

Dikegulac Promotes Abscission in Citrus

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Abstract. Two formulations of the plant growth regulator dikegulac (2,3:4,6-di-O-isopropylidene- α -L-xylo-2-hexulofuranosic acid), consisting of dikegulac-sodium (Atrimmec) or dikegulac:ascorbic acid (1:1) (DAA), as well as 5-chloro-3-methyl-4-nitro-pyrazole at 200 mg·L⁻¹, were applied as foliar sprays to 'Hamlin' and 'Valencia' orange trees (*Citrus sinensis* L. Osbeck) at two dates during the harvest season for each cultivar (11 Nov. and 10 Jan. for 'Hamlin', 22 Mar. and 25 May for 'Valencia'). Fruit detachment force was evaluated 10 days after application, whereas cumulative leaf abscission was monitored up to 60 days after application. In both cultivars, Atrimmec and DAA at 3,000 mg·L⁻¹ induced moderate fruit loosening when applied at the earlier application date, but fruit loosening improved when applied at the later application date. In 'Hamlin', both formulations caused higher leaf abscission when applied at the later date. DAA applications resulted in low leaf loss in 'Valencia' regardless of application time, whereas Atrimmec caused unacceptably high leaf loss at either application date. No differences in internal fruit quality were found as a result of any abscission material treatment. The results indicate that DAA could be a promising option to induce fruit loosening in late harvested 'Valencia' orange trees with minimal undesirable side effects.

The adaptation of mechanical harvesting to processing oranges is an important goal of the Florida citrus industry. However, the high fruit detachment force (FDF) of commercial orange varieties (Brown, 1998; Bukovac, 1979) has been a significant impediment for reaching high mechanical harvesting efficiencies in Florida citrus. Earlier work with fruit crops such as cherries demonstrated a close relationship between the efficiency of mechanical harvesting devices and FDF (Cain, 1967). The use of abscission agents will increase mechanical harvesting efficiency by uniformly reducing FDF; however, currently no compounds are registered for use in citrus.

The success of an abscission material for citrus depends on its ability to selectively loosen mature fruit of all commercial oranges with little or no phytotoxicity associated with application. This is especially true for 'Valencia' orange, since flowers, young fruit, young developing shoots and mature fruit can be found on the tree at the time of harvest. In general, mechanical harvesters operating without abscission agents can selectively harvest about 90% of the mature fruit from 'Valencia' trees during the early and midharvest season. However, as the season advances and young fruit enlarge, mechanical harvesters remove

increasing numbers of immature fruit, thereby negatively impacting yield the following year. Typically, mechanical harvesting ends during the first week in May, even though up to 6 weeks of additional harvesting may be necessary. When this occurs, hand harvesting is done until harvesting is complete. Hand harvesting can be costly at the end of the season, and more importantly, labor becomes increasingly scarce as crews are lost to crops north of Florida. A selective, nonphytotoxic abscission material is needed for use at the end of the harvest season to enable mechanical harvesting into June. A number of compounds have been evaluated in Florida's citrus groves for the purpose of increasing mechanical harvesting efficiency. Despite over 30 years of research, none have been registered because of poor and/or inconsistent loosening, phytotoxicity, unknown toxicology, and economic concerns (Kender et al., 1999; Wilson, 1978).

Dikegulac is the common name of a monosaccharide-related compound (2,3:4,6-di-O-isopropylidene- α -L-xylo-2-hexulofuranosic), whose sodium salt is an intermediate in the commercial synthesis of L-ascorbic acid (Bocion et al., 1975). Dikegulac has been shown to be a potent plant growth regulator, and its sodium salt, dikegulac-sodium, is used to promote lateral branching in plants by disrupting apical dominance (pinching agent) in azaleas and other herbaceous perennial crops (Bocion et al., 1975; Hodges et al., 1995; Latimer, 2001; Orson and Kofranek, 1978). Dikegulac-sodium, registered under the brand name Atrimmec, is reported to be a relatively non-phytotoxic plant growth regulator (Tew, 1997). During our screening trials, we found that Atrimmec caused mature fruit

drop. However, little or no information was available concerning dikegulac's potential effects on fruit abscission. The objective of this study was to determine the efficacy and phytotoxicity of two effective formulations of dikegulac as mature fruit looseners of 'Hamlin' and 'Valencia' oranges.

Materials and Methods

Plant material and abscission compounds. *Citrus sinensis* L. Osbeck 'Valencia', (10 to 13 years of age), 'Hamlin' (8 to 10 years of age), and 'Navel' (15 years of age) orange trees in groves located at the Citrus Research and Education Center, Lake Alfred, Fla., were used in the experiments. Two formulations of dikegulac were tested, consisting of the chemical mixture dikegulac (free acid) and ascorbic acid, each at 100 g·L⁻¹ (DAA), and dikegulac sodium salt, 20 g·L⁻¹ (Atrimmec). Both formulations were provided by PBI/Gordon Corporation (Kansas City, Mo.). Applied concentrations of abscission materials ranged from 0 to 5000 mg·L⁻¹ (a.i.) for the dikegulac formulations. A model abscission agent, 5-chloro-3-methyl-4-nitro-pyrazole (CMN-P), that causes mature fruit-specific abscission was used as a positive control. Kinetic, an organosilicate adjuvant (Setre Chemical Co., Memphis, Tenn.) was applied at 0.15% and included in all treatments or by itself as a negative control. All treatments were dissolved or dispersed in water before application. For branch or canopy section tests, treatments were applied using a pressurized 1-L hand sprayer. For whole tree applications, treatments were applied using a 50-L capacity air-blast sprayer. In all cases, applications were made at an equivalent rate of 5 L per tree (runoff).

Preliminary tests. In 2000 and 2001, the following exploratory tests were conducted with 'Valencia' and 'Navel' oranges during each harvest season:

TEST 1. The effect of Atrimmec on reduction in FDF during the less responsive period of 'Valencia' orange was examined. The less responsive period occurs in mid-April to early May and is associated with reduced abscission material efficacy due to endogenous hormonal fluxes (Yuan et al., 2001). Atrimmec concentrations ranging from 0 to 5000 mg·L⁻¹ (a.i.) were applied on 18 Apr. 2000. Three replicates per treatment were used, consisting of 1 m³ canopy sections, and bearing 30 to 50 fruit each. Additional treatments, consisting of 200 g·L⁻¹ CMN-P and water-adjuvant spray solutions were applied as positive and negative controls, respectively. FDF was evaluated three days (CMN-P) or 10 d (DAA) after application using a digital force gauge (Force Five Wagner Instruments, Greenwich, Conn.). Fruit were clipped to include 3.0 cm of stem, inserted into the gauge, and the stem was pulled parallel to the fruit axis until it separated from the fruit. The force necessary to remove the fruit from the stem was measured in kilograms. Leaf abscission was determined using one tagged branch per canopy section and was recorded at various times up to 60 d after application. Results were expressed as percent cumulative loss.

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TEST 2. A comparative study of Atrimmec and DAA efficacy was performed during late harvest season (May) of 'Valencia' orange. Atrimmec and DAA, each at 3000 mg·L⁻¹ (a.i.), were applied on May 4, 2001 to three replications of 1-m³ canopy sections. CMN-P at 200 g·L⁻¹ and water-adjuvant controls were applied as positive and negative controls. FDF and leaf abscission were evaluated as indicated above.

TEST 3. The effect of Atrimmec on flower abscission was investigated. Atrimmec was applied at 0, 2000, 3500 and 5000 mg·L⁻¹ (a.i.). Four replications per treatment were used, consisting of 1-m³ canopy section of 'Navel' orange trees, each section bearing 300 to 500 reproductive structures (buds, open flowers and fruitlets) and no less than 50 leaves. The applications were carried out on 2 Mar. 2000 during full bloom. Leaf and flower abscission were evaluated by counting leaves and reproductive structures before, and 5 and 13 d after application. The results were expressed as percent flower and leaf abscission.

Experiment 1. Comparative effects of DAA and Atrimmec on mature fruit loosening in 'Hamlin' orange. One canopy section test was initiated in 'Hamlin' orange on each of two dates: 10 Nov. 2001 (midseason application) and 11 Jan. 2002 (late-season application). Each test consisted of three randomly selected 1.0-m³ canopy section replicates that contained at least 20 mature fruit for FDF measurements. One branch within each 1-m³ section with at least 25 leaves was flagged to evaluate cumulative leaf abscission. Treatments consisted of DAA and Atrimmec, each at 3000 mg·L⁻¹ (a.i.), 200 g·L⁻¹ CMN-P and a water-adjuvant control. Average minimum and maximum temperatures at application and 10 d thereafter were 14.9 and 25.3 °C for the earlier application date and 11.2 and 24.1 °C for the later application date. FDF was measured 3 d (CMN-P) and 10 days (DAA, Atrimmec, adjuvant control) after application, and leaf abscission was evaluated at various times up to 60 d after application.

Experiment 2. Comparative effects of DAA and Atrimmec on mature fruit loosening in 'Valencia' orange. Two whole tree tests were initiated on 22 Mar. 2002 (early-season application) and 25 May 2002 (late-season application). Twelve trees were selected for treatment at each application date. Three trees within the 12-tree block were randomly selected for each treatment and application date. Treatments consisted of DAA and Atrimmec, each at 3000 mg·L⁻¹ (a.i.), 200 g·L⁻¹ CMN-P and a water-adjuvant control. Ninety uniform, mature fruit were labeled per tree to evaluate FDF; in addition, 3 branches, each bearing no less than 25 leaves. Where available, flowers, and/or fruitlets were tagged on individual branches to determine leaf, flower, and developing fruit abscission by counting the floral and young fruit structures at 0, 5, 13, and 60 d after application. Average minimum and maximum temperatures at application and 10 d thereafter were 15.8 and 29.4 °C for the earlier application date and 19.9 and 30.6 °C for the later application date. FDF and leaf abscission were determined as de-

scribed above. Fruit quality was determined in a subset of 20 fruit/replication used for FDF analysis. After FDF measurements were taken, fruit were transported to the laboratory where fruit weight (g), juice content (%), juice acidity (% citric acid), soluble solids (°Brix) and maturity ratio (°Brix:% citric acid) were evaluated. Visual observations were made 6 and 12 months after treatment to note intermediate or long term effects of each application on tree health.

Statistics. Data were computed and, if necessary, transformed using arcsin transformation in MS-Excel functions (Microsoft, Redmond, Wash.). Data of Expts. 1 and 2 (including juice analysis) were analyzed as a 4 × 2 factorial. Data from preliminary tests were analyzed as a single factor design. Analysis of variance and Duncan's multiple range test were performed on all data using the SAS statistical package (SAS Institute Inc., 1996).

Results

Preliminary tests 1, 2, and 3. The purpose of these branch tests was to obtain preliminary information on efficacy and selectivity of Atrimmec compared with the model abscission agent CMN-P. The results demonstrated that Atrimmec at 5000 mg·L⁻¹ and the model abscission compound CMN-P at 200 mg·L⁻¹ significantly reduced FDF (Table 1). However, both 3500 and 5000 mg·L⁻¹ Atrimmec concentrations significantly increased leaf loss. Based on our previous experience with CMN-P, the reduction in FDF was not as great as expected, and this indicated that the trees had entered the less responsive period (Hartmond et al., 2000). Nevertheless, the results indicated that Atrimmec applications could affect citrus abscission. Additional trials conducted to determine efficacy of Atrimmec or DAA after the less responsive period demonstrated that 3000 mg·L⁻¹ consistently reduced FDF while keeping leaf loss to lower levels (Table 2). Application of either compound at higher concentrations resulted in peel damage manifested as surface pitting (data not shown). When applied to flowering 'Navel' orange trees, Atrimmec caused floral abscission and loss of young leaves from developing shoots in a concentration-dependent manner (Table 3). Within 13 d, floral and young leaf abscission reached 100% with 3500 and 5000 mg·L⁻¹ Atrimmec. Additional trials established that 3000 mg·L⁻¹ Atrimmec was as efficacious at 3500 mg·L⁻¹ Atrimmec (data not shown). Based on the results of these preliminary trials, experiments were conducted to establish efficacy and selectivity of Atrimmec and DAA

Table 3. The effect of Atrimmec applied on 2 Mar. 2000 on abscission of flowers and leaves of developing vegetative shoots of Navel orange.

Atrimmec concn (mg·L ⁻¹)	Flower abscission (%)		Abscission (%) of leaves of developing shoots	
	Day 5	Day 13	Day 5	Day 13
0	10.4 b ²	37 b	19.2 b	19.7 b
2000	27.3 b	80.4 a	91.0 a	90.6 a
3500	78.9 a	100 a	90.0 a	100 a
5000	93.1 a	100 a	100 a	100 a

²Means of treatments within the same column followed by the same letter are not significantly different from each other, $P \leq 0.05$.

at 3000 mg·L⁻¹ when applied during two times of the harvest season corresponding to early or midseason and late season application dates of 'Hamlin' and 'Valencia' orange.

Experiments 1 and 2. In 2001 and 2002, applications were made to both 'Hamlin' and 'Valencia' orange at times corresponding to either mid-or-late-harvest season ('Hamlin') and either early-or-late-harvest season ('Valencia'). Generally speaking, both dikegulac formulations reduced FDF and increased % cumulative leaf loss as compared with the adjuvant controls, but these effects were greater for the late season application in both cultivars (Figs. 1 and 2). 'Hamlin' appeared to be particularly responsive to the abscission materials. When applied at the late season date, both DAA and Atrimmec were as effective in reducing FDF as the model abscission compound CMN-P (Fig.

Table 1. The effect of Atrimmec and CMN-P applied 18 Apr. 2000 on fruit detachment force (FDF) and cumulative leaf abscission during the less responsive period in 'Valencia' orange.

Treatment and concn	FDF (kg) ²	Cumulative leaf abscission (%) ³
Atrimmec		
500 mg·L ⁻¹	9.36 b ²	9.80 c
2000 mg·L ⁻¹	9.47 b	9.80 c
3500 mg·L ⁻¹	10.24 a	19.41 b
5000 mg·L ⁻¹	6.62 c	37.43 a
CMN-P		
200 mg·L ⁻¹	7.08 c	4.00 c
Adjuvant control	10.39 a	9.82 c

²FDF was measured 10 d after Atrimmec application and 5 d after CMN-P application.

³Cumulative leaf abscission (%) was determined 21 d after abscission material application.

⁴Means of treatments within the same column followed by the same letter are not significantly different from each other, $P \leq 0.05$

Table 2. Comparative effects of Atrimmec, DAA, and CMN-P applied on 4 May 2001 on fruit detachment force (FDF) and % cumulative leaf abscission of 'Valencia' orange.

Treatment	FDF (kg) ²	Cumulative leaf abscission (%) ³
Atrimmec 3000 mg·L ⁻¹	5.49 b ²	1.80 b
DAA 3000 mg·L ⁻¹	5.82 b	7.95 a
CMN-P 200 mg·L ⁻¹	3.42 b	5.94 ab
Adjuvant control	10.32 a	1.85 b

²FDF was measured 10 d after Atrimmec application and 5 d after CMN-P application.

³Cumulative leaf abscission (%) was determined 21 d after abscission material application.

⁴Means of treatments within the same column followed by the same letter are not significantly different from each other, $P \leq 0.05$.

Hamlin

materials. Leaf abscission in Hamlin was also higher when applied during the late season; however, 'Valencia' leaves did not respond in the same way. Little seasonal variation in leaf loss was measured in trees treated with DAA; in contrast, leaves were more sensitive early in the season when treated with Atrimmec. The differential response between early or midseason application and late season application in both cultivars is not an effect of increased temperatures later in the season, as temperatures during the treatment and subsequent measurement period were relatively similar during these times.

Ascorbic acid, a component of the DAA formulation, appeared to mitigate the effect of the active ingredient dikegulac on leaf loss. Ascorbic acid is known to participate in detoxification of reactive oxygen species (ROS) in plant systems (del Rio et al., 1998; Thompson et al., 1987), and it is postulated that ROS generation is an intrinsic feature of abscission (Burns, 2002; Roberts et al., 2002). ROS could play a role in the initiation of membrane breakdown leading to the promotion or acceleration of abscission. Therefore, preventing or greatly reducing the accumulation of ROS during abscission may lessen the action of an abscission agent. Citrus leaves appear to be more sensitive to most abscission agents than mature fruit, since application of compounds such as ethephon cause marked leaf loss with little effect on reduction of FDF (Burns 2002; Kender et al., 2000).

Abscission of young vegetative organs resulting from Atrimmec application suggests that these structures are particularly sensitive to this compound. In 'Hamlin', 60 to 90 d after application, new flushing shoots appeared to be inhibited or were significantly delayed in emergence, and in some cases, twig necrosis was evident. When applied late in the season, little or no young fruit developed. Late season application of either Atrimmec or DAA to 'Valencia' orange, however, showed minimal or no phytotoxicity symptoms 90 d after application. This suggests that late season applications show promise for loosening mature 'Valencia' fruit.

Atrimmec is classified as a chemical pinching agent. In preliminary work, we determined that other pinching agents, Off-Shoot-O (methyl decanoate) and Bonzai (paclobutrazol), were unable to cause mature fruit loosening (Kender et al., 2000; data not shown). Thus, dikegulac is a chemical pinching agent that appears to effectively target metabolism that ultimately induces or accelerates abscission. The active ingredient of Atrimmec, dikegulac, has been shown to destroy apical dominance and encourage lateral branching (Bocion et al., 1975; Lih-Jyu et al., 1981; Orson and Kofranek, 1978), suggesting that the mode of action is related to auxin balance. Auxin is known to delay abscission (Brown, 1997), and the alteration of endogenous auxin balance, such as the disruption of transport with auxin transport inhibitors, promotes mature citrus fruit abscission (Yuan et al., 2001, 2003).

In conclusion, we have shown that the dikegulac formulations used in this study

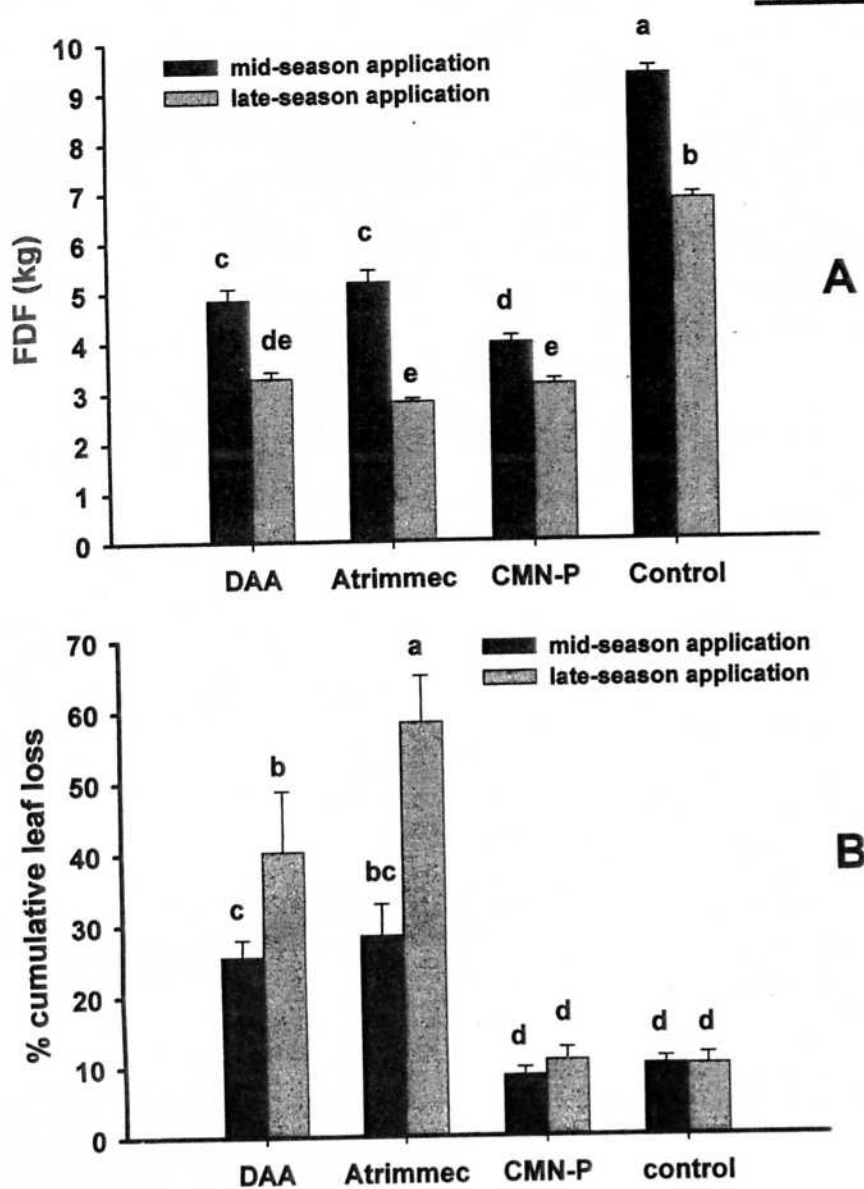


Fig. 1. Fruit detachment force (FDF) (A) and cumulative leaf loss (%) (B) in 'Hamlin' orange trees after mid- and late-season application of DAA, Atrimmec, CMN-P, or adjuvant alone (control) to the canopy. SE means are shown as thin lines through the top of each bar. Mean separation by Duncan's multiple range test, $P < 0.05$. Spray treatments were applied at a rate of 5 L per tree.

IA), but leaf loss was unacceptably high. At the same concentration in 'Valencia', DAA, and Atrimmec did not reduce FDF as much as in 'Hamlin' (Fig. 2). DAA did not cause significant leaf abscission in 'Valencia'; however, Atrimmec caused high leaf loss, especially when applied in the early-harvest season (Fig. 2B). Atrimmec and DAA applied during bloom of 'Valencia' orange significantly increased floral and young fruit abscission when compared with CMN-P and control trees 60 d after application (Table 4). In contrast, treatment differences in young fruit retention were not found when any abscission agent was applied later in the season. For each cultivar, Atrimmec, DAA, or CMN-P did not significantly affect juice quality at either application date; nor they cause any visible intermediate or long term negative effects on tree health (data not shown).

Discussion

We have shown that late season applications of DAA may reduce FDF in 'Valencia' orange to values necessary (up to 50% or greater reduction in FDF) to increase mechanical harvesting efficiency (Wilson, 1978), while minimizing leaf loss associated with Atrimmec application. The seasonal variation found in this study for 'Valencia' and 'Hamlin' orange responses to dikegulac formulations was previously shown in citrus cultivars such as 'Valencia' when sprayed with abscission materials such as metsulfuron-methyl and CMN-P in Florida (Hartmond et al., 2000). In this study, late season applications of Atrimmec or DAA appeared more efficacious than early season applications, suggesting that fruit maturity may play a role in response to these abscission

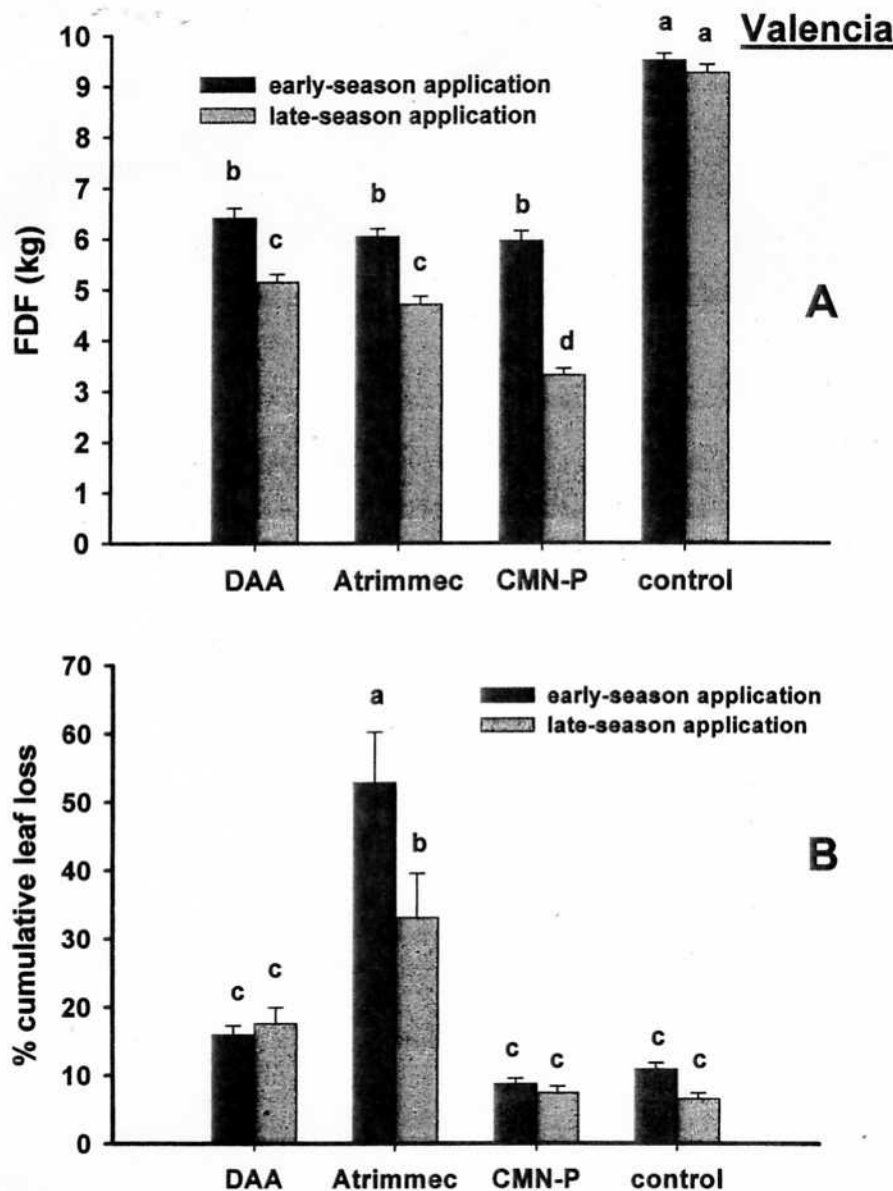


Fig. 2. Fruit detachment force (FDF) (A) and cumulative leaf loss (%) (B) in 'Valencia' orange trees after early and late-season application of DAA, Atrimmec, CMN-P, or adjuvant alone (control) to the canopy. SE means are shown as thin lines through the top of each bar. Mean separation by Duncan's multiple range test, $P < 0.05$. Spray treatments were applied at the rate of 5 L per tree.

Table 4. Comparative effects of Atrimmec, DAA, and CMN-P applied during full bloom on 22 Mar. 2002 or after bloom and fruit set on 25 May 2002 on developing fruit retention (%) of 'Valencia' orange 60 d after application.

Treatment	Retention of young developing fruit (%)	
	22 Mar. 2002	25 May 2002
Atrimmec 3000 mg·L ⁻¹	0.89 c ^a	84.07 ^{ns}
DAA 3000 mg·L ⁻¹	0.26 d	83.33 ^{ns}
CMN-P 200 mg·L ⁻¹	2.60 b	83.33 ^{ns}
Adjuvant control	3.87 a	87.96 ^{ns}

^aMeans of treatments within the same column followed by the same letter are not significantly different from each other, $P \leq 0.05$.

caused abscission. However, only the DAA formulation applied late in the 'Valencia' harvest season loosened mature fruit with no effect on young developing fruit and minimal effect on leaf drop and long-term tree health. Thus, DAA may fill a niche that would enable mechanical harvesters to continue to operate in 'Valencia' groves when young developing fruit would otherwise be harvested without an abscission agent. Although dikegulac has not been cleared for use in food crops, the fact that the mammalian toxicity of dikegulac is reportedly low (Bocion et al., 1975) could reduce concerns about dikegulac's outlook as an aid for mechanical harvesting citrus.

Further research will focus on increasing efficacy of DAA with combinations of other abscission materials.

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