J. ISSAAS Vol. 23, No. 1: 90-102 (2017)

EFFECT OF 1-MCP ON ETHYLENE REGULATION AND QUALITY OF APPLE, APRICOT AND ASPARAGUS

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(Received: September 25, 2016; Accepted: December 23, 2016)

ABSTRACT

The effect of 1-methylcyclopropene (1-MCP) on fruits and vegetables has always been a topic of interest. The current study was conducted to investigate the effect of 1-MCP on ethylene production and on quality of apple (*Malus domestica*) during ripening, of Japanese apricot (*Prunus mume*) harvested at green and yellow stages, of and asparagus (*Asparagus officinalis*) spears. The apple fruit, Japanese apricot fruit, and asparagus spears were treated with 1-MCP for 24 h. Ethylene, CO_2, color, firmness, and upward growth (bending upward when stored at a horizontal position due to negative geotropism) of the asparagus spears were measured sequentially at the laboratory of Tropical Horticulture, Department of International Agricultural Development, Tokyo University of Agriculture (Setagaya campus) from 2014-2015. Ethylene production reduced significantly in apple, was unaffected in Japanese apricot, and increased in asparagus. Respiration significantly decreased in apple and Japanese apricot during green and yellow stages, but no significant changes were observed in asparagus spears. Quality parameters, such as color, firmness and weight loss (apple) have improved in all the tested fruits and vegetable, suggesting that 1-MCP could maintain quality during storage under ambient conditions. Asparagus spears in a horizontal position stopped upward growth when these were treated with 1-MCP, suggesting it could improve asparagus post-harvest quality

Key words: firmness, respiration, upward growth

INTRODUCTION

Fruits and vegetables are important sources of vitamins, minerals, sugar, fiber, and other nutrients. As recommended by the World Health Organization (WHO), an adult needs to consume at least 400 g of fruits and vegetables daily (WHO, 2003). Although Southeast Asian countries have experienced significant economic progress, rapid growth still has not improved the livelihood and nutrition in these regions. These countries still deal with poor nutrition (i.e. deficiencies in carbohydrates, proteins, essential vitamins and minerals) and infectious diseases (AVRDC, 2014-16). To provide a variety of fruits and vegetables throughout the year, there are various activities which deliver adequate nutrients, from the introduction of new vegetable varieties, encouraging traditional vegetable production at home and school gardening to appropriate postharvest technologies (AVRDC, 2016).

On the other hand, maintaining quality of fruits and vegetables is critical, as they are prone to lose postharvest freshness and decay faster than other agricultural commodity. The loss of fruits and vegetable in Southeast Asian countries are generally high, ranging from 10 to 50% (FAO, 2011). For instance, postharvest loss was reported to range from 10-30 % in Japan, 12-60% in Thailand, 28-42 % in Philippines, and 10 % in Taiwan respectively (FFTC, 1993).

Ethylene is a gaseous hormone, which has been known to promote ripening and accelerate senescence, leading to fruits and vegetables losses (Taiz and Zeiger, 2002). In order to improve postharvest freshness of fruits and vegetables in Southeast Asia, controlling ethylene metabolism with simple and effective methods might be an essential key in providing nutritious commodities for consumers, especially for infants, elderly, and/or reproductive women.

The discovery of 1-methylcyclopropene (1-MCP) as an ethylene inhibitor goes back to the 1980s by Blankenship and Sisler (Blankenship and Dole, 1993). 1-MCP is a gas, which is commercially stored as a stable powder that is dissolved in water to start gas production. It has a higher affinity to ethylene binding sites, 10 times more than ethylene itself, and is released easily. 1-MCP can be a safe and cheap alternative to promote postharvest quality of fruits and vegetables, especially in developing countries.

This research therefore sought to investigate the effect of 1-MCP on ethylene regulation on the quality of apple (*Malus domestica*), Japanese apricot (*Prunus mume*), and asparagus (*Asparagus officinalis*) to provide basic information useful for practical applications.

MATERIALS AND METHODS

Plant materials

The experiments were conducted at Tokyo University of Agriculture (Setagaya campus), under ambient temperature from 2014 to 2015. Apple c.v. "Fuji", Japanese apricot c.v. "Nanko", and asparagus c.v. "UC157" were brought to the Laboratory of Tropical Horticulture, Tokyo University of Agriculture. Forty fresh mature apples (2 treatments with 20 replications for each treatment), 80 Japanese apricot fruits (2 treatments with 10 replications for each treatment during each stage) harvested at green and yellow stages, and 20 asparagus spears (5 for each treatment) were prepared for the experiments mentioned below. Uniform materials were selected by checking initial ethylene production. All plant material was stored under ambient conditions.

1-MCP applications

1-MCP solution was prepared by dissolving 8.184 mg powdered 1-MCP into 1.56 ml of water, then placed into a 6.8-liter container. All experimental materials mentioned above were treated with 1µl L^{-1} concentration of 1-MCP using Smart Fresh® made by Agro Fresh, Inc. for 24 h, while the control was kept without any treatment in airtight chambers, as suggested by Blankenship and Dole (2003), Watkins (2006) and Guillen (2006).

Measurement of ethylene production, CO_2 release, hardness, color, weight, and rising angles

Ethylene and CO_2 production were analyzed in apple, asparagus, and Japanese apricot at 0, 1, 3, 6, 9, 12, 24 h after treatment with 1-MCP. In apples, ethylene and CO_2 production were continuously measured every 5 days by gas chromatography using a flame ionization detector (Shimadzu) and TCD (Shimadzu) using headspace method, respectively. Each material was placed inside a 550-ml glass jar and incubated in the dark for 1 h at room temperature and 1 ml headspace gas was removed using a plastic syringe for analyzing ethylene and CO_2 production. Both GCs were equipped with a Sunpack A column (Shinwa Kako). The parameters were set at: injector 180°C, column 80°C, detector 200°C for ethylene analysis, and at injector 150°C, column 40°C, detector 150°C for CO_2 analysis.

Fruit firmness was measured with a non-destructive firmness meter Multilateral Tester Model 2519-104 (INSTRON), indicating the force (N) required to press the fruit skin randomly (usually in the middle part of the fruit's skin) at 1 mm/sec using a non-destructive Φ 1 cm metal plug. The Multilateral Tester was calibrated with a computer through which firmness was calculated and presented in excel sheet.

A portable colorimeter (NR-3000, Nippon Denshoku Ind.Co.Ltd., Japan) with D65 standards illumination and a 2° standard observer were used to determine color parameters (L = lightness, a = positive values for red color intensity and negative values for green color intensity, and b = positive values for yellow color intensity and negative for blue color intensity). Weight loss for apple was recorded by an electric balance (HF-4000, Japan) from each replication in all treatments at periodical intervals, and cumulative losses in weight were calculated and expressed in percent.

Asparagus spears were usually stored in a vertical position, because spears are prone to upward growth due to negative geotropism when placed horizontally, resulting in a loss of energy and freshness. Considering this, experiments were conducted to see whether 1-MCP could help overcome such a phenomenon. There were four treatments, including a control and a 1-MCP treatment positioned horizontally, and a control and a 1-MCP treatment positioned vertically, with 5 replications each. The 20 spears treated with or without 1-MCP (5 replications in each treatments) were laid horizontally or vertically to check if it affected the rising angle of asparagus when stored horizontally. The rising angle of the asparagus spears was measured everyday using a protractor.

RESULTS AND DISCUSSION

Apple

Ethylene and CO_2 production. CO_2 production suggested that 1-MCP significantly reduced respiration (at 5%) on a continuous basis compared with the control from 1 to 25 days after treatment. Ethylene production in apple decreased with 1-MCP treatment (Fig. 1 A and B). It was significantly different at 5% from 3 h to 25 days after continuous treatment, compared with a control (Fig. 1 A). Similarly, treating apple fruit with 1-MCP reduced respiration significantly than the control at one day after treatment (Fig.1 B). Experimental results show that ethylene production in Fuji apple fruits has dramatically reduced upon treatment with 1-MCP, compared with a control.

Ethylene production was dramatically reduced in Fuji apple fruits treated with 1-MCP, as compared with a control. Usually, lower ethylene production is reported in different cultivars of apple fruits, including Gala, Fuji, Golden delicious, McIntosh, Granny Smith, Red Chief Delicious, Law Rome, Jona gold, and Empire as pointed out by Valero and Serrano (2010). We can say that 1-MCP has different effects on ethylene biosynthesis, depending on variety and stage of fruit and vegetables. Results obtained are in accordance with other studies, which showed the influence of 1-MCP on ethylene biosynthesis through feedback inhibition (Blankenship and Dole, 2003).

The effect of 1-MCP on apple respiration suggests that treatment with 1-MCP might constantly decrease respiration (CO_2), due to a decrease in ethylene sensitivity caused by inhibition of ethylene binding to its receptors by 1-MCP. The affinity of 1-MCP is 10 times greater than ethylene. Thus, the respiration of the fruits and vegetable tested were all inhibited, regardless of any positive and negative change in ethylene production upon 1-MCP treatment, since 1-MCP inhibits ethylene by blocking its receptors. Usually, lower ethylene production is reported in different cultivars of apple fruits, including Gala, Fuji, Golden delicious, McIntosh, Granny Smith, Red Chief Delicious, Law Rome, Jona gold, and Empire as pointed out by Valero and Serrano (2010).

Color, firmness and loss in weight. A significant weight loss (at 5%) in fruits treated with 1-MCP was observed from 5 to 25 days after continuous storage, compared with a control. Although apple was harvested at maturity, a delay in color change was still observed in fruits treated with 1-MCP at 1 or 5% compared with untreated fruits (Fig. 1; C, D and E) at 1, 2, and 3 weeks after treatment as compared with a control. However, there were no significant differences between control and 1-MCP treated fruits at 4 and 5 weeks of storage (Fig. 1; C). The results on firmness showed that 1-MCP-treated fruits were significantly harder than untreated fruits at 1 to 25 days after treatment (Fig. 1 D).

In addition, the inhibition of ethylene production and its action by 1-MCP was found to cause some changes in quality parameters, such as color, firmness and loss in weight.

Color change is an important quality for customers, and several researches pointed out the effectiveness of 1-MCP on the color of apple, pear, green plum, kiwifruit, and avocado (Valero and Serrano 2010). Our results also support the same tendency, but the precise mechanism of 1-MCP on anthocyanin or carotenoid pigmentation, as well as on chlorophyll decomposition might be studied further.

Fruit softening in apple could be prevented or delayed by 1-MCP treatment. The softening process is closely associated with ethylene production (Valero and Serrano, 2010). In plum, the activity of exo-PG and EGase was lower when treated with 1-MCP (Watkin, 2006). Similarly, the effectiveness of 1-MCP on apple firmness in our experiment is consistent with previously published reports by Mir *et al.* (2001), Fan *et al*. (1999), Rupasinghe *et al.* (2000), and Watkins *et al*. (2000).

Our results suggest that 1-MCP prevents weight loss in apple. The effect of 1-MCP on weight loss has been reported in tomato (Guillen *et al*. 2007), plum (Valero *et al*. 2003), and avocado (Watkin 2006).

Japanese apricot

1. Green stage

Ethylene and CO_2: There was no significant difference in ethylene production between fruits during green stage (Fig. 2; A). Respiration records showed that fruits treated with 1-MCP significantly produced less CO_2 than the control (Fig. 2; B) from 48 to 72 h.

In case of Japanese apricot, however, our records show that there was no significant difference in ethylene production between 1-MCP treatment and control during both green and yellow stages of harvest. For tomato fruit, ethylene production in young green fruit was inhibited, but was promoted in mature pink tomato by 1-MCP treatment, indicating that 1-MCP blocks either the self-promoting effect of system I ethylene or self-inhibiting effect of system II ethylene (Poyesh *et al*. in press). The results obtained are in accordance with other studies, which showed the influence of 1-MCP on ethylene biosynthesis through feedback inhibition (Blankenship and Dole, 2003). Martinez-Romero *et al.* (2007a) reported that the effect of 1-MCP on stone fruits generally depended on the dose of 1-MCP application, so the dose-response curve should also be considered.

Data on the effect of 1-MCP on Japanese apricot respiration suggests that 1-MCP treatment may constantly decrease respiration (CO_2). This may be due to the decrease in ethylene sensitivity by the inhibition of ethylene binding to its receptors by 1-MCP. Abdi *et al.* (1998) also reported the reduction in CO_2 in plum while Watkin (2006) reported a reduction in respiration of apple fruit due to 1-MCP treatment.

Color and firmness: Delay in color change was significantly obvious (at 5%) in 1-MCP treated fruit from 24 to 72 h of continuous treatment, compared to a control (Fig. 2; C). Data related to firmness showed that 1-MCP could prevent softening of fruits, as 1-MCP treated fruits were firmer than untreated ones (Fig. 2; D). Firmness values were significantly higher (at 5%) in fruits treated with 1-MCP than a control from 24 to 96 h of continuous treatment.

2. Yellow stage

Ethylene and CO_2: Results from ethylene production showed no significant difference between yellow fruits treated with 1-MCP and untreated fruits throughout the study (Fig 2; E). However, fruits treated with 1-MCP produced less ethylene than the control, but was not significant. On the other hand, CO_2 production was significantly less in yellow fruits treated with 1-MCP at 0, 6, 12, and 48 h after treatment while at 72 h; 1-MCP treated fruit produced significantly higher CO_2 compared with a control (Fig. 2; F). Respiration decreased due to 1-MCP treatment up to 48 hours after treatment.

Our records show that there was no significant difference in ethylene production between 1-MCP treated Japanese apricot and untreated ones during both green and yellow stages of harvest.

Color and firmness: Data on color change revealed no significant difference between Japanese apricot during the yellow treated with 1-MCP a control under ambient conditions (Fig. 2; G). However, fruits treated with 1-MCP exhibited a slight delayin color change than a control based on data at 72 h after treatment. The data related to the softening of Japanese apricot at yellow stage revealed that fruits treated with 1-MCP were significantly firmer (at 5%) than the control at 24 to 96 h after storage (Fig. 2; H).

Asparagus

Asparagus spears are transported and stored in a horizontal position. Since it rises up when it positioned horizontally, this can cause problems. Earlier research on rice seedlings showed a positive relationship between rice ethylene production with an increasing propensity towards horizontally placed seedlings (Koshio *et al*. 2010). Hence, asparagus spears were treated with 1-MCP and positioned horizontally and vertically.

Ethylene CO_2 production: Ethylene production from both horizontally and vertically positioned spears was significantly higher (at 5%) than a control from 3 to 96 h after treatment (Fig 3;A and B). No significant differences in CO_2 production between asparagus spears treated with 1-MCP and a control, positioned either horizontally or vertically (Fig 3; C and D).

Since the asparagus spears were at a mature stage, during the present experiment, 1-MCP might block self-inhibition of ethylene production to promote ethylene production. We can say that 1-MCP has different effects on ethylene biosynthesis, depending on the variety and stage of fruit or vegetable. The results obtained are in accordance with other studies, that show the influence of 1-MCP on ethylene biosynthesis through feedback inhibition (Blankenship and Dole, 2003). Results are somehow in accordance with the study of Zhang and Zhang (2007), indicating that the quality of asparagus improved after the 1-MCP application, with a reduction in respiration.

Upward growth: Asparagus spears treated with 1-MCP had significantly slower upward growth (at 1%) than the control from 24 to 96 h after treatment. In this case, upward growth refers to the plant bending upward once stored in a horizontal position due to geotropism.

The effect of 1-MCP on asparagus spears suggests that 1-MCP could affect its upward growth when placed in a horizontal positions. In the case of rice seedlings, Koshio *et al.* (2010) reported a positive relationship between rice ethylene production and upward growth of horizontally positioned seedlings. The involvement of ethylene production in expressing negative gravitropism of rice and other plants was also mentioned by Abe *et al.* (1998), Blancaflor and Masson (2003), Horton

(1993) and Lu *et al*. (2001). 1-MCP might be useful in preventing asparagus bending and lowering respiration. Hence, our results suggest that 1-MCP could preserve quality by blocking ethylene receptors, since it blocks the action of ethylene, rather that production. A similar mechanism was reported by Sisler and Blankenship (1996) and Blankenship and Dole (2003).

CONCLUSION

Our experimental results demonstrate that in apple, Japanese apricot and asparagus, 1-MCP could improve some quality-related parameters. 1-MCP is approved by the European Union as non-toxic in 2005, by the Environmental Protection Agency (EPA) for use on ornamentals in 1999, and on edibles in 2002, followed by approval by Japan and more than 40 countries by 2010. Current experimental results demonstrate that 1-MCP could prolong storage of tested fruits and vegetables, helping farmers, traders and consumers in Southeast Asian countries to overcome the postharvest problems of their products, especially when cold storage and other facilities are not available.

ACKNOWLEDGEMENT

The authors humbly thank JICA for providing the scholarship for the first author and their kind PEACE Project team members, and the laboratory members of Tropical horticulture for their support, as without their support it was impossible to achieve this accomplishment.

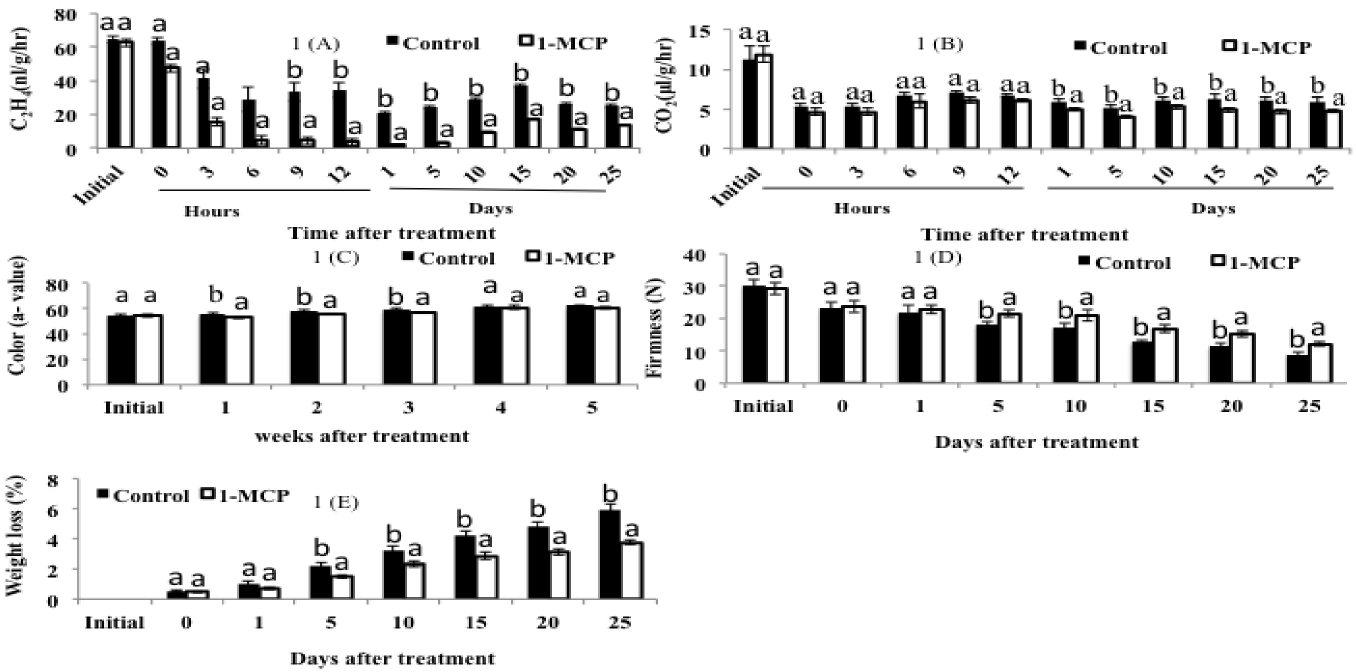

Fig. 1. Effect of 1-MCP on ethylene 1(A), respiration 1(B), color 1(C), firmness 1(D) and weight loss 1(E) on apple. Results represent means ± SE (n=10). Values with different superscripts indicate significant difference ($p < 0.05$) using Tukey test.

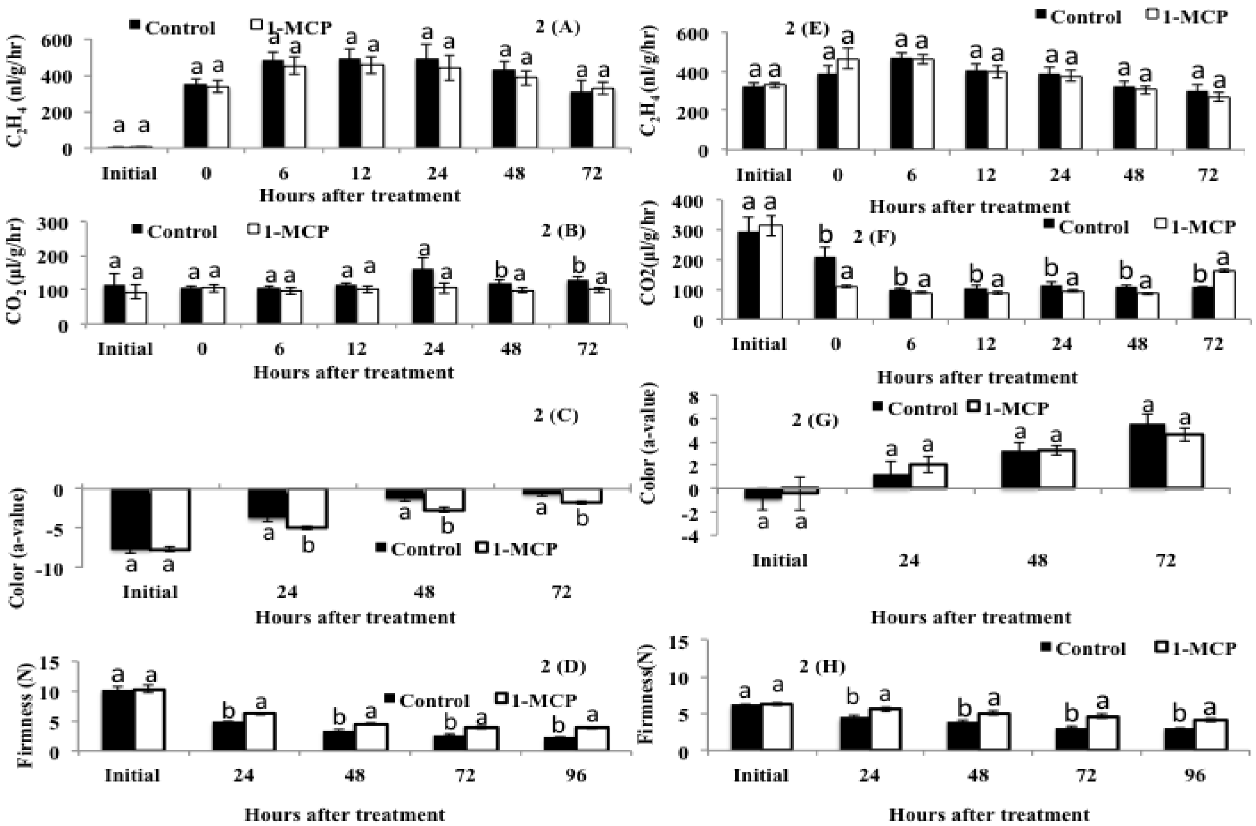

Fig. 2. Effect of 1-MCP on ethylene production 2 (A, E), respiration 2 (B, F), color 2 (C, G) and firmness 2 (D, H) on green and yellow stages of Japanese apricot. Results represent means ± SE (n=10). Values with different superscripts indicate significant difference ($p < 0.05$) using Tukey test.

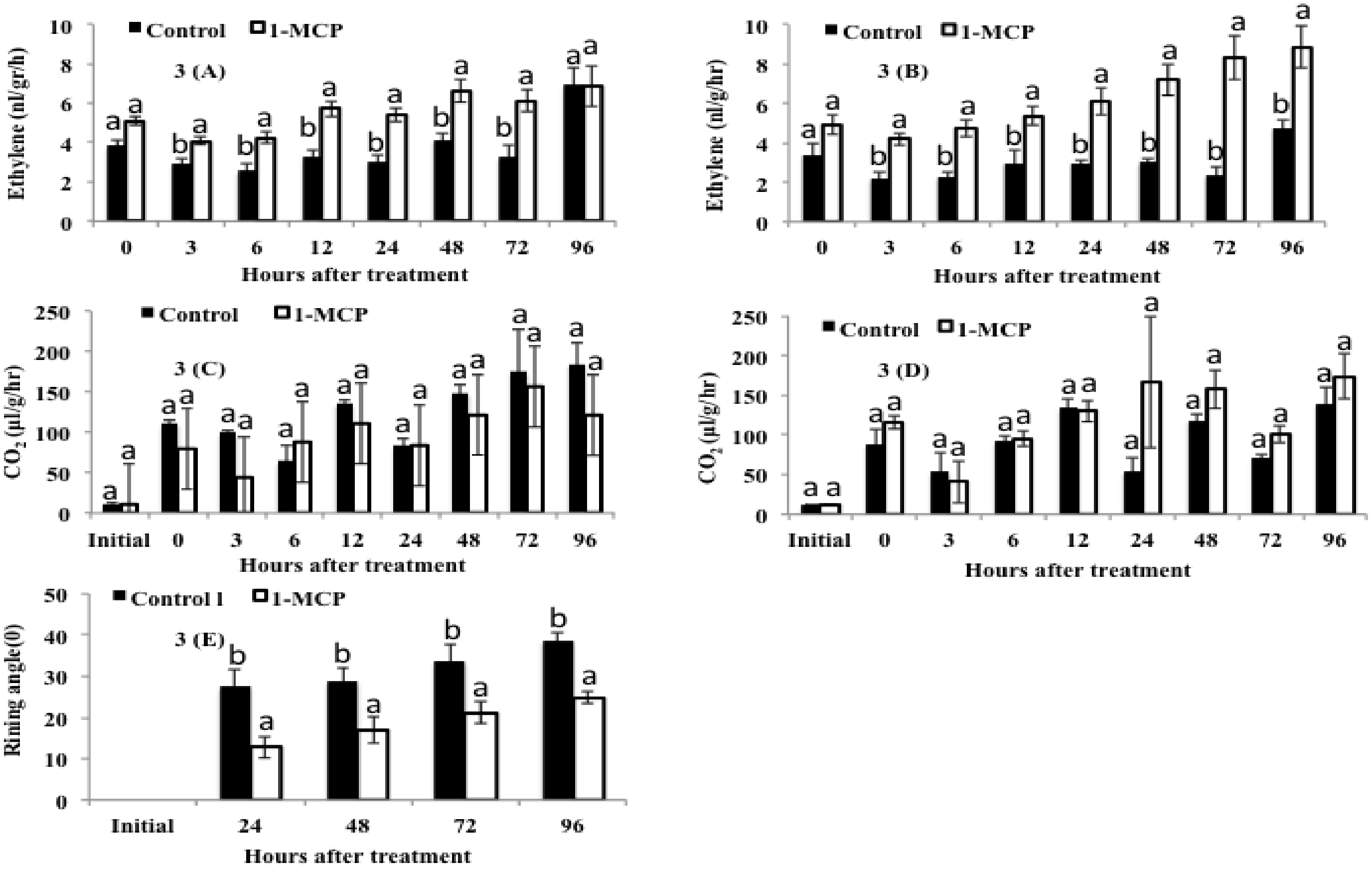

Fig. 3. Effect of 1-MCP on ethylene 3 (A, B), respiration 3 (B, C) of vertically and horizontally positioned asparagus spears and the rising angle of asparagus when horizontally positioned. Results represent means ± SE (n=10). Values with different superscripts indicate significant difference ($p < 0.05$) using Tukey test.

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