

FLORAL ORGANS REMOVAL IN AVOCADO: OPENING AND CLOSING CONTROL

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Avocado flower opens twice: first as functional female, second acting as male. These phases of flower opening and closing control the orderly exposure of mature female and male organs to pollinators. Disorders of this flower behavior, as partial and single flower aperture, have been previously documented. However, little is known about the factors controlling movements of tepals and the consequences of their malfunctioning on the function of pistil and stamens. By selective removal of tepals, stamens, nectaries, staminodes, and pistil, we explored organ-control of the avocado flower cycle. Nectary and staminode removal did not change the flower cycle. Early pistil removal advanced first closing, but no other changes were noticed later. Pistil removal during the male phase did not alter the flower cycle. Removal of tepals during the female phase conspicuously modified the angle of the corresponding stamen beyond horizontal and consequently delayed or made retraction incomplete during first closing. Lesser changes were detected when tepals removal was performed during the male phase. Stamen removal also modified movement of tepals. In a few cases when the stamen was cut in the female phase, the tepals did not open in the male phase. If the stamen was eliminated during the second opening, the tepals closing was advanced. These modifications suggest that female and male reproductive organs control the corresponding flower opening, whereas closure is mostly lead by tepals. Our observations also suggest that endogenous factors related to reproductive organ maturation rather than environmental cues control avocado flower cycle.

Key words: sexual phases, tepals, pistil, stamens, staminodes, nectaries.

CONTROL DE LA APERTURA Y CIERRE DE LA FLOR DICÓGAMA DEL AGUACATE

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El aguacate es dicógamo. Su flor abre primero como femenina y, tras un cierre, lo hace como masculina. Estas fases de apertura y cierre de la flor regulan la exposición ordenada de los órganos reproductivos a insectos vectores. Aunque se conocen diferentes alteraciones del ciclo, escaso es el conocimiento sobre los órganos reguladores del movimiento de los tépalos y las consecuencias de su mal funcionamiento. En este trabajo analizamos estas consecuencias mediante

la mutilación de nectarios, estaminodios, tépalos, estambres y pistilo. Los resultados indican que la mutilación de nectarios y estaminodios no alteró el ciclo dicógamo. La eliminación del tépalo afectó al estambre que se extendió más allá de la horizontal durante la primera apertura; consecuentemente este estambre retrasó su retracción, en ocasiones incompleta. Los cambios fueron menores durante la fase masculina. La eliminación del estambre alteró asimismo el comportamiento del tépalo. En algunas flores se observó que en ausencia de estambre en fase femenina, el tépalo no abría en fase masculina, mientras que adelantaba su cierre si el estambre se mutilaba en fase masculina. La eliminación temprana del pistilo adelantó el primer cierre, aunque no hubo cambios en la fase masculina. Las observaciones sugieren que el pistilo y los estambres controlan la apertura de la flor en sus respectivas fases, mientras que la posición y movimiento de los estambres durante los cierres estarían bajo control de los tépalos. Nuestras observaciones apuntan a factores endógenos ligados a la maduración de los órganos reproductivos como reguladores del ciclo floral del aguacate.

Palabras clave: fases sexuales, tépalos, pistilo, estambres, estaminodios, nectarios.

INTRODUCTION

Avocado presents protogynous dichogamy with synchronous daily complementarity. In this unusual breeding system every avocado flower opens twice. First day the flower opens as female, second day the flower reopens as male. Tepals close between female and male phases. Conspicuous movements of the reproductive organs accompany the opening and closure of tepals (Ish-Am and Eisikowitch, 1991). During the female phase, the stigma is receptive and able to adhere pollen grains carried out by insects, but the stamens, placed in the horizontal just above the tepals, are immature and do not deliver pollen. Nectar in this period is secreted by staminodes. In the next male phase, the three inner stamens position surrounding the pistil, while the outer six keep an angle of about 45°. In this phase, the anthers dehisce by four valves offering pollen as reward to pollinators. The stigma is usually wilted. Nectar is secreted by three pairs of nectaries in the male phase.

During previous observations carried out on 'Hass', 'Fuerte', 'Bacon' and 'Zutano', we have detected deviations of their floral cycle (Cabezas *et al*, 2003a), being the most outstanding flowers opening three times or just one, and flowers with incomplete tepal aperture or lacking synchronization between the aperture of tepals and stamens' dehiscence. At this regard, many flowers, specially in 'Zutano', entered male phase without the preceptive closing of tepals. Flowers in the female phase of 'Hass' were found to develop occasionally with an incomplete aperture of tepal. More often, 'Hass' flowers in their male phase lacked synchronization among tepals and stamens. In these last flowers, only some tepals and their correspondent stamens open fully. Remaining tepals continue attached to the pistil and their annexed stamens remain immature below

tepals (Figure 1). These tepals and stamens unfolded next day, extending male phase one more day.

In this work, we explored the changes occurring in tepal display and fertile organs exposure to insects by means of the selective removal of tepals, stamens, nectaries, staminodes, and pistil in flowers of the cultivar 'Hass' with the aim of bringing some insight into the endogenous and environmental factors controlling the unique floral behavior of avocado.

MATERIAL AND METHODS

Selective removal of floral organs was achieved in flowers of cultivar 'Hass' trees cultivated in Almuñécar (Granada, Spain). The removal affected to a single tepal, nectary, stamen, staminode, or pistil. Only one organ was cut in each flower. A control intact flower was left nearby. Following organ removal, the subsequent changes in activity and position of the remaining flower organs were monitored and recorded until definitive flower closing. Special attention was taken in the changes expressed by organs occupying equivalent position to the organ removed in adjacent whorls compared with left intact organs. Organs of the same whorl were identified based on its position in relation to panicle axis. A schedule of the procedure is presented in Figure 2.

Mutilations were commonly repeated in a sample of 16 flowers in four moments of the flower cycle, either at the beginning or at the end of the female or male phase (phenostages F1f, F2f, F1m, and F4m, respectively; Cabezas *et al*, 2003). The comparison of the responses during flower opening versus closing pretends to determine if a different mechanism control tepal aperture and closure. The comparison of the response during female versus male phase explores changes in the identity of the organs controlling flower cycle in both phases. It should be noticed that an intervention during female phase also allows more time for changes to be expressed. Flowers were tagged using pink and blue threads according to the moment of intervention.

For tepals and stamens a distinction between the removal of organs in internal and external whorls was done, and in the case of stamens a comparison was performed between the complete removal of the whole stamen versus the elimination of the anther; in a reduced sample of flowers the whole pistil was cut while in other sample only the stigma was removed, leaving intact style and ovary. The trial was carried out during the period of full bloom of 2004 (mid-April). Organ removal was achieved using a sharp razor trying not to affect adjacent organs.

RESULTS

The mutilation of nectaries and staminodes did not change tepal aperture or the expression of female and male organs when compared to control nearby flowers. This situation was expressed in all samples with no differences depending on the phenostage in which the removal was performed. No increase in the nectar

produced was noticed in remaining nectar producing-organs when a single nectar or staminode was cut.

The removal of the tepal conspicuously modified the angle of stamen positioning during the opening of the female phase (F1f). In this moment in absence of the corresponding tepal, the stamen went beyond horizontal (Figure 3), needing more time to come back in the first flower closure. In some flowers, the stamen did not complete the retraction. This response affected only to the stamen occupying an equivalent position to that of the tepal; all other stamens remained unaffected. If tepal removal was achieved later during first closing (F2f), the stamen retraction was delayed and commonly incomplete (Figure 4). Minor changes were detected when tepal mutilation was performed in the male phase, with only one exception in which the stamen remained out at the moment of the definitive flower closure. The dehiscence of the anthers seemed to progress as did in control flowers. On the other hand, the removal of external tepals produced greater changes than the elimination of the internal ones. Not all flowers expressed changes of the same magnitude. In some occasions a weak response could be due to an incomplete tepal removal, although in other cases no reason for the lack of response could be tracked. It is also important to underline that in all cases the movement of the stamen, although altered, took place.

Stamen removal during female phase did also modify tepal movement but the effects were not immediate. At this regard, when the stamen was cut at the beginning of the female phase (F1f), tepals open and close without noticeable changes with respect to control flowers, but in the next male phase the corresponding tepal scarcely moved lacking synchronization with other tepals (Figure 5). The filament seems to play a major role in this response because the removal of the anther scarcely modified the movement of the corresponding tepal. On the contrary, when the stamen was eliminated during male opening, the only effect observed was an advancement in the closure of tepal. The filament seems to play a major role in this response because the removal of the anther scarcely modified the movement of the corresponding tepal.

The cut of the whole pistil or of just the stigma during the female phase advanced first closing, but no other changes were noticed later. Neither nectar secreting organs, nor stamens expressed any change in response to pistil mutilation. The advancement of first flower closure was simultaneous, indicating that pistil removal affected all tepals equally. Pistil removal during male phase did not alter flower cycle.

DISCUSSION

Despite that the flower behavior of avocado was described long time ago (Nirodi, 1922; Stout, 1923), our knowledge about the factors governing its flower cycle is very limited. At this regard, the cycle of flower aperture and closure and the daily pattern of avocado pollinators' activity dictate that light and temperature might control avocado flower behavior (Stout, 1927). Papademetriou (1976), on the contrary, found doubtful light influence on flower opening since flowers of

'Hashimoto' and other cultivars may start to open at darkness. Lesley and Bringhurst (1951) observed some relations between temperature and first flower opening in 'Fuerte' (type B). Furthermore, Gazit and Degani (2002) state that detached flowers kept in the dark close and reopen at about the same speed that in the field if the same temperatures are provided. Low temperatures are known to extend avocado flower cycle and caused repeated male phase openings (Sedgley and Grant, 1982), but the interpretation of this fact commonly does not go apart from thermal effects (Stout, 1923) and no questioning of the last reasons of tepal aperture delay is the rule in the literature.

In the few cases in which flower movement has been study in nature, temperature and no light has been found the main environmental factor controlling the diurnal pattern of petal opening and closing (Tanaka *et al*, 1987; Von Hase *et al*, 2006). In this last study, in several Aizoaceae and Neurodeceae has been found that air temperature rise correlates well with flower opening and temperature decrease with closing. In vespertine flowers (often pollinated by moths) the response to temperature is the opposite, although we have not found any paper in the literature analyzing this aspect. Interestingly, Von Hase *et al*, (2006) found that petal closing showed a lower relationship with temperature than flower opening, suggesting for that reason that endogenous factors are more important for petal closing.

Our results suggest, on the contrary, that the movements that characterize the unique avocado flower cycle are instead under endogenous control. At this regard, it can be concluded that the removal of pistil shortened female phase (advancing tepal closure), but left unaffected male phase. On the contrary, the removal of the stamen during male phase provoked the shortening of pollen donation period, but did not change female phase. Furthermore, when the stamen removal was achieved early enough (in first opening) changes were not immediate but affected profoundly tepal movement during next male phase. The restrained movement of tepals in response to early stamen mutilation agrees with previous observations in which tepal movement was refrained when the corresponding stamen remained immature and not dehiscing during the male phase (Cabezas *et al*, 2003). So contrary, to our expectations, there is no one single floral organ controlling flower cycle, but pistil seems to govern female phase and stamens the male one; tepals being important elements limiting stamen folding and unfolding.

It could not be other way. As emphasized by Van Hase *et al*, (2006) petal movement has to be analyzed in the context of its function. The sequence of avocado tepal aperture and closure obviously controls the orderly exposition of mature female and male organs to insect visitors. In female phase when the pistil is mature and capable of adhering pollen grains is important that tepals and stamens move further and left fully exposed the pistil to insect activity. On the contrary, during male phase the position of the inner stamens surrounding the pistil and the 45° angle of the outer six facilitate the contact of anthers with pollinator body, therefore an efficient transfer of pollen during male phase involves a repositioning of the stamen. In both phases the tepals, positioned in

the horizontal are the landing area for insects, and its folding and unfolding fit the pattern of activity of avocado pollinators.

Once the main organs implicated in the characteristics movements of avocado flowers have been identified a more ample research is possible to study the effects of the alterations of flower cycle on avocado reproductive success. For instance, the effects on male fitness due to the opening delay of some anthers is uncertain because although it may extend male phase, also diminish initially the amount of pollen available for pollinators. Interference between male and female functions is probably increased in avocado flowers without intermediate closure. Lastly, the consequences of repeated flowers aperture on pollen removal and deposition in the stigma also merit further studies.

CONCLUSIONS

Flower sequence of aperture and closure controls the orderly exposition of mature female and male organs to insect visitors. As a whole our results suggest that the presence of the corresponding reproductive organs control each flower phase while tepals control stamens folding and unfolding. Our observations also suggest that endogenous messengers related to reproductive organs maturation rather than environmental cues rule avocado flower display. The correspondence between tepal and stamen movements corroborated in this study indicates a close communication between neighboring organs. Short life of avocado flowers and dichogamy may explain why the exposure of fertile organs to visitors through appropriate tepal display relies on endogenous factors linked to pistil and stamen maturation rather than on environmental cues as it has been observed in other species.

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Figure 1. Partial flower aperture during male phase of 'Hass'. Some tepals remain closed when the stamens are immature and not dehiscing.

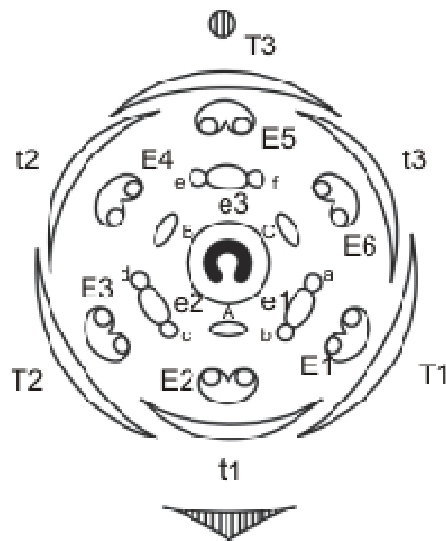


Figure 2. Avocado floral diagram (modified from Gaillard, 1987). Outer tepals (T1, 2, 3), inner tepals (t1, 2, 3), stamen in outermost whorl (E1, 2, 3, 4, 5, 6), stamens in innermost whorl (e1, 2, 3), nectaries (a, b, c, d, e, f) and staminodes (A, B, C).



Figure 3. Flower in which an external tepal was removed at the beginning of the female phase (F1f). Observe the corresponding stamen going beyond horizontal.



Figure 4. Flower in which an external tepal was removed at the end of the female phase (F2f). Inner and outer stamens remain out at first closure.



Figure 5. Flower in which a stamen was removed at the end of the female phase (F2f). The corresponding tepal did not unfold during second flower aperture.

