

Screening Fruit Loosening Agents for Black Ripe Processed Table Olives

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Abstract. The California table olive (*Olea europaea* L.) industry relies exclusively on hand harvesting of its primary Manzanillo cultivar. Increased harvesting costs have intensified industry interest in identifying an abscission agent that can be used with developing mechanical harvesting technologies to increase removal rates. Table olives are harvested immature green at horticultural maturity but before physiological maturity. The goal of this research was to reevaluate the potential of ethylene-releasing compounds (ERCs) as olive-loosening agents and to screen additional candidates previously shown to accelerate citrus fruit abscission. Eleven compounds were screened at two separate table olive-growing sites (Fresno and Tehama counties) in California in September until Nov. 2006. Compounds were applied at various concentrations alone or in combination. Fruit detachment force (FDF) and percent fruit drop were measured and leaf loss assessed. Of the compounds evaluated, the ERC ethephon (2-chloroethyl phosphonic acid) and 1-aminocyclopropane-1-carboxylic acid were the most efficacious. In whole tree applications, concentrations of ethephon or 1-aminocyclopropane-1-carboxylic acid above 1000 mg·L⁻¹ reduced FDF to less than 50% of the untreated control within 17 days, but leaf drop increased with increasing concentrations. Addition of 1-methylcyclopropene reduced efficacy of ethephon and delayed leaf drop. Monopotassium phosphate + ethephon (4% and 1000 mg·L⁻¹, respectively) reduced FDF and leaf loss was equivalent to the ethephon alone treatment. Compounds such as methyl jasmonate, coronatine, dikegulac, MAXCEL, traumatic acid, and 5-chloro-3-methyl-4-nitro-1H-pyrazole were not efficacious.

Olives (*Olea europaea* L.) are commercially grown throughout the world in areas of Mediterranean climate. Cultivation is primarily for oil and table olive products. In the United States, California is the only state with commercial olive orchards and in 2000 to 2002 accounted for ≈7% of total world table olive production (Connell, 2005). The California olive industry relies almost exclusively on the table olive.

Table olives are harvested immature green at horticultural maturity but before physiological maturity (Ferguson et al., 2005). Harvest is done by hand labor over a period of ≈2 months. Increased labor costs and low labor availability have intensified

industry interest in mechanical harvesting. Unusually low temperatures during flower bud initiation in Feb. 2006 in California's Central Valley olive production areas markedly reduced yield in a year when lower yield was expected as a result of alternate bearing. Extremely low yield combined with labor pressures increased harvesting costs further and strained olive inventories at the processing plant. To ensure future economic stability for the olive grower and consistent product flow at the processing plant, the California table olive industry has viewed the development of a successful mechanical harvesting system, that is, mechanical harvesting combined with abscission agent application (Burns et al., 2005), as a high priority.

Trunk-shaking mechanical harvesters were evaluated in the past but unable to harvest table olive fruit efficiently and without damage (Ferguson et al., 2005). Canopy-shaking mechanical harvesters are being developed currently and show promise. How-

ever, a fruit-specific abscission agent would allow for higher fruit removal. Moreover, an abscission agent would enable lower mechanical forces to be applied during harvest, thus minimizing fruit damage. Fruit damage is an industry concern because bruising may compromise quality of the final canned product, although this remains to be tested.

Abscission agents were applied experimentally to olive canopies in the past to improve fruit removal by trunk-shaking mechanical harvesters (Martin, 1994). Of those tested, only ethylene-releasing compounds (ERCs) such as ethephon (2-chloroethyl phosphonic acid) achieved consistent fruit loosening (Hartmann et al., 1970; Martin et al., 1981). ERC use in many crops, including olive, has been problematic as a result of attendant leaf loss coincident with fruit loosening. Treatments designed to minimize unwanted defoliation such as calcium application (Martin et al., 1981), or alteration in ERC application timing and duration (Lang and Martin, 1985, 1989), were promising in a laboratory setting but unpredictable in the field (Martin, 1994). Recent work in Florida identified compounds that accelerated mature and immature fruit abscission when applied to citrus canopies in the field (Burns, 2002). In this work, we describe efficacy of several of these compounds and reevaluate the potential of ethephon for olive fruit loosening.

Materials and Methods

Olive screening trials were located at two separate sites in California. At each site, fruit were deemed ready for harvest as table olives based on color change from green to straw and appearance of characteristic white milky juice from fruit when squeezed (Ferguson et al., 2005). Fruit detachment force (FDF) was measured at various times up to 17 d after application using an Imada DPA-11 digital force gauge (Imada, Northbrook, IL). At various times, treated branches were removed and brought to the laboratory. Olive fruit were clipped from branches to include at least 1-cm pedicel, inserted into the gauge, and the pedicel pulled parallel to the fruit axis until it separated from the fruit. The force necessary to remove fruit from the pedicel was measured in grams of force.

Fresno county site

Trials were initiated on four separate dates in late September to mid-Oct. 2006 at an experimental orchard located at the University of California's Kearney Agricultural Center, Parlier, CA. Trees were 12 years of age. Varieties used were 'Rigali' on its own roots, 'Manzanillo' on 'Rigali' rootstock, and 'Mission' on 'Manzanillo' rootstock. For each of the four trials described below, three replicate branches were selected on one tree of each variety, giving an overall total of nine replicates for each treatment. Each branch was considered an experimental unit and contained at least eight fruit and 25 leaves. Treatments were randomly assigned

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Table 1. The effect of putative abscission agents on fruit detachment force (FDF, grams of force) and fruit drop (%) in olive treated on the dates indicated in 2006^a.

Date	Treatment	FDF (grams of force)	Fruit drop (%)
21 Sept.	Methyl jasmonate		
	2 mM	456.2 ^w	3.4 ^w
	10 mM	448.4	4.8
	20 mM	443.6	4.0
	Propyldihydrojasmonate		
	200 mg·L ⁻¹	461.3	3.1
	1000 mg·L ⁻¹	450.6	4.7
	2000 mg·L ⁻¹	449.3	3.3
	Control	458.1	4.6
25 Sept.	MAXCEL		
	200 mg·L ⁻¹	530.3	3.2 c
	1000 mg·L ⁻¹	532.2	0.0 c
	2000 mg·L ⁻¹	517.1	3.2 c
	ACC ^y		
	500 mg·L ⁻¹	538.1	28.4 b
	1000 mg·L ⁻¹	519.7	54.2 a
	Coronatine		
	200 mg·L ⁻¹	544.1	1.5 c
27 Sept.	Control	554.6	3.5 c
	Ethephon		
	500 mg·L ⁻¹	407.4 ab	12.9 b
	1000 mg·L ⁻¹	261.1 b	46.4 a
	Traumatic acid (TA)		
	1000 mg·L ⁻¹	419.6 a	0.0 b
	Ethephon + TA		
	1000 + 1000 mg·L ⁻¹	368.4 ab	44.9 a
	Control	492.6 a	2.5 b
8 Oct.	Dikegulac		
	200 mg·L ⁻¹	468.0	0.0
	1000 mg·L ⁻¹	511.7	1.3
	2000 mg·L ⁻¹	489.7	2.6
	CMNP ^x		
	1000 mg·L ⁻¹	469.6	0.0
	2000 mg·L ⁻¹	476.0	0.0
	Control	527.4	2.8

^aBranch tests were conducted in Fresno county, CA.^yACC = 1-aminocyclopropane-1-carboxylic acid.^xCMNP = 3-chloro-5-methyl-4-nitro-1H-pyrazole.^wMeans within each date and column followed by the same letter are not significantly different ($P \leq 0.05$). Absence of letters within each date and column indicates no statistical significance found.

to branches, and the number of fruit on each branch was recorded. Abscission compounds were dissolved in water containing 0.05% Kinetic organosilicate adjuvant (Setre Chemical Co., Memphis, TN). A water control was included in all trials. Treatments were applied between 1030 and 1400 HR with a handheld 1.5-L pressurized sprayer until runoff. FDF was measured 7 or 12 d after application. Percentage fruit drop was computed by counting the number of fruit that dropped from the branch at the end of the experiment, dividing by the total number of fruit at the beginning of the trial, and multiplying by 100. Data were analyzed as a completely randomized design. Analysis of variance and Duncan's mean separation were used to test significance between treatment means. Percentage data were transformed using arcsine transformation when necessary to stabilize variance.

Trial 1. On 21 Sept. 2006, branches were sprayed with 2, 10, or 20 mM methyl jasmonate (MJ; Bedoukian Research, Inc., Danbury, CT) or 200, 1000, or 2000 mg·L⁻¹ propyldihydrojasmonate (Valent BioSciences Corp., Libertyville, IL). Maximum, minimum, and average temperatures on the day of application were 85, 54, and 69 °F (29, 12,

and 20 °C), respectively. Average maximum, minimum, and overall average temperatures for the duration of the trial were 83, 53, and 67 °F (28, 11, and 19 °C), respectively. FDF and fruit drop measurements were taken 12 d after application.

Trial 2. On 25 Sept. 2006, branches were sprayed with 200, 1000, or 2000 mg·L⁻¹ 6-benzyladenine (MAXCEL; Valent BioSciences Corp.); 500 or 1000 mg·L⁻¹ 1-aminocyclopropane-1-carboxylic acid (ACC; Sigma-Aldrich, St. Louis, MO.); or 200 mg·L⁻¹ coronatine [fermented, extracted, and quantified according to Burns et al. (2003)]. Maximum, minimum, and average temperatures on the day of application were 92, 48, and 69 °F (33, 8, and 20 °C), respectively. Average maximum, minimum, and overall average temperatures for the duration of the trial were 79, 52, and 65 °F (26, 11, and 18 °C), respectively. FDF and fruit drop measurements were taken 12 d after application.

Trial 3. On 27 Sept. 2006, branches were sprayed with 1000 mg·L⁻¹ traumatic acid (TA; Sigma-Aldrich Co.); 500 or 1000 mg·L⁻¹ ethephon (Ethrel; Aventis Crop Science, Research Triangle Park, NC); or TA + ethephon (1000 + 1000 mg·L⁻¹). Maximum,

minimum, and average temperatures on the day of application were 90, 53, and 69 °F (32, 11, and 20 °C), respectively. Average maximum, minimum, and overall average temperatures for the duration of the trial were 78, 52, and 64 °F (25, 11, and 17 °C), respectively. FDF and fruit drop measurements were taken 12 d after application.

Trial 4. On 8 Oct. 2006, branches were sprayed with 200, 1000, or 2000 mg·L⁻¹ dikegulac (Atrimmec; PBI/Gordon Corp, Kansas City, MO); or 1000 or 2000 mg·L⁻¹ 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMNP; previously formulated by Abbott Laboratories, Libertyville, IL). Maximum, minimum, and average temperatures on the day of application were 78, 49, and 62 °F (25, 9, and 16 °C), respectively. Average maximum, minimum, and overall average temperatures for the duration of the trial were 78, 52, and 64 °F (25, 11, and 17 °C), respectively. As a result of time constraints, FDF and fruit drop measurements were taken 7 d after application.

Tehama county site

A trial was initiated on 24 Oct. 2006 in a commercial olive orchard located 5 miles (8 km) northwest of Corning, CA. Forty-seven uniform 7-year-old 'Manzanillo' trees on their own roots were selected and treatments randomly assigned. Fifteen treatments were applied to three trees each. All spray treatments included 0.05% Silwet adjuvant. Spray applications were done using a 200-gallon (757-L) tank equipped with a pressurized handgun until runoff. Treatments were 500, 1000, 1500, or 2000 mg·L⁻¹ ethephon; 5 mM 1-MCP (as SmartFresh; AgroFresh, Philadelphia, PA); 1000, 1500, or 2000 mg·L⁻¹ ethephon + 5 mM 1-MCP; 500, 1000, or 2000 mg·L⁻¹ ACC, 4% monopotassium phosphate (MPK; MORA-LEAF P&K; Wilbur Ellis, Fresno, CA) with and without 1000 mg·L⁻¹ ethephon; and a water and untreated control. Maximum, minimum, and average temperatures on the day of application were 86, 46, and 63 °F (30, 8, and 17 °C), respectively. A representative branch from each replicate tree was removed 3, 6, 10, 13, and 17 d after application. Branches were transported to the Glenn County Cooperative Extension Office in Orland, CA, and FDF was measured as described above. Defoliation was evaluated in the orchard using a subjective leaf abscission score (LAS) of 0 (no defoliation), 1 (light defoliation), 2 (moderate defoliation), and 3 (severe defoliation, greater than 50% canopy volume). Average maximum, minimum, and overall average temperatures for the duration of the trial were 74, 49, and 61 °F (23, 9, and 16 °C), respectively. Data were analyzed as a completely randomized design. Analysis of variance and Duncan's mean separation were used to test significance between treatment means.

Results

Fresno county site. The varietal effect was not significant in each trial, so data from

the three varieties were pooled. MJ, propyl-dihydrojasmonate, MAXCEL, coronatine, TA, and CMNP had no effect on FDF or percent fruit drop at any concentration tested (Table 1). Only treatment with 1000 mg·L⁻¹ ethephon significantly reduced FDF when compared with control fruit. Fruit drop was significantly increased in fruit treated with 1000 mg·L⁻¹ ethephon and the combination treatment of ethephon and TA at 1000 mg·L⁻¹ each. Although 500 and 1000 mg·L⁻¹ ACC significantly increased fruit drop, FDF was not altered. In this case, loosening and fruit drop likely occurred before FDF was measured, and the remaining fruit either did not respond to the compound or the partially digested abscission zones reformed or “healed”.

Tehama county site. Application of 1500 and 2000 mg·L⁻¹ ethephon reduced FDF as days after application increased (Fig. 1A). Concentrations below 1500 mg·L⁻¹ were not as effective. By day 17, FDF in fruit treated with 1500 and 2000 mg·L⁻¹ were statistically lower than any other treatment, whereas lower ethephon concentrations were no different from the control. ACC reduced FDF as days after application increased (Fig. 1B). Like ethephon, ACC application at 2000 mg·L⁻¹ gave significantly lower FDF values when compared with other ACC concentrations. After day 6, LAS increased in all ethephon and ACC treatments (Fig. 1C–D). LAS was significantly greater at the highest concentration compared with the other concentrations tested. At comparable concentrations of 500 and 1000 mg·L⁻¹, ACC application resulted in significantly lower FDF readings than ethephon (Table 2). However, no statistical difference between ACC and ethephon was measured when either was applied at 2000 mg·L⁻¹. Despite higher efficacy attributed to ACC at comparable concentrations, there was no statistical difference in LAS. Ethephon at 1000 mg·L⁻¹ + 4% MPK significantly lowered FDF when compared with ethephon or MPK alone after 17 d; however, LAS was not significantly different (Fig. 2).

To reduce unwanted leaf drop associated with ethephon applications, 5 mM 1-MCP was combined with ethephon in the spray tank and applied to olive canopies. At all ethephon concentrations, 1-MCP delayed ethephon-induced leaf drop (data not shown). Leaf drop 17 d after application was low when 1000 or 1500 mg·L⁻¹ ethephon was used, and 1-MCP had no effect (Table 3). However, 1-MCP significantly lowered leaf drop associated with 2000 mg·L⁻¹ ethephon application. Ethephon-induced olive fruit loosening was delayed by 1-MCP (data not shown). After 17 d, however, there were no significant differences in FDF when 1-MCP was applied.

Discussion

ERCs such as ethephon have been used in an attempt to accelerate fruit abscission. After decades of research with ethephon done

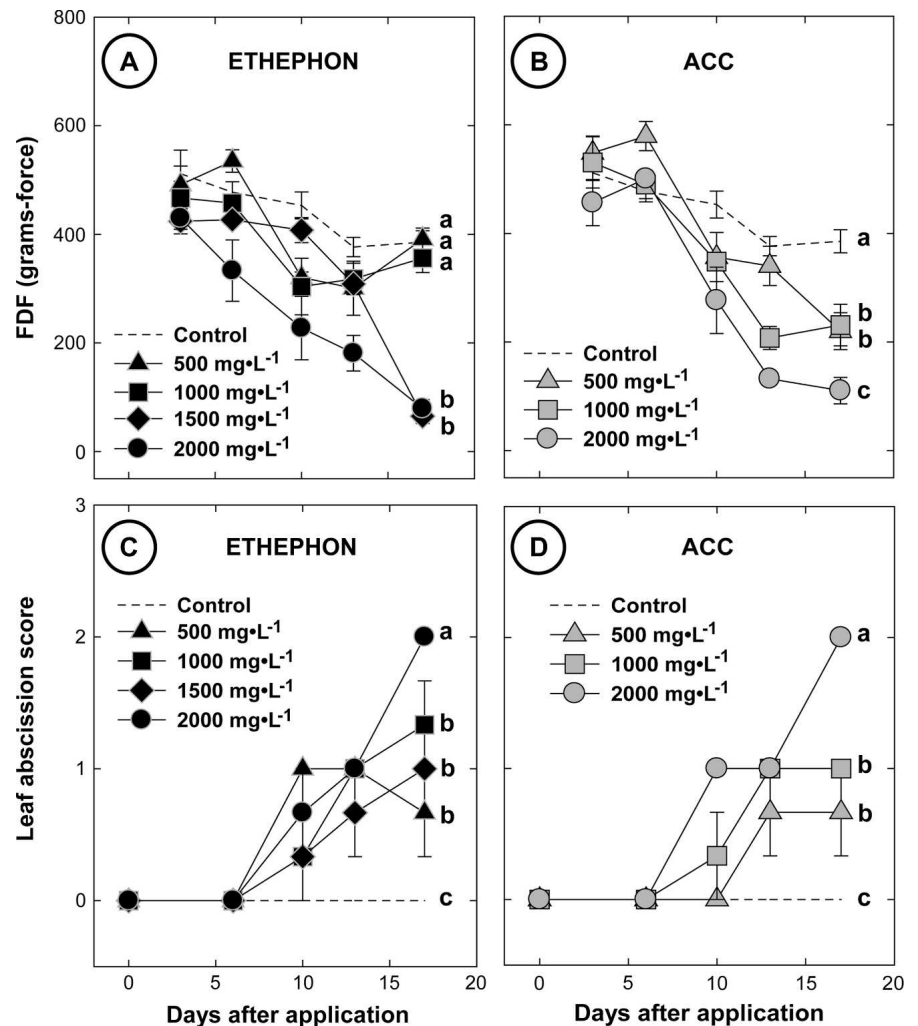


Fig. 1. Change in fruit detachment force (FDF in grams of force, A–B) and leaf abscission score (C–D) of olive up to 17 d after treatment with indicated concentrations of (A and C) ethephon and (B and D) 1-aminocyclopropane-1-carboxylic acid (ACC). Adjuvant-treated trees served as controls. Leaf abscission scores range from 0 (no defoliation) to 3 (severe defoliation). Whole tree tests were conducted in Tehama county, CA. Application date was 24 Oct. 2006. Data plotted are the means ± SE. Analysis of variance and Duncan’s mean separation test performed on d 17 data. Means followed by the same letter are not significantly different ($P \leq 0.05$).

for the purpose of adapting fruit crops for mechanical harvesting, commercial application remains restricted to cherries (Bukovac, 1979) and walnuts (Ramos, 1997). Cherries and walnuts grown for processed markets can be sprayed with ethephon before mechanical harvesting to loosen fruit and increase fruit removal. Significant research effort was spent on adapting olives for mechanical harvesting using ethephon. Although mechanical fruit removal approached 85% in some cultivars, the ‘Manzanillo’ olive, important to the current California table olive industry, was particularly prone to excessive leaf loss at ethephon concentrations necessary to accelerate fruit loosening (Martin et al., 1981). Furthermore, ethephon-dependent fruit loosening and leaf loss in olive was influenced by temperature and irrigation practices (Klein et al., 1978; Martin et al., 1981). Excessive temperatures and water stress at the time of application improved ethephon efficacy but also accelerated unwanted leaf loss and phytotoxicity (Bukovac, 1979;

Table 2. Comparison of ethephon and 1-aminocyclopropane-1-carboxylic acid (ACC) application at indicated concentrations on fruit detachment force (FDF, grams of force) and leaf abscission score (LAS) of olive 17 d after application².

Concn (mg·L ⁻¹)	Abscission agent	FDF (grams of force)	LAS ³
500	ACC	219.9 b [*]	0.7 [*]
	Ethephon	391.1 a	0.7
	Water control	401.2 a	0.0
1000	ACC	231.3 b	1.0 a
	Ethephon	356.3 a	1.3 a
	Water control	401.2 a	0.0 b
2000	ACC	110.5 b	2.0 a
	Ethephon	79.3 b	2.0 a
	Water control	401.2 a	0.0 b

²Whole tree tests were conducted in Tehama county, CA. Application date was 24 Oct. 2006.

³LAS range from 0 (no defoliation) to 3 (severe defoliation).

^{*}Means within each concentration and column followed by the same letter are not significantly different ($P \leq 0.05$). Absence of letters within each date and column indicates no statistical significance found.

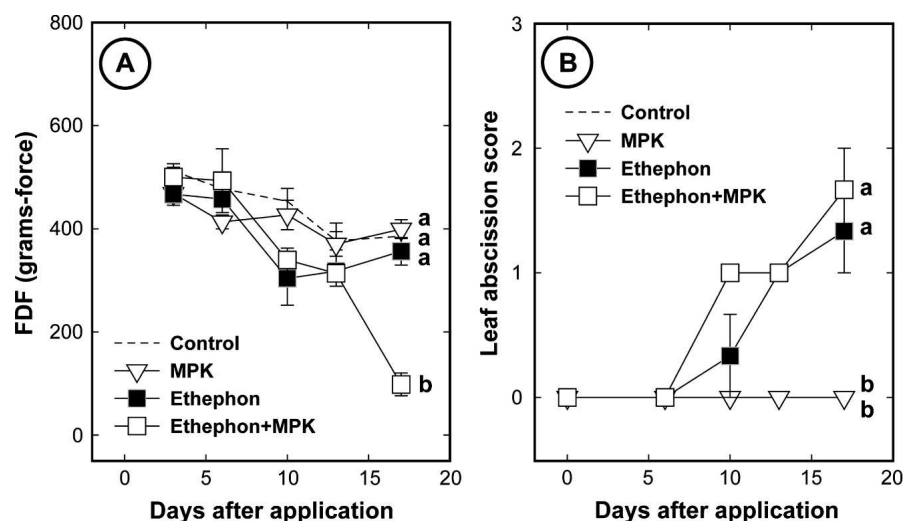


Fig. 2. Change in fruit detachment force (FDF in grams of force, **A**) and leaf abscission score (**B**) of olive up to 17 d after treatment with 4% monopotassium phosphate (MPK), 1000 mg·L⁻¹ ethephon, or 4% MPK + 1000 mg·L⁻¹ ethephon. Adjuvant-treated trees served as controls. Leaf abscission scores range from 0 (no defoliation) to 3 (severe defoliation); MPK alone and adjuvant controls had a leaf abscission score of 0 throughout the course of the experiment. Whole tree tests were conducted in Tehama county, CA. Application date was 24 Oct. 2006. Data plotted are the means \pm SE. Analysis of variance and Duncan's mean separation test performed on d 17 data. Means followed by the same letter are not significantly different ($P \leq 0.05$).

Table 3. Comparison of ethephon at indicated concentrations alone or in combination with 5 mM 1-methylcyclopropene (1-MCP) on fruit detachment force (FDF, grams of force) and leaf abscission score (LAS) of olive 17 d after application².

Concn (mg·L ⁻¹)	Abscission agent	FDF (grams of force)	LAS ³
1000	Ethephon	356.3 ^a	1.3 a ^x
	Ethephon + 1-MCP	387.8	1.0 a
	Water control	401.2	0.0 b
1500	Ethephon	65.2 b	1.0 a
	Ethephon + 1-MCP	174.3 b	1.0 a
	Water control	401.2 a	0.0 b
2000	Ethephon	79.3 c	2.0 a
	Ethephon + 1-MCP	147.7 b	0.3 b
	Water control	401.2 a	0.0 b

²Whole tree tests were conducted in Tehama county, CA. Application date was 24 Oct. 2006.

³LAS range from 0 (no defoliation) to 3 (severe defoliation).

^xMeans within each concentration and column followed by the same letter are not significantly different ($P \leq 0.05$). Absence of letters within each date and column indicates no statistical significance found.

Pozo and Burns, 2000; Yuan and Burns, 2004).

In the work reported here, only the ERC ethephon or the ethylene biosynthetic precursor ACC significantly reduced olive FDF in the field. ACC is converted to ethylene rapidly upon uptake, providing a significant but transient source of ethylene to treated tissues. ACC was shown to promote leaf ethylene production without causing significant leaf loss in olive explants (Martin et al., 1981), suggesting that canopy applications may minimize leaf loss while at the same time accelerate fruit abscission. Although ACC was as effective as or better than ethephon in promoting fruit loosening, leaf loss was high with both compounds.

The ethylene perception inhibitor 1-MCP was shown to reduce unwanted defoliation caused by ethephon without impacting desirable fruit loosening in citrus (Pozo et al., 2004b). This differential action of 1-MCP on mature fruit and leaves may be the result of duration of 1-MCP exposure or differential

sensitivity (Burns, 2007). In olive, no benefit of 1-MCP application was seen at low ethephon concentrations, but leaf loss was significantly reduced when 1-MCP was included in the 2000 mg·L⁻¹ ethephon application. Whether defoliation was inhibited or delayed by 1-MCP beyond the trial reported here was not evaluated per se, but visual assessment of the site after 6 months indicated no further leaf loss or longlasting effect on tree health. FDF was reduced by combined ethephon + 1-MCP treatment, but for the purpose of mechanical harvesting, the minimal effect on efficacy may not impair the potential for mechanical fruit removal. A 50% reduction in FDF was necessary to maximize machine removal of citrus (Burns et al., 2005). Based on these results, further research with ethephon + 1-MCP combinations is warranted.

Banno et al. (1993) demonstrated that phosphorus-containing compounds such as potassium-phosphate buffer accelerated olive fruit abscission with minimal leaf loss.

Goren et al. (1998) attributed phosphorus-containing buffer-induced abscission to ethylene production by treated tissues, most notably with leaves. MPK at 3% combined with 500 mg·L⁻¹ ethephon reduced olive FDF by 40% and increased machine removal of oil olives in Spain (Barranco et al., 2004). In our work with California table olives, greater than 75% reduction in FDF was achieved with combination application of 4% MPK + 1000 mg·L⁻¹ ethephon, and leaf loss was no greater than 1000 mg·L⁻¹ ethephon alone. These results indicate that additional trials should focus on MPK + ethephon combinations that maximize FDF and minimize defoliation.

Traumatic acid, an oxidation product of polyunsaturated fatty acids with plant growth regulator activity (Zimmerman and Coudron, 1979), was shown to induce abscission in cotton leaf explants (Strong and Krutwagen, 1967). TA application alone slightly reduced citrus FDF, but efficacy was improved in a synergistic manner when combined with ethephon (Burns and Pozo, unpublished results). TA had no effect on olive FDF, and combining TA with ethephon did not improve efficacy compared with ethephon alone.

Other abscission agents screened in this work were not efficacious. Methyl jasmonate and coronatine were efficacious in citrus (Burns et al., 2003; Hartmond et al., 2000) and grape (Fidelibus et al., 2007) but not olive. Dikegulac and CMNP caused mature fruit abscission in citrus (Burns, 2002; Pozo et al., 2004a) but had little effect on grape (Fidelibus et al., 2007) and no effect on olive. The basis of the differential response of these fruit types is unknown; however, the table olive is harvested immature, whereas grape and citrus are harvested at, or close to, physiological maturity (Ferguson et al., 2005; Spiegel-Roy and Goldschmidt, 1996; Winkler et al., 1974). It is well known that induction and acceleration of abscission is dependent upon the appropriate developmental and environmental cues (Bleeker and Patterson, 1997). The anatomical development of the immature olive fruit abscission zone at the time of commercial harvest may be incomplete (Reed and Hartmann, 1976) or have reduced tissue sensitivity and, thus, the ability to respond to abscission agents (Trewavas, 1986), thereby attenuating or inhibiting response to applied abscission agents. Furthermore, uptake may be influenced by physiological maturity, fruit position, and tissue composition (Ben-Tal, 1992).

In conclusion, trials were conducted at two separate California table olive-growing sites in 2006. We demonstrated that ACC and ethephon alone or in combination with MPK loosened olive fruit destined for the table olive market. Our future work will focus on maximizing the potential of these agents for olive mechanical harvesting and further screening of additional candidates. In this context, an acceptable abscission agent would selectively and predictably loosen

olive fruit and minimize leaf loss with no phytotoxicity and no impact on quality of the final product.

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