

Implications of starch content in the flower at anthesis on final fruit set in avocado

M.L. Alcaraz¹, J. Rodrigo², J.I. Hormaza²

¹Instituto de Hortofruticultura Subtropical y Mediterránea la Mayora (IHSM–UMA-CSIC). Apdo 29750 Algarrobo-Costa, Málaga, Spain

²Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Av. Montañana 930, 50059 Zaragoza, Spain

Abstract

In avocado, only a very small fraction of the flowers initially produced are able to set fruits due to a huge drop of flowers and developing fruits. The objective of this work was to analyze the implications of the nutritive status of the flower at anthesis in the reproductive success. For this purpose, a total of 600 flowers from 'Hass' were hand-pollinated during the female stage using pollen from 'Fuerte' flowers at the male stage. After pollination, each flower was labeled. The day following pollination, once the pollen tubes had grown along the style and reached the ovary, the style of each flower was excised and fixed for microscope analysis. To link microscopical observations of the style in each pistil with the behaviour of the corresponding ovaries that remained in the trees, fruit set and fruit abscission were sequentially examined in the field up to harvest. To relate the starch content in the flower at anthesis to the capacity to set fruit, two populations of flowers were established: flowers and fruits that dropped and those that remained in the trees up to harvest. Once the fate of each flower was known, starch was quantified in each style with the help of an image analysis system attached to the microscope. The flowers that successfully developed into fruits presented significant higher starch content in the style than those that abscised. These results suggest that the capacity of a flower to become a fruit could be preconditioned by its starch content at anthesis.

Keywords: anthesis, avocado, fertilization, fruit set, starch

Resumen

En aguacate, solo una pequeña proporción de flores se transforma en fruto debido a una caída masiva de flores y frutos en desarrollo. El objetivo de este trabajo fue analizar las implicaciones del estado nutritivo de la flor en antesis sobre el éxito reproductivo. Con este propósito, se polinizaron manualmente 600 flores de 'Hass' en estado femenino usando polen de 'Fuerte'. Después de la polinización, se marcó individualmente cada flor. El día siguiente a la polinización, una vez que el tubo polínico había crecido a través del estilo, se cortó y fijó el estilo de cada flor para su análisis posterior al microscopio. Para poder relacionar las observaciones microscópicas del estilo con el comportamiento de su ovario correspondiente que permaneció en el árbol, se llevó a cabo un seguimiento en el campo de la caída y cuajado de frutos hasta el momento de la cosecha. Para relacionar el contenido de almidón de la flor en antesis con su capacidad de convertirse en fruto, se establecieron dos poblaciones de flores: flores que finalmente no se desarrollaron en frutos maduros y flores que dieron lugar a frutos que permanecieron en el árbol hasta el momento de la cosecha. Una vez conocido el destino final de cada flor, se cuantificó el contenido de almidón en el estilo con un analizador de imagen acoplado al microscopio. El contenido en almidón fue significativamente más alto en las flores que se desarrollaron en frutos lo que sugiere que la capacidad de la flor para convertirse en fruto podría estar condicionada a priori por su contenido nutritivo en antesis.

Palabras clave: aguacate, almidón, antesis, cuajado, fecundación

Introduction

A main limitation for avocado production worldwide is the massive abscission of flowers and developing fruits resulting in less than 1% of the flowers initially produced able to set fruits (Cameron et al, 1952; Garner et al., 2008; Garner and Lovatt, 2008). Although numerous factors could influence avocado fruit set rate, information is still not available to explain why most flowers prematurely abscise while some of them remain in the trees since no morphological differences are apparent between the flowers that will finally drop and those that will be retained in the trees (Sedgley, 1980). Flower and fruit abscission are the result of different events that take place during the progamic phase and early fruit development. However, a common observation in different plant species is that, even after an adequate pollination and pollen tube growth, often the percentage of fruits developed is very small compared to the initial number of flowers produced (Charlesworth, 1989; Wilcock and Neiland, 2002). Therefore, factors inherent to the pistil could play an important role in flower fate. Among those factors, it has been known for quite a long time that the nutritive reserves present in the tree play a major role in final yield and the term flower quality has been frequently used to explain differences in fruit set (Williams, 1965; Goldwin, 1981), although the significance of the concept of flower quality and the causes behind it are still largely unknown. The fact that both pollen tube growth (Herrero and Dickinson, 1979) and ovule development (Arbeloa and Herrero, 1991) are highly dependent on the starch reserves previously accumulated in the female tissues of the flower, suggest that the starch reserves present at flower opening could play a major role in fruit set and they could indeed be one of the main factors determining flower quality (Rodrigo et al., 2000)..

In avocado, the presence of starch in the ovary has been reported at anthesis (Sedgley, 1979), but the implications of starch reserves within the flower in the reproductive process have not yet been analyzed with detail. In order to evaluate if differences among flowers could help to explain the low fruit set in avocado, starch content of individual flowers was measured. Later, to explore the implications of the nutritive status of the flower in the reproductive process, the starch content in avocado pistils has been examined in pollinated and nonpollinated flowers at anthesis and during the days following anthesis in avocado orchards in Spain, the only European country with a significant commercial production of avocados.

Materials and methods

The work was carried out in an experimental orchard at the E.E. La Mayora in Málaga (Spain) (36° 45' N). As a first step, to analyze the presence of starch in the ovary 30 inflorescences were selected in three trees and approximately 5 open flowers were collected daily. Approximately 600 flowers fixed in FAA were dehydrated in a tertiary butyl alcohol, embedded in paraffin wax and sectioned transversally at 10 μm . The samples were stained with I_2KI during 5 minutes, observed under the microscope and the images were collected using a digital camera connected to the microscope and processed using a Quantimet Q550 Image Analysis System according the method described by Rodrigo et al. (1997) with modifications. The dimension of the frame used to quantify the starch on each section was 1337 μm^2 . Starch content was measured in three ovary tissues: cortical tissue, integument and nucellus. Moreover, to explore the implications of the nutritive status of the flower in the reproductive process, starch content was examined in pollinated and nonpollinated flowers during the nine days following anthesis. Measurements of the starch content were performed in 4-7 flowers per day. Pollen tube growth were monitored in 10 pistils under a microscope with UV epifluorescence during two days after pollination staining with aniline blue in 0.3 M K_3PO_4 for callose (Linskens and Esser, 1957).

As a second step we developed a system to correlate the starch content at anthesis with individual flower fate by cutting the styles once the pollen tubes had reached the ovary but prior the achievement of fertilization; the ovary remains on the tree and it can develop into a fruit (Rodrigo et al., 2009). For this purpose, a total of 600 flowers from 'Hass' were hand-pollinated during the female stage using pollen from 'Fuerte' (Fig. 1a). After pollination, each flower was individually labeled. The next day, once the pollen tubes had grown along the style, the style of each flower was excised and fixed for microscope analysis (Fig. 1b). To link microscopical observations of the style in each pistil with the behaviour of the corresponding ovaries that remained in the trees, fruit set and fruit abscission were sequentially examined in the field up to harvest in the labeled flowers (Fig.1c). To relate the starch content in the flower at anthesis to the capacity to set fruit, two populations of flowers were

established: flowers and fruits that dropped and those that remained in the trees up to harvest. Once the final fate of each flower was known, starch was quantified in each style with the help of an image analysis system attached to the microscope as described previously.

Results and discussion

Several factors, such as temperature (Sedgley, 1977, 1987; Sedgley and Annells, 1981), outcrossing rate (Degani et al., 1989, 1990, 1997; Garner et al., 2008), alternate bearing (Hodgson, 1947; Hodgson and Cameron, 1935), overlap in flowering stages (Alcaraz and Hormaza, 2009, 2011) or nutritional deficiencies (Embleton et al., 1959) have been proposed to influence avocado fruit set rate. However, information is still elusive to explain why most flowers prematurely abscise while some of them remain in the tree (Alcaraz et al., 2010).

The results obtained in this work show that, while no external differences are observed among avocado flowers at anthesis, a high variability is present in starch content in the cortical tissue of the ovary, following a normal distribution, with a very small proportion of the flowers presenting high starch content (Fig. 2). The starch is widely compartmentalized into the different pistil tissues: cortical tissue, integument and nucellus, being the content higher in the nucellus than in the cortical tissue and integuments.

The starch reserves in the cortical tissue of the ovary followed a similar pattern of mobilization in pollinated and nonpollinated flowers during the days following anthesis. The initial depletion and later accumulation of starch in the ovary in the days following anthesis appears to be a conserved process, independent of pollination, since it occurs in both pollinated and nonpollinated flowers. But differences were observed in the starch accumulation within the integuments between both types of flowers. The rapid decline of starch content in the integuments, which took place two days after anthesis in pollinated but not in nonpollinated flowers, occurred concomitantly with the start of the progamic phase suggesting that starch reserves in the ovule could play a main role in the final outcome of the reproductive process in avocado.

Once a positive correlation between starch content in the cortical tissue of the style and starch content in the ovary was found at anthesis, we followed the fate of individual ovules that remained in the trees and relate it with the nutritive reserves in the flowers at anthesis. The optical density in the styles excised ranged from 200 to 496 pixels with highly significant differences in starch content among styles (Fig. 3). The results obtained showed a significant correlation between starch content in the styles at anthesis and likelihood of developing into a fruit: the flowers with styles that presented higher starch content at anthesis had higher chances to develop into fruits whereas those with the lowest starch content were more likely to drop during the days and weeks following anthesis. Therefore, the capacity of a flower to become a fruit could be preconditioned by the nutritive status of the flower. The results obtained indicate that, although fertilization is a requirement for flower development into a fruit in avocado, other factors independent of pollination as the nutritive status of the pistil at anthesis could be significant to determine the subsequent flower fate.

Conclusions

Although fertilization is a requirement for fruit set, additional factors inherent to the flower, such as the nutritive status of the ovary at anthesis, seem to play a determinant role in final fruit set in avocado.

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Figures

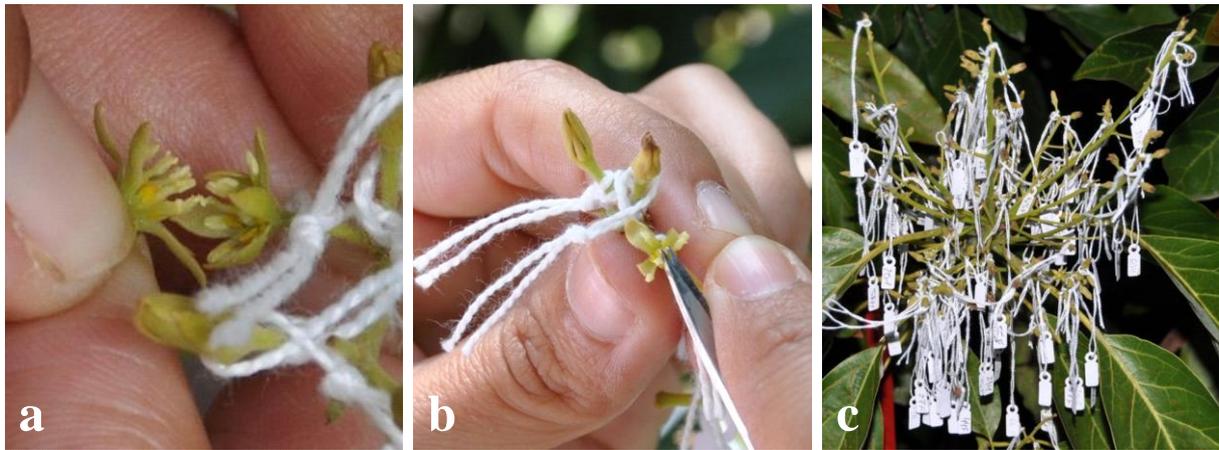


Figure 1. Image showing the process of pollinating 'Hass' flowers in the female stages with anthers from 'Fuerte' collected in the male stage (a), removing the style one day after pollination (b) and the inflorescence (c) with labeled ovaries after the styles were removed.

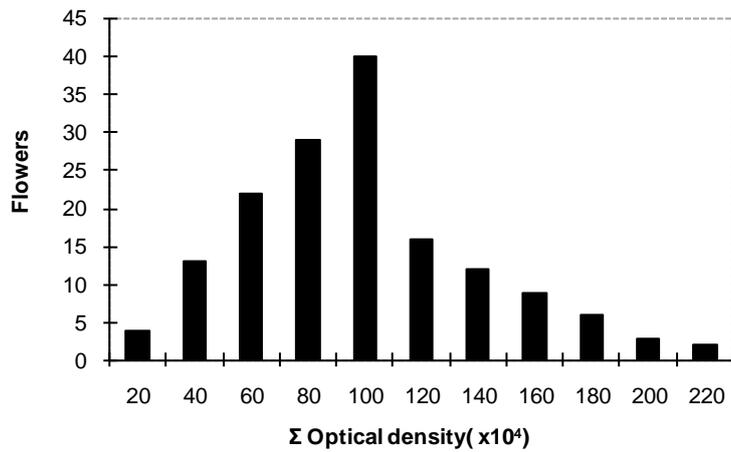


Figure 2. Distribution of avocado flowers according to their ovary starch content at anthesis

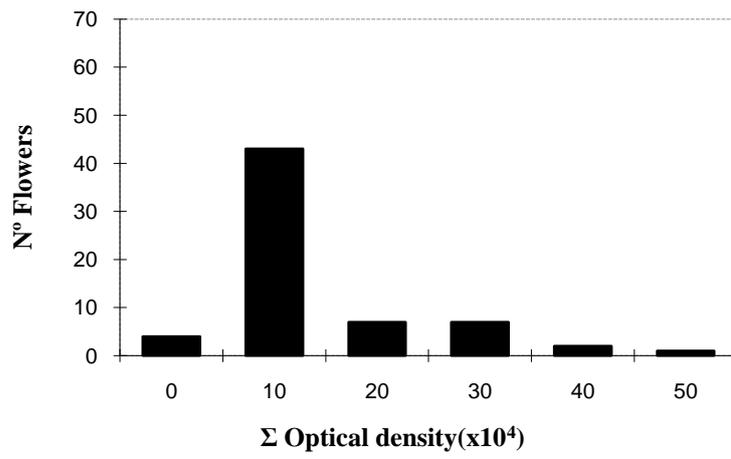


Figure 3. Distribution of hand-pollinated avocado flowers according to their starch content in the style the day after anthesis.