The Role of Phosphorus as an Abscission-inducing Agent for Olive Leaves and Fruit

Kiyoshi Banno[1], George C. Martin, and Robert M. Carlson
Department of Pomology, University of California, Davis, CA 95616

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Abstract. Citrate-phosphate buffer induced olive (*Olea europaea* L.) leaf abscission at pH 3, 5, or 7. Of several sources of P-induced leaf abscission, NaH_2PO_4 was the most effective. Sensitivity to P was shown by leaf abscission, leaf desiccation, or both among 32 species representing 22 genera. Applied P accumulates in petioles, but its effect on abscission does not seem to depend on ethylene production. The low P content in the ethephon molecule may have an additive effect in ethephon-induced abscission. Stem-fed NaH_2PO_4 or ethephon resulted in total leaf and fruit abscission. Of the chemicals applied as a foliar treatment, only NaH_2PO_4 caused fruit abscission with minimal leaf loss. Adding Al_2O_3 to adsorb P in treatment solutions delayed the abscission effect of ethephon and NaH_2PO_4. Adding glycerol to NaH_2PO_4 increased fruit abscission from 50% to ≈80% and leaf abscission from ≈9% to 18%. The presumed effect of glycerol is from slowing the drying rate and thereby increasing P penetration into the fruit abscission zone. The pedicel-fruit cavity is a collection basin for spray accumulation that is not present in the leaf petiole attachment to the stem. This morphological difference probably leads to greater absorption of abscission-inducing materials by fruit. Chemical name used: (2-chloroethyl)phosphonic acid (ethephon).

Ethephon is the most widely used ethylene-releasing compound in agriculture to induce fruit abscission (Ben-Tal and Lavee, 1976; Daniel and Wilkinson, 1972; Martin et al., 1981). Mechanically harvesting olives removes only 60% of the fruit, an unacceptable degree of removal. Also, the remaining fruit lead to alternate bearing. For olive, all ethylene-releasing compounds, including ethephon, have been tested extensively as fruit looseners for mechanical harvest, but their acceptance for olive has been precluded because of excessive leaf loss (Martin et al., 1981). Leaf loss in olive at harvest leads to the entry of olive knot bacteria (*Pseudomonas syringae* pv. *savastanoi),* reduced crop the next year, and tree decline (Martin, 1986).

Potential fruit- and leaf-abscission-inducing chemicals are tested routinely in our laboratory using an olive explant system. Olive shoots containing six nodes and 12 leaves are taken from shoots in the field and placed with their cut ends in vials of solution for testing (Lang and Martin, 1985). Using the explant system, we accidentally found that citrate-phosphate buffer induced leaf abscission. This serendipitous finding has led to extensive investigations into new strategies for obtaining olive fruit abscission. The purpose of this investigation was to identify the abscission-inducing agent in citrate-phosphate buffer; explore its range of influence on several species, including our target species *Olea europaea;* and explore the mechanism of abscission.

Materials and Methods

Vegetative shoots of 'Manzanillo' olive were collected as needed from the field during the growing season at the Univ. of California, Davis. Excised shoots were placed immediately in buckets of water so that cut ends were immersed for transport to the laboratory. There, shoots containing six nodes with pairs of 1 -year-old leaves were cut under water to ≈20 cm long and placed

into 50-ml test tubes of either distilled water or treatment solutions.

Phosphorus-induced leaf abscission using stem-fed explants. In the pH experiment, citrate-phosphate buffer solutions were mixtures of 100 mM citric acid and 200 mM NaH_2PO_4 to achieve pH 3.0, 5.0, and 7.0. In the initial experiments, citrate-phosphate buffer induced leaf toxicity and extensive defoliation; thus, all subsequent experiments reported here were conducted at 10% of the original strength (10 mM citric acid and 20 mM NaH_2PO_4).

To clarify which component of the citrate-phosphate buffer caused abscission, citric acid and NaH_2PO_4 were tested separately at 0.25, 2.5, and 25 mM. After finding that NaH_2PO_4 was the active component, sodium chloride, sodium carbonate, monobasic calcium phosphate, and monobasic potassium phosphate were compared at 0.25, 2.5, and 25 mM to determine which ion in NaH_2PO_4 induced leaf abscission. Solution pH was adjusted routinely to 5 with 0.1 N HC1.

In the stem-fed explant system, 200 mg ethephon/liter (≈ pH 3.0) resulted in complete leaf abscission within a few days. Leaf abscission with ethephon at 200 mg·liter[-1] was compared with 25 mM NaH_2PO_4, and each compound was tested further by adding 500 mg Al_2O_3/ml to adsorb phosphate (Elliot et al., 1983). Al_2O_3 is not soluble in water, and the ratio of solid to solution provides a superabundant surface for adsorption of phosphate at 25 μM inorganic P (Elliot et al., 1983).

The location of P accumulation in the explants was estimated by measuring total P after acid digestion (Skinner et al., 1987). Phosphorus content was compared in stem bark and wood and in leaf petioles and blades during the first 4 days of P stem feeding. The notion that P may induce leaf abscission via elevated ethylene production was tested by measuring ethylene daily in P-treated explants (Lavee and Martin, 1981a).

The range of P-induced leaf abscission was tested on 22 genera representing 32 tree species using 25 mM NaH_2PO_4, pH 5, stem-fed to excised shoots during the last week of Sept. 1991. Each shoot without fruit or flowers was cut to ≈25 cm long, placed in water, carried to the laboratory, recut under water, and placed in a vial containing phosphate. Leaf abscission was evaluated for 10 days.

Phosphorus-induced leaf and fruit abscission using stem-fed and foliar-sprayed explants. Olive explants maintain water use and "normal" appearance for 7 days in the laboratory. By day 10,

however, the explants take up little water per day, begin to show leaf chlorosis, and leaf abscission may begin. All experiments reported in this segment were terminated 7 days after treatment so that comparisons of treatment effects would be made before control explant deterioration was apparent.

Fruiting shoots of 'Manzanillo' olive were collected and treated monthly starting from middle Aug. to early Oct. 1991. Shoots containing six nodes and six to eight fruit were chosen and recut under water to ≈20 cm long and placed into 50-ml test tubes as described before.

Olive leaf and fruit abscission rates in stem-fed explants were compared among 2.5 and 25 mM NaH_2PO_4, 200 mg/liter ethephon, 200 mg ethephon/liter with ethylene removed (ethephon at pH 7 adjusted with NaOH), and 200 mg ethephon/liter with 500 mg Al_2O_3/ml to adsorb phosphate.

Sodium dihydrogen phosphate at several concentrations was applied as a foliar spray to the fruiting shoots with 0.25% Regulaid (Kalo Agricultural Chemicals, Petaluma, Calif.) added as a wetting agent. Adding 1% glycerol in the spray solution was tested to prolong drying time and promote phosphate penetration (Fisher and Walker, 1955), and two sprays (separated by 4 h) of NaH_2PO_4 at 50 mM were compared to one spray at 100 mM. In a final experiment, one droplet (≈30 µl) of 200 mg ethephon/liter or 100 mM NaH_2PO_4 was applied to the pedicel-fruit cavity and the leaf axil, and the leaf and fruit abscission response was compared to that obtained with foliar sprays of 200 mg/liter ethephon and 100 mM NaH_2PO_4.

In each experiment, 12 replications were used in a randomized complete-block design. Leaf and fruit abscission was determined daily by touching the explants lightly with a finger. All data were tested by analysis variance and the means were compared by Duncan's multiple range test.

Results and Discussion

All initial experiments were continued until all leaves abscissed. At pH 3, 5, and 7, citrate-phosphate buffer stimulated leaf abscission compared with the control, but there was no significant difference among the citratephosphate buffer treatments (Fig. 1). Although no evidence was found for pH to influence abscission, it was unclear which component (citric acid or NaH_2PO_4) of the buffer solution induced abscission. A comparison at three concentrations showed that NaH_2PO_4 influenced leaf abscission more than citric acid (data not shown). Citric acid up to 2.5 mM did not
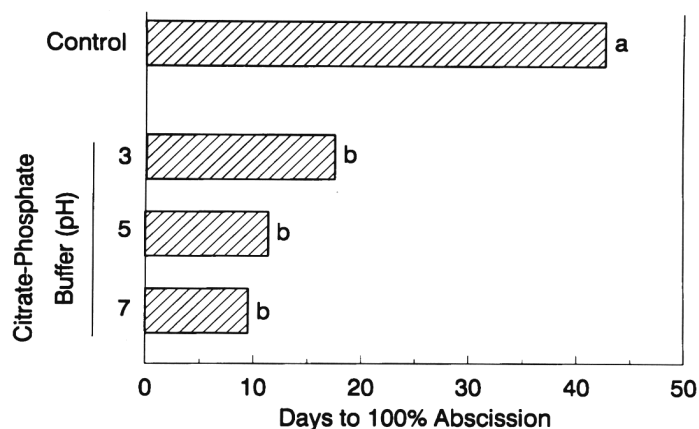

Fig. 1. Effect of stem feeding citrate–phosphate buffer at three pHs and a pH 5 water control on 'Manzanillo' olive leaf abscission from excised shoots. Mean separation by Duncan's multiple range test at $P \le 0.05$.
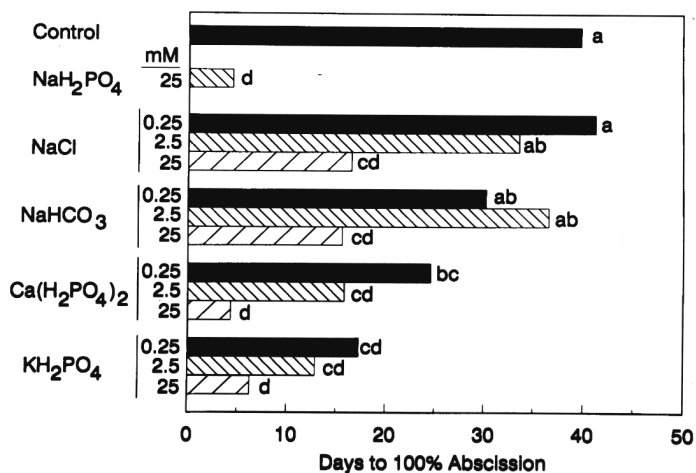

Fig. 2. Effect of stem feeding several Na and phosphate compounds on leaf abscission from excised 'Manzanillo' olive shoots; pH of all solutions was adjusted to 5. Mean separation by Duncan's multiple range test at $P \le 0.05$.

significantly affect leaf abscission in the controls, whereas even 0.25 mM NaH_2PO_4 significantly induced leaf abscission more than citric acid or control treatments.

Table 1. Leaf abscission response to 25 mM NaH_2PO_4 stem-fed to excised shoots of selected tree species.

Leaf desiccation and death, no abscission	Leaf desiccation and abscission	Leaf abscission without desiccation	No leaf desiccation or abscission, but white exudate from leaf petiole
Eucalyptus polyanthemos Schauer	Celtis occidentalis L.	Actinidia chinensis Planch.	Buxus microphylla Siebold and Zucc.
Ficus carica L.	Diospyros kaki L.f.	Feijoa sellowiana O. Berg	Euonymus japonica Thumb.
Juglans hindsii Jeps.	Eriobotrya japonica (Thumb.) Lindl.	Olea europaea L.	Ginkgo biloba L.
Liriodendron turipifera L.	Malus pumila Mill.	Populus alba L.	Nerium oleader L.
Malus baccata (L.) Borkh.	Prunus avium L.	Ulmus americana L.	
Pistacia vera L.	Prunus cerasus L.		
Prunus armeniaca L.	Prunus cerasifera J.F. Ehrh.		
Prunus dulcis Mill.	Prunus domestica L.		
Prunus persica (L.) Batsch.	Pyrus calleryana Decne.		
Prunus salicina Lindl.	Pyrus communis L.		
	Pyrus pyrifolia (Burm.f.) Nakai		
	Quercus suber L.		
	Vitis vinifera L.		

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Sodium dihydrogen phosphate greatly advanced the onset of leaf abscission, but the ion that was most active in the abscission response remained unknown. To determine the active ion, several forms of Na and P were tested at 0.25, 2.5, and 25 mM. Although all compounds tested at 25 mM induced olive leaf abscission, only P sources gave the rapid and significant effects at 0.25 or 2.5 mM (Fig. 2).

Thirty-two species representing 22 genera were treated with stem-fed 25 mM NaH_2PO_4. The most rapid leaf abscission-occurring without deleterious visual symptoms such as chlorosis, desiccation, or leaf curling-was found with *Actinidia, Feijoa, Olea, Populus,* and *Ulmus* (Table 1). All other species showed a range of responses including leaf desiccation, death, white exudate on leaf petiole, and no leaf abscission. In these other species, 25 mM NaH_2PO_4 could have been phytotoxic; lower concentrations were not tested, nor was xylem plugging determined.

We hypothesized that the rapid olive leaf abscission that occurs without leaf chlorosis after P is stem-fed was due to its accumulation in the explant and its subsequent induction of ethylene synthesis. With 25 mM NaH_2PO_4, olive leaf abscission occurs within 2 days of stem feeding (Fig. 3). Phosphorus concentration in bark and wood below leaves reached a maximum within 2 days of P stem feeding (Fig. 4). In leaf blades, P concentration leveled off by day 3. In contrast, petiole P accumulation continued linearly for 4 days to the end of the experiment. Because the leaf abscission zone is found where the petiole attaches to the shoot, the amount and rate of P accumulation in the petiole was thought to bear directly on the abscission mechanism.

If ethylene production in P stem-fed olive explants caused leaf abscission, then the elevated ethylene evolution should occur well before abscission. We have shown previously that explants must be exposed to ethylene gas ≈96 h before leaf abscission (Lavee and Martin, 1981a-c). Also, in field experiments, fruit or leaf abscission is not apparent for ≈7 days after ethephon treatment (Martin et al., 1981). Measuring ethylene evolution after P stem feeding did not correlate closely with the leaf abscission response (Figs. 3 and 5). In fact, ethylene evolution seems to parallel rather than precede leaf abscission. Even more important, the amount of ethylene measured (<50 ppb per container with leaves enclosed) may have been too low to influence leaf abscission. Our previous experiments have shown that 3 to 5 ppm ethylene are required in a closed system for at least 28 h to induce leaf abscission, which would occur ≈96 h later (Lang and Martin, 1989); thus, rates at ≤50 ppb in an open system are unlikely to induce leaf abscission. Even when explants were stem-fed 500 mg/liter ethephon for 6 h, ≈54 h elapsed before all leaves abscissed (Lavee and Martin, 1981a). Other researchers using tomato pericarp disks, apple slices, or microorganisms showed that phosphate concentrations greater than ≈200 mM inhibited ethylene production (Fuchs et al., 1981; Sobolewska and Plich, 1986).

Ethephon contains P, and we were interested in determining to what extent the P in ethephon might contribute to olive leaf abscission. Al_2O_3 was added to NaH_2PO_4 and ethephon solutions to adsorb phosphate in stem-feeding experiments. When either 25 mM NaH_2PO_4 or 200 mg ethephon/liter was used alone and fed continuously, all leaves abscissed by 6 and 3 days, respectively (Fig. 6). When 500 mg Al_2O_3/ml was added to each compound, all leaves abscissed in 13 and 11 days, respectively. The Al_2O_3 reacts with phosphate; thus, we assumed that Al_2O_3 adsorbed phosphate as ethephon broke down to release ethylene. Clearly, P, even in ethephon, contributes to olive leaf abscission. Additional evidence of the role of P from ethephon in leaf abscission was gained by adjusting ethephon to pH 7 with NaOH to drive off the ethylene
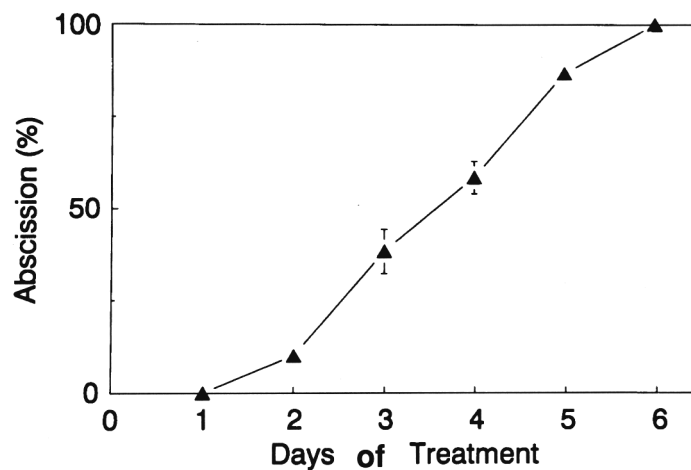

Fig. 3. Effect of stem feeding 25 mM NaH_2PO_4 at pH 5 on 'Manzanillo' olive leaf abscission from excised shoots. Vertical bars indicate an SE that exceeds the height of the markers at days 3 and 4 only.
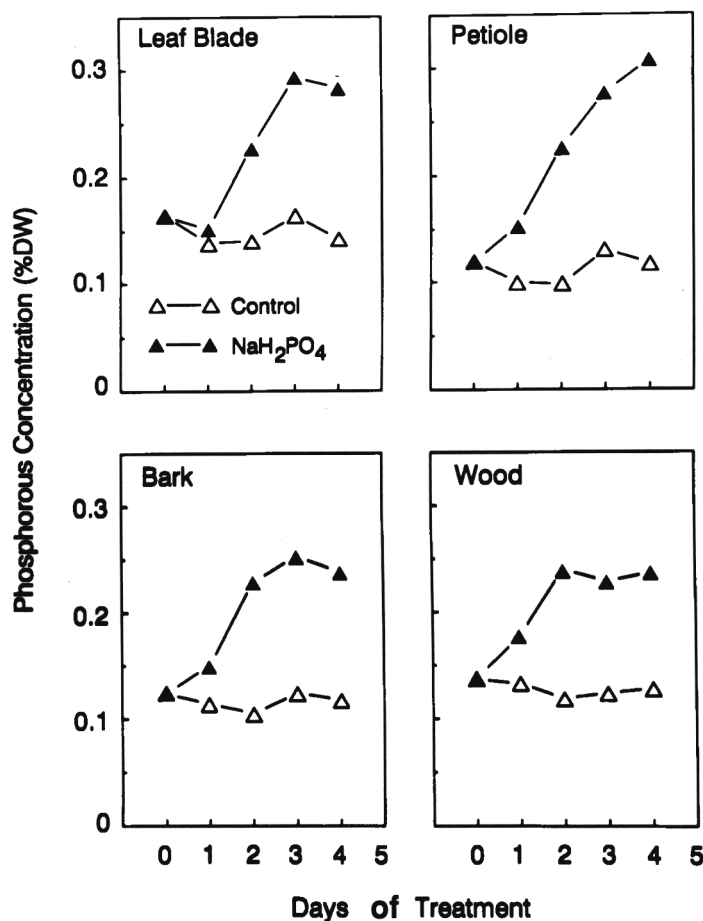

Fig. 4. Effect of stem feeding 25 mM NaH_2PO_4 at pH 5 on P concentration in portions of excised olive shoots. SEs were smaller than the height of the triangle markers.

(ethylene in ethephon is stable at pH 3). Ethephon was allowed to evolve ethylene for 4 days after being adjusted to pH 7 before the solution was stem-fed to explants. The amount of P remaining in the ethephon solution after driving off ethylene was calculated at 1.38 mM, if all ethylene was evolved from the ethephon molecules. Even this low amount of P was sufficient to induce leaf abscission (Fig. 6). Although not shown, 500 mg Al_2O_3/ml did not induce leaf abscission.
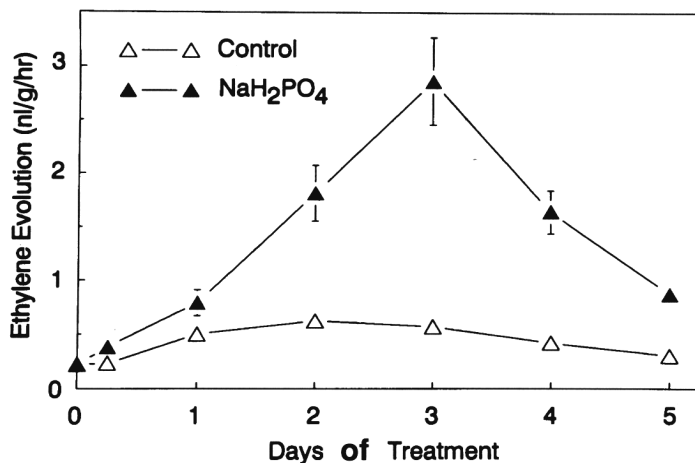
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Fig. 5. Effect of stem feeding NaH_2PO_4 at pH 5 to excised shoots on ethylene evolution from 'Manzanillo' olive leaves. Vertical bars indicate an SE that does not exceed the height of the triangle markers in some cases.
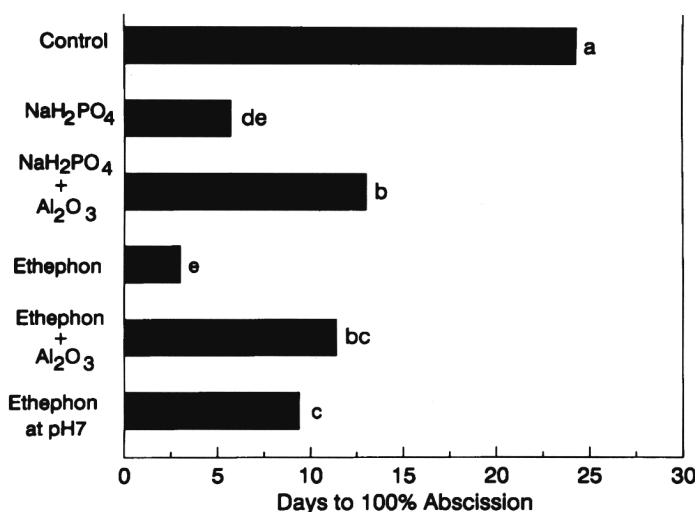

Fig. 6. Effect of stem feeding 25 mM NaH2PO4 at pH 5 or 200 mg ethephon/liter at pH 3 with and without adding 500 mg/ml Al_2O_3 on 'Manzanillo' olive leaf abscission from excised shoots. Mean separation by Duncan's multiple range test at $P \leq 0.05$.

Explants with fruit were sampled and treated monthly starting Aug. 1991. The data presented in Figs. 7-10 are from late September and early October and are typical of results from similar experiments repeated in each one of the 3 months. The data selected for publishing represent the normal harvest months for California olive (Sibbett et al., 1986).

When stem-fed, 25 mM NaH_2PO_4 or 200 mg ethephon/liter led to rapid leaf and fruit abscission (Fig. 7). At a lower NaH_2PO_4 concentration or with Al_2O_3 added to ethephon, leaf and fruit abscission was delayed (Fig. 7). When ethylene was driven from ethephon by 4 days of treatment at pH 7 (NaOH), subsequent explant treatment with the ethylene-free solution led to delayed leaf and fruit abscission. In the latter case, we suspect abscission was induced by the phosphate residue in the original ethephon solution and any remaining ethylene. Note that the amount of leaf and fruit abscission from the ethephon at pH 7, with a calculated 1.38 mM P content, and that of 2.5 mM NaH_2PO_4 was similar (Fig. 7).

Explants with fruit were sprayed with 100, 250, or 500 mM NaH_2PO_4 or 500 mg/liter ethephon. These concentrations were

chosen after preliminary experiments showed that higher concentrations of the chemicals were required to cause abscission via foliar sprays than stem-feeding. The higher concentrations may be required, since the thick waxy overlapping peltate trichomes may reduce the penetration of the chemicals into olive leaves or fruit (Weis et al., 1988). Extensive leaf and fruit abscission resulted from ethephon sprays (Fig. 8). In contrast, even 500 mM NaH_2PO_4 induced <25% leaf abscission, while fruit abscission was equal to that obtained with ethephon. This result supports our notion that NaH_2PO_4 and ethephon may induce fruit abscission by collecting in the pedicel-fruit basin, resulting in greater absorption, whereas no such pooling site is available on a leaf. Also, we conjectured that the polar NaH_2PO_4 molecules would penetrate the leaf surface less than the nonpolar ethephon molecules. The contrast in leaf and fruit morphology may be exploited, as the anatomical details of olive leaf and fruit abscission processes are similar (Polito and Lavee, 1980; Reed and Hartmann, 1976; Weis et al., 1988, 1991).

Even 25% olive leaf abscission at harvest is thought to be excessive, to reduce flowering the next year, and to increase the entry of olive knot bacteria at leaf scars (Martin, 1986). Lower concentrations of NaH_2PO_4 as single and double sprays (after the first spray dried) and adding glycerol to NaH_2PO_4 to prolong drying time and increase the opportunity for chemical penetration
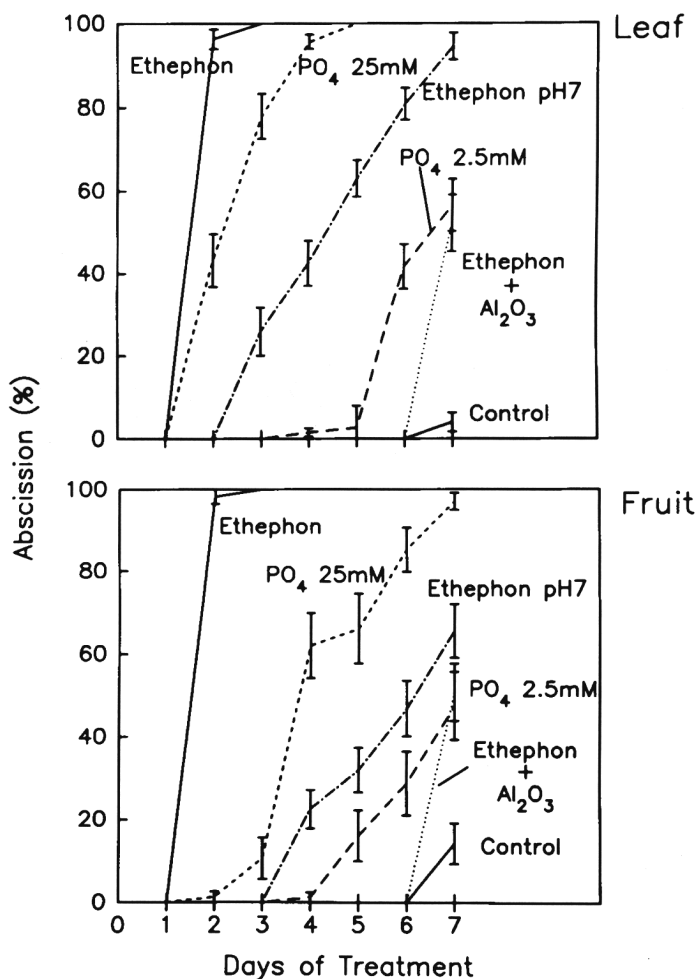

Fig. 7. Effects of stem feeding 25 mM NaH_2PO_4 at pH 5 and 200 mg ethephon/liter at pH 3 on 'Manzanillo' leaf and fruit abscission from excised shoots. Vertical bars indicate the SE of the mean.

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into the fruit abscission were tested. Whereas 500 mg ethephon/liter led to extensive leaf and fruit abscission (Fig. 8), 50 or 100 mM NaH$_2$PO$_4$ caused <10% leaf abscission and ≈50% fruit abscission in 7 days (Fig. 9). Adding glycerol to 100 mM NaH$_2$PO$_4$ increased leaf and fruit abscission to ≈10% and 75%, respectively. Although not shown on the figure because of data overlap, 50 mM NaH$_2$PO$_4$ sprayed twice gave results similar to those obtained with the single 100 mM NaH$_2$PO$_4$ treatment.

These results were promising, as it began to appear that a commercial treatment strategy to treat olive trees in the field using NaH$_2$PO$_4$ could be designed for minimal leaf loss and sufficient fruit loosening. To further test our hypothesis regarding spray accumulation in the pedicel-fruit cavity, ethephon and NaH$_2$PO$_4$ were applied as sprays or by filling the pedicel-fruit cavity with 30 µl of spray solution. Both (as spray or cavity treatment) 500 mg/liter ethephon and 100 mM NaH$_2$PO$_4$ led to 80% and 60% fruit abscission, respectively (Fig. 10). In contrast, 30 µl of 500 mg/liter ethephon or 100 mM NaH$_2$PO$_4$ solution applied to the petiole led to 20% and 10% leaf abscission, respectively. The solution-collection advantage of the pedicel-fruit cavity and its subsequent greater absorption clearly led to greater fruit than leaf abscission (Fig. 10).

There are several practical reasons to continue this research. Phosphorus routinely is used as a fertilizer and a food additive; thus, Environmental Protection Agency clearance should be easy to obtain. The cost of P treatment for olive fruit loosening will not be prohibitive. The polar nature of inorganic P allowed it to penetrate better in the pedicel-fruit attachment zone than in the leaf, as shown in our results. Still, it is evident that, should sufficient P penetrate leaf surfaces, excessive leaf abscission could occur. Devising means to maximize fruit abscission while minimizing leaf abscission remains to be achieved.

The response range of plant species to P should be explored in detail. Selective breeding for plants able to cope with low or high P may be possible. Also, unaccountable flower abscission reported for many crops may be an expression of sensitivity to high P content during the bloom and postbloom periods of development. The mechanism of P leaf abscission remains obscure.
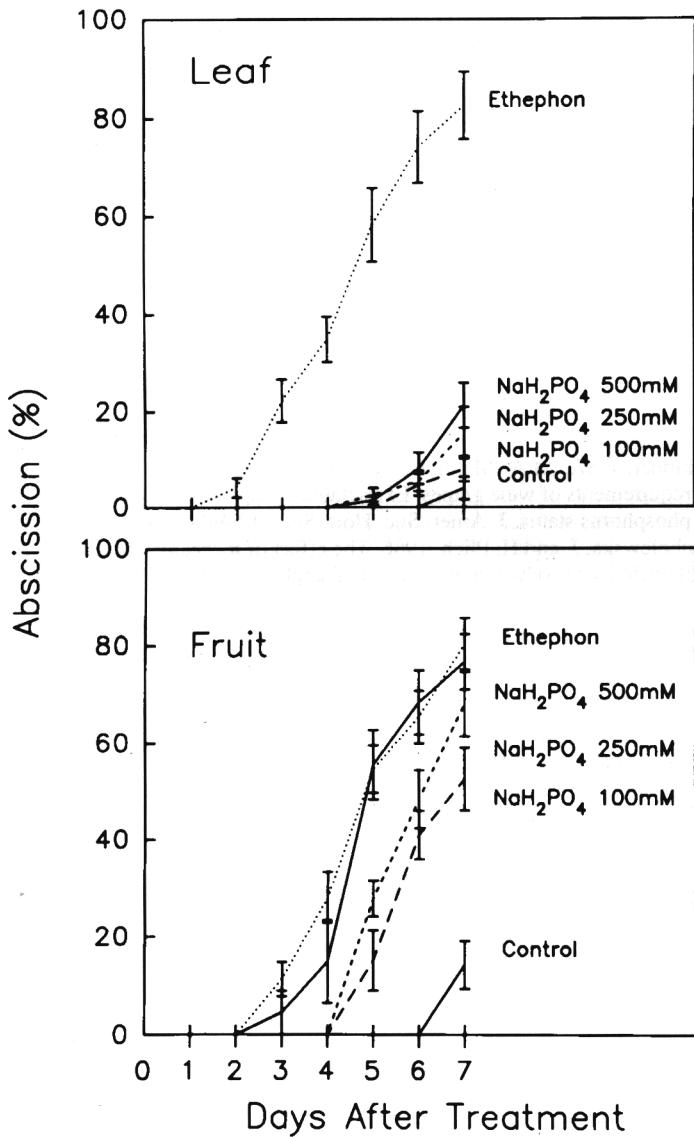

Fig. 8. Effects of foliar application of 500 mg ethephon/liter at pH 3 and NaH$_2$PO$_4$ at several concentrations at pH 5 on 'Manzanillo' leaf and fruit abscission from excised shoots. Vertical bars indicate the SE of the mean.
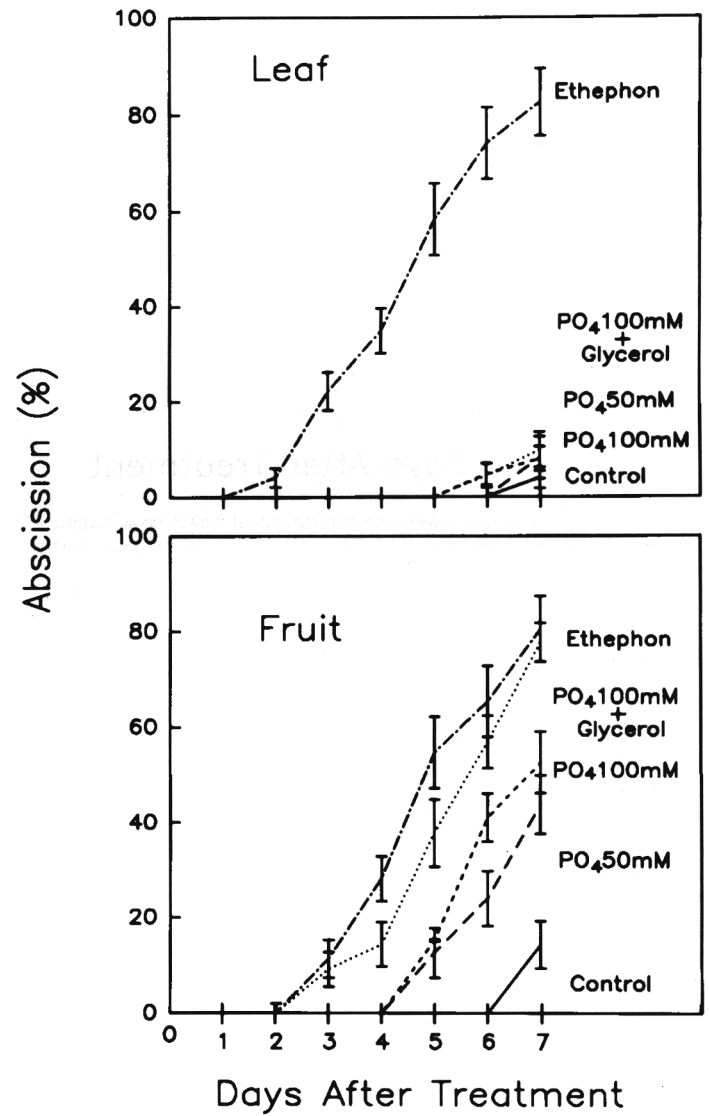
Fig. 9. Effects of 500 mg ethephon/liter compared to glycerol and single or double applications of NaH$_2$PO$_4$ as foliar sprays on leaf and fruit abscission from excised 'Manzanillo' olive shoots. Vertical bars indicate the SE of the mean.
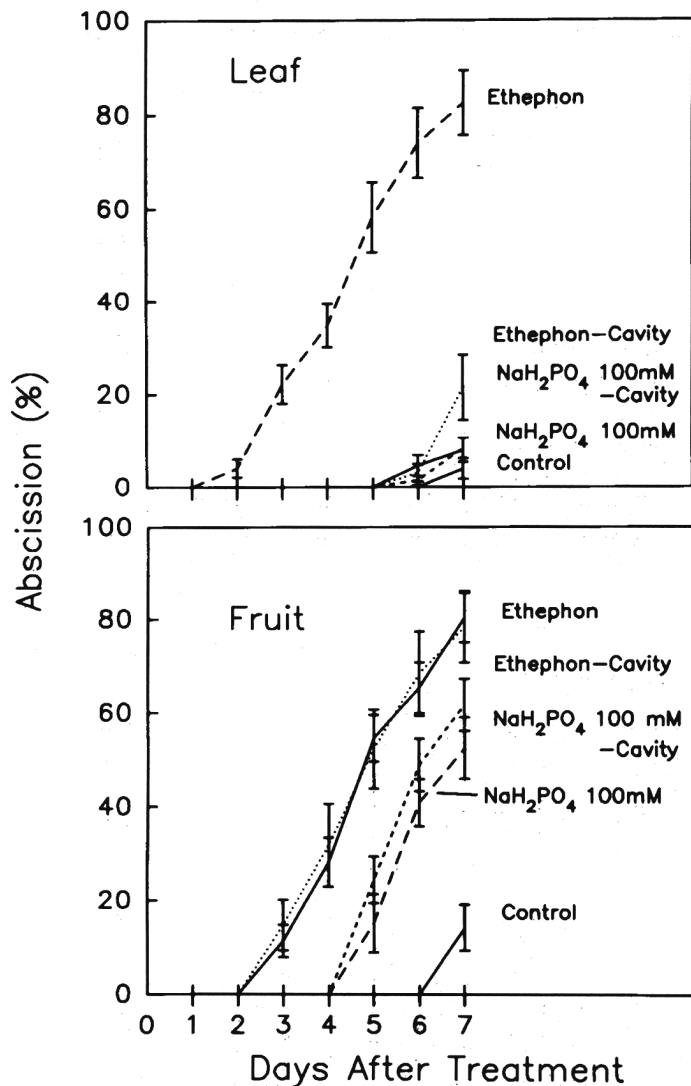
Fig. 10. Effects of foliar application or treatment of the pedicel–fruit cavity with either 500 mg/liter ethephon at pH 3 or 100 mM NaH$_2$PO$_4$ at pH 5 on leaf and fruit abscission from excised 'Manzanillo' olive shoots. Vertical bars indicate the SE of the mean.

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