Promotion of Ethylene Evolution and Fruit Abscission in the Olive by 2-Chloroethanephosphonic Acid and Cycloheximide[1]

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Abstract. Applications of 2-chloroethanephosphonic acid (Ethrel) at 2,250 ppm to olive (*Olea europaea* L.) trees under warm, low humidity conditions caused a considerable reduction in fruit removal force along with marked increases in leaf abscission and in ethylene evolution from both leaves and fruit. The addition of urea at 1.35% to the spray formulations caused a strong increase in ethylene production and in abscission effects. Definite cultivar differences appeared in response to Ethrel. An Auxin, NAA, blocked Ethrel activity, particularly if applied before, or simultaneously with, Ethrel. If applied 2 or 3 days after Ethrel, NAA reduced leaf abscission while maintaining fruit abscission. Rains following shortly after Ethrel application greatly reduced its activity in both ethylene production and abscission induction. Abscission effects from Ethrel were greater under warm than cool conditions.

Cycloheximide at 50, 100, and 200 ppm applied during cool, humid weather caused ethylene production from olive leaves and fruits as well as a reduction in fruit removal force and an increase in leaf abscission. However, ethylene evolution and abscission effects were much less than those obtained with Ethrel.

To facilitate mechanical harvesting of olives there has been intensive work (6, 9, 10, 11, 13, 14, 15) in various olive growing countries to develop spray formulations that induce fruit abscission. Studies on chemical acceleration of fruit abscission, including those with the olive, were reviewed by Cooper et al. (1). Chemicals used experimentally on olives include maleic hydrazide, glycerine, ascorbic acid, iodoacetic acid, salicylic acid, and 2-chloroethylphosphonic acid (Ethrel). The latter breaks down to yield ethylene in an alkaline solution (16, 20) and causes ethylene effects in plants. Each of these chemicals has been effective on olives under certain conditions but none, so far, has shown sufficient promise to justify its use commercially. For example, ascorbic acid and maleic hydrazide (11), have caused olive fruit abscission but only at high ambient air moisture levels due, presumably, to humidity effects on absorption. Based on previous studies (9, 10, 11) in California with all these chemicals, Ethrel is the most effective material in causing fruit abscission under the warm, low humidity conditions which occur during the table olive harvest season. However, excessive leaf drop with subsequent deleterious effects on fruit set may preclude its use.

The present study was designed to test several new materials, to obtain further information on the use of Ethrel on olives, and to develop methods for preventing excessive leaf drop.

Materials and Methods

Trials were conducted with several olive cultivars during the 1969 harvest season at Davis, California, using orchard trees as well as fruiting trees in containers. Limb segments of orchard trees, comprising about 1/4 of the fruiting area and bearing 20 to 30 lbs of fruit, were used as experimental units, with 3 such replicates per treatment, each on a different tree. Each container-grown tree, bearing 10 to 15 lbs of fruit, was sprayed as a separate unit, with 3 such replicate trees per treatment. Sprays were applied to run-off with a small power sprayer. A surfactant, X-77 at 0.1%, was added to all applications. Urea, at 1.35% was added to some Ethrel applications to further decrease fruit removal force (11).

The force required to pull the fruits from the intact shoots on the trees was determined with powered equipment described previously (10). Twenty-five fruits were pulled and the results averaged for each measurement. Leaf abscission was determined by calculating the percentage of leaves dropping after vigorously shaking the branches by hand.

For ethylene determinations, samples of leaves and fruits were collected from experimental branches at the times fruit removal force was measured, then taken immediately to the laboratory where they were placed in glass jars at room temperature under a measured air flow. Ethylene in the airstream was determined at intervals for several days by gas chromatography in an Aerograph A-600C Hy-Fi flame ionization unit fitted with a 152 × 0.16 cm column packed with 60/80 mesh alumina.

Table 1 lists materials used in a preliminary application to determine their possible effectiveness. These had been reported elsewhere as promoting fruit abscission but had not been used previously in California on olives; e.g., cycloheximide (2); hexamic acid, (cyclohexanesulfamic acid) (18); and oleic acid.[4] Ethrel 68–240, at 3 concentrations, at 3 pH levels, and with urea at 2 concns as an additive, was included for comparison, as well as abscisic acid at 5 and ascorbic acid at 1 concn.

Ethrel 68–240. Table 2 lists 3 concns of Ethrel with and without urea applied to 'Manzanillo' olives and Table 3 lists 5 olive cultivars sprayed with Ethrel plus urea.

Leaf abscission in olives tends to be excessive following Ethrel applications. Previous work (11) showed that naphthaleneacetic acid (NAA) counteracts to some extent abscission effects of Ethrel. Table 4 lists spray treatments in which Ethrel and NAA, with and without urea, are combined in applications to the same tree, with NAA applied either 2 days before Ethrel, in a simultaneous application, or following Ethrel by either 2 or 3 days.

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[4]Used experimentally on olives in Italy. Results reported at the XXXIII Fiera del Levante, XIV Giornata della Meccanica Agraria, Bari, Italy. September 20, 1969.

Ethylene was determined from leaves and fruits on branches sprayed with some of these combinations.

In a separate experiment to study the influence of NAA applied following Ethrel + urea, 'Mission' trees were sprayed with Ethrel + urea, then NAA was applied to the same trees either 2, 3, or 4 days later (Fig. 1).

The effectiveness of Ethrel + urea in lowering the fruit removal force was greatly reduced by rain (0.45 in) the day following application (Fig. 2). This could have been due to the rain, to the lower temperatures accompanying the rain, or to both. To examine these alternatives, a test was conducted with 'Ascolano' trees in containers. Two

Table 1. Effect of several abscission-promoting chemicals on fruit removal force, leaf abscission, and ethylene production, 'Manzanillo' olives.

Material and Concentration	Reduction in fruit removal force, percent	Leaf abscission, percent	Ethylene production, μl/kg/hr	
			Leaves	Fruits
Sprays applied Sept. 23; data taken Oct. 13, 1969				
Cycloheximide 20 ppm	15.2	1.91	1.75*	0.10*
Cycloheximide 10 ppm	15.1	2.12	2.08*	0.11*
Cycloheximide 5 ppm	2.1	0.76	1.36*	0.09*
Hexamic acid 2%	25.0	2.44	6.22*	0.07*
Hexamic acid 1%	14.9	1.06	1.80*	0.04
Hexamic acid 0.5%	21.1	1.38	1.30*	0.02
Ethrel 2000 ppm	42.7*	20.59*	93.32*	10.38*
Ethrel 1000 ppm	11.5	6.08	34.59*	2.76*
Ethrel 500 ppm	9.0	7.62	24.94*	2.49*
Ethrel 1000 ppm pH 4	11.4	22.27*	46.26*	6.40*
Ethrel 1000 ppm pH 7	6.9	15.80*	113.53*	9.51*
Ethrel 1000 ppm pH 10	14.3	20.28*	84.52*	7.20*
Oleic acid 4%	(4% incr.)	3.17	4.27*	0.20*
Ethrel 1000 ppm + urea 0.68%	(8% incr.)	14.29	26.33*	4.83*
Ethrel 1000 ppm + urea 1.35%	0	25.99*	67.24*	6.60*
Ascorbic acid, 1% + Orchex N-795, 1.5%	(11% incr.)	3.98	3.36*	0.08*
Control	–	2.04	0.79	0.03
Sprays applied Oct. 6; data taken Oct. 20, 1969				
Abscisic acid 400 ppm	0	0	1.53*	0.05
Abscisic acid 200 ppm	5.9	0	–	–
Abscisic acid 100 ppm	0	0	–	–
Abscisic acid 50 ppm	1.8	0	–	–
Abscisic acid 25 ppm	13.8	0	–	–
Control	–	0	0.77	0.04

*Significantly different from control at 5% level.

Table 2. Effect of Ethrel, with and without urea (1.35%) on fruit removal force, leaf abscission, and ethylene production. 'Manzanillo' olives. Sprays applied October 21, 1969. Average values for 3 sampling dates: October 24, 27 and 31.

Treatment	Fruit removal force, grams[x]		Leaf abscission, percent[x]		Ethylene production μl/kg/hr (without urea)	
	Without urea	With urea	Without urea	With urea	Leaves[x]	Fruits[x]
Ethrel, 2000 ppm	514 c	482 c	27.4 c	35.9 b	36.93 a	8.01 a
Ethrel, 2250 ppm	433 c	210 a	34.7 c	57.6 a	–	–
Ethrel, 2500 ppm	308 b	166 a	40.0 b	73.1 a	–	–
Control	597 d	597 d	0.5 d	0.5 d	0.75 b	0.036 b

[x]Numbers followed by different letters are significantly different at the 5% level as determined by Duncan's multiple range test (4).

Table 3. Effect of Ethrel + urea sprays on fruit removal force and leaf abscission of 5 olive cultivars. Sprayed November 11; measurements taken November 17, 1969.

	Fruit removal force, grams		Percent reduction from control[x]	Leaf abscission, percent[x]	
	Control[x]	Ethrel 2250 ppm + urea 1.35%		Control	Ethrel 2250 ppm + urea 1.35%
Sevillano	899 a	475	46.93 bc	1.84 e	32.89 c
Mission	543 b	139	73.70 a	1.04 e	56.18 a
Barouni	911 a	369	59.33 ab	0.87 e	38.89 bc
Manzanillo	571 b	192	66.33 a	0.65 e	44.09 b
Ascolano	707 ab	447	36.66 c	0.88 e	18.73 d

[x]Numbers followed by different letters are significantly different at the 5% level as determined by Duncan's multiple range test (4).

Table 4. Effect of Ethrel (2250 ppm) sprays, with and without urea (1.35%), preceded by, with, or followed by NAA (50 ppm) sprays, on fruit removal force, leaf abscission, and ethylene production. 'Manzanillo' olives. 1969.

Treatments	Fruit removal force,[xy] grams		Leaf abscission,[xy] percent		Ethylene production[z], µl/kg/hr			
					Leaves[x]		Fruit[x]	
Materials and dates applied	With urea	Without urea	With urea	Without urea	With urea	Without urea	With urea	Without urea
NAA (Oct. 28) + Ethrel (Oct. 31)........	502 c	528 abc	6.8 d	5.0 d	114.6 b	46.4 c	6.3 cd	5.4 d
NAA (Oct. 28) + Ethrel (Oct. 28)........	463 c	541 abc	6.1 cd	5.0 d	180.8 a	64.7 c	14.1 a	6.7 cd
Ethrel (Oct. 28) + NAA (Oct. 30).........	333 d	479 c	11.1 bc	9.8 bc	–	–	–	–
Ethrel (Oct. 28) + NAA (Oct. 31, 9 a.m.)..	299 d	469 c	39.7 a	4.3 d	218.1 a	67.3 c	11.0 b	8.3 c
Ethrel (Oct. 28) + NAA (Oct. 31, 5 p.m.)..	317 d	519 bc	14.4 b	10.8 b	–	–	–	–
Control...............	612 ab	623 a	0.8 e	1.1 e	–	1.8 d	–	0.2 d

[x]Numbers followed by a different letter are significantly different at the 5% level as determined by Duncan's multiple range test (4).
[y]Average of measurements taken on 4 dates (Oct. 31, Nov. 1, Nov. 4, and Nov. 18) following spraying on Oct. 28.
[z]Average of determinations made each day for 7 days after spraying.

groups of trees were placed in a heated greenhouse and 2 groups were left out-doors where temperatures averaged 20°F lower. In each situation, 1 group received a 5-hr simulated rain 6 hr after spray treatments. For each environmental condition there was a spray treatment consisting of Ethrel + urea, another consisting of Ethrel + urea followed in 2 days by NAA, and a control. These treatments are listed in Table 5. Fruit removal force, leaf abscission, and ethylene production were determined for each treatment.

Cycloheximide. Following the cycloheximide applications at 5, 10, and 20 ppm in September (Table 1), sprays of this material were also applied December 2 at 50, 100, and 200 ppm to 'Manzanillo' trees. At the latter date the fruits were almost mature, red to black in color; the weather was cool (approximately 30°F min. and 60°F max.) with high relative humidity. Fruit removal force, leaf abscission, and ethylene production from leaves and fruits were determined at intervals until December 29 (Figs. 3 and 4).

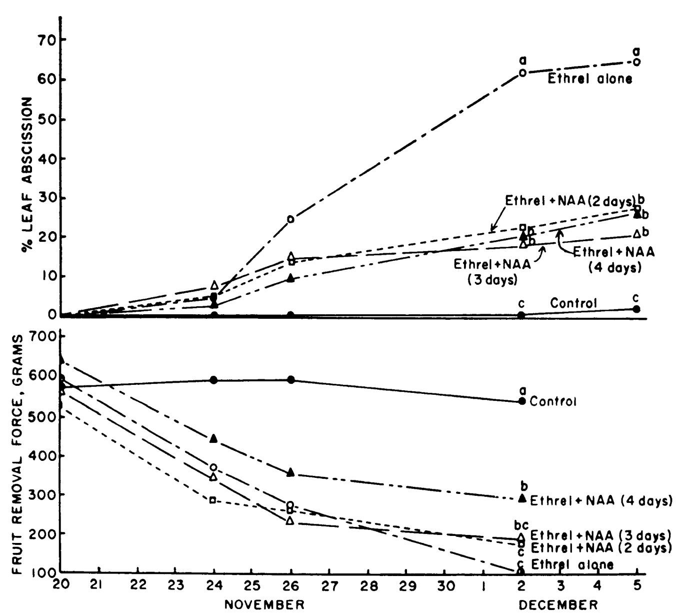

Fig. 1. Effect of Ethrel sprays applied November 18, 1969, to 'Mission' olives on leaf abscission and fruit removal force as influenced by subsequent applications of NAA, 2, 3, or 4 days later. All Ethrel sprays were at 2250 ppm + urea at 1.35%. NAA was applied at 50 ppm. Means followed by different letters are significantly different at the 5% level.

Results and Discussion

Ethrel at 2,000 ppm was the only treatment listed in

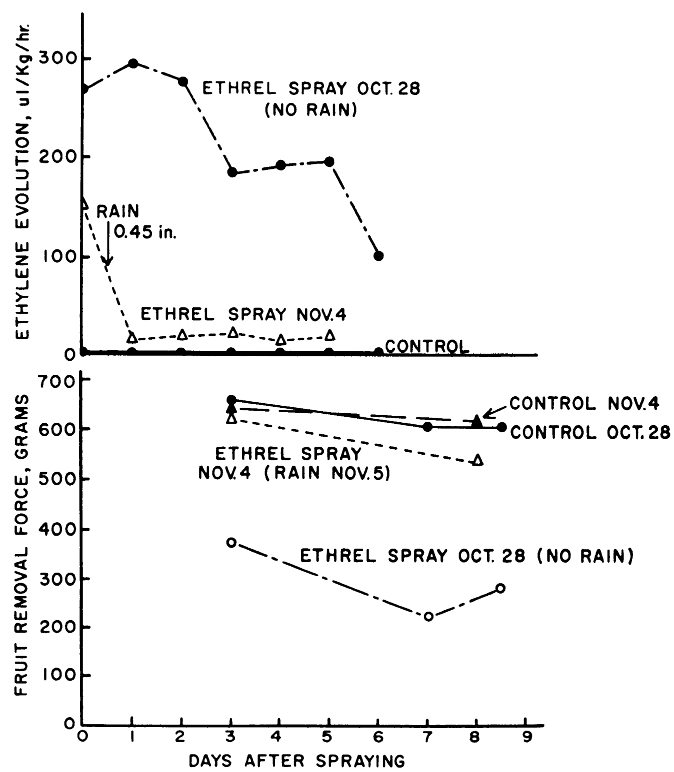

Fig. 2. Effect of rain one day after spraying (Ethrel, 2250 ppm + urea, 1.35%) on ethylene evolution and fruit attachment force in 'Manzanillo' olives. Each Ethrel spray was followed by an NAA spray after 2 days (October 28 application), or 3 days (November 4 application).

Table 5. Influence of temperature and simulated rain following Ethrel + urea sprays on fruit removal force, leaf abscission, and ethylene production by leaves of 'Ascolano' olive trees in containers.[x]

Treatment	Warm (greenhouse, 60° to 80°F)[y]		Cool (out-doors, 35° to 65°F)[y]	
	Dry	5 hrs simulated rain 6 hrs after spraying with Ethrel + urea	Dry	5 hrs simulated rain 6 hrs after spraying with Ethrel + urea
Fruit removal force (grams)				
Ethrel, 2250 ppm + urea, 1.35%	344 e	467 d	463 d	598 ab
Ethrel, 2250 ppm + urea, 1.35% + NAA 50 ppm (after 2 days)	281 e	490 cd	453 d	569 bc
Control	572 bc	666 a	590 ab	498 cd
Leaf abscission, percent				
Ethrel, 2250 ppm + urea, 1.35%	19.0 a	8.1 c	9.8 bc	3.6 de
Ethrel, 2250 ppm + urea, 1.35% + NAA, 50 ppm (after 2 days)	22.4 a	20.3 a	12.9 b	4.9 cd
Control	0.5 f	1.9 ef	1.4 ef	1.3 ef
Ethylene production by leaves, µl/kg/hr				
Ethrel, 2250 ppm + urea, 1.35%	100.7 a	28.8 b	116.0 a	24.4 b
Ethrel, 2250 ppm + urea, 1.35% + NAA, 50 ppm (after 2 days)	116.5 a	34.5 b	103.7 a	17.4 bc
Control	1.6 c	1.3 c	2.3 c	1.9 c

[x]Values for fruit removal force and leaf abscission are means of measurements taken 2, 4 and 8 days after treatment. Values for ethylene release are means of measurements taken 0, 1, 2, 3, 4, 5 and 6 days after treatment.
[y]Numbers followed by different letters are significantly different at the 5% level as determined by Duncan's multiple range test (4).

Table 1 that caused a significant reduction in fruit removal force over the control. Ethrel at that and lower concentrations significantly increased leaf abscission over the control; this was not true for any other treatment. Ethylene production from leaves was significantly in-

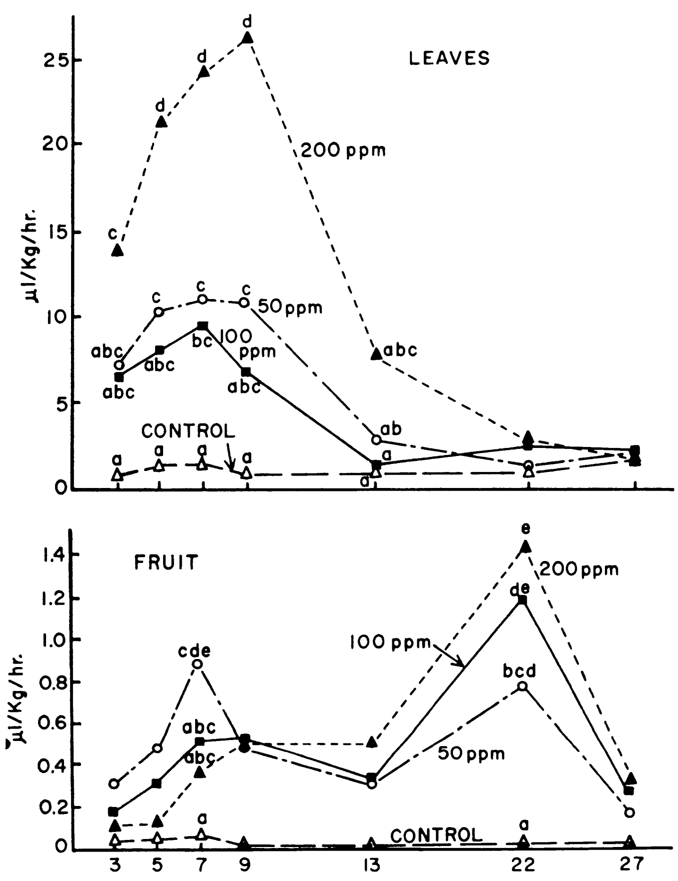

Fig. 3. Effect of 3 concentrations of cycloheximide sprays on ethylene evolution by leaves *(above)* and fruits *(below)*. Sprays applied December 2, 1969. Means followed by different letters are significantly different at the 5% level.

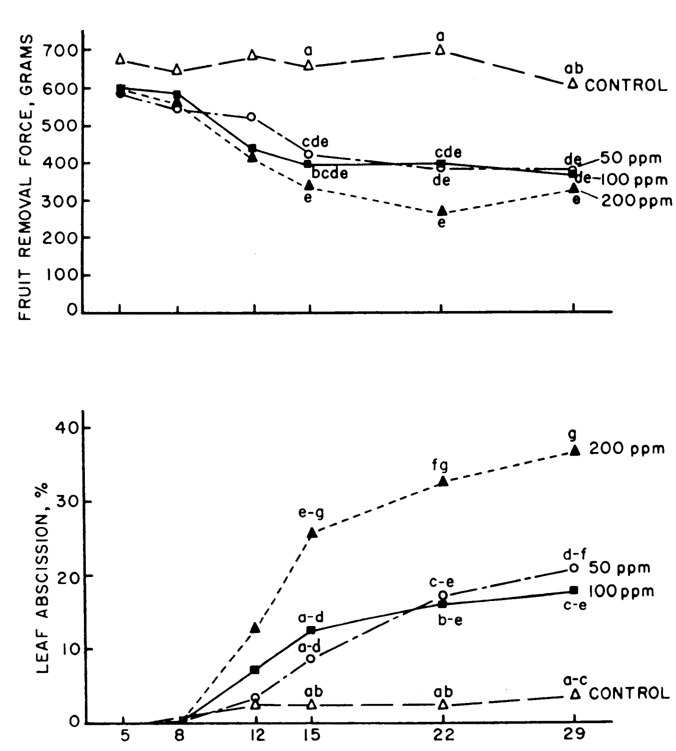

Fig. 4. Effect of various concentrations of cycloheximide sprays (applied December 2, 1969) on fruit removal force *(above)* and leaf abscission *(below)*. Means followed by different letters are significantly different at the 5% level.

creased over the control for every treatment and fruits for every treatment except the 2 lowest levels of hexamic acid and from abscisic acid at 400 ppm. Ethylene production from leaves on Ethrel-treated branches was much greater than from fruits. It generally caused leaf abscission and, in one case, fruit abscission. Although other materials significantly increased ethylene production, it was presumably at a level too low to cause abscission. Abscisic acid at 400 ppm caused a significant increase in ethylene production but still far too low to cause leaf abscission. Ascorbic acid at 1% + Orchex N-795[5] spray additive caused a low but significant increase in ethylene production by leaves and fruits although the amount was insufficient to cause fruit abscission.

Ethrel 68–240. Previous experience showed that if fruit removal force of olives is about 250 g, a limb or trunk shaker will readily harvest the fruit. This level was attained with Ethrel 68–240 at 2,250 ppm active ingredient concn when urea at 1.35% was added (Table 2). However, about half the leaves dropped. This would have a deleterious effect on flower initiation for the following season's crop (6).

There is a pronounced cultivar difference in olives in response to Ethrel + urea (Table 3). 'Mission' had the greatest reduction in fruit removal force and the highest percentage leaf drop, followed in order by 'Manzanillo', 'Barouni', 'Sevillano', and 'Ascolano'.

In the efforts to obtain separation of leaf and fruit abscission, the greatest reduction in fruit removal force was obtained from treatment with Ethrel on October 28 followed by NAA on October 31 at 9:00 AM (Table 4). This treatment also gave the highest leaf abscission and the greatest ethylene release from both leaves and fruits. NAA applied before or simultaneously with Ethrel reduced ethylene release from both leaves and fruit; it also reduced leaf and fruit abscission in comparison with treatments where NAA was applied following Ethrel sprays. The influence of urea as an additive to Ethrel on ethylene release and on leaf and fruit abscission became greater with the delayed application of NAA. Figure 5 shows the pattern of ethylene production from leaves of 'Manzanillo' trees sprayed with Ethrel alone or Ethrel + urea, plus a spray, either before, at the same time, or later, with NAA. The addition of urea to Ethrel always caused a marked stimulation in ethylene production, as much as a 3-fold increase. Sprays of NAA reduced ethylene production from the beginning if applied simultaneously with Ethrel, or later, if applied following the initial Ethrel application.

Using the very responsive 'Mission' cultivar, separation of leaf and fruit abscission was obtained (Fig. 1) by a subsequent spray of NAA 2 or 3 days after Ethrel + urea was applied. A spray of Ethrel + urea reduced the fruit removal force to 100 grams in 14 days and to almost as low a level (about 180 grams) from a similar spray treatment followed by an NAA application 2 or 3 days later. On leaf abscission, Ethrel + urea alone resulted in 65% leaf drop after 17 days. A similar spray followed by NAA after 2 or 3 days caused 22 to 26% leaf drop. This influence of auxin on ethylene activity agrees with work of Hall (8), which showed a blockage of ethylene-induced abscission by prior treatments with exogenous auxin; and with a similar auxin-ethylene relationship in cotton abscission demonstrated by Morgan (17).

Ethylene production dropped rapidly, almost to the level of the control following a rain (0.45 in) which occurred shortly after a spray application. The fruit removal force stayed at about the same level as the control. (Fig. 2).

Simulated rain markedly reduced ethylene output under both warm and cool conditions, but ethylene release showed no relationship to the temperature difference (Table 5). Possibly as a reflection of the ethylene differences, leaf abscission decreased and fruit removal force increased under the simulated rain treatment at both warm and cool conditions. Trees given these same treatments but with an added spray of NAA 2 days after the Ethrel + urea spray, generally behaved the same as trees sprayed only with Ethrel + urea.

There was no significant difference in ethylene release associated with temperature differences in trees sprayed with Ethrel + urea, but leaf abscission was significantly higher and fruit removal force significantly lower under warm compared to cool conditions. Possibly factors other than ethylene production, having a temperature dependency, were involved. Hield et al. (12), have reported increased leaf abscission following Ethrel sprays on citrus under warm (70° to 85°F) compared to cool (45° to 66°F) conditions.

Ethrel sprays did not cause any deleterious effects on raw olive fruits. 'Mission' fruits harvested from trees sprayed with Ethrel at 2,250 ppm + urea (1.35%) had normal flavor and texture after processing by the California black ripe method (3). However, such fruits were slightly darker in color and had more prominent lenticels than fruits from unsprayed trees. Ethrel spray treatments which caused heavy defoliation also resulted in nonblooming the following spring.

The reason for the pronounced increase in ethylene release and the marked lowering of the fruit removal force due to the addition of urea to Ethrel is not clear. Ethrel activity is strongly dependent upon pH level (5) but pH measurements of Ethrel spray formulations with and without urea were the same. It may be that urea facilitated absorption of Ethrel through the cuticular

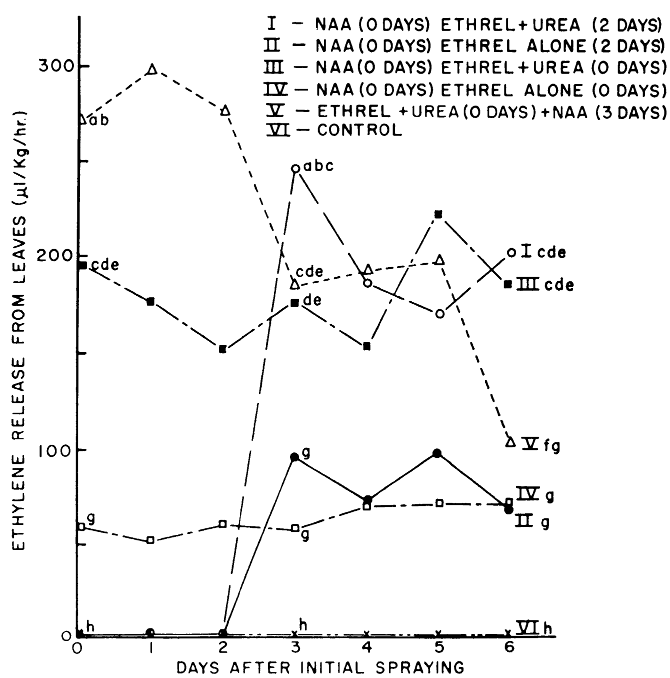

Fig. 5. Effect of various combinations of spray applications of NAA (50 ppm) and Ethrel (2250 ppm), with and without urea (1.35%), on ethylene release from leaves of 'Manzanillo' olive trees. Means followed by different letters are significantly different at the 5% level.

[5]Mfg. by Humble Oil & Refining Co.

membrane. There is evidence (19) that urea is a strong promoter of permeability, not only for itself but for other materials applied simultaneously.

Cycloheximide. This material, which is an antibiotic and an inhibitor of protein synthesis, caused ethylene production in olives when applied at 200 ppm, reaching a peak with leaves (26.3 μl/kg/hr) in 5 to 7 days, and with fruit (1.4 μl/kg/hr) after 25 days (Fig. 3). Pronounced leaf abscission subsequently resulted (Fig. 4). A slow but significant reduction in fruit removal force occurred; this slow response differs from that obtained with citrus where the fruit removal force was lowered from 8.1 kg to 3.0 kg in 2 days (1). In olives ethylene output following cycloheximide sprays was far lower than that obtained with Ethrel, and the effect on fruit removal force was much less. Cycloheximide caused pronounced leaf abscission; it also caused some development of corky tissue around the lenticels on olive fruits as well as some fruit pitting.

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Root-Knot Nematode Resistance in Snap Beans: Breeding and Nature of Resistance[1]

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Abstract. Bush type snap beans *(Phaseolus vulgaris* L.) with resistance to the root-knot nematode, *Meloidogyne incognita* (cotton strain) are being developed by using PI-165426 as the resistant parent. PI-165426 (resistant), 'Black Valentine' (susceptible) and F_5 breeding line B3864 (resistant) were inoculated with second-stage larvae. There were no significant differences in larval penetration of roots. Root tips showed slight swellings at infection loci of resistant and susceptible plants. Necrosis was evident in the resistant lines 4 days after inoculation. Histological studies of early infections showed that resistance was due to absence of adequate giant cell development and to hypersensitive reaction within the infected portion of the root. When soil temperature was changed from 16 to 28°C, galling, female development, and egg mass production in the resistant plants were increased.

Nematodes account for an estimated annual reduction in bean yields to over $4 million in the United States (20). Effective seasonal control can be obtained with the application of nematicides, but the cost is high. Resistant bean cultivars could provide the least expensive method of controlling nematode species for which resistance is available. They are potentially important to some emerging nations where beans are an important source of protein in the diet.

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Root-knot nematodes are widely distributed in the Southeastern United States. The symptoms of infection on beans are familiar to many growers; roots generally contain large compound galls and are often invaded by secondary organisms that cause premature and extensive root-rot. Plants grown in heavily infested soil can have infections on the stem, cotyledons and leaves (21).

In 1931 Isbell (17) reported resistance in 2 strains of pole beans, 'Alabama No. 1' and 'Alabama No. 2' to root-knot nematodes *Heterodera marioni* (Cornu) Goodey, 1932. After the taxonomic revision of nematode spe-