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Title is in **bold**, except for the scientific names; for plants include family (for animals, Order: Family).

## **Inverse leafing phenology of the dry forest understory shrub *Jacquinia nervosa* (Theophrastaceae)**

Óscar M. Chaves<sup>1</sup> & Gerardo Avalos<sup>1,2\*</sup>

Authors' names are spelled out, family name and addresses are numbered

<sup>1</sup>Escuela de Biología, Universidad de Costa Rica, 2060 San José, Costa Rica; ochaves@costarricense.cr

<sup>2</sup>The School for Field Studies, Center for Sustainable Development Studies, 10 Federal St., Salem, MA 01970 USA; avalos@fieldstudies.org

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**Abstract:** In the dry forest of Santa Rosa National Park, Costa Rica, the understory shrub *Jacquinia nervosa* presents an inverse pattern of phenology that concentrates vegetative growth and reproduction during the dry season. In this study, we tested the “escape from herbivory” hypothesis as a potential explanation for the inverse phenological pattern of *J. nervosa*. We monitored leaf, flower and fruit production in 36 adult plants from October 2000 to August 2001.

“Abstract:” is written in **bold** and starts with an introductory paragraph, objective; a summary of methods, results and main conclusions; all in a single paragraph.

Leaves of six randomly selected branches per plant were marked and weeks to measure the cumulative loss in leaf area. To analyze relation we collected 15 fruits per plant and counted the total number of seeds, as well as the number and type of seed predators found in the fruit. Flower, and fruit production occurred during the first part of the dry season (September to February). The cumulative herbivory levels were similar to other tropical dry forest tree species that concentrate leaf production during the dry season and were concentrated on young leaves, which lost an average of 36.77 % of their area (SD = 34.35, N = 195). Chewing beetles of the genus *Epicauta* (Meloidae) were the most important herbivores. In mature leaves, most of the damage was caused by the beetle *Coptocycla rufonotata* (Chrysomelidae). Fruits took 4 months to develop during the dry season (January-March 2001) but continue increasing in size well into the first 3 months of the wet season (May-July). Average seed number per ripe fruit was 9 (SD = 5, N = 500). Seed predation in mature fruits was 42 % (SD = 47, N = 122). Most seeds were damaged by moth larvae of the family Tortricidae. Only 3 % of the flowers became fruits. This was influenced by the low level of flower synchrony ( $0.38 \pm 0.26$ , N = 36 plants), but neither leaf synchrony ( $0.88 \pm 0.06$ , N = 36 plants) nor plant size

influenced fruit numbers. The significant damaged produced by insect herbivores in young leaves, fruits, and seeds, as well as the low reproductive index observed in *J. nervosa*, shows that the inverse leafing phenology of this species is not consistent with the “escape hypothesis” since *J. nervosa* was considerably attacked during the dry season. Considering the strong seasonality of the tropical dry forest and the heliophyte character of *J. nervosa*, it is more likely that this phenological strategy evolved in response to seasonal fluctuations in light availability, light quality, and daylength.

Include 5-7 **general** subject words, in lower case and separated by semicolon.

**Key words:** escape hypothesis; *Jacquinia nervosa*; inverse phenology; insect herbivory; tropical dry forest.

**Total word: 5209**

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Insect herbivores significantly influence plant fitness across ecological and evolutionary scales (Marquis, 1984; Aide, 1988; Cunningham, 1997; Strauss & Armbruster, 1997; Maron, 1998). In tropical forests, leaf damage by herbivores can reach up to 7-20% of total leaf area (Edwards, 1977; Dirzo, 1984; Coley, 1990; Coley & Barone, 1996) although there is considerable variation in levels of damage (Braker & Chazdon, 1993; Dirzo & Domínguez, 1995). Even under moderate damage, herbivory, combined with other stress factors such as competition or water shortages, imposes important limits on plant survival and reproduction (Brown et al., 1987). For instance, moderate levels of leaf herbivory retarded vegetative growth and flowering in the understory shrub *Piper arieianum* (Marquis, 1988), and damaged individuals of *Gutierrezia sarothrae* produced up to five times less seeds per branch relative to control plants (Wisdom et al., 1989). However, under certain circumstances, herbivory may have positive effects. Morrison and Reekie (1995) observed increased photosynthetic rates of undamaged leaves that compensated for the leaf area removed in *Oenothera biennis*. In addition, leaf herbivory could weed out the less resistant individuals in a population (Maschinski & Whitham, 1989).

Citations are in parentheses, starting with the oldest and ending in the newest. Comas are used between name and year. Two author citations are written with “&”, and more than three authors use all the names in the first citation, then use “et al.” More than six authors use always “et al.” several publications per year as Smith (1995, a, b).

## MATERIALS AND METHODS

**Study site:** This study was carried-out for 12 months (May-July 2000, and November 2000 to August 2001) in the “Indio Pelado” trail in Santa Rosa National Park, Guanacaste, Costa Rica. Santa Rosa is located between the Gulf of Papagayo and the Interamerican Highway (10° 44'13" - 11°00'37" N and 85°34'48" - 85°58'51" W) at 290m in elevation.

Geographic coordinates are written exactly in this abbreviated way: (10°44'13" - 11°00'37" N and 85°34'48" - 85°58'51" W).

The upper plateau maintains tropical premontane forests, whereas the coastal lowlands present a large extension of Tropical Dry Forests (Tosi, 1969). Average annual rainfall is 1 423 mm, being September and October the wettest months. Average annual temperature is 25.7 °C, and relative humidity is 81 % (Santa Rosa National Park Climatic Records). The dry season extends from late November through April. During the dry season, nearly 70 % of the canopy trees are deciduous (Hartshorn, 1983). The “Indio Pelado” trail is dominated by secondary forests composed primarily of seedlings and saplings of canopy species and understory shrubs (i.e., *Cochlospermum vitifolium*, *Semialarium mexicanum*, *Jacquinia nervosa*), trees of 10-20 m in height (i.e., *Spondias mombin*, *Bursera simaruba*), and a few

Write temperatures in this format: 27.5 °C.

20-35 m old trees remaining from the original forests (i.e., *Pachira quinata*, *Manilkara chicle*, *Hymenaea courbaril* and *Pseudobombax septenatum*).

**Study system:** *Jacquinia nervosa* (The plant in deciduous and semideciduous forests of from southern Jalisco, Mexico, to northeast 0.1 to 60 adults per ha has been registered (Janzen, 1970b). Adults vary 1.5-6 m in height with a diameter at breast height (DBH) of 1.5-20 cm.

Subheadings are in bold, with colon, and immediately followed by text.

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**Measurement of leaf herbivory:** To measure average leaf area lost to herbivores we randomly chose six branches per plant of the above-mentioned individuals, and marked every damaged leaf in all the branches. Leaf loss was quantified every 15 days during the study period. To estimate leaf area we measured different individuals, and measured their length and width. Leaf area was measured with a LI-Cor LI-3100 leaf area meter (Li-Cor Inc., Lincoln, Nebraska). Based on these results we calculated a linear regression to predict leaf area based on leaf length and width measurements non-destructively in the field ( $r^2 = 0.97$ ,  $F = 36.5$ ,  $N = 125$ ,  $P = 0.001$ ). In addition, we used a 10x10 cm grid to quickly estimate the leaf area removed. We divided the leaf area into seven categories of damage relative to the percentage of leaf area lost: 0 (0 %), 1 (1-10 %), 2 (11-25 %), 3 (26-35 %), 4 (36-50 %), 5 (51-75 %) and 6 (76-99 %) following Morrow (1984). In addition, we performed bi-weekly observations of the presence and identity of herbivores on eight external branches in each of 20 *J. nervosa* mature plants. Observations were done early in the morning (6:00-8:00) and at night (17:30-19:30). We registered and collected the type of herbivores present on the plant with an aspirator and an entomological net. We identified herbivores to species, genus, or family and classify them according with the most important damage they inflicted on the plant (Table 1).

Numbers under 11 are written in words, when not followed by units.

Hours are given in 24 hour format. Hour ranges are written with

Mathematical formulas are written in Word and their components are described in the text. Do not use spaces within mathematical formulas.

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**Measurement of phenological synchrony and reproductive success:** We estimated synchrony in leaf, flower and fruit production following Augspurger (1983):  $X_i = \sum e_{j-i} / (n-1) * f_i$ , where  $e_i$  represents the days when individuals  $i$  and  $j$  produced leaves synchronically,  $f_i$  represents leaf life span in individual  $i$ ,  $n$  represents sample size, and  $X_i$  represents the magnitude of population synchrony. This index ranges from 0 (total asynchrony) to 1 (100 % synchrony in the population). Individual reproductive success ( $E_i$ ) was determined following Fuchs (2000):  $E_i = (f_1/f_2) * 100$ , where  $f_1$  is the number of mature fruits and  $f_2$  is the total number of flowers per plant. Reproductive success at the population level corresponds to the average value of the  $E_i$  across all individuals.

Statistical analyses are included at the end of the section, without a specific subtitle.

We compared herbivory levels among young and mature leaves using a t-test. A Kruskal-Wallis analysis was used to compare temporal changes in herbivore damage among mature leaves produced in different dates, as well as levels of seed predation by date.

For comparing synchrony level between leaves, flowers, and fruits we used one factor ANOVA. A multifactorial ANOVA was used to determine how leaf and flower synchrony, as well as plant size, influenced the level of reproductive success with the following model:  $Y = \mu + x_1 + x_2 + x_3 + e$ , where Y represents reproductive success,  $x_1$  is leaf synchrony,  $x_2$  is flower synchrony,  $x_3$  is plant size, and e is the residual error. Data were log-transformed to correct for deviations from the normal distribution. All the analyses were done using SYSTAT 9.0 (SYSTAT, 1999).

Section headings (MATERIAL AND METHODS, RESULTS, DISCUSSION, etc.) are in upper case, not in bold.

## RESULTS

**Phenology:** Leaf, flower and fruit production took place in the first part of the dry season (end November to February). In most plants, leaf flushing occurred simultaneously with the production of flower buds, although the magnitude and temporal trajectories were different (Fig. 1A). Anthesis began in mid January and reached a peak in the first week of February, when the first fruits were also formed. The main fruiting peak occurred in mid February; there was also a slight increase in fruit numbers by the end of February (Fig. 1B). Only 12 % of a total of 440 immature fruits developed into mature fruits by March (see peak in fruit production Fig. 1C). Fruits had an average diameter of 1.80 cm (Standard Deviation, SD = 0.37, Number of cases, N = 500), and an average of 9.11 (SD = 5.36, N = 500) seeds per fruit.

The word Figure is abbreviated to "Fig."

Statistical information is abbreviated after the first time: 1.80 (median), SD = 0.37, N = 500; simplify afterwards.

**Synchrony in leaf, flower, and fruit production:** Average synchrony at the population level in leaf, flower and fruit production showed significant differences ( $F_{1,32} = 122.7$ ,  $P < 0.001$ ). Leaf production was the most synchronic phenological trait (0.88, SE = 0.012), whereas fruit production (0.18, SE = 0.03) showed the lowest synchrony followed by flower production (0.39, SE = 0.05).

**Remotion of leaf area by herbivores:** Significant differences were found in herbivory levels among young leaves produced at the start of the growing season (late November-early December 2000) and mature leaves (t-test = 44.1, d.f. = 28,  $P = 0.04$ ). Young leaves lost an average of 36.77 % of their area, with significant within plant variation. Herbivory levels of mature leaves changed little throughout the growing season although they steadily increased over time (Kruskal-Wallis = 6.12, d.f. = 3,  $P = 0.11$ ; Fig. 2). Main herbivores of young leaves were chewing insects, especially the beetle *Epicauta* sp. (Meloidae). Although mature leaves were less damaged, the most important damage was caused by the beetle *Epicauta* sp. (Chrysomelidae), which removed the

Statistical results are presented briefly in parentheses, with test name and P value separated by commas (Degrees of Freedom, are optional).

vascular system exposed. In addition, 8.22 % of the plants had damage caused by larvae of an unidentified hymenopteran leaf miner (Table 1).

**Reproductive success:** This parameter was low showing an average of 3.4 %, although variation among plants was high (SD = 7.6, N = 36). Fruit production was considerably affected by flower synchrony ( $F_{3,14} = 2.01$ ,  $P = 0.027$ ), but neither by the synchrony of leaf production ( $F_{1,3} = 1.08$ ,  $P = 0.32$ ) nor by plant size ( $F_{1,2} = 0.28$ ,  $P = 0.76$ ).

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## DISCUSSION

No subheadings; this section must match with the results.

The timing of leaf production in tropical plants is associated with favorable periods for growth and carbon fixation. In weakly seasonal forests leaf, flower and fruit production is concentrated in the driest time of the year to take advantage of peaks in light availability (Janzen, 1967; Gentry, 1974; Wright & Cornejo, 1990; van Shaik et al., 1993). In the dry forest, the intensive and long dry season provokes water stress, high irradiation, high temperatures, and higher evapotranspiration, limiting vegetative growth, and triggering flower and fruit production. In *J. nervosa*, both leaf and flower production, as well as the bulk of the fructification period, were concentrated during the dry season, whereas plants become deciduous and remain dormant during the wet period. The same phenological pattern has been described for *Faidherbia albida* in the dry forests and savannas of West Africa (Wickens, 1969; Roupsard et al., 1999). In this legume, the phreatophytic character facilitates survival during droughts, as well as leaf, flower and fruit production during this very stressful period. Leaves are dropped after the start of the rains possibly due to light reduction and canopy closure, analogous to *J. nervosa*.

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In summary, the “escape” hypothesis is not congruent with the phenological pattern of *J. nervosa*. This dry forest understory shrub was affected by a diverse assemblage of leaf herbivores active during the dry season. Considering the strong seasonality of tropical dry forests, and the heliophyte character of *J. nervosa*, it is more likely that abiotic factors, such as seasonal changes in light availability, light quality and photoperiod could have worked more effectively as selective factors in the evolution of the inverse leafing phenology.

## ACKNOWLEDGMENTS

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Acknowledgments are brief and only people who provided significant assistance is included. Do not use titles (Dr., Mr., Mss., etc.).

The *Resumen* (Spanish *Abstract*) has the same information and structure as the *Abstract*. If you are not fluent, ask the journal staff (vnielsen.rbt@gmail.com) to do it for you (no charge).

## RESUMEN

Insectos herbívoros influyen significativamente adecuación de las plantas a través de escalas ecológicas y evolutivas. Analizamos la hipótesis de “escape de la herbivoría” como explicación para la fenología foliar inversa del arbusto de sotobosque del bosque seco *Jacquinia nervosa*, el cual produce sus hojas durante la estación seca y se mantiene sin ellas durante la estación lluviosa. Medimos la producción de hojas, flores y frutos, el daño por herbivoría en hojas y semillas, y la fauna de herbívoros en 36 plantas adultas de octubre 2000 hasta agosto 2001 en Santa Rosa, Costa Rica. La herbivoría foliar acumulada durante la estación seca fue similar a la herbivoría de otras especies que concentran la producción foliar en la estación lluviosa. En las hojas maduras la mayor parte del daño fue causado por el escarabajo *Coptocycla rufonotata* (Chrysomelidae). La depredación de semillas predisposición fue de 42 % (DE = 47 %, N = 122) y es causada por larvas de polilla de la familia Tortricidae. Los niveles de daño indican que la fenología foliar de *J. nervosa* no coincide con la hipótesis de “escape”. Considerando la fuerte estacionalidad del bosque seco y el carácter heliófito de *J. nervosa*, es probable que esta estrategia fenológica evolucionara en respuesta a cambios estacionales en la disponibilidad y calidad de la luz, y duración del fotoperíodo.

**Palabras clave:** hipótesis de escape, *Jacquinia nervosa*, fenología inversa, herbivoría por insectos, bosque seco tropical.

## REFERENCES

Present the list of references in APA 6<sup>th</sup> edition format (like in the examples below).

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Pimenta, P. F., Saraiva, E. M., Rowton, E., Modi, G. B., Garraway, L. A., Beverley, S. M., Turco, S. J., & Sacks, D. L. (1994). Evidence that the vectorial competence of phlebotomine sand flies for different species of *Leishmania* is controlled by structural polymorphisms in the surface lipophosphoglycan. *Proceedings of the National Academy of Sciences United States*, 91(2), 9155-9159.

The word “TABLE” is not in bold and is written in full upper case. Table heading is brief and in normal letters (scientific names are written in italics), centered and with no period at the end.

TABLE 1  
List of insect herbivores found in *J. nervosa*

Group	Number of Species	of Stage <sup>1</sup>	Diet <sup>2</sup>	Damage category	Damage level <sup>3</sup>
Acari	1	a, j	ml	sucker	U.D.
Afidae	2	a, j	jl	sucker	U.D.
Coptocycla	2	a	ml	scraper	3
Hemiptera	1	a	ml	sucker	1
Cicadellidae	4	a, j	ml	sucker	2
Curculionidae	2	a, j	fr	driller	2
Hymenoptera	1	j	jl, ml	miner	1
Grillidae	4	a	jl, fl	chewer	2
Lepidoptera	1	j	fr, s	chewer	3
Epicauta	1	a	jl	chewer	4
Miridae	1	a	ml	sucker	1
Pentatomidae	3	a	ml, fr	sucker	1
Tortrichidae	1	j	fr, s	chewer	5

No bold or full upper case is used inside tables.

<sup>1</sup> a = adult, j = juvenile.

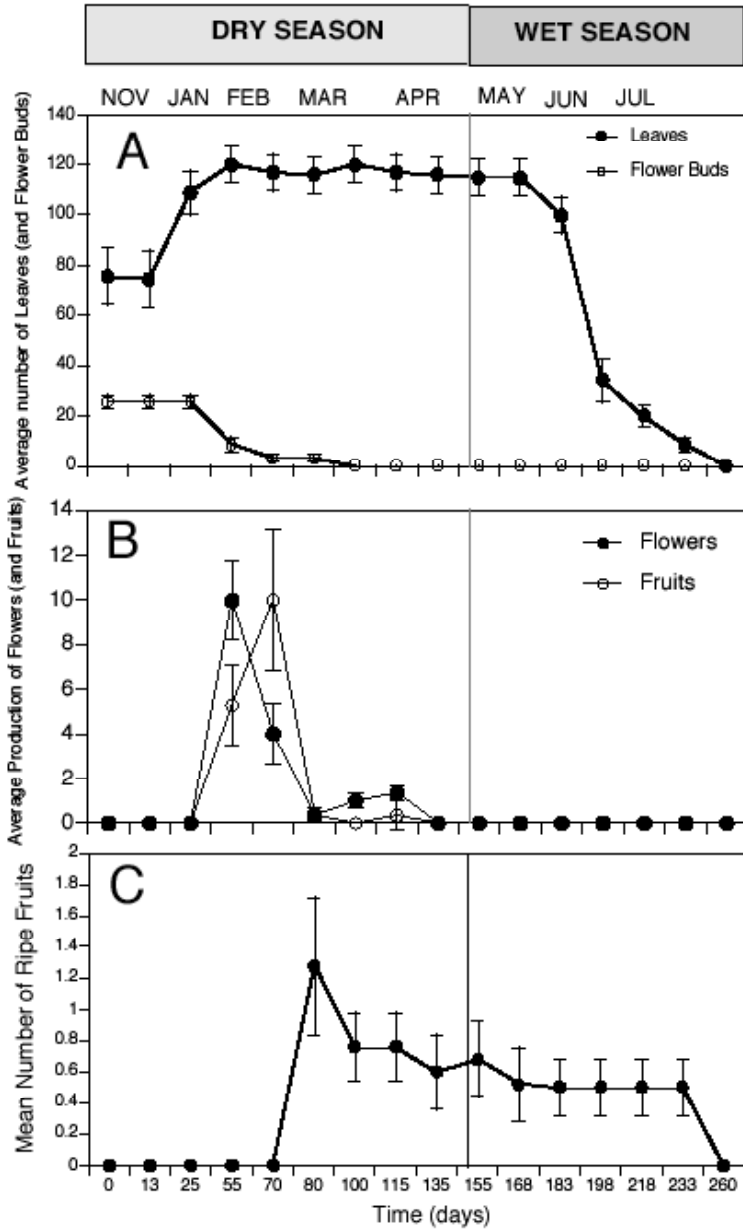
<sup>2</sup> jl = juvenile leaves, ml = mature leaves, fr = fruits, fl = flowers, s = seeds.



<sup>3</sup> **U.D.** = undetermined, 1 = light, 2 = moderate, 3 = moderate to high, 4 = high, 5 = very high.

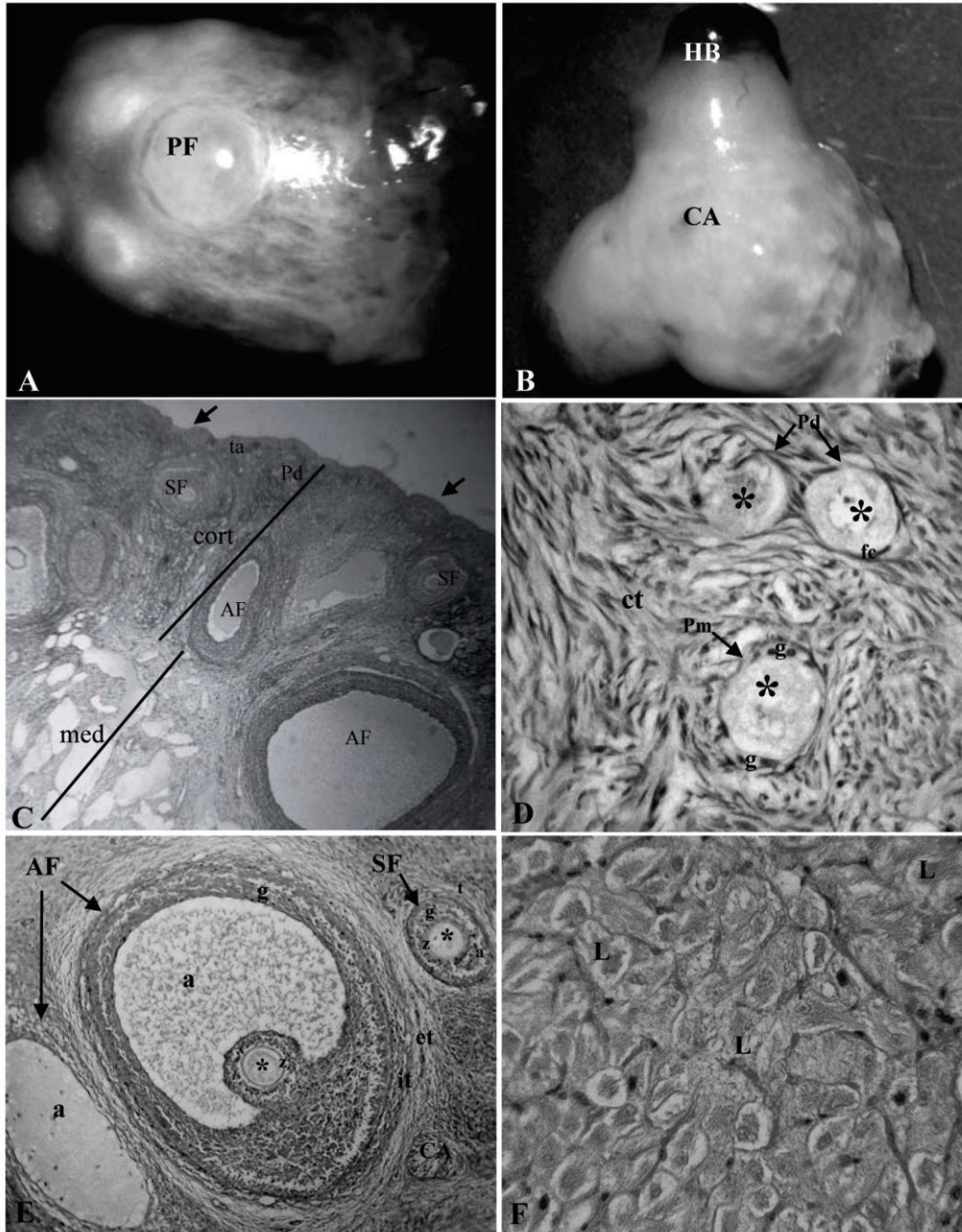
Data obtained from periodic observations made on 36 adult plants from November 2000 through June 2001.

Symbols and explanations appear under table and bold is used if necessary for clarity.



Graphs should be black and white. Whenever possible (a free guide to graphs and figures is available in the journal website)

**Fig. 1.** A. Average number of leaves and flower buds ( $\pm 1$  SE); B. number of flowers and immature fruits; and C. ripe fruits in 36 adult individuals of *J. nervosa*, November through August 2001, Santa Rosa National Park, Costa Rica.



The photographs must be attached at the end of the Word document and if they are too large attached as separate JPG or TIFF (100 % quality compression) file. They must have a high resolution quality. There is a simple quality check that you can do: enlarge them in your screen. If they still look well, then they will probably be acceptable. Remember that normal figures will be reduced to a width of 6-12 cm: label lettering must be easy to read even after that reduction.