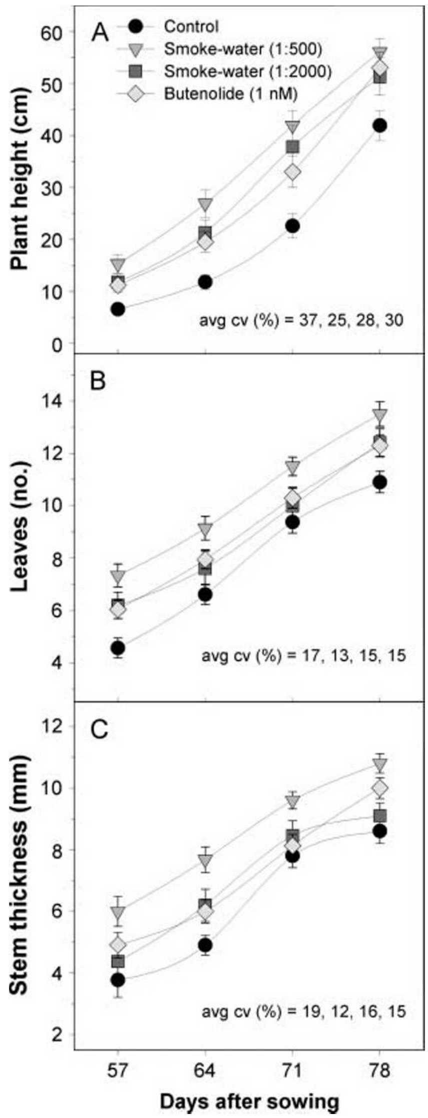

Fig. 1. (A–C) Effect of smoke treatments on growth parameters of 'Heinz-1370' tomato under greenhouse conditions. The smoke extract [500 mL (1 mL = 0.0338 fl oz)] prepared from burning red grass was filtered through filter paper and was used as stock solution. Smoke-water concentrations were prepared by diluting 1 mL of stock solution with 500 or 2000 mL of tap water for soil drenching. The treatment pots were irrigated twice weekly with smoke-water or butenolide solution and once with water until they reached field capacity. The control pots were irrigated with water three times weekly (n = 15 plants). The symbols are ±SE and the average CV on each figure is according to label sequence (1 mm = 0.0394 inch, 1 cm = 0.3937 inch, 1 nM = 1 ppb).

Effect on fruit yield and nutritional composition. Smoke-water (1:500) application significantly improved the average and total number of marketable tomato fruit

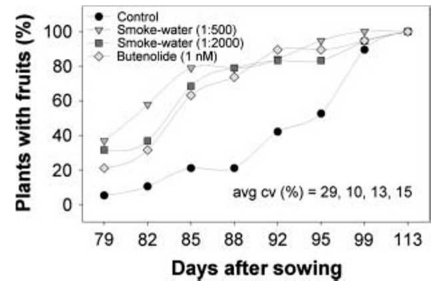

Fig. 2. Effect of smoke treatments on percentage of 'Heinz-1370' tomato plants with fruit under greenhouse conditions. The smoke extract [500 mL (1 mL = 0.0338 fl oz)] prepared from burning red grass was filtered through filter paper and was used as stock solution. Smoke-water concentrations were prepared by diluting 1 mL of stock solution with 500 or 2000 mL of tap water for soil drenching. The treatment pots were irrigated twice weekly with smoke-water or butenolide solution and once with water until they reached field capacity. The control pots were irrigated with water three times weekly (n = 15 plants). Average CV on figure is according to label sequence (1 nM = 1 ppb).

(Table 1). In comparison with control plants, about three extra fruit per plant were harvested from this treatment. The butenolide and a low concentration of smoke-water (1:2000) also yielded higher numbers of fruit than the control (Table 1; i.e., 36 and 25 more fruit, respectively, per 15 plants). However, these results were not statistically significant. These findings suggest that higher concentrations of smoke-water may positively enhance tomato yield. Interestingly, when studying the traditional plant cultivation practices of Alyawara-speaking Australian aborigines, Latz (1995) and O'Connell et al. (1983) documented that bush tomato populations were fire-dependent with an 80-fold increase in the productivity on a site that was recently burnt. Ahmed et al. (2006) showed that smoke enhanced the germination of the australian bush tomato (*Solanum centrale*), suggesting that the increase in productivity of bush tomato may be from the promoting effect of smoke on seed germination. In this study, smoke treatments increased the number of fruit per plant, which augmented the total weight of fruit. Consequently, smoke-water and butenolide treatments significantly

Table 1. Effect of smoke treatments on yield of 'Heinz-1370' tomato under greenhouse conditions. The smoke-extract (500 mL)[z] prepared from burning red grass was filtered through filter paper and was used as stock solution. Smoke-water concentrations were prepared by diluting 1 mL of stock solution with 500 or 2000 mL of tap water for soil drenching. The treatment pots were irrigated twice weekly with smoke-water or butenolide solution and once with water until they reached field capacity. The control pots were irrigated with water three times weekly (n = 15 plants).

Treatment[y]	Fruit (no./plant)	Total fruit (no.)	Mean fruit wt (g)[x]	Mean fruit diam (mm)[x]	Biomass (g/plant)	Harvest index[w]
	Mean ± SE					
Control	8.27 ± 0.80 b[v]	124 ± 0.80 b	72.8 ± 4.3	54.0 ± 0.7	780 ± 63	0.694 ± 0.7 b
Smoke-water (1:500 v/v)	11.2 ± 1.11 a	168 ± 1.11 a	67.3 ± 3.6	54.4 ± 0.6	976 ± 93	0.762 ± 0.8 a
Smoke-water (1:2000 v/v)	9.94 ± 0.96 ab	149 ± 0.96 ab	68.4 ± 2.5	53.3 ± 0.6	854 ± 81	0.733 ± 0.7 a
Butenolide (1 nM)	10.6 ± 0.81 ab	160 ± 0.81 ab	73.6 ± 3.1	54.0 ± 0.6	944 ± 70	0.750 ± 0.8 a

[z]1 mL = 0.0338 fl oz.
[y]1 nM = 1 ppb.
[x]1 g = 0.0353 oz; 1 mm = 0.0394 inch.
[w]Ratio of fruit yield to total aboveground biomass yield on a fresh weight basis.
[v]Values in the column with different letter(s) are significantly different at $P \leq 0.05$ by least significant difference.

achieved better HI than the control, with no change in fruit weight and size (Table 1). This finding clearly reveals that the smoke-water and butenolide solution, without compromising the fruit weight and size, helped in improving tomato yield. In spite of positive effects on growth and yield, the smoke-water and butenolide treatments did not affect the nutritional value of tomatoes. Total soluble solids, pH, ascorbic acid, lycopene, and β-carotene content were similar in all treatments (Table 2).

Many studies have suggested that plant-derived smoke and butenolide can be of potential in seed pretreatment to improve germination and seedling vigor of horticultural crops (Brown and Van Staden, 1998; Light and Van Staden, 2004; Merritt et al., 2005; Van Staden et al., 2006). The findings of this study indicate that smoke can also improve yield. A smoke-water concentration of 1:500 was more effective than other treatments in enhancing the growth and marketable fruit yield of tomato. Although tomato plants treated with smoke-water concentration of 1:2000 and butenolide solution of 1 nM recorded more fruit, these results were not statistically different from the control. This suggests that the concentrations of smoke-water and butenolide are important factors that should be considered when crop yield is studied. Butenolide can be effective at very low concentrations for promoting seed germination (Flematti et al., 2004; Van Staden et al., 2004). However, on the basis of this study, higher concentrations of butenolide than those used for germination studies may be beneficial for growth and yield. Higher concentrations in this trial were not tested because of the limited extraction of butenolide from plant-derived smoke-water. Therefore, commercial production of butenolide is essential to test a wide range of concentrations. Butenolide is not toxic or genotoxic and can be safely used (Verschaeve et al., 2006). Recently, Rokich and Dixon (2007) suggested that between 1 and 20 g of pure butenolide can likely cover 1 ha of land, which could be economical. However, at present, the use of smoke-water is favored as it is a very inexpensive technique for resource-limited tomato growers.

Conclusions

This work shows that smoke-water had a positive influence on the growth and yield of tomatoes under greenhouse conditions. The HI was significantly promoted with all smoke treatments. This finding clearly reveals that there was an increase in the number of marketable tomato fruit, which is an important factor in commercial production. At the same

Table 2. Effect of smoke treatments on nutritional composition of 'Heinz-1370' tomato fruit that were allowed to ripen at room temperature. The smoke-extract (500 mL)[z] prepared from burning red grass was filtered through filter paper and was used as stock solution. Smoke-water concentrations were prepared by diluting 1 mL of stock solution with 500 or 2000 mL of tap water for soil drenching. The treatment pots were irrigated twice weekly with smoke-water or butenolide solution and once with water until they reached field capacity. The control pots were irrigated with water three times weekly (n = 15 plants). Five fully ripe tomatoes at five different harvests were used and the estimation procedure was repeated five times. Mean values are with ±SE.

Treatment[y]	Ascorbic acid (mg/100 g)[x]	β-Carotene (mg/100 g)	Lycopene (mg/100 g)	pH	Total soluble solids (%)
		Mean ± SE			Mean ± SE
Control	57.1 ± 7.1	6.77 ± 1.4	30.7 ± 5.9	4.0	5.7 ± 0.09
Smoke-water (1:500 v/v)	87.4 ± 8.5	7.27 ± 0.3	29.8 ± 2.0	4.0	5.6 ± 0.15
Smoke-water (1:2000 v/v)	75.0 ± 16.0	6.56 ± 1.3	29.7 ± 7.8	4.0	5.4 ± 0.09
Butenolide (1 nM)	62.0 ± 23.4	6.97 ± 1.3	33.3 ± 5.1	4.0	5.4 ± 0.11

[z]1 mL = 0.0338 fl oz.
[y]1 nM = 1 ppb.
[x]1 mg/100 g = 10 ppm.

time, these treatments did not imbalance major nutritional contents of tomato fruit, indicating the safe use of diluted smoke-water concentrations. In fact, when fields are burnt in traditional practices, the smoke concentration in the soil is much higher. In spite of this, the burnt fields do not show harmful effects on crops. Smoke-water can be useful for tomatoes that are grown hydroponically and in polyhouses. Greenhouse production of tomatoes is more expensive than cultivation in the fields (Peet and Welles, 2005). Smoke technology may help tomato growers in minimizing running costs by early harvesting and increasing number of fruit.

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