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Comparison of Methods to Study Plant Phenological Patterns. The Case of *Halimium atriplicifolium* (*Cistaceae*)

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Summary

CASTRO-DÍEZ P., MILLA-GUTIÉRREZ R. & MONTSERRAT-MARTÍ G. 2003. Comparison of methods to study phenological patterns. The case of *Halimium atriplicifolium* (*Cistaceae*). – Phyton (Horn, Austria) 43 (1): 59–78, 3 figures. – English with German summary.

The methods to study plant phenology described in the literature vary widely. The aim of this article is to compare the phenological information gathered through different methods on the same population of *Halimium atriplicifolium* (LAM.) SPACH (*Cistaceae*), a Mediterranean evergreen shrub, so that the advantages and disadvantages of different methods can be discussed. The first method, called semi-quantitative (SQT), was based on a monthly estimation of each phenophase incidence through a visual inspection of ten whole plants. The second one, called quantitative (QT) was based on a monthly monitoring of all the leaves, buds, flowers and fruits borne on five tagged branches throughout an annual cycle. Both methods allowed to draw the calendar of leaf production and shedding of brachyblasts, dolichoblasts and reproductive shoots, development of inflorescence and flower buds, flowering, fruit setting and seed dispersal. In addition, leaf shedding was also studied using ten litter

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collectors, placed below the SQT-sampling plants (LC method). The mean dates of each phenophase's beginning, maximum incidence, ending and the duration obtained from the different methods were calculated for both methods. The 95% confidence limits of the variable means were calculated, together with the minimum sampling size necessary to get a ±30 days 95% confidence limit in each variable. It has been estimated that, to get similar confidence limits, the QT method requires 4,5 hours of field work per sampling date, versus only one hour with the SQT one. We found a good agreement between the methods for most of the phenophases. The main inter-method differences appeared in phenophase duration, which tended to be longer on the basis of the SQT method. Both the lower sensitivity of the QT method to unusual events and the lower reliability of the SQT one on the definition of inconspicuous phenophases, may account for such a discrepancy. The QT method is advisable for inconspicuous phenophases, such as leaf production by brachyblasts; LC gets the best calendar for leaf shedding with the least sampling effort, and the SQT is recommended for the remaining more conspicuous phenophases.

Zusammenfassung

CASTRO-DÍEZ P., MILLA-GUTIÉRREZ R. & MONTSERRAT-MARTÍ G. 2003. Vergleich der Methoden zum Studium phänologischer Muster. Der Fall von *Halimium atriplicifolium* (*Cistaceae*). – Phyton (Horn, Austria) 43 (1): 59–78, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die verschiedenen, in der Literatur beschriebenen Methoden zum Studium der Phänologie von Pflanzen variieren sehr. Das Anliegen dieser Arbeit ist es, die mittels zweier Methoden an ein und derselben Population von *Halimium atriplicifolium* (LAM.) SPACH (*Cistaceae*), einem mediterranen, immergrünen Strauch, ermittelten Daten zu vergleichen, um die Vor- und Nachteile der Methoden diskutieren zu können. Die erste Methode, als semiquantitativ (SQT) bezeichnet, beruht auf der Schätzung der Phänophasendauer durch visuelle Beobachtungen ganzer Pflanzen. Die zweite Methode, quantitativ (QT) genannt, basiert auf dem monatlichen Erheben aller Blätter, Knospen, Blüten und Früchte an fünf markierten Zweigen während eines Jahreszyklus. Beide Methoden führen zu einem Kalender für Blattproduktion, Abwurf von Brachyblasten, Dolichoblasten und reproduktiven Sprossen, Entwicklung von Blütenständen und Blütenknospen, Blüte, Fruchtansatz und Samenfreisetzung. Zusätzlich wurde Laubwurf durch 10 Laubfallen unterhalb der SQT-Pflanzen erfasst (LC-Methode). Die mittleren Daten des Beginnes, des Höhepunktes, des Endes und der Dauer jeder Phänophase wurden für beide Methoden berechnet. Die 95% Konfidenzwahrscheinlichkeiten der Variablenmittelwerte wurden berechnet, ebenso wie die minimale Stichprobengröße die nötig ist, um eine ± 30 Tage 95% Konfidenzwahrscheinlichkeit zu erhalten. Es wurde geschätzt, daß, um gleiche Konfidenzwahrscheinlichkeiten zu erhalten, die QT-Methode 4,5 Stunden Feldarbeit pro Probenahmetag benötigt. Dem steht ein Aufwand von nur einer Stunde mit der SQT-Methode gegenüber. Für die meisten Phänophasen fanden wir gute Übereinstimmung zwischen beiden Methoden. Die wesentlichsten Unterschiede zwischen den Methoden ergaben sich in der Phänophasendauer, die dazu tendiert, nach der SQT-Methode länger zu erscheinen. Sowohl die geringere Empfindlichkeit der QT-Methode gegenüber ungewöhnlichen Ereignissen, als auch die geringere Zuverlässigkeit der SQT-Methode beim Abgrenzen unauffälliger Phänophasen, mögen für diese Diskrepanz

verantwortlich sein. Die QT-Methode empfiehlt sich für unauffällige Phänophasen, wie die Blattproduktion an Brachyblasten; LC ergibt die besten Daten für Laubwurf mit den geringsten Aufwand. SQT ist für die verbleibenden, auffälligeren Phänophasen geeignet.

1. Introduction

Plants living in seasonal climates distribute their life cycle events (shoot growth, flowering, fruit setting, seed dispersal, leaf shedding, etc.) through different times of the year. The phenological pattern, i.e. the annual distribution of these events, is an important component of the plant's strategy to deal with seasonal climates (BEATLEY 1974, MOONEY 1983, ORSHAN 1989), and its knowledge contributes towards an understanding of the ecosystem function (KÖRNER 1994). The study of plant phenology has to face different methodological adversities: Field observations should last for at least one year to ensure a satisfactory analysis, and sometimes they have to be repeated over further years to gain certainty. Additionally, between-year variations in plant phenology complicate the comparisons of different sets of observations. Furthermore, asynchrony of phenological events within and between individuals is another handicap to the drawing of a single-species phenological diagram (RATHCKE & LACEY 1985, BOLMGREN 1998). The consequence of these problems is a lack of a generally-accepted methodology of phenological data gathering and analysis (SCHIRONE & al. 1990).

Different authors have developed different methods to study plant phenology, depending on the aim of the research, the type of plant and climatic conditions. These methods vary widely as regards the time required for data gathering and the precision of the information. Sampling units may be single branches (BAKER & al. 1982, GILL & MAHALL, 1986, NILSEN 1986, NEGI & SINGH 1992, OLIVEIRA & al. 1994, DHAILA & al. 1995, NITTA & OHSAWA 1997) or whole plants (FRANKIE & al. 1974, ARROYO & al. 1981, BERTILLER & al. 1991, NÉEMAN 1993). The observation intervals vary from a few days (for example, ESTABROOK & al. 1982, SHUKLA & RAMAKRISHNAN 1984, WRIGHT & CALDERON 1995) to one month (for example, LOWMAN 1992, KAPLAN & GUTMAN 1999). Some authors just monitor one or a few individuals, which are considered as prototypes of the species or populations (BAKER & al. 1982, NILSEN 1986, ORSHAN 1989, NITTA & OHSAWA 1997). Others select a representative number of samples to get the frequency of phenological stages across the population (SHUKLA & RAMAKRISHNAN 1982, KAPLAN & GUTMAN 1999). Depending on how the sampling unit is assigned to each phenological category, methods can be classified into qualitative or quantitative. In the former, each life cycle event or phenophase, is considered to be present or absent from the sample unit, on the basis of visual inspection (MEDWAY 1972, FRANKIE & al. 1974,

MOONEY & al. 1980, ARROYO & al. 1981, BAKER & al. 1982, ORSHAN 1989, BERTILLER & al. 1991, NÉEMAN 1993). Quantitative methods label all plant elements in the sampling unit, and follow their fate during the observation period. Therefore, the incidence of each phenophase is quantified through the number of elements appeared or shed, and/or by their size increase between observation dates (BOOJH & RAMAKRISHNAN 1982a, 1982b, ESTABROOK & al. 1982, OHSAWA & al. 1983, GILL & MAHALL 1986, NILSEN 1986, SCHIRONE & al. 1990, OLIVEIRA & al. 1994, SEIWA & KIKUZAWA 1996).

The aim of this work is to compare the phenological information gathered on the same population through different methods, so that the reliability of each method to describe each life cycle event could be discussed. We selected a population of *Halimium atriplicifolium* (LAM.) SPACH (*Cistaceae*), which is a Mediterranean evergreen shrub. The phenological methods to be compared differ in time investment, in sampling size and in precision: The first one is based on the observation of whole individuals and assignation of an index of phenophase frequency. The second one consists of monitoring every organ borne on some selected branches, therefore being quantitative. In addition, leaf shedding is also monitored through litter collectors placed below the selected plants. The questions adressed are the following:

1. With the current sampling effort, which method gets the best confidence to define the calendar of each phenophase?
2. How big should be the sampling size and the sampling effort to get a pre-established confidenece limit for each phenophase calendar?
3. In what events do the different methods agree and disagree?
4. In case of disagreement, what are the reasons for it? which method can be recommended and why?

2. Materials and Methods

2.1. Description of the Studied Species

Halimium atriplicifolum (*Cistaceae*) is a Mediterranean shrub growing in central and SW Spain. It has a height up to 1.75 m. Different kind of shoots can be identified in this plant. Long vegetative branches with distinct internodes (dolichoblasts) possess pairs of opposed petioled-leaves, with a lamina of 6–55 × 3–33 mm, in whose axils arise sylleptic short branches nearly without internodes inbetween (brachyblasts). As brachyblasts may eventually grow into dolichoblasts (they are 'partial brachyblasts', ORSHAN 1989) we only considered those short shoots as brachyblasts when they possess three or less leaf pairs and no further brachyblasts in their axils, the rest being counted as dolichoblasts. Both kinds of shoots terminate in a meristem covered by a pair of unfolded leaves. Shoots bearing reproductive organs differ morphologically from vegetative ones in that they are covered by glandular hairs, possess sessile leaves of

10–50 × 6–34 mm, and they end in an inflorescence of 2–8 flowers (CASTROVIEJO & al. 1995).

2.2. Location

The study site is located in the municipality of Arganda del Rey, SE Madrid, Central Spain, at 640 m of altitude, 40° 18′ of latitude and 00° 15′ of longitude. The mean annual precipitation and temperature are 461 mm and 13.5°C, respectively (DE LEÓN-LLAMAZARES 1989). The landscape is hilly, the bedrock being limestones and gypsum. We selected an homogeneous population which extends along the north side of a hill, with a gentle slope.

2.3. Phenological Methods

Phenological observations began on November 1999 and extended till October 2000, the frequency of data collection being every month. Two types of sampling methods were applied. The first one was based on the qualitative method of ORSHAN 1989, but modified to get a within-population phenophase frequency at every sampling date (CASTRO-DÍEZ & MONTSERRAT-MARTÍ 1998). Ten adult and healthy individuals were randomly selected from the population and labelled before the beginning of the sampling. During each visit we carefully examined each individual to determine the degree of incidence of the following phenophases: leaf production and shedding from dolichoblast, brachyblast and reproductive shoots, development of inflorescence and flower buds, flowering, fruit setting and seed dispersal. When a phenophase was present on a plant, we assigned it a frequency index, depending on the percentage of the crown where it appeared: 1 = presence less than 5%, 2 = presence between 5–25%, 3 = presence over 25% of the crown. The incidence of a phenophase in the whole population was calculated for each date as the average of the ten sampled plants' frequency indexes. In addition, 3–5 representative branches were collected during each visit from neighbouring plants and preserved in a herbarium for future verifications. A few phenophases (leaf production, leaf shedding on reproductive shoots, and development of inflorescence and flower buds), were finally described on the basis of this herbarium material due to identification problems in the field. This method has been named "semiquantitative" (SQT), as it is based on qualitative observations, but accounts for the phenophase frequency both within individuals and within the population.

The other sampling method was a quantitative one (QT). Sampling units were five south-exposed one-year-old branches, coming from five out of the ten individuals labelled for the previous sampling. Each branch was tagged and carefully drawn, taking into account the number and position of each element (brachyblasts, leaves, vegetative buds, inflorescence buds,

flower buds, flowers, closed capsules, open capsules). Every month we annotated in the previous-month's graph all changes (elements appeared or shed), so we ended up with a series of 12 monthly graphs per branch. From this information we constructed one matrix per branch and sampling date, where all shoots (dolichoblasts, brachyblasts and reproductive shoots) appeared in rows, and their number of leaves, buds, flowers, and fruits (closed or open) appeared in columns. The cumulative number of elements appeared or shed per branch at the end of the observation period was used to calculate the percentage of change between consecutive sampling dates (see Table 1). In this way we defined the same phenophases as before.

Table 1. – Data matrix obtained through the quantitative method (QT) for one of the sampled branches of *Halimium atriplicifolium*. Figures are the percentage of elements appeared or shed on each sampling date with respect to the whole sampling period. Leaves appeared and shed are shown for each shoot type (B-brachyblasts, D-dolichoblasts, RS- reproductive shoots). The rest of elements belonged to reproductive shoots.

	New leaves			Shed leaves			Inflo.	Flower		Closed	Open
DATE	B	D	RS	B	D	RS	buds	buds	Flowers	capsules	capsules
19-11-99	0	0		0	0						
22-12-99	0	0		2	0						
19-01-00	0	0		2	0						
16-02-00	2	0		2	0						
22-03-00	8	0		0	4						
26-04-00	18	0	59	12	0	0	11	0	0	0	0
26-05-00	19	33	29	0	0	0	35	58	20	0	0
27-06-00	25	67	12	17	15	50	54	42	80	25	0
20-07-00	28	0	0	27	41	27	0	0	0	55	11
31-08-00	0	0	0	19	30	23	0	0	0	0	22
30-09-00	0	0	0	13	3	0	0	0	0	10	34
30-10-00	0	0	0	6	7	0	0	0	0	10	33

Finally, an additional method was applied to quantify leaf shedding. Ten litter collectors (LC) were constructed with a piece of a PVC tube 12.5 cm diameter and 10 cm long. The bottom of the tube was covered with a mesh of 1 mm. Each collector was placed under the crown of each of the 10 SQT-sampling plants and fixed to the ground with a metal bar. In every visit the leafy litter was collected, kept in paper bags, labelled and brought to the lab where bags were left for 48 h in an oven at 60° C before weighing. The calendar of bulk leaf shedding was determined as the percentage of leaf dry mass collected each month with respect to the annual total. To compare this result with those obtained by the other methods for each shoot type, we calculated both a SQT- and a QT-bulk leaf shedding as the average between the frequency index of all shoot types, and by summing

up the number of leaves shed from brachyblasts, dolichoblasts and reproductive shoots, respectively.

2.4. Phenological Variables

The phenophase calendars obtained with each method were drawn by representing in abscissas the sampling dates and in ordinates the frequency index, percentage of elements appeared/shed, or percentage of dry mass, for SQT, QT and LC methods, respectively. These graphs allow a visual comparison of results from each method.

In order to represent numerically the phenological information, we obtained from each sampled plant or branch the beginning, ending and modal dates of all phenophases. The dates of beginning and ending were those of the first sampling when the phenophase was present and absent, respectively. In the LC sampling, we considered the leaf abscission to be present when the dry mass of the leaf material collected exceeded 0.5 g in order to eliminate accidental leaf fall. The modal date was that with the highest value in ordinates, the earlier peak being considered if more than one equivalent peaks appeared. All dates were expressed as number of days since the first of January 2000, and so the two dates of 1999 possessed a negative value. By subtracting the date of beginning from the date of ending we obtained the duration of each phenophase in days. However, sometimes we found a month with phenophase presence in the middle of a long phenophase absence period, or the reverse. In these cases we added or subtracted 30 days to the duration figure. In those branches where a phenophase exhibited no clear final point within the year, we neither established the beginning nor the ending dates. For those phenophases in which we registered the end of the 1999 cycle and the beginning of the 2000 one, we assumed no between-year calendar change in order to calculate the phenophase length.

2.5. Assessment of Sampling Effort and Sampling Sizes

The sampling sizes (5, 10 and 10 for QT, SQT and LC, respectively), were a priori defined. The average time required to collect phenological information from each sample unit was assessed for each method. In order to compare the reliability of the dates which describe each phenophase calendar, the 95% confidence limits for each mean were calculated (Zar 1996). Afterwards, the minimum sampling size to get a confidence limit of ± 1230 days for each date was calculated for each method using the iterative method described in Zar 1996.

2.6. Comparison Between Methods

The figures of initiation, maximum, ending and duration of each phenophase obtained by the different sampling methods, were compared by

means of t-Student or U-Man-Whitney tests. For bulk leaf shedding comparison a Kruskall-Wallis test was used. Leaf production and shedding of reproductive shoots, as well as development of inflorescence and flower buds, were not statistically compared because SQT information was based only on 3–5 herbarium branches.

3. Results

3.1. Sampling Effort and Sampling Size

The flowering phenophase was not observed in three out of the 10 SQT plants and in one out of the five QT ones, reducing the initial sampling sizes to seven and four, respectively. The same was true for fruit setting in one individual of both samplings. Dates of beginning and ending of dolichoblast leaf shedding were calculated just in 8 plants with the SQT method, as the two left individuals did not show a clear date neither of beginning nor ending.

As an average, the sampling of each unit took 5, 30 and 3 minutes of field work for SQT, QT and LC respectively, although the last method required 10 additional minutes in the lab per sample. Therefore, the sampling effort to perform a whole data collection in the field per month consisted on 50, 150 and 30 minutes, respectively, plus 100 additional minutes for LC processing and weighting in the lab. However, this sampling effort varied widely along the year, with maximum values in spring, when most phenophases were active, and minimum in summer and winter.

The 95% confidence limits ($\pm$d) of the mean dates of beginning, ending and maximum, plus the duration of each phenophase are represented in Table 2. The variables with no value could not be normalised, so the confidence limits could not be calculated. Most of the non-normal variables exhibited a binomial distribution, with just two values, while others exhibited a constant value (in these cases d = 0). The confidence limits were narrower for the SQT than for the QT variables, except for leaf shedding of dolichoblasts and duration of the flower bud development. This was expected on the basis of the smaller sampling size of the second method.

Table 3 shows the sampling size required to get a 95% confidence interval of $\pm$30 days in the phenological variables. In most cases the obtained values were above the current sampling size, varying from 5 to 26 for the SQT method (average = 12) and from 3 to 40 for the SQT one (average = 9). However, when comparing the sampling size required by both methods for the same variable, only seven pairs were found to be available: three of them exhibited lower sampling size for QT than for SQT, the reverse being true for the four left.

Intra-method differences in confidence limits and required sampling sizes also came out from tables 2 and 3. Phenophases concerned with reproduction (inflorescence and flower bud formation, flowering, and fruit

Table 2. – The numbers reported for each phenological variable obtained through the different methods are the 95% confidence limits of the means (d-values) expressed in days, so that the probability that the population mean of each variable fall in the range x ± d is 95%. SQT- semiquantitative method, QT- quantitative method and LC- litter collectors method. Zero values mean that the variable was a constant. Missing values correspond to variables which could not be normalised. Reported values are natural ones, although some of them were transformed before calculations.

			SQT		
	N	Beginning	Ending	Maximum*	Duration*
Leaf production on brachyblasts	10	–	–	–	22,3
Leaf production on dolichoblasts	10	–	–	–	–
Development of flower buds	10	–	20,04	–	35,34
Flowering	7	–	24,28	–	22,43
Fruit setting	9	0	–	–	–
Seed dispersal	10	–	53,78	–	41,76
Leaf shedding on brachyblasts	10	39,26	–	–	19,51
Leaf shedding on dolichoblasts	8/10*	53,95	24,37	–	38,41

			QT		
	N	Beginning	Ending	Maximum	Duration
Leaf production on brachyblasts	5	49,4	0	56,14	49,4
Leaf production on dolichoblasts	5	40,38	–	38,5	56,51
Leaf production on reproductive shoots	5	–	29,26	33,43	33,63
Development of inflorescence buds	5	–	32,05 (2)	32,06	28,59
Development of flower buds	5	–	–	–	19,9
Flowering	4	–	–	0	–
Fruit setting	4	–	–	–	–
Seed dispersal	5	35,73		31,17	
Leaf shedding on brachyblasts	5	79,65	35,97 (1)	57,11	116,02
Leaf shedding on dolichoblasts	5	45,43	31,17	-	69,83
Leaf shedding on reproductive shoots	5	–	–	35,73	–

			LC		
	N	Beginning	Ending	Maximum	Duration
Bulk leaf shedding	10	29,03 (3)	–	–	–

(1) 1/x transformation
(2) log(x) transformation
(3) $x^{0,5}$ transformation
* The number of cases differed between variables (see text)

Table 3. – Sampling sizes required for each variable and method to get a 95% confidence limit of ±30 days using different methods.

	SQT			
N	Beginning	Ending	Maximum	Duration
Leaf production on brachyblasts	–	–	–	6
Leaf production on dolichoblasts	–	–	–	–
Development of flower buds	–	6	–	13
Flowering	–	6	–	5
Fruit setting	–	–	–	–
Seed dispersal	–	26	–	17
Leaf shedding on brachyblasts	15	–	–	6
Leaf shedding on dolichoblasts	20	6	–	15

	QT			
N	Beginning	Ending	Maximum	Duration
Leaf production on brachyblasts	9	–	11	9
Leaf production on dolichoblasts	7	–	7	11
Leaf production on reproductive shoots	–	5	6	6
Development of inflorescence buds	–	5	5	5
Development of flower buds	–	–	–	3
Flowering	–	–	–	-
Fruit setting	–	–	–	–
Seed dispersal	6		5	
Leaf shedding on brachyblasts	24	6	12	40
Leaf shedding on dolichoblasts	8	5	–	16
Leaf shedding on reproductive shoots	–	–	6	–

	LC			
N	Beginning	Ending	Maximum	Duration
Bulk leaf shedding	9	–	-	–

setting) seemed to be the most reliable (narrow confidence limits and low sampling size), although seed dispersal exhibited a higher uncertainty when studied through the SQT method. Leaf shedding from different shoots required the biggest sampling sizes on the basis of SQT (up to 20); however, the QT method only required high sampling sizes for brachyblast leaf shedding and for the duration of dolichoblast leaf shedding. The mean date for the beginning of bulk leaf shedding obtained with the LC method fall within the ±30 days interval with the current sampling effort (n=10). Leaf production of the different shoots studied through QT required sampling sizes between 5 and 11 to get a ±30 days 95% confidence limit for their means; however, we could calculate almost no value for the same phenophases studied through the SQT method.

3.2. Comparison of Methods

The phenological information obtained through the different methods can be graphically compared in Figs. 1 and 2. Additionally, Fig.3 compares the dates of beginning, ending, maximum and duration of the phenophases.

The brachyblast leaf production's curve was narrower when studied with the QT method as compared with the SQT one, mainly due to a later beginning (Fig. 1a). Significant inter-methods differences were found in the dates of beginning and maximum, as well as in phenophase duration (Fig. 3a).

The shape of the leaf production curves from both dolichoblasts and reproductive shoots exhibited little between-method differences. Statistical comparisons of dates did not detect any difference in dolichoblast leaf production. (Fig. 1b,c and 3b).

Inflorescence and flower bud development were found to start one month earlier by the SQT method than by the QT one (Fig. 1d,e). Flower bud formation started and peaked one month earlier on the basis of the SQT. Accordingly, duration of this phenophase was one month longer when monitored with the SQT method.

The patterns of flowering shown by both methods were quite similar, both in the beginning and peaking dates. The ending date of this phenophase varied between August (QT) and September (SQT), although the difference was not statistically significant. On the contrary, the average duration of flowering significantly differed between methods, being longer for the SQT one (Figs. 1f and 3c).

No relevant difference in the fruit setting calendar appeared between the QT and SQT methods (Figs. 1g and 3d). Seed dispersal exhibited no significant inter-method difference for the starting and peaking dates (Figs. 1h and 3e). We have not registered the end of this phenophase by the QT method, as we did not distinguish between open and closed capsules until the second half of the sampling period (Fig. 1h).

Leaf shedding of brachyblasts exhibited a similar pattern on the basis of both methods (Figs. 2a and 3f). The same was not true for the ending date of dolichoblasts' leaf shedding, which was later when monitored by the SQT method. The duration of this phenophase tended to be longer on the basis of the SQT method ($p = 0.07$, Mann-Whitney's U test) (Figs. 2b and 3g). Leaf shedding of reproductive shoots also coincided between methods, peaking in June, together with the other two shoot types (Fig. 2c). The pattern of bulk leaf shedding found by the LC method was similar to that obtained through the other methods (Fig. 2d). However, statistical comparisons revealed that the ending date and the duration accorded more with the QT figures (Fig. 3h).

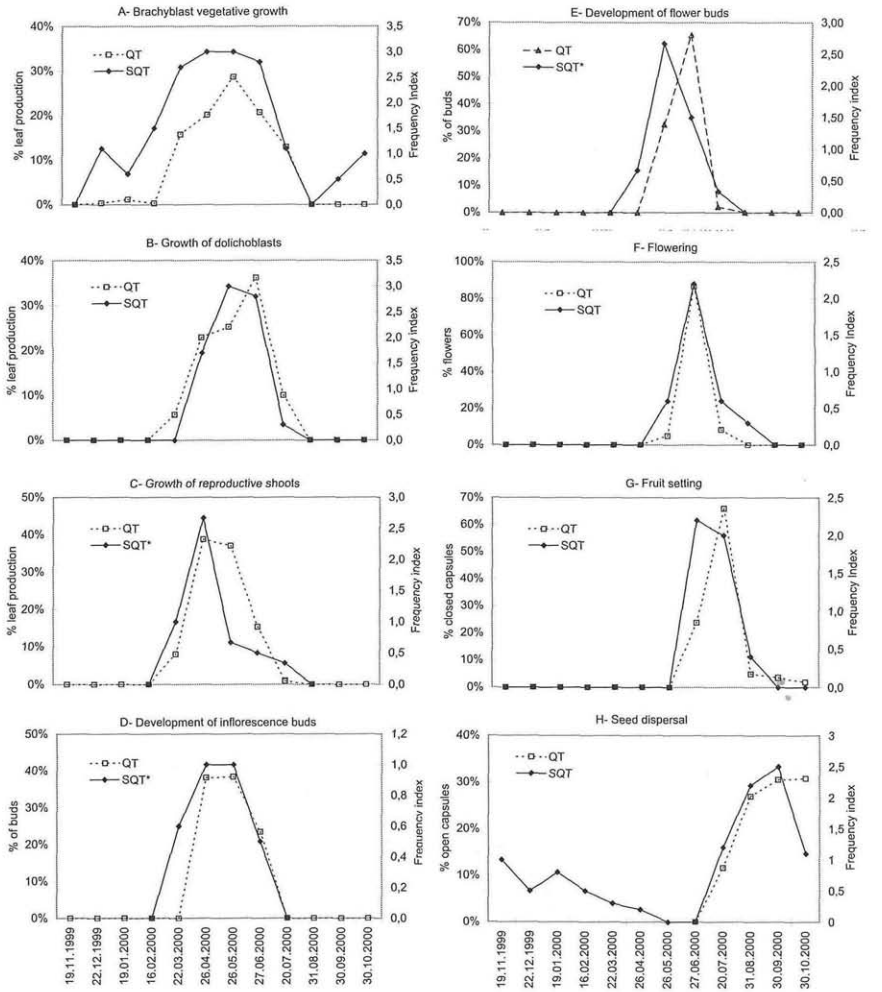

Fig. 1. – Development of vegetative and reproductive phenophases of *Halimium atriplicifolium* between November 1999 and October 2000, on the basis of the quantitative (QT) and semiquantitative method (SQT). Each QT point is the mean of five sampling units, representing the percentage of elements produced or shed each month with respect to the whole sampling period. SQT points are the averaged frequency index between 10 plants, except those with an asterisk, which came from 3–5 herbarium-preserved branches (see methods).

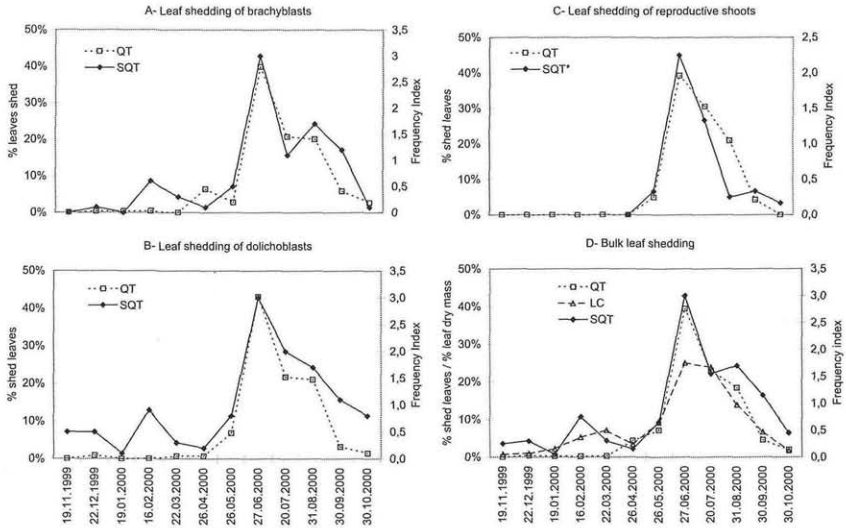

Fig. 2. – Development of leaf shedding of *Halimium atriplicifolium* between November 1999 and October 2000, on the basis of the quantitative (QT), semiquantitative (SQT) and litter collector (LC) methods. Each QT point is the mean of five sampling units, representing the percentage of leaves shed each month with respect to the whole sampling period. SQT points are like in Fig. 1. LC points represent the average percentage of leaf dry mass collected in 10 litter traps each month with respect to the annual cumulative leaf dry mass.

4. Discussion

4.1. Sampling Effort and Sampling Size

Our data revealed that, in average, the current sampling size of both the QT and the SQT samplings were too small, resulting in many non-normal variables, and 95% confidence limits of the means usually lasted over one month, which tended to be wider for the QT method. By stating arbitrarily a confidence limit of ± 30 days for all the variable means, the required sampling size varied both between methods and between phenophases. When comparing methods, we found the average sampling size required for SQT to be 12, while that for QT was nine. Although the reliability of this values is partial, due to the high number of missing values, it could be a first approach to calculate the mean sampling effort necessary to get a similar confidence, which results in a sampling time of one hour to process the 12 SQT samples and 4,5 hours to process the nine QT ones, per sampling date. The only normal LC variable gave a sampling size of nine, which requires a field work of 0.5 hours, plus 1.5 hours in the lab.

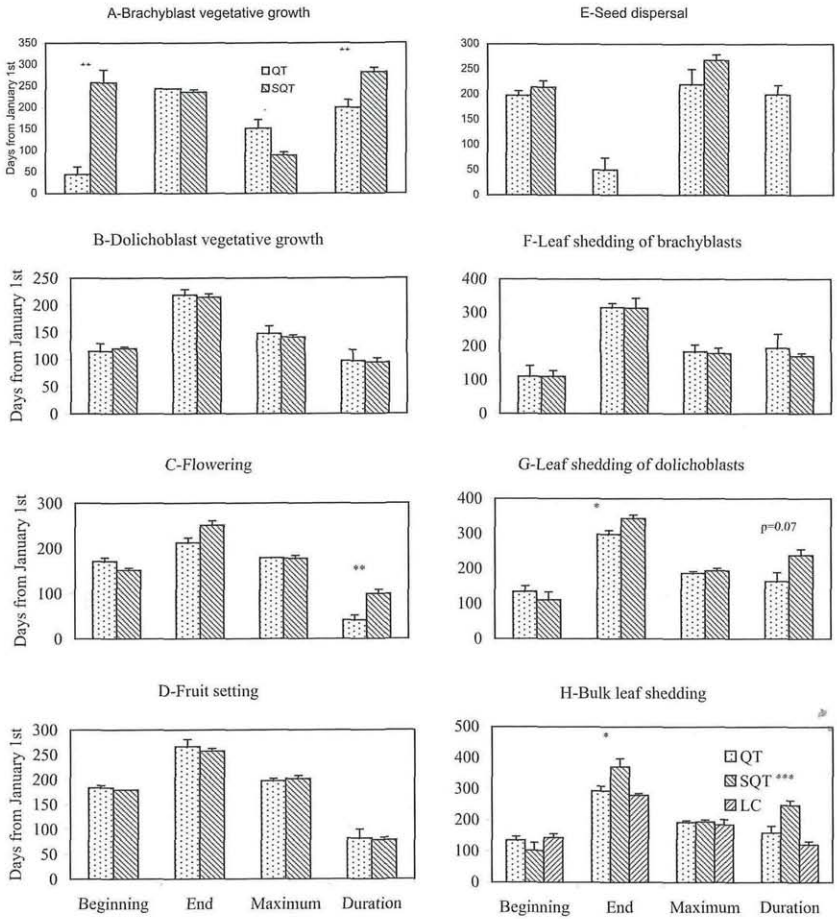

Fig. 3. – Comparison of dates of phenophase beginning, end, and maximum, plus duration calculated through the quantitative (QT), semiquantitative (SQT) and litter collector (LC) methods. Dates are expressed in days as from the first of January. Bars represent the standard error. Asterisks over a pair of columns mean significant differences on the basis of Student t-test (duration of both brachyblasts' vegetative growth and flowering), a Kruskall-Wallis test (for bulk leaf shedding comparison) or Mann-Whitney U-test (rest of comparisons). * $0.01<p\leq0.05$, ** $0.001<p\leq0.01$, *** $p\leq0.001$

Sampling sizes should be proportional to the data variance. Different components contribute to increase the variance in phenological data sets: The foremost is the intrinsic asynchrony of phenophases, both within and between plants. The second is the degree of uncertainty in the assignation of a frequency value to each phenophase, which is a methodological com-

ponent. The SQT method did not account for intra-plant phenological asynchrony, as the sampling unit is the whole plant. On the contrary, data variance accounted by the QT method is the sum of both within and between-plant asynchrony. The methodological contribution to the variance is higher when presence or frequency of phenophases is assigned by eye (i.e. SQT method), as it implies a wider degree of uncertainty, which may result in an artificial increase of the data variance.

Both components of the data variance have a differential contibution in each phenophase. The shortest phenophases, which in this species coincided with those related with reproduction (development of flower and inflorescence buds, flowering and fruit setting), exhibited the lowest variance on the basis of both methods. This is probably due to a high intra- and inter-plant synchrony, and to a low SQT uncertainty, as these phenophases are very conspicuous. On the contrary, leaf shedding of brachyblasts and dolichoblasts exhibited the highest variances. This event has been reported to be quite long and variable in evergreen species, in contrast to deciduous ones (ADDICOTT & ADDICOTT 1982, ORSHAN 1989, CASTRO-DÍEZ & MONTSERRAT-MARTÍ 1998, ESCUDERO & DEL ARCO 1987), so a large proportion of its variance might be due to asynchrony, especially for QT. In addition, the SQT method estimated the frequency of leaf abscision from the eye-estimated proportion of dried leaves attached to the shoots, but such leaves may remain for some time in the plant. Therefore, methodological bias might consistently contribute to increase the variance of the SQT leaf shedding calendar. The variance of the dates of leaf production by both kinds of shoots exhibited an intermediate value.

In summary, the optimum sampling size for phenological studies vary between methods and phenophases. It is advisable to have previous information about phenophase synchrony on the study species before making a decision. In addition, if a qualitative method is selected, the sampling size should be inversely proportional to the facility to observe the phenophases' presence.

4.2. Comparison Between Methods

In spite of the methodological and sampling-size differences, the phenophase patterns resulting from both methods were in good agreement. The statistical comparison revealed that the main difference appeared in the duration of a few phenophases (brachyblast leaf production, flowering and leaf shedding of dolichoblasts), which was longer on the basis of the SQT method. The QT method is more prone to underestimate phenophases duration as both, the smaller sampling unit and the sample number make it less sensitive to infrequent events. On the other hand, the eye-assignation of frequency indices by SQT, makes its results less reliable than those of QT. The importance of each advantage and disadvantage differs between

phenophases, depending on their synchrony and the facility to be eye-observed, as reported before.

The leaf production occurs by unfolding of small leaves wrapping the shoot apex, and keeps on until the last pair fails to expand and remains covering the apical meristems. The lack of scale-protected buds make this process quite unconspicous. In dolichoblast and reproductive shoots, leaf production is accompanied by a conspicuous shoot elongation, but the same is not true for brachyblasts, where this phenophase was difficult to observe by eye. We might have overestimated this phenophase with the SQT method by assigning low frequency indices whenever we were not aware of the phenophase presence. Therefore, in spite of the tendency of the QT method to underestimate phenophase duration, for this particular phenophase we are more aware of the calendar reported by the QT method.

On the contrary, the longer duration of flowering on the basis of the SQT method seems more reliable than the shorter calendar reported by QT, as this phenophase is very conspicuous. It probably happened so that one plant bearing several flowers on a particular date yielded a flowering index above zero through the SQT method, but no precence with the QT one, if the selected branch was lacking flowers. However, our result contrasts with those obtained by WRIGHT & CALDERON 1995, who compared the pattern of flowering of a tropical forest community obtained through a qualitative and a quantitative method and found an agreement in the phenophase's midpoint, but a smaller phenophase length on the basis of the qualitative method.

The earlier detection of inflorescence and flower buds through the SQT method again suggests that we miss the beginning of these phenophases with QT method, as both kinds of buds are easily observed.

Leaf shedding exhibited a consistent pattern across the different methods, mainly in the modal dates. However, the SQT method seems more prone to overestimate the importance of leaf shedding during periods of low incidence (see fig. 2). This may be explained by considering how each method detect leaf abscission: while both the LC and the QT quantify the amount of leaves actually fallen during the time lag between two sampling dates, the SQT method estimates the frequency of senescent leaves still attached to the plant, but these leaves may still remain for a longer period on the plant. Therefore, LC and QT methods appear to be more reliable for this phenophases. The use of litter collectors has the advantages of being more time-saving in the field and allowing a broader sampling size. Therefore, the LC method would exhibit a higher sensitivity to detect leaf fall out of the main abscission period. In fact, the LC graph revealed a small peak of leaf shedding in February and March (Fig. 3l), which was not detected by the QT method. The simplicity of the LC method has encouraged many authors to use litter collectors to establish calendars of leaf

shedding (ESCUDERO & al. 1987, MAYA & ARRIAGA 1996, WILLIAMS & al. 1997) and flowering (WRIGHT & CALDERON 1995), to quantify fruit production (RAPP 1969, HERNÁNDEZ & al. 1992), to calculate leaf turnover (ESCUDERO & DEL ARCO 1987, LOWMAN 1992) and primary production (BELLOT & al. 1992). The drawback of this method is that it does not allow to distinguish leaf functional classes unless they differ in morphology, this information being necessary to understand leaf dynamics of heteroblastic plants (ORSHAN 1963, WESTMAN 1981).

4.3. Sampling Frequency

Although this study has not tested the sampling frequency as a methodological variable, it is important to achieve an agreement between the sampling method and the sampling frequency. Studies focused on short phenophases (for example, flowering), require a high frequency to get a representative observation number of the process. However, the closer the sampling dates were, the smaller were the differences observed in the plant, so that a quantitative sampling might be necessary to detect such differences. On the other hand, long phenophases can be satisfactorily described through a low sampling frequency schedule, in which greater differences are likely to be found between consecutive observations. Therefore, a qualitative method may detect them. An exception should be noted for long but very slow phenophases, where the low between-dates differences may require a more sensitive quantitative method. The sampling frequency in the current study (around 1 month) was satisfactory for most of the phenophases, although the shortest ones (development of flower buds and flowering) would be better described with a higher frequency.

5. Conclusion

In summary, the QT method has the disadvantage of the high time consumption, which severely limits the sampling size, as compared to the SQT one. This makes the QT method more prone to overlook infrequent events. On the other hand, the QT method has the advantage of a higher precision, as data are reported in numerical frequencies, rather than in semi-quantitative frequency indices. In addition, monthly variation of samples was kept in drawings, so that phenological events can be defined at the end of the sampling and reanalysed whenever necessary. This was not the case of the SQT method, in which phenological events were defined before the sampling, and the phenological diagram was constructed throughout the visits to the field. Reanalysis of the phenophase calendar was also possible on the basis of the herbarium-preserved branches, but frequency indices assigned to this material were a poor representation of the population. In spite of these differences, just a few discrepancies came

out from comparison of the phenophase calendars obtained through both methods. In the case of vegetative growth of brachyblasts, we recommend the QT method, whenever the study species lacks conspicous scale-protected vegetative buds. For leaf abscission we advise the use of litter collectors designed for evergreen species. The rest of phenophases could be satisfactorily described with a SQT method.

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