Pak. J. Bot., 47(6): 2181-2188, 2015.

REPRODUCTIVITY AND PHENOLOGY OF ARGAN (*ARGANIA SPINOSA* (L.) SKEELS) A RARE TREE ENDEMIC TO THE WEST OF ALGERIA

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Abstract

Argania spinosa in its ecological interest is threefold: forest, forage and fruit (oil production). The regeneration of the argan tree, it is important to know the phenology of this species. The study of phenology helps to know precisely the periods of activity of vegetation for productivity measures (Embryogenesis of the pollen grain, pollen deposition, pollen adhesion, fluorescence microscopy of pollen tube growth and Pollen grain size). Our results on the study of the phenology of two taxa of *Argania spinosa* (Mascara taxa and Mostaganem taxa), Regarding the phenological behavior of the argan under the effect of environmental conditions, we can mention that some argan trees (of Oggaz and one of Stidia) are early trees bloom twice a year in October and in spring, the author trees are late trees manifest activity during periods of the year. Begin to bloom from February until spring, this period is characterized by the breakdown of flower buds and the appearance of flowers on the twigs of the previous year and those of the current year; the fall of their ripe fruit takes place in June of the following year. Argan trees wore two generations of fruit, fruit knotted this season and last season tied fruit maturing. The length of the cycle is detected 9 and 16 months, we believe that a time synchronization in the evolution of physiological behavior argan trees of the two stations with 90%.

Key words: Argan tree, Productivity, Pollen, Phenology, Early, Late trees.

Introduction

In southern Algeria the forest based on endemic heritage: the argan tree (*Argania spinosa* L. Skeels). It occupies 90.644 hectares (Ouled safi, 2014). Argan inflorescence is a glomerule of up to 15 pentamerous hermaphroditic flowers. They are grouped in the axils of the leaves or on the nodes of the shoots. An average of 9.9 from 10 stigmas was bearing pollen at the blooming flower phase (FE), whereas 6.5 stigmas were covered with pollen at the flower bud with an emerging style phase (BFS). Although the number of deposited or adhered pollen grains did not differ between both phenological phases, only an average of 1.6 pollen grains would germinate on BFS stigmas as compared to 6.4 on FE. Moreover, highly significant differences were observed between phenological phases for stigma height, pollen tube number per stigma, maximum pollen tube length and maximum pollen tube length to stigma height ratio, FE being the phase showing the highest values for these variables. No pollen germination was observed on the flower bud (BF) stigmas, and pollen tubes, although present on BFS, never attained the base of its style reaching the ovule. However pollen tubes reached the base of the style in 25% of FE (Benlahbil & Bani-Aameur, 2015). For successful cultivation and conservation of plants a detailed knowledge of their reproductive biology is required (Moza & Bhatnagar, 2007). Phenology is a bio-indicator of climate evolutions. Measurements of phenological stages on perennial species provide actually significant illustrations and assessments of the impact of climate change. Phenology is also one of the main key characteristics of the capacity of adaptation of perennial species, generating questions about their consequences on plant growth and development or on fruit quality (Garcia de Cortazar, 2015). The application of phenological studies to detect biological responses to changing climate is grounded in the adaptations of terrestrial organisms to the 12-month climate cycle (Cloern, 2015). Argan phenology varies greatly among trees, total fruit drop spreading from late March to mid-July; moreover, an exceptional fructification may occur in autumn in some trees. From February to about May, the infestation rate is low except in fruit produced during autumnal fructification in June and July (Mazih, 2015). As part of the regeneration of the argan tree, it is important to know the phenology of this species in a simple and understandable way in both Oggaz (Mascara) and Stidia (Mostaganem) stations, we chose to present the results by morphological behavior; that is to say, depending on the time of onset and develop parts of the tree: leaves, buds, flowers, and fruits. Observations on the evolution of the biological activities of the argan tree are performed during different times of the year to better monitor all physiological changes characterizing the active period since the foliation to the ripening and fruit drop. The study of phenology is large, the need to know precisely the periods of activity of vegetation for productivity measures, characterization of relations linking the periodicity of vegetation bills environment to determine the discriminating factors phenological behavior of species and predict their responses to changes in environmental variables, the role of the argan tree in animal feed (Grouzis *et al.,* 1991). The importance of phenology can be summarized into: The good knowledge of the periods of vegetation activity is necessary for the productivity measures, the comprehension and mastery of the plant's conditions of growth in function to the setting, and The argan tree provides fodder which represents food for animals in arid and semi arid areas especially

Materiel and Methods

Study site: Forest nursery Oggaz area Mascara (Oggaz) is located in north-western Algeria at an altitude of 600m average. the semi-arid bioclimatic cool winter, its geographical localization by GPS (35 ° 43, 588 'N, 00 ° 15,331' W), forest home in the region of Mostaganem (Stidia) is located in the north-west of Algeria on the Mediterranean 350 km west of Algiers, its geographical localization by GPS (35 ° 48, 170 'N, 00 ° 03,994' W) (Belhaoua & Besselma, 2009), (Fig. 1).

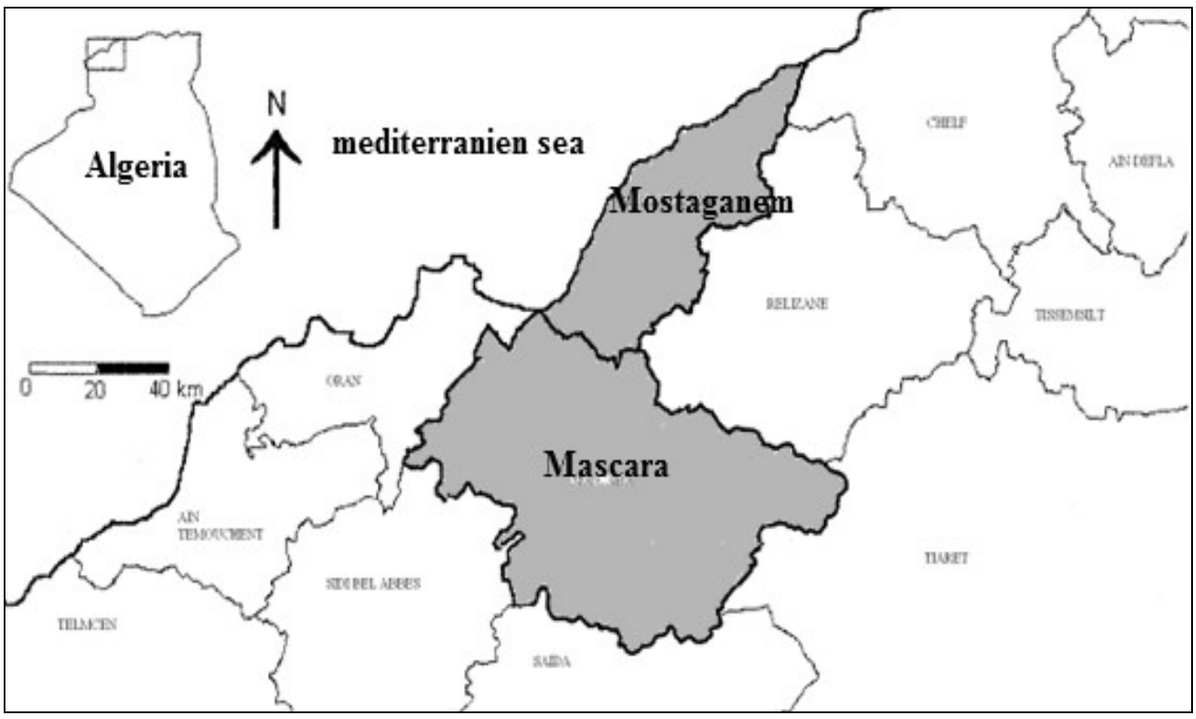

Fig.1. Representative situation map of the study area.

Study species: The argan tree is the only species in the genus Argania of the family "Sapotaceae" and order "Ebénales" Argan in French takes its name from the tree "Argan" , the origin of the Arabic name probably lies in the word " Irgen " which means in Berber "tachelhait", which is the core hardwood fruit tree , where the Berber derive a reputable oil argan (Rouhi, 1991), shows a typical tree structure dicotyledonous family "Sapotaceae" gender "Argania" is highly polymorphic , it has some analogy with the olive, but found only on large areas in southern Morocco and the south west of Algeria (in Tindouf), The argan tree is a monoecious species, with hermaphrodite flowers appear in March, they have axillary clusters with five hairy sepals, rounded, white, somewhat round at the base, five petals green bell flower after fertilization gives a yellowish green drupe about the size of a large olive called argan (Emberger, 1925; Mensier, 1957).

Floral biology: Length of the sepals, petals and stamens were measured. Number of stamens, number of pollen grains per flower was measured by various methods.

Embryogenesis of the pollen grain of the argan: To better identify the life cycle of the argan tree, we proceeded to identify the stages of development of the male gametophyte (pollen grain). Were collected buds of various stages of development ranging from the appearance of the button (1 mm) to the development of the flower (4 mm). We tested two mounting solutions: solution Carnaoy I (3: 1 absolute ethanol, glacial acetic acid) and FAE solution (1: 1: 8 ° 37 formaldehyde, glacial acetic acid, ethanol 80°) at 4°C and stored in 70° alcohol.

In situ observation of pollen deposition: To examine the degree of exposure of phonological phases to pollen reception, we examined in the field the presence or the absence of pollen on the stigma. Four replications of each of 10 BFS and 10 FE flowers of three random trees were observed using a field magnifier (G x 20).

Light microscope observation of pollen adhesion: Pistils of ten BFS (flower bud with an emerging style) flowers and ten FE (flower bud) flowers from three random trees were excised using a laboratory magnifier (G x 20). The stigmas were agitated in a drop of acetocarmine on a slide to separate pollen covering the stigma but not adhering to it. The pistils were cut longitudinally with a razor blade and the stylar canal was stained with acetocarrnine under alight microscope (G x 40).Then it was possible to count both adhered and germinating pollen grains (Ascher & Peloquin, 1968; Ramsey & Vaughton, 2000).

Pollen grain size: Pollen grains were collected by dividing the flower on a glass drag. Were screened for fertility the number of pores and size using an ocular micrometer optical microscope (G x 40).

Phenology: Observations and measurements were performed of two taxa of *Argania spinosa* (Oggaz taxa and Stidia taxa). They are seen as productive subjects in the shrub combination of argan. We used calipers to measure the elongation of the fruit in question, and a camera to take pictures. The observations were made on the leaves, twigs of the current year and the previous year, fruits, flowers and bud formation, taking into account color variation of these bodies during the various stages

phenological. The work is to divide the tree habit selected based on the exposure of the tree in the light of day: Portion of the shaft (8 branches), and West part of the shaft (8 branches). This division allows us to see the relationship phenology and climate for both stations, perform the observations in the same day, for two stations (Stidia and Oggaz).

Results

Floral biology: The total number of flowers per inflorescence ranged from 175±25. Inflorescence had several small branches, The argan tree is a monoecious species, with hermaphrodite flowers zygomorphic, bisexual, pentamers, polypetalous, they have axillary clusters with five hairy sepals, rounded, white, somewhat round at the base, five petals green bell flowers, are greenish yellow or sometimes white, they are grouped in small axillary clusters, the calyx of the flower is composed of five sepals pubescent succeeding two bracts, corolla, bell, consists of five petals obtuse and rounded with an average length of 2 mm. The flower has a pleasant smell odor. Calyx consists of five sepals free, with an average length of 0.5 mm. corolla consists of five petals with an average length of 1 mm. The androecium consisted of five stamens present in the form of a ring around the carpel and they were present at the same level of stigma. Average length of the cheesecloth is 4 mm, and 0.05 mm of anthers.

Embryogenesis of the pollen grain of the argan: Our results illustrate the different stages of development of the pollen grain. And microspore mother cell undergoes meiosis to result in a tetrad of four microspores wrapped in a bag. These microspores after magnification are released. Thereafter, each undergoes a series of asymmetric mitotic divisions, producing a mature pollen grain with two sperm cells to ensure double fertilization the embryo sac at the time of fertilization.

In situ observation of pollen deposition: Phenological phase was a highly significant main factor for the number of stigmas bearing pollen, whereas neither tree nor interaction was significant mean number of stigmas bearing pollen was 10 for FE contrasting with 7 stigmas for BFS.

Light microscope observation of pollen adhesion: Phenological stage was a highly significant main factor for the number of pollen grains germinating on the stigma, both BFS and FE received similar amounts of pollen deposited, although these varied between 16 and 70 grains digits. However, an average of 7 grains germinates on the stigma FE (flower bud) from 2 to BFS (flower bud with an emerging style).

Observation of fluorescence microscopy of pollen tube growth: Phenological stage was a highly significant main factor for the number of tubes of high stigma, pollen stigma, the maximum length of the pollen tube and the maximum length of the pollen tube to the stigma height ratio. Height means style was 3900 pm FE and 3500 pm for BFS where it varied significantly between trees 3300 pm to 3700 pm. On average, 10 grains of pollen tubes on formed pollen FE stigma

Pollen grain size: Argan fertile pollen grains colored with aceto-carmine are uniformly red and round. Values are mean percentage of pollen grains 100, most of the grains having two pores were intermediate in size (25-30 pm).

Phenology: From the results obtained on all the observations, we note the existence of morphological differences between the two subjects argan tree color, fruit size and abiotic environmental conditions. The Oggaz tree (Mascara) is dark green, stronger in height and width and the fruits obtained also larger (5cm) than those of about Stidia (Mostaganem) (3cm), which is characterized by a bright green color, earliness of opening of buds on the branches of the year compared to the subject Oggaz, and even the appearance of small leaves at the end of the branches of the year during the spring period (March to June) (Fig. 2).

The rest of the phenological characteristics are almost identical in both sources (or Oggaz Stidia), the results of different observations made in situ on the two subjects of the study: the experimental shaft of the Oggaz station (Mascara) and experimental forest tree house Stidia (Mostaganem) of June of the current year to June of the following year for two consecutive years (2010-2011 and 2011-2012). The 4 stages of phenology are shown in the following illustrations:

Fig. 2. A: mature fruit, fleshy pericarp (pulp) was dried fruit and seeds of the two subjects (Oggaz and Stidia). B: Stidia tree; C: Oggaz tree.

Flowering: Period from October, Oggaz argan and a one of Stidia are early trees bloom twice a year in October and in spring. Period from February to March**,** late trees are flowering, begin to take on a yellowish color, with a well developed morphology, they continue their growth to become rigid. The buds are bursting flowers on the branches of the year and those of the previous year, the flowers are distinct bouquets, we can count and observe the floral organs to the naked eye, and they have 5 sepals with a shaped corolla of welded petals. Inside, one can identify the stamens which are inserted and have a light yellow color, there should be noted the presence of insects on good sunny strata. These insects insure the pollination in the argan tree. The density of broken buttons is higher on February, then, the appearance of new flowers continues with low density for the months that follow until the end of May. Period from April to May, we note the appearance of yellow tips on the branches of the tree that open, this is the end stage of budding, most flowers open marking the end of the flowering stage and the beginning of the fruiting phase. The branches of the tree are quite long, grayish at the base, reddish brown towards the end, and less apical buds ensure growth in length. We talk about correlation of growth inhibition when the growth of apical buds are intensified along the axillaries buds are inhibited. The internodes are significant and twigs of the previous year are lignified (Fig. 3).

Fructification: Period from May to July, the appearance of berries of few millimeters in dark green color on the branches of the year. But the density of the new fruit is very low; the tree possesses some branches that are deprived of leaves to the inside of the crown shaft. The density of fruits of the preceding year is important especially at the side that is exposed to the sun. This density varies from an orientation to another. We see that the density at the west side is higher than at the east. The variations are also observed at the level of fruits that take a greenish color underside for the west expositions due to a high luminosity. Contrary to the east expositions where there is no change. The number of fruits varies from 1 to 5 on a branch of 1 to 2 per knot, the fruits become yellowish progressively. The fruits fall is nearly total: It is the stage of maturation. The preceding year branches witness the fall of the first fruits they have become mature especially those exposed to the west. Period from August to September, the branching of the previous year is rigid and lignified. However, those of the year are green to reddish range and reach a length of 20 cm, the new fruits finish their growth until the end of the year (December), grouped by 1-5 on the twigs, branches of the year have a brown color and become progressively stiffer. Period from October to December, we find that the appearance of berries starts in September. The fruit from the fertilization of spring flowers from the previous year that have remained dormant until autumn start fruiting Fruit size increases and takes the green color, and reaches a length of a few cm, it is useful to note that the density of fruit is important especially in the parts exposed to the sun; the number of fruit varies from 1 to 5 on the node. The branches of the year present a brown color and become progressively rigid. At this period, the argan tree continues losing its yellowish leaves (Fig. 4).

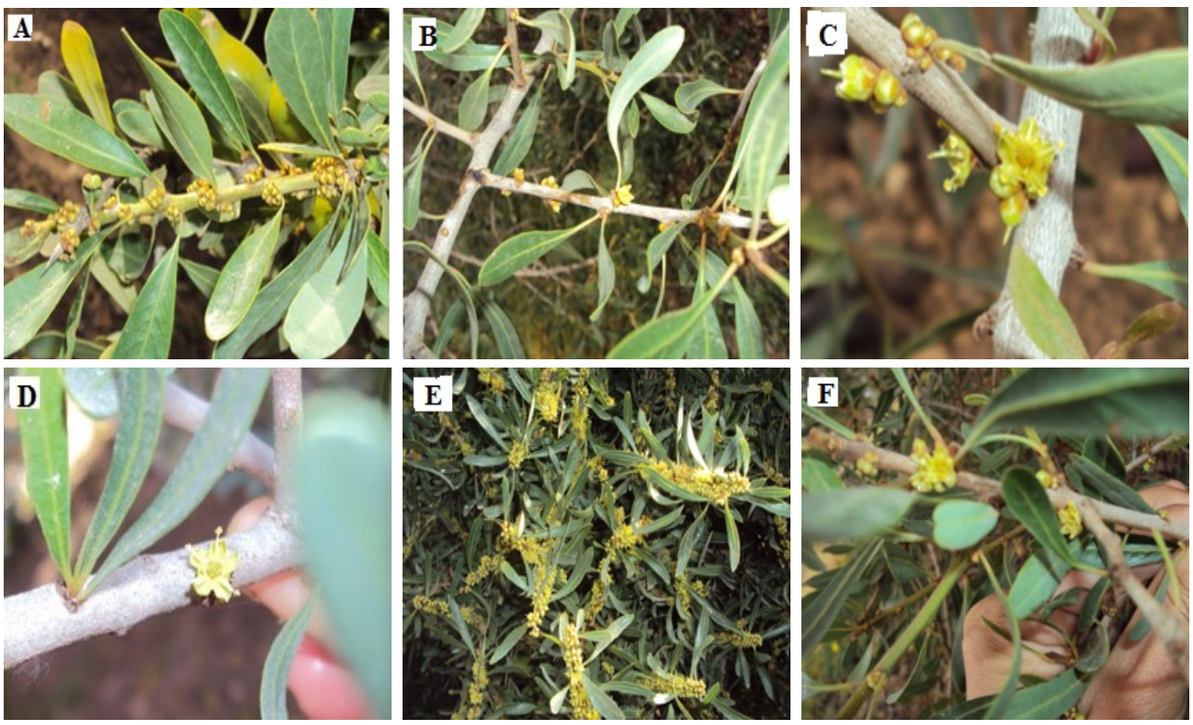

Fig. 3. Flowering argan. A: buttons (Stidia argan); B: buttons (Oggaz argan); C: flower (Stidia argan), The argan tree is a monoecious species, with hermaphrodite flowers, they have axillary clusters with five sepals; D: flower (Oggaz argan); E: complete breakdown of buttons (Stidia argan); F: complete breakdown of buttons (Oggaz argan).

Fig. 4. Fructification. A: early fruiting (Stidia argan); B: early fruiting (Oggaz argan); C: new green fruit finish their magnification in Winter (Stidia argan); D: new green fruit finish their magnification in Winter (Oggaz argan); E: fruits in spring (Stidia argan); F: fruits in spring (Oggaz argan); G: fruit ripening in May (Stidia argan); H: fruit ripening in May (Oggaz argan).

Fig. 5. Seed set. A: leaves turn yellow (Stidia argan); B: leaves turn yellow (Oggaz argan); C: fall fruits and leaves in June.

Seed set: Period from June to September, during the summer period, some leaves turn yellow in almost all strata making. A relatively high leaf fall begins, unlike shady giving a less dense green port reddish colors because all the fruit to reach maturity, which is called the stage of maturation. At this stage the branches of the year (fertile branches) appear as branching of the previous year, as the flowers finish their pollination produce berries dark green color with thorny ends in late July. The beginning of August is the stage of fruit set; the branching of the previous year becomes naked, because almost all fruit fell to the ground for all strata studied the tree, except some one remaining in the form of nuts (Fig. 5).

Foliation: Period from June to September, this period represents an important loss of adult leaves of a yellowish coloration, we have observed a relatively high loss of the yellow leaves that are exposed to a high luminosity (west exposition), and contrary to those that is under umber (east orientation). At the same time, there is the appearance of youth leaves at the branches extremity; they are of a light green color. The old branches conserve yet leaves larger in surface, lengthened and of a dark green color. Also some green buds appear at the lignified branches extremities which end by opening. According to the observations of each month, we always the presence of light green small

leaves in a weak density in relation to autumn months because the argan tree has persistent leaves; Period from October to December, the apical parts of the branches of the current year and also of the preceding one possess little leaves grouped by 3 to 4 leaves (beginning of foliation). The leaves continue growing in width, length and thickness, they are grouped from 1 to 5 on the same knot at the extremity of branches. Their color becomes completely dark green and the superior side becomes soft, the new leaves continue growing and are lignified. For both expositions (east and west), no variation has been registered on the leaves (Fig. 6).

Fig. 6. Foliation argan. A: foliation early in October (Stidia argan); B: foliation early in October (Oggaz argan); C: open green buttons and leaves formation (Stidia argan); D: open green buttons and leaves formation (Oggaz argan); E: wide and fully developed leaves in spring (Stidia argan); F: wide and fully developed leaves in spring (Oggaz argan); G: drop adult yellow leaves on the ground (Stidia argan); H: drop adult yellow leaves on the ground (Oggaz argan).

Discussion

The basic knowledge on reproductive biology is not only essential for evolutionary and systematic studies, but also important for effective conservation strategies. When stigma was exposed to pollen, both BFS and FE may have the same number of pollen grains deposited and bonded. Argan is a cross-pollinated species these results are compatible with Benlahbil results in 2015, argan has a dry stigma. Although out crossing species would show strong adhesion to enforce their authority to fix the pollen, dried stigmas, as has been observed for Arabidopsis Heyneh., Are generally barriers to mass pollination (Zinkl & Preussd, 2000). They promote the adhesion of pollen, pollen hydration and penetration of the pollen tube, the large variability between trees can suggest a cytogenetic basis phenomenon that may be interesting to study. (Cavalcante *et al*., 2000). According to the results of observations on the phenological behavior of the argan tree effected by the conditions of the natural environment, we can report that the argan actively manifest during periods of the year. From February until the spring period is characterized by the bursting buds and the appearance of flowers on the twigs of the previous year and those of the current year, and the fruit is started from the month of May. Thus we find that the maturation and fruit drop from the previous year took place during the summer period, also berries of the year continue to grow, young leaves appear at the ends of branches of the current year and those of the previous year. Our results on the study of the phenology of the argan Oggaz, work Mebarki (2001), Belkhodja *et al*. (2006) and Dif (2004), the work of Ben Chettouh on the phenology of the argan tree of Stidia in 1999, and Kechairi work (2009) on the phenology of the argan Tindouf provide the same data on the evolution over time of the various life stages of the species, we note that there is a morphological difference between the two topics of argan, we see early opening buds in argan Stidia compared to the adult Oggaz and the appearance of small leaves at the ends of branches of the year during the spring period in the argan tree of Stidia. For the rest of phenological data on flowering stage, fruit maturation, growth and lignification of new branches and falling leaves become yellowish, we believe that a time synchronization in the evolution of physiological

behavior argan trees of the two stations. In Algeria, Kechairi (2009) describe the different phenological stages of the argan Tindouf according to the following scheme: The completion of fruit growth in March and full maturation with yellowish gold was observed in June. Flowering was observed in November and it was completed in March, the foliation is sub-persistent peaks where argan take the greenish color during all the year. In Morocco, Metro (1953) describe the different phenological stages of the argan tree arboretum Oued Cherrate according to the following scheme: The period from June to September is characterized by falling ripe fruit of the year and the appearance of some small fruits especially in the sunny side. Foliage Part turns yellow, dries and eventually falls. Foliation begins with the appearance of small leaves at the ends of branches of the year at the same time, the green buttons at the end of the year lignified branches open indicating the end stage of fruit set. During the period from October to December, new branches of the summer season continued growth and become woody. Leaves complete maturity whose limb becomes wide, elongate and color is dark and complete the smooth upper face green. From January to March, we see the emergence of new branches on the tree that continue their growth gradually. The flowers appear on the branches of the year and those growing. Finally, during the period from April to May. In comparison with the results of observations of Metro (1953), Ferradous (1996) and Montoya (1984) on the phenology of the argan Oued Cherrate (Morocco) Kechairi of the argan Tindouf and Benchattouh phenological study on Mostaganem argan in Algeria and our phenological data both Oggaz stations (Mascara) and Stidia (Mostaganem), Unless the two trees, one of Stidia and the other Oggaz are early trees bloom twice a year in October and during the spring, as they carried small fruit of the same size in October. The two trees show the same phenological behavior despite the differentiation of its abiotic factors, climatic data and soil type, in these circumstances, we believe that the type of early or late tree and phenological behavior of the tree is related not only to climatic factors but also the genotype of the tree. It seems useful to note that there is a general similarity in the progression over time of different life stages of the species. We believe that this is linked to the observed early favorable natural environment is summarized by the Mediterranean marine humidity because Stidia tree not far from the sea only a few meters.. It is also important to add that the argan tree is still green or loses a part of its foliage even in the Tindouf region where drought is very strong as observation Kechairi (2009), with its biological mechanisms the argan tree regulates its biological functions to better withstand difficult conditions very favorable environment and waits for it to trigger its physiological functions change. Finally, as noted by Dupin (1949), it is worth noting the biennial nature of the formation and maturation of fruit; therefore we believe that the argan tree also has this "biennial character" because young late fruits complement their growth in the presence of the following autumn rains.

Conclusion

Some argan trees (of Oggaz and one of Stidia) are early trees bloom twice a year in October and in spring, the author are late trees manifest activity during periods of the year; the fall of their ripe fruit takes place in June of the following year. They bloom once a year in spring, wore two generations of fruit, fruit knotted this season and last season tied fruit maturing. The length of the cycle is found 9 and 16 months. Starting in June, fruit ripening and leaf yellowing beginning occur. Thus, the tree's fruit experiencing almost total fall leaves becoming yellow and eventually fall regularly; defoliation of trees is more intense, the trees keep their leaves longer, falling yellow leaves is relatively low, and followed by the emergence of new leaves.

Acknowledgments

The author thank the Pr. Nadjraoui Dalila, The authors are also grateful to Pr. Mderbal Khalladi, Mr. Kies Kada, students of Mekkioui M: Seddiki S, Adda R, Benmira Y, Belmokhetar A, an all teachers USTHB and Mascara University.

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(Received for publication 28 August 2014)