Application of the Abscission Agent 5-Chloro-3-methyl-4-nitro-1*H*-pyrazole Does Not Affect Peel Integrity or Postharvest Decay of Mechanically Harvested Late-season Fruit of 'Valencia' Orange during the Normal Commercial Harvest-to-processing Period

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Abstract. In Florida, the combined use of mechanical harvesters and the abscission agent 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP) for late-season harvesting (May to June) of fruit of 'Valencia' orange is effective at removing mature fruit with minimal adverse effects on the subsequent season's crop. However, CMNP can cause fruit peel scarring, and no data were available on how this affects peel integrity and potential losses resulting from fruit crushing and/or decay before processing. In this study, two late-season harvest dates were tested in commercial orchards during 2009 and 2010. Harvesting treatments consisted of combinations of two mechanical harvester ground speeds (0.8 and 1.6 km·h⁻¹), two harvester shaker head frequencies (185 and 220 cycles/min), and CMNP foliar applications (4 days before harvesting) at 250 and 300 mg·L⁻¹ in a spray volume of 2810 L·ha⁻¹ plus mechanically-harvested and hand-picked controls. After harvesting, fruit samples were randomly collected from each block for peel resistance and postharvest decay evaluations. Peel resistance was determined by measuring both peel puncture force and fruit crush force. Fruit used to study postharvest decay were stored at 27 °C and 50% relative humidity or ambient conditions and evaluated daily for 8 days. Peel resistance was unaffected by mechanical harvesting combinations or CMNP application. No significant effects on postharvest decay were found among treatments for at least 3 days after harvest. However, a significant increase in postharvest decay between CMNP-treated and untreated fruit began between 4 and 6 days after harvest such that by 8 days after harvest, decay was as high as 25% in CMNP-treated fruit. The results indicate that CMNP can be safely used in combination with late-season mechanical harvesting under the conditions described in this study without losses resulting from fruit crushing or decay for at least 3 days, a time period well within the normal commercial harvest-to-processing time of ≈36 h.

Florida produced 6.5 million t of sweet oranges [*Citrus ×sinensis* (L.) Osbeck] during the 2008–2009 harvest season from ≈200,000 ha (Florida Agricultural Statistics Service, 2010). More than 95% of this fruit is used to produce orange juice, making Florida the second largest orange juice producer in the world. Harvesting is the single largest cost associated with citrus production in Florida, accounting for up to 50% of the total cost of production (Muraro, 2009). This is in contrast with Brazil, the largest producer of orange juice in the world, where harvesting costs are much lower such that during the 2000–2001 harvest season, Brazilian citrus growers were able to deliver frozen concentrated orange juice to the port of Tampa, FL, for only $0.0757 more per pound soluble solids than Florida growers. This discrepancy in production costs has been a major driving force in Florida's efforts to develop mechanical harvesting technology (Florida Department of Citrus, 2010; Whitney, 1995).

Adoption of mechanical harvesting systems has been slow. During the 2008–2009 harvest season, less than 15,000 ha (7% of total acreage) of citrus was mechanically harvested in Florida (Florida Department of Citrus, 2009). A number of issues have contributed to the slow adoption of mechanical harvesting technology, including processor concerns about the quantity of debris mixed with mechanically harvested fruit (Spann and Danyluk, 2010), production manager concerns about the effects of mechanical harvesting on tree health (Li et al., 2005), and issues related to the late-season harvesting of 'Valencia' oranges (Melgar et al., 2010).

'Valencia' orange trees typically bloom in late February through March in Florida and the mature crop is harvested beginning in March and extending into mid-June. Thus, there is an overlap between the young growing fruit and mature fruit. Studies have shown a significant reduction in the subsequent year's yield when 'Valencia' orange trees are mechanically harvested after ≈1 May (Hedden et al., 1984; Roka et al., 2005; Whitney, 1975). This is approximately the point when the young developing fruit have obtained a large enough size (≈2.5 cm diameter) and mass that they will be removed by the canopy shaker mechanism along with the mature fruit. Because of the ever-growing demand for not-from-concentrate (NFC) orange juice, processors require that growers hold fruit on the trees later into the season to provide fresh fruit as long as possible. Thus, efforts have been underway to find a viable way to harvest 'Valencia' orange fruit after 1 May without impacting subsequent year yields.

The abscission agent CMNP effectively loosens mature citrus fruit without affecting immature fruit, allowing mechanical harvest of 'Valencia' orange fruit after 1 May without impacting subsequent year yields (Burns et al., 2006). However, CMNP can sometimes cause peel injury consisting of a brown ring of depressed flavedo tissue around the stylar end of fruit where the CMNP spray solution accumulates (Alferez et al., 2006). This injury, commonly referred to as peel scarring, is associated with phospholipase A2 (PLA2) activity and can be prevented by inhibiting PLA2 with aristolochic acid (Alferez et al., 2006). No further characterization of this scarring has been conducted and processors are concerned that it may reduce peel integrity and/or increase fruit decay before processing.

In Florida, harvested oranges for processing are transported to the processing plant in open-topped semitrailers that hold 27 t of fruit. Fruit are stored until processing in these trailers under ambient conditions. Generally, fruit are transported from the orchard to the processing plant the day of harvest and are processed within 24 h. However, processors are concerned that fruit treated with CMNP, because of the scarring that can occur, may have weaker peels and be more susceptible to damage during transport and storage or may begin to decay more quickly than untreated fruit.

Our objective was to determine if fruit treated with CMNP and subjected to mechanical harvesting have reduced peel integrity, measured as peel puncture force and fruit crush force, or are more susceptible to post-harvest decay within the commercial time-frame of normal harvest to processing.

Materials and Methods

Plant material and culture. The trials were conducted in a 21-year-old commercial citrus orchard of 'Valencia' sweet orange trees on 'Carrizo' citrange rootstock (*C. sinensis × Poncirus trifoliata*) near Immokalee, FL, managed according to standard practices (Obreza and Morgan, 2008; Parsons and Morgan, 2010). The trees were spaced $3.0 × 6.5$ m (500 trees/ha) with an average canopy height of 4.2 m and width of 3.8 m maintained by annual mechanical hedging. The lower limbs of the canopy had been mechanically skirted to ≈1 m above the soil. Fruit was harvested at two dates during the late-season time period in two consecutive seasons, 8 and 26 May 2009 and 4 and 29 May 2010.

5-Chloro-3-methyl-4-nitro-1H-pyrazole treatments and harvesting. Four d before each harvest date, the trees were sprayed with the abscission agent CMNP at 250 mg a.i./L (2009) or 300 mg a.i./L (2010) in a spray volume of 2810 L·ha⁻¹ using a multifan vertical airblast sprayer (Model 4000L; Oxbo International, Clear Lake, WI) as described by Ebel et al. (2009). Unsprayed trees served as controls.

Trees were harvested 4 d after CMNP application using a tractor-powered continuous travel canopy shake harvester (Model 3210; Oxbo International). In 2009, the harvester was operated at a ground speed of 0.8 or 1.6 km·h⁻¹ in combination with two shaker head frequencies (185 and 210 cycles/min) for a total of four ground speed × shaker head frequency combinations. In 2010, the harvester was operated at 1.6 km·h⁻¹ with a shaker head frequency of 220 cycles/min. In 2009 only, both CMNP-treated and untreated trees were harvested by hand as additional controls. Four eight-tree plots of each treatment combination (ground speed × shaker head frequency × CMNP treatment for 2009; CMNP treated and untreated for 2010) were harvested each year at both harvest times.

Fruit puncture and crush force. Sixty fruit were randomly sampled from each plot at the time of harvest and transported to the laboratory. All data collection (fruit crush force, peel puncture force, and peel deflection) was performed the day after harvest. Two 10-fruit subsamples from each plot were used for fruit puncture and crush force measurements, respectively. Fruit puncture force was measured at one point per fruit along the fruit equator (stem axis oriented perpendicular to the punch) using a digital texture analyzer (Model TA-XTZi; Texture Technologies Corp., Scarsdale, NY) equipped with a 0.632-cm diameter punch and operated at a speed of 5 mm·s⁻¹. Puncture force (Newtons) and maximum fruit peel deflection (millimeters) before puncture were recorded.

The digital texture analyzer used for fruit puncture force did not have sufficient capacity to crush whole citrus fruit. Thus, to measure fruit crush force, a device was built to consistently squeeze each fruit against a flat surface at a uniform speed while continuously recording the force applied until each fruit ruptured. The device consisted of a box ≈0.5 × 0.3 × 0.6 m (width × depth × height) constructed from plywood (20 mm thick). Inside the box was a plywood platform that held a digital scale (Model SVI 200F; Acculab USA, Edgewood, NY) that could be raised and lowered using a pneumatically powered hydraulic jack (Model 94487; Central Hydraulics, Camarillo, CA). The platform and scale moved up and down on a set of heavy-duty ball bearing drawer glides. The jack was operated using compressed air at 0.6 MPa that allowed the platform and scale to travel at 5 mm·s⁻¹. The scale was connected to a field computer (Model Allegro CX; Juniper Systems, Inc., Logan, UT) through an RS232 cable and data were recorded continuously. Each fruit was placed on the scale with the stem axis parallel to the direction of the force, the scale was tared, and pressure was applied to the jack so that the stem end of each fruit was pressed against the top of the box. As each fruit made contact with the top of the box, the force applied (Newtons per fruit) was recorded. When each fruit ruptured, there was an immediate drop in the force readings. The highest reading before the drop was recorded as the fruit crush force. Crush force data are reported as Newtons per fruit.

The stem and stylar ends of citrus fruit represent natural weak areas on the fruit surface. Because our objective was to determine peel integrity changes, our tests were designed to minimize the effect of these natural weak points on our results. Thus, fruit puncture force was determined along the equator of each fruit, a point approximately equidistant between the two natural weak points. Similarly, orienting the fruit with the stem axis parallel to the direction of force application supported these natural weak points. Thus, the peel usually ruptured along the equator of the fruit giving us a truer representation of peel strength than if the fruit had been oriented perpendicular to the force, which would have given us a reading indicative of the stem or stylar end scar strength.

Decay studies. Fruit were not washed or treated in any way before storage to best simulate actual conditions experienced between harvesting and processing. In 2009, the 40 remaining fruit per sample were placed into perforated plastic bins (60 × 40 × 30 cm, length × width × depth) and stored in the dark at 27 °C and 50% relative humidity (RH) for 8 d. In 2010, to better simulate the conditions fruit are exposed to between harvesting and processing, the samples were stored outside with no covering under ambient temperature and RH in the plastic bins as described for the 2009 study. Average temperature ± SD and RH ± SD for the 8-d storage period were

26.1 ± 3.7 °C and 74.5% ± 15.1% RH in early May and 26.2 ± 4.0 °C and 74.4% ± 16.9% RH in late May as recorded by the Florida Automated Weather Network Lake Alfred weather station located ≈250 m from the storage location (Florida Automated Weather Network, 2010). The fruit were checked daily and the number of fruit showing decay symptoms was recorded. Decaying fruit were not removed after each count.

Data analysis. Analysis of variance for a randomized block design was performed for the 2009 fruit crush force, puncture force, deflection, and decay data using Prism 5.0 (GraphPad Software, La Jolla, CA) followed by means separation by Tukey's honestly significant difference ($P = 0.05$). Fruit crush force, puncture force, deflection, and decay data were analyzed by *t* test in 2010. Percent decay data were arcsine transformed and analysis was performed on the transformed data.

Results

In 2009, harvester ground speed and shaker head frequency did not affect the variables measured so data were pooled across these factors. Thus, data presented are for mechanical harvesting with and without CMNP application and hand harvest with and without CMNP application.

Harvest method and CMNP application had no effect on fruit crush force at either harvest date in 2009 (Fig. 1, top) or 2010 (Fig. 2, top). Fruit crush forces ranged from 376.6 to 418.7 N and 348.1 to 380.5 N at the early and late harvests of 2009, respectively. In 2010, fruit crush force values were similar, ranging from 374.6 to 403.0 N and 359.9 to 362.8 N at the early and late harvests, respectively.

Peel puncture force was similarly unaffected by harvest method or CMNP application at either harvest time in 2009 (Fig. 1, middle) or 2010 (Fig. 2, middle). Peel puncture force ranged from 28.4 to 32.4 N and 27.5 to 30.4 N for the early and late harvests of 2009, respectively, and 24.5 to 27.5 N and 30.4 N for the early and late harvests of 2010, respectively.

Peel deflection was also unaffected by harvest method or CMNP application in 2009 and 2010 (Figs. 1 and 2, bottom). Peel deflection ranged from 8.3 to 9.4 mm and 7.8 to 8.2 mm at the early and late harvests of 2009, respectively. In 2010, peel deflection ranged from 6.3 to 7.0 mm and 7.9 to 8.0 mm at the early and late harvests, respectively.

From 1 to 3 d after harvest in 2009, fruit decay was low (less than 2.5%) and there was no difference among any of the treatments at either harvest date (Fig. 3). However, by 6 d after harvest for the early May harvest, decay was significantly higher for CMNP-treated fruit than non-CMNP-treated fruit. At 7 and 8 d after harvest, hand-harvested CMNP-treated fruit separated from mechanically harvested CMNP-treated fruit and had significantly greater decay. Fruit decay for the late May 2009 harvest followed a similar trend
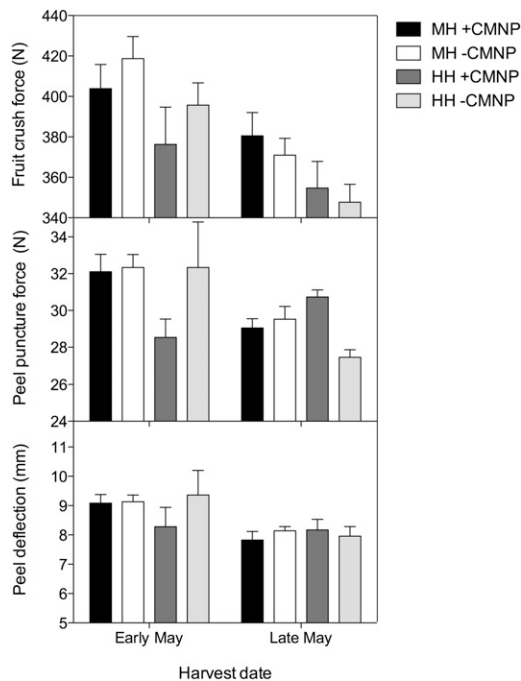
Fig. 1. Fruit crush force, puncture force, and peel deflection for mechanically harvested (MH) and hand-harvested (HH) 'Valencia' sweet oranges treated with and without the abscission agent 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP) in early and late May 2009. CMNP was applied 4 d before harvest at 250 mg·L^{-1} in a spray volume of 2810 L·ha^{-1}. Data represent means ± SD, n = 40–160.
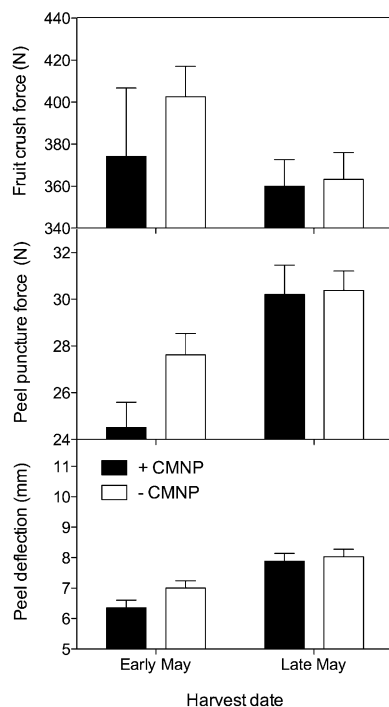

Fig. 2. Fruit crush force, puncture force, and peel deflection for mechanically harvested 'Valencia' sweet oranges treated with and without the abscission agent 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP) in early and late May 2010. CMNP was applied 4 d before harvest at 300 mg·L^{-1} in a spray volume of 2810 L·ha^{-1}. Data represent means ± SD, n = 40.
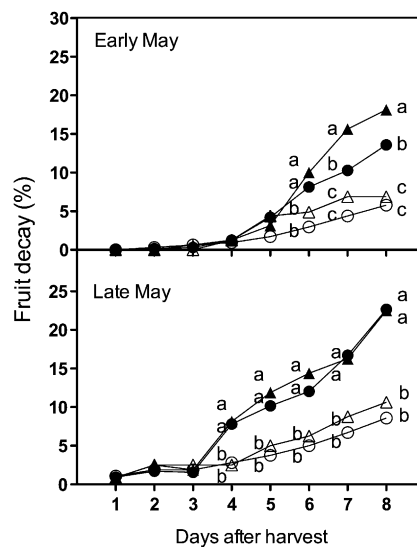

Fig. 3. Fruit decay for mechanically harvested (circles) and hand-harvested (triangles) 'Valencia' sweet oranges treated with (solid symbols) and without (open symbols) the abscission agent 5-chloro-3-methyl-4-nitro-1*H*-pyrazole (CMNP) in early (top) and late (bottom) May 2009. CMNP was applied 4 d before harvest at 250 mg·L^{-1} in a spray volume of 2810 L·ha^{-1}. Fruit were stored at 27 °C and 50% relative humidity after each harvest. Lowercase letters indicate significant differences between treatment means; days after harvest without letters are not significantly different (Tukey's honestly significant difference, $P = 0.05$). Data represent means ± SD, n = 4–16.

to the early May harvest with CMNP-treated fruit having significantly more decay beginning 4 d after harvest compared with non-CMNP-treated fruit. There were no statistical differences in decay among hand and mechanically harvested fruit in the late May harvest.

In 2010, there was slightly more decay than in 2009, increasing to 3% and 5% by 3 d

after harvest for the early and late harvest times, respectively (Fig. 4). However, similar to the 2009 data, no significant differences between CMNP-treated and untreated fruit arose until at least 5 d after harvest.

Discussion

In the present study, we determined that the force required to crush mature 'Valencia' orange fruit ranged from ≈350 to 400 N. In a study to determine the maximum allowable forces for robotic harvesting of citrus, Flood et al. (2006) reported a burst force (crush force) of 289 and 459 N for 'Valencia' orange fruit harvested on 15 May and 16 June, respectively. However, in their study, the fruit were stored at 4 °C for 1 week after harvest and before testing. Furthermore, they oriented the fruit so that the stem axis of the fruit was perpendicular to the force applied, whereas in our tests, the stem axis was parallel to the direction of the force. In a multiyear study to determine citrus fruit strength characteristics to determine the potential for damage from mechanical harvesting, Churchill et al. (1980) found that the 3-year average burst force for 'Valencia' orange fruit was 419 N, similar to our results. In addition, Churchill et al. (1980) treated some 'Valencia' orange fruit with the abscission agent Release® (Abbott Laboratories, Abbott Park, IL), an early commercial formulation of CMNP no longer available. They stated that the non-sprayed fruit required "slightly higher average force" to burst but do not give any numerical values or statistical analyses. We found no significant differences in fruit crush force between CMNP-treated and non-treated fruit at either harvest date in both years of the study.

Our results showed that 'Valencia' orange fruit had a puncture force of ≈30 N regardless of harvest date, CMNP treatment, or harvest method. This value is substantially lower than that reported by Churchill et al. (1980) who report a range from 50 to 60 N for 'Valencia' orange fruit harvested from 10 to 20 May and Flood et al. (2006) who report an average value of 43.9 N for 'Valencia' orange fruit harvested on 15 May. However, these previous studies used a punch speed of 3.3 mm·s^{-1}, whereas we used a speed of 5 mm·s^{-1}, which may account for the lower puncture force in our study. Churchill et al. (1980) do not report any difference in puncture force between abscission agent-treated and untreated fruit, similar to our results.

Although the feed rate of the punch differed by 50% between our study and the previous studies by Churchill et al. (1980) and Flood et al. (2006), all of the studies report similar peel deflection before puncture, ranging from 8 to 10 mm.

Postharvest storage of fruits and vegetables is generally done at low temperature and high humidity to reduce respiration and maintain hydration (Kader, 1992). The U.S. Department of Agriculture-recommended storage conditions for citrus fruit are temperatures of 0 to 1 °C and 85% to 90% RH (Ritenour, 2004). However, processing oranges are stored
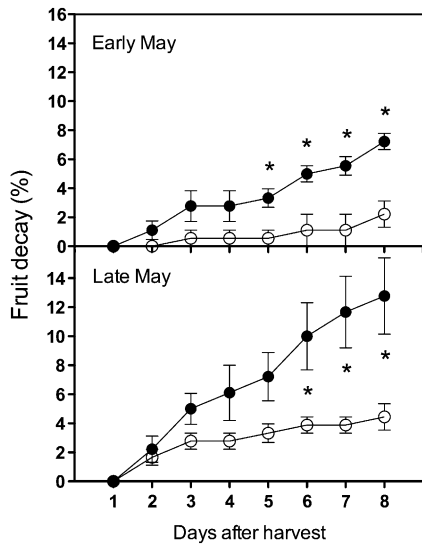
Fig. 4. Fruit decay for mechanically harvested 'Valencia' sweet oranges treated with (solid symbols) and without (open symbols) the abscission agent 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMNP) in early (top) and late (bottom) May 2010. CMNP was applied 4 d before harvest at 300 mg·L^{-1} in a spray volume of 2810 L·ha^{-1}. Fruit were stored at ambient temperature and relative humidity (RH) after each harvest (26.1 ± 3.7 °C, 74.5% ± 15.1% RH for early May; 26.2 ± 4.0 °C, 74.4% ± 16.9% RH for late May). Asterisks indicate significant differences between treatment means (t test, $P = 0.05$). Data represent means ± SD, n = 4.

at ambient temperature and RH from the time of harvest until processing. The 3-year average temperature and RH in May (late-season 'Valencia' harvest season) for Bradenton, Lake Wales, and Clewiston, FL, the locations of three major citrus processing plants, are 25 °C and 75%, 24.5 °C and 73%, and 25.5 °C and 74%, respectively, but maximum temperatures of 35 °C are not uncommon (Florida Automated Weather Network, 2010). In 2009, our storage conditions were of slightly higher temperature (27 °C) but lower RH (50%) than these averages. Thus, in 2010, we decided to use ambient conditions, which fell well within the range of the 3-year average at the processing locations mentioned. These somewhat different conditions between 2009 and 2010 did not affect the overall conclusions of the experiment, but the higher RH in 2010 may have contributed to the slightly higher, and

possibly more realistic, decay from Day 1 to 3 in that year compared with 2009.

Under our experimental conditions, there was no difference in decay among any of the treatments for at least 3 d after harvest; however, beginning between 4 and 6 d after harvest, CMNP-treated fruit decayed more quickly than non-treated fruit. We did not determine the specific reason that CMNP-treated fruit decayed more quickly beyond 3 d after harvest. First, the peel scarring described by Alferez et al. (2006) may have sufficiently disrupted cellular integrity of the flavedo to allow pathogen entry. Second, in CMNP-treated fruit, the calyx, commonly referred to as the "button," abscises cleanly from the fruit, whereas in non-treated mechanically and hand-harvested fruit, the calyx usually remains attached to the fruit. This difference may result in a point of entry for postharvest decay pathogens in CMNP-treated fruit. Generally, oranges destined for processing are processed within 24 to 36 h of harvest; thus, the greater decay on CMNP-treated fruit beyond 3 d after harvest is of little importance to the citrus processing industry. However, if CMNP or other abscission agents are ever developed for fresh fruit harvesting, this will be an important factor to consider.

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