Leaf Primordium Initiation and Expanded Leaf Production are Co-ordinated through Similar Response to Air Temperature in Pea (*Pisum sativum* L.)

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Received: 9 December 1996 Accepted: 26 March 1997

Accurate prediction of the timing of leaf area development is essential to analyse and predict the responses of crops to the environment. In this paper, we analyse the two processes determining the chronology of leaf development—initiation of leaf primordia by the shoot meristem and production of expanded leaves out of the shoot tip—in several pea (*Pisum sativum* L.) cultivars in response to air temperature and plant growth rate. Contrasting levels of air temperature and plant growth rate during leaf development were induced by a wide range of sowing dates and plant densities in glasshouse or field experiments. Full leaf expansion was found to occur one phyllochron after full leaf unfolding, whatever the leaf nodal position. Primordium initiation and expanded leaf production rates presented similar quantitative responses to air temperature (linear response and common x-intercept), whatever the plant growth rate, cultivar or period of cycle. As a consequence, they were co-ordinated and the numbers of initiated primordia or expanded leaves were easily deduced from simple visual observation of leaf unfolding. The change, over time, of the numbers of initiated leaf primordia and fully expanded leaves correlated with cumulated degree-days, with stable relationships in a wide range of environmental conditions. Two phases, with different production rates, had to be considered. These results allowed us to predict accurately the beginning and the end of individual leaf development from daily mean air temperatures. The relationships obtained here provide an effective way of analysing and predicting leaf development responses to the environment.

Key words: *Pisum sativum* L., pea, number of leaf primordia, number of leaves, temperature, modelling.

INTRODUCTION

Leaf area plays a key role in crop biomass accumulation because it determines the amount of solar radiation intercepted by the crop. This in turn determines the amount of dry matter accumulated by a crop which explains a large part of the yield variation. Therefore, progress in understanding crop production in response to the environment requires a quantitative understanding of leaf area development. Accurate prediction of the timing of leaf area development is essential to analyse and predict the responses of plants to the environment. For example, the determination of the beginning and the end of individual leaf development appeared central to the analysis and prediction of the effect of environmental conditions such as soil water deficit on leaf expansion in pea (Lecoeur et al., 1995; Lecoeur, Wery and Sinclair, 1996).

The pea shoot is made up of a succession of elementary units, phytomers, each carrying one leaf (Nougare dé and Rondet, 1973a). Two stages delimit the development of each leaf: primordium initiation by the meristem, and full leaf expansion after emergence from the shoot tip.

In many species, predictors have been built including environmental factors such as temperature, light or day length to predict the rate of plant development (Rickman and Klepper, 1995). Generally, average air temperature accumulated in the form of a degree-day sum is found to be linearly correlated with various developmental descriptors (e.g. unfolded leaves in pea; Truong and Duthion, 1993). If the change over time of the number of initiated leaf primordia and fully expanded leaves on the stem can be correlated with average temperature sums, the two lines form a diagram which describes the chronology of development of individual leaves (Rickman and Klepper, 1995). In the same manner, Ney and Turc (1993) described the chronology of development of pea reproductive organs.

Several studies on the functioning of the apical meristem during one plastochron have been made in pea (Lyndon, 1968; Nougaréde and Rondet, 1973a); but there are no data concerning the prediction of the rhythm of initiation of successive leaf primordia in response to environmental factors on this species at the whole plant level. In wheat, for example, leaf primordium initiation rate seems to depend mainly on temperature, irrespective of daylength, sowing time and moderate nitrogen or water stress (Miglietta, 1989; Jamieson et al., 1995).

The rate of leaf appearance integrates meristem functioning and leaf expansion. It is generally more sensitive to the environment than is initiation of leaf primordia [e.g. Kirby (1995) on wheat; NeSmith and Ritchie (1992) on maize]. Truong and Duthion (1993) reported that the rate of leaf appearance was related to temperature and dry matter.
growth rate in pea. In all studies, leaf appearance was characterized by visual signs (leaf tip appearance on cereals, leaf unfolding on pea) and was not related to the time of full leaf expansion.

The aim of this paper is twofold. Firstly to analyse, in a wide range of growing conditions, the two phenomena determining chronology of leaf development: the rhythm of initiation of new leaf primordia by the shoot meristem and the rhythm of production of expanded leaves from the shoot tip. These rhythms will be analysed in response to air temperature and plant growth rate in order to propose a prediction of leaf development from air temperature, eventually corrected with plant growth rate. The second aim is to examine the co-ordination between these events (primordium initiation and full leaf expansion) and phenological stages such as the nodal stage defined by Maurer, Jaffray and Fletcher (1966), and so to determine whether they can be deduced from these simple visual observations and air temperature.

**MATERIALS AND METHODS**

*Plant material and growing conditions*

In order to obtain a wide range of growing conditions in terms of temperature and plant growth (Table 1), two experiments using the pea cultivar Messire were carried out in a glasshouse (expts 1 and 2) and two in the field (expts 3 and 4), with various sowing dates. An additional field experiment (expt 5), including four cultivars (Alex, Baccara, Messire and Solara) sown at five successive dates, was conducted in 1996. Messire is a leafed genotype whereas Alex, Baccara and Solara are semi-leafless. Mean temperatures during the vegetative phase (between emergence and flowering) and during the reproductive period (between the beginning of flowering and the cessation of leaf emergence from the shoot top) are shown in Table 1. All treatments were irrigated, every day in glasshouse and every third day in field experiments, in order to maintain soil water potential above $-10$ kPa at a depth of 20 cm and $-50$ kPa at a depth of 25 cm in the glasshouse and field, respectively. Previous work indicated that neither transpiration nor leaf expansion were affected at such levels of soil water availability (Lecoeur and Sinclair, 1996).

Pot experiments were conducted in a glasshouse in Montpellier on cultivar Messire. Air temperature was regulated in order to avoid values above $25 \, ^\circ \text{C}$ in expt 1 and $28 \, ^\circ \text{C}$ in expt 2. Additional light was supplied to obtain a photoperiod of at least 14 h to prevent problems of delayed flower initiation. Twelve pots per treatment were filled with a mixture of loamy soil and organic compost. Sixteen seeds were sown per pot, thinned to 12 after emergence and eight at the beginning of flowering. Pots were 0-30 m-diameter (35 l) in expt 1 and 0-20 m-diameter (12 l) in expt 2. Lateral branches were removed as soon as they became visible. Experiment 1 included two treatments: (1) ‘control’ plants, in which reproductive organs were allowed to develop

<table>
<thead>
<tr>
<th>Expt</th>
<th>Location</th>
<th>Sowing date</th>
<th>Cultivar</th>
<th>Flowers removed</th>
<th>Branches removed</th>
<th>Plant population*</th>
<th>Mean temperature (°C)</th>
<th>Dry matter accumulation (g per plant) [Duration (d)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glasshouse</td>
<td>27 Jan. 1995</td>
<td>Messire</td>
<td>No</td>
<td>Yes</td>
<td>50</td>
<td>16.2 (16) 17.3 (17)</td>
<td>2.43 (45) 4.80 (15)</td>
</tr>
<tr>
<td>2</td>
<td>Glasshouse</td>
<td>13 May 1994</td>
<td>Messire</td>
<td>No</td>
<td>Yes</td>
<td>120</td>
<td>21.0 (21) 24.4 (24)</td>
<td>1.40 (34) 1.14 (9)</td>
</tr>
<tr>
<td>3</td>
<td>Field</td>
<td>3 Feb. 1994</td>
<td>Messire</td>
<td>No</td>
<td>No</td>
<td>75</td>
<td>11.7 (11) 15.7 (15)</td>
<td>4.03 (58) 6.35 (27)</td>
</tr>
<tr>
<td>4</td>
<td>Field</td>
<td>28 Mar. 1994</td>
<td>Messire</td>
<td>No</td>
<td>No</td>
<td>75</td>
<td>14.2 (14) 18.6 (18)</td>
<td>3.50 (43) 4.87 (21)</td>
</tr>
<tr>
<td>5</td>
<td>Field</td>
<td>12 Mar. 1996</td>
<td>Alex</td>
<td>No</td>
<td>No</td>
<td>73</td>
<td>43 (43) 36 (36)</td>
<td>40 (34) 14 (9)</td>
</tr>
<tr>
<td>6</td>
<td>Field</td>
<td>27 Mar. 1996</td>
<td>Messire</td>
<td>No</td>
<td>No</td>
<td>75</td>
<td>14.4 (14) 18.1 (18)</td>
<td>2.22 (42) 6.44 (21)</td>
</tr>
<tr>
<td>7</td>
<td>Field</td>
<td>10 Apr. 1996</td>
<td>Baccara</td>
<td>No</td>
<td>No</td>
<td>78</td>
<td>15.5 (15) 19.7 (19)</td>
<td>2.21 (39) 3.68 (18)</td>
</tr>
<tr>
<td>8</td>
<td>Field</td>
<td>13 May 1996</td>
<td>Messire</td>
<td>No</td>
<td>No</td>
<td>78</td>
<td>15.5 (15) 19.2 (19)</td>
<td>2.39 (38) 4.16 (17)</td>
</tr>
<tr>
<td>9</td>
<td>Field</td>
<td>30 May 1996</td>
<td>Baccara</td>
<td>No</td>
<td>No</td>
<td>78</td>
<td>15.5 (15) 18.9 (18)</td>
<td>2.31 (39) 4.74 (14)</td>
</tr>
<tr>
<td>10</td>
<td>Field</td>
<td>13 May 1996</td>
<td>Messire</td>
<td>No</td>
<td>No</td>
<td>80</td>
<td>16.1 (16) 21.4 (21)</td>
<td>3.75 (37) 2.56 (16)</td>
</tr>
<tr>
<td>11</td>
<td>Field</td>
<td>30 May 1996</td>
<td>Solara</td>
<td>No</td>
<td>No</td>
<td>68</td>
<td>16.1 (16) 21.7 (21)</td>
<td>2.89 (37) 4.05 (17)</td>
</tr>
<tr>
<td>12</td>
<td>Field</td>
<td>13 May 1996</td>
<td>Alex</td>
<td>No</td>
<td>No</td>
<td>77</td>
<td>22.0 (22) 21.6 (21)</td>
<td>1.76 (28) 3.17 (12)</td>
</tr>
<tr>
<td>13</td>
<td>Field</td>
<td>30 May 1996</td>
<td>Messire</td>
<td>No</td>
<td>No</td>
<td>83</td>
<td>22.0 (22) 21.6 (21)</td>
<td>2.99 (28) 3.11 (12)</td>
</tr>
<tr>
<td>14</td>
<td>Field</td>
<td>13 May 1996</td>
<td>Baccara</td>
<td>No</td>
<td>No</td>
<td>73</td>
<td>21.7 (21) 22.0 (22)</td>
<td>1.81 (26) 2.92 (13)</td>
</tr>
<tr>
<td>15</td>
<td>Field</td>
<td>30 May 1996</td>
<td>Messire</td>
<td>No</td>
<td>No</td>
<td>76</td>
<td>22.3 (22) 25.3 (25)</td>
<td>1.43 (33) 2.69 (11)</td>
</tr>
</tbody>
</table>

VP, vegetative period: from emergence to beginning of flowering; RP, reproductive period: from beginning of flowering to cessation of leaf expansion. *Plant population, number of plants m$^{-2}$ at beginning of flowering.
normally; and (2) a flower-removing treatment, where newly opening flowers were removed every second day. In expt 2, all plants were used as the ‘control’ of expt 1. Pots were smaller than those of expt 1 and plant density was high (approx. 120 plants m\(^{-2}\) at flowering, compared to 50 plants m\(^{-2}\) in expt 1). Lower growth rates were thus induced through higher competition for light (Table 1).

Field experiments were conducted near Montpellier (France) on a sandy loam soil (fluvio-calcic Cambissol). Peas, cv Messire (expts 3 and 4) and cv Alex, Baccara, Messire and Solara (expt 5), were sown at a density of 80 seeds m\(^{-2}\) and a 0.25 m row spacing on 4.5 × 4.0 m (expts 3 and 4) or 4.0 × 3.5 m (expt 5) plots. Sowing dates are indicated in Table 1. All plants were treated as the ‘control’ of expt 1, except that branching was allowed to occur normally. Due to genotypic differences, the number of stems varied between 72 (cv Alex, sowing date 1) and 158 (cv Baccara, sowing date 1). Seeds of cv Solara were unavailable for the first 1996 sowing date, and plots of cv Baccara and Solara at the latest sowing date were removed after emergence because of low plant density.

### Measurements

Air and soil (5 cm depth) temperatures were continuously recorded with thermistor probes connected to a data logger. Cumulated degree-days after emergence were calculated by daily integration of air temperature and a base of 3 °C (see below). Cumulated degree-days before emergence were calculated using soil temperature (5 cm depth).

Six plants per treatment in expts 1, 2 and 5 (12 in expts 3 and 4) were harvested twice a week. The total number of unfolded leaves on the main stem, or ‘nodal stage’, was counted according to Maurer et al. (1966). During its development a pea plant has a number of fully unfolded leaves and one visible partially unfolded leaf. Younger leaves are enclosed inside the stipules of the unfolding leaf, forming the apical bud. The so-called nodal stage considers only the visible leaves and is equal to the number of fully unfolded leaves plus a decimal notation (from 0.1 to 1.0) for the unfolding leaf according to the progress of leaflet and tendril unfolding and emergence above the stipules (Maurer et al., 1966).

The number of initiated leaf primordia was determined on the main stem by dissection of the shoot tip under a microscope (×10–×80). Time zero of initiation of leaf primordium \(n\) was determined by an increase in height of the meristematic dome above the last visible leaf primordium \(n − 1\) (Nougarède and Rondet, 1973b). Leaf primordium at time zero of initiation was included in the counting. Two intermediate stages, denoted \(n + 0.3\) and \(n + 0.6\), were defined between initiation of leaf primordia \(n\) and \(n + 1\) according to the size of leaf primordium \(n\). The first two basal organs above the cotyledonary node, corresponding to vestigial leaves, were not taken into account either for the number of leaf primordia nor for the number of unfolded leaves. The dry weight of aerial parts was measured on the same six (or 12) plants in expts 1 to 4 after drying for 48 h at 80 °C. In expt 5, additional plants were harvested once to three times weekly on 0.5 m\(^{2}\) to measure total aerial dry matter accumulation. The number of unfolded leaves (Maurer et al., 1966) was counted once a week on the main stem of all harvested plants.

### Determination of the number of fully expanded leaves

The number of fully expanded leaves was only directly measured in expt 1. Stipule and leaflet length of each growing leaf was measured daily with a rule on one marked plant per pot. Stipule and leaflet area have been shown to be highly correlated to the square of their length (Lecoeur and Sinclair, 1996). A leaf was counted as fully expanded when its stipule and leaflet lengths ceased to increase. Measurements began when stipules of the eighth leaf emerged from the apical bud and ended when leaflets of the eighteenth leaf ceased to grow.

Because direct measurement was time consuming, an indirect method was developed to determine full leaf expansion based on the correspondence between the number of expanded leaves and the number of unfolded leaves. The nodal stage (Maurer et al., 1966) was systematically measured at the same time and on the same plants as stipule and leaflet length in expt 1. Stipule length and nodal stage were also simultaneously measured at five dates during the cycle on 24 plants of cultivars Messire and Solara in the first sowing date of expt 5.

In order to compare leaves of different size and nodal position, stipule area and nodal stage were expressed in relative units. Relative nodal stage (RNS) was equal to the difference between plant nodal stage and leaf nodal position. For a given leaf \(n\), RNS equalled \(-1\) when its leaflets or tendrils became visible out of the apical bud, \(0\) at full unfolding (when compound leaf \(n + 1\) emerged from the bud), and \(1\) when leaf \(n + 1\) was totally unfolded. Stipule area relative to its final value (RSA) was linearly related to relative nodal stage. All the data fitted into a common regression:

\[
\text{RSA} = 0.29 \text{RNS} + 0.70 r^2 = 0.934 \quad n = 520.
\]

Relative leaflet area (RLA) was related to relative nodal stage by the equation:

\[
\text{RLA} = 0.30 \text{RNS} + 0.53 r^2 = 0.955 \quad n = 650.
\]

Distinguishing between genotypes or leaf nodal positions did not improve the model. Calculations were made with Table Curve 2D software (Jandel Scientific Software GmbH, Erkrath, Germany).

The relative nodal stage at which stipules (resp. leaflets) reached full expansion was calculated as RNS when RSA (resp. RLA) equalled \(1\), i.e. RNS = \(1.0 \pm 0.4\) (resp. RNS = \(1.6 \pm 0.4\)) (confidence interval at \(P = 0.05\)). Those values of RNS corresponded to the mean difference between the number of leaves with fully expanded stipules (resp. leaflets) and the number of unfolded leaves. For the semi-leafless genotype Solara, full stipule expansion corresponded to full leaf expansion. For the leafed genotype Messire, full expansion of the whole leaf was slightly delayed after full stipule expansion because leaflets continued to grow for a
RESULTS

Number of initiated leaf primordia and expanded leaves against time

The change over time of the number of initiated leaf primordia and the number of expanded leaves in expt 1 are presented in Fig. 1A. Four initiated leaf primordia, besides the primordia of the two vestigial leaves, were present in the seed; the fifth was at time zero of initiation at sowing and began to develop after seed imbibition. The apex regularly produced new primordia at the same rate in the two treatments. When flowers were removed, the meristem continuously produced leaf primordia until the end of the experiment. On plants with developing pods, the meristem ceased to produce leaf primordia during the period of flowering.

The production of expanded leaves showed similar trends. Several leaves emerged from the apical bud after cessation of primordium initiation by the meristem. However, most of the leaf primordia present in the shoot tip when the meristem stopped did not develop as expanded leaves. Approximately eight leaf primordia remained undeveloped in the shoot tip after cessation of leaf emergence in all experiments (Table 2). On the other hand, when leaf primordia were continuously produced by the meristem due to removal of flowers, the number of developing leaves in the shoot tip was maintained above 13 until the end of the experiment (Table 2).

Co-ordination between primordium initiation and expanded leaf production

In Fig. 1B, the ratio between the rate of leaf primordium initiation and the rate of expanded leaf production, calculated over short periods (6–10 d) in expt 1, is plotted against time. Two phases had to be distinguished. During the first phase, the initiation of leaf primordia was higher than the production of expanded leaves, with a ratio of approx. 1.6. Thereafter, the two production rates remained more or less equal. This second phase extended until the end of the experiment for plants with flower removal. The ratio fell rapidly to zero about 60 d after planting for podded plants.

As a consequence the difference between the number of initiated leaf primordia and the number of expanded leaves, which equals the number of developing leaves, increased during the first phase to reach a plateau that was maintained during the second period (Fig. 2A). This number then decreased on podded plants because several leaves reached full expansion after cessation of primordium initiation. The same pattern occurred in all experiments. When the number of developing leaves was expressed against the number of expanded leaves (Fig. 2B), all data from the five experiments and four cultivars before the cessation of initiation fitted a common regression with an accuracy of ±0.6 leaf primordia, corresponding to the mean confidence interval of those measurements. The transition between phase 1 (leaf primordium initiation faster than expanded leaf production) and phase 2 (synchronism between the two processes) occurred when the number of expanded leaves reached six.

Fig. 1. A, Change with time of the numbers of initiated primordia (\(\nabla, \blacktriangle\)) and expanded leaves (\(\bigcirc, \bullet\)) in expt 1. Vertical bars correspond to confidence intervals at \(P = 0.05\). B, Change with time of the ratio of number of leaf primordia/number of expanded leaves produced during short periods (6–10 d). \(\bigcirc\), ‘Control’ plants; \(\bullet\), Plants with flowers removed. Vestigial leaves at the first two nodes were excluded from the analysis.

short time. Nevertheless, total leaf area reached more than 90% of its final value at RNS = 1.

In the following text the term ‘number of fully expanded leaves’ refers to the number of leaves with fully expanded stipules, in order to be consistent between leafed and semi-leafless cultivars; it was measured in expt 1 (Fig. 1), but calculated as the number of unfolded leaves (Maurer nodal stage) minus 1 at any given time in all other experiments. Cultivars Baccara and Alex were assumed to behave as Solara and Messire.
Tables 1. Number of undeveloped leaves present in the shoot tip above the last expanded leaf at the time of the cessation of meristem activity and at the end of the experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cultivar</th>
<th>Number of undeveloped leaves at the time of the cessation of meristem activity (confidence interval at ( P = 0.05 ))</th>
<th>Number of undeveloped leaves at the end of experiment (confidence interval at ( P = 0.05 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Messire</td>
<td>13.1 (0.2)</td>
<td>7.8 (0.3)</td>
</tr>
<tr>
<td></td>
<td>Messire (Flower removed)</td>
<td>13.4 (0.3)</td>
<td>13.2 (0.3)</td>
</tr>
<tr>
<td>2</td>
<td>Messire</td>
<td>13.6 (0.3)</td>
<td>8.6 (0.3)</td>
</tr>
<tr>
<td>3</td>
<td>Messire</td>
<td>13.2 (0.4)</td>
<td>8.6 (0.4)</td>
</tr>
<tr>
<td>4</td>
<td>Messire</td>
<td>13.1 (0.2)</td>
<td>7.8 (0.4)</td>
</tr>
<tr>
<td>5</td>
<td>Messire (Date 1)</td>
<td>13.1 (0.2)</td>
<td>7.8 (0.4)</td>
</tr>
</tbody>
</table>

Fig. 2. Number of developing leaves (calculated as the difference between the number of initiated leaf primordia, \( N_{PI} \), and the number of fully expanded leaves, \( N_{LE} \)) according to the number of expanded leaves. A. Data from ‘control’ plants (○) and plants with flowers removed (●) in exp 1. B. Data from all experiments. Cultivar Messire, in exp 1 (○), 2 (●), 3 (△), 4 (■) and 5 (■); cvs. Alex (▽), Baccara (□) and Solara (△) in exp 5. Equations of linear regressions were \( y = 10.0 + 0.6x (r^2 = 0.86) \) for \( x < 6 \), and \( y = 13.5 \) for \( x \geq 6 \).

Equations describing the difference between the number of initiated primordia \( (N_{PI}) \) and the number of expanded leaves \( (N_{LE}) \) were:

less than six expanded leaves:

\[ N_{PI} - N_{LE} = 0.6N_{LE} + 10.0 \quad (r^2 = 0.856) \]

i.e. \( N_{PI} = 1.6N_{LE} + 10.0 \) \hspace{1cm} (1)

more than six expanded leaves:

\[ N_{PI} - N_{LE} = 13.5 \]

i.e. \( N_{PI} = N_{LE} + 13.5 \) \hspace{1cm} (2)

Response of the production rates of leaf primordia and expanded leaves to temperature

The response of the production rates of leaf primordia and fully expanded leaves, calculated over short periods (6–10 d), to mean air temperature during these periods varied according to the two phases of the cycle mentioned above (Fig. 3). Considering each phase individually, the rate of leaf primordium initiation \( (R_{PI}) \) varied linearly with temperature \( (\theta) \) within the range of temperatures covered in the different experiments. Equations of linear regressions were:

\[ R_{PI1} = 0.0361\theta - 0.1067 \quad (r^2 = 0.920) \] \hspace{1cm} (3)

\[ R_{PI2} = 0.0263\theta - 0.0846 \quad (r^2 = 0.947) \] \hspace{1cm} (4)

for phase 1 (less than six expanded leaves), and 2 (more than six expanded leaves), respectively. The base temperature (x-intercept) was similar and close to 3 °C for the two phases.

The rate of production of expanded leaves \( (R_{LE}) \) was also linearly related to temperature with two different relationships during the two phases mentioned above (Fig. 3B). Linear regressions were:

\[ R_{LE1} = 0.0222\theta - 0.0690 \quad (r^2 = 0.895) \] \hspace{1cm} (5)

\[ R_{LE2} = 0.0264\theta - 0.0767 \quad (r^2 = 0.867) \] \hspace{1cm} (6)

for phase 1 and 2, respectively. Again base temperatures were close to 3 °C for both phases. Considering 3 °C as the common base temperature of the two processes and two phases, eqns (3) to (6) can be written as follows:

\[ R_{PI1} = R'_{PI1}(\theta - 3) \] \hspace{1cm} (3')

\[ R_{PI2} = R'_{PI2}(\theta - 3) \] \hspace{1cm} (4')

\[ R_{LE1} = R'_{LE1}(\theta - 3) \] \hspace{1cm} (5')

\[ R_{LE2} = R'_{LE2}(\theta - 3) \] \hspace{1cm} (6')

where \( R'_{PI1}, R'_{PI2}, R'_{LE1} \) and \( R'_{LE2} \) are the rates of primordium initiation and expanded leaf production during the two phases expressed on a thermal time basis, that is as numbers of organs produced per degree-day. Numerical values were 0.0362, 0.0258, 0.0222 and 0.0263 leaves per degree-day, respectively. The plastochrons (reciprocals of the regression
Fig. 3. Response of rates of primordium initiation (A) and expanded leaf production (B) to air temperature. Data from the five experiments and four cultivars Messire (○), Alex (▽, ▼), Baccara (□, ■) and Solara (△, ▲) are included. Open symbols refer to the beginning of the cycle, before there were six expanded leaves; closed symbols refer to the later part of the cycle. Regression equations were: \( y = 0.0361x - 0.1067 \) \((r^2 = 0.92)\) and \( y = 0.0263x - 0.0846 \) \((r^2 = 0.95)\) for primordium initiation rate (A); \( y = 0.0222x - 0.0690 \) \((r^2 = 0.89)\) and \( y = 0.0264x - 0.0767 \) \((r^2 = 0.87)\) for expanded leaf production rate (B), before and after six expanded leaves, respectively. The x-intercept of the lines corresponds to the base temperature, close to 3°C in all cases (3.0, 3.2, 3.1 and 2.9°C, respectively).

Fig. 4. Response of rates of primordium initiation to plant growth rate before (A) and after (B) six expanded leaves, and expanded leaf production before (C) and after (D) six expanded leaves. Symbols as in Fig. 3.
slopes $R_{PI1}^0$ and $R_{PI2}^0$ were $27.6 \pm 0.6$ and $38.7 \pm 1.1$ degree-days for the early and late part of the cycle, respectively. During phase 1, the phyllochron (reciprocal of $R_{PI1}^0$) was equal to $45.1 \pm 1.2$ degree-days, i.e. approx. 1.6 plastochrons in accordance with eqn (1). During the second phase, the phyllochron (reciprocal of $R_{PI2}^0$) was equal to $38.1 \pm 0.8$ degree-days and not significantly different from the plastochron.

**Response to plant growth rate**

The response of $R_{PI1}$ and $R_{PI2}$ to plant growth rate was analysed considering each phase separately. The rates of leaf primordium initiation and expanded leaf production (per degree-day) calculated over short periods were independent of plant growth rate (g dry matter degree-day$^{-1}$) calculated over the same period (Fig. 4). Although plant growth rates varied within a range larger than one to ten across sampling dates and treatments, no tendency was observed for primordium or leaf production rates to increase with plant growth rate (Fig. 4). On the other hand, within the range of plant growth rates common to the two phases (0.002 to 0.008 g dry matter degree-day$^{-1}$), mean values of $R_{PI1}$ (resp. $R_{PI2}$) of plants after six expanded leaves were significantly lower (resp. higher) than those of younger plants, despite same mean values of plant growth rate. So, values of $R_{PI1}$ and $R_{PI2}$ appeared to be linked with the phase of the cycle and not with plant growth rate.

**Relationships between numbers of initiated leaf primordia and expanded leaves, and cumulated degree-days**

Numbers of initiated leaf primordia and expanded leaves correlated with cumulated degree-days with two different linear regressions corresponding to the two phases (before and after six expanded leaves) mentioned above (Fig. 5A). Provided that the date when $N_{LE}$ equalled 0 was used as the thermal time origin, all the experimental data fitted into common regressions.

Equations for production of expanded leaves corresponding to the two phases are:

$$N_{LE1} = (R_{LE1}^0)(CDD) \quad (7)$$

$$N_{LE2} = R_{LE2}^0(CDD - CDD_s) + N_{LEa} \quad (8)$$

where CDD are the cumulated degree-days from the beginning of expanded leaf production, with a base of 3 °C, and CDD$_s$ and $N_{LEa}$ are values of CDD and $N_{PI}$ at the shift. $N_{LEa}$ is derived from eqns (1) and (2), and CDD$_s$ from eqn (7).

$$N_{LEa} = (13.5 - 10)/0.6$$

$$CDD_s = N_{LEa}/R_{LE1}^0.$$  

Including numerical values of eqns (5') and (6') in eqns (7) and (8):

$$N_{LE1} = 0.0222CDD \quad (9)$$

$$N_{LE2} = 0.0263CDD - 1.1. \quad (10)$$

The number of initiated primordia is then calculated using eqns (1), (2) and (3'):

$$N_{PI1} = 0.0362CDD + 10.0 \quad (11)$$

$$N_{PI2} = 0.0263CDD + 12.4. \quad (12)$$

For all cultivars in the study, $N_{PI1}$ equalled five at the beginning of the experiment when the meristem began to initiate new primordia after seed imbibition. $N_{PI2}$ at emergence ranged from 8.4 to 9.3, with an average value of 8.8, in accordance with Etévé and Derieux (1982) on other pea cultivars. The time intervals between seed imbibition, or emergence, and the beginning of expanded leaf production can be estimated by replacing $N_{PI1}$ with corresponding values in eqn (11) in order to include emergence or imbibition as CDD origin into eqns (9) to (12).

CDD (imbibition) = $(5 - 10)/0.0362 = -138.1$ degree-days

CDD (emergence) = $(8.8 - 10)/0.0362 = -33.1$ degree-days.
DISCUSSION

Stable response to temperature of production rates of leaf primordia and expanded leaves in a wide range of environmental conditions

As proposed for cereals by Rickman and Klepper (1995), leaf development in pea can be described by a diagrammatic representation as shown in Fig. 5. The change in the numbers of initiated leaf primordia and fully expanded leaves over time are represented as linear functions of cumulated degree-days. The same relationships remained valid across sowing dates and genotypes (Fig. 5), whatever the plant growth rate. In the absence of mineral or water stress, plant growth rate is linked with intercepted radiation. So, in a wide range of environmental conditions, the rates of production of primordia and expanded leaves were independent of the amount of radiation intercepted per plastochron or phylochron (Fig. 4). Foliar morphology of genotypes had no effect on those rates. It was simply necessary to add a delay of 0.6 phylochron in the fully expanded leaf stage to take into account the expansion of leaflets on leafed cultivars compared to semi-leafless ones. Reproductive development did not change the rhythms of primordium or leaf production. Removing the flowers induced prolonged production of primordia and expanded leaves compared with podded plants, but at the same rates.

Two successive phases during expanded leaf production

Two periods of leaf production were distinguished with different rates. During the first phase, primordium initiation was faster than expanded leaf production (Fig. 1B) and the number of developing leaves increased (Fig. 2). This behaviour is common among determinate species such as wheat (Kirby, 1990; Rickman and Klepper, 1995), maize (Warrington and Kanemasu, 1983; Zur, Reid and Hesketh, 1989) or sunflower (Yegappan et al., 1980). As a consequence the duration of leaf development increases with leaf nodal position (Fig. 5B). This could contribute to the increase in leaf area from the bottom to the mid-part of the stem, giving the vertical distribution of leaf area widespread among agronomic species (e.g. Dwyer et al., 1992 on maize; Lecoeur et al., 1995 on pea) adapted to rapidly maximizing radiation interception. During the second period, plastochron and phylochron were equal. The number of developing leaves inside the apical bud remained constant until the cessation of meristem activity. This steady-state of the apical bud has been observed on white clover, a typically indeterminate plant that maintains this pattern during several months (Belaygue et al., 1996). The succession of two patterns was probably linked with the indeterminate habit of pea. The shift between the two behaviours occurred when the number of developing leaves reached the critical value of the steady-state period. This value, and the number of expanded leaves at the shift, were unaffected across cultivars and treatments, and seemed to be genetically determined. Reproductive development did not seem to be involved, because the number of expanded leaves at flower initiation (first floral primordium visible under apical meristem) ranged from two in expts 3 and 4, to five in expt 4, whereas the number of expanded leaves at the shift remained unchanged. These results have now to be tested on a wider genetic background.

Decorrelation between leaf production rate and plant growth rate

Contrary to our results, Truong and Duthion (1993) mentioned a positive correlation between leaf production rate and plant growth rate of pea. But this apparent discrepancy could be due to different analyses of similar results. As a matter of fact, our conclusion is based on an analysis with two phases, evidenced by the change, over time, of the ratio $R_{\text{PI}}/R_{\text{LE}}$ and of the number of developing leaves (Figs 1B and 2). Without such data, we could also conclude with our results that the rate of expanded leaf production increased with plant growth rate; taking into account all our pre-flowering field data would lead to a significant positive correlation between leaf production rate and plant growth rate ($r^2 = 0.574$). This correlation, and that found by Truong and Duthion (1993), could be due to the similar global tendency of the two processes to increase with time more than a direct effect of biomass accumulation on leaf production rate. In standard field conditions, high growth rates and the later part of the cycle are always associated because of the increase of plant leaf area with time and, in most cases, of daily solar radiation. Our analysis with two distinct phases, and our results in a glasshouse with low plant growth rates during phase 2 associated with high leaf production rates (expt 2), allowed us to decorrelate the two phenomena (Fig. 4).

Co-ordination between primordium initiation and expanded leaf production

Our results indicate a strong correlation between the number of initiated primordia and the number of expanded leaves [Fig. 2 and eqns (1) and (2)], which remained unchanged across cultivars and experimental treatments, indicating a co-ordination between primordium initiation and expanded leaf production in a wide range of conditions. Such a co-ordination has been underlined in wheat, where phenological stages of the apex can be directly related to leaf appearance (Kirby, 1990). In our study, the two processes of leaf primordium initiation and expanded leaf production presented similar quantitative responses to air temperature: linear relationships with a common base temperature. The relationships remained stable in all our experiments during the same two phases of the cycle for the two processes. The co-ordination between the two processes appears as a consequence of those common responses to temperature. Cell expansion and cell division in the elongating zone of maize leaves appeared to be co-ordinated in the same way through common temperature responses (Ben Haj Salah and Tardieu, 1995). Production of leaf primordia is relevant to cell division, and that of expanded leaves depends on both cell division and expansion. Co-ordination between apex stages and leaf production observed in pea or wheat could be due to a common response to temperature of the basic processes of cell division and cell expansion.
CONCLUSIONS

Prediction of the chronology of leaf development

As shown in Fig. 5, the production of leaf primordia and expanded leaves on pea plants can be described as linear functions of thermal units. Two phases, with different production rates, had to be considered. Because of the stability of the responses to air temperature, only mean daily air temperatures were necessary to construct the diagram. The dates of the beginning (primordium initiation) and end (full expansion) of development of individual leaves according to their nodal position, are deduced from this diagram (Rickman and Klepper, 1995) (Fig. 5B). Relationships remained valid for cultivars differing in foliation morphology.

Calculation of phytomer age from visual stages

The co-ordination between primordium initiation and expanded leaf production allowed us to estimate accurately the number of initiated primordia from the number of expanded leaves (Fig. 2B). Because the latter was directly related to the nodal stage of Maurer et al. (1966), the simple measurement of that visual stage is sufficient to calculate the numbers of initiated primordia and expanded leaves on the plant. Using Fig. 5B, it is then possible to calculate precisely the age of each developing leaf, according to its nodal position, as the thermal time from its initiation.

These results supply a powerful framework, both in the analysis and the prediction of leaf growth responses to the environment. Knowing phytomer age at any given date is crucial in analysing the effect of environment on leaf growth through cell number and cell size (Lecoeur et al., 1995). Coupled with a quantitative model of leaf area expansion in response to the environment, the present model is an effective way of predicting the temporal development and the final area of all individual leaves on pea plants, with required inputs reduced to daily climatic variables (Lecoeur et al., 1996).

ACKNOWLEDGEMENTS

We thank L. Guilioni, S. Combaud and E. Pic for their help in data collection; P. Naudin for technical assistance and Pr J. Wery for fruitful discussions. This work was partly supported by the European Union Agro-Industry-Research Programme (contract AIR3-CT92-0279).

LITERATURE CITED


