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Naphthalene Acetic Acid and 2,3,5-triiodobenzoic Acid Affect the Response of Mature Orange Fruit to Abscission Chemicals

Rongcai Yuan, Ulrich Hartmond, and Walter J. Kender[1]

University of Florida, Institute for Food and Agricultural Sciences, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred FL 33850-2299

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Abstract. **Effects of NAA, TIBA, ethephon, and CMN-Pyrazole on fruit detachment force (FDF) of mature 'Valencia' and 'Hamlin' orange [*Citrus sinensis* (L.) Osb.] fruit were examined in 2000 and 2001. NAA effectively inhibited the reduction in FDF or fruit abscission caused by ethephon when applied to the abscission zone 24 hours before ethephon application, but had no significant effect when applied to the fruit without contacting the abscission zone, or to the peduncle ≈4 cm above the abscission zone. TIBA, an auxin transport inhibitor, decreased FDF of mature fruit and promoted fruit abscission when applied alone as a spray to the canopy or directly to the fruit peduncle. This response was dependent on TIBA concentration. TIBA was more effective when applied in combination with ethephon or CMN-Pyrazole than alone. These results are consistent with our previous data that endogenous auxin concentration in the abscission zone of mature 'Valencia' orange fruit is one of the factors controlling the sensitivity and thus the responsiveness of the abscission zone of mature fruit to abscission chemicals. Chemical names used: 5-chloro-3-methyl-4-nitro-pyrazole (CMN-Pyrazole); 2-chloroethylphosphonic acid (ethephon); naphthalene acetic acid (NAA); 2,3,5-triiodobenzoic acid (TIBA).**

Due to increasing labor costs, development of an effective abscission chemical to facilitate mechanical harvesting of citrus used for processing is a high research priority in the Florida citrus industry. It is especially challenging to find a suitable abscission chemical for the late-season orange cultivar 'Valencia' because new flushes and young fruit of the next year's crop, which are very sensitive to abscission chemicals, are rapidly growing during the harvest season. In addition, during the harvest season there is a period of less responsiveness, starting from late April or early May and lasting for ≈2 to 6 weeks, during which time mature fruit respond poorly to abscission chemicals (Hartmond et al., 2000; Wheaton et al., 1977; Yuan et al., 2001a). The factors responsible for the "less responsive period" are unclear.

It has been suggested that the balance between plant growth promoters and inhibitors controls fruit abscission (Addicott, 1982; Brown, 1997; Goren, 1993). Auxin (IAA) plays a very important role in the control of leaf and fruit abscission (Goren, 1993; Osborne, 1989). Application of auxin to bean leaf explants prior to exposure to ethylene inhibited leaf abscission (Tucker et al., 1988). Spring flushes, young fruit of the following year's crop, and roots growing rapidly during most of the 'Valencia' harvest season (Bevington and Castle, 1985; Wheaton et al., 1977), are rich sources of endogenous plant hormones such as IAA, cytokinins (CTKs), and gibberellins (GAs) (Goldschmidt, 1976; Hofman, 1990; Plummer et al., 1991). Our previous results showed that the abscission zone of mature 'Valencia' orange fruit had about twice the IAA concentration during the "less responsive period" than during the "responsive period" (Yuan et al., 2001b). On the contrary, ethylene induced by wounding or abscission chemicals, such as CMN-Pyrazole, which stimulates wound ethylene production by injuring the peel, and ethephon, an ethylene-releasing compound, promotes fruit or leaf abscission by increasing the activity of two hydrolytic enzymes, cellulase and polygalacturonase (PG), in the fruit abscission zone (Kossuth and Biggs, 1977; Wheaton et al., 1977). These two hydrolytic enzymes were found to be responsible for the degradation of cell walls and subsequent fruit abscission (Goren, 1993; Rasmussen, 1973).

The purpose of this investigation was to study the hormonal mechanism controlling the response of the mature 'Valencia' and 'Hamlin' orange fruit to abscission chemicals. Two abscission chemicals, ethephon and CMN-Pyrazole, were studied in relation to NAA, an artificial auxin, and TIBA, an inhibitor of auxin transport, during both the "responsive" and the "less responsive periods."

Materials and Methods

Six experiments were conducted with 11-year-old 'Valencia' orange trees on rough lemon (*Citrus jambhiri* Lush) rootstock (Expts. 1, 2, 3, 4, and 5) or 11-year-old 'Hamlin' orange trees on 'Carrizo' citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] rootstock (Expt. 6) located at the Citrus Research and Education Center, Lake Alfred, Fla.

Expt. 1. CMN-Pyrazole and TIBA treatments. Six fruiting branches of 20 uniform mature fruit each were selected and marked on each of three 'Valencia' orange trees on 19 Apr. 2000. A randomized complete-block design with three replications of 20 mature fruit each was used with one replication on each tree. CMN-Pyrazole at 0 or 100 mg·L^{-1} and TIBA at 0, 1, or 5 mM were applied in combination to one fruiting branch of 20 marked fruit in each tree. TIBA solutions containing 0.1% of the adjuvant Tween 20 were applied by saturating an absorbent twisted Kimwipe tissue paper collar tied around the peduncle, ≈2 cm above the abscission zone, every 3–4 d from 20 Apr. to 3 May 2000. CMN-Pyrazole containing 0.1% of Tween 20 was applied on 5 May 2000 by dipping the entire fruit, including the abscission zone, into the treatment solution. Subsequently, the number of dropped fruit was counted, and the fruit detachment force (FDF) of the remaining fruit was measured using a digital force gauge (Force Five, Wagner Instruments, Greenwich, Conn.) 7 d after the application of CMN-Pyrazole. The weight (in newtons) of abscised fruit was included in the calculation of mean FDF values.

Expt. 2. Application of ethephon and NAA to the fruit abscission zone. Sixty-four small fruiting branches of 10 mature fruit each were selected on 17 'Valencia' orange trees, marked, and separated into four blocks of 16 fruiting branches each on 23 May 2000. A randomized complete-block design with four replications of 10 fruit each was used. Combinations of NAA at 0, 2, 20, or 200 mg·L^{-1} and ethephon at 0, 10, 100, or 500 mg·L^{-1} were applied to one fruiting branch of 10 mature fruit in each block. The absorbent twisted tissue paper collar tied around the abscission zone of mature fruit received a solution of NAA containing 0.1% of Tween 20 on 24 May 2000, followed by a solution of ethephon containing 0.1% of Tween 20 on 25 May 2000. Fruit drop and FDF were determined 8 d after the application of ethephon as described above.

Expt. 3. Canopy sprays of TIBA and ethephon. Thirty canopy sections with 20 marked, uniform mature 'Valencia' orange fruit each were selected and grouped into three blocks of 10 sections each. A randomized complete-block design with three replications of 20 fruit each was used. A section in each block was sprayed with a combination of ethephon at 0 or

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[1]To whom reprint requests should be addressed. Phone: (863) 956-1151; fax: (863) 956-4631; e-mail: kender@lal.ufl.edu

100 mg·L⁻¹, and TIBA at 0, 1, 5, 10, or 20 mM on 23 May 2000. TIBA solutions containing 0.1% Kinetic (Setre Chemical Co., Memphis, Tenn.) as an adjuvant were applied ≈3 h before the application of the ethephon solutions including 0.1% Kinetic. Fruit drop and FDF were determined 10 d after treatment according to the method described previously. Leaf abscission was determined 10 d after applications by visually rating the canopy defoliation on a scale from 0 (no leaf abscission) to 10 (complete loss of leaves).

Expt. 4. NAA, ethephon, and TIBA treatments. Thirty-two small fruiting branches of 10 uniform mature fruit each were selected from five 'Valencia' orange trees, and grouped into four blocks of eight small fruiting branches each. A randomized complete-block design with four replications of 10 fruit each was used. A small fruiting branch of 10 uniform mature fruit in each block was treated with a combination of ethephon at 0 or 350 mg·L⁻¹, NAA at 0 or 200 mg·L⁻¹, and TIBA at 0 or 5 mM (total of eight treatment concentrations). All solutions contained 0.1% of Tween 20 adjuvant, and were applied by saturating an absorbent collar as described in Expt. 1. TIBA was applied to the peduncle ≈2 cm above the abscission zone on 8 June 2000. Twenty-four hours later, NAA was applied to the peduncle ≈4 cm above the abscission zone or 2 cm above the absorbent collar saturated with TIBA. Ethephon solution was applied to the abscission zone on 10 June 2000. Fruit abscission and FDF were determined 9 d after the application of ethephon as described in Expt. 1.

Expt. 5. NAA fruit dip, and ethephon abscission zone applications. Four fruiting branches of 10 uniform mature fruit each were selected on each of four 'Valencia' orange trees. A randomized complete-block design with four replications of 10 mature fruit each was used. A small fruiting branch of 10 mature fruit in each block was treated with a combination of NAA at 0 or 200 mg·L⁻¹, and ethephon at 0 or 350 mg·L⁻¹. The lower 95% of the fruit was dipped into a NAA solution containing 0.1% Tween 20, avoiding any contact with the abscission zone and peduncle, on 15 June 2000. Twenty-four hours later, ethephon solutions containing 0.1% Tween 20 were applied to the abscission zone using an absorbent collar as described in Expt. 1. Fruit abscission and FDF were determined 7 d after the application of ethephon.

Expt. 6. Application of ethephon and NAA to the fruit abscission zone. Twenty groups of 10 mature fruit each were selected on 20 'Hamlin' orange trees, marked, and separated into four blocks of five groups each on 29 Jan. 2001. A randomized complete-block design with four replications of 10 fruit each was used. One group of 10 mature fruit each from each block received one of five treatments. Treatments consisted of 1) untreated control; 2) NAA at 200 mg·L⁻¹; 3) ethephon at 500 mg·L⁻¹; 4) NAA at 200 mg·L⁻¹ 24 h prior to ethephon at 500 mg·L⁻¹; 5) NAA at 200 mg·L⁻¹ 24 h after ethephon at 500 mg·L⁻¹. A solution of ethephon or NAA containing 0.1% of Tween 20 was applied to the absorbent twisted tissue

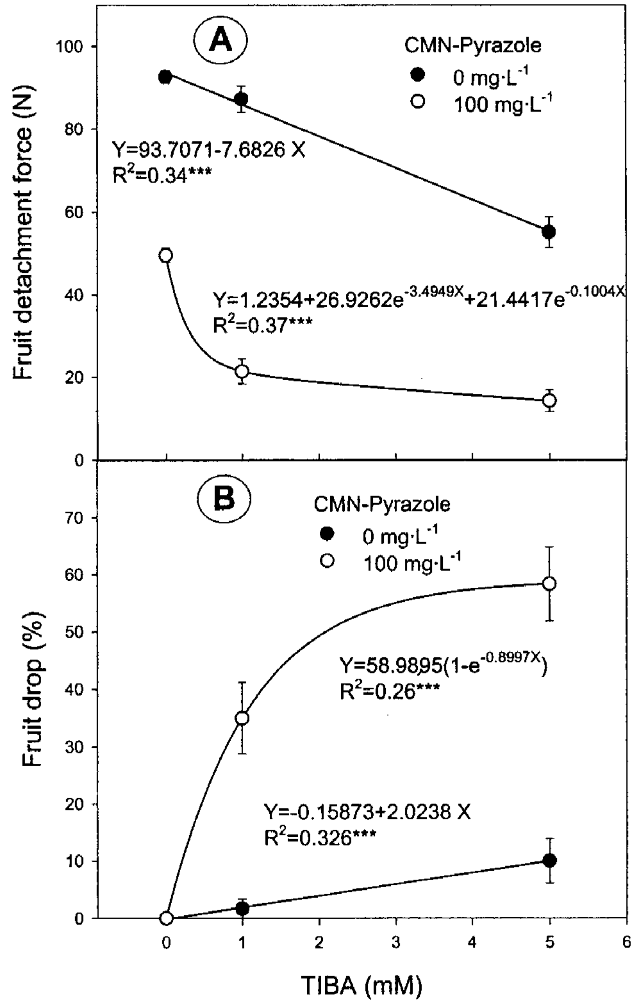

Fig. 1. Effects of TIBA and CMN-Pyrazole on (**A**) fruit detachment force and (**B**) fruit abscission of mature 'Valencia' oranges in 2000. Data are means ± SE (n = 60).

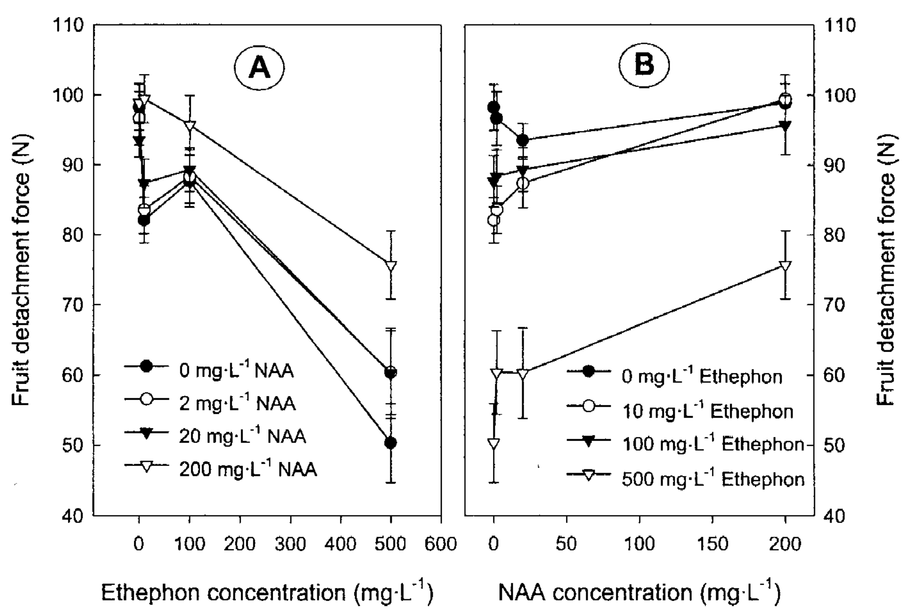

Fig. 2. Effect of NAA and ethephon on the fruit detachment force of mature 'Valencia' oranges in 2000. Data are means ± SE (n = 40).

paper collar tied around the abscission zone of mature fruit. Ethephon was applied on 30 Jan. 2001. Fruit drop and FDF were determined 9 d after the application of ethephon as described above.

Statistical analyses. Statistical analyses included analysis of variance, Duncan's multiple range test, and regression analysis. Statistical Analysis System for PC (SAS Institute, Cary, N.C.) was used to analyze these data.

Results

Expt. 1. Effect of CMN-Pyrazole and TIBA on FDF and fruit abscission. FDF was decreased and fruit abscission was increased with increasing concentrations of TIBA regardless of CMN-Pyrazole (Fig. 1 A and B). CMN-Pyrazole at 100 mg·L^{-1} effectively reduced FDF irrespective of TIBA; however, it promoted fruit drop only when applied in combination with TIBA.

Expt. 2. Influence of application of ethephon and NAA to the fruit abscission zone on FDF and fruit abscission. Overall, FDF decreased in response to increasing ethephon concentrations regardless of NAA treatment (Fig. 2A), but increased with increasing concentrations of NAA (Fig. 2B). Only ethephon at 500 mg·L^{-1} induced fruit drop (Fig. 3A). Increasing concentrations of NAA decreased ethephon-induced fruit drop (Fig. 3B).

Expt. 3. Effect of sprays of TIBA and ethephon on FDF, fruit abscission, and leaf abscission. FDF was decreased, and fruit and leaf abscission increased with increasing concentrations of TIBA irrespective of ethephon application (Fig. 4 A, B, and C). Ethephon at 100 mg·L^{-1} reduced the FDF, and promoted leaf abscission regardless of TIBA. Fruit drop was increased by ethephon only when the concentration of TIBA was 10 mM.

Expt. 4. Effect of NAA, ethephon, and TIBA on FDF and fruit abscission. Ethephon at 350 mg·L^{-1} applied to the abscission zone of mature 'Valencia' orange fruit, and TIBA at 5 mM applied to the peduncle effectively reduced FDF, and promoted fruit drop (Table 1). NAA at 200 mg·L^{-1} applied to the peduncle had no significant effect on FDF or fruit drop. There was no significant interaction between chemicals.

Expt. 5. Effect of NAA fruit dip, and ethephon abscission zone applications on FDF and fruit abscission. Ethephon at 350 mg·L^{-1} when applied directly to the abscission zone effectively reduced FDF, and caused fruit abscission regardless of NAA application (Fig. 5 A and B). Dipping only the lower 95% of the fruit without contacting the abscission zone had no influence on either FDF or fruit abscission.

Expt. 6. Application of ethephon and NAA to the fruit abscission zone. Ethephon application decreased FDF and promoted fruit abscission regardless of NAA application (Fig. 6 A and B). NAA alone had no influence on FDF or fruit drop. NAA applied 24 h prior to, but not 24 h after ethephon application, significantly inhibited the ethephon loosening effect.

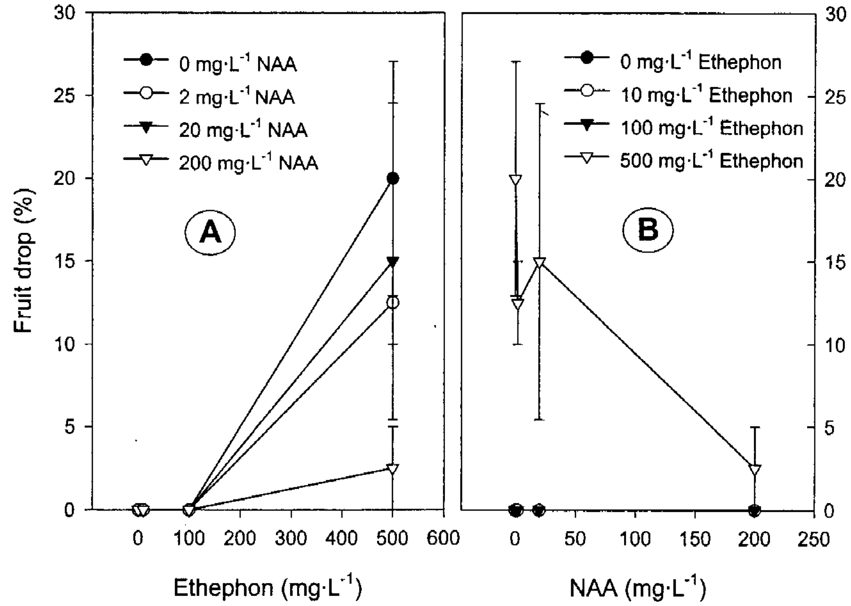

Fig. 3. Ethephon and NAA applied to abscission zone on fruit drop of mature 'Valencia' oranges in 2000. Data are means ± SE (n = 40).

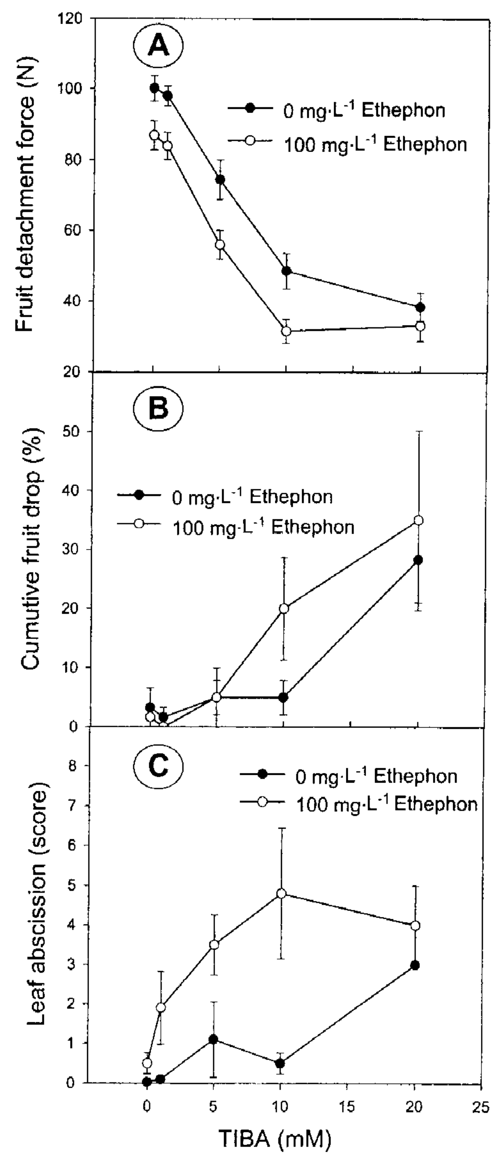

Fig. 4. Effects of TIBA and ethephon on (**A**) fruit detachment force, (**B**) fruit abscission, and (**C**) leaf abscission of 'Valencia' oranges in 2000. Data are means ± SE (n = 60 for **A** and **B**, 3 for **C**, respectively).

Table 1. Effects of NAA, TIBA, and ethephon on FDF and fruit abscission of mature 'Valencia' orange fruit.[z]

Ethephon (mg·L⁻¹)[y]	NAA (mg·L⁻¹)[y]	TIBA (mM)[y]	FDF (N)[x]	Fruit abscission (%)[x]
0	0	0	94.17	0.0
0	0	5	84.57	5.0
0	200	0	94.91	0.0
0	200	5	88.33	2.5
350	0	0	70.50	7.5
350	0	5	60.18	15.0
350	200	0	79.57	0.0
350	200	5	65.65	17.5
Significance				
Ethephon			***	*
NAA			NS	NS
TIBA			**	*
Ethephon × NAA			NS	NS
Ethephon × TIBA			NS	NS
NAA × TIBA			NS	NS
Ethephon × NAA × TIBA			NS	NS

[z]Mean of 40 observations; FDF = fruit detachment force.
[y]Ethephon, NAA, and TIBA were applied on 10, 9, 8 of June 2000, respectively.
[x]FDF and fruit abscission were determined 9 d after the application of ethephon.
NS, *, **, ***Nonsignificant or significant at $P = 0.05, 0.01, 0.001$, respectively.

Discussion

Okuda and Hirabayashi (1998) reported that TIBA was an effective inhibitor of auxin transport in citrus. Reduction of the levels of endogenous auxin in the abscission zone leads to the increased sensitivity of the abscission zone to ethylene (Huberman et al., 1997; Osborne, 1989). Our previous results (Yuan et al., 2001b) demonstrated that the balance between endogenous IAA and abscisic acid (ABA) in the abscission zone of mature fruit might be a crucial factor controlling the sensitivity and thus the response of mature 'Valencia' orange fruit to abscission chemicals. In the present study, TIBA alone (spray or direct application to the peduncle) effectively decreased FDF and promoted fruit abscission during the harvest season (Figs. 1 and 4; Table 1). The responsiveness to TIBA was dependent on the concentration, and combinations of TIBA with ethephon or CMN-pyrazole loosened fruit more effectively than each chemical applied alone. These results can be explained in terms of hormone balance. The

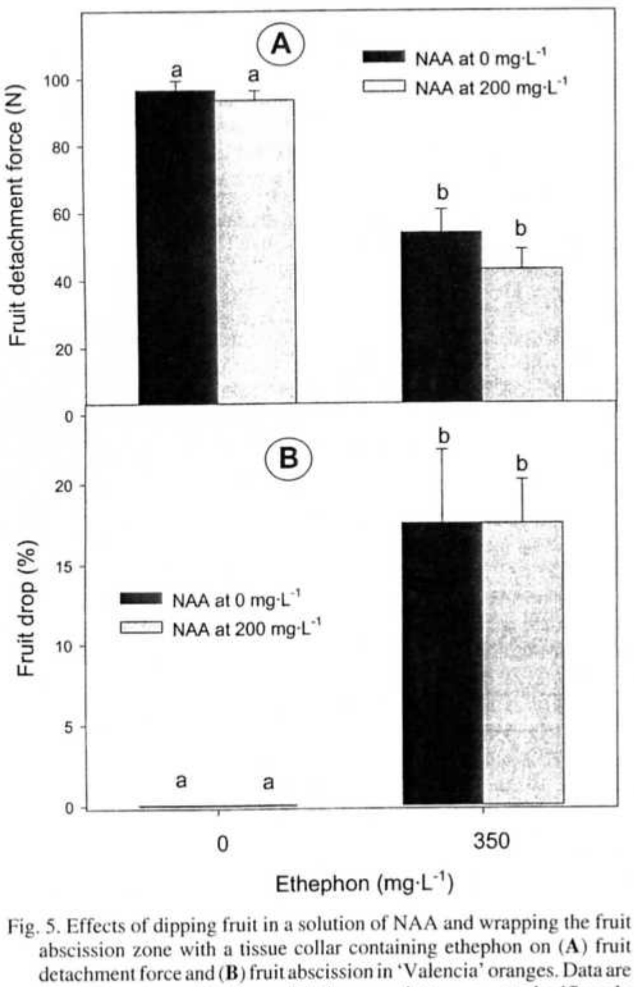

Fig. 5. Effects of dipping fruit in a solution of NAA and wrapping the fruit abscission zone with a tissue collar containing ethephon on (**A**) fruit detachment force and (**B**) fruit abscission in 'Valencia' oranges. Data are means ± SE (n = 40). Bars with the same letter are not significantly different from one another at 5% level by Duncan's multiple range test.

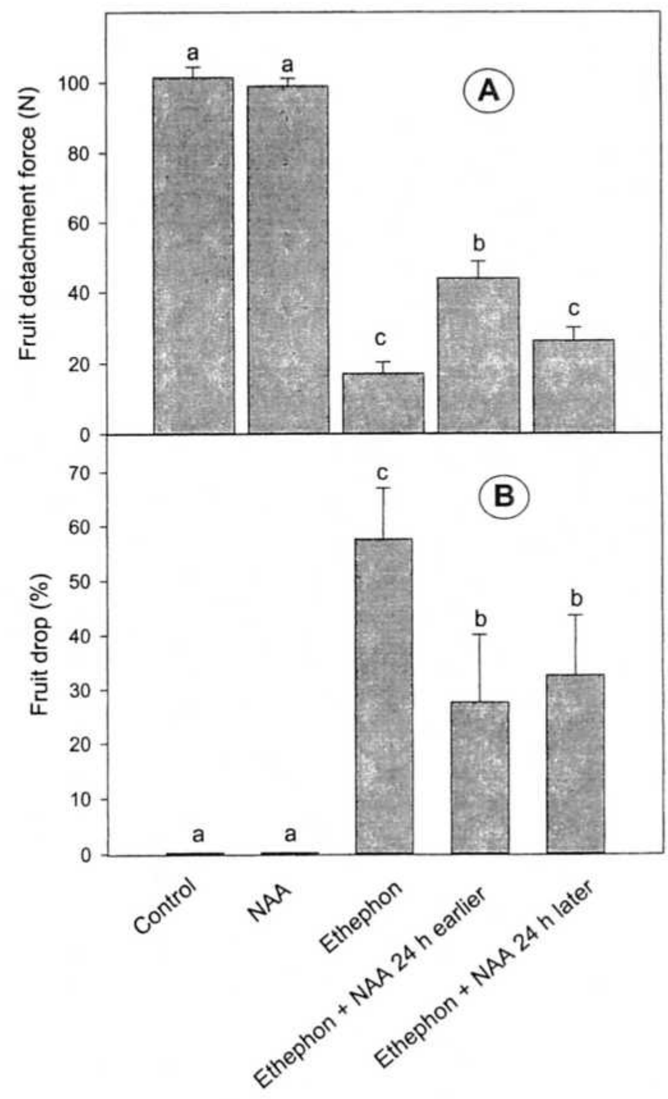

Fig. 6. Ethephon and NAA applied to the fruit abscission zone on (**A**) fruit detachment force and (**B**) fruit drop of mature 'Hamlin' oranges in 2001. Data are means ± SE (n = 40). Bars with the same letter are not significantly different from one another at 5% level by Duncan's multiple range test.

response of mature 'Valencia' orange fruit to abscission chemicals was positively related to the ratio of IAA to ABA in the fruit abscission zone (Yuan et al., 2001b). Application of TIBA inhibited IAA movement from the young fruit or other organs to the fruit abscission zone, thereby reducing the ratio of IAA to ABA, and thus leading to the increased sensitivity of the abscission zone to ethylene. Therefore, application of abscission chemicals, which release ethylene or promote wound ethylene, further reduced the FDF and caused more fruit abscission.

Our previous results showed that during the period of the "less responsiveness" of mature 'Valencia' oranges to abscission chemicals starting 25 Apr. and ending 25 May 2000 (Yuan et al., 2001a), the fruit abscission zone had high endogenous IAA concentrations and low ABA concentrations (Yuan et al., 2001b). The high endogenous auxin concentrations in the abscission zone of mature fruit might move from the young fruit that are on the trees at the same time (Yuan et al., 2001a). In the present study, application of TIBA to the peduncle every 3 or 4 d from 20 Apr. until 3 May may have blocked the auxin movement from young fruit to the abscission zone of mature fruit. Therefore, the ratio of IAA to ABA was decreased and the sensitivity of mature fruit to ethylene increased. As a result, the responsiveness of mature fruit to abscission chemicals was increased and thus the "less responsive period" was overcome. These results support our previous suggestion that the high ratio of IAA to ABA in the abscission zone is one of the factors responsible for the less responsiveness of mature fruit to abscission chemicals (Yuan et al., 2001b).

Ethephon can effectively loosen mature fruit in citrus (Kender et al., 2000; Kossuth and Biggs, 1977; Wheaton et al., 1977; Wilson et al., 1981), but other growth regulators have effects on its efficiency. In our study, NAA at 200 $mg \cdot L^{-1}$ effectively reduced ethephon-induced fruit loosening when applied directly to the abscission zone 24 h before application of ethephon, but it had no effect when applied 24 h after ethephon application. NAA is a synthetic auxin. Using bean leaf explants, Tucker et al. (1988) found that application of auxin prior to exposure to ethylene inhibited the accumulation of cellulase mRNA and thus blocked leaf abscission. However, NAA had no significant effect when applied either to fruit without contacting the abscission zone or to the peduncle ≈4 cm above the abscission zone (Fig. 5; Table 1). This may be due to insufficient translocation of NAA from the peel of the fruit or the peduncle to the abscission zone at the time when the ethephon was applied to the abscission zone.

In conclusion, application of NAA, an artificial auxin, to the abscission zone increased the ratio of auxin to abscisic acid and resulted in the decreased sensitivity of the abscission zone to ethylene, thereby reducing the fruit loosening caused by ethephon or CMN-Pyrazole. In contrast, reduced auxin levels in the abscission zone as a result of TIBA application, an auxin transport inhibitor, increased the sensitivity of the abscission zone to ethylene, and thus promoted the response to abscission chemicals and fruit abscission even in the "less responsive period."

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