

1-METHYLCYCLOPROPENE REDUCES FRUITLET LOSS CAUSED BY ETHEPHON FOLIAR SPRAYS

LUIS V. POZO AND JACQUELINE K. BURNS*

University of Florida, IFAS

*Horticultural Sciences Department
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850*

Additional index words. 5-chloro-3-methyl-4-nitro-1*H*-pyrazole, abscission agent, fruit detachment force, leaf abscission, mature fruit loosening

Abstract. 1-Methylcyclopropene (1-MCP) is a gaseous ethylene binding inhibitor used to control or delay ethylene-related postharvest problems in a range of horticultural commodities. To evaluate the potential of 1-MCP to prevent unwanted defoliation and fruitlet loss when using ethephon to loosen mature citrus fruit, canopy sector tests were conducted in 'Valencia' orange [*Citrus sinensis* (L.) Osb.] using 400 mg·L⁻¹ ethephon (2-chloro-ethyl-2-phosphonic acid) in combination with 1, 2.5 and 5 mM concentrations of 1-MCP. Although there was no difference in loosening of mature fruit by ethephon and ethephon + 1-MCP treatments, 1-MCP reduced defoliation caused by ethephon. Studies on the effect of 1-MCP + ethephon application on flower and fruitlet abscission demonstrated that unwanted abscission in these organs could also be reduced. The results suggest that ethephon-induced phytotoxicity can be reduced with 1-MCP, but the gaseous nature of 1-MCP is an impediment to uniform application. A sprayable 1-MCP formulation is needed for preharvest use in many horticultural crops.

A selective abscission compound is considered crucial for the adoption of year-round mechanical harvesting of Florida's citrus 'Valencia' crop. To date, no abscission agents are registered for use in citrus. A number of potential abscission agents have been tested in Florida during the past 10 years, such as 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP), methyl jasmonate (MeJA), 2-chloro-2-ethyl-phosphonic acid (ethephon), and coronatine (Burns, 2002; Burns et al., 2003; Hartmond et al., 2000). Among them, only CMNP has proven to selectively loosen mature fruit when used over wide concentration ranges. Ethephon is registered for use on several food crops and relatively inexpensive to purchase and apply. Thus, this product could be an attractive abscission agent candidate, particularly if unwanted defoliation can be reduced.

Ethephon is an ethylene-releasing agrochemical used to enhance mature fruit abscission and aid mechanical harvesting in several horticultural crops (Bukovac et al., 1969; Cooper and Henry, 1968; Kender, 1998; Kender and Hartmond, 2000; Martin et al., 1981; Perez et al., 1981; Rasmussen, 1976). Its potential as an abscission agent in citrus harvesting remains limited unless adverse side effects, such as abscission of young developing fruitlets, excessive leaf abscission, terminal

dieback, and gummosis (Burns, 2002; Kender et al., 2000) can be effectively minimized.

The ethylene binding inhibitor 1-methylcyclopropene (1-MCP) has been effective in controlling or reducing ethephon-related disorders associated with senescence. These include pre- or post-harvest problems on climacteric fruits, abscission in floricultural commodities (Blankenship and Dole, 2003), as well as fruit decay during postharvest storage of apples (Mattheis et al., 2001; Saftner et al., 2003), peaches (Kluge and Jacomino, 2002), avocado, tomatoes (Huber et al., 2003; Nakatsuka et al., 1997), bananas (Golding et al., 1998; Harris et al., 2000), and papaya (Jacomino et al., 2002). Abscission in citrus leaf and fruit explants could be reduced by 1-MCP treatment (Porat et al., 1999; Zhong et al., 2001). Leaf abscission, gummosis, and twig dieback were successfully controlled in *Citrus madurensis* and *Citrus sinensis* treated with a simultaneous application of 1-MCP + ethephon, with little effect on ethephon-induced reduction of fruit detachment force (Pozo and Burns, 2000; Pozo et al., 2004). Application of 1-MCP was more effective when combined with ethephon in the spray tank rather than applying it 24 h before or after ethephon treatment (Pozo et al., 2004). We hypothesized that 1-MCP could reduce ethephon-induced loss of fruitlets in citrus. The objective of this work was to investigate the effect of 1-MCP + ethephon spray solutions on fruitlet retention and abscission of mature fruit and leaves at full bloom and during young fruit growth in 'Valencia' orange.

Materials and Methods

Plant material, compounds and application procedures. *Citrus sinensis* L. Osbeck cv. 'Valencia', grafted on rough lemon rootstock were located at the Citrus Research and Education Center, Lake Alfred, Fla., and used for this study. Trees were between 15 and 18 years of age. Experiments were conducted in March through June 2006. 1-Methylcyclopropene (1-MCP) was obtained from Rohm and Haas, Inc., Philadelphia, Pa., as a 3.3% active ingredient formulation ('SmartFresh®'). Abscission agents used in this work were ethephon, 2-chloro-ethyl-2-phosphonic acid ('Ethrel®', 21.7% ethephon w/v, Aventis Crop Science, Research Triangle Park, N.J.) and CMNP (5-chloro-3-methyl-4-nitro-1*H*-pyrazole, 17.2% active ingredient, as previously formulated by Abbott Laboratories, Libertyville, Ill.). Spray solutions contained the organosilicate adjuvant Kinetic® (Setre Chemical Co., Memphis, Tenn.) at 0.15% (v/v). Treatments were applied to canopy sections (approximately 1 m³) using a pressurized 1-L capacity hand sprayer. Spray solutions were applied until runoff. Rainfall did not occur for 3 d after application in all trials reported.

Fruitlet retention, mature fruit, and leaf drop measurements. Trees within a 30-tree row were used in the trial. One-hundred canopy sections were marked within this row and randomly assigned treatments. Each section contained over 30 mature fruit and fruitlets, and more than 100 leaves. Equatorial diameter (mm) of at least 10 fruitlets was monitored on a weekly basis within these sections from day 0 (16 Mar. 2006) through 111 d (5 Jul. 2006) after full bloom (DAB) using a

This research was supported by the Florida Agricultural Experiment Station and Florida Citrus Initiative Funds. We thank AgroFresh, Inc. for supplying 1-MCP in the form of SmartFresh®.

*Corresponding author; e-mail: jkbu@ufl.edu

digital caliper ('Digimatic' caliper, Model CD-6"CS, Mitutoyo Products Corporation, Japan). At fruit developmental stages corresponding to 0, 26, 35, 63, and 81 DAB (avg. diameters of 2, 5, 10, 20, and 30 mm; Fig. 1), four canopy sections were sprayed with either 5 mM 1-MCP + 400 mg·L⁻¹ ethephon, 400 mg·L⁻¹ ethephon, 5 mM 1-MCP, 200 mg·L⁻¹ CMNP, or only Kinetic plus water as a control. Thus, each treatment was replicated four times on each of the five spray dates. Fruit detachment force (FDF, kg) was measured 5 d after each application with a digital force gauge ('Force Five', Wagner Instruments, Greenwich, Conn.; Pozo et al., 2004). Cumulative percent of leaf abscission and fruitlet retention was measured by counting these organs immediately prior to and 30 d after each application. Results are expressed as a percentage of the total organ number at the beginning of the experiments.

Statistics. Percentage data were transformed to stabilize variance using arcsin transformation in MS-Excel functions (Microsoft, Redmond, Wash.). Data were analyzed within each spray date as a 1-way factorial with spray treatment as the independent variable. Analysis of variance, regression analysis, and Duncan's multiple range tests were performed using the SAS statistical package (SAS Inst., Inc., Cary, N.C.).

Results

Defoliation and FDF of mature fruit. As expected, ethephon application at 0, 26, 35, 63, and 81 DAB resulted in high defoliation (Fig. 2). The most severe defoliation was observed when treatments were applied at 26 DAB. The absolute values of ethephon-induced leaf drop during the treatment period ranged from 48%–82%. Application of 1-MCP + ethephon markedly reduced ethephon-induced leaf abscission. FDF remained unaffected by 1-MCP, with little or no difference between 1-MCP + ethephon and ethephon treatments 5 d after application (Fig. 3). CMNP reduced FDF to consistently lower values than other treatments.

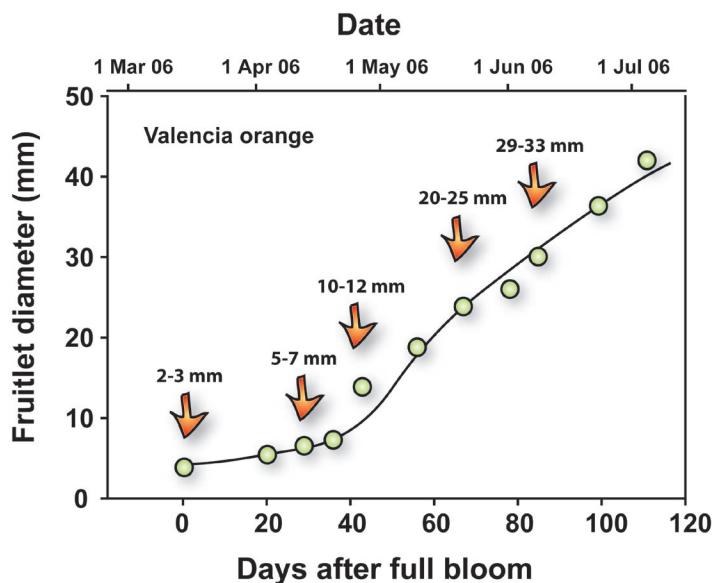


Fig. 1. Fruitlet diameter of 'Valencia' oranges from full bloom (16 Mar 2006) to 111 d after full bloom (5 Jul. 2006). Arrows indicate date of spray. Range of fruitlet diameters on each spray date are indicated above arrows. Standard error bars are smaller than mean markers. The equation for the line is $y = 64[1+e^{-(x-78)/28}]$, $r^2 = 0.98$.

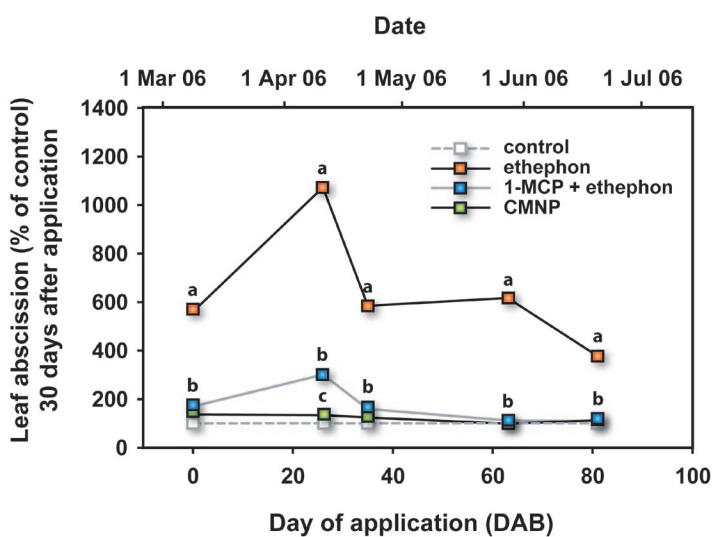


Fig. 2. Effect of 5 mM 1-MCP + 400 mg·L⁻¹ ethephon, 400 mg·L⁻¹ ethephon, and 200 mg·L⁻¹ CMNP on leaf abscission 30 d after spray application. There was no difference observed between 1-MCP and adjuvant alone treatments so only the adjuvant alone is plotted (control). Data are presented as percent of control. Points (alone or cluster) at the same application date with different letters are statistically different at $P < 0.05$.

Fruitlet retention. When applied from 0 to 63 DAB, ethephon caused nearly all flowers and/or fruitlets to abscise within 30 d after application (Fig. 4). At the last spray date (81 DAB), green fruit retention in ethephon-sprayed canopy sections approached that of the control, but was still significantly lower. In general, abscission occurred at the pedicel abscission layer for the first four application dates, whereas at the last application date, fruitlets abscised either at the calyx or pedicel abscission zones (data not shown). Fruitlet abscission decreased in branches sprayed with 1-MCP + ethephon, but average retention remained below the control in all but the last application date. Application of 1-MCP alone numerically

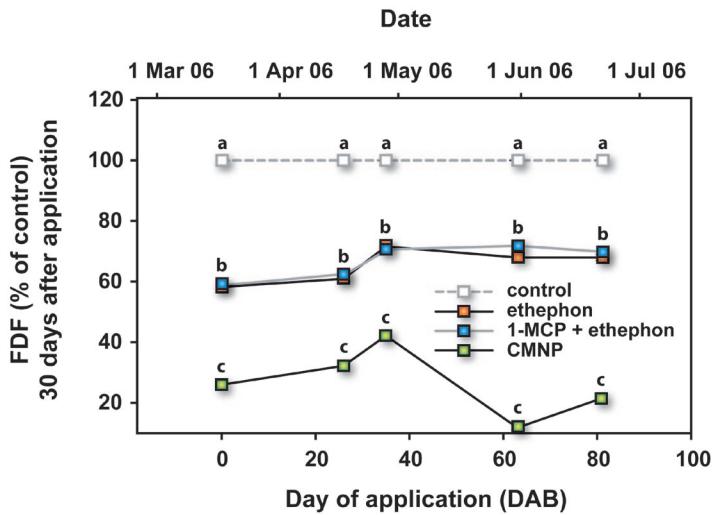


Fig. 3. Effect of 5 mM 1-MCP + 400 mg·L⁻¹ ethephon, 400 mg·L⁻¹ ethephon, and 200 mg·L⁻¹ CMNP on fruit detachment force (FDF) 5 d after spray application. There was no difference observed between 1-MCP and adjuvant alone treatments so only the adjuvant alone is plotted (control). Points (alone or cluster) at the same application date with different letters are statistically different at $P < 0.05$.

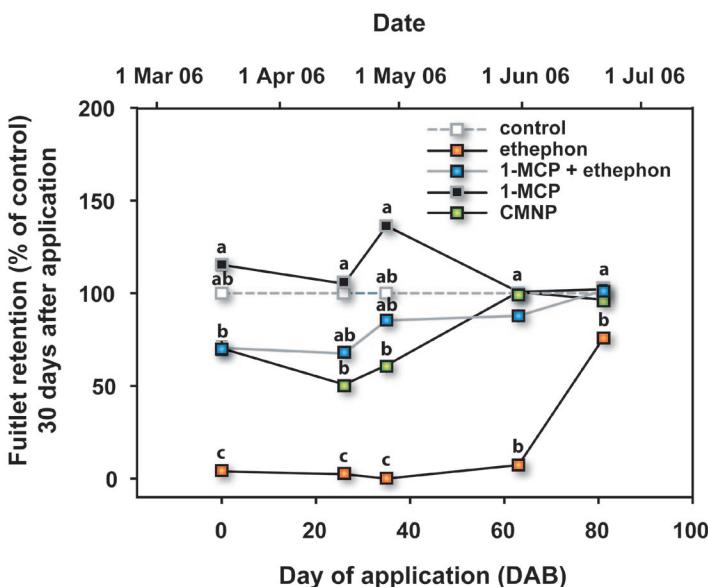


Fig. 4. Fig. 2. Effect of 5 mM 1-MCP, 5 mM 1-MCP + 400 mg L⁻¹ ethephon, 400 mg L⁻¹ ethephon, 200 mg L⁻¹ CMNP, and adjuvant alone (control) on fruitlet retention 30 d after spray application. Data are plotted as percent of control. Points (alone or cluster) at the same application date with different letters are statistically different at $P < 0.05$.

reduced natural fruitlet drop at the first and third application dates. Interestingly, CMNP application significantly reduced fruitlet retention when applied at the first three dates. By 63 DAB, there was no difference between fruit retention of CMNP-treated and the adjuvant control.

Discussion

Our previous work demonstrated that the ethylene binding inhibitor 1-MCP applied as a gas to potted *Citrus mandarinensis* trees before ethephon treatment or as a spray solution containing ethephon +1-MCP, greatly reduced defoliation and gummosis associated with ethephon applications. Ethephon-induced mature fruit loosening was either not altered or only partially reduced by 1-MCP (Pozo and Burns, 2000; Pozo et al., 2004). Yuan et al. (2005) demonstrated that up to 25% annual defoliation caused no adverse effect on tree growth and return yield in 'Valencia' when defoliated before bloom. Taken together, these results suggested that ethephon could be considered as an abscission agent candidate for Florida citrus, provided that fruitlet abscission was not markedly increased with ethephon sprays. Retention of young fruit is especially important if ethephon is to be used for late season 'Valencia' harvest in combination with mechanical harvesting.

The focus of the current work was to determine the effect of ethephon and 1-MCP + ethephon applications on flower and fruitlet retention during and after bloom in 'Valencia'. Our results confirmed that 1-MCP could reduce ethephon-induced flower and fruitlet abscission. However, fruitlet loss remained greater than the control at most application dates. In some cases, fruitlet retention was increased with 1-MCP alone, suggesting that subsequent yield could be improved. Surprisingly, CMNP application increased fruitlet loss in this study, implying that next year's yield would be negatively impacted. In previous trials, CMNP application to 'Valencia'

canopies containing fruitlets from 4 to 26 mm in diameter did not reduce yield the following season (Whitney, 1975; Whitney et al., 1986). Whether yield would be altered by treatments described in our study will require increased sample sizes, whole tree application, and evaluation of yield the following season. As fruitlets developed, sensitivity to ethephon was greatly reduced. Altered sensitivity of developing organs to ethylene has been well documented (Abeles et al., 1992).

Ethephon + 1-MCP applications, although promising, remain experimental. Since 1-MCP is a gas in its active form, field application remains problematic. In this work, 1-MCP was dissolved in the spray tank, the spray tank capped, and sprays were applied within 10 min. Any further delay in applying the spray mixture, once the combined solution was prepared, could lead to loss of the gaseous active ingredient. Furthermore, combined sprays should be applied in large droplet sizes under relatively low pressure to reduce loss of the volatile active ingredient. The volatility of 1-MCP is the reason why a relatively large concentration (5 mM) was needed to counteract unwanted side effects of ethephon.

In conclusion, ethephon-induced fruitlet and leaf loss can be mitigated by including 1-MCP in the spray without compromising effective loosening of mature fruit. Successful field application will require development of a sprayable formulation or a non-gaseous 1-MCP analog that will minimize volatile loss of the active ingredient.

Literature Cited

- Abeles, F. B., P. W. Morgan, and M. E. Saltveit. 1992. Ethylene in plant biology. Academic Press, Inc., San Diego, CA.
- Blankenship, S. and J. M. Dole. 2003. 1-Methylcyclopropene: a review. Post-harvest Biol. Technol. 28(1):1-25.
- Bukovac, M. J., F. Zucconi, R. P. Larsen, and C. D. Kesner. 1969. Chemical promotion of fruit abscission in cherries and plums with special reference to 2-chloroethylphosphonic acid. J. Amer. Soc. Hort. Sci. 94:226-230.
- Burns, J. K. 2002. Using molecular biology tools to identify abscission materials for citrus. HortScience 37:459-464.
- Burns, J. K., L. V. Pozo, C. R. Arias, and B. Hockema. 2003. Coronatine and abscission in citrus. J. Amer. Soc. Hort. Sci. 128:309-315.
- Cooper, W. C. and W. H. Henry. 1968. Field trials with potential abscission chemicals as an aid to mechanical harvesting of citrus in Florida. Proc. Fla. State Hort. Soc. 81:62-68.
- Golding, J. B., D. Shearer, S. G. Wyllie, and W. B. McGlasson. 1998. Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. Postharvest Bio. Technol. 14(1):87-98.
- Harris, D. R., J. A. Seberry, R. B. H. Wills, and L. J. Spohr. 2000. Effect of fruit maturity on efficiency of 1-methylcyclopropene to delay the ripening of bananas. Postharvest Biol. Technol. 20(3):303-308.
- Hartmond, U., R. Yuan, J. K. Burns, A. Grant, and W. J. Kender. 2000. Citrus fruit abscission induced by methyl-jasmonate. J. Amer. Soc. Hort. Sci. 125(5):547-552.
- Huber, D., J. Jeong, and M. Ritenour. 2003. Use of 1-Methylcyclopropene on tomato and avocado fruits: potential for enhanced shelf life and quality retention. Fla. Coop. Ext. Serv., IFAS. January 2003.
- Jacomino, A. P., R. A. Kluge, A. Brackmann, and P. R. DeCamargo. 2002. Amadurecimiento e senescencia de mamao com 1-metilciclopropeno. Sci. Agricola 59(2):303-308.
- Kender, W. J. 1998. The Florida experience with citrus abscission chemicals, pp. 52-57. In: W. J. Kender and J. K. Burns (eds.). Proc. Citrus Abscission Workshop, 3-4 Feb. 1998. CREC, Lake Alfred, FL.
- Kender, W. J. and U. Hartmond. 2000. Abscission materials as aid for harvesting Florida citrus. Proc. Intl. Soc. Citricult. IX Congr. 1:285-287.
- Kender, W. J., U. Hartmond, R. Yuan, L. Pozo, and A. Grant. 2000. Factors influencing the effectiveness of ethephon as a citrus fruit abscission agent. Proc. Fla. State Hort. Soc. 113:88-92.
- Kluge, R. A. and A. P. Jacomino. 2002. Shelf life of peaches treated with 1-methylcyclopropene. Sci. Agricola 59(1):69-72.
- Martin, G. C., S. Lavee, and G. S. Sibbett. 1981. Chemical loosening agents to assist mechanical harvest of olive. J. Amer. Soc. Hort. Sci. 106:325-330.

- Mattheis, J., X. Fan, and L. Argenta. 2001. Responses of pacific northwest apples to 1-methylcyclopropene. Proc. Wash. Tree Fruit Postharvest Conf. Wenatchee, WA, 13-14 Mar. 2001.
- Nakatsuka, A., S. Shiomi, Y. Kubo, and A. Inaba. 1997. Expression and internal feedback regulation of ACC synthase and ACC oxidase genes in ripening tomato fruit. *Plant Cell Physiol.* 38:1103-1110.
- Perez, M. C., I. Quintero, and A. Martinez. 1981. Combination of abscission-inducing chemicals and mechanical harvesting on Valencia late oranges in Cuba. *Proc. Intl. Soc. Citricult.* 1:285-288.
- Porat, R., B. Weiss, L. Cohen, A. Dauss, R. Goren, and S. Drob. 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest quality of 'Shamouti' oranges. *Postharvest Biol. Technol.* 15:155-163.
- Pozo, L. and J. K. Burns. 2000. Ethylene action inhibitors reduced Ethrel-induced leaf drop and gummosis in citrus. *Proc. Intl. Soc. Citricult.* IX Congr. 1:578-579.
- Pozo, L., R. Yuan, I. Kostenyuk, F. Alferez, G. Zhong, and J. K. Burns. 2004. Differential effects of 1-methylcyclopropene on citrus leaf and mature fruit abscission. *J. Amer. Soc. Hort. Sci.* 129(4):473-478.
- Rasmussen, G. K. 1976. Effect of abscission-inducing chemicals on mandarin oranges. *Proc. Fla. State Hort. Soc.* 89:39-41.
- Saftner, R. A., J. A. Abbott, W. S. Conway, and C. L. Barden. 2003. Effects of 1-methylcyclopropene and heat treatments on ripening and postharvest decay in 'Golden Delicious' apples. *J. Amer. Soc. Hort. Sci.* 128:120-127.
- Whitney, J. D. 1975. Orange yield and removal studies with air and trunk shakers using two abscission chemicals. *Proc. Fla. State Hort. Soc.* 8:120-124.
- Whitney, J. D., B. B. Churchill, and S. L. Hedden. 1986. A five-year study of orange removal with trunk shakers. *Proc. Fla. State Hort. Soc.* 99:40-44.
- Yuan R., F. Alferez, I. Kostenyuk, S. Singh, J. P. Syvertsen, and J. K. Burns. 2005. Partial defoliation can decrease average leaf size but has little effect on orange tree growth, fruit yield and juice quality. *HortScience* 40:2011-2015.
- Zhong, G., Y. Huberman, M. Fend, X. Q. Sisler, D. Holland, and R. Goren. 2001. Effect of 1-methylcyclopropene on ethylene-induced abscission in citrus. *Physiol. Plant.* 113:134-141.