Analysis of leaf appearance, leaf death and phoma leaf spot, caused by *Leptosphaeria maculans*, on oilseed rape (*Brassica napus*) cultivars

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Abstract

Development of phoma leaf spot (caused by *Leptosphaeria maculans*) on winter oilseed rape (canola, *Brassica napus*) was assessed in two experiments at Rothamsted in successive years (2003-2004 and 2004-2005 growing seasons). Both experiments compared oilseed rape cultivars Eurol, Darmor, Canberra and Lipton, which differ in their resistance to *L. maculans*. Data were analysed to describe disease development in terms of increasing numbers of leaves affected over thermal time from sowing. The cultivars showed similar patterns of leaf spot development in the 2003-2004 experiment when inoculum concentration was relatively low (up to 133 ascospores m^{-3} air), Darmor developing 5.3 diseased leaves per plant by 5 May 2004, Canberra 6.6, Eurol 6.8 and Lipton 7.5. Inoculum concentration was up to 7-fold greater in 2004-2005, with Eurol and Darmor developing 2.4 diseased leaves per plant by 16 February 2005, whereas Lipton and Canberra developed 2.8 and 3.0 diseased leaves, respectively. Based on three defined periods of crop development, a piece-wise linear statistical model was applied to progress of the leaf spot disease (cumulative diseased leaves) in relation to appearance ('birth') and death of leaves for individual plants of each cultivar. Estimates of the thermal time from sowing until appearance of the first leaf or death of the first leaf, the rate of increase in number of diseased leaves and the area under the disease progress line (AUDPL) for the first time period were made. In 2004-2005 Canberra (1025 leaves × °C d) and Lipton (879) had greater AUDPL values than Eurol (427) and Darmor (598). For Darmor and Lipton the severity of leaf spotting could be related to the severity of stem canker at harvest. Eurol had less leaf spotting but severe stem canker, whereas Canberra had more leaf spotting but less severe canker.

Keywords Disease assessment; epidemic development; multiple responses; phoma stem canker; repeated measures; statistical model.

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Introduction

To aid understanding of the factors affecting severity of foliar disease epidemics in arable crops, it can be helpful to describe their progress using models (e.g. Evans *et al.* 2008; Lovell *et. al.*, 2004b; Papastamati *et al.* 2002). Models can also be used to assess effectiveness of different treatments (e.g. crop cultivar resistance, fungicide regime) and so help to make recommendations for disease control. To model temporal progress of non-biotrophic foliar diseases, it is necessary to accommodate production of new, healthy leaves, the development of disease symptoms on leaves after infection, and death of healthy or diseased leaves at different rates (Fig. 1). The corresponding data collected may be numbers of healthy, diseased and dead leaves, assessed on plants sampled from crops.

(Fig. 1 near here)

Phoma leaf spot (*Leptosphaeria maculans*) is an example of a non-biotrophic foliar disease whose dynamics can be studied on different oilseed rape cultivars to assess their influence on disease development. For *L. maculans*, the symptoms on leaves of young oilseed rape plants at the rosette stage of growth are spots initiated by air-borne ascospore inoculum produced on the diseased debris of previous crops (Fig. 2). The development of leaf spots (one or more spots per leaf) is followed by a period of asymptomatic, systemic growth along the leaf petiole to the stem where the pathogen causes damaging cankers (Evans *et al.*, 2008; West *et al.*, 2001). In the UK, the disease is monocyclic, with one cycle per growing season and there is little evidence for secondary spread from leaf to leaf by splash-dispersed conidia (Fitt *et al.*, 2006a). However, the ascospores that initiate leaf spots can be released from pseudothecia maturing consecutively in debris over a long period of time (Huang *et al.*, 2007). Thus the number of diseased leaves can increase at a steady rate, albeit dependent on occurrence of weather conditions favouring ascospore release and dispersal. However, the date of onset of leaf spotting in autumn can be predicted accurately by a weather-based model, without using ascospore data (Evans *et al.*, 2008).

(Fig. 2 near here)

L. maculans produces damaging epidemics of phoma stem canker in the oilseed rape crop world-wide (Fitt *et al.*, 2006a, 2008; West *et al.*, 2001), seriously affecting crop yield (Zhou *et al.*, 1999), with losses estimated at over US$1000M each growing season at a price of US$300 t^{-1} (Fitt *et al.*, 2008). Variability between different years (growing seasons) in weather conditions and concentrations of inoculum has a major influence on observed disease severity (Huang *et al.*, 2005). Cultivars of winter oilseed rape show a range of resistance/susceptibility to *L. maculans*, expressed both at the phoma leaf spot stage and during development of the stem canker stage of the disease. Husbandry of cultivars grown (Aubertot *et al.*, 2006; West & Fitt, 2005) and genes for resistance to *L. maculans* in these cultivars (Delourme *et al.*, 2004; Fitt *et al.*, 2006a; Rouxel *et al.*, 2003; Stachowiak *et al.*, 2006)

combine to enhance disease control. Management strategies include use of quantitative resistance that operates during colonisation of *B. napus* stems after leaf spots are observed (Huang *et al.*, 2009).

In autumn-sown ('winter') oilseed rape crops in the UK, from the seedling stage the plants develop to a rosette stage (GS 2,0, using the growth stage coding of Sylvester-Bradley, 1985) and the number of leaves then remains fairly constant ('birth' and 'death' rates of leaves being similar) over the winter period. Following this, in the spring the numbers of leaves on plants increase as they start stem extension (GS 2,1-2,5, Fig. 2). Crop growth and leaf production are largely temperature-dependent (Brisson *et al.*, 2003). Then the crop starts to flower and the number of leaves on the plant may increase a little more or start to decrease, depending on the characteristics of the particular cultivar.

With sufficient inoculum available, the number of diseased leaves (i.e. with spots) on a plant increases throughout the rosette stage of growth. This number may reach a maximum when the plant starts stem extension, because both diseased and symptomless leaves are dying and being replaced by new leaves. Using this information, modelling of the *L. maculans* leaf spot epidemic affords a description of the host-pathogen interaction and the influence of environment on disease progress (Evans *et al.*, 2008; Salam *et al.*, 2007; Steed *et al.*, 2007; Sun *et al.*, 2000). Therefore, to investigate the development of the phoma leaf spot stage of *L. maculans*, in two separate field experiments at Rothamsted, Harpenden, UK, individual plants of four different cultivars were monitored over a thermal time course (from sowing) and repeated assessments of the numbers of healthy, dead and diseased leaves were made to study the rates of increase in these numbers of leaves, as categorised for individual plants.

For describing progress of *L. maculans*, the symptoms may be assessed non-destructively on the same plants over time, so that the disease progress may be compared across a number of individual (replicate) plants for each treatment. A direct approach to modelling data may be taken (empirical statistical modelling) to find a parsimonious model (e.g. Evans *et al.*, 2008). Alternatively, more complex mechanistic mathematical models can be developed. However, such models could involve the indexing of many parameter values with known or estimated values from literature, and estimating only a few parameters from the fitting process, as for modelling light leaf spot (*Pyrenopeziza brassicae*, e.g. Papastamati *et al.*, 2002). It is important to consider development of the disease and growth of the host plant concurrently, to study how plants react to the pathogen in relation to a chosen measure of time. The problems of using actual time rather than some form of 'environment-time', such as thermal time, have been documented (Lovell *et al.*, 2004a). Specifically, the continuous monitoring of symptoms on individuals with respect to a time-scale based on accumulation of the factor influencing both plant growth and disease development gives the investigator a more appropriate assessment of the rate of disease progress. Furthermore, use of thermal time makes it easier to compare field trials at different stages in the growing season (Lovell *et al.*, 2004b). The type of disease under investigation will often determine the 'environment-time'

that should be used. Thermal time is appropriate for diseases such as wheat leaf blotch (*Septoria tritici*) and *L. maculans*, where development of spots is dependent on temperature (Toscano-Underwood *et al.*, 2001; Huang *et al.*, 2007; Lovell *et al.*, 2004b) if inoculum is present.

The aim of this work is to compare four oilseed rape cultivars within and across two growing seasons (years) by fitting a simple plant-specific statistical model to the numbers of different types of leaves to estimate parameters relating to the host-pathogen interaction.

Methods

Data for development of phoma leaf spot over thermal time

To study the development of phoma leaf spotting throughout the growing season, two winter oilseed rape field experiments were done at Rothamsted, in the 2003-2004 (Experiment 1) and 2004-2005 growing seasons (Experiment 2) (Pirie, 2007). In both seasons, cultivars Eurol, Darmor, Canberra and Lipton were grown. Seeds were hand-sown in single row plots (2 m by 25 cm) on 12 or 17 September 2003 or 3 September 2004, as part of larger experiments (including more cultivars) using a randomised block design with three blocks. These four cultivars were selected for study because of differences in their 'field' resistance (polygenic) to *Leptosphaeria maculans* in the UK national recommended list (www.hgca.co.uk). Recommended list trials assess this resistance by recording the severity of stem canker just before harvest to produce a resistance scale ranging from 1 (susceptible) to 9 (resistant). Cultivars Canberra and Darmor were rated 7, and Eurol 5, whereas Lipton had a rating of 4. These cultivars also carry a range of major genes ('*R*' genes) conferring resistance to leaf infection by strains (races) of *L. maculans* with particular avirulent alleles (Rouxel *et al.*, 2003; Delourme *et al.*, 2006; Fitt *et al.*, 2006a; Stachowiak *et al.*, 2006). Canberra has resistance gene *Rlm1*, Darmor has *Rlm9*, Eurol has *Rlm2* and Lipton has *Rlm3*. However, the *L. maculans* population at Rothamsted is 100% virulent against *Rlm9*, *Rlm2* and *Rlm3* and 80% virulent against *Rlm1* (Stachowiak *et al.*, 2006).

In each season, rainfall (mm) and temperature (^{o}C) data were collected daily (from 0900 h GMT to 0900 h GMT the next day) by a synoptic weather station at Rothamsted situated approximately 1 km distant from the field. These data were recorded from sowing (12 September 2003 for Experiment 1 and 3 September 2004 for Experiment 2) and the mean daily temperature above 0^{o}C was accumulated over days as a measure of thermal time (°C d). The base temperature for growth of oilseed rape has been estimated as 4.5 °C (Gabrielle *et al.*, 1998) and there is evidence that the base temperature for *L. maculans* growth is not likely to be less than 0 °C (Biddulph *et al.*, 1999). The temperature data were recorded by a 107 thermistor probe (Campbell Scientific, Loughborough, UK) and rainfall by a 0.2 mm ARG100 tipping bucket rain gauge (Campbell Scientific, Loughborough, UK).

Stem base debris colonised by *L. maculans* from the previous season's oilseed rape crop (cv. Apex) at Rothamsted was spread around the field plots after plant emergence to provide inoculum to initiate phoma leaf spot development in both experiments. Release of *L. maculans* ascospores from debris collected from the same Rothamsted source was monitored using a Burkard seven-day recording volumetric spore sampler (Burkard Manufacturing, Rickmansworth, UK). Trays of the stem debris were placed around the spore sampler. The sampler has a vacuum pump that takes in air at a rate of 10 L min^{-1}, and the air-borne particles drawn in are impacted onto a wax (Vaseline)-coated Melinex tape attached to a drum that rotates at a speed of 2 mm h^{-1} past the opening of the sampler (Lacey & West, 2006), of width 14 mm. Drums were replaced at seven-day intervals. After exposure, each tape was divided into pieces of length 48 mm, each piece corresponding to collection of air spora over a 24 h period. Each piece was mounted onto a microscope slide and stained with 0.1% trypan blue in lactophenol (w/v). This slide was examined under a light microscope (250× magnification) with field diameter 0.88 mm; the numbers of spores along the length of the tape were counted in two longitudinal transects and the mean calculated. The mean number of spores was multiplied by a conversion factor of 2.09 m^{-3} to obtain a measurement of spores per cubic metre of air sampled (McCartney *et al.*, 1997). These data were recorded from 1 September 2003 to 1 May 2004 for Experiment 1 and from 1 August 2004 to 1 April 2005 for Experiment 2.

Ten plants randomly selected per plot (30 plants per cultivar) were marked, and each leaf was numbered on the underside in sequence as it appeared (leaf length *ca.* 3 cm) using a permanent marker pen. On each assessment date, the appearance ('birth') of each leaf, whether a leaf was diseased (with at least one phoma leaf spot caused by *L. maculans*) and the fall ('death') of each leaf was recorded. Only leaves with spots caused by *L. maculans*, rather than due to any other foliar pathogen such as the related *L. biglobosa*, were counted. As well as counts of numbers of leaves, the numbers of angular, grey spots (lesions) produced on leaves by *L. maculans* were counted weekly. A given leaf could belong to only one category (healthy, diseased or dead), but healthy and diseased leaves could both move into the dead leaves category. The total number of leaves was the sum of healthy, diseased and dead leaves. To illustrate the development of the disease, cumulative numbers of diseased leaves were calculated. The number of cumulative diseased leaves is the running total of the number of infections (one per leaf) on a plant by the disease. Modelling the accumulation of disease enables comparison of cultivars in terms of their resistance to leaf spotting over (thermal) time. Assessments were done weekly in autumn/winter and monthly in spring. Since phoma leaf spot is a monocyclic disease, with little secondary disease spread in the UK, it is unlikely that the act of assessing plants influenced the progress of the disease. There were 16 assessments for Experiment 1 (from 2 December 2003 to 5 May 2004) and 11 assessments for Experiment 2 (from 14 October 2004 to 16 February 2005). There were fewer assessments for Experiment 2 due to constraints on resources. The experiments received no fungicide treatments. They were combine harvested (no yields taken) on 27 July 2004 and 30 July 2005, respectively.

Procedure for statistical modelling

Statistical modelling of the data was done to allow simultaneous assessment of differences between cultivars in terms of plant growth and disease progress. As the experiment was repeated in successive years, it was also possible to compare cultivars within and between growing seasons. A two stage modelling approach was used. Firstly, each plant was modelled separately, using all three variates (numbers of healthy, cumulative diseased and cumulative dead leaves) together. Secondly, the sets of estimated parameters were analysed (Mead *et al.*, 1993). Rather than considering the death of diseased and healthy leaves separately, the two rates (as proposed in Fig. 1) are combined as a common rate of leaf death for the present modelling. In the first stage of modelling, each variate within each plant was treated as piece-wise linear (i.e. a set of lines with break-points between them; Sprent, 1961). Models were fitted using least squares regression. Taking each experiment separately, a series of models was fitted, beginning with a 'maximal' model that allowed separate thermal time break-points for all three variates. Successive models combined break-points and rate parameters (within thermal time periods), as appropriate both for the development of the model in terms of the biological information and for producing a statistically acceptable (parsimonious) representation of the data. Assessment of the best model for all individuals was done using the F-test.

The cumulative total number of leaves (healthy plus diseased and dead leaves) over thermal time was analysed separately using ordinary linear fits for each plant with subsequent analysis of sets of estimated parameters. Therefore the same modelling approach was used, firstly to estimate the predicted thermal time from sowing to the (theoretical) appearance of the first leaf and the rate of leaf production, and then to analyse the sets of these two estimated parameters for the comparison of cultivars. For each plant, the first of these parameters is the thermal time from sowing until the regression line through cumulative total leaves crosses the thermal time axis (when number of leaves is zero). This is an extrapolative estimate, outside the thermal time range of the data, so inference should be made with caution.

The piece-wise linear models (the first stage of modelling) were constructed and fitted using GenStat® (2007) with reference to Payne *et al.* (2007). The sets of estimated parameters were analysed (the second stage of modelling) by using the method of Residual Maximum Likelihood (REML) (Patterson & Thompson, 1971) to take account of the design structure and provide predicted means without the influence of missing plants from blocks. When significant differences ($P < 0.05$) between cultivars were found, these were investigated using approximate t-tests on the appropriate degrees of freedom from the REML model.

Results

Development of phoma leaf spot epidemic

The pattern of changes in healthy, cumulative diseased and cumulative dead leaves observed in plants was generally divided into three periods of thermal time (Table 1), although in 2004-2005 data were observed in only the first two periods. These three periods of thermal time correspond approximately to the rosette, stem extension and flower bud development growth stages of winter oilseed rape crops (Sylvester-Bradley, 1985; Table 1). In 2003-2004, air-borne *Leptosphaeria maculans* ascospores were first detected in large numbers in early December 2003 (Fig. 3a), following a period of very dry summer weather that delayed ascospore maturation, with total rainfall from August to October being only 55.2 mm. This corresponded to the time of onset of phoma leaf spots in December. The period of ascospore release lasted until mid-March 2004, and during this period changes in the total number of phoma leaf spots per plant (mean of the four cultivars) reflected the pattern of ascospore release. In 2004-2005, ascospore release began much earlier, in October 2004 (Fig. 3c), and reached a maximum in early November with inoculum concentration generally being (up to seven-fold) greater than in 2003-2004. There was greater autumn rainfall in 2004-2005, with total rainfall from August to October 2004 being 264.0 mm. The changes in numbers of leaf spots per plant followed a similar pattern to the previous growing season. In both growing seasons, it is noted that the first leaf spots are observed before or around the same time as the first detection of airborne ascospores. As the spore sampler was sited approximately 1 km away from the site of the experiment, inoculum other than that collected by the spore sampler would have been encountered by the experimental crop.

(Table 1, Figure 3 around here)

The relationships between the mean numbers of healthy, cumulative diseased and cumulative dead leaves for each cultivar and thermal time are shown in Fig. 4. The rate of increase in mean cumulative diseased leaves was greater for all cultivars in 2003-2004 than in 2004-2005. The cultivars showed similar patterns of leaf spot development in the 2003-2004 experiment when inoculum concentration was relatively low, with Darmor developing 5.3 diseased leaves per plant by 5 May 2004, Canberra 6.6, Eurol 6.8 and Lipton 7.5. In 2004-2005 Eurol and Darmor developed 2.4 diseased leaves per plant by 16 February 2005, whereas Lipton and Canberra developed 2.8 and 3.0 diseased leaves, respectively. Mean total numbers of leaves showed a more rapid increase for Canberra and Lipton than for Eurol and Darmor. Although three (two) periods of growth could be distinguished in 2003-2004 (2004-2005) in plots of the data for individual plants, as can be seen in eight plants selected at random (one for each cultivar in each experiment, Fig. 5), mean numbers of healthy and cumulative diseased leaves (Fig. 4) obscured the changes in individual plants. Therefore, differences between individual plants in the pattern over thermal time for each variate, and inter-relationships between variates, were investigated to obtain a common, parsimonious model for all plants for each experiment separately (as the plants were not assessed beyond 1388 °C d after sowing in 2004-2005).

(Figures 4 and 5 around here)

Results of statistical modelling of phoma leaf spot progress

Thermal-time break-points for the regression lines can be related to the starts of the three stages of crop development over the thermal time course (Table 1, Fig. 2). Although there was a period of growth from the seedling to rosette stage (before 500 °C d, GS 1,0-1,5), data were not collected on plants at this time. Examples for individual plants in 2003-2004 (Fig. 5a-d) and 2004-2005 (Fig. 5e-h) suggest that the disease progress can be divided into periods relating to the stages of development of the oilseed rape crop. The cumulative numbers of diseased and dead leaves increased with plateaux indicating that there were periods when leaves were not dying and new leaves were not developing phoma leaf spot symptoms. Data from all plants in 2003-2004, when assessments stopped in May 2004, suggested that the phoma leaf spot epidemic could be divided into three distinct periods, but data from 2004-2005 could be divided into only two periods because assessments stopped in February 2005 (Fig. 5b, d) at 1388 °C d (Fig. 2).

To illustrate the results of the modelling procedure, the outcome for Experiment 1 (2003-2004) is described. For each plant separately, the variate modelled was the stacked response of live leaves, cumulative diseased leaves and cumulative dead leaves over thermal time, using an indicator variable to denote type of leaves. A 'maximal' model for each plant was produced, with 15 parameters denoting the thermal time break-points between periods, the rates of increase (or decrease) in numbers of leaves in each thermal time period and the number of healthy leaves in the first period. The parameters in this model were:

Thermal time break-points: tt_{1d}, tt_{1di}, tt_{2h}, tt_{2d}, tt_{2di}, tt_{3h}, tt_{3d}, tt_{3di}

Rates: b_{1d}, b_{1di}, b_{2h}, b_{3h}, b_{3d}, b_{3di}

Plateau: h_1

where parameter *tt* is a thermal time break-point, *b* a rate of change in leaf numbers, and *h* is a plateau for healthy leaves. The subscript numbers *1*, *2* and *3* indicate the thermal time period to which the parameter refers for *b* and *h* parameters, and to the start of the period for *tt* parameters (cf. Table 1); *h* is number of healthy leaves, *di* is cumulative number of diseased leaves and *d* is cumulative number of dead leaves. Thus, for example, the parameters tt_{1di} and tt_{1d} are the respective thermal times when accumulation of diseased and dead leaves started.

Consecutively simpler models were then fitted, to determine which thermal time break-points could be combined across the three periods, using the F-test to compare the nested models. Only 11% of plants required within-plant tt_{1d} and tt_{1di} to be estimated separately, so a common tt_1 was estimated for plants. Furthermore, only 13% of plants required tt_{2h} and tt_{2d} parameters to be estimated separately within plants, whereas 30% of plants required tt_{2di} to be estimated separately. Hence, a common tt_2 was estimated for healthy and cumulative dead leaves but separate tt_{2di} parameters were retained for cumulative diseased leaves. As only 5% of plants required separate tt_{3h}, tt_{3d} and tt_{3di} parameters, a

common tt_3 parameter was estimated for each plant. Since it was not possible to combine any of the rates of leaf production (b parameters) across the variates within the thermal time periods, the best model for Experiment 1 data had parameters (see Fig. 5a-d):

Thermal time break-points: tt_1, tt_2, tt_{2di}, tt_3

Rates: b_{1d}, b_{1di}, b_{2h}, b_{3h}, b_{3d}, b_{3di}

Plateau: h_1

The equation of this best model was:

$$y(tt) = \begin{cases} \left.\begin{array}{ll} h_1 & tt \le tt_2 \\ h_1 + b_{2h}(tt - tt_2) & tt_2 < tt \le tt_3 \\ h_1 + b_{2h}(tt_3 - tt_2) + b_{3h}(tt - tt_3) & tt > tt_3 \end{array}\right\} \textit{healthy} \\ \left.\begin{array}{ll} 0 & tt \le tt_1 \\ b_{1d}(tt - tt_1) & tt_1 < tt \le tt_2 \\ b_{1d}(tt_2 - tt_1) & tt_2 < tt \le tt_3 \\ b_{1d}(tt_2 - tt_1) + b_{3d}(tt - tt_3) & tt > tt_3 \end{array}\right\} \textit{cumulative dead} \\ \left.\begin{array}{ll} 0 & tt < tt_1 \\ b_{1di}(tt - tt_1) & tt_1 < tt \le tt_{2di} \\ b_{1di}(tt_{2di} - tt_1) & tt_{2di} < tt \le tt_3 \\ b_{1di}(tt_{2di} - tt_1) + b_{3di}(tt - tt_3) & tt > tt_3 \end{array}\right\} \textit{cumulative diseased} \end{cases}$$

where the response variate (y) is given by the (stacked) numbers of healthy, cumulative dead and cumulative diseased leaves at observed values of thermal time. The model fitted the data for each plant well, except when recorded data ended abruptly as a result of premature plant death caused by factors other than the disease (e.g. grazing by animals or birds). Therefore, six, one, seven and five plants were omitted from the analysis for cvs Eurol, Canberra, Lipton and Darmor, respectively. Plant death was not related to position in the design.

Further sets of parameters were calculated from those estimated. These were:

$dislag = tt_2 - tt_{2di}$ — *Disease lag*: difference between thermal times when healthy leaves start to increase and when cumulative diseased leaves reach a plateau.

$$ttdisplat = \begin{cases} (tt_3 - tt_2) & tt_2 \ge tt_{2di} \\ (tt_3 - tt_{2di}) & tt_2 < tt_{2di} \end{cases}$$

Thermal time disease plateau: thermal time for which healthy leaves increase whilst cumulative diseased leaves remain constant.

$areadis = 0.5b_{1di}(tt_{2di} - tt_1)^2$ *Area of disease*: area under the disease progress line (AUDPL) for cumulative number of diseased leaves before it reaches a plateau.

A similar modelling procedure was done for Experiment 2 (2004-2005), where the individual plant model (Fig. 5e-h) differed in that the third period of crop growth was not assessed and the increase in cumulative dead leaves was consistently linear for the majority of plants, because either it did not reach the plateau or else presented insufficient observations to detect it statistically. In this case, five, six, seven and five plants were omitted from the analysis for cvs Eurol, Canberra, Lipton and Darmor respectively, because the data ended prematurely. Using the F-test, separate tt_1 parameters were required for cumulative diseased and dead leaves for 26% of the plants, but there was a common tt_2 parameter for healthy and cumulative diseased leaves implying no disease lag. No further reduction in the number of parameters was possible. As there were no data on the third period of growth, the *ttdisplat* parameter was calculated as $1388 - tt_2$, this being the thermal time at the final observation of plants minus the thermal time when healthy leaves began to increase and cumulative diseased leaves reached a plateau. A full explanation of all the parameters in the models used for both experiments is given in Table 2 and some are illustrated in Figure 2.

(Table 2 near here)

For both experiments, inspection of residuals showed that the assumptions of the analysis were satisfied. An assumption of Normality was made and this was found to be acceptable, without transformation. It was subsequently found that estimated parameters did not vary greatly for plants within cultivars and that the conclusion about which plant-specific model to use for each experiment remained the same when a Poisson distribution was used, so the results obtained using the Normal distribution were retained. Furthermore, for each experiment the residual mean squares (s^2 values) of the final model applied to all plants were assessed using the REML method. From this analysis, no problem of variability in the fit across blocks and no significant differences ($P > 0.150$) between cultivars were observed, so the plant-to-plant variability for the fit of the model was acceptable. The average R^2 value for plants in 2003-2004 was 89% (range 74 – 98%) whereas in 2004-2005 it was 94% (range 82 – 98%).

Analysis of estimated parameters from the model of phoma leaf spot progress

The REML predicted mean values for each parameter, derived from the best model for each experiment, and the resulting calculated parameters differed between cultivars (Table 3). The simplest measure of disease was the cumulative number of diseased leaves, and comparison of parameters relating to this variable revealed significant differences ($P<0.05$) between cultivars in both experiments but particularly in 2004-2005. As an overall measure of epidemic severity in the rosette stage of crop growth, the area under the (fitted) disease progress line (AUDPL) in the first thermal

time period of the model (*areadis*) was calculated (Table 2, Fig. 2). Differences in this parameter between cultivars were statistically significant only in 2004-2005 (P = 0.006), with Canberra (1025 leaves × °C d) and Lipton (878.8 leaves × °C d) having most disease. However, the value for cv. Lipton was much greater than that for the other cultivars in 2003-2004 (706.9 leaves × °C d). In this experiment, the parameter tt_{2di}, the thermal time point at which the cumulative number of diseased leaves reached a plateau, did not differ significantly between cultivars (P = 0.521), although it was smaller for cv. Darmor.

(Table 3 near here)

The parameter *dislag* can be interpreted as the thermal time from when cumulative number of diseased leaves starts to remain constant (as proposed by the model) to when the number of healthy leaves starts to increase, or as the thermal time difference between when cumulative number of dead and cumulative number of diseased leaves reach their respective plateau (Table 2, Fig. 2). A large value for *dislag* indicates a long period of thermal time for which diseased leaves have stopped increasing whilst healthy leaves have not yet started to increase. This could indicate a degree of resistance to leaf spotting for a cultivar, albeit dependent on the rate of disease accumulation (b_{1di}) that had already occurred. By contrast, a small or negative *dislag* indicates that diseased leaves are still being accumulated until or after the thermal time when the number of healthy leaves start to increase. In 2003-2004, there were significant differences (P = 0.017) in this parameter between cultivars and its value was greater for cv. Darmor (184 °C d), because the thermal time at which healthy leaves began to increase and cumulative dead leaves reached a plateau (tt_2) was later for this cultivar (1337 °C d) than the others. As a measure of disease severity, the maximum of the ratio of the cumulative number of diseased leaves to *cumulative* number of healthy leaves was 0.62 (Darmor), 0.66 (Lipton), 0.68 (Canberra) and 0.76 (Eurol). The accumulated thermal time at which the cumulative numbers of dead and diseased leaves began to increase again (tt_3) was later for cv. Darmor than for cvs Canberra or Eurol. Thus, at least in 2003-2004, the estimated parameters suggest there was more resistance to leaf spotting for Darmor.

The initial rate of increase in cumulative number of dead leaves (b_{1d}) was significantly different (P < 0.023) and greater for cv. Darmor than for cvs Canberra and Eurol, indicating that there was a greater rate of leaf shed for Darmor during the rosette stage of plant growth. The secondary rate of increase in cumulative number of dead leaves (b_{3d}) was significantly different (P < 0.001) and greater for cv. Lipton than the other cultivars. This second rate of increase in cumulative number of dead leaves was generally greater than the first rate. The first rate of increase in healthy leaves (b_{2h}) was greatest for cv. Eurol, followed by Lipton. The second rate of change for the number of healthy leaves (b_{3h}) was either an increase (cvs Canberra and Darmor) or a decrease (cvs Eurol and Lipton).

Analysis of the thermal time period for which the number of healthy leaves increased whilst numbers of cumulative diseased leaves were estimated to remain constant (*ttdisplat*), cv. Eurol had a significantly different (P < 0.018) and smaller value (58.6 °C d) than cvs Darmor (100.5 °C d) and

Lipton (114.1 °C d), indicating that there was less thermal time for new leaves of this cultivar to accumulate before further phoma leaf spots developed. Although the initial number of healthy leaves at the beginning of the assessment period was about four for all cultivars, it was significantly different ($P < 0.025$) and smaller for cv. Darmor than for cvs Canberra and Lipton.

In Experiment 2, in the 2004-2005 growing season when number of *L. maculans* ascospores was greater than in 2003-2004, the modelling showed that the pattern of disease progress over thermal time differed from 2003-2004, with different sets of thermal time break points being required for the different types of leaves. The thermal time at which the cumulative number of diseased leaves started to increase (tt_{1di}) was later than the thermal time that number of cumulative dead leaves began to increase (tt_1) for all cvs except Lipton. However, tt_{1di} was significantly different ($P < 0.005$) and greater for cvs Eurol and Darmor than for cvs Canberra and Lipton. The thermal time for which healthy leaves increased whilst cumulative diseased leaves were estimated to remain constant (*ttdisplat*) was greater for cvs Eurol and Darmor than for cvs Canberra and Lipton. Cultivar Lipton had a significantly different ($P = 0.013$) and greater tt_2 than cv. Eurol, and therefore accumulated diseased leaves for longer than cv. Eurol. Although the rates of increase in numbers of healthy leaves were similar for the cultivars in this experiment, the rate of increase in diseased leaves was greater for cv. Eurol than for cvs Canberra or Lipton. The number of healthy leaves at the start of assessments (h_1) was similar in this experiment to that observed in 2003-2004, with three to five leaves present. The area under the fitted line for cumulative diseased leaves (*areadis*) was significantly different ($P = 0.001$) and smaller for cv. Eurol (426.7 °C d) than for cv. Canberra (1025.1 °C d). The maximum of the ratio of the cumulative number of diseased leaves to cumulative number of healthy leaves was 0.32 (Darmor), 0.35 (Eurol), 0.42 (Canberra) and 0.54 (Lipton).

Total number of leaves

Changes in cumulative total leaves (healthy plus diseased and dead) from the experiments were modelled separately for each plant and increased linearly with thermal time for all plants. Thermal time to leaf appearance differed for all cultivars in both experiments, with Darmor having the shortest thermal time to leaf appearance in 2003-2004 (Table 4). Eurol, Darmor and Canberra had much shorter thermal times to leaf appearance in 2004-2005, with Canberra being significantly different ($P < 0.001$) from Lipton. This suggests that different environmental conditions in the two years may have affected these cultivars more than Lipton. The greatest difference in rates of leaf production between cultivars was in 2004-2005, with Canberra having a much smaller rate. The other cultivars had similar rates of leaf production in both experiments. Fig. 6 shows the increase in total leaf number for each cultivar in each experiment, using the REML predicted means, but also plotting the maximum and minimum possible rates of leaf production, given all the values of intercepts and slopes of the fitted linear regression models. These were used instead of confidence intervals because of the plant-specific method of modelling; although this is extrapolative and may produce unlikely

patterns of plant growth, it illustrates the variability that may be encountered. In particular, it shows the greater variability for Canberra than other cultivars in 2004-2005.

(Table 4 and Figure 6 near here)

Discussion

These results show how this two-stage modelling approach has been used to study differences in development of *Leptosphaeria maculans* epidemics on different oilseed rape cultivars growing in different seasons, and it is clear that this approach could be applied to data (numbers of leaves) from experiments studying foliar diseases with similar epidemiological characteristics. In 2003-2004, low summer rainfall did not favour seedling emergence or release of *L. maculans* ascospores in autumn 2003. This was reflected in the greater thermal time estimate for the start of leaf growth (Table 4) in this season. In 2004-2005, in autumn 2004 cv. Lipton established more slowly than the other cultivars. Huang *et al.* (2005) found that wetness provided by rainfall was particularly important for the release of ascospores. The variability in the estimated parameters from the model in both growing seasons shows how changing environmental factors affected cultivars differently. In 2003-2004, there was little rainfall from late February to early March 2004 (Fig. 3a), suggesting that this dry period (with low temperatures at this time) may partially explain the plateau observed in cumulative diseased leaves during stem extension, because such conditions would have made it difficult for the pathogen to infect leaves. However, in 2004-2005 there was less evidence of the importance of such an effect, as there was more rainfall during the stem extension growth stage in 2005 and yet the plateau in diseased leaves was still well-defined. This suggests the importance of the cultivar-specific rates of leaf production during stem extension allowing plants to 'keep ahead' of the disease.

The model describes the disease progress and plant growth simultaneously, and provides a good description of these two processes, along with parameters that can be compared across cultivars to consider how they differ within and across growing seasons (years). Counts of dead previously healthy, and dead previously diseased, leaves (Fig. 1) were not modelled separately, a common rate of death being estimated. Although an analysis using these separate variates would have yielded further information, it would not have differed in ability to compare cultivars. Previous modelling to study *L. maculans* has described the relationship between leaf spotting and the severity of the resulting stem canker (Sun *et al.*, 2000) or predicting the onset of ascospore release (Salam *et al.*, 2007) rather than the progress of leaf spotting. Rather than applying a complex mechanistic model, such as that developed by Papastamati *et al.* (2002) for the progress of light leaf spot, possibly involving many input parameters, a simple model based on the observed trends in the leaf number data that relate to the known stages of oilseed rape plant growth (Sylvester-Bradley, 1985) over thermal time is used. Other statistical modelling has focussed on predicting the severity of phoma stem canker in the future (Evans *et al.*, 2008) but does not address the interaction between leaf production and leaf spotting. A

different approach for analysis of these data would be to apply simple temporal models based on survival theory (Box-Steffensmeier & Jones, 2004) or to use a generalised linear mixed model (GLMM) (Gueorguieva, 2001). However, this latter approach could not easily be applied to these data as the variates were particularly complex over the thermal time course.

The current modelling avoids the problem of non-independence of observations by fitting data from individual plants and then analysing the sets of individual parameters (independent observations) as its second stage. Furthermore, it was possible to estimate 'hidden' parameters, such as the thermal time break-points that may relate to biologically important stages in crop growth or epidemic development; it is not possible to obtain such information if the data are analysed across all plants (Fig. 4). These results show that the rate of increase in numbers of leaves could be assumed to be linear with thermal time, which is acceptable (Trudgill *et al.*, 2005) when temperatures are not at the extremes for plant growth. Although observations were taken for a longer period of time in 2003-2004 than in 2004-2005, using *thermal* time allows the responses from the two growing seasons to be modelled on the same (thermal time) axis (cf. Lovell *et al.*, 2004b), to then compare the estimated parameters (Table 3) from the first two periods of development (Table 1). Although rainfall is an alternative additional explanatory variable to temperature, the explanation using a model based on thermal time only was acceptable. Incorporating rainfall to give a "developmental unit" (see, for example, Powers *et al.*, 2003) against which to model the data was therefore not considered. Similarly, the concentration of ascospores in the air was not used in the modelling.

Although presence of leaf spots indicates that infection has occurred, the extent of leaf spotting is not necessarily correlated to the extent of final stem canker disease (Sun *et al.*, 2000; Huang *et al.*, 2009). By harvest in the two growing seasons, stem canker was most severe on cvs Eurol (76% of plants with cankers in 2003-2004 and 98% in 2004-2005) and Lipton (79% and 91%), less severe on cv. Darmor (54% and 74%) and least severe on cv. Canberra (38% and 62%) (Pirie, 2007). Although the relative severities of leaf spotting and stem canker for cvs Darmor and Lipton were comparable, the low severity of leaf spotting on cv. Eurol did not relate to the severe stem canker observed on this cultivar and, conversely, there was more leaf spotting on cv. Canberra but low final stem canker severity. Differences between the cultivars in terms of plant growth were observed with Eurol and Lipton (on average) having a decrease in healthy leaves in the flower bud development period of growth. Such a response could relate to leaf-shed (Bashi *et al.*, 1983; Guyot *et al.*, 2001; van den Berg & van den Bosch, 2004) as a reaction to disease. In contrast, Canberra and Darmor had an increase in leaf production, which could also enhance disease-escape (Garcia-Guzman & Burdon, 1997; Lovell *et al.*, 1997). These responses were related to the inoculum concentration encountered by the cultivars, as Lipton had a greater rate of decrease in healthy leaf number than Eurol, which coincided with more disease being observed on Lipton. The severity of stem canker is independent of the severity of leaf spotting but there is a relationship between the timing of leaf spotting and the severity of canker (West *et al.*, 2001; Steed *et al.*, 2007). However, our results

1 suggest that there is some evidence that leaf retention could increase incidence of stem canker in
2 susceptible cultivars.
3

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Table 1 Winter oilseed rape crop growth stages, with approximate thermal time ranges, in relation to numbers of healthy, diseased and dead leaves and concentration of air-borne *Leptosphaeria maculans* ascospore inoculum for two experiments at Rothamsted, in the 2003-04 and 2004-05 growing seasons.

Thermal time range (°C d)[a]	**Crop growth stage (GS)**[b]	**Number of healthy leaves**	**Cumulative number of dead leaves**	**Cumulative number of diseased leaves**	**Inoculum concentration (spores m^{-3})**
500-1000	Rosette (2,0)	Roughly constant	Increasing	Increasing	High
1000-1500	Stem extension (2,0 – 2,5)	Increasing	Roughly constant (2003-04), increasing (2004-05)	Roughly constant[c]	Medium
1500-2000 (2003-04)	Flower bud development (3,0 – 3,7)	Increase/decrease	Increasing	Increasing[c]	Low

[a]Approximate ranges using 0 °C as the base temperature, thermal time is accumulated from sowing.

[b]Taken from Sylvester-Bradley (1985).

[c] New diseased leaves occurring at these stages do not usually contribute to the development of severe basal stem canker but do produce less damaging upper stem lesions (Sun *et al.,* 2000).

Table 2 Explanation of thermal time break-points (°C d, base temperature 0 °C), rates (leaves (°C d)$^{-1}$), and number of leaves (and other parameters calculated from these) from the three-part model (i.e. two break-points) of numbers of healthy leaves, dead leaves (cumulative) and diseased leaves (cumulative) fitted to data for individual plants of four winter oilseed rape cultivars in the 2003-04 (Experiment 1) growing season, and from the two-part model (i.e. one break-point) in the 2004-05 (Experiment 2) growing season at Rothamsted, for development of phoma leaf spotting (*Leptosphaeria maculans*) in relation to leaf birth and death on winter oilseed rape.

Parameter	Explanation
tt_1	Leaves start to die; and become diseased (2003-04).
tt_{1di}	Leaves start to become diseased (2004-05).
tt_2	Healthy leaves start to increase and cumulative number of dead leaves reaches a plateau; cumulative diseased leaves reach a plateau (2004-05).
tt_{2di}	Cumulative diseased leaves reach a plateau (2003-04).
tt_3	Cumulative dead and diseased leaves start to increase, healthy leaves start to increase or decrease (2003-04).
b_{1d}	Increase in cumulative dead leaves in the first period.
b_{1di}	Increase in cumulative diseased leaves in the first period.
b_{2h}	Increase in healthy leaves in the second period.
b_{3h}	Increase or decrease in healthy leaves in the third period (2003-04).
b_{3d}	Increase in cumulative dead leaves in the third period (2003-04).
b_{3di}	Increase in cumulative diseased leaves in the third period (2003-04).
h_1	Number of healthy leaves in the first period.
dislag	*Disease lag*: difference in thermal times when healthy leaves start to increase and when cumulative diseased leaves reach a plateau ($tt_2 - tt_{2di}$) (2003-04).
ttdisplat	*Thermal time disease plateau*: thermal time for which healthy leaves increase whilst cumulative diseased leaves remain constant: the difference between tt_3 and tt_2 when $tt_2 > tt_{2di}$ or the difference between tt_3 and tt_{2di} when $tt_2 < tt_{2di}$ (2003-04); difference between the thermal time at the last assessment and tt_2 (1388.2 – tt_2) (2004-05).
areadis	*Area of disease*: area under the disease progress line (AUDPL) for cumulative number of diseased leaves before it reaches a plateau ($0.5b_{1di}(tt_{2di} - tt_1)^2$) (2003-04); $0.5b_{1di}(tt_2 - tt_{1di})^2$ (2004-05).

Table 3 Predicted mean values of parameters from a REML analysis of thermal time break-points (°C d, base temperature 0 °C), rates (leaves (°C d)$^{-1}$), and number of leaves (and other parameters calculated from these) from the three-part model (i.e. two break-points) of numbers of healthy leaves, dead leaves (cumulative) and diseased leaves (cumulative) fitted to data for individual plants of four winter oilseed rape cultivars in the 2003-04 (Experiment 1) growing season, and from the two-part model (i.e. one break-point) in the 2004-05 (Experiment 2) growing season at Rothamsted describing development of phoma leaf spotting (*Leptosphaeria maculans*) in relation to leaf birth and death on winter oilseed rape.

Year	**2003-04**					**2004-05**				
Parameter[a]	**Darmor**	**Canberra**	**Eurol**	**Lipton**	**SED**[c] **(94 df)**	**Darmor**	**Canberra**	**Eurol**	**Lipton**	**SED**[c] **(76 df)**
tt_1	829.3	826.9	827.4	823.5	17.97	692.3	559.9	639.5	686.4	25.36[b]
tt_{1di}						819.5	638.5	879.6	672.5	84.32[b]
tt_2	1337	1276	1281	1279	19.7[b]	1189	1198	1142	1223	29.31[b]
tt_{2di}	1153	1184	1196	1182	29.8					
tt_3	1439	1371	1380	1411	24.3[b]					
b_{1d}	0.013	0.011	0.011	0.012	0.00068[b]	0.01	0.0099	0.0093	0.0095	0.00047
b_{1di}	0.012	0.0098	0.0086	0.011	0.0014	0.0091	0.0065	0.016	0.0060	0.0039[b]
b_{2h}	0.038	0.048	0.097	0.051	0.034	0.014	0.015	0.011	0.020	0.003
b_{3h}	0.0044	0.00021	-0.00076	-0.0023	0.0032					
b_{3d}	0.013	0.013	0.013	0.017	0.0010[b]					
b_{3di}	0.008	0.0083	0.0098	0.010	0.0013					
h_1	4.07	4.56	4.37	4.57	0.21[b]	4.89	4.71	5.2	3.25	0.5
dislag (°C d)	184	92.1	82.1	100.5	36.37[b]					
ttdisplat (°Cd)	100.5	86.3	58.6	114.1	17.62[b]	199.6	190.4	245.8	164.8	29.31[b]
areadis (leaves×°C d)	582.5	587	579.4	706.9	101.2	597.8	1025.1	426.7	878.8	238.1[b]

[a]see Table 2 for explanation of parameters.

[b]differences between cultivars significant ($P < 0.05$).

[c]Although REML provides a standard error of the difference (SED) for each pair of means, for convenience the average SEDs are presented. This is justified because the range of SEDs for a particular parameter was always small (< 15% of the average SED), suggesting that the imbalance caused by missing plants was within acceptable limits.

Table 4 Means of predicted thermal time from sowing to the appearance[a] of the first leaf for each plant [estimated using linear regression of total number of leaves (cumulative) against accumulated thermal time (°C d, base temperature 0 °C); this is the thermal time when the regression line crosses the thermal time axis (when number of leaves is 0)] and rate of increase in number of leaves per unit thermal time. Values in the table are predicted means from the REML analysis of the set of estimated values for this parameter, from analyses of data from individual plants of each of four winter oilseed rape cultivars (Darmor, Canberra, Eurol, Lipton). See Fig. 6 for mean lines of best fit using these values.

	Darmor	Canberra	Eurol	Lipton	SED	df
Thermal time from sowing to appearance of leaf 1 (°C d)						
2003-04	415.5	564.0	542.4	556.5	26.67[b]	103
2004-05	349.8	186.7	228.8	584.5	113.8[b]	97
Rate of increase in number of leaves per unit thermal time [(°C d)$^{-1}$]						
2003-04	0.013	0.015	0.014	0.016	0.00065[b]	103
2004-05	0.013	0.011	0.012	0.016	0.0019[b]	97

[a] appearance ('birth') of the leaf is defined as thermal time when it reached a length of approximately 3 cm.

[b] significant difference between cultivars ($P < 0.05$).

Figure Legends

Figure 1 Diagram illustrating the relationships between the processes of leaf birth (production of new healthy leaves), infection by a non-biotrophic pathogen (resulting in development of diseased leaves) and death of healthy or diseased leaves for a model of leaf dynamics in relation to development in time of a foliar disease epidemic.

Figure 2 Stages in the development of winter oilseed rape crops (rosette, stem extension, flower bud development) in relation to development of phoma leaf spot epidemics (release of air-borne ascospores, leaf infection through stomata, increase in number of leaf spots) and thermal time (from sowing in September,°C d, base temperature 0 °C) parameters used in describing them. For full details of parameters, see Table 2. Approximate thermal times for the start and end of growth stages are given. The last assessment for 2004-05 was at 1388 °C d.

Figure 3 Changes in observed mean number of phoma leaf spots (●) per plant on four cultivars of field-sown winter oilseed rape and numbers of air-borne *Leptosphaeria maculans* ascospores (—) detected by a Burkard spore sampler (spores m^{-3} air) in the 2003-04 (Experiment 1) (a) and 2004-05 (Experiment 2) (c) growing seasons at Rothamsted. Rainfall (mm) (bars) and average temperature (^{o}C) (—) at Rothamsted over the same period in 2003-04 (b) and 2004-05 (d). Ascospores were viewed with a light microscope on stained Melinex tapes recovered from the spore sampler (Lacey & West, 2006).

Figure 4 Development of phoma leaf spot (*Leptosphaeria maculans*) in relation to leaf birth and death on four cultivars of field-sown winter oilseed rape, Darmor (a, e), Canberra (b, f), Eurol (c, g) and Lipton (d, h), in the 2003-04 (Experiment 1) (a-d) and 2004-05 (Experiment 2) (e-h) growing seasons at Rothamsted. Observed data for mean numbers of healthy (○), diseased (cumulative, ∆), dead (cumulative, ●) and total leaves (cumulative, ▼) plotted against thermal time (°C d, base temperature 0 °C) from sowing. Numbers are means for 10 marked plants in each of three replicate plots (i.e. 30 plants). In Experiment 2, data were not recorded for cv. Lipton at the first five time points. Fitted data for total number of leaves are shown in Fig. 6.

Figure 5 Development of phoma leaf spot (*Leptosphaeria maculans*) in relation to leaf birth and death for cv. Darmor, block 2, plot 60, plant 10 in the 2003-04 (Experiment 1)

growing season (a), and block 2 plot 55, plant 8 in the 2004-05 (Experiment 2) growing season (e); cv. Canberra, block 3 plot 120, plant 6 in 2003-04 (b), and block 4, plot 124, plant 3 in 2004-05 (f); cv. Eurol, block 3, plot 124, plant 3 in 2003-04 (c) and block 2, plot 44, plant 3 in 2004-05 (g); cv. Lipton, block 3, plot 113, plant 6 in 2003-04 (d), and block 1, plot 48, plant 2 in 2004-05 (h); against thermal time (^{o}C d, base temperature 0°C) from sowing, as examples. Numbers of healthy (○, —), diseased (cumulative, Δ, ···), dead (cumulative, ●, - - -) leaves, showing how a piece-wise linear model fits the data. Parameters are number of healthy leaves at the start of monitoring (h_1), thermal times when leaves start to die (tt_1) or become diseased (tt_{1di}), when number of healthy leaves increases and cumulative number of dead leaves reaches a plateau (tt_2), when number of diseased leaves reaches a plateau (tt_{2di}), when numbers of dead and diseased leaves start to increase again after their plateaux (tt_3), rates of increase (or decrease) in numbers of leaves in relevant sections for numbers of healthy (b_{2h}, b_{3h}), cumulative dead (b_{1d}, b_{3d}) or cumulative diseased (b_{1di}, b_{3di}) leaves. Data were fitted by a model with two-break-points in 2003-04 (assessments from 2 December to 5 May) and by a model with one break-point in 2004-05 (assessments from 14 October to 16 February).

Figure 6 Means of fits for linear regressions for each plant of total number of leaves (cumulative) against thermal time (°C d, base temperature 0 °C) from sowing for cvs Darmor (a, e), Canberra (b, f), Eurol (c, g) and Lipton (d, h) in the 2003-04 (Experiment 1) (a-d) and 2004-05 (Experiment 2) (e-f) growing seasons, using mean parameter values in Table 4. Means of fits to individual plant data for all plants (—), maximum and minimum rates of increase in total number of leaves (- - -), given all the values of intercepts and slopes of the fitted linear regression models. These were used due to the plant-specific method of modelling. Observed data (means) are shown in Fig. 4 (▼). The estimated thermal time to appearance of the first leaf is the thermal time from sowing when the regression line for cumulative total leaves crosses the thermal time axis (when number of leaves is zero).

Figure 1

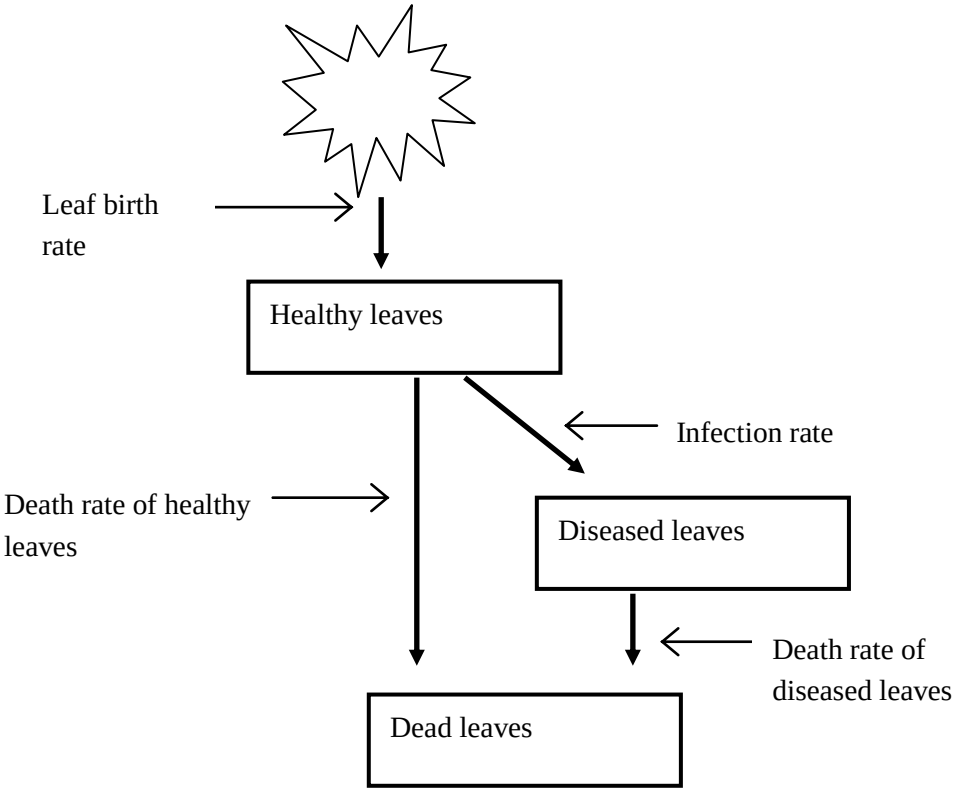

Leaf birth rate
Healthy leaves
Infection rate
Death rate of healthy leaves
Diseased leaves
Death rate of diseased leaves
Dead leaves

Figure 2

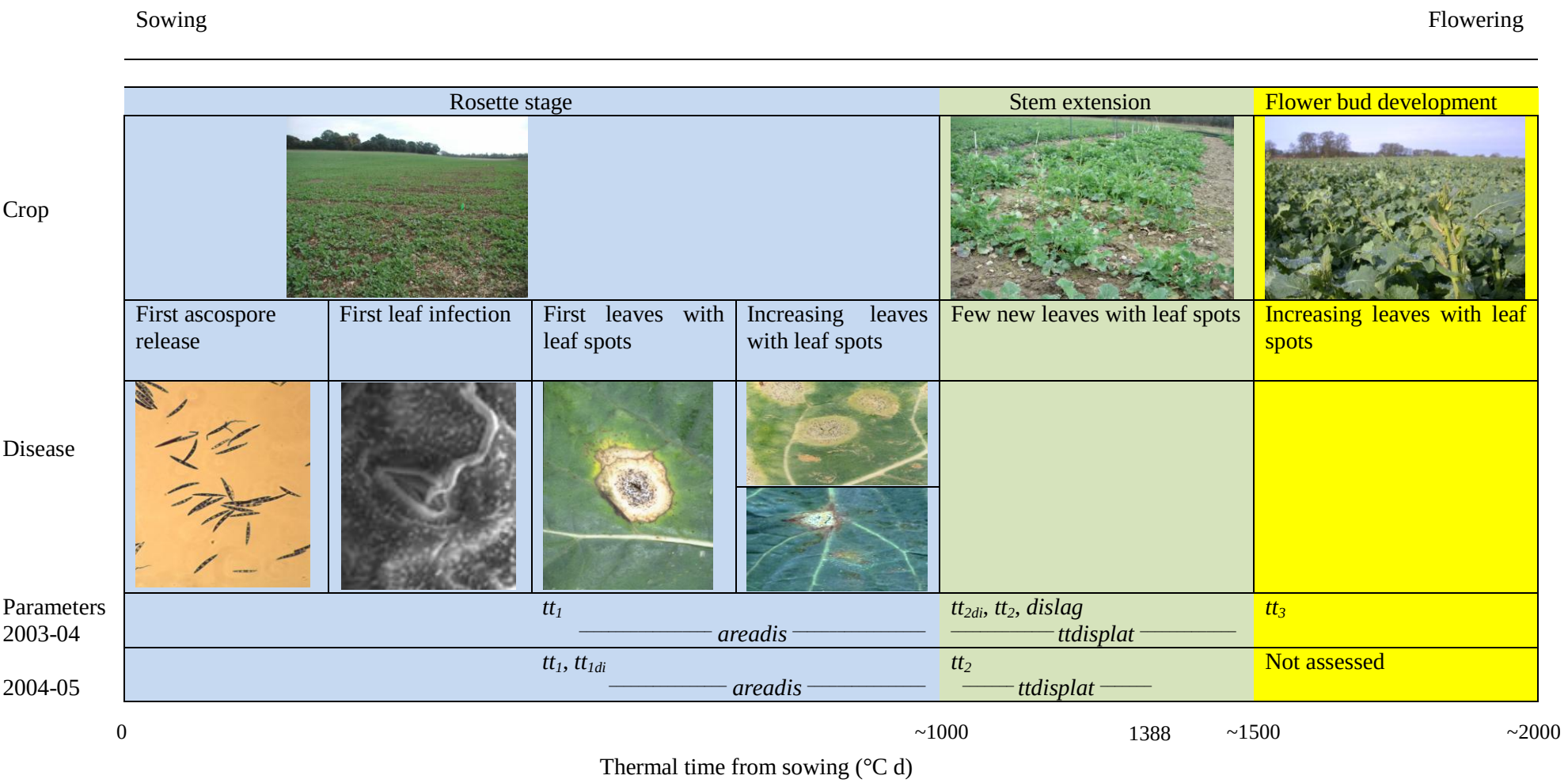

Sowing
Flowering
Rosette stage
Stem extension
Flower bud development
Crop
First ascospore release
First leaf infection
First leaves with leaf spots
Increasing leaves with leaf spots
Few new leaves with leaf spots
Increasing leaves with leaf spots
Disease
Parameters 2003-04
tt1
areadis
tt2di, tt2, dislag
ttdisplat
tt3
2004-05
tt1, tt1di
areadis
tt2
ttdisplat
Not assessed
0
~1000
1388
~1500
~2000
Thermal time from sowing (°C d)

Figure 3

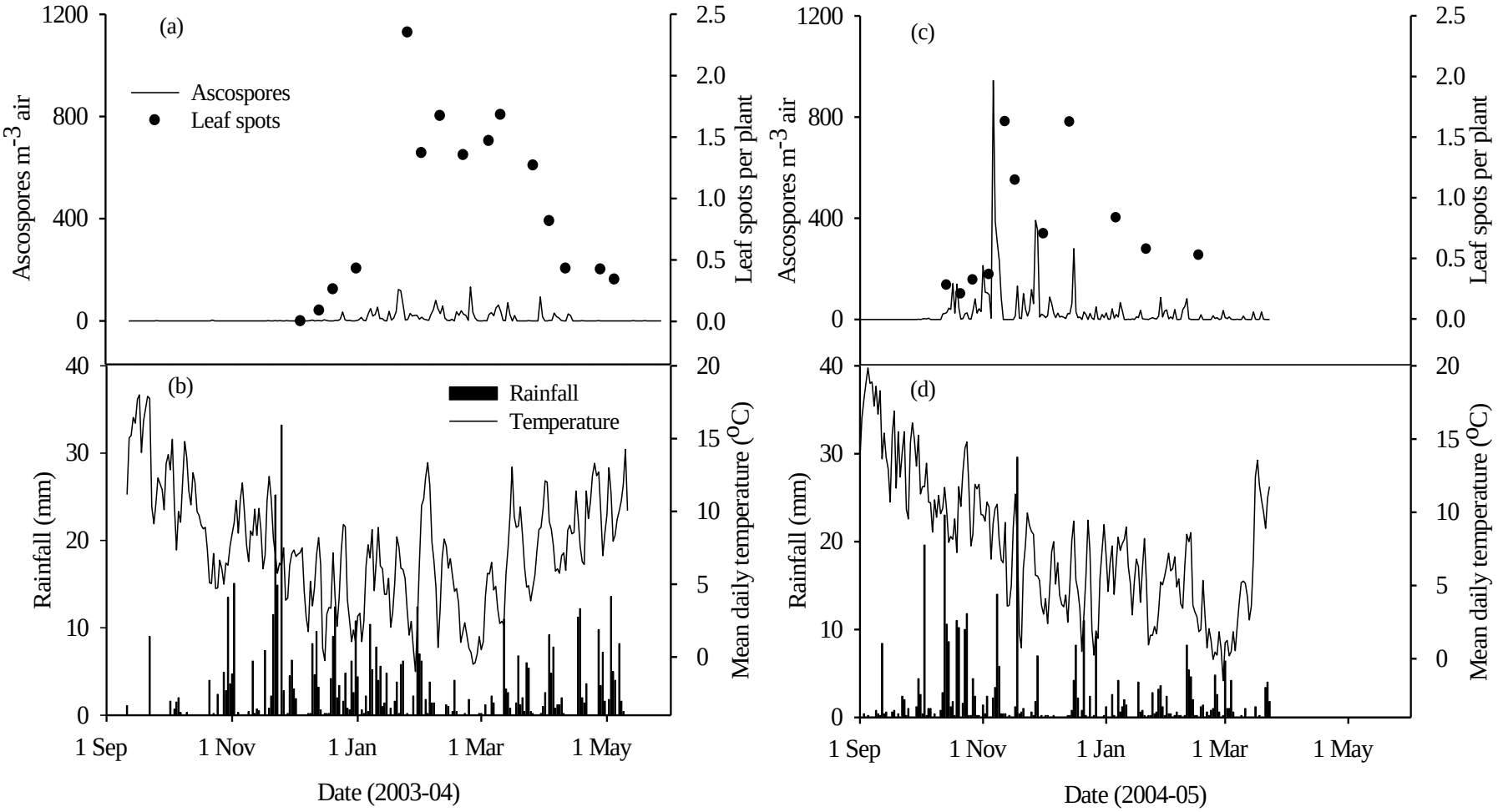

(a)
Ascospores
Leaf spots
Ascospores m-3 air
Leaf spots per plant
(b)
Rainfall
Temperature
Rainfall (mm)
Mean daily temperature (oC)
1 Sep
1 Nov
1 Jan
1 Mar
1 May
Date (2003-04)
(c)
(d)
Date (2004-05)

Figure 4

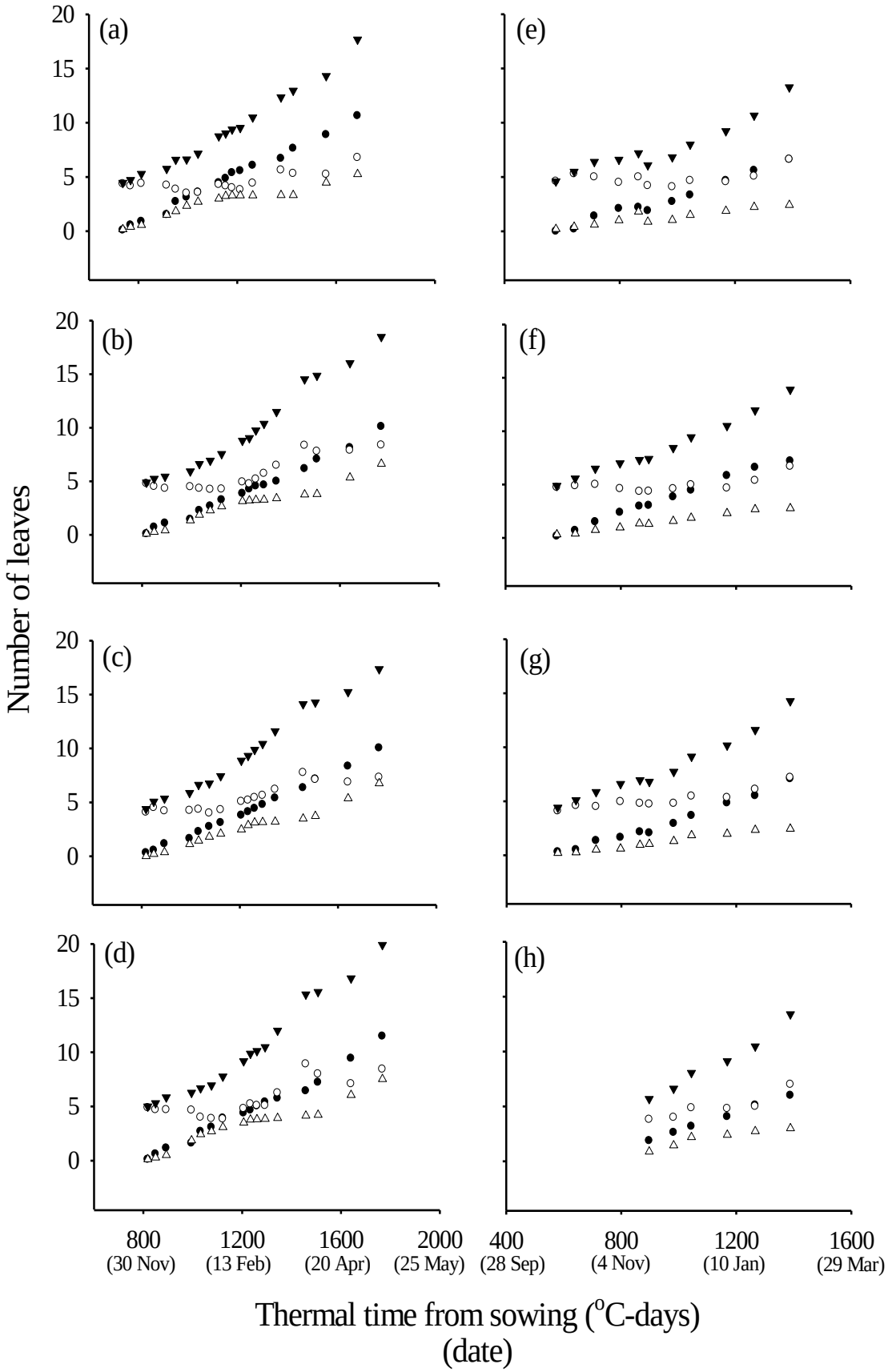

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Number of leaves
0
5
10
15
20
800
(30 Nov)
1200
(13 Feb)
1600
(20 Apr)
2000
(25 May)
400
(28 Sep)
800
(4 Nov)
1200
(10 Jan)
1600
(29 Mar)
Thermal time from sowing (°C-days)
(date)

Figure 5

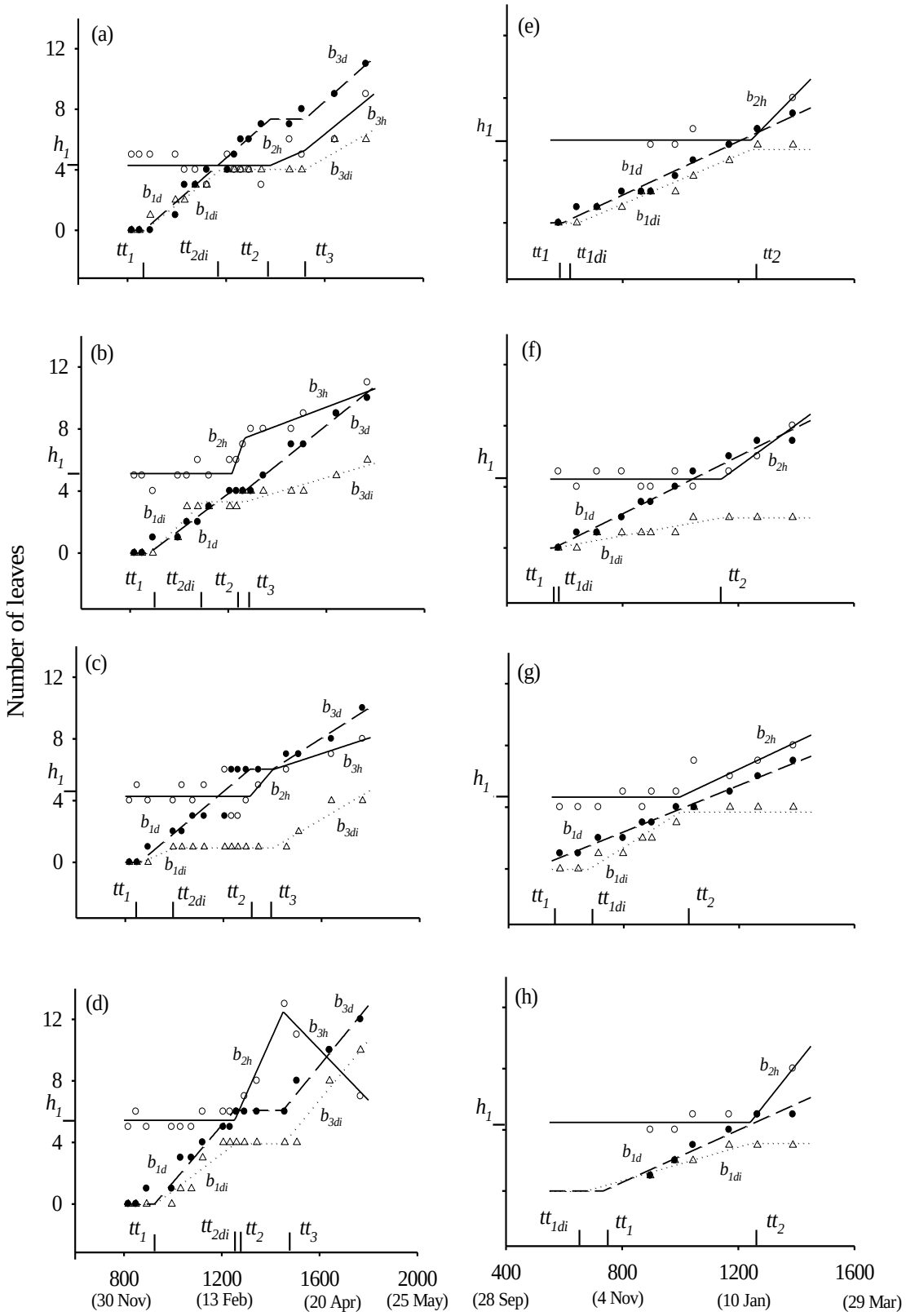
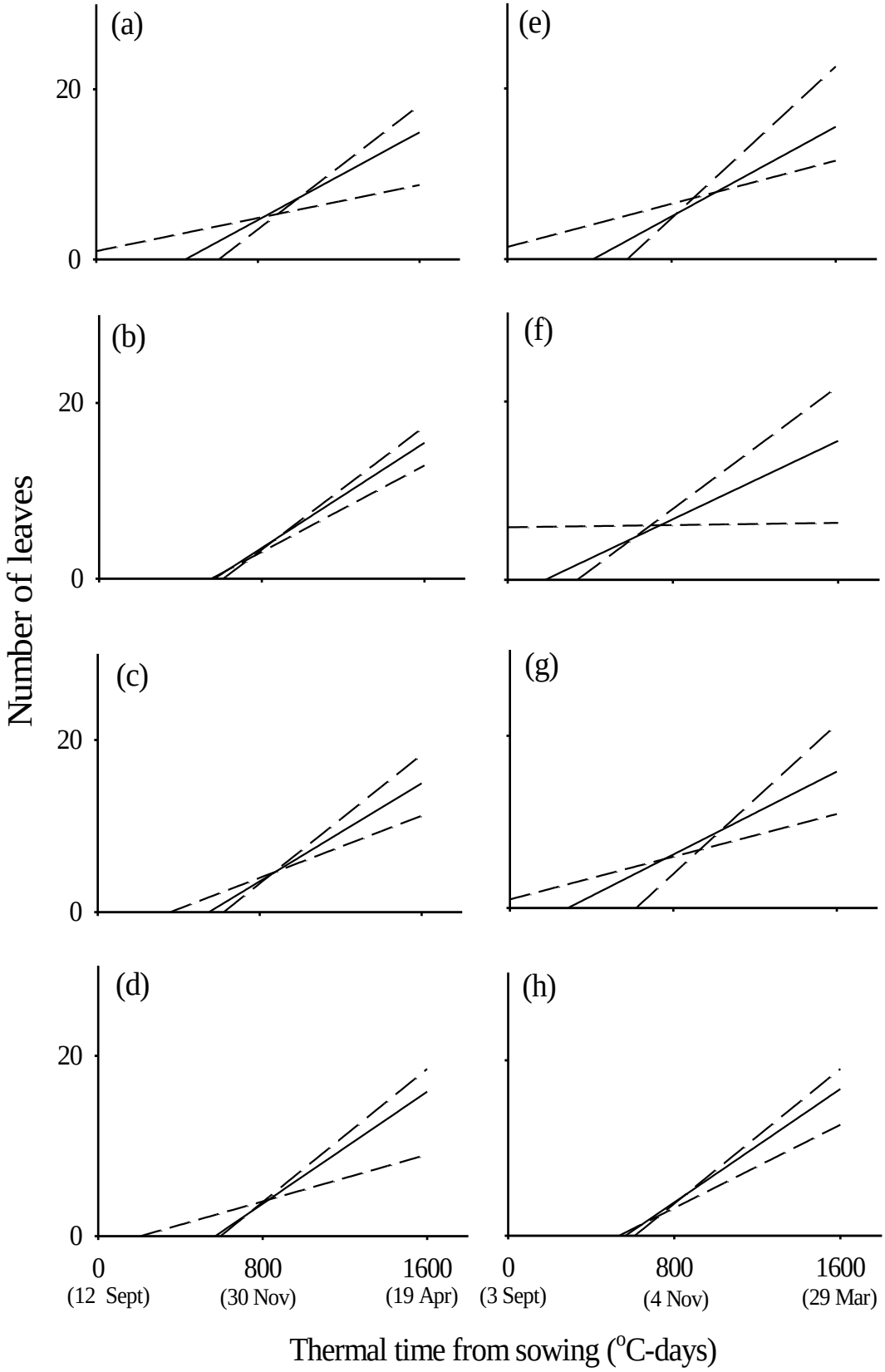

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Number of leaves
Thermal time from sowing (°C d)
800
(30 Nov)
1200
(13 Feb)
1600
(20 Apr)
2000
(25 May)
400
(28 Sep)
800
(4 Nov)
1200
(10 Jan)
1600
(29 Mar)

Figure 6


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
20
0
Number of leaves
0
(12 Sept)
800
(30 Nov)
1600
(19 Apr)
0
(3 Sept)
800
(4 Nov)
1600
(29 Mar)
Thermal time from sowing (°C-days)