

# Differential Effects of 1-Methylcyclopropene on Citrus Leaf and Mature Fruit Abscission

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**ABSTRACT.** 1-MCP is a gaseous ethylene binding inhibitor that controls or delays ethylene-related postharvest problems in a range of horticultural commodities. Our previous work demonstrated that exposure of calamondin to 1-MCP 16 hours before canopy sprays of ethephon greatly reduced unwanted leaf drop while only partially inhibiting the ability of ethephon to cause fruit loosening. The objective of this work was to determine whether formulated 1-MCP (SmartFresh) could be used in the field to stop defoliation caused by abscission agent applications without significantly altering abscission agent-induced fruit loosening. Spray solutions containing 400 mg·L<sup>-1</sup> ethephon with 0, 1, 2.5, and 5 mM 1-MCP were applied to canopies of 'Hamlin' and 'Valencia' (*Citrus sinensis*). Timing of 1-MCP applications was a) 24 hours before, b) in combination with, or c) 24 hours after ethephon. Ethephon at 400 mg·L<sup>-1</sup> significantly reduced fruit detachment force (FDF) but caused >70% leaf drop within 15 days after application in both cultivars. Applications of 1-MCP reduced ethephon-associated leaf abscission but had little effect on the ability of ethephon to reduce FDF. Timing of 1-MCP applications did not affect the ability of ethephon to cause fruit loosening; however, the best consistent treatment for control of leaf drop was achieved with the combined application of 5 mM 1-MCP and 400 mg·L<sup>-1</sup> ethephon. 1-MCP was used in combination with the abscission agents coronatine, methyl jasmonate (MeJa) and 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP) to determine its effect on leaf drop and fruit loosening. Leaf drop in trees treated with ethephon, coronatine, and MeJa was reduced by addition of 1-MCP. However, fruit loosening was largely prevented when 1-MCP was used in combination with coronatine or MeJa. Like ethephon, CMNP-induced fruit loosening was not affected by 1-MCP. The results demonstrate the ability to control ethephon-induced leaf abscission without affecting mature fruit loosening by targeting ethylene binding in citrus.

Citriculture remains a dominant economic force in Florida. Crop estimates indicate that the state will produce 246 million 40.8-kg (90-lb) boxes of oranges in the 2003–04 season (Florida Agricultural Statistics Service, 2003). On a 40-kg-box basis, the 2000–01 production cost of oranges destined for processing averaged \$1.87, while the cost of harvesting operations, on the other hand, rose to \$2.10 (Muraro et al., 2003). In contrast, the cost of production and harvesting in Brazil were \$1.37 and \$0.53, respectively. To remain competitive in a global market, Florida's citrus industry must become more efficient; in such a context, the mechanization of orange harvesting has become a priority.

Mechanical harvesting technologies such as trunk and canopy shakers are operational in Florida today, and fruit removal averages between 70% and 90% depending on factors such as grove conditions and tree architecture (Brown 2002). The development of abscission agents for use in combination with mechanical harvesting is expected to increase fruit removal percentages and speed of harvest while decreasing overall harvesting cost. An effective abscission agent can consistently loosen mature fruit without causing phytotoxicity to leaves and young developing fruit (Burns 2002). Ethephon (2-chloro-2-ethyl-phosphonic acid), methyl jasmonate (MeJa), 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP) and coronatine have been tested in Florida as abscission

agents (Burns, 2002; Burns et al., 2003; Hartmond et al., 2000). These agents successfully loosen mature fruit, but only CMNP is specific for mature fruit within a wide concentration range. Ethephon chemically degrades to ethylene directly after uptake into alkaline plant tissue (Bukovac et al., 1969; Warner and Leopold, 1969), and CMNP, MeJa and coronatine induce ethylene evolution in responding tissues (Burns et al., 2003; Hartmond et al., 2000). It is thought that ethylene produced as a result of application of these agents promotes abscission.

Currently, no abscission agents are registered for use in citrus. It would be advantageous to adapt ethephon for use as an abscission agent because it is already registered for use on several food crops and is relatively inexpensive to apply. Unfortunately, under Florida's environmental conditions, ethephon causes unacceptably high leaf abscission when applied at concentrations effective to loosen mature citrus fruit (Burns, 2002). The action of ethephon is greatly affected by temperature, and high temperatures throughout the harvest season can increase efficacy of the material and consequently decrease the consistency of the response (Bukovac et al., 1969).

In recent years, the ethylene binding inhibitor 1-methylcyclopropene (1-MCP) has been used to delay or prevent senescence symptoms including abscission in floricultural commodities (Blankenship and Dole, 2003) and ethylene-related disorders associated with postharvest ripening, senescence, handling and decay of climacteric fruit such as apples (Saftner et al., 2003), peaches (Kluge and Jacomino, 2002), avocado and tomatoes (Jeong et al., 2002; Nakatsuka et al., 1997) and papaya (Jacomino et al., 2002). Although 1-MCP can inhibit or delay degreening in citrus, its application enhanced postharvest chilling injury and decay

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(Porat et al., 1999). Abscission in citrus leaf and fruit explants stored for various lengths of time can be prevented by the use of 1-MCP (Porat et al., 2001; Zhong et al., 2001), but work on effect of 1-MCP on whole citrus plants has not been extensively reported. In our previous work, we found that ethylene binding or perception inhibitors, such as 1-MCP and silver thiosulfate, reduced ethephon-induced leaf drop and gummosis in *Citrus madurensis* but only partially inhibited the ability of ethephon to loosen mature fruit (Poza and Burns, 2000). The objective of this work was to determine if 1-MCP could be used in the field in combination with ethephon and other abscission agents to reduce defoliation while maintaining mature fruit loosening associated with the abscission agent.

## Materials and Methods

**PLANT MATERIAL.** *Citrus sinensis* L. Osbeck cv. 'Valencia', grafted on rough lemon rootstock, and *Citrus sinensis* L. Osbeck 'Hamlin', grafted on 'Carrizo' citrange rootstock, were located at the Citrus Research and Education Center, Lake Alfred, Fla. Trees were between 15 and 18 years of age. Experiments were conducted in two harvest seasons.

**ABSCISSION AGENTS AND APPLICATION PROCEDURES.** 1-Methylcyclopropene (1-MCP) was obtained from Rohm and Haas, Inc., in either 0.14% or 3.3% active ingredient formulations (Smart-Fresh). Abscission agents used in this work were 1) ethephon [(2-chloroethyl) phosphonic acid (Ethrel, 21.7% ethephon w/v, Aventis Crop Science, N.J.)], 2) coronatine, a natural product obtained by fermentation with *Pseudomonas syringae* (Burns et al., 2003), 3) methyl-jasmonate (MeJa) (Bedoukian Research Inc., Conn.), and 4) 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMNP, 17.2% active ingredient, as previously formulated by Abbott Laboratories, Ill.). All abscission agents were dissolved or dispersed in distilled water and contained the organosilicate adjuvant Kinetic (Setre Chemical Co., Tenn.) at 0.15% (v/v). Treatments were applied to canopy sections ( $\approx 1 \text{ m}^3$ ) using a pressurized, 1-L capacity hand sprayer. For whole tree canopy treatments, a 12-L capacity pressurized back-pack sprayer was used. Spray solutions were applied until runoff. Rainfall did not occur for 3 d after application in all trials reported.

**EXPERIMENT 1: LEAF ABSCISSION AND FRUIT LOOSENING WITH ETHEPHON.** Spray solutions containing ethephon at 0, 200, 250, 300, 350, 400, or 450  $\text{mg}\cdot\text{L}^{-1}$  were applied to canopy sections bearing 80 to 300 leaves and no fewer than 10 mature fruit each. The experiment containing treatments described above were repeated twice in November to December ('Hamlin', 18°C avg. trial temps.) and twice in February to March ('Valencia', 23°C average trial temps.). The same row of 15 uniform trees was used for duplicated experiments with each cultivar. Four different randomly distributed sections were used per treatment at each time. In all cases, fruit detachment force (FDF) was measured 5 d after application with a digital force gauge (Force Five, Wagner Instruments, Greenwich, Conn.). Mature fruit with stems attached were clipped  $\approx 2.5 \text{ cm}$  above the fruit abscission zone, inserted into the gauge, and the stem pulled parallel to the fruit axis until it separated from the fruit. Results are expressed in kilograms. Cumulative percent leaf abscission was measured by counting leaves before and after periods up to 15 d postapplication. Results are expressed as a percentage of the starting leaf number.

**EXPERIMENT 2: TIMING OF 1-MCP APPLICATION.** Spray solutions containing 1-MCP at 0, 1, 2.5, and 5 mM were applied either 24 h before, in combination with, or 24 h after foliar sprays of 400

$\text{mg}\cdot\text{L}^{-1}$  ethephon. 1-MCP as SmartFresh was dissolved directly into the spray tank containing either water or ethephon and water, and applied within 5 min. Additional treatments included 400  $\text{mg}\cdot\text{L}^{-1}$  ethephon, 200  $\text{mg}\cdot\text{L}^{-1}$  CMNP and water as controls. Three randomly distributed canopy sections of 'Hamlin' orange, in a row of 15 trees, were used for each treatment. Each section contained 80 to 300 leaves and no fewer than five mature fruit. The experiment was repeated three times in November and December (17.8°C average trial temps.) using different canopy sections within the 15-tree row. FDF and cumulative percent leaf abscission were measured as described above, except leaf abscission was evaluated up to 30 d postapplication.

**EXPERIMENT 3: 1-MCP/ETHEPHON COMBINATION APPLICATION ON WHOLE CANOPIES OF 'HAMLIN' ORANGE.** The optimal timing and concentration of 1-MCP/ethephon applications was determined to be 5 mM 1-MCP applied in combination with ethephon to citrus canopies. Therefore, solutions containing 5 mM 1-MCP and 400  $\text{mg}\cdot\text{L}^{-1}$  ethephon were prepared as described above and applied until runoff to entire canopies of 'Hamlin' orange trees in January and February (14.6°C average trial temps.). Additional treatments consisted of 400  $\text{mg}\cdot\text{L}^{-1}$  ethephon, 200  $\text{mg}\cdot\text{L}^{-1}$  CMNP, and water. In all treatments, three replicate trees were used. FDF was taken on 10 randomly selected fruit/tree and was monitored as described above. Cumulative percent leaf abscission was evaluated on four tagged branches/tree on no fewer than 150 leaves/branch. Leaf abscission was monitored as described above for periods up to 30 d after application. Juice analysis was determined on fruit used for FDF analysis. Acid (percent, as citric acid), Brix, and acid/Brix ratio of mature fruit at harvest were determined using standard procedures.

**EXPERIMENT 4: FRUIT LOOSENING AND LEAF ABSCISSION WITH 1-MCP IN COMBINATION WITH CORONATINE, MEJA, CMNP, AND ETHEPHON.** Spray solutions containing 200  $\text{mg}\cdot\text{L}^{-1}$  coronatine, 15 mM MeJa, 200  $\text{mg}\cdot\text{L}^{-1}$  CMNP, and 400  $\text{mg}\cdot\text{L}^{-1}$  ethephon were applied alone or in combination with 5 mM 1-MCP to canopy sections of 'Valencia' orange. Three canopy sections per treatment were used in a 15-tree row, each containing 50 to 200 leaves and no fewer than 10 mature fruit. FDF was monitored as described above. Cumulative percent leaf abscission was evaluated as described above for periods up to 30 d after application. The experiment was repeated three times in March and April (19.4°C average trial temps.).

**STATISTICS.** Data were computed and, if necessary, transformed to stabilize variance using arcsin transformation in MS-Excel functions (Microsoft, Redmond, Wash.). Data of Expt. 1 were analyzed as a one-way factorial, with ethephon as variable. Data of Expt. 2 were analyzed as a three-way factorial, with ethephon concentration, 1-MCP concentration, and spray timing as variables. Data of Expt. 3 were analyzed as a one-way factorial, with treatment as variable. Data of Expt. 4 were analyzed as a two-way factorial, with abscission agent and 1-MCP application as variables. Analysis of variance, regression analysis and Duncan's multiple range tests were performed on data of experiments 1, 2, 3, and 4 using the SAS statistical package (SAS Inst. Inc., Cary, N.C.).

## Results

**EXPERIMENT 1: HIGH LEAF ABSCISSION IS INDUCED BY ETHEPHON.** After 15 d, leaf abscission in control trees was <10% in both 'Hamlin' and 'Valencia' (data not shown). Leaf abscission was increased by ethephon in a concentration-dependent manner in both cultivars (Fig. 1). In control trees, FDF was  $\approx 10 \text{ kg}$  (data not

shown). A concentration of 400 mg·L<sup>-1</sup> ethephon was required to reduce the FDF below 50%; however, at this concentration, leaf abscission was >70% in both varieties.

**EXPERIMENTS 2 AND 3: COMBINED APPLICATIONS OF 1-MCP AND ETHEPHON REDUCE DEFOLIATION BUT HAVE NO EFFECT ON ETHEPHON-INDUCED REDUCTION IN FDF.** Cumulative leaf abscission was over 90% with 400 mg·L<sup>-1</sup> 30 d after application (Fig. 2). Defoliation was greatly reduced when 1-MCP was combined with ethephon and applied to citrus canopy sections (Fig. 2, Table 1). Applying 1-MCP 24 h before or after ethephon application was not as effective, although 5 mM 1-MCP applied 24 h before ethephon significantly reduced defoliation (Table 1). Ethephon combined with either 1 or 2.5 mM 1-MCP controlled excessive leaf abscission in this trial; however, additional trials with 'Valencia' orange later in the season confirmed that 5 mM 1-MCP was the most effective (data not shown). Consistency of response to 1-MCP was less perhaps because average temperatures were at least 10 °C higher later in the season. Despite the positive effect of 1-MCP on leaf abscission, ethephon-induced reduction in FDF was not affected by 1-MCP applied at any concentration or timing (Fig. 3, Table 2). Whole tree canopy applications of 5 mM 1-MCP in combination with 400 mg·L<sup>-1</sup> ethephon were also effective in reducing defoliation, but had minimal effect on ethephon-induced reduction in FDF (Table 3). Juice quality of mature fruit at harvest was unaffected by any abscission agent and/or 1-MCP.

**EXPERIMENT 4: EFFICACY OF SEVERAL ABSCISSION AGENTS IS DIFFERENTIALLY AFFECTED BY 1-MCP.** Coronatine, MeJa, ethephon, and CMNP were applied to canopy sections of 'Valencia' orange

alone or in combination with 1-MCP to compare the abscission response of leaves and mature fruit. Cumulative percent leaf abscission induced by coronatine and MeJa alone was ≈20% (Fig. 4A). Combining the application of 1-MCP with these two abscission agents did not significantly affect leaf abscission, although the amount was numerically less. Leaf abscission induced by CMNP either alone or with 1-MCP was no different than the control. In contrast, 1-MCP significantly reduced defoliation induced by ethephon. Coronatine, MeJa, CMNP, and ethephon applications alone significantly reduced FDF (Fig. 4B). The reduction in FDF induced by CMNP or ethephon was not affected by 1-MCP treatment. However, little reduction in FDF occurred in the coronatine and MeJa treatments when 1-MCP was included in the spray application.

## Discussion

The effect of the ethylene binding inhibitor 1-MCP on leaf and mature fruit abscission induced by ethephon and other agents has been investigated in citrus. In previous work under greenhouse conditions, we demonstrated that exposure of calamondin (*Citrus madurensis*) trees to 1-MCP applied as a gas 16 h before ethephon application or silver thiosulfate at the time of ethephon application greatly reduced gummosis and the defoliating effects of ethephon sprays, while only partially inhibiting ethephon-induced reduction of FDF (Poza and Burns, 2000). This work has now been extended to the field with the commercial sweet orange cultivars 'Hamlin' and 'Valencia' using 1-MCP formulated as SmartFresh.

Dissolving SmartFresh powder into the spray tank containing the abscission agent immediately before application and applying to citrus canopies reduced leaf abscission with little effect on ethephon-induced reduction in FDF. This is the first report demonstrating the use of SmartFresh powder for the purpose of specifically controlling leaf abscission in whole trees in the field. The selective effect of 1-MCP was optimal and consistent when 1-MCP was combined with the ethephon solution and immediately applied. It is unclear why 1-MCP application 24 h before ethephon treatment was not as effective, especially since it is thought that 1-MCP binding is irreversible (Blankenship and Dole, 2003). A one-time, 'single-burst' application before ethephon sprays may not be adequate to reach and saturate all receptors in mature fruit. Application of 1-MCP 24 h after ethephon sprays was not sufficient to prevent abscission events once advanced by ethephon.

After SmartFresh is dissolved, application should not be delayed, as the gaseous active ingredient is quickly lost. Delays of >5 min or more reduced the effectiveness of 1-MCP (data not shown). The volatility of the active ingredient is likely the primary reason why

Fig. 1. Effect of ethephon concentration applied as foliar sprays on FDF (in kilograms, 5 d after application) and leaf abscission (percent, 15 d after application). Upper graphs: 'Hamlin' orange, branch application on 25 Nov. 2002 (n = 4). Lower graphs: 'Valencia orange', branch application on 7 Feb. 2003 (n = 4). The SE mean is indicated as bars through mean symbols. \*\*Significant at  $P \leq 0.01$ .



