Oecologia (2003) 136:244–251
DOI 10.1007/s00442-003-1267-7

PLANT ANIMAL INTERACTIONS

Olli-Pekka Tikkanen · Riitta Julkunen-Tiitto

Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operophtera brumata*

Received: 17 December 2002 / Accepted: 19 March 2003 / Published online: 1 May 2003

Abstract Phenological synchrony between budburst and emergence of larvae is critical for the fitness of many spring-feeding insect herbivores. Therefore, large intraspecific variation in timing of budburst of the host may have a negative effect on the herbivore. We studied how asynchrony between emergence of larvae and budburst affects the fitness of *Operophtera brumata* (Lepidoptera: Geometridae), a major defoliator of *Quercus robur*, which can adapt to the phenology of a single tree. It is known that, in maturing leaves of *Q. robur*, accumulation of condensed tannins has a negative effect on growth of *O. brumata*. However, there is no information about the effect of hydrolysable tannins and other phenolics that are potential antifeedants. In this study, we also analysed changes in secondary chemistry of the foliage of *Q. robur* and how different compounds are correlated with growth and survival of *O. brumata*. The effect of asynchrony on *O. brumata* was studied in rearing experiments. The neonate larvae were incubated without food for different periods of time. The decline in nutritional quality of foliage was estimated by rearing cohorts of larvae with manipulated hatching times on the leaves of ten individual *Q. robur* trees. For the chemical analysis, the foliage of these trees was sampled at regular intervals. In the absence of foliage, mortality of neonate larvae started to increase exponentially soon after the larvae emerged. If the larvae missed budburst, the decline in nutritional quality of the foliage led to increased mortality and lower body mass (= fecundity). Hydrolysable tannins were not significantly correlated with performance of the larvae. Only condensed tannins were found to correlate negatively with the growth and survival of *O. brumata*. Certain individual trees were unsuitable hosts for *O. brumata* because the decline in quality of the foliage was very rapid. Based on regression equations for increasing rate of mortality and decreasing fecundity, we calculated that a relatively small mismatch of ±30 degree days between budburst and hatching of larvae leads to a 50% decrease in the fitness of *O. brumata*. Thus, large phenological variation within a *Q. robur* stand can limit the colonisation of neighbouring trees by dispersing larvae. Furthermore, the hybridisation of moths adapted to phenologically different trees may lead to maladapted phenology of their offspring.

Keywords Host colonisation · Herbivore fitness · Insect herbivory · Plant phenolics · Tannins

O.-P. Tikkanen (✉) · R. Julkunen-Tiitto
Department of Biology,
University of Joensuu,
P.O. Box 111, 80101 Joensuu, Finland
e-mail: otikkane@cc.joensuu.fi
Tel.: +358-13-2514550
Fax: +358-13-2513590

Introduction

Phenological variation can function as an effective mechanism for hosts to avoid spring-active defoliating insects. As a result of variation in budburst times, "the window of high acceptability", i.e. the period when the quality of the foliage is favourable for successful colonisation and later development of emerging individuals (sensu Hunter and Lechowicz 1992), occurs at different times in different trees. When phenological variation is large, there is less overlap among the windows of high acceptability and the defensive effect can be strong enough to limit colonisation by insect herbivores. The phenological stage of the host at the time of emergence of insects has been found to affect performance (Hough and Pimentel 1978; Quiring 1992, 1994; Tikkanen and Lyytikäinen-Saarenmaa 2002) and colonisation (Du Merle 1988; Kolb and Teulon 1991; Hunter 1992; Quiring 1994; Mopper and Simberloff 1995; Chen et al. 2001) of herbivores in many plant-insect herbivore systems. The colonisation is limited, most probably because the budburst period within the host species is longer than the period of herbivore emergence. However, there are also cases where phenology does not affect distribution of spring-feeding insects in the canopy

(Marino and Cornell 1993; Connor et al. 1994) or the effect does not last the whole season (Fox et al. 1997).

Time of budburst of individual trees of *Quercus robur* L. (Fagaceae) can vary by as much as 3 weeks, and among different phenotypes the differences are consistent between years (Crawley and Akhteruzzaman 1988; Van Dongen et al. 1997; see also Fox et al. 1997). *Operophtera brumata* L. (Lepidoptera: Geometridae) is one of the most abundant insect herbivores on *Q. robur*. The larvae emerge in early spring when the nutritional quality of the leaves is highest (Feeny 1968, 1970). If larvae emerge too late after bud burst, their future growth will be much lower (Tikkanen and Lyytikäinen-Saarenmaa 2002). However, the eggs must also not hatch too early, because neonate larvae do not tolerate starvation for long periods (2–5 days according to Hunter 1990). Therefore, close synchrony between budburst of the host tree and hatching of larvae is critical for the fitness of the moth (Wint 1983; Van Dongen et al. 1997; Tikkanen and Lyytikäinen-Saarenmaa 2002).

Compared to the other host-insect systems cited above, the special characteristic of *O. brumata* is its ability to adapt to the budburst of a single host tree. Low and ineffective mobility of *O. brumata* compared to many other lepidopteran species may facilitate formation of adaptive demes (Edmunds and Alstad 1978; but see Van Zandt and Mopper 1998). Early instar larvae can balloon on air currents (Edland 1971; Holliday 1977), but this is a passive mechanism and larvae settle on vegetation haphazardly. Larvae are likely to pupate near the base of the host tree (Embree 1965). Adult females are flightless and tend to climb up to the nearest tree trunk to copulate and lay eggs (Graf et al. 1995). Adult males also remain in a relatively small area (Van Dongen et al. 1996). In accordance with the theory of adaptive deme formation, Van Dongen et al. (1997) found a significant positive correlation between budburst and time of hatching of eggs in oak stands. In addition, the range of hatching times of egg clutches was the same as the observed variation in time of budburst for *Q. robur*. Thus, because *O. brumata* apparently is able to adapt to the budburst of individual trees, large variation in budburst per se may not protect hosts.

The observations of Embree (1965) and of Varley and Gradwell (1968), who found very high mortality of first-instar larvae, are not in accordance with the apparent ability of *O. brumata* to adapt to the phenology of single host trees. They suggested that the reason for this was phenological asynchrony. High intraspecific phenological variation may provide an explanation for this contradiction. The dispersion of male *O. brumata* is not as limited as that of females (Van Dongen et al. 1996). Therefore, hybridisation between moths originating from different locally adapted demes (of single trees) can take place. If there is sufficient phenological variation in the host population, this could lead to maladapted hatching phenology of the offspring. The distribution of egg-hatching times in a single brood is also an important factor, because early instar larvae have the potential to colonise neighbouring trees (Holliday 1977). However, if the range of hatching times within a brood is considerably smaller than the phenological variation in budburst of the host species, this limits the number of suitable host trees available for colonisation by dispersing larvae.

The negative effect of the phenological variation in *Q. robur* on populations of *O. brumata* should be strongly dependent on the rate of decline in quality of the foliage in spring (due to an increase in secondary chemicals and leaf toughness, Hunter and Lechowicz 1992). Condensed tannins, which accumulate rapidly after leaf expansion, are known to affect the growth of *O. brumata* larvae negatively (Feeny 1968, 1970). There are reports on North American *Quercus* species which suggest that hydrolysable tannins may also have a negative effect on growth of insect herbivores (Rossiter et al. 1988; Lill and Marquis 2001). Hydrolysable tannins are also abundant in the leaves of *Q. robur* (Scalbert and Haslam 1987). However, it is not known whether they affect the performance of *O. brumata*.

Our aim was to determine whether large phenological variation of *Q. robur* could have a negative effect on the life of a spring-feeding defoliator, *O. brumata,* assuming that it is able to adapt locally to the phenology of a single host tree. By measuring the effect of declining quality of foliage on the fitness of *O. brumata*, we estimated how large this variation should be in order to protect *Q. robur.* This was done by rearing cohorts of larvae with manipulated hatching times on the leaves of ten individual *Q. robur* trees. In addition, we studied the effect of starvation on survival of neonate larvae. Based on the data of these two experiments, we were able to predict the effect of asynchrony between budburst and egg hatching on the fitness of the moth and to estimate the critical amount of variation in budburst among trees. We also measured changes in the secondary chemistry of leaves, including condensed and hydrolysable tannins, and analysed the relationship between the concentration of different compounds and the growth and survival of larvae.

Materials and methods

Study site

In spring 2001, well before budburst, 28 15- to 30-year-old *Quercus robur* trees growing in a 0.5 ha mixed stand near Banchory, NW Scotland (57°05′N, 2°30′W) were selected for monitoring (all trees were higher than 3 m and had a fully developed crown). After the buds of the earliest trees had started to swell and the green parts of the bud scales became visible, the phenology of the trees was observed daily. When the buds of a tree were predominantly open (leaves 5–10 mm long and clearly out of bud scales), budburst was considered to have taken place. Ten of 28 trees, which represented the whole range of budburst phenology of the stand, were selected systematically for more specific analyses and experiments. From the beginning of February, the air temperature of the stand was recorded at 1-h intervals, and cumulative temperature sums [degree-days (dd), threshold +5°C] were used in all time-related measurements. A Hobo Pro Series data-logger, which was protected from direct sunlight and mounted on top of a 2.5-m-high pipe, was

used for temperature recordings. The range of budburst times was 64 dd (n=28, mean 135 dd; SD±20).

Analysis of leaf secondary chemistry

The foliage of the ten trees was sampled for chemical analysis at the budburst of each tree, followed by four to five further samplings. At each sampling, five current-year shoots per tree were clipped randomly from a height of 1.5–3.5 m and were dried at room temperature. Changes in secondary chemistry of the foliage during leaf maturation were analysed in three samples per tree at the Natural Product Research Laboratory, University of Joensuu. The stems and petioles were removed from the leaf material. The remaining material was milled and mixed together. The first sample analysed for HPLC-phenolics was taken at budburst (0 dd), and the second and third at ≥100 and ≥200 dd, respectively, after budburst of that tree. Because condensed tannins were found to correlate negatively with growth of larvae, the tannins from all samples were analysed in order to obtain a better picture of their rate of accumulation.

For soluble phenolics, 10 mg of milled leaf tissue was extracted in an Eppendorf vial with 600 μl of methanol (100%) using an Ultra-Turrax homogeniser for 20 s; after that, the sample was left to stand on ice for 15 min. The sample was re-homogenised for a further 20 s and centrifuged (13,000 rpm, for 3 min). The extraction procedure was repeated three more times with only 2 min on ice. The methanol in the combined extracts was evaporated under nitrogen. The samples were re-dissolved in 0.6 ml of methanol: H_2O (1:1) for HPLC runs. For condensed tannins (soluble and insoluble), a 5–10 mg sample of dried leaf powder was weighed, and oxidative de-polymerisation for condensed tannins was processed in acid butanol, as described by Porter et al. (1986). Condensed tannins were quantified using tannin from *Salix purpurea* L. leaves purified by Sephadex LH20. Soluble phenolics were quantified by HPLC-DAD as reported by Julkunen-Tiitto and Sorsa (2001). HPLC-MS was used to identify the components. HPLC/API-ES (positive/negative ions) conditions were as follows: the column was Rp C_{18}, ID 2 mm, 10 cm in length; ES fragmentor voltage was 80–100 depending on the components; flow rate was 0.4 ml/min, injected volume 5 μl.

Quantification of the components was as follows: gallic acid derivatives and ellagitannins were based on pentagalloylglucose; salidroside was based on salidroside, catechin-derivatives on (+)-catechin, ellagic acid derivatives on ellagic acid, quercetin derivatives on hyperin, kaempferol derivatives on kaempferol-3-glucoside and isorhamnetin derivatives on isorhamnetin. HPLC/API-ES produced the following molecular weights: salidroside 323 (M+23), (+)-catechin 291 (M+1), hyperin 487 (M+23), quercetin-3-glucoside 487 (M+23), quercetin-3-glucuronide 479 (M+1), kaempferol-3-glucoside 471 (M+23), isorhamnetin 501 (M+23), kaempferol-3-(diacetyl)-glucoside 557 (M+23) and ellagitannin (vescalagin/castalagin) 933 (M-H).

Tolerance of starvation

In the absence of new foliage, the starvation rate of larvae was estimated by keeping neonate larvae in 200 ml transparent plastic vials with only a disc of moistened tissue paper. On five mornings, freshly emerged neonates were placed in three vials, 30 larvae in each, and the vials were placed in an incubator (+10.5°C). On the sixth morning, the last group of neonates was placed in vials together with small twigs of flushing foliage. Simultaneously, fresh twigs were added to all other vials. Thus, there were six groups of larvae that had spent 0, 5.5, 11, 22, 27.5 and 33 dd without food (there was a 1-day break in the middle of the series). The vials were kept in the incubator for 6 more days before the number of survivors was counted. All *O. brumata* eggs and larvae used in rearing experiments originated from laboratory stock (the Turku-population, see Tikkanen and Lyytikäinen-Saarenmaa 2002).

Temporal decline in leaf quality

The effect of leaf maturation on development of *O. brumata* was studied by rearing cohorts of larvae with manipulated emergence times on foliage from the ten *Q. robur* trees. Each cohort consisted of 15 randomly picked larvae, which came from 40 different egg clutches. The clutches were kept separately in vials in a shed, which was slightly warmer than ambient temperature. When neonates started to emerge, vials with eggs were moved to a refrigerator (+2°C). In the evenings before rearing of new cohorts was to be initiated, a random set of ten vials was taken back to the shed. The vials were checked and "old" larvae were discarded from the following experiments.

The larvae were reared in the insectary of Centre for Ecology and Hydrology, Banchory at ambient temperature, 2 km from the stand where the trees grew. Rearing was initiated at budburst of a tree when the first cohort of 15 neonates was placed on fresh foliage (because of variation in budburst, the initiation time also varied among different trees). The second cohort was placed on foliage after 3–5 days and the third and fourth etc. at intervals of 3–5 days. Freshly emerged neonates were placed singly in 200-ml transparent plastic vials with an open bud or a few leaves, as in the case of the latter cohorts. In each vial, to prevent wilting of the foliage, there was a disc of moistened tissue paper. New leaves were added and the old leaves were removed at intervals of 3–4 days. When a larva reached the fifth and final instar stage, 1/4 of the volume of the vial was filled with peat debris. These vials were monitored daily, and the day when a larva had burrowed into the peat was considered the end of the larval period. The pupae were weighed 1 month after pupation, at the end of July or early August, depending on the time the cohort was initiated. The growth rate of the larvae (GR) of the first two cohorts per tree was calculated by dividing pupal weight by degree-days needed to complete the larval period. In the third and fourth cohorts mortality was very high, and therefore their GR was not calculated. The correlation (r_s) of larval performance (survival and growth rate, trees as replicates) of the first two cohorts with concentration of identified secondary compounds was analysed at the middle (120 dd) and end (200 dd) of the larval period.

Because some of the trees were relatively small, there was a danger that clipping large quantities of twigs for the rearing experiment may have had unwanted and uncontrolled effects on the quality of the foliage. Therefore, only two or three cohorts were reared on the foliage of these smaller trees.

Consequences of asynchrony to fitness of *O. brumata*

Tolerance to starvation was considered to be the predominant component of fitness if larvae emerged before budburst. After budburst, the declining-rate of fitness was estimated by multiplying the rate of pupal survival by the declining rate of fecundity. Survival of pupae in a cohort was recorded as the proportion of healthy pupae of the initial number of larvae per cohort per tree (n=15). Because adult *O. brumata* do not feed, the reproductive capacity is determined by larval size. Therefore, the pupal weights of the females are valid indicators of fecundity. Mean fecundity of the cohorts was calculated using the equation of Roland and Myers (1987): number of eggs =9.14× fresh weight of pupa −101. Because female moths are distinctly larger than males (Tikkanen et al. 2000), the heaviest 50% of the pupae per cohort were used to calculate cohort fecundity (in the laboratory stock, the female: male ratio was 1:1; O.-P. Tikkanen, unpublished data). The change in pupal mortality and fecundity as a function of degree-days from budburst was estimated using linear regression analysis.

The survival values before budburst, predicted by the regression equation for starvation rate of neonate larvae, were calculated with 5-dd intervals. Then the calculated values were related to the highest value at 0 dd, which received a fitness value of 1. The survival values for pupae obtained with the mortality regression equation were multiplied by the values obtained with the fecundity regression equation at 5-dd intervals after budburst. These products

were related to the highest value at 0 dd. Finally, the results of all these calculations, the predicted values for fitness before and after budburst, were plotted on the same graph, which shows the effect of asynchrony on the fitness of *O. brumata.*

Distribution of hatching times within a brood

The frequency distribution of the hatching times of the offspring of *O. brumata* was determined for 17 broods. Samples of 100–170 eggs from each brood were incubated at a constant temperature of +12°C, and the number of emerged larvae was recorded daily. The median day of brood hatching (the day when 50% of the larvae in a brood had emerged) was assigned the number 0. The frequency distributions (%) of emerged larvae of the 17 broods was plotted as a function of degree-day deviation from the brood median, and a gaussian normal distribution equation

$$Y = ae^{\left[-0.5\left(\frac{X}{b}\right)^2\right]} \quad (1)$$

was fitted to the hatching time data using the "regression wizard" of the SigmaPlot 5.0 graphic software (SigmaPlot 1999). In all statistical tests, the SPSS 10.1 for Windows software package was used (SPSS 2000).

Results

Leaf secondary chemistry

We identified eleven different phenolic compounds (monogalloylglucose, salidroside-derivative, (+)-catechin, ellagic acid-monoglycoside, hyperin, quercetin-3-glucoside and 3-glucuronide (coeluted), kampferol-3-glucoside, isorhamnetin-3-glucoside, kaempferol-3(diacetyl)-glycoside and isorhamnetin-glycoside) and two groups of phenolic compounds (ellagic tannins and condensed tannins) from the leaves of *Quercus robur*. There were 15 different compounds in the ellagic tannins; but because none of them was significantly correlated with the growth

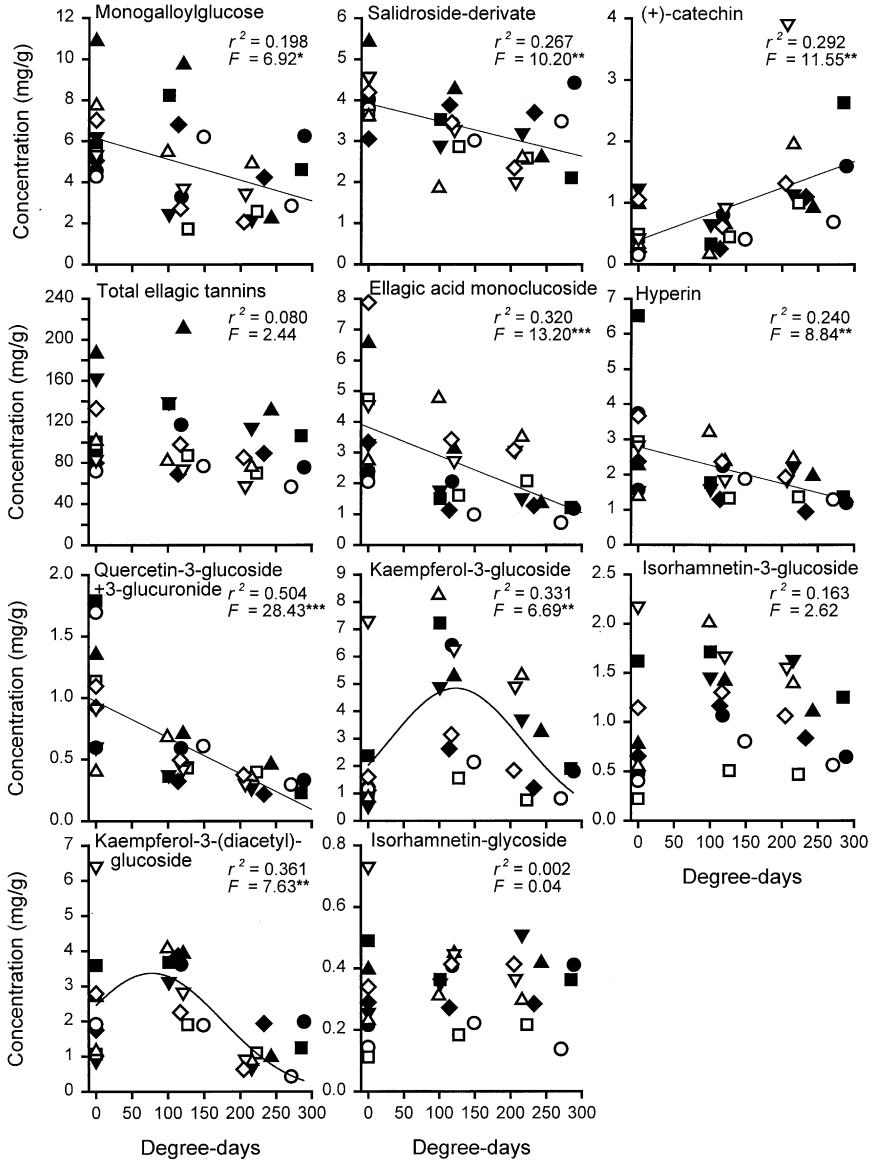

Fig. 1 Change in the secondary chemistry of the foliage of *Quercus robur* in relation to leaf development in spring and early summer. Zero degree-days indicates budburst. The different symbols refer to the same individual trees in all figures

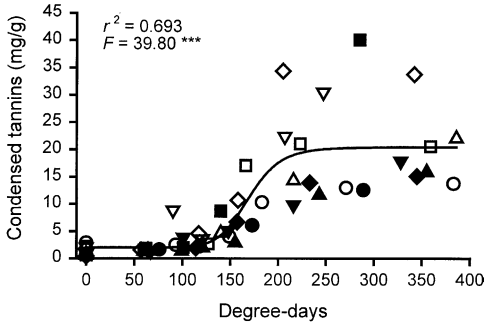

Fig. 2 Changes in the amount of condensed tannins in the foliage of *Quercus robur* in relation to leaf development in spring and early summer. Regression line, $Y = 2 + \frac{18.4}{1+e^{\frac{169.6-X}{10.3}}}$, shows the accumulation rate of condensed tannins as the function of degree-days from budburst (dd =0)

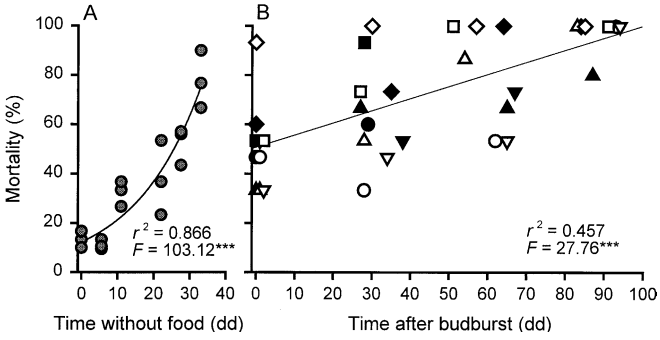

Fig. 3 Increase in the rate of mortality of larvae of *Operophtera brumata* **A** due to starving when they emerge before budburst and **B** due to maturation of foliage when they emerge after budburst (*dd* degree-days). In **B**, overlapping symbols at 0 dd have been uncovered by moving them horizontally 1 or 2 dd (symbols as in Fig. 1). Equations for regression lines are: Y=12.37 e $^{0.055\ X}$ for (**A**) and Y=50.88+0.492X for (**B**)

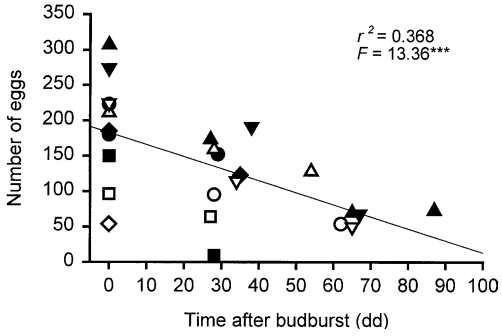

Fig. 4 Decline in the fecundity of *O. brumata* females in relation to the delay between budburst of *Q. robur* and initiation of feeding. Equation for regression line is: Y=182.9–1.7X. Symbols as in Fig. 1

traits of *O. brumata* (results not shown), only the total amount of ellagic tannins is reported here.

The amount of all compounds, except the ellagic tannins, isorhamnetin-3-glucoside and isorhamnetin-derivative, changed considerably during leaf development (Figs. 1, 2). The amount of monogalloylglucose, salidroside-derivative, ellagic acid monoglucoside, hyperin and quercetin-3-glucoside+3-glucuronide declined linearly while the amount of (+)-catechin increased. The amount of kaempferol-3-glucoside and kaempferol-3(diacetyl)-glucoside peaked in the middle of the sampling period. At the beginning of leaf development, the amount of condensed tannins was low, but 150 dd after budburst it started to rise rapidly. At 200 dd after budburst, the accumulation of condensed tannins reached a plateau of, on average, 20 mg/g. All quantified compounds also showed considerable variation among individual trees (Figs. 1, 2).

Tolerance of starvation and decline of leaf quality

Mortality of first- instar larvae increased exponentially with the number of degree-days spent without food. The larvae that had spent the longest time without food (33 dd) suffered >70% mortality (Fig. 3A). The effect of maturation of foliage on mean survival of cohorts was also significant; from the 50% mortality of the first cohort, the mortality increased linearly and in the fourth cohorts was almost 100% (Fig. 3B). Both tree identity and cohort affected the GR of the larvae, but there was no Tree × Cohort interaction (2-way ANOVA; Tree, $F_{9,107}$=3.12, P=0.002; Cohort, $F_{1,107}$=23.01, P=0.001; T×C, $F_{8,107}$=0.57, P=0.8). Pupal weight and, consequently, potential number of eggs declined linearly as the foliage matured (Fig. 4). In the third cohorts, fecundity was twice as low as in the first cohorts.

Only the concentration of condensed tannins at 200 dd after budburst correlated significantly and negatively with GR of the larvae in the second cohorts, after a sequential Bonferroni test (n=10, r_s=–0.84, P=0.025; P-values corrected with the total number of compounds (12), see Rice 1989). Also, there were negative correlations between concentration of condensed tannins at 200 dd after budburst with survival of larvae of the second cohorts (r_s=–0.74) and with growth of larvae in the first cohorts (r_s=–0.76) but these correlations appeared to be non-significant after the Bonferroni test (P>0.1). For the other compounds there were no signs of any relationship (all $|r_s|$ <0.55, P>0.1). The low amount of condensed tannins in the middle of the larval period (at 120 dd) apparently had no effect either on survival or growth. There was no statistical difference in mean GR of larvae reared on foliage of the five smallest and the five large trees (t-test; t=1.23, df=8, P=0.254). This indicates that variation in size (age) of the trees had no marked influence on the results.

Phenological synchrony and fitness

According to the equation that predicts starvation of neonate *O. brumata*, in the absence of foliage, all larvae

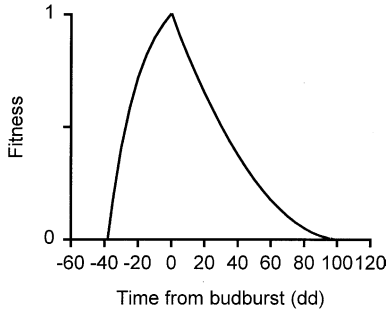

Fig. 5 Relative fitness of *O. brumata* as influenced by the degree of a synchrony between budburst (dd =0) and emergence of larvae (for details see Materials and methods)

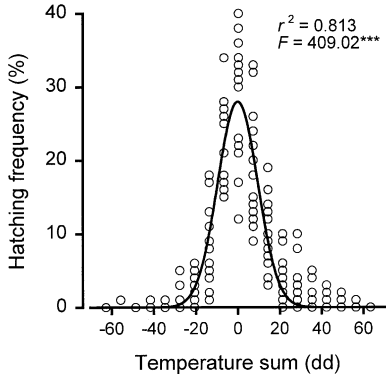

Fig. 6 Frequency distributions of the egg hatching of 17 broods of *O. brumata* at constant temperature. Medians of broods are standardised (0 dd). Equation for regression line is:

$$Y = 0.28e^{\left[-0.5\left(\frac{X}{9.528}\right)^2\right]}$$

perish if they emerge –40 dd before budburst (Fig. 5). Consequently, the fitness of such larvae was zero. At –25 dd before budburst, the fitness (survival) of larvae was 50% less than at the time of budburst. At +30 dd after budburst, the estimated fitness of *O. brumata* was 50% less than if larvae had emerged in synchrony with budburst (Fig. 5).

Distribution of brood hatching times

From the 17 broods, the total number of hatched eggs was 2191. The distribution of hatching frequencies, analysed from the total standardised egg population, was leptokurtic (Shapiro-Wilk W=0.941, P<0.001; mean 1.1 dd, SD±11.7 dd; kurtosis =1.69) and slightly skewed to the right (skewness =0.56). However, the equation

$$Y = 0.28e^{\left[-0.5\left(\frac{X}{9.528}\right)^2\right]} \quad (2)$$

fitted the plotted frequencies (%) of the brood hatching times very well (r^2=0.813, F=409.02, P<0.001; Fig. 6). This model predicts that, in conditions used here, 90% of the eggs within the broods will hatch within a period of 28 dd and 95% will hatch within 42 dd.

Discussion

Both too early and too late emergence strongly reduce fitness of *O. brumata*. At –10 dd before budburst, the mortality of the neonates, due to starving, starts to rise rapidly (Fig. 3A). If larvae emerge too late after budburst, the quality of the foliage has declined so much that a significant number of larvae do not survive (Fig. 3B); and the body mass and fecundity of the surviving individuals is much lower (Fig. 4). Our model predicts that a difference greater than ±30 dd reduces the fitness of *O. brumata* by ≥50% compared to individuals that have hatched in synchrony with budburst (Fig. 5). At the research site, the mean daily temperature of the hatching period (the 2nd half of May) was +11.8°C. In these conditions, the mismatch of 30 dd is equivalent to 4.4 calendar days.

Larvae that miss budburst cannot compensate for the decline in foliage quality by increasing the length of the feeding period. The first cohorts had already reached the end of their larval period when the concentration of condensed tannins in the leaves increased. However, the larvae of the later cohorts had to complete their fourth and fifth instars feeding on leaves rich in condensed tannins. Tammaru (1998) showed that in another geometrid, *Epirrita autumnata* L., the decline in nutritional quality of the food of the middle instars leads to a cumulative decrease in pupal weights in the last instars. Therefore, even a relatively small mismatch of few days between budburst and hatching can crucially affect the fitness of *O. brumata.*

Based on our fitness model (Fig. 5) and distribution of hatching times within an egg clutch (Fig. 6), we can estimate that if a certain genotype is locally adapted to budburst of the tree where its parents have dwelled, 90% of the offspring emerge during the time when the predicted average fitness is high (between 1 and 0.75). This may be one reason why successive generations of adults of *O. brumata* tend to stay in the vicinity of their pupation site (Graf et al. 1995; Van Dongen et al. 1996). After all, individual mature *Q. robur* trees are reasonably stable patches for *O. brumata*.

The critical question is how well *O. brumata* is able to match its hatching to small-scale phenological variation in a forest stand. Although *O. brumata* has good phenological adaptability (Van Dongen et al. 1997), perfect phenological synchrony can be challenged by a heterogeneous environment. The variation in timing of egg hatching is apparently under genetic control (Tikkanen and Lyytikäinen-Saarenmaa 2002), and offspring express a hatching time that is intermediate of the parental phenotypes (Speyer 1941). If there is large phenological variation in budburst times of neighbouring trees, hybridisation between adults originating from different trees can lead to significant phenological asynchrony and lowered

fitness of offspring as well as lowered fecundity of the local moth population. In addition, adult females of *O. brumata* are non-selective in terms of their oviposition site (Graf et al. 1995; O.-P. Tikkanen, personal observation). Therefore, accidental choices of "incorrect" host trees by adults are unavoidable and increase in numbers as the diversity and the stem density of stands increase.

If larvae of *O. brumata* do not find suitable foliage on their natal tree, they are forced to disperse. Early instars are able to drift in wind some 50 m in favourable conditions (Edland 1971), and they can colonise neighbouring trees if they manage to land on foliage that is in suitable phenological stage (Holliday 1977). However, drifting of larvae is passive ballooning in the air. The larvae cannot select the direction of movement and, inevitably, the mortality of dispersing larvae is very high (Varley and Gradwell 1968). According to Fig. 6, approximately 90% of a brood of *O. brumata* hatched within 30 dd. In spring weather, 30 dd is approximately 5 calendar days but the range of budburst of different individuals of *Q. robur* can be as much as 3 weeks (Crawley and Akhteruzzaman 1988; Van Dongen et al. 1997). Thus, the high variation in budburst of *Q. robur* is enough to have a negative effect on the moth population by limiting the number of trees available for colonisation by drifting larvae.

Although we found 28 different phenolic compounds in the leaves of *Q. robur*, only the amount of condensed tannins correlated negatively with growth and survival of the larvae of *O. brumata* (see also Feeny 1968). This is in contrast to reports in which hydrolysable tannins of *Quercus* spp. have been found to limit the growth of moth larvae (Rossiter et al. 1988; Lill and Marquis 2001). In *Q. robur*, the effect of condensed tannins may be so strong that the possible negative effect of hydrolysable tannins (total ellagic tannins + monogalloyl glucose) cannot be detected. Alternatively, hydrolysable tannins of European *Q. robur* may not have the same defensive function as the hydrolysable tannins in its North American congener. For example, Ayres et al. (1997) found that the defensive effect of condensed tannins varied between congeneric tree species.

The extremely high mortality of larvae on certain trees suggests that the chemical defences of *Q. robur* can be very effective alone (Fig. 3B). For growth of larvae, the differences among trees were dramatic; and on one tree (depicted by a clear diamond), of a total number of 60 larvae, only 1 larva pupated successfully. Thus, trees in which condensed tannins start to accumulate early and in great quantity are likely to be poor hosts for *O. brumata*. This could explain why, from year to year, certain *Q. robur* individuals suffer from heavier defoliation than others (Crawley and Akhteruzzaman 1988; Humphrey and Swaine 1997).

Obviously, however, the rate of accumulation of condensed tannins is not the ultimate defensive mechanism of *Q. robur* against *O. brumata*. The studies of Feeny (1970) and other researchers (e.g. Scriber and Slansky 1980; Ayres and MacLean 1987; Kause et al. 1999) show how increasing leaf toughness and decreasing water content are associated with poorer performance of folivorous larvae. Therefore the ultimate defensive trait is most likely, the rate of maturation of the foliage as a whole, where accumulation of condensed tannins, decline in water content and increase in leaf toughness are all connected in the same process (Scalbert and Haslam 1987).

In conclusion, the observed high intraspecific phenological heterogeneity in *Q. robur* can be an effective mechanism for protecting this tree species against *O. brumata* and it might explain the very high mortality of early instar larvae found by Embree (1965) and Varley and Gradwell (1968). According to our model, within a *Q. robur* stand, a variation of 30 dd or greater in budburst is enough to limit the success of dispersing larvae. In addition, 90% of the eggs in a brood of *O. brumata* hatch within 30 dd. Therefore, many trees are beyond the reach of offspring of a single female. Furthermore, hybridisation of genotypes that are phenologically adapted to trees with substantially different budburst times should reduce the fitness of the offspring. Our study is one step towards a more advanced model that could help us to understand better the effects of gene flow, adaptive deme formation and host quality variation on the *Q. robur/O. brumata*-system and, in general, on plant-insect interactions.

Acknowledgements We thank the personnel of the Centre for Ecology and Hydrology Banchory, for their hospitality during the field work. Special thanks to Outi Nousiainen for making the chemical analyses. Susanna Puustinen, Tiina Ylioja and an anonymous referee are thanked for critical reviewing of our manuscript that helped us greatly to improve the text. A grant from the Academy of Finland (71961) made possible the work of O.-P.T. in Scotland. The CEH Banchory provided facilities for the field work and the University of Joensuu for the chemical analyses.

References

Ayres MP, MacLean SF Jr (1987) Development of birch leaves and growth energetics of *Epirrita autumnata* (Geometridae). Ecology 68:558–568

Ayres MP, Clausen TP, MacLean SF Jr, Redman AM, Reichardt PB (1997) Diversity of structure and antiherbivory activity in condensed tannins. Ecology 78:1696–1712

Chen Z, Kolb TE, Clancy KM (2001) Mechanisms of Douglas-fir resistance to western spruce budworm defoliation: bud burst phenology, photosynthetic compensation and growth rate. Tree Physiol 21:1159–1169

Connor EF, Adams-Manson RH, Carr TG, Beck MW (1994) The effects of host plant phenology on the demography and population dynamics of the leaf-mining moth, *Cameria hamadryadella* (Lepidoptera: Gracillariidae). Ecol Entomol 19:111–120

Crawley MJ, Akhteruzzaman M (1988) Individual variation in the phenology of oak trees and its consequences for herbivorous insects. Funct Ecol 2:409–415

Du Merle P (1988) Phenological resistance of oaks to the green leafroller, *Tortrix viridiana* (Lepidoptera: Tortricidae). In: Mattson WJ, Levieux J, Bernard-Dagan C (eds) Mechanisms of woody plant defences against insects. Springer, Berlin Heidelberg New York, pp 215–226

Edland T (1971) Wind dispersal of the winter moth larvae *Operophtera brumata* L. (Lep., Geometridae) and its relevance to control measures. Nor Entomol Tidsskr 18:103–107
Edmunds GF, Alstad DN (1978) Coevolution in insect herbivores and conifers. Science 199:941–945
Embree DG (1965) The population dynamics of the winter moth in Nova Scotia, 1954–62. Mem Entomol Soc Can 46:1–57
Feeny P (1968) Effect of oak leaf tannins on larval growth of the winter moth *Operophtera brumata*. J Insect Physiol 14:805–817
Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51:565–581
Fox CW, Waddell KJ, Groeters FR, Mosseau TA (1997) Variation in budbreak phenology affects the distribution of a leafmining beetle (*Brachys tessellatus*) on turkey oak (*Quercus laevis*). Ecoscience 4:480–489
Graf B, Borer F, Höpli HU, Höhn H, Dorn S (1995) The winter moth, *Operophtera brumata* L. (Lep., Geometridae) on apple and cherry: spatial and temporal aspects of recolonization in autumn. J Appl Entomol 119:295–301
Holliday NJ (1977) Population ecology of winter moth (*Operophtera brumata*) on apple in relation to larval dispersal and time of bud burst. J Appl Ecol 14:803–813
Hough JA, Pimentel D (1978) Influence of host foliage on development, survival and fecundity of gypsy moth. Environ Entomol 7:97–102
Humphrey JW, Swaine MD (1997) Factors affecting the natural regeneration of *Quercus* in Scottish oakwoods. II. Insect defoliation of trees and seedlings. J Appl Ecol 34:585–593
Hunter AF, Lechowicz MJ (1992) Foliage quality changes during canopy development of some northern hardwood trees. Oecologia 89:316–323
Hunter MD (1990) Differential susceptibility to variable plant phenology and its role in competition between two insect herbivores on oak. Ecol Entomol 15:401–408
Hunter MD (1992) A variable insect-plant interaction: the relationship between tree budburst phenology and population levels of insect herbivores among trees. Ecol Entomol 16:91–95
Julkunen-Tiitto R, Sorsa S (2001) Testing the drying methods for willow flavonoids, tannins and salicylates. J Chem Ecol 27:779–789
Kause A, Ossipov V, Haukioja E, Lempa K, Hanhimäki S, Ossipova S (1999) Multiplicity of biochemical factors determining quality of growing birch leaves. Oecologia 120:102–112
Kolb TE, Teulon DAJ (1991) Relationship between sugar maple budburst phenology and pear thrips damage. Can J For Res 21:1043–1048
Lill JT, Marquis RJ (2001) The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia 126:418–428
Marino PC, Cornell HV (1993) Adult feeding and oviposition of *Phytomyza ilicicola* (Diptera: Agromyzidae) in response to leaf and tree phenology. Environ Entomol 22:1294–1301
Mopper S, Simberloff D (1995) Differential herbivory in an oak population: the role of plant phenology and insect performance. Ecology 76:1233–1241
Porter LJ, Hrstich LN, Chan BG (1986) The conversion of proanthocyanidins and prodelphinidins to cyanidins and delphinidins. Phytochemistry 25:223–230
Quiring DT (1992) Rapid change in suitability of white spruce for a specialist herbivore, *Zeiraphera canadensis*, as a function of leaf age. Can J Zool 70:2132–2138
Quiring DT (1994) Influence of inter-tree variation in time of budburst of white spruce on herbivory and the behaviour and survivorship of *Zeiraphera canadensis*. Ecol Entomol 19:17–25
Rice WR (1989) Analyzing tables of statistical tests. Evolution 42:223–225
Roland J, Myers JH (1987) Improved insect performance from host-plant defoliation: winter moth on oak and apple. Ecol Entomol 12:409–414
Rossiter MC, Schultz JC, Baldwin IT (1988) Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology 69:267–277
Scalbert A, Haslam E (1987) Polyphenols and chemical defence of the leaves of *Quercus robur*. Phytochemistry 26:3191–3195
Scriber JM, Slansky F Jr (1981) Nutritional ecology of immature insects. Annu Rev Entomol 26:183–211
SigmaPlot (1999) SigmaPlot 5.0 Users Guide. SPSS, Chicago
Speyer W (1941) Weitere Beiträge zur Biologie und Bekämpfung des Kleinen Frostspanners (*Cheimatobia brumata* L.). Arb Physiol Angew Entomol Berlin-Dahlem 8:245–261
SPSS (2000) SPSS Base 10.0 Users Guide. SPSS, Chicago
Tammaru T (1998) Determination of adult size in folivorous moth: constraints at instar level? Ecol Entomol 23:80–89
Tikkanen O-P, Lyytikäinen-Saarenmaa P (2002) Adaptation of a generalist moth, *Operophtera brumata*, to variable budburst phenology of host plants. Entomol Exp Appl 103:123–133
Tikkanen O-P, Niemelä P, Keränen J (2000) Growth and development of a generalist insect herbivore, *Operophtera brumata*, on original and alternative host plants. Oecologia 122:529–536
Van Dongen S, Matthysen E, Dhondt AA (1996) Restricted male winter moth (*Operophtera brumata* L.) dispersal among host trees. Acta Oecol 17:319–229
Van Dongen S, Backeljau T, Matthysen E, Dhondt AA (1997) Synchronization of hatching date with budburst of individual host trees (*Quercus robur*) in the winter moth (*Operophtera brumata*) and its fitness consequences. J Anim Ecol 66:113–121
Van Zandt PA, Mopper S (1998) A meta-analysis of adaptive deme formation ín phytophagous insect populations. Am Nat 152:595–604
Varley GC, Gradwell GR (1968) Population models for the winter moth. In: Southwood TRE (ed) Symposia of the royal entomological society of London No. 4. Blackwell, Oxford, pp 132–142
Wint W (1983) The role of alternative host-plant species in the life of a polyphagous moth, *Operophtera brumata* (Lepidoptera: Geometridae). J Anim Ecol 52:439–450