The Role of Chilling in Releasing Olive Floral Buds from Dormancy

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Abstract. Experiments with olive (*Olea europaea* L.) shoot explants were carried out to determine the influence of winter chilling on the release of axillary buds from dormancy. This investigation was designed to explore an alternative explanation for the confusing concept surrounding the role of chilling in olive floral induction. Leafy explants collected from 10 Nov. to 6 Mar. were grown in a greenhouse under mist at 13/24C (night/day) and in a growth chamber at 10/21C (night/day) to determine the end of dormancy. Growth of floral buds from leafy explants was first recorded from 5 Jan. samples. After that date the percentage of developing floral buds and rate of their development increased. Floral bud abscission, increase in bud fresh weight, and simultaneous decrease of relative bud dry weight were associated with growth initiation of floral buds. Manual defoliation of adult trees during the period of shoot explant collection indicated that leaves play a critical role in development once the floral buds had completed dormancy. Supplementary chilling of isolated shoots collected 20 Jan. demonstrated that 7.2C was sufficient to complete chilling requirements, while 12.5C allowed both the completion of chilling requirements and the proper temperature for subsequent floral bud growth. Winter chilling is required to release previously initiated floral buds from dormancy, and we question the previous concept that the role of chilling is to induce olive floral initiation.

Chilling has been thought to cause olive floral induction since the studies of Hartmann and coworkers (Hackett and Hartmann, 1963, 1964, 1967; Hartmann, 1953; Hartmann and Porlingis, 1957). These studies were conducted with potted olive plants or with individual shoots on potted trees exposed to chilling for 10 to 12 weeks. Only under those conditions were axillary buds, morphologically initiated to floral buds, able to grow (Hackett and Hartmann, 1963, 1964). These authors based their determination of flower initiation on anatomical observation of both the broad dome and formation of the first sepal primordium at the apical meristem of the axillary buds (Almeida, 1940; Hackett and Hartmann, 1963, 1964; Hartmann, 1951). The proposed inductive role of chilling in flowering of potted olive plants was also supported by experiments of Hartmann and Whisler (1975) in which 12 weeks of specific chilling treatment induced flowering, when applied starting any time during the year.

In the intervening years, field observations as well as recent experimental data contradict the proposition that chilling results in olive floral induction. We now contend that floral induction occurs before winter, based on the following evidence: The alternate bearing habit of the olive implies that most of the buds in bearing (On-year) plants could not have been induced by chilling treatments in the previous growing season. Further, the presence of fruitlets (Lavee et al., 1986) or their seeds (Stutte and Martin, 1986) as early as 40 days after full bloom, i.e., some 5 months before the chilling period, prevents flower induction and therefore flowering the following year. Recently, it was found that early summer trunk injections of GA₃ in non-bearing trees prevent flowering the next year (Navarro et al., 1990a). However, morphological and histochemical (Pinney and Polito, 1990) and biochemical and morphological (Navarro et al., 1990b) changes in the buds suggest that floral induction is

already accomplished by midautumn. Finally, shading experiments (Tombessi and Cartechini, 1986) show that reduction of the density of radiation by 60% prevents flowering the next year when done before midautumn.

The experiments presented in this paper were carried out to test the hypothesis that winter chilling is required in olive to release already-induced floral buds from dormancy, much as occurs in temperature-zone deciduous fruit trees.

Materials and Methods

The response to natural winter chilling. Shoots grown during 1989 were collected throughout the 1989-90 winter from adult trees of 'Ascolano' and 'Manzanillo' olive grown under irrigation in one experimental plot of the Univ. of California at Davis. Type of shoots, preparation and manipulation of explants, and other environmental conditions in both greenhouse and growth chamber are described by Rallo and Martin (1991). In brief, shoots with ≈ 18 nodes were cut in the field and immersed in water for transport to the laboratory. There, six-node explants were prepared and placed in vials containing 8-hydroxyquinoline citrate (8-HQC) for subsequent experiments. The explants from 'Manzanillo' belonged to 1989 bearing (On) and nonbearing (Off) trees, whereas the explants from 'Ascolano' were from trees in the Off season during 1989. Explants were grown in a greenhouse under mist at 13/24C (night/day) under ambient light or in a growth chamber at 10/21C (night/day) with a photosynthetic photon flux density of 260 to 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The explants in the growth chamber were placed in vials with a 200-ppm aqueous solution of 8-HQC to prevent xylem plugging (Halevy and Mayak, 1981).

The greenhouse experiment was designed as a split plot where the main plots were assigned to collecting dates and the subplots to the three treatments: 'Ascolano' Off, 'Manzanillo' Off, and 'Manzanillo' On in 1989. There were four explants per subplot and three blocks. A randomized block with three replicates and four explants per replication (plot) was designed for each collecting date in the growth chamber experiment. The treatments were the same as those for the greenhouse experiment.

Once development of the axillary buds was apparent, the number of developing buds and their developmental stage were

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recorded every 2 weeks. Bud abscission occurred in the greenhouse explants and was recorded at the conclusion of the experiment, on 4 Apr. 1990. The fresh and dry weight of a sample of 15 to 20 buds per shoot in six shoots was also recorded on all collecting dates. The number of hours below $7.2 \pm 0.5^\circ\text{C}$ was computed from the weather station records kept in the Dept. of Pomology.

The response to supplementary artificial chilling. Explants of 'Manzanillo' Off in 1989 were taken on 20. Jan. 1990 from shoots of mature trees to test their response to supplementary differential chilling. At the time of collection, 800 h below 7.2°C had accumulated in the field. Populations of explants were randomized in two growth chambers set at $7.2 \pm 0.5^\circ\text{C}$ and $12.5 \pm 0.5^\circ\text{C}$ (Hackett and Hartmann, 1967; Hartmann and Whisler, 1975) for 4 and 6 weeks and then transferred to another growth chamber with 10 h of light and set at $10/21^\circ\text{C}$ (night/day) where they were grown until 10 weeks had elapsed from their collection in the field. A control set of explants was grown in the latter chamber during the entire 10 weeks of the experiment. The experiment was designed as a completely randomized block with three replicates and four explants per replication (plot). The number of growing axillary buds per explant was recorded at 6, 8, and 10 weeks.

Defoliation. This experiment tested the growth and flowering response of the axillary buds in single shoots to various dates of defoliation in mature trees from November to March, i.e., during the hypothesized floral induction-initiation and/or dormancy period of the olive. Six shoots per tree in three trees of each 'Ascolano' Off, 'Manzanillo' Off, and 'Manzanillo' On in 1989 were completely defoliated on the same dates (treatments) that the shoots were collected for explants in the previously outlined experiments. The shoots from these mature trees, for each treatment, were chosen at random in six outside areas of the canopy between 1.5 and 2.5 m from the ground. The dates of defoliation were assigned at random within any of the six similar outside areas of the canopy described. The number of growing vegetative and reproductive axillary buds was recorded on 30 Apr. 1990.

For all experiments when the variance for different treatments was heterogeneous, the data are presented as the average $\pm$ SE per treatment. Bud development is presented as percentages of vegetative or floral buds based on total buds. For analysis of variance, the original data were arcsin transformed.

Results

Influence of shoot collection date on growth of axillary buds.

For all experiments conducted in the greenhouse or growth chamber using olive explants, only floral axillary buds developed ('Ascolano' Off, 'Manzanillo' Off, and 'Manzanillo' On). No floral bud development occurred from explants collected before 5 Jan. (Fig. 1). Following 5 Jan., percent floral bud development increased steadily to the maximum at the final date, 6 Mar. (Fig. 1). Differences between those treatments were due primarily to the previous season bearing status of the trees as evidenced by buds developing in 'Manzanillo' Off and 'Manzanillo' On explants in both environmental conditions. 'Manzanillo' Off bud development occurred earlier than 'Ascolano' Off in the greenhouse and growth chambers.

Floral bud abscission was evident in the explants of all three treatments (cultivars and/or bearing conditions in 1989) grown in the greenhouse (Fig. 2). Differences among dates of collection ($P < 0.01$) showed that percent bud abscission increased with later dates of collection (Fig. 2). More abscission was

Fig. 1. Floral bud development of olive shoot explants collected from 5 Jan. to 6 Mar. from 'Ascolano' Off, 'Manzanillo' Off and 'Manzanillo' On. Explants were placed in growth chambers at $10/21^\circ\text{C}$ (night/day) or in a greenhouse under mist at $13/24^\circ\text{C}$ (night/day) where they were held for 9 or 12 weeks, respectively. Bars equal $\pm$ SE.

Fig. 2. Floral bud abscission on 4 Apr. in 'Ascolano' Off, 'Manzanillo' Off, and 'Manzanillo' On olive shoot explants collected at various dates and grown in a greenhouse at $13/24^\circ\text{C}$ (night/day) in ambient light. Differences among cultivar-bearing status $\times$ date ($P < 0.01$), cultivar-bearing status ($P < 0.01$), and date ($P < 0.01$) were significant for arcsin-transformed data.

apparent after 6 Feb. in 'Ascolano' than 'Manzanillo'. The differences in bud abscission due to the bearing condition of 'Manzanillo' in 1989 were also related to the percentage of developing buds between Off and On trees in this cultivar.

Seasonal changes in bud fresh and dry weight. Average fresh weight increased and the percentage of dry weight decreased simultaneously in the samples of axillary buds taken from the shoots collected after 6 Feb., in trees of 'Manzanillo' Off and 'Ascolano' Off. In contrast, buds collected from 'Manzanillo' On trees did not show such a pattern (Fig. 3).

Response to supplementary artificial chilling. The response of explants taken on 20 Jan. depended on temperature. Ten weeks at $10/21^\circ\text{C}$ resulted in intermediate bud growth compared

Fig. 3. Seasonal changes of average fresh weight and percent dry weight of axillary buds of 'Ascolano' Off, 'Manzanillo' Off, and 'Manzanillo' On olive from explants collected at various dates. Bars equal $\pm$ SE.

with the transfer treatments described below (Fig. 4A). A constant 7.2C prevented bud development for 4 weeks, whereas by 6 weeks some development occurred (Fig. 4 B, C). Transfer from 7.2C after 4 or 6 weeks at that temperature to 10/21C resulted in rapid bud development (Fig. 4 B, C). A constant 12.5C promoted bud development. By 6 weeks (Fig. 4 D, E) $\approx$ 90% of the buds had emerged and no additional development occurred once the explants were transferred to 10/21C (Fig. 4E).

Response to defoliation. A gradual increase in percentage of floral bud development was observed for all three treatments ('Ascolano' Off, 'Manzanillo' Off, and 'Manzanillo' On) when defoliation was finished by January (Fig. 5). Differences in floral budbreak were significant among treatments ($P < 0.01$), dates of defoliation ($P < 0.001$), and for the corresponding interaction ($P < 0.001$). The number of developing floral buds of the defoliated shoots approached that of the untreated control the later the date of defoliation. Nevertheless, some floral bud development occurred even at the earliest date of defoliation, i.e., 13 Nov., in all three treatments (Fig. 6).

Bud development of axillary vegetative buds was minimal in 'Ascolano' Off, minor in 'Manzanillo' Off, but substantial in 'Manzanillo' On (Fig. 6). For 'Manzanillo' On, the percentage of vegetative bud development was significantly higher than for 'Manzanillo' Off ($P < 0.01$) and higher than the percentage of developing floral buds for any defoliation date (Fig. 6). There was no significant difference in developing vegetative buds between the control and the dates of defoliation for any cultivar-bearing status treatment.

Discussion

The results of our experiments do not agree with those of previous olive literature. Our results with olive are similar to those commonly associated with temperate-zone deciduous fruit trees; that is, floral buds develop when excised shoots collected during the winter from outdoor trees are placed in favorable growth conditions (Fig. 1). This response is commonly interpreted as indicative of release from dormancy of previously initiated floral buds (Saure, 1985). The olive floral bud development response, not observed in vegetative buds, was characterized by a higher percentage of buds that developed and by more rapid bud development the later the date of collection. Moreover, supplementary artificial chilling increased and accelerated floral bud development from explants that were partially chilled outdoors (Fig. 4). The observed abscission of axillary floral buds (Fig. 2) has also been associated with poorly devel-

Fig. 4. Floral bud development of olive in response to temperature. Shoots collected 20 Jan. Buds refers to the average percent of developing buds per explant; explant refers to the percent of explants with at least one developing bud under the various temperatures illustrated. Differences among treatments for angular transformed data at each time of recording were significant as indicated. [Explants ($\square$) P 0.008, 0.054, 0.125, 0.165; Buds ($\bullet$) P 0.018, 0.038, 0.135 at 4, 6, 8, and 10 weeks].

oped buds or buds that are fully developed but not released from dormancy (Millella and Deidda, 1968).

Previous authors reported a relationship between accumulated chilling hours and the number of developing olive inflorescences (Hackett and Hartmann, 1963, 1967; Hartmann and Porlingis, 1957; Hartmann and Whisler, 1975); however, these authors assumed that chilling brought about floral initiation. One reason for this assessment lies in the criterion used to establish the date of floral initiation (Hackett and Hartmann, 1963, 1964), which was based on the morphological changes in the apex of the axillary buds observed between mid-February and mid-March in the northern hemisphere (Almeida, 1940; Hartmann, 1951; Hackett and Hartmann, 1963, 1964; King, 1938). However,

Fig. 5. Shoots of 'Ascolano' Off (left), 'Manzanillo' Off (middle) and 'Manzanillo' On (right) olives showing bud development on 20 Apr. Dates indicate defoliation times.

recent morphological and histochemical observations suggest that floral initiation in olive occurs by midautumn (Pinney and Polito, 1990). Therefore, the morphological changes observed by Hackett and Hartmann (1963, 1964), which were associated with an increase in bud fresh weight and a concomitant decrease in the percentage of bud dry weight, could simply indicate the simultaneous resumption of development and the morphological differentiation of already initiated floral buds after their release from dormancy. Our data support that contention. Similar changes in bud fresh and dry weight in 'Ascolano' Off and 'Manzanillo' Off but not in 'Manzanillo' On (Fig. 3) occurred as floral bud development took place (Fig. 1).

We suggest that an alternate interpretation can be given the results of the papers claiming that chilling is required for floral induction in olive (Badr and Hartmann, 1971; Hackett and Hartmann, 1963, 1964, 1967; Hartmann, 1953; Hartmann and Porlingis, 1957; Hartmann and Whisler, 1975), namely, that chilling in olive is required to release initiated floral buds from dormancy. In fact, only in the papers of Hackett and Hartmann (1963, 1964) were both developing floral and vegetative buds measured. The data of the 1963 paper show that only 7.7% of the axillary buds (6.1% floral and 1.6% vegetative) were able to develop when chilling was insufficient, whereas as many as 74% of the axillary buds (73.6% of floral and 0.4% of vegetative) developed when the plants were well chilled (1862 h < 7.2°C); the relevant differences being, therefore, in developing floral buds. Our data (Fig. 1) show similar differences between unchilled and progressively chilled shoots, suggesting that the observed differences in the number of developing floral buds in the above papers could have been dormant floral buds.

A differential action of the temperature during winter was observed in our experiments (Figs. 1 and 4); 12.5°C was more effective than 10/21°C in promoting floral bud development of the shoots collected 20 Jan. In contrast, the 10/21°C regime strongly induced floral bud development when shoots were collected after 21 Feb. or when they were collected on 20 Jan. and

given supplementary chilling at 7.2°C. It seems, therefore, that 7.2°C effectively met chilling requirements and that 12.5°C met both chilling requirements of dormant buds and the temperature to promote growth of chilled buds (Fig. 4). Thus, for olive, hours < 7.2°C may not be the best indicator for bud chilling requirements. The 10/21°C regime or a greenhouse at 13/24°C best served the temperature needs for forcing chilled buds. Differential chilling action, even negative effects, for specific ranges of temperature is reported in many fruit trees (Saure, 1985). Olive, too, may have a differential chilling and growth response during the winter. Such could explain, in part, the results of others (Badr and Hartmann, 1971; Hackett and Hartmann, 1967; Hartmann and Whisler, 1975). Denny and MacEachern (1983) reported that the number of days with means between 12.2 and 13.3°C was a common factor for flowering in the best olive producing areas of the world.

As many as 30% of floral buds were recorded as being developing in shoots defoliated 13 Nov. when < 100 h below 7.2°C had accumulated. Later defoliation led to increasing percentages of floral bud that developed. In contrast, defoliation did not influence the percentage of developing vegetative buds the following spring (Fig. 6). These data contrast with those of Hackett and Hartmann (1964), who reported that minimum leaf area and low temperature were required concurrently for inflorescence formation. Even so, those authors found that 50% of buds formed inflorescences when shoots were defoliated on 26 Nov. Only when defoliation was complemented with girdling was flowering prevented the next spring. According to our defoliation studies, leaf area and chilling were not simultaneously required for floral bud formation. Further, when floral initiation was completed between 1 and 15 Nov. (Pinney and Polito, 1990), and then those buds became dormant, later floral bud development under warm conditions could be explained by the availability of photoassimilates from nearby foliage, without which growth would be minimal. This latter argument, relating photoassimilates to development, could also justify the differences in the

Fig. 6. Percentage of developing reproductive and vegetative buds of shoots defoliated in the field at various dates during dormancy for 'Ascolano' Off, 'Manzanillo' Off, and 'Manzanillo' On olive trees. Bud development was recorded on 20 Apr. Differences of arcsin-transformed data for developing floral buds were significant among dates ($P < 0.001$), among cultivar-bearing status treatments ($P < 0.01$), and for the interaction ($P < 0.001$). Differences of arcsin-transformed data for vegetative buds were only significant among cultivar-bearing status treatments ($P < 0.01$).

total quantity of developing floral buds between 'Manzanillo' Off and On trees.

In summary, all of the results of the experiments presented here—1) bud development in naturally and artificially chilled explants, 2) fresh and dry weight changes in buds during winter, 3) bud abscission in explants at the time of bud development, and 4) bud development in shoots defoliated at different dates during winter—provide evidence of release from dormancy of previously initiated floral buds and contradict the current concept that chilling in olive results in floral induction.

Literature Cited

- Almeida, F.J. 1940. Safra e contrasafra na oliveira. Boletim Ministerio de Agricultura de Portugal. 7:154.
- Badr, S.A. and H.T. Hartmann. 1971. Effect of diurnal fluctuating vs. constant temperatures on flower induction and sex expression in the olive. *Physiol. Plant.* 24:40-45.
- Denney, J.O. and G.R. MacEachern. 1983. An analysis of several climatic temperature variables dealing with olive reproduction. *J. Amer. Soc. Hort. Sci.* 108:578-581.
- Hackett, W.P. and H.T. Hartmann. 1963. Morphological development of olive as related to low temperature requirement for inflorescence formation. *Bot. Gaz.* 124:383-387.
- Hackett, W.P. and H.T. Hartmann. 1964. Inflorescence formation in olive as influenced by low temperature, photoperiod, and leaf area. *Bot. Gaz.* 125:65-72.
- Hackett, W.P. and H.T. Hartmann. 1967. The influence of temperature on floral initiation in the olive. *Physiol. Plant.* 20:430-436.
- Halevy, A.H., and S. Mayak. 1981. Senescence and postharvest physiology of cut flowers. Part 2. *Hort. Rev.* 3:59-143.
- Hartmann, H.T. 1951. Time of floral differentiation of the olive in California. *Bot. Gaz.* 112:323-327.
- Hartmann, H.T. 1953. Effect of winter chilling on fruitfulness and vegetative growth in the olive. *Proc. Amer. Soc. Hort. Sci.* 62:184-190.
- Hartmann, H.T., and I. Porlingis. 1957. Effect of different amounts of winter chilling in fruitfulness of several olive varieties. *Bot. Gaz.* 119:102-104.
- Hartmann, H.T., and J.E. Whisler. 1975. Flower production in olive as influenced by various chilling temperature regimes. *J. Amer. Soc. Hort. Sci.* 100:670-674.
- King, J.R. 1938. Morphological development of the fruit of the olive. *Hilgardia* 11:437-454.
- Lavee, S., H. Harshemesh, and N. Avidan. 1986. Phenolic acids possible involvement in regulating growth and alternate fruiting in olive trees. *Acta Hort.* 179:317-328.
- Millella, A. and P. Deidda. 1968. La esigenze in freddo dell'olivo. *Studdi Sassaressi. Sez III Annali della Facolta di Agraria dell'Universita di Sassari* 16:1-22.
- Navarro, C., R. Fernández-Escobar, and M. Benlloch. 1990a. Flower bud induction in 'Manzanillo' olive. *Acta Hort.* 286:195-198.
- Navarro, C., M. Benlloch, and R. Fernández-Escobar. 1990b. Biochemical and morphological changes related to flowering in the olive. XXIII Intl. Hort. Congr. Florence, Italy Abstr. 3196.
- Pinney, K. and V.S. Polito. 1990. Flower initiation in "Manzanillo" olive. *Acta Hort.* 286:203-206.
- Rallo, L. and G.C. Martin. 1991. Determinación de la salida del reposo de yemas de olivo en ramos aislados. *Actas Hort.* (In press.)
- Saure, M.C. 1985. Dormancy release in deciduous fruit trees. *Hort. Rev.* 7:239-300.
- Stutte, G.W. and G.C. Martin. 1986. Effect of killing the seed on return bloom of olive. *Scientia Hort.* 29:107-113.
- Tombessi, A. and A. Cartechini. 1986. L'effetto dell'ombreggiamento della chioma sulla differenziazione delle gemme a fiore dell'olivo. *Rivista di Ortoflorofrutticoltura Italiana* 70:277-285.