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Ethephon Tissue Penetration and Harvest Effectiveness in Olive as a Function of Solution pH, Application Time, and BA or NAA Addition

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Abstract. **Fruit removal force (FRF) and percent leaf drop (LD) of fruit-bearing olive (*Olea europaea* L.) shoots were examined 120 hours after being sprayed with ethephon at 600 mg·liter^{-1} and held under controlled-environmental conditions analogous to those found in the field in California at harvest time in mid-October. FRF was not significantly affected by solution pH, but FRF of all treated shoots was significantly lower than that of the untreated controls. Only at pH 5 was percent LD significantly greater than that of the controls, but, of the shoots treated with ethephon, the lowest percent LD occurred at pH 3. Percent LD after treatment with ethephon at pH 3 was not affected by application time, but FRF was significantly less than the controls' when shoots were treated at 7 AM or 12 PM but not at 5 PM or 10 PM. Adding NAA to the ethephon solution raised FRF and adding BA lowered FRF compared to ethephon alone. Adding NAA or BA did not mitigate percent LD significantly. Adding BA advanced anthocyanin production in fruit. Ethephon penetration of rachides was ≈70% that of petioles. Correlation between ethephon penetration of petioles and percent LD was greater than that between penetration of rachides and FRF. Correlation was significant for both tissues only in the 12 PM pH 3 treatment; correlation was also significant for petiole penetration and percent LD at pH 5. Autoradiographic studies of the ^{14}C-ethephon penetration showed no pH effect, greater penetration into petioles than rachides, and that radioactivity was limited largely to intercellular spaces, with accumulation in vascular bundles, especially xylem. Regardless of treatment, FRF and percent LD are negatively correlated ($r^2 = 0.615$). Mean results to be expected using ethephon as an olive harvest aid under these conditions are an FRF of ≈3 N and a percent LD of ≈15%. The desired low FRF and percent LD were obtained by applying ethephon alone at pH 3 at 7 AM. Raising ethephon solution pH does not increase harvest effectiveness. Chemical names used: (2-chloroethyl)phosphonic acid (ethephon), naphthalene acetic acid (NAA), 6-benzylaminopurine (BA).**

Because harvest costs can account for as much as 60% of production costs, olive producers worldwide have long been interested in the possibility of using mechanical harvesters (Fridley et al., 1973; Hartmann, 1973; Lamouria and Hartmann, 1959; Lang and Martin, 1985; Martin et al., 1981). Mechanical olive harvest can be more efficient using chemical loosening agents to reduce fruit-removal force (FRF). Using such chemicals is especially important in California, where most olives are harvested before reaching physiological maturity, and, as a consequence, FRF is high (Hartmann et al., 1968; Hartmann et al., 1975; Martin et al., 1981). Several chemicals have been tested as fruit-abscission promoters, and the best results have been with ethylene-releasing chemicals (ERCs) (Hartmann, 1973; Hartmann et al., 1968; Hartmann et al., 1970, 1975, 1976; Lavee and Haskal, 1976; Jacobini and Tombesi, 1973; Martin et al., 1981; Polito and Stallman, 1981; Vitagliano, 1969). Only the ERC ethephon, which has been licensed for use in the United States on many edible crops (Kays and Beaudry, 1987), is likely to be approved for use on olive under present regulatory conditions. Despite its widespread use, ethephon, especially as used on perennial fruit trees, has plagued growers with inconsistent results (Beaudry and Kays, 1987).

When applied to olive, ethephon affects fruit and leaf abscission (Lavee and Haskal, 1976; Lang and Martin, 1985, 1987, 1989). Leaf abscission is a serious problem because a loss >25% can adversely affect return bloom (Hartmann, 1973) and the presence of leaf scars permits infection by *Pseudomonas syringae* pv. *savastanoi* Smith, the causal pathogen of olive-knot disease (Hewitt, 1938). Hartmann et al. (1970) applied NAA with ethephon to olive under field conditions and successfully decreased leaf abscission with less fruit loosening. Lang (1987) noted that, of the plant growth regulators, only cytokinins have not been tested for effects on olive fruit abscission. Martin et al. (1981) applied various forms of Ca in ethephon solutions to mitigate leaf loss; leaf loss was reduced, but FRF was increased.

Abscission studies conducted in the field under naturally variable environmental conditions are neither controlled nor repeatable and often lack statistical verification. Likewise, abscission studies conducted in the laboratory under controlled and repeatable conditions usually lack analogy to conditions under which the ERCs would be applied in the field. In the case of ethephon, this is a particularly critical problem. Temperature, relative humidity (RH), and the pH of the water available for mixing the chemical can affect ethylene evolution rate as a result of ethephon decomposition and the extent of tissue penetration (Beaudry and Kays, 1987; Ben-Tal and Lavee, 1976a; Bukovac et al., 1969; Cooke and Randall, 1968; Flore and Bukovac, 1982; Kays and Beaudry, 1987; Klein et al., 1978, 1979; Lavee and Haskal, 1976; Maynard and Swan, 1963; Olien and Bukovac, 1978, 1982; Warner and Leopold, 1969; Yang, 1969).

Ethephon solutions have been buffered to pH 6.3 and 7 to speed up ethylene release and mitigate olive leaf loss (Ben-Tal and Lavee, 1976a; Klein et al., 1979). Lavee and Haskal (1976) examined ethephon application in the field at different times of the

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day, but without conclusive results. Laboratory studies by Lang and Martin (1985, 1987) approached the problem of field analogy indirectly by examining ERC (including ethephon) ethylene-release characteristics and organ response with precise application and using pulses of ethylene gas to model ethylene release from ERCs in the field.

Penetration of ERCs into plants and their subsequent movement within plant tissues can influence their effectiveness (Kays and Beaudry, 1987). Epstein et al. (1977) studied penetration of ^{14}C-ethephon at pH 7 in phosphate buffer into attached and detached olives and rachides (pedicels). More radiolabeled material was found in the fruit when ^{14}C-ethephon was applied at the distal end of the fruit than when it was applied to the pedicel–fruit cavity. These authors concluded that ethephon movement was unidirectional out of the fruit and rachis toward the leaves. Lang and Martin (1986) studied stem-fed or sprayed ^{31}P-ethephon penetration into olive leaves using nuclear magnetic resonance (NMR). Ethephon peaks disappeared and phosphate peaks appeared in a negative linear relationship. However, the lower limit for detecting ethephon using NMR was several times the concentration used in agricultural situations.

This research was undertaken with three purposes in mind. The first purpose was to develop an experimental system that was controlled and analogous to field conditions. The second was to test the following hypotheses: a) solution pH affects olive FRF and percent LD, b) application time (as an expression of temperature and RH) affects FRF and percent LD, c) ethephon penetration influences ethephon's effects on FRF and percent LD, and d) applying NAA or BA with ethephon can help mitigate percent LD. The third was to define the harvest effectiveness of ethephon on olive.

Materials and Methods

The olive inflorescence is complex; its structure has been redefined by Weis et al. (1988, 1991). In the present discussion, rachis is the fruit stem, the pedicel is the point of attachment of the rachis to the fruit, and the peduncle is the thickened portion of the rachis where it attaches to the stem.

Experiments were conducted at 3- to 4-week intervals beginning in late August and ending in early December 1990 and 1991. Fruit-bearing 'Manzanillo' olive shoots were excised in the early morning from trees of the Univ. of California Dept. of Pomology research orchards at Davis, and the proximal ends were placed in a bucket of water. In 1990, each shoot bore at least six rachides; because of the reduced yields following a damaging freeze in December 1990 (Denney et al., 1993), the lower limit was reduced to four rachides in 1991. Most rachides bore one fruit, but many rachides bore two or more fruit. After transport to the growth chamber facility, each shoot was processed to reduce transpiration by removing all tissue beginning at the second node distal to the fruiting area. An upper limit of 10 rachides per shoot was used for 1990 and 1991: any rachides or leaves proximal to the selected fruiting area were removed, but the main stem was retained. The bases of these shoots were recut under running deionized water, then placed in 40-ml vials containing a solution of 8-hydroxyquinoline citrate (8-HQC) at 200 mg·liter^{-1}. 8-HQC is a bactericide used to improve shoot quality and holding time.

Each treatment unit contained six shoots, with a mean of 53.9 fruit and 49.1 nodes per treatment unit. Generally, there were two leaves per node, but there were occasionally three. The treatments were as follows.

Solution pH. The treatment units were sprayed with a solution of ethephon in deionized water at a concentration of 600 mg·liter^{-1} to discern differences among experimental conditions. Concentrations of 900 to 1200 mg·liter^{-1}, which are used in the field, usually reduced mean FRF to below 1.5 N and it was difficult to differentiate between the various treatments being tested. The pH of each solution to be applied was raised from 2.5 using crushed solid KOH and adjusted with 1 N HCl to pH 3, 5, and 7. The three solutions were sprayed on the shoots in the appropriate treatment unit at 5 PM using a hand-pumped household spray bottle in 1990 and an aerosol-spray bomb in 1991. In 1990, cuttings were sprayed to run-off. In 1991, exactly 40 ml of solution was applied to each treatment unit, resulting in complete coverage as indicated by drip-off. A control unit of six shoots was left untreated.

Application time. Using methods described above, a solution of ethephon at 600 mg·liter^{-1} and pH 3 was sprayed on separate treatment units at 5:00 PM, 10:00 PM, 7:00 AM, and 12:00 PM. Two control units of six shoots each were left untreated for processing after 120 h at 5:00 PM and 12:00 PM.

BA or NAA addition. Using methods described above, solutions of ethephon at 600 mg·liter^{-1} and pH 3 were prepared from solutions of BA at 25, 50, and 100 mg·liter^{-1} solubilized in warm distilled water. Concentrations of BA at 125, 200, 250, and 500 mg·liter^{-1} were achieved by adding small amounts of BA solubilized in warm 80% methanol to an ethephon solution at pH 3. The K salt of NAA (NAA-800) at concentrations of 125, 250, 500, 1000, 2000, or 2500 mg·liter^{-1} was added to solutions of ethephon at pH 3 and a concentration of 600 mg·liter^{-1}. Final solution pH was monitored for all solutions. The sprays with these added chemicals were applied on two dates at 5:00 PM and on three dates at 12:00 PM. Control units of six shoots were left untreated at each application time. Additional controls using solutions of BA and NAA alone—that is, without ethephon—were also used.

Control chamber to simulate field conditions. Olive shoots were placed in a growth chamber fitted with a temperature controller. This controller has ramp-and-soak functions that allow the chamber temperature to follow a diurnal sine wave pattern , with a nighttime minimum of 10C at 5:00 AM and a daytime maximum of 30C at 2:00 PM. This diurnal pattern is about the average found at olive-producing sites in California's Central Valley during mid-October, the optimum time for olive harvest (Denney, 1982; Sibbett et al., 1986). In 1990, RH was controlled using the built-in growth chamber controls, with an abrupt change from a nighttime RH of 80% to a daytime RH of 30% and back again at 7:00 AM and 7:00 PM, respectively. In 1991, a temperature controller with ramp-and-soak functions was installed on the growth chamber for RH functions. This allowed the programming of a RH pattern that was also a diurnal sine wave—but the mirror image for that for temperature—with a nighttime maximum of 80% at 5:00 AM and a daytime minimum of 30% at 2:00 PM, also about that found in California's Central Valley in mid-October. Chamber temperature and RH were monitored and recorded using a datalogger equipped with a temperature–RH probe. Incandescent and fluorescent lamps in the growth chamber were turned on at 7:00 AM and turned off at 7:00 PM.

Treated units and untreated controls were harvested after 120 h. The six shoots of each treatment were placed in a plastic bag for transport to the laboratory. In the laboratory, FRF was measured by attaching a hood connected to a hand-held dynamometer to each fruit, which was then pulled off its rachis. FRF was measured as kilograms of force, which was later converted to Newtons. Any fruit that dropped without being attached to the dynamometer were also assigned an FRF of 0.005 kg each. Most of these dropped fruit could not be assigned with any certainly to a particular shoot of the

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six in the treatment unit. LD was calculated as a percent of leaves present. After fruit removal, a cupped hand was passed up and down the stem twice. The number of leaves that dropped was recorded and added to the number of leaves that had dropped of their own accord.

Ethephon tissue penetration. After FRF and percent LD were measured, all rachides and leaves not already removed were taken off the shoots, and the denuded shoots were discarded. Separately, leaves and rachides for each solution pH and application time were washed to remove external ethephon residues by swirling for 1 min in a 0.05% solution of Tween-20 detergent in deionized water and rinsing thoroughly in running deionized water. Each group of leaves and rachides was placed in separate plastic bags and refrigerated at ≈4C overnight for processing the next day. In 1990, two 0.5-g petiole and rachide samples were taken. For the rachis, larger portions of the peduncle and pedicel ends, where abscission zones form, were taken than of the middle of the rachis. Each 0.5-g sample was macerated in 10 ml distilled water using a polytron. In 1991, a 1-g sample of each tissue type from the treatments was macerated in 20 ml distilled water. The macerated mixture was centrifuged at ≈2500 rpm for 3 min. The supernatant was filtered under vacuum using Whatman's no. 1 paper. In 1990, three 1-ml aliquots of the filtrate were taken for each 0.5-g sample. In 1991, four 1-ml aliquots of the filtrate were taken for each 1-g sample. Each aliquot was placed in a 50-ml culture tube, and 1 ml of a 1 M KOH solution was added to each to raise the pH to ≈11.5, at which level virtually all ethephon molecules are in the dianion form and ethephon decomposes and ethylene evolves rapidly (Biddle et al., 1976). The culture tubes were covered with rubber-septum stoppers and allowed to incubate at ≈20C for 24 h. After thorough mixing, a 10-ml gas sample was taken to estimate ethylene using a gas chromatograph fitted with a 2-ml loop, an alumina 60/80 mesh column at 80C, and a single flame ionization detector.

Autoradiography. Nine shoots containing at least four fruit-bearing rachides each were selected from field trees once in January 1992 and processed as described above. Ethephon spray solutions were prepared by mixing a solution of 1,2-^{14}C-(2-chloroethyl)phosphonic acid (Amersham Lot CFQ 5661, dated 12 Oct. 1990; 85 µl; 55 µCi; 46 mCi·mM^{-1}; radiopurity >97.5%) into a regular solution of ethephon in deionized water to produce an effective concentration of 600 mg·liter^{-1}. Three 40-ml samples of this ^{14}C-ethephon-spiked solution were taken, and pH was adjusted to 3, 5, and 7, respectively, using crushed solid KOH and 1 N HCl. Three shoots each were sprayed at each pH using a hand-pumped sprayer inside a plastic bag at 4:00 PM. After spraying, shoots were returned to the 8-HQC solution. The tub containing the nine shoots was placed inside a containment chamber through which air was drawn using a vacuum pump at a flow rate of 300 ml·min^{-1}. Air exiting the containment chamber was passed over granules coated with potassium permanganate to scrub radiolabeled ethylene before venting into the environment. Temperature and RH were monitored using a datalogger equipped with a temperature–RH probe installed in the inlet to the containment chamber. Growth chamber lights were left off during the experiment to avoid any greenhouse effect in the containment chamber.

Shoots were harvested after 120 h, in keeping with the pH experiments described above. Shoots were removed from the containment chamber and immediately placed in plastic bags for disposal. Each of the three groups of three shoots was washed separately for 2 min in a solution of 0.05% Tween-20 in deionized water, then rinsed twice in deionized water. The cuttings were refrigerated overnight for processing the next day. The following afternoon, distal portions of the rachides including pedicels and proximal portions of the petioles still attached to the stem were excised from the washed shoots. The abscission zones form in these areas in the olive. These portions were cut into sections ≈1 mm long.

Samples were divided into groups by tissue (rachis, pedicel, petiole, or petiolar abscission zone), solution pH (3, 5, or 7), and fixation treatment with OsO_4 vapor from OsO_4 dissolved in water held in a film cartridge capsule lid in a covered petri dish with a moist filter paper on the bottom (to prevent excessive tissue dehydration) for 0.5 h. Osmium tetroxide is a powerful oxidizer that has a special attraction to lipids; it is used in electron microscopy to provide detailed staining of membranes, which contain phospholipids. Osmium tetroxide will also stain a variety of other tissues; it is effective in attacking double bonds, such as those found in ethephon and ethylene.

After fixation, samples were dehydrated in a 10% to 100% ethanol series twice, then in a 3 propylene oxide : 1 ethanol (v/v) series, and finally in propylene oxide and resin using a Spurr's resin kit. Resin embedding with continuous mixing continued for 24 h before the samples in resin were allowed to harden in aluminum pans. Sections (2 µm thick) of the various tissue samples were taken using an automated microtome. Sections were applied to drops of distilled water on 80-mm glass slides with 20-mm etched glass ends. No bonding material was used.

The slides were coated with a specialized autoradiographic emulsion diluted 1:1 (v/v) with 0.6 M ammonium acetate dissolved in double deionized water in complete darkness and placed in light-tight boxes with a desiccator material. The boxes were covered with aluminum foil and placed in a refrigerator at ≈4C for 3 weeks to expose the emulsion. In complete darkness, the slides were developed using developer diluted 1:1 (v/v) with double-deionized water and fixed. Slides were counterstained using Toluidine Blue O, rinsed in deionized water, and mounted in glycerol. Slides were viewed and photographed using a photomicroscope.

Results and Discussion

Solution pH. Ethephon solution pH did not significantly affect FRF of the treated shoots, but all were significantly different from the control (Table 1). Percent LD was significantly different from control only at pH 5. Ethephon penetration of rachides was variable, and none of the treated shoots was different from the untreated control (Table 1). There was increasing mean penetration of ethephon in petioles with increasing pH (r = 0.99), but only pH 7 was significantly different from the control. Overall, rachis penetration was only ≈70% of the petiole penetration, a fact that seems to agree with studies indicating that penetration is generally less in fruit tissue than in vegetative tissue (Kays and Beaudry, 1987). This also agrees with the work of Reed and Hartmann (1976) and Polito and Lavee (1980) concerning the different surface characteristics of the areas at which petiole and pedicel abscission zones form. As expected, a negative relationship existed between ethephon penetration and FRF. Likewise, the correlations for percent LD and the petiole penetration showed no particular pattern, but there is a significant correlation (r = 0.92) at pH 5. This result reinforces the significance of the increase in percent LD at pH 5 seen in Table 1. Why this is the case is unclear, but it may relate to the pH of intercellular spaces, normally ≈4.5, which is closer to 5 than to 3 or 7.

These data contradict much of the literature associated with ethephon use on olive. Researchers have reported that raising pH from 3 to ≈7 decreased FRF, presumably because of the more rapid release of ethylene from ethephon at that pH (Ben-Tal and Lavee,

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Table 1. Effect of solution pH of ethephon at 600 mg liter^{-1} applied at 5 PM on fruit-removal force (FRF), percent leaf drop (LD), and ethylene evolved as a measure of ethephon penetration of target organ. Values are ±SD.

	FRF	LD	Ethylene evolved (μl·liter·h^{-1}·g^{-1} fresh weight)	
pH	(N)	(%)	Rachis	Petiole
3	3.2 ± 1.7 b[z]	9.7 ± 6.7 ab	5.7 ± 7.6 a	4.5 ± 3.7 ab
5	2.9 ± 2.1 b	20.0 ± 21.7 a	3.3 ± 2.2 a	6.1 ± 4.5 ab
7	2.7 ± 1.3 b	15.0 ± 14.1 ab	5.0 ± 3.2 a	8.6 ± 6.7 a
Control	5.0 ± 0.7 a	3.3 ± 3.0 b	1.0 ± 1.2 a	1.1 ± 1.1 b

[z]Mean separation within columns at $P = 0.05$ using Duncan's multiple range test.

1976a, 1976b). A body of literature has evolved based on this point, and the presumed pulse of ethylene that comes from ethephon decomposition as the daily temperature rises in the field increases the decomposition rate (Epstein et al., 1977; Klein et al., 1978, 1981). In most cases in which ethephon was used, the pH was raised using phosphate buffers. Klein et al. (1978) used either phosphate buffer or NaOH to raise the pH, but they did not distinguish results based on this characteristic. Recent work in our laboratory indicated that phosphate alone has a pronounced effect on olive leaf and fruit abscission (Banno et al., 1993; Tiefengraber et al., 1994), and we conclude that the decrease in FRF seen at the higher pH under these circumstances is due, at least in part, to the action of phosphate in conjunction with that of ethylene from ethephon, rather than increased ethephon decomposition.

Autoradiography. In the autoradiographic studies, the presence of ^{14}C exclusively in ethephon was not established, therefore the term radioactivity will be used when referring to presence of label in autoradiographs. No differences in ethephon penetration as a function of solution pH could be determined. Tissue type was more important for determining extent of penetration. Most radioactivity seemed to be associated with vascular tissue. Radioactivity was found in the intercellular spaces of petiolar abscission zone (Fig. 1A) and was associated with xylem tracheids in attending tissue (Fig. 1B). Radioactivity was also found in the intercellular spaces of mostly xylem tissue in the vascular bundles of pedicels (Fig. 1C) with less found in the pith (Fig. 1D). Overall, the most radioactivity was found in pedicels rather than in other tissue types. These studies make clear what has long been suspected. Best results in terms of reducing FRF are obtained when ethephon is applied to the cavity at the pedicel–fruit junction, and ethephon solution naturally accumulates in this spot when the chemical is sprayed on the tree (Epstein et al., 1977; Lang and Martin, 1987). This accumulation likely means that more chemical penetrates into the tissue at this place than in other tissues.

Epstein et al. (1977) found that there was movement of ethephon in the olive rachis, and they theorized that movement was unidirectional from the fruit to the leaves. The clear association of ethephon with vascular tissues in the present study supports this theory. However, our study shows a clear association of ethephon with the xylem. It is possible that radioactivity seen in the xylem tissue remains there because of lack of ethephon transport. It seems far more likely, however, that the presence of ethephon in the xylem indicates that these tissues are involved in the chemical's transport. It is possible, but not likely, that movement from the fruit to the leaves as proposed by Epstein et al. (1977) takes place in the xylem. However, these authors point out that olives shrivel under water stress, and they imply that loss of water to the leaves rather than through fruit lenticels is the cause of this shrivel. This notwithstanding, the movement of materials away from a strong sink such as the fruit would more likely take place in the phloem. However, no support that phloem transports ethephon emerged in this study.

Application time. None of the four timed applications of ethephon at pH 3 differ from each other in terms of FRF, but two of these, 7 AM and 12 PM, were different from the controls (Table 2). Despite the wide disparities in the percent LD means, there were no significant treatment effects. Ethephon tissue penetration as gauged by ethylene evolution did not differ from treatment to treatment, but rachis penetration was only ≈70% of that of petioles (Table 2).

Correlations between tissue penetration and FRF and percent LD are significant only at 12 PM ($r = –0.95$ and 0.98, respectively). The low FRF and high percent LD at 12 PM and the close correlation of these parameters with ethephon penetration may be the result of higher temperature or lower RH. The results tend to agree with those of Klein et al. (1978), who showed acceptable FRF and moderate leaf loss at moderate temperatures and higher RH than at higher temperatures and low RH. The key to ethephon's penetration may be the level of daily hydration. Water accumulates repeatedly in the pedicel–fruit cavity at 7 AM when RH is high, thereby allowing dried ethephon to redissolve on the surface and increasing the probability of its penetration. However, this same rehydration occurs on the petiole when the cuticle is most resistant, decreasing the probability of its penetration. This process may repeat for both tissues day after day as the pattern of low temperature and high RH is repeated. Nevertheless, the difference in response to the application time suggests that the environmental conditions under which the initial application is made are most important. That stomata are more likely to be open at 12 PM and 7 AM may also affect ethephon leaf penetration and degree of abscission (Flore and Bukovac, 1982). However, the data presented here (Table 2) showing a similar change in degree of ethephon penetration of rachis and petiole at 12 PM indicate that changes in surface characteristics are probably more important.

Addition of BA or NAA. FRF for treatments with NAA or BA added to ethephon solution were not different from treatments with ethephon alone (Table 3). However, since the treatments with NAA and BA added are significantly different from each other, some effect on fruit and leaf abscission is apparent. BA decreased and NAA increased FRF compared to ethephon alone. Neither NAA or BA affected percent LD. Overall, NAA or BA concentration seems to have made little difference in terms of FRF or percent LD.

To summarize results with regard to our experimental hypotheses, 1) pH of applied ethephon solutions did not affect FRF, but pH 5 negatively affected percent LD compared to the control; 2) applying ethephon at 7 AM or 12 PM lowers FRF significantly but applying it at 5 PM or 10 PM does not lower FRF significantly compared to the control; percent LD was not significantly affected by application time; 3) adding BA or NAA to the ethephon solution did not mitigate percent LD significantly compared to ethephon alone, and 4) penetration studies did not clarify the effect of this factor in ethephon use on olive, but significant correlations with percent LD at pH 5 and with FRF and percent LD at pH 3 applied

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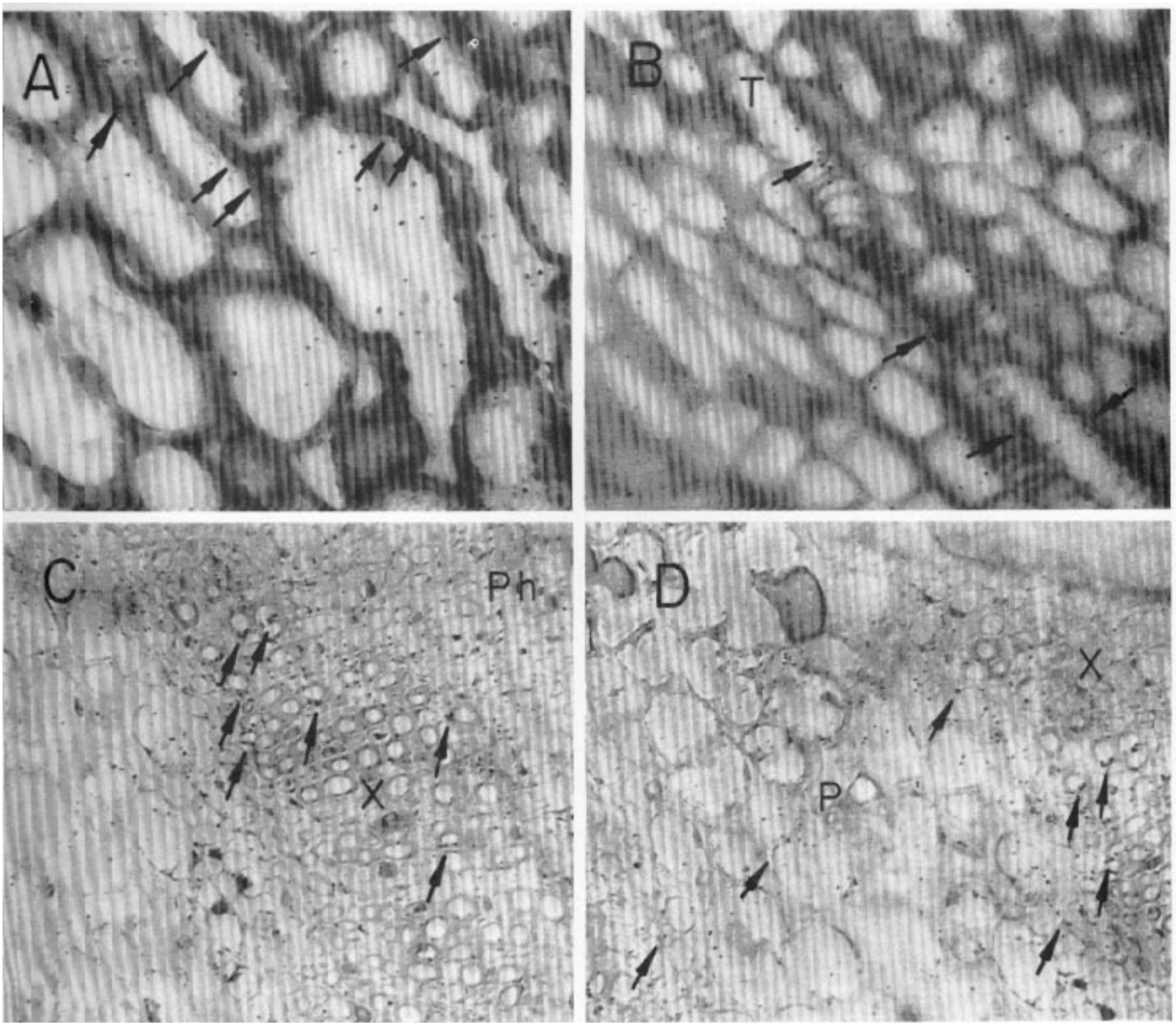

Fig. 1. (**A**) Ethephon in intercellular spaces in a petiolar abscission zone (×157). Some silver grains, especially those in the cell lumens (bound ethylene?) are probably from background, but others in groups (arrows) are signs of radiolabeled ethephon. (**B**) Longitudinal section of a petiolar abscission zone xylem tracheid (T) (×157). Some of the silver grains seen in the print are probably from background, but the groups of silver grains along the tracheid running from top left to bottom right (arrows) are from radiolabeled ethephon. (**C**) Cross section of a pedicel (×98). More silver grains are found around the xylem vessels (X) (large lumens) running from right to left than in the phloem (Ph) (**D**) Cross section of a pedicel (×98). Relatively few silver grains (arrows) indicating the presence of radiolabeled ethephon appear in the pith (P) in the center as compared to the xylem (X) and other vascular tissue at top and bottom.

Table 2. Effect of application time of ethephon at 600 mg·liter^{-1} and pH 3 on fruit-removal force (FRF), percent leaf drop (LD), and ethylene evolved as a measure of ethephon penetration of target organ. Values are ±SD.

	FRF	LD	Ethylene evolved (µl·liter·h^{-1}·g^{-1} fresh weight)	
Time (h)	(N)	(%)	Rachis	Petiole
5 PM	3.2 ± 1.9 ab[z]	17.5 ± 17.1 a	1.4 ± 0.7 a	2.2 ± 0.7 a
10 PM	3.8 ± 0.8 ab	16.2 ± 11.2 a	1.6 ± 1.4 a	2.3 ± 1.7 a
7 AM	2.9 ± 0.9 b	10.2 ± 10.6 a	1.2 ± 0.7 a	2.1 ± 0.7 a
12 PM	2.4 ± 1.1 b	23.1 ± 4.4 a	2.5 ± 2.0 a	3.2 ± 0.9 a
Control	4.8 ± 0.7 a	6.0 ± 11.0 a	1.3 ± 1.7 a	1.4 ± 1.6 a

[z]Mean separation within columns at $P = 0.05$ using Duncan's multiple range test.

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Table 3. Effect of adding NAA or BA to ethephon at 600 mg·liter^{-1} and pH 3 on fruit-removal force (FRF) and percent leaf drop (LD).Values are ±SD.

Treatment	FRF (N)	LD (%)
Ethephon + BA (50 mg·liter^{-1})	2.0 ± 1.7 c[z]	17.3 ± 11.7 a
Ethephon + BA (all concentrations)	2.0 ± 1.3 c	17.0 ± 14.6 a
Ethephon	2.8 ± 1.6 bc	15.8 ± 10.8 a
Ethephon + NAA (500 mg·liter^{-1})	3.8 ± 1.0 ab	7.6 ± 7.7 a
Ethephon + NAA (all concentrations)	3.9 ± 0.9 ab	9.5 ± 8.9 a
Control	4.8 ± 0.7 a	5.4 ± 8.6 a

[z]Mean separation at $P = 0.05$ using Duncan's multiple range test.

at 12 PM suggest that it is an important factor. Autoradiographic studies suggest that xylem transports ethephon in olive and that ethephon accumulation is greater in pedicel tissue than in other tissues.

Harvest effectiveness. The literature on ethephon as an olive harvest aid generally deals in terms of reduced FRF, and percent LD is either ignored completely or discounted (Ben-Tal and Lavee, 1976a, 1976b; Klein et al., 1978). Leaf drop may not even be mentioned, and, if it is, it is rarely quantified. An exception is the work of Lang and Martin (1987, 1989), who used fruit-to-leaf abscission ratios when evaluating ERCs and gaseous ethylene treatments. Percent LD is an important factor in deciding the effectiveness of chemical harvest aids on olive because of the importance of leaves for return bloom and because of the threat of olive-knot disease infection (Hartmann, 1973, Hewitt, 1938; Lang and Martin, 1987). In our results, different treatments that produced similar results in terms of FRF did not have similar results in terms of percent LD. This was especially true when the pH of the ethephon solution is considered (Table 1).

There is, however, another important reason to include percent LD along with FRF in any definition of harvest effectiveness of ethephon on olive. These two parameters are inextricably related. For example, FRF declines as percent LD increases in controls compared over time (Fig. 2). Also, when all the treatments tested in the present studies are considered together, a very clear pattern emerges: there is a negative linear relationship between FRF and percent LD ($r^2 = 0.615$). This relationship is true for the controls for treatments using ethephon alone or in treatments using ethephon with growth regulators added (Fig. 3).

At a FRF of 1 N, 100% fruit drop does not result in 100% leaf drop because leaf petioles and fruit rachides do not respond identically to ethylene. Lang and Martin (1985) previously suggested that pedicel and petiole tissues have different sensitivities to ethylene, with the pedicel tissues responding more quickly and for less time than the leaf tissue. It may also be that the more conservative response to ethylene found in leaf tissue allows for a wider variation in responsiveness brought about by other factors, such as tissue age and environmental conditions.

Harvest effectiveness under the conditions established for these experiments can be seen as a convergence between decreasing FRF and increasing percent LD. The point of convergence that emerges from our data is a FRF of 3.0 ± 0.6 N and a percent LD of 14.7 ± 4.4%. A FRF of ≈3 N should allow for ≈85% fruit removal, an acceptable level for commercial production. It also seems likely that anything added to mitigate leaf abscission, such as auxins or Ca compounds, will have a similar effect on fruit abscission (Hartmann et al., 1970;

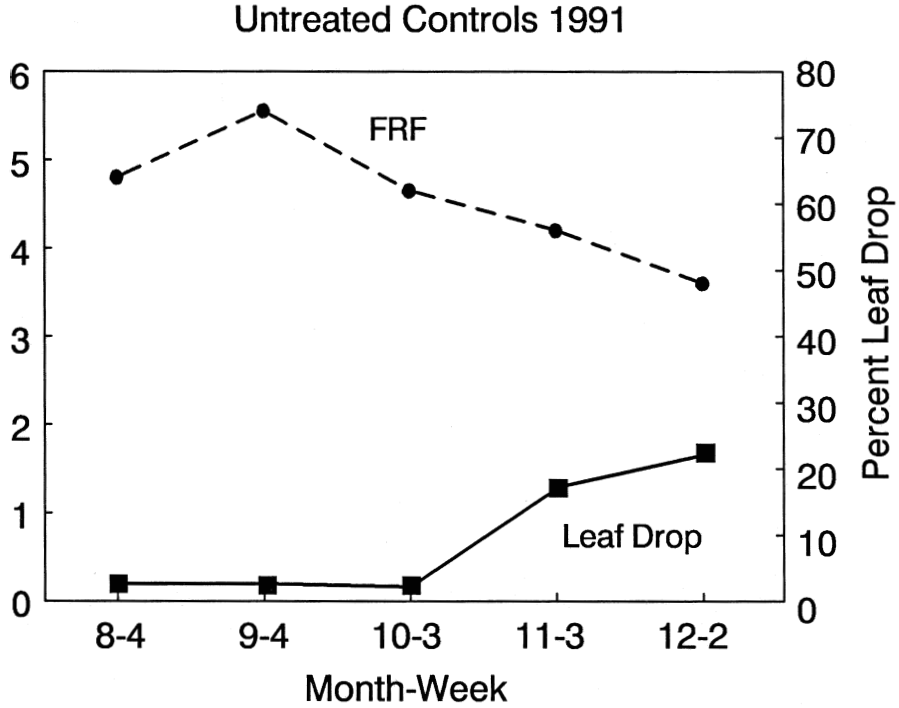

Fig. 2. Fruit-removal force (FRF) and percent leaf drop for controls, 1991. In the olive shoots not treated with ethephon, mean FRF declined slowly from late summer to late autumn, whereas mean percent leaf drop increased over the same period.

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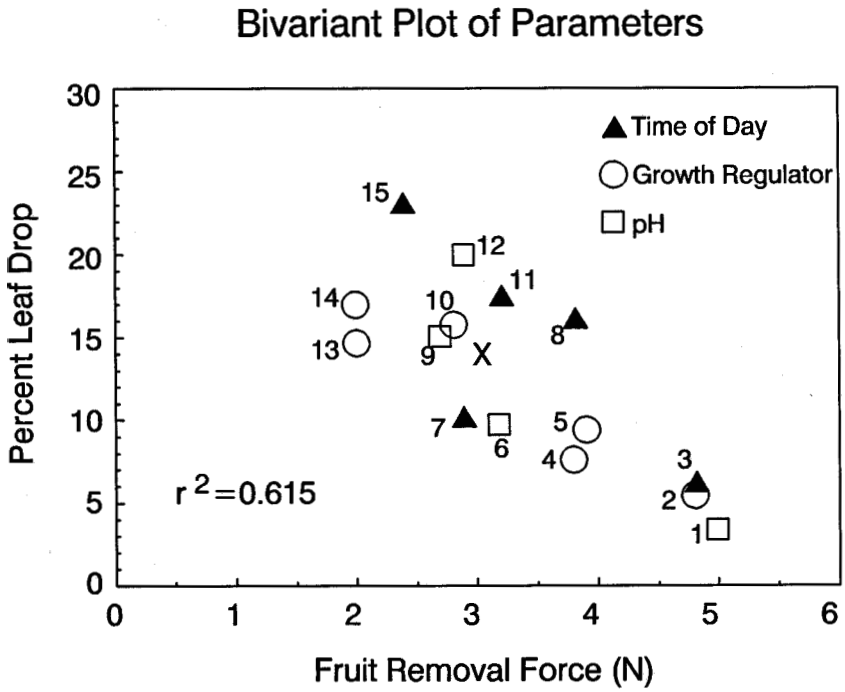

Fig 3. Bivariant plot of all treatments. There is a strong, negative linear relationship between declining fruit-removal force and increasing percent leaf drop ($r^2 = 0.615$). The two processes seem to be interconnected, but pedicels may be more sensitive to ethylene than are petioles. Treatments: 1) pH control, 2) growth regulator control, 3) application time control, 4) ethephon + NAA (500 mg·liter^{-1}), 5) ethephon ± NAA (all concentrations), 6) ethephon pH 3, 7) ethephon 7 AM, 8) ethephon 10 PM, 9) ethephon pH 7, 10) ethephon alone (growth regulator experiments), 11) ethephon 5 PM, 12) ethephon pH 5, 12) ethephon + BA (all concentrations), 14) ethephon + BA (50 mg·liter^{-1}), 15) ethephon 12 PM; X) mean of all treatments containing ethephon.

Martin et al., 1981). Any factor that reduces FRF or percent LD from this point will add to ethephon's effectiveness.

Increasing harvest effectiveness of ethephon on olive will probably be achieved by studying the surface characteristics of petioles and rachides, with an eye toward decreasing petiole penetration or increasing rachis penetration. Progress may be made using surfactants. Our research suggests that a low solution pH is best; higher pH results in higher petiole penetration and percent LD. Our research and that of others indicate that applying ethephon under relatively cool, moist conditions is more effective than under warmer, drier conditions.

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