Citrus Fruit Abscission Induced by Methyl-jasmonate

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ABSTRACT. Methyl jasmonate (MJ) was tested as a potential abscission chemical to enhance mechanical harvest of ‘Hamlin’ and ‘Valencia’ orange [Citrus sinensis (L.) Osb.]. In field experiments, a solution of 1, 5, 10, 20, or 100 mM MJ was applied either as a stem wrap to individual fruit or as a spray to entire trees or canopy sectors. Solutions of 10, 20, and 100 mM MJ resulted in significant and consistent reduction of fruit detachment force and caused fruit drop within 7 to 10 days. Fruit loosening was preceded by an increase in the internal ethylene concentration of fruit similar to that of other experimental abscission compounds. While concentrations of 10 mM and less caused no or negligible phytotoxicity, solutions exceeding 10 mM MJ induced unacceptable levels of leaf abscission.

Currently, the Florida citrus industry is introducing commercial mechanical harvesting of processing oranges (Citrus sinensis) (Brown, 1998). Without the aid of fruit loosening agents, mechanical harvesting systems fail to recover all of the fruit from the tree (Whitney and Harrell, 1989). An effective abscission material has to significantly loosen fruit consistently during the entire harvest season from October to June without phytotoxic effects on leaves and reproductive organs (Kender, 1998). Various abscission-inducing chemicals for citrus have been evaluated over the last decade for this purpose (Kender, 1998), and more recently by our research team (Hartmond et al., 2000a). However, to date all tested materials proved to be either ineffective, phytotoxic, or toxicologically unacceptable (Kender et al., 1999).

Jasmonates have been described as novel plant hormones involved in numerous physiological plant processes, specifically stress responses, senescence, and leaf abscission (Gross and Parthier, 1994). In previous experiments, Hartmond et al. (2000a) observed that sprays of 1 mM jasmonate (MJ) can also loosen ‘Valencia’ orange fruit, but that increased concentrations were necessary for consistent loosening. Therefore, the objective of this study was to determine the abscission activity of MJ applied to citrus fruit over a range of concentrations in order to evaluate the potential of MJ as an abscission material for ‘Hamlin’ and ‘Valencia’ orange, the two major cultivars grown for processing in Florida.

Materials and Methods

Field experiments were conducted during the 1998–99 harvest season in one ‘Hamlin’ and two ‘Valencia’ orange groves at the Citrus Research and Education Center in Lake Alfred, Fla. ‘Hamlin’ orange trees (Expts. 1, 3, and 4) on ‘Carrizo’ citrange [Citrus sinensis (L.) Osb. × Poncirus trifoliata (L.) Raf.] rootstock were 13 years old and averaged 2.4 m in height (spaced 4.5 × 6.0 m). ‘Valencia’ trees on ‘Carrizo’ citrange rootstock were either 7 years old (Expt. 2; 2.4 m high, spaced at 2.2 × 5 m) or 12 years old (Expts. 5 and 6; 4.2 m high, spaced at 4.5 × 6.3 m).

All experiments were conducted after the fruit achieved physiological maturity (sugar to acid ratio of >13). Before applications, the ground under each tree was cleared to monitor fruit and leaf drop during the course of the experiments. Detachment force (DFD) of mature fruit was measured using a digital force gauge (Force Five, Wagner Instruments, Greenwich, Conn.). Fruit were clipped with 3 cm of stem, inserted into the gauge, and the stem pulled parallel to the fruit axis until it separated at the abscission zone. In experiments where only a limited number of marked fruit were treated, the weight (in N) of abscised fruit was included in the calculation of mean FDF values. In all experiments, Kinetic (Setre Chem. Co., Memphis, Tenn.) was included in all solutions as an adjuvant at 0.125% to allow dispersion of MJ.

EXPERIMENT 1. A solution of 0 or 10 mM MJ was applied to individual, mature fruit and branches of six ‘Hamlin’ orange trees on 20 Jan. 1999. Ninety marked fruit (three groups of 30 fruit) were treated with each application method. Fruit were treated by either dipping the entire fruit into the solution, including the abscission zone, or by dipping only the lower 95% of the fruit into the solution, avoiding contact with the peduncle and abscission zone. In addition, entire branches with fruit were sprayed with a pump-up hand sprayer. Alternatively, 500 L of each of the solutions was applied directly to the abscission zone or 2 cm above the abscission zone of individual fruit by saturating an absorbent collar (twisted tissue paper) that was tied around the peduncle. Fruit drop was recorded daily for 14 d, after which FDF was measured on all remaining fruit.

Ethylene production of 10 fruit and seven leaves each from selected treatments was measured 1, 2, 3, 4, and 7 d after treatment. Ethylene production in the fruit was measured by submerging individual excised fruit in degassed water and evacuating and collecting the internal air (Burns et al., 1999). Individual leaves were cut from the branch, their petioles placed into 1.5-mL Eppendorf tubes containing water, and enclosed in 60 mL test tubes. After a 2 h incubation period at 28°C, 1 mL head-space air was sampled with a syringe. Ethylene concentrations were measured with a gas chromatograph (Hewlett-Packard, Avondale, Pa.) equipped with an alumina column and flame ionization detector (Sawamura et al., 1978).

EXPERIMENT 2. Using the previously described tissue paper wraps, a solution of 0, 1, 5, 10, 20, or 50 mM MJ was applied to the abscission zone of 20 mature ‘Valencia’ orange fruit per treatment on 21 May 1999. Fruit drop was recorded over 14 d, and the detachment force of fruit that remained attached to the tree was measured 14 d after application.

In Expts. 3 to 6, solutions were applied at a rate of 5 L/tree.
using an electric 50-L field sprayer (Chemical Containers, Lake Wales, Fla.) equipped with a hand-held spray boom with flat-fan nozzles (R&D Sprayers, Opelousas, La.). The boom length was adjusted to the tree height and used vertically permitting applications with minimal spray drift or overlap (Hartmond et al., 2000b).

**Experiment 3.** Each of three ‘Hamlin’ trees was divided into three equal canopy sections (southwest, east, and northwest) that were used as experimental units. Previous experiments had shown no indication of significant translocation of MJ within the canopy (Expt. 1 and unpublished data). MJ at 0, 10 or 100 mM was applied to each of three replicate sections on 9 Dec. 1998. FDF of fruit from the middle of each section was measured 6, 10, and 14 d after spray and fruit drop was recorded twice weekly over 21 d. Phytotoxicity was determined by visually rating the canopy defoliation on a scale from 0 (no phytotoxicity symptoms) to 10 (complete loss of leaves) after 21 d.

**Experiment 4.** An additional 20 ‘Hamlin’ trees were sprayed on 8 Jan. 1999 with MJ at 0, 1, 5, 10, or 20 mM. Each treatment was applied to four whole trees. FDF, fruit drop, and leaf loss were recorded from 3 to 21 d after application. Additionally, ethylene production in the fruit and leaves was sampled 8, 24, and 72 h after spray treatment and quantified as described previously.

**Experiment 5.** On 10 Mar. 1999, a solution of 0, 5, 10, or 20 mM MJ was sprayed on three replicate whole ‘Valencia’ orange trees. FDF, fruit drop, and leaf loss were evaluated twice weekly over 21 d. Since trees were blooming at application time, flower abscission was determined by counting the number of flowers on four tagged branches per tree over the experimental period.

**Experiment 6.** Expt. 5 was repeated during early fruit development. Canopy halves of six trees were sprayed with a 0, 5, 10, or 20 mM solution on 12 Apr. 1999, in three replicates. In addition to measuring mature fruit loosening and leaf loss, we counted fruitlet drop on two branches per canopy sector monitoring at least 50 fruitlets per replication.

All experiments were arranged in a randomized complete block design. All data were subjected to analysis of variance and regression analysis using SAS procedures (SAS Institute, Cary, N.C.).

**Results**

**Experiment 1.** Dipping the entire fruit, including the abscission zone, in a 10 mM MJ solution resulted in reduced FDF from 70 to 50 N within 4 d (Fig. 1A). Dipping fruit, excluding the abscission zone, or treating only the peduncle above the abscission zone with 10 mM MJ reduced FDF to a similar degree after 14 d. The greatest FDF reduction was achieved by treating the fruit abscission zone directly. Within 4 d, the abscission zone treatment caused fruit drop which increased to >70% by day 10 (Fig. 1B). Treatment of the peduncle caused a gradual increase in fruit drop. Dipping fruit or spraying branches caused very little fruit drop. Fruit internal ethylene production was high 24 h after dipping and remained high when the abscission zone was included in the dip (Fig. 2A). Applying MJ only to the peduncle above the abscission zone had no effect on ethylene production in the fruit. Leaf sprays resulted in high ethylene production within 24 h that decreased to control levels after 48 h (Fig. 2B).

**Experiment 2.** In ‘Valencia’ oranges, increasing the concentration of MJ above 5 mM strongly increased fruit abscission when applied directly to the abscission zone. At concentrations ≥10
mm, FDF was significantly reduced to <40 N by day 11 (Fig. 3A). Direct abscission zone treatments induced significant fruit drop after 3 d and reached 100% with 50 mM on day 11 (Fig. 3B). Fruit treated with 10 or 20 mM MJ responded less rapidly and showed 70% to 80% abscission by day 11. MJ at ≤5 mM was not effective in loosening the abscission zone of ‘Valencia’ oranges.

**Experiment 3.** Detachment forces of ‘Hamlin’ fruit were significantly reduced with 10 or 100 mM MJ within 6 d after application and remained lower than controls for the measurement period of 14 d (Fig. 4A). At 100 mM, FDF averaged below 20 N which was associated with significant fruit drop (Fig. 4B). Fruit drop at 10 mM was insignificant. Likewise, leaf loss was insignificant at 10 mM, but severe at 100 mM (Fig. 5A).

**Experiment 4.** FDF of ‘Hamlin’ oranges was significantly reduced by 10 or 20 mM MJ after 3 d and decreased from 50 N to ≈30 N 14 d after application with no significant change after day 11 (Fig. 6A). MJ at 1 or 5 mM had no effect on FDF. Likewise, fruit drop at 1 or 5 mM MJ was similar to the control, but was significantly higher with MJ at 10 or 20 mM (Fig. 6B). Leaf drop increased with the concentration of MJ, but was significant only...
Increased ethylene production in fruit was similar for 10 and 20 mM treatments. Initial peak levels were measured 24 h after spraying, which, after being lower at 48 h, increased further at 72 h (Fig. 7A). Ethylene production in leaves was significantly higher with 20 mM as compared to 10 mM treatments and peaked 8 h after application. After 24 h, leaf ethylene production decreased to near control levels (Fig. 7B).

**EXPERIMENT 5.** All MJ treatments reduced FDF below control levels 5 and 9 d after application (Fig 8A). While 5 and 10 mM concentrations loosened fruit to similar FDF, increasing the concentration to 20 mM significantly improved efficacy of MJ by day 5 and 9. Loosening was insufficient to cause any fruit drop (data not presented), but MJ caused some defoliation even at the 5 mM level (Fig. 5A). Leaf drop caused by 10 mM MJ increased until 21 d and reached levels similar to the 20 mM treatment, which caused rapid leaf drop within 5 d. Flower loss occurred naturally during the experimental period and was only increased by the 20 mM treatment (Fig. 8B).

**EXPERIMENT 6.** Within 7 d after treatment, the FDF of ‘Valencia’ oranges was reduced by 5, 10, and 20 mM sprays of MJ (Fig. 9A). However, differences from the control were not always observed. Likewise, mature fruit drop was minimal and only increased slightly with 20 mM MJ (data not presented). MJ at 20 mM resulted in significant defoliation within the first week after application, while 5 mM MJ had no effect (Fig. 5A). Leaf drop caused by 10 mM MJ slowly increased up to day 21, reaching a level similar to 20 mM. At this time in the ‘Valencia’ season, MJ appeared to have no effect on abscission of fruitlets that had set. Fruitlet drop was equal to the control during the 3-week period (Fig. 9B).

In all experiments, regression analysis of FDF data showed improved fruit loosening with an increase in the MJ concentration (Fig. 5B). In addition, leaf loss significantly increased with the MJ concentration of the spray solutions in all experiments (Fig. 5A).
Fig. 9. Effect of methyl jasmonate sprays at 0, 5, 10, or 20 mM on (A) mature FDF and (B) fruitlet loss of ‘Valencia’ orange (Expt. 6). Vertical bars represent SE [n = 30 (FDF) or 3 (drop)].

Discussion

**FRUIT LOOSENING.** Our preliminary experiments using 0.7 and 1 mM MJ sprays demonstrated abscission activity in ‘Valencia’ oranges, but fruit loosening was inconsistent at the low concentrations (Hartmond et al., 2000a). Higher MJ concentrations used in the present study improved fruit loosening significantly and consistently. Application of MJ directly to the abscission zone was particularly effective in reducing FDF and causing fruit drop. MJ applied to the abscission zone at 10 mM reduced FDF to <40 N, which is close to the estimated threshold of 25 to 50 N for complete fruit removal by mechanical harvesters (Wilson, 1973). While the technique of directly treating the abscission zone may be a useful research tool for screening potential abscission chemicals for uniform and rapid fruit loosening activity, it has no practical application.

Spray applications to the canopy were generally less effective than abscission zone treatments. Loosening and fruit drop from concentrations of 1 and 5 mM was minimal and may be too low to significantly affect harvester performance. At 10 and 20 mM, MJ consistently loosened fruit of both cultivars effectively by day 10. Concentrations of 50 or even 100 mM caused a very high portion of the fruit to completely abscise and drop. MJ was effective throughout the harvest season of ‘Hamlin’ orange. However, the relatively weak loosening effects observed in ‘Valencia’ orange in April (Expt. 6) even at 20 mM indicated failure of MJ to effectively loosen fruit during the ‘less responsive period’ associated with early development of ‘Valencia’ fruitlets (Hartmond et al., 2000b; Wheaton et al., 1977). If MJ is not effective throughout the entire harvesting season of ‘Valencia’ orange, its usefulness as an abscission chemical would be severely limited.

**ETHYLENE PRODUCTION.** Ethylene is known to be involved in mature citrus fruit abscission (Burns et al., 1999; Kossuth and Biggs, 1977; Sexton, 1995). In our study, ethylene production in fruit and leaves was measured within 8 h and remained high for up to 3 d (Fig. 2). Increasing MJ concentrations caused increased ethylene production in fruit and leaves (Fig. 7). The unusual second peak measured at 72 h in Expt. 4 was possibly the result of chilling injury due to the combination of MJ with cold night temperatures (5 to 7 °C) during that period (Cooper et al., 1969). Previously, MJ application was shown to cause increased ethylene production in tomato (Lycopersicum esculentum Mill.) and apple fruit (Malus sylvestris (L.) Mill. var. domestica (Borkh.) Mansf.) (Saniewski et al., 1987a, 1987b) and in leaves of cucumber (Cucumis sativus) (Abeles et al., 1989). However, studies in other crops found no increase (Beltran et al., 1998; Ueda et al., 1996) or even a decrease in ethylene production (Sanz et al., 1993). These studies concluded that the abscission processes initiated by MJ were due to a direct effect of MJ on polysaccharide metabolism in the abscission zone (Ueda et al., 1996) and the mechanical weakening of cell walls (Miyamoto et al., 1997), and were independent of ethylene production within the tissue (Abeles et al., 1989). Based on our results, using relatively high concentrations of MJ (≥10 mM versus <0.1 mM), we cannot exclude the effect of ethylene on leaf abscission. However, fruit ethylene production was 10-fold greater following applications of another abscission material, CMN-pyrazole (unpublished data), which loosened fruit very effectively but caused no leaf abscission (Biggs and Kossuth, 1980; Hartmond et al., 2000b); this implies that other mechanisms independent of ethylene effects are likely to contribute to the observed abscission response of citrus to MJ.

Controlled applications only to the fruit, the peduncle, or the abscission zone showed the need for MJ to be deposited near the target tissue for effective loosening. Treating the abscission zone directly was most effective in dropping fruit. Dipping the fruit resulted in strong ethylene production that persisted longer if the abscission zone was included in the treatment. When MJ was applied only to the peduncle above the abscission zone, little increase in fruit ethylene was detected, yet MJ triggered an abscission response (Fig. 1). This may either indicate limited transport of ethylene from the peduncle into the fruit or confirm that MJ does not exert its effect through ethylene production. Alternatively, it may directly affect auxin transport to the abscission zone and alter cell wall metabolism (Sexton, 1995).

**PHYTOTOXICITY.** Leaf abscission was observed in all experiments where the entire canopy or branches were sprayed with MJ. Treatments of up to 10 mM MJ caused acceptable levels of leaf drop. However, at 20, 50, or 100 mM concentrations, sprays were highly phytotoxic and abscised >50% of all leaves. Natural flower abscission and initial fruitlet drop were unaffected by 5 and 10 mM sprays that were applied to ‘Valencia’ orange during bloom and fruit set. Only the 20 mM concentration significantly increased phytotoxicity in floral tissue.

The extensive tests in ‘Hamlin’ and ‘Valencia’ oranges reported herein confirm that MJ induces citrus fruit abscission. Abscission zone treatments with ≥25 mM MJ and sprays of ≥10 mM effectively reduced FDF and caused fruit drop. As with previously tested abscission chemicals (Burns et al., 1999; Holm and Wilson, 1977; Sexton, 1995), fruit ethylene production was associated with the abscission activity of MJ. While concentrations ≥20 mM MJ gave the greatest and most consistent effects on fruit loosening, they also caused severe or excessive defoliation. While 10 mM MJ did not cause significant phytotoxicity, fruit loosening should be improved for practical applications in me-
Mechanical harvesting systems. Further experiments will explore the use of different adjuvants and combinations of MJ with other abscission compounds to enhance its efficacy and to assure adequate improvement of harvesting efficiency particularly during the ‘Valencia’ harvest.

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