

# Physiological Factors Affecting Response of Mature 'Valencia' Orange Fruit to CMN-Pyrazole. I. Effects of Young Fruit, Shoot, and Root Growth

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**ABSTRACT.** Influence of young fruit, shoot, and root growth on response of mature 'Valencia' oranges [*Citrus sinensis* (L.) Osbeck] to the abscission chemical CMN-pyrazole was examined in 1999 and 2000. CMN-pyrazole dramatically increased ethylene production in mature fruit and reduced the fruit detachment force (FDF), except during a period of reduced response to CMN-pyrazole in early May when spring vegetative growth, young fruit of the following year's crop, and mature fruit were all on the trees. Removal of spring flushes, which included spring vegetative shoots and leafy and leafless inflorescences, prevented any young fruit and shoot growth, but did not inhibit root growth. However, trunk girdling in combination with removal of spring flushes not only prevented growth of young fruit and shoots but also inhibited root growth. During the responsive period, there were no differences in either ethylene production or FDF of CMN-pyrazole-treated mature oranges between 1) the nonmanipulated trees and those manipulated by either 2) removal of spring flushes alone, or 3) in combination with trunk girdling. However, during the less responsive period, ethylene production in CMN-pyrazole-treated mature oranges was significantly lower while the FDF was higher in nonmanipulated trees than in trees treated by either removal of spring flushes alone, or in combination with trunk girdling. There was no difference in either fruit ethylene production or FDF between trees manipulated by (2) removal of spring flushes alone, and (3) removal of spring flushes in combination with trunk girdling regardless of CMN-pyrazole application. Shoot growth terminated at least 2 weeks before the onset of the less responsive period. Removal of young fruit increased response of mature fruit to CMN-pyrazole during the less responsive period. This suggests that hormones from rapidly growing young fruit may be responsible for the occurrence of the less responsive period. Chemical name used: 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMN-pyrazole).

Orange (*Citrus sinensis*) fruit have a very high detachment force at harvest (Brown, 1998). Successful mechanical harvesting of orange fruit is enhanced by use of abscission chemicals that reduce fruit detachment force (FDF). Early and midseason oranges usually mature in November or December, and appear to respond readily to the effective abscission chemicals. However, for the widely grown, late season, processing cultivar Valencia, the harvest season begins in early April ( $\approx 1$  year after full bloom) and extends through June. Spring vegetative growth, young developing fruit of the following year's crop, and mature fruit are all on the trees during most of the 'Valencia' harvest season. The response of mature fruit to abscission chemicals seems to be inconsistent (Cooper and Wilson, 1971; Hartmond et al., 2000a; Holm and Wilson, 1977; Wheaton et al., 1977). Mature 'Valencia' fruit respond poorly to abscission chemicals starting from late April or early May, and lasting for about 2 to 6 weeks (Hartmond et al., 2000a; Holm and Wilson, 1976; Wheaton et al., 1977). This condition is referred to as the less responsive period (Hartmond et al., 2000a). Normal response of mature 'Valencia' to abscission chemicals resumes following this period. The factors responsible for the less responsive period are unclear.

Hormones have been implicated in immature or mature fruit abscission. Young leaves and fruit are rich sources of auxin, gibberellins (GAs), and cytokinins (CTKs) (Davies, 1995). Based on these facts, it has been speculated that reduced responsiveness

of mature fruit to abscission chemicals is associated with the regreening of the fruit peel that may occur in May and June (Jahn and Young, 1976; Wheaton et al., 1977), and is caused by endogenous hormones such as GAs moving from young leaves of the spring flush (Holm and Wilson, 1976; Rasmussen, 1973) or auxin moving from young developing fruitlets to mature fruit (Wheaton et al., 1977). Roots also synthesize endogenous hormones such as CTKs and abscisic acid (Davies, 1995), and root growth of 'Valencia' orange is very active in spring (Bevington and Castle, 1985; Elezaby, 1989; Menocal-Barberena, 1999). Previous reports showed that root growth was inhibited by trunk girdling performed before bloom in litchi (*Litchi chinensis* Sonn.) trees (Yuan and Huang, 1993).

The abscission chemical CMN-pyrazole is the only known chemical that has the ability to loosen mature 'Valencia' orange fruit without causing injury to bloom, young fruit, or foliage (Holm and Wilson, 1977; Kender, 1998; Wilson, 1973). Therefore, the goal of our investigation was to study the influence of young fruit, shoot, and root growth on response of mature 'Valencia' orange fruit to the abscission chemical CMN-pyrazole.

## Materials and Methods

All field experiments were conducted with 10-year-old (1999) or 11-year-old (2000) 'Valencia' orange trees grafted on rough lemon (*Citrus jambhiri* Lush) rootstock located at the Citrus Research and Education Center, Lake Alfred, Fla. in the 1999 and 2000 harvest seasons. Full bloom occurred approximately in late March in Lake Alfred, Fla. All experiments were arranged in a randomized complete block design.

**TIME COURSE FOR THE RESPONSE OF MATURE FRUIT TO CMN-PYRAZOLE (EXPT. 1).** Two groups of 180 uniform mature fruit were

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selected and marked on each of four trees. Each tree was a block. One group of marked fruit in each tree was sprayed on 29 Mar. 1999 with a solution of CMN-pyrazole at 150 mg·L<sup>-1</sup> containing 0.125% of the adjuvant Kinetic (Setre Chem. Co., Memphis, Tenn.) using a pressurized hand sprayer. The other group of fruit in each tree was sprayed only with 0.125% Kinetic and served as a control. Ethylene production of five fruit from each group on each of four trees was determined 0, 1, 2, 3, 4, and 7 d after application of CMN-pyrazole as described in Hartmond et al. (2000b). Briefly, internal air was evacuated by vacuum from fruit submerged in degassed water and collected. Ethylene concentration was measured with a gas chromatograph (Hewlett-Packard, Avondale, Pa.) equipped with an alumina column and flame ionization detector. FDF was measured at the same time using a digital force gauge (Force Five, Wagner Instruments, Greenwich, Conn.) (Hartmond et al., 2000a).

**SEASONAL VARIATION IN THE RESPONSE OF MATURE FRUIT TO CMN-PYRAZOLE IN 1999 (EXPT. 2).** Starting on 9 Mar. 1999 and ending on 2 June 1999, two groups of 20 uniform mature fruit were selected and marked on each of four trees on each date at 7 to 10 d intervals. Each tree was a block. One group of marked fruit in each tree was

sprayed with a solution of CMN-pyrazole at 150 mg·L<sup>-1</sup> containing 0.125% of the adjuvant Kinetic using a pressurized hand sprayer. The other group of fruit in each tree was sprayed only with 0.125% Kinetic and served as a control. A total of 11 spray treatments were applied during the harvest season. FDF was measured 7 d after each spray application as described previously. Ethylene production of five fruit from each group on each of four trees was determined 2 d after application of CMN-pyrazole as described in Expt. 1.

Fruit maturity was measured at 1 to 2 week intervals from 13 Jan. to 16 June 1999 using standard juice analysis procedures for soluble solids and titratable acids (Wardowski et al., 1991).

**EFFECTS OF REMOVAL OF FLUSHES AND TRUNK GIRDLING ON THE RESPONSE OF MATURE FRUIT TO CMN-PYRAZOLE (EXPT. 3).** Twelve uniform trees were selected and grouped into four blocks of three trees each. One tree in each block was manipulated by either 1) removing flushes alone, or 2) in combination with trunk girdling. The third tree in each block was not manipulated and served as a control. The flushes that were removed included spring vegetative shoots, and leafy and leafless inflorescences. All new shoots, leafy and leafless inflorescences were removed from the beginning of spring growth until mid-June 1999. Therefore, no young fruit and new shoots were found on the trees whose flushes were removed. Trunks were girdled on 16 Feb. 1999 by removing a 7-mm-wide strip of bark including phloem and cambial tissue. The girdle was monitored periodically for cambial growth and was scraped to prevent phloem reconnection until mid-June. Each block was a replication.

A 30 × 60 × 60 cm root observation chamber was constructed and buried beneath the canopy at the dripline ≈120 cm from the trunk with a root observation window of 26 × 53 cm width facing the trunk of each of nine trees. The gap in front of the root observation window was filled with sieved soil to obtain good contact and allow regrowth of roots. The main frame of the chambers was constructed from 2 × 3.5 cm timbers, the observation window of the chambers was made from 3-mm-thick clear polycarbonate sheets, and the other sides of the chambers were made of 1-mm-thick transparent polyvinyl chloride plastic sheets. The chambers were covered with an insulated board to exclude light and limit temperature changes. Root growth was traced on the observation window with a marker and measured weekly. Twenty young fruit on each tree were tagged and measured weekly for diameter. Four branches of ≈5 to 6 mm in diameter from each tree were tagged and their shoot growth was measured weekly.

Two groups of 20 uniform mature fruit were selected and marked on each of the 12 manipulated and nonmanipulated trees on 9 Mar., 4 May, 11 May, 18 May, and 25 May 1999, respectively. One group from each tree was sprayed with a solution of CMN-pyrazole at 0 or 150 mg·L<sup>-1</sup> containing 0.125% of Kinetic using a pressurized hand sprayer. FDF was measured 7 d after spraying, and ethylene production of 5 fruit from each group on each of four trees was determined 2 d after application of CMN-pyrazole as described previously.

**SEASONAL VARIATION IN THE RESPONSE OF MATURE FRUIT TO CMN-PYRAZOLE IN 2000 (EXPT. 4).** Seasonal variation in the response of mature 'Valencia' orange fruit to CMN-pyrazole was determined again in 2000. Starting on 29 Mar. 2000 and ending on 2 June 2000, two groups of 20 uniform mature fruit were selected and marked on each of four trees on each date at 7

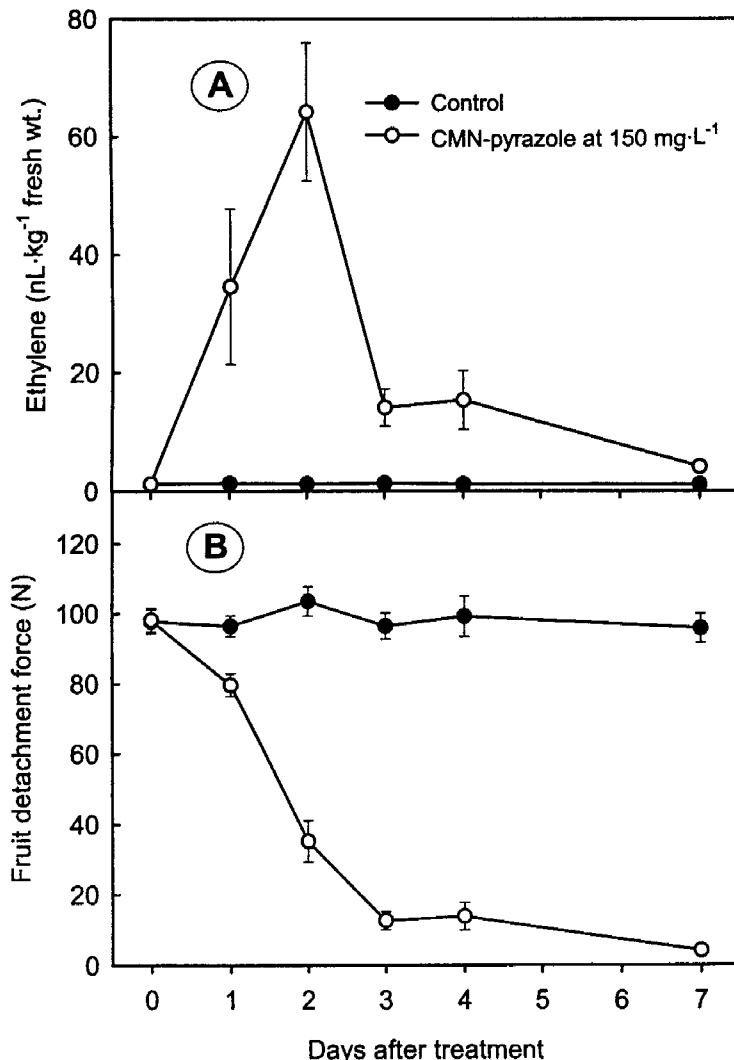


Fig. 1. Effects of CMN-pyrazole at 150 mg·L<sup>-1</sup> on (A) fruit ethylene production and (B) fruit detachment force of 'Valencia' oranges. Treatments were applied 29 Mar. 1999. Data are means ± SE (n = 80).

to 10 d intervals. Each tree was a block. One group of marked fruit in each tree was sprayed with a solution of CMN-pyrazole at 100 mg·L<sup>-1</sup> containing 0.125% Kinetic using a pressurized hand sprayer. The other group of fruit in each tree was sprayed only with 0.125% Kinetic and served as a control. A total of eight spray treatments were applied during the harvest season. FDF was measured 7 d after treatment as described in Expt. 1. In addition, 10 young fruit on each of four trees were tagged and measured weekly for diameter.

**EFFECTS OF YOUNG FRUIT ON EFFICACY OF CMN-PYRAZOLE (EXPT. 5).** Six mature trees were selected and grouped into three blocks. Flowers and young fruit on one tree from each block were completely removed on 30 Mar. 2000. The new vegetative shoots or leaves were not removed. Another tree from each block was not manipulated and served as a control. Two groups of 20 uniform mature fruit from each tree were selected and marked. One group of 20 mature fruit in each tree received a spray of CMN-pyrazole on 5 May 2000 at 0 or 100 mg·L<sup>-1</sup> containing 0.125% of the adjuvant Kinetic. FDF was measured 7 d after treatment. Additional samples of 10 mature fruit from each tree were harvested, washed, dried with paper towels, and used to determine fruit color on 29 Mar., 3 May, 23 May, and 8 June 2000, respectively. Peel color (chroma and hue angle) at the top of mature fruit around the stem and abscission zone was measured at three positions along the shoulder of each fruit using a chroma meter (CR200; Minolta Co., Asaka, Japan) measuring in CIE 1976 (L\*, a\*, b\*) (McGuire, 1992) [CIE = Commission Internationale de l'Eclairage (International Commission on Illumination)]. Chroma (C\*), calculated as  $(a^{*2} + b^{*2})^{1/2}$ , is an index of color saturation or intensity (degree of departure from gray toward pure chromatic color). Hue angle (h°) (0° = red-purple, 90° = yellow, 180° = bluish green, 270° = blue) is calculated from the arctangent of a\*/b\*.

**STATISTICAL ANALYSES.** Statistical analyses included analysis of variance, Duncan's multiple range test, orthogonal polynomial comparison, and regression. When interactions of main effects were significant, sums of squares were partitioned into sums of squares of one main effect nested within each level of the other main effect involved in the interaction. Statistical Analysis Systems Software for PC (SAS Institute Inc., Cary, N.C.) was used for all data analyses.

## Results

**TIME COURSE FOR THE RESPONSE OF MATURE FRUIT TO CMN-PYRAZOLE (EXPT. 1).** Applications of CMN-pyrazole at 150 mg·L<sup>-1</sup> to 'Valencia' orange fruit dramatically increased fruit ethylene production (Fig. 1A). Ethylene production peaked 2 d after application of CMN-pyrazole. Applications of CMN-pyrazole at 150 mg·L<sup>-1</sup> also effectively reduced FDF which decreased from ≈100 to 13 N, 3 d following application (Fig. 1B).

**SEASONAL VARIATION IN THE RESPONSE OF MATURE FRUIT TO CMN-PYRAZOLE IN 1999 (EXPT. 2).** Mature 'Valencia' orange fruit responded variably to CMN-pyrazole over the 3-month observation period (Fig. 2A and B). Compared with the nontreated

control, FDF of treated fruit was extremely low and fruit ethylene production was extremely high before 27 Apr. CMN-pyrazole then became less effective in reducing FDF of mature fruit and increasing fruit ethylene production. After 25 May, FDF of treated fruit decreased and fruit ethylene production increased again. Soluble solids concentration to acid ratio of nontreated fruit increased from

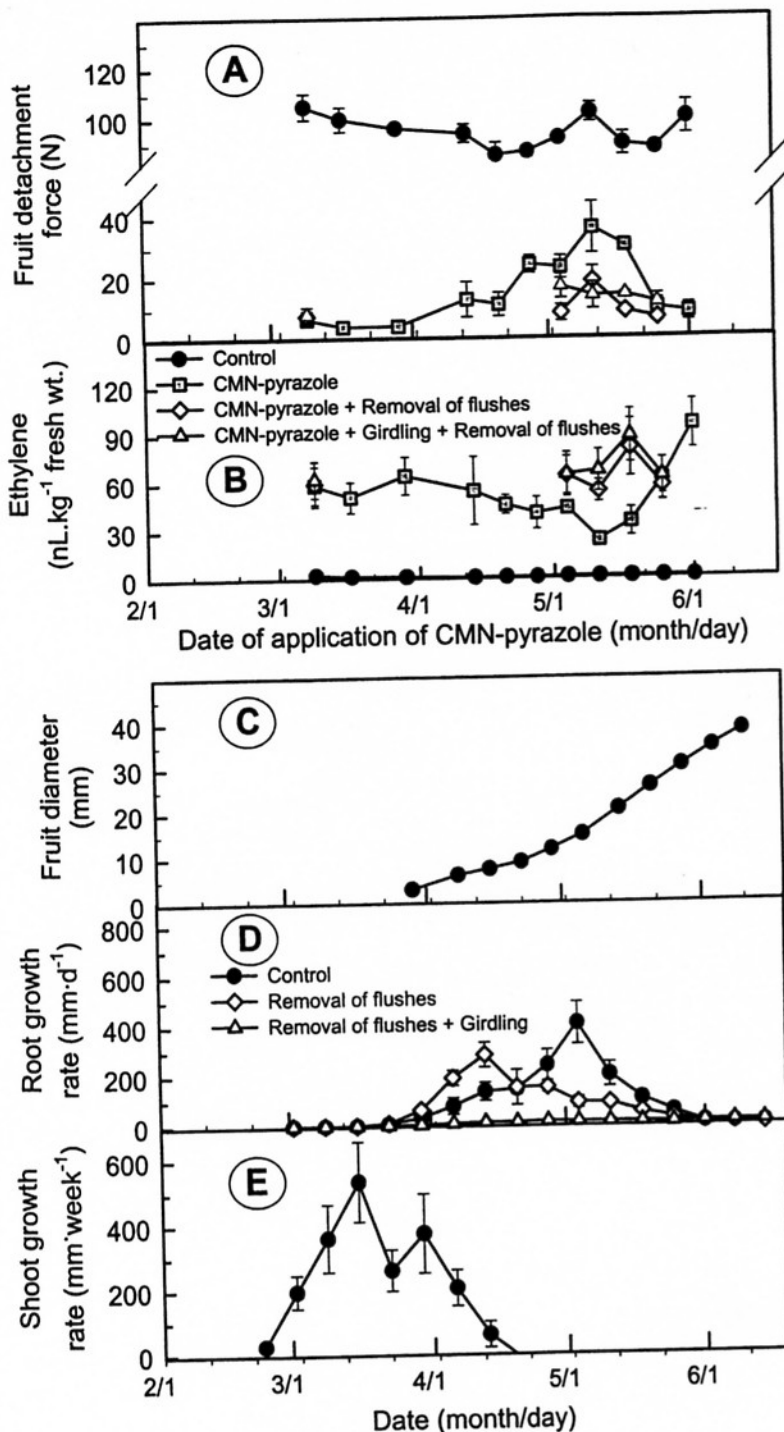


Fig. 2. Effects of CMN-pyrazole at 150 mg·L<sup>-1</sup>, removal of flushes, and trunk girdling on (A) FDF and (B) ethylene production of mature fruit, and on growth of (C) young fruit, (D) roots, and (E) shoots in 'Valencia' oranges. FDF and ethylene production were measured 7 and 2 d after application of CMN-pyrazole, respectively. Trunk girdling and removal of flushes were applied on 16 Feb. 1999. Data are means ± SE (n = 80 in A, 20 in B, and 3 in C, D, and E, respectively).

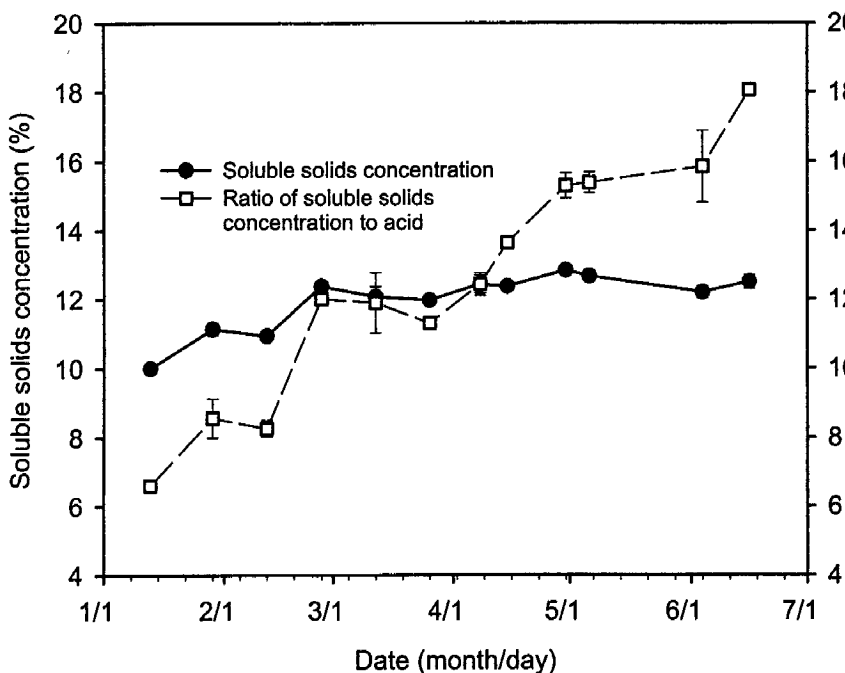


Fig. 3. Seasonal fluctuation in soluble solids concentration, and ratio of soluble solids concentration to % acid of nontreated mature 'Valencia' oranges in Lake Alfred, Fla., 1999. Data are means  $\pm$  SE ( $n = 3$ ).

$\approx 6.6$  in mid-January to 18 in mid-June (Fig. 3).

**EFFECTS OF REMOVAL OF FLUSHES AND TRUNK GIRDLING ON THE RESPONSE OF MATURE FRUIT TO CMN-PYRAZOLE (EXPT. 3).** Nonmanipulated control trees bloomed in late March. The young fruit were  $\approx 10$  mm in diameter at the beginning of the less responsive period (27 Apr.). Due to removal of all flowers, no young fruit were found on the trees manipulated by either removing flushes alone or in combination with trunk girdling (Fig. 2C). Root growth of nonmanipulated control trees began immediately after full bloom in late March, peaked in early May, and terminated in late May (Fig. 2D). Root growth on trees with only the flush removed started at the same time as the nonmanipulated control trees, but reached its maximum earlier. Trunk girdling in combination with removal of flushes inhibited root growth. There were two waves of spring shoot growth on nonmanipulated control trees over the 4-month observation period (Fig. 2E). Shoot growth on nonmanipulated control trees stopped in mid April, whereas no shoot growth was recorded on the trees manipulated by either removing flushes alone or in combination with trunk girdling.

Overall, application of CMN-pyrazole at  $150 \text{ mg}\cdot\text{L}^{-1}$  reduced FDF of mature fruit and increased fruit ethylene production (Fig. 2A and B). Without application of CMN-pyrazole, there was no significant difference in FDF and fruit ethylene production between manipulated and nonmanipulated trees (data not presented). With application of CMN-pyrazole, the mature fruit on nonmanipulated trees had higher FDF and lower ethylene production than those on trees manipulated by flush removal alone or in combination with trunk girdling on 4, 11, and 18 May (during the less responsive period), but not on 9 Mar. (before the less responsive period), and 25 May (after the less responsive period).

Fig. 4. Seasonal variation in (A) FDF of mature fruit in response to CMN-pyrazole at  $100 \text{ mg}\cdot\text{L}^{-1}$ , and (B) young fruit growth in 'Valencia' oranges in 2000. FDF was measured 7 d after treatment. Data are means  $\pm$  SE ( $n = 80$  in A, and 40 in B, respectively). Full bloom was late Mar. 1999 for mature fruit, and late Mar. 2000 for young fruit.

young fruit removed. Trees with flowers and young fruit removed

