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Changes of Respiration Rate, Ethylene Evolution, and Abscisic Acid Content in Developing Inflorescence and Young Fruit of Olive (*Olea europaea* L. cv. Konservolia)

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Abstract. Simultaneous measurements of respiration, ethylene production, and abscisic acid (ABA) concentrations, as well as the growth parameters length, fresh weight (FW), and dry weight (DW) of olive (*Olea europaea* L. cv. Konservolia) inflorescence were carried out at short intervals (3–7 days) during the period from bud burst until the 3rd week after full bloom (AFB), when young fruit reached 8 mm in length. The axis of inflorescence elongated remarkably during the 3rd week after bud burst (ABB), massive bract shedding occurred during the 4th week ABB, full bloom (FB) was observed 7 weeks ABB, and massive floral organ abscission 1 week AFB. The results showed a continuous increase in inflorescence FW and DW from bud burst until 4 days before FB. Respiration rate, ethylene production, and levels of ABA were relatively high during the first 3 weeks ABB. After this period, respiration and ethylene followed a similar pattern of changes, inversely to that of ABA concentration. An accumulation of inflorescence ABA 6 and 4 days before FB was associated with the minimum values of respiration and ethylene production on the same dates. The sharp decrease in the ABA concentration during FB and 3 days later was followed by a high rise in ethylene and an increase in respiration rate, which both rose further 1 week AFB. The results suggest a possible correlation of ABA with the early stage of floral abscission, whereas ethylene production seems to be correlated with the terminal separatory activity in olive inflorescence abscission processes.

Key Words. Abscisic acid—Abscission—Ethylene—Inflorescence—Olive—Respiration

Abbreviations: ABA, abscisic acid; FB, full bloom, FW, fresh weight; DW, dry weight; ABB, after bud burst; AFB, after full bloom.

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A prolonged period of anthesis (6–7 weeks) is characteristic of olive. A series of morphologic changes of inflorescence takes place (e.g., size, shape, color) during anthesis. During this period, floral organ abscission occurs on olive trees with abscission zones located mainly in bracts, petals, individual flowers, and peduncles (Weis et al. 1988, 1991).

Abscission is a complex process and is usually initiated by increased abscisic acid (ABA) levels some days before the final shedding, as well as increased ethylene production (Addicott 1982, Moore 1989, Reid 1985, Sexton and Roberts 1982). ABA has been reported to promote ethylene production in many tissues (Abeles 1967, Cooper and Henry 1973, Riov et al. 1990, Sagee et al. 1980, Sexton and Roberts 1982, and many others). Ethylene has induced a rapid increase of ABA in citrus flavedo (Goldschmidt et al. 1973) and in rose petals (Mayak and Halevy 1972). Sargent et al. (1984), however, found that in Gramineae the only regulator to accelerate abscission is ABA.

The start of the abscission process, whether natural or stimulated by the application of ethylene, is marked by increased respiration that stimulates higher rates of RNA and protein synthesis (Addicott 1982, Sexton and Roberts 1982). Vemmos et al. (1994) also found that an increased respiration rate preceded inflorescent bud abscission in pistachio.

The role of ethylene in the abscission process in olive flowers, leaves, and fruits has been studied before (Lavee and Martin 1981, Martin et al. 1981, Weis et al. 1988, 1991), particularly from a commercial perspective. Lavee and Martin (1981) measured the ethylene production by inflorescence at only three different stages (young, growing, mature). However, endogenous ethylene production has not been studied thoroughly during the long period of anthesis and flower abscission. Kitsaki et al. (1995) found that the ABA content of inflorescence was relatively high from early anthesis until full bloom

(FB), reaching a maximum 1 week before FB and then decreasing sharply.

The purpose of this study was to measure simultaneously the internal factors (ABA, ethylene, and respiration) related to abscission during the whole period of anthesis within short time intervals; the results might be important for both scientific and practical considerations.

Materials and Methods

Plants

The work was carried out in 1995 in the olive orchard of the Agricultural University of Athens. Three 25-year-old fruit-bearing olive trees (*Olea europaea* L. cv. Konservolia) were selected. Reproductive shoots 15–25 cm long, with ten or more inflorescences, were cut from positions distributed symmetrically around the crown of each tree from the beginning of bud burst (April 1) until early fruit development, when young fruit had attained a length of 8 mm (June 8). The samples were taken at 9 a.m. at weekly intervals during the first stages of inflorescence development and twice a week from 3 weeks before FB until 2 weeks after full bloom (AFB). The cut ends of shoots were immersed immediately in water, and the samples were transferred quickly to the laboratory. The sample from each tree was divided into three groups, and inflorescences were taken from the middle region of the shoots for respiration rate, ethylene production, and ABA concentration measurements. All of the events that took place during anthesis were written down in detail; changes in development and morphology of inflorescence were recorded with photographs of representative inflorescences from experimental trees. Length as well as fresh weight (FW) and dry weight (DW) of inflorescence and young fruit were determined by measuring the length and weight of ten inflorescences per tree. FB, determined according to Rappoport and Rallo (1991), was observed 47 days after bud burst (ABB).

Respiration Rate

A portable infrared gas analyzer system (LI-COR, LI-6200 model) was used, and the CO_2 released by the tissue was measured in a 0.25-liter chamber. The tissue and air temperatures and the relative humidity in the chamber were also recorded. Because the measurements were taken in a temperature-controlled cabinet, the tissue temperature was kept at 27°C so the air temperature in the chamber was very similar to that of the tissue. The relative humidity in the chamber was influenced by the FW of the inflorescence and was 60 ± 2%. The measurements were made under weak light conditions (PAR = 30 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The air flow in the infrared gas analyzer was about 500 $\mu mol \cdot s^{-1}$. The inflorescences were removed from the shoots, weighed immediately, and inserted into the chamber for three continuous measurements that lasted only 30–60 s to avoid the effect of tissue drying. The respiration rate was expressed as μmol of $CO_2 \cdot g^{-1}$ FW $\cdot s^{-1}$.

Ethylene Production

The rate of ethylene production was measured using a gas chromatography system (Perkin Elmer Sigma 300 dual FID chromatograph) equipped with an alumina column (6 feet long, ⅛-inch diameter/183 cm long, 0.32 cm diameter, and 80–100 mesh). The data were recorded by a Perkin Elmer LCI-100 laboratory computing integrator. For ethylene determinations, inflorescences were placed in 38-mL volume flasks that were sealed to prevent air exchange. Preliminary tests showed that there was a linear relation between the amount of ethylene evolution and the duration of enclosure between 1 and 2 h. Thus, an enclosure time of 2 h was used. The ethylene produced by the tissue was expressed as $\mu l \cdot g^{-1}$ FW $\cdot h^{-1} \cdot 10^{-4}$.

Abscisic Acid Content

Endogenous ABA was determined according to Kitsaki et al. (1995). In brief, each sample was homogenized in cold methanol (80% v/v), and ABA was extracted overnight twice. Samples were purified by partitioning with diethyl ether followed by thin layer chromatography on Silica Gel HF254 (parallel with authentic ABA), methylated with diazomethane, and analyzed with a Varian (model 3700) GLC-EC gas chromatograph. A glass column (200 cm × 0.23 cm × 0.60 cm) packed with 3% DC 200/500 on Chromosorb W 100–200 mesh was used. Nitrogen was used as carrier gas at a flow rate of 30 $ml \cdot min^{-1}$, and injector, column, and detector temperatures were 220, 200, and 250°C, respectively. The ABA concentration was expressed in $ng \cdot g^{-1}$ of tissue FW.

Results

Morphological Aspects of Olive Inflorescence Development

The developmental stages of olive inflorescence from bud burst to fruit set, at weekly intervals, are presented in Fig. 1. One week ABB inflorescences looked like small berries. The small flowers were completely covered by bracts (Fig. 1*a*). By the end of the second week rachillas and flowers became more visible (Fig. 1*b*). In the 3rd week the axis of inflorescence had increased remarkably, and bracts were more open compared with the previous week (Fig. 1*c*). The swollen flowers, which became spherical, was the main characteristic of the 4th week; sepals covered the greatest part of the corolla (Fig. 1*d*), and a significant number of bracts had already dropped from the base of the rachilla. One week later, the swelling flowers appeared bigger, particularly the corolla. The separation among the flowers was more advanced (Fig. 1*e*) in the 6th week, and the swollen flowers had increased in size. Petals turned to a yellowish (color not visible in Fig. 1*f*), and the beginning of their separation was clear in the 7th week (Fig. 1*g*). Finally, Fig. 1*h* shows despoiled inflorescences with the majority of flowers abscised. It is interesting to note that bracts began to drop during the 3rd week ABB, and the drop continued during the whole period of inflorescence development. The total number of flowers per inflorescence (on sixth node from the top) was 26.7 ± 2 and the percentage of perfect flowers 25.5 ± 4%. A small percentage (1–2%) of corollas started to drop during the 5th week ABB. This drop continued until FB. Massive flower abscission occurred the week after full bloom (AFB); the fertilized ovaries remaining on inflorescence

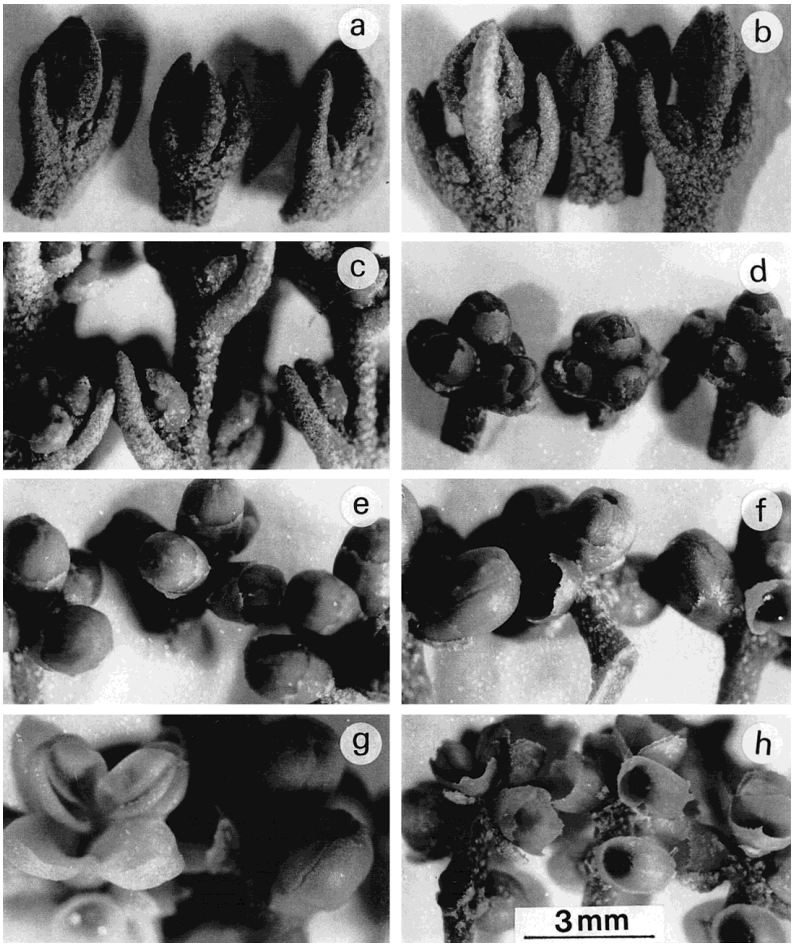

Fig. 1. Different developmental stages of olive (cv. Konservolia) inflorescence from bud burst to fruit set at weekly intervals in 1995. *Panel a;* 1st week after bud burst (ABB); *panel b;* 2nd week ABB . . . *h;* 8th week ABB. *Panels a* and *b* show the whole inflorescence; *panel c* shows the central part of inflorescence; and *panels d, e, f, g,* and *h* show the first rachilla from the base of the inflorescence.

were 10.5 ± 2% of the total number of flowers 7 days AFB; 22 days AFB the remaining young fruit fell to 5.33 ± 0.5%.

In the absence of any pests or diseases and in view of the normal climatic conditions that prevailed, such high abscission must be considered natural.

Inflorescence Development

Inflorescence length increased continuously with inflorescence development and reached its maximum 1 week before FB (Fig. 2).

The FW and DW of inflorescence also increased continuously from bud burst until 43 days ABB (3–4 days before FB), when they reached maximum values (about 300 mg and 90 mg per organ, respectively). From FB on, a sharp decrease occurred for both FW and DW until 1 week AFB when they started rising again with young fruit growth. In contrast to FW and DW, the DW/FW ratio decreased from bud burst until 2 weeks before FB and started increasing 1 week before FB, while decreasing slightly after fruit set. Thus the decrease was caused mainly by flower drop.

Respiration Rate

The respiration rate (Fig. 3) remained relatively high during the first 3 weeks of inflorescence development, reaching its maximum (about 24 μL of $CO_2 \cdot g^{-1}$ $FW \cdot min^{-1}$) at the end of the 1st week. After this period the respiration rate decreased continuously until 4 days before FB, when it reached a minimum value (about 8 μL of $CO_2 \cdot g^{-1}$ $FW \cdot min^{-1}$). From FB on, respiration increased continuously for a period of 2 weeks and started decreasing again when the young fruit reached about 8 mm in length.

Ethylene Production

The ethylene production of inflorescence (Fig. 3) rose from bud burst until 22 days ABB, when it reached its maximum value (about 45 $\mu l \cdot g^{-1}$ $FW \cdot h^{-1}10^{-4}$). After

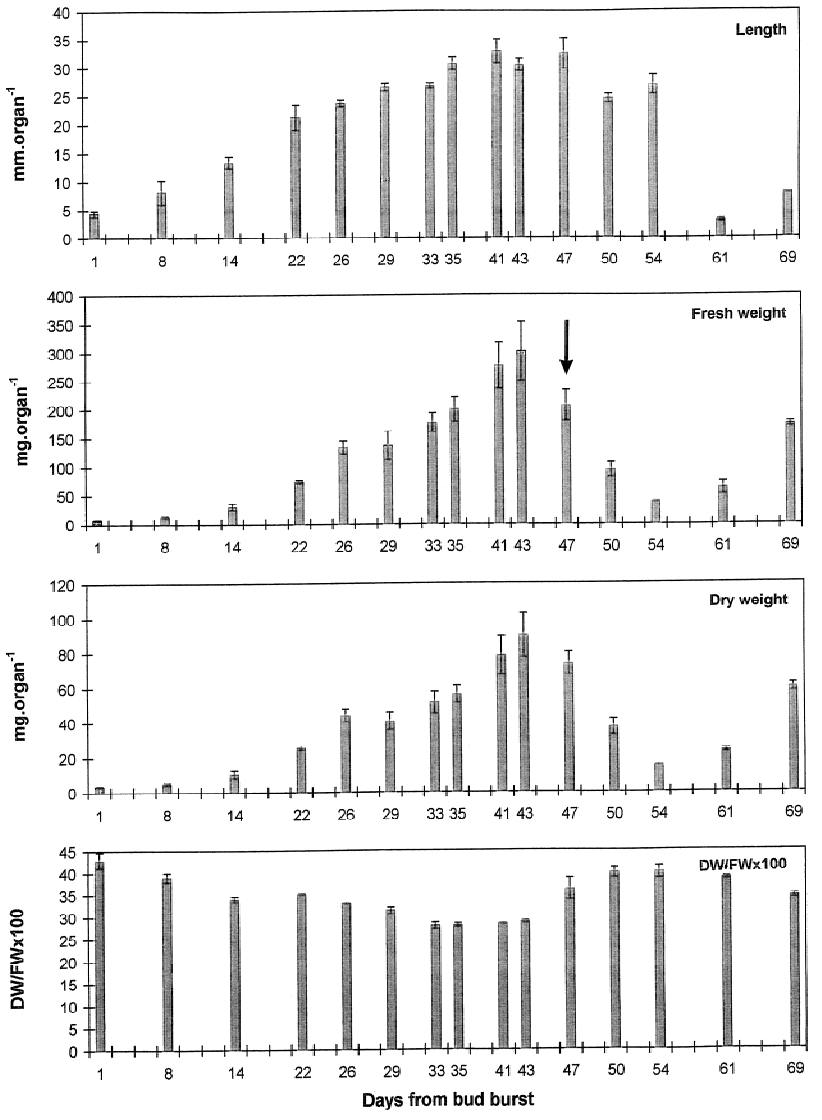

Fig. 2. Changes in length, FW, DW, and the DW/FW ratio in olive (cv. Konservolia) inflorescence and young fruit from bud burst to early fruit development. The *arrow* indicates full bloom. *Bars* represent ±SE.

this period ethylene production decreased continuously, parallel to the reduction in respiration, until 1 week before FB. Then it increased again and reached high levels at 3 and 7 days AFB, the period of organ (e.g., petals, stamens) and flower abscission. From the 2nd week AFB ethylene dropped to low levels and reached a minimum (about 2.7 $\mu l \cdot g^{-1}$ FW $\cdot h^{-1} \cdot 10^{-4}$) when the young fruit were 8 mm long.

Abscisic Acid Content

The ABA content of inflorescence (Fig. 3) was relatively high from 1 week ABB until 4 days before FB. The highest values, however, were detected 22 days ABB and 6 and 4 days before FB (820, 835, and 980 $ng \cdot g^{-1}$ FW, respectively). ABA concentrations fell significantly at FB, except for a temporal increase, occurring 1 week AFB.

Discussion

Olive inflorescence is a panicle arising as a central axis terminated by a flower, with lateral axes branching from the peduncle and containing several bracts and flowers at different developmental stages (Weis et al. 1988). Because of the variety in the developmental stages of olive inflorescence organs, a precise physiological interpretation is difficult. The prolonged period of anthesis which has been reported for other olive cultivars (Weis et al. 1991) was also confirmed in this study for Konservolia because it lasted almost 7 weeks (Fig. 2). The shedding of inflorescence organs also lasted for a long period;

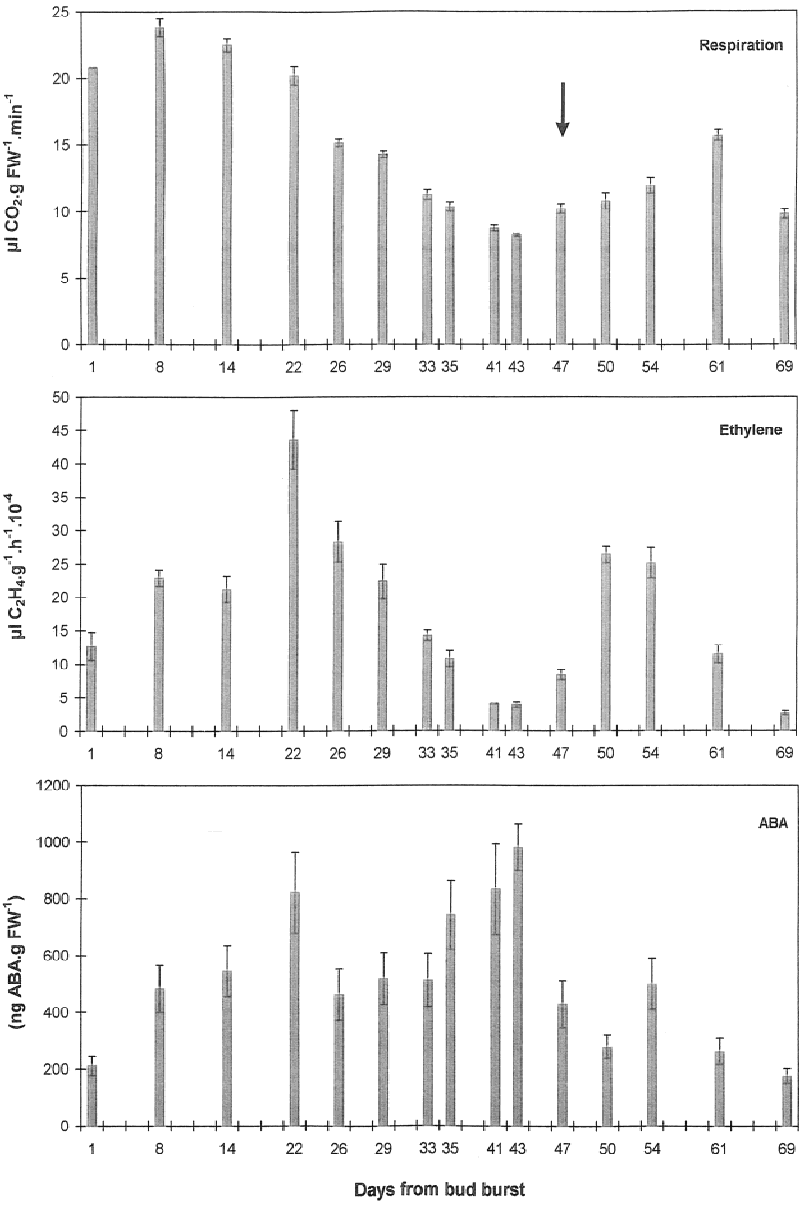

Fig. 3. Changes in respiration rates, ethylene production, and ABA concentration in olive (cv. Konservolia) inflorescence and young fruit from bud burst to early fruit development. The *arrow* indicates full bloom. *Bars* represent ±SE.

bracts started to shed before flowers during early anthesis, whereas massive flower abscission occurred during the 1st week AFB, as has been reported for other olive cultivars (Rallo and Fernandez-Escobar 1985, Weis et al. 1991). In addition, a small percentage of corollas started to drop 2 weeks before FB, when petals were still closed.

The increase in FW and DW after the completion of inflorescence elongation can be attributed to the swelling flowers and particularly to that of the corollas, stamens, and ovaries. The period of lower values of DW/FW (from 33 up to 43 days ABB) suggests a higher ratio of water over assimilates transport to inflorescence tissues compared with the remainder of the experiment. Concerning the role of water stress on inflorescence development, the water status of the parent plant must be considered crucial at this period.

Respiration rates of inflorescence remained at high levels during the first 3 weeks ABB (Fig. 3). Moreover, the high rates of respiration were generally associated with low rates of growth and vice versa throughout the experiment. This was confirmed by linear regression analysis between respiration and FW and DW (Table 1) which revealed a negative correlation ($r = -0.82$ and -0.84, respectively). Similar results (high respiration and low increase in DW) have been reported for young fruit development in sweet and sour cherry (Blanpied 1972). The high respiratory levels in the early stage of flowering probably reflect the intense rate of physiological activity

Table 1. Statistically significant linear correlation coefficients among length, FW, DW, respiration rate, ethylene, and ABA in developing olive inflorescence.

Parameters	Regression coefficient[a]	Degrees of freedoms
Length-FW[b]	0.821^{***}	12
Length-DW[b]	0.845^{***}	12
Length-Respiration[b]	-0.904^{***}	12
FW-DW[b]	-0.988^{***}	12
FW-Respiration[b]	-0.820^{***}	12
FW-Ethylene[b]	-0.642^{*}	12
DW-Respiration[b]	-0.841^{***}	12
DW-Ethylene[b]	-0.605^{*}	12
Respiration-Ethylene[c]	0.799^{**}	11

[a] $p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.
[b] Data were analyzed from bud burst (March 1) until 1 week after AFB (May 24).
[c] Data were analyzed from 3 weeks ABB (April 22) until young fruit development (June 8).

in meristematic cells of young developing inflorescences.

The high increase in ethylene production during the early flowering period and the relatively high ABA levels 22 days ABB are possibly related to the massive bract abscission during this time. Similar results for ethylene in this stage of flowering have been reported for apple, cherry (Blanpied 1972), and young olive inflorescence (Lavee and Martin 1981), whereas high levels of ABA have also been found in *Pyrus communis* (Browning 1989). In addition to any effect on bract abscission, the role of ABA in the control of dry matter accumulation must also be considered (Beruter 1983, Browning 1980, Dunlap and Robacker 1990, Kojima et al. 1993).

From 22 days ABB until 3 days AFB both ethylene and respiration follow a similar pattern of changes (significant linear correlation at $p < 0.01$, Table 1), suggesting a possible interaction between them, which differs from that of ABA. Both ethylene and respiration had their minimum values 4 days before FB, while ABA level was at its maximum. The increased respiration rate from 4 days before FB is probably related to the increased ethylene production during FB and flower abscission. Similar results have been found in cotton explant abscission (Marynick 1977) and coleus explants (Reid 1985). However, the increased rates of respiration during the 2nd week AFB may be partially a result of the early fruit development.

Respiration and particularly ethylene increased again AFB. The sharp increase in ethylene production (fivefold) from 4 days before FB to 7 days AFB coincides with the period of massive flower shedding. This might also be associated with pollination and petal wilting and abscission, as has been suggested by other investigators for other kinds of flowers (Blanpied 1972, Hall and Forsyth 1967, Morgan et al. 1973, Reid 1985, Swanson et al. 1975). The results for ethylene in this study, combined with those of Weis et al. (1991), indicate that flower abscission in olive is related to ethylene production.

The results of increased ABA levels 12, 6, and 4 days before FB, when it reached its maximum, and the following drop to about one third of its previous level 3 days AFB are very similar to those found in flowers of tomato (Kojima et al. 1993), in stamens of citrus (Kojima 1996), and upper flowers of lupin (Porter 1977).

ABA accumulation and its peak preceded the increased ethylene production by about 1 week. The flower abscission observed later suggests a possible initiative effect of ABA on flower abscission. Whether ABA is responsible or not for the increased ethylene production must be investigated further. The concept that ABA is responsible for initiating the abscission processes and ethylene for the terminal separatory activity as has already been suggested for several plants (Addicott 1982, Cooper and Henry 1973, Moore 1989, Riov et al. 1990) might also be applicable to olive flower abscission. The increased ethylene production and its relation to flower abscission in olive must be taken into account if ethylene inhibitors or promoters are to be applied for increasing fruit set or thinning flowers.

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References

Abeles FB (1967) Mechanism of action of abscission accelerators. Physiol Plant 20:442–454

Addicott FA (1982) Abscission. University of California Press, Berkeley, pp 98–151

Beruter J (1983) Effects of abscisic acid on sorbitol uptake in growing apple fruits. J Exp Bot 34:737–743

Blanpied GD (1972) A study of ethylene in apple, red raspberry, and cherry. Plant Physiol 49:627–630

Browning G (1980) Endogenous *cis,trans*-abscisic acid and pea seed development: Evidence for a role in seed growth from changes induced by temperature. J Exp Bot 31:185–197

Browning G (1989) The physiology of fruit set. In: Wright CJ (ed) Manipulation of fruiting. Butterworth, London, pp 195–217

Cooper WC, Henry WH (1973) Abscisic acid, auxin, and gibberellic acid in wound ethylene production and abscission of fruit of *Citrus sinensis* (L.) Osbeck on two rutstocks. In: Sumiki Y (ed) Plant growth substances. Hirokawa Publishing, Tokyo, pp 1052–1061

Dunlap JR, Robacker KM (1990) Abscisic acid alters the metabolism of indole-3-acetic acid in senescing flowers of *Cucumis melo* L. Plant Physiol 94:870–874

Goldschmidt EE, Goren R, Even-Chen Z, Bittner S (1973) Increase in free and bound abscisic acid during natural and ethylene-induced senescence of citrus fruit peel. Plant Physiol 51:879–882

Hall IV, Forsyth FR (1967) Production of ethylene by flowers follow-

ing pollination and treatments with water and auxin. Can J Bot 45:1163–1166

Kitsaki CK, Drossopoulos JB, Terzis S (1995) Endogenous free abscisic acid in floral, bark, and leaf tissues of olive during anthesis and early fruit development. Sci Hort 64:95–102

Kojima K (1996) Changes of abscisic acid, indole-3-acetic acid and gibberellin-like substances in the flowers and developing fruitlets of citrus cultivar "Hyuganatsu." Sci Hort 65:263–272

Kojima K, Kuraishi S, Sakurai N, Fusao K (1993) Distribution of abscisic acid in different parts of the reproductive organs of tomato. Sci Hort 56:23–30

Lavee S, Martin GC (1981) Ethylene evolution from various developing organs of olive (*Olea europaea* L.) after excision. Physiol Plant 51:33–38

Martin GC, Lavee S, Sibbert GS (1981) Chemical loosening agents to assist mechanical harvest of olive. J Am Soc Hort Sci 106:325–330

Marynick MC (1977) Patterns of ethylene and carbon dioxide evolution during cotton explant abscission. Plant Physiol 59:484–489

Mayak S, Halevy AH (1972) Interrelationships of ethylene and abscisic acid in the control of rose petal senescence. Plant Physiol 50: 341–346

Moore TC (1989) Biochemistry and physiology of plant hormones. 2nd ed. Springer-Verlag, New York, pp 217–218

Morgan PW, Durham JI, Lipe JA (1973) Ethylene production by the cotton flowers: Role and regulation. Proceedings of the 8th International Conference on Plant Growth Substances. Science Council of Japan International Plant Growth Substances Association, pp 1062–1068

Porter NG (1977) The role of abscisic acid in flower abscission of *Lupinus luteous.* Physiol Plant 40:50–54

Rallo L, Fernadez-Escobar R (1985) Influence of cultivar and flower thinning within the inflorescence competition among olive fruit. J Am Soc Hort Sci 110:303–308

Rappoport H, Rallo L (1991) Postanthesis flower and fruit abscission in "Manzanillo" olive. J Am Soc Hort Sci 116:720–723

Reid MS (1985) Ethylene and abscission. HortScience 20:45–50

Riov J, Dagan E, Goren R, Yang SF (1990) Characterization of abscisic acid-induced ethylene production in citrus leaf and tomato fruit tissues. Plant Physiol 92:48–53

Sagee O, Goren R, Riov J (1980) Abscission of citrus leaf explants. Interrelationships of abscisic acid, ethylene, and hydrolytic enzymes. Plant Physiol 66:750–753

Sargent JA, Osborne OJ, Dunford SM (1984) Cell separation and its hormonal control during fruit abscission in the Gramineae. J Exp Bot 35:1663–1674

Sexton R, Roberts JA (1982) Cell biology of abscission. Annu Rev Plant Physiol 33:133–162

Swanson B, Wilkins HF, Weiser CF, Klein I (1975) Endogenous ethylene and abscisic acid relative to phytogerontology. Plant Physiol 55:370–376

Vemmos SN, Pontikis CA, Tolia-Marioli AP (1994) Respiration rate and ethylene production in inflorescence buds of pistachio in relation to alternate bearing. Sci Hort 57:165–172

Weis KG, Goren R, Martin GC, Webster BD (1988) Leaf and inflorescence abscission in olive. I. Regulation by ethylene and ethephon. Bot Gaz 149:391–397

Weis KG, Webster BD, Goren R, Martin GC (1991) Inflorescence abscission in olive: Anatomy and histochemistry in response to ethylene and ethephon. Bot Gaz 152:51–58