The effect of temperature on the development and life cycle regulation of the pine weevil *Hylobius abietis* and the potential impacts of climate change

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Abstract

1. The pine weevil *Hylobius abietis* is widely distributed in the Palaearctic region where it is a major pest. Although predominantly semi-voltine, with a 2-year life cycle, the generation time across its range can vary from 1 to 4 years. The duration of the life cycle and the seasonal timing of weevil activity affect the economic impact and management of this pest, all of which are likely to change in a warming climate.

2. To determine the effect of temperature and tree species on weevil growth and development, laboratory experiments were performed with eggs, larvae, prepupae, pupae and adults, using, as appropriate, the host species Scots pine *Pinus sylvestris* L. and Sitka spruce *Picea sitchensis* (Bong.) Carr. under constant or alternating temperatures.

3. The development rate was linearly related to temperature, with developmental thresholds for eggs, larvae and pupae of 8, 4.5 and 7.3 °C, respectively. Day-degrees were estimated for each life stage. Larval development was affected by tree species, being slower on Sitka spruce than on Scots pine, and was faster under alternating than constant temperatures.

4. The development time for prepupae was highly variable, with an apparent facultative prepupal diapause initiated by temperature. The temperature range 20–17.5 °C marked the transition between median prepupal development times of approximately 25 and 90 days. The prepupal stage may serve to minimize the risk of overwintering mortality in the pupal stage and help to synchronize the life cycle.

5. Larval and adult mass was positively related to developmental temperature, demonstrating an inverse temperature size rule, and weevils were heavier when developing on Scots pine than Sitka spruce. Development in alternating temperatures reduced weevil mass on Scots pine. The influence of temperature on weevil mass is likely to have a positive effect on fecundity and overwintering survival. The effects of climate change on development, voltinism and weevil mass are discussed.

Keywords Climate change, day-degrees, developmental thresholds, prepupal diapause, temperature size rule, thermal threshold, voltinism.

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Introduction

The pine weevil *Hylobius abietis* is a widely distributed species that is associated with conifer forests across the northern Palaearctic region and is a major pest of European plantation forestry (Leather *et al.*, 1999; CAB International, 2003). Only the adult weevils are of economic significance, when feeding on young trees planted after clearfelling. Although weevil abundance in managed forests is an important factor

in determining economic impact, it is nevertheless often considered to be a low density pest. This is largely because a single adult can damage or kill several young plants (Eidmann & Lindelöw, 1997; Wainhouse *et al.*, 2004), and so small numbers of adults emerging from root-stumps can have a significant impact on sites that have been replanted. As a consequence, the duration of the life cycle, the number of seasons over which emergence occurs and its seasonal timing can also have a significant influence on the amount of post-planting damage (Moore *et al.*, 2004; Wainhouse *et al.*, 2007).

The range of *H. abietis* extends latitudinally from the Mediterranean area to northern Scandinavia. The weevils are predominantly semi-voltine, although the life cycle can vary from 1 year in the south to ≥4 years in northern forests and, at any one site, adult emergence may occur over ≥2 years (Bejer-Petersen *et al.*, 1962; Bakke & Lekander, 1965; Bejer-Petersen, 1975; Moore *et al.*, 2004). The main part of the life cycle is spent below ground where larvae feed and develop within the bark of conifer root-stumps of recent clearfells (Leather *et al.*, 1999). Adult weevils typically emerge in the autumn to feed for a variable period before overwintering in the soil or litter layer and then re-emerging in the spring. Adult-acquired resources are essential for reproduction, and probably also for wing muscle development (Örlander *et al.*, 2000; Wainhouse *et al.*, 2004; Tan *et al.*, 2010) during 'maturation feeding'. The main period of feeding, however, occurs in the spring on the bark of seedling trees or twigs of mature trees (Day *et al.*, 2004). Adults subsequently disperse to new clearfells where they oviposit in the bark of root-stumps below ground or in the surrounding soil (Nordlander *et al.*, 1997), typically between May and August.

The geographical variation in voltinism indicates that the weevil has a flexible life cycle responsive to local conditions, of which temperature is likely to be the dominant factor. This is supported by the observation that development in the field is much faster on sunny than on shaded sites (Bakke & Lekander, 1965; Bejer-Petersen, 1975). Factors other than temperature can also contribute to variation in the timing of emergence. *Hylobius abietis* utilizes a number of naturally occurring host conifers across its range, most importantly species of *Pinus* (pines) and *Picea* (spruces). Development on pine, for which the weevil shows a preference, is faster than on spruce (Bakke & Lekander, 1965; Thorpe & Day, 2002) and silvicultural operations can alter the timing of oviposition by influencing the seasonal availability of root-stumps (Lekander *et al.*, 1985; Moore *et al.*, 2004), and therefore whether development occurs over two or more seasons. The thermal requirements of the different developmental stages of pine weevil are largely unknown, in part because the relatively long life cycle is difficult to study. Nor is it known what cues in the subterranean environment influence the seasonal timing of development and adult emergence.

The influence of geographical location and host species on voltinism and seasonal emergence patterns is an important factor in the management of this pest (Örlander *et al.*, 1997; von Sydow, 1997) and understanding how this might be affected by climate change is an important focus of current research. Determining the influence of temperature on development is an essential part of predicting how life cycle duration and seasonal timing of emergence will be affected as the climate warms. Development in a warmer climate is also likely to affect weevil size because, for most insects, size decreases as the temperature during development increases (Angilletta & Dunham, 2003; Kingsolver & Huey, 2008). The effects of climate change on insects will be strongly influenced by their life-history characteristics; for example, whether their life cycle is linked to the availability of transitory food resources or constrained by an obligatory diapause (Bale *et al.*, 2002; Harrington, 2002). A number of forest pests, such as bark and wood boring insects have, like the pine weevil, uni- or semi-voltine life cycles, much of which is spent in a 'protected' microenvironment. Under suitable conditions, these insects may be capable of continuous development on a food resource that is available throughout the year. Within their microenvironment, they may also avoid the increased mortality that could result from large fluctuations in temperature or extreme weather events such as drought or intense rainfall that could occur in a warmer but more unstable climate (Ayres & Lombardero, 2000; Bale *et al.*, 2002; Jenkins *et al.*, 2009).

The present experiments aimed to determine the thermal requirements for pine weevil development on two important conifer species: the native European Scots pine (SP) *Pinus sylvestris* L. and the introduced North American Sitka spruce (SS) *Picea sitchensis* (Bong.) Carr, to provide data for modelling geographical variation in development under current and future climates.

Materials and methods

Collection of adults

Recently-emerged adult *H. abietis* were collected from SP clearfell sites located at Thetford Forest, Norfolk, U.K. (52°26′N; 00°40′E) (August 2008) and Ringwood Forest, Dorset, U.K. (50°53′N; 01°52′W) (August to September 2008; April to May 2009). Clearfells were approximately 12–18 months old and weevils attracted to SP log traps were collected every few days and stored at 2.5 °C without food, for up to 3 months.

Experimental treatments

The lower developmental threshold temperatures, growth and development rates, and day-degree (DD) requirements of different developmental stages of *H. abietis*, were determined at constant and alternating temperatures. Experiments were performed in incubators from which light was excluded but with developmental stages briefly exposed to ambient laboratory light conditions at intervals during assessments. Incubator temperature was monitored using Tinytag temperature data loggers (TGP-4520; Gemini Data Loggers Ltd, U.K.) to confirm accuracy within a range of ± 0.2 °C. To obtain eggs for experiments, adults were removed from storage and kept in mixed sex populations in 1.2-L plastic boxes at ambient laboratory conditions, and allowed to feed and oviposit in the stem bark of young (<5 years) SP twigs. All experimental weighings were carried out on a microbalance (Sartorius Ltd, U.K.) with an accuracy of ± 0.001 mg.

Development of eggs

To minimize variation in the age of eggs at the start of experiments, the stems of young SP in which females oviposited were replaced with fresh stems each morning and evening at intervals of 8–14 h, and eggs were dissected from beneath the bark of the old stems. The time of oviposition was estimated as the half-observation interval. Eggs were transferred to moist filter paper in clear plastic boxes and randomly allocated to a temperature treatment as they became available on different dates during October 2009. In total, approximately 100 eggs were assigned to each of five constant temperatures: 12.5, 15, 17.5, 20 and 25 °C. The eggs were checked for hatching three times daily, at intervals of 4–13 h. Time of hatch was recorded as the half observation interval, and the newly-emerged larvae were weighed.

Development of larvae, prepupae and pupae

For experiments on the development of larvae and subsequent prepupal and pupal stages, eggs were collected daily from the stem material in the oviposition boxes, placed on moist filter paper in clear plastic boxes at room temperature, and examined twice daily for hatching. Larvae that had hatched within the last 12 h were weighed and used in the experiments. The bark on which larvae were fed was taken from newly-felled trees, approximately 20–30 years old, of either SP or SS. Bark (approximately 3–6 mm thick) was removed in sections from most of the trunk up to the lower crown but avoiding the thicker bark on the lowest part of the trunk. Trees of SS with heavily lignified bark were avoided because of its detrimental effects on developing larvae (Wainhouse *et al.*, 1998; Thorpe & Day, 2002). Bark was stored in sealed plastic bags at 2.5 °C for up to 8 weeks for use in experiments, before replacement with newly-collected bark. For experiments, two pieces of bark, up to approximately 9 × 6 cm in size, were held together with their inner, phloem, surfaces in contact by a paper-binding ('bulldog') clip attached along one edge. Experimental larvae were placed individually between the two pieces of bark at a distance from the clip that minimized the risk of crushing while giving appropriate thigmotactic stimulation during feeding on the inner bark. At the start of the experiment, these bark units were placed on moist filter paper in clear plastic containers and transferred to a constant temperature of 17.5 °C for 24 h to allow the first-instar larvae to begin feeding before transfer to experimental temperatures.

For experiments on SP, larvae were randomly allocated as they became available to one of five constant temperatures (12.5, 15, 17.5, 20 or 25 °C) or two alternating temperatures (10/15 °C and 15/20 °C under a 12 : 12 h temperature cycle) where the respective daily temperature accumulations were equivalent to the constant 12.5 and 17.5 °C regimes. Approximately 40 larvae were set up at each temperature in October to November 2008, and a further 50 in June to August 2009. For experiments with SS, to investigate the effect of an alternate host plant on development, 50 larvae were allocated at random to each of the constant temperatures 15, 17.5 and 20 °C in June to August 2009. For all experimental larvae, the bark was replaced at intervals of approximately 10 days before it became

limiting or discoloured in any treatment. From 10–14 days before the end of the larval feeding period, larvae were weighed every 4–5 days to determine the time of maximum mass, which indicated that larval development was complete and that they had stopped feeding. Maximum larval mass (max-mass) was defined as the mass of larvae at the time of the first weighing to be followed by two further weighings showing no increase in mass. This cessation of feeding was also indicated by attempts to construct a pupal cell, and these larvae were termed prepupae. The time of max-mass was determined as the end of the larval and beginning of the prepupal stage. To facilitate construction of the pupal cell at the same time as allowing monitoring of further development, prepupal larvae were placed in predrilled 'cells' in thin sapwood sections from the appropriate tree species covered with a section of fresh bark clipped to the wood. The prepupal larvae were left undisturbed without bark changes and monitored every 4–5 days for pupation and, subsequently, adult eclosion. Newly-eclosed adults were sexed and weighed.

Larval and adult mass, growth rates and survival

Data on the sex of adults, larval and adult mass, development time and mortality was used in a further analysis of the effects of temperature and tree species on mass, growth rate and percentage survival. The growth rate of larvae was determined from their max-mass and development time. Percentage survival was determined for the larval, prepupal and pupal stages.

Statistical analysis

Development of eggs. The effect of temperature on development rate, the reciprocal of the mean development time ($1/d$, where d = days), was modelled using a nonlinear mixed model of the form $y_{ij} = ([\text{temp}_{ij} - (t_0 + \mu_i)] \times b_0) + e_{ij}$, where y_{ij} represents the development rate of the jth egg from the ith date ($i = 1, \ldots, 16$), temp_{ij} is the constant temperature, t_0 and b_0 are, respectively, the fixed-effects parameters for the lower developmental threshold temperature and development rate, μ_i is the random-effect parameter associated with variation between set-up dates, and e_{ij} is the residual error independent of the μ_i, both of which are assumed to be independent and normally distributed. The DD for development above the threshold temperature was determined as $1/b_0$, in this and subsequent experiments. A weighting function proportional to the reciprocal of temperature was applied to the data to ensure that residual errors were normally distributed and similar across the experimental range of temperatures.

Development of larvae, prepupae and pupae. Both larval and pupal development were analyzed using a model similar to that used for eggs. In a preliminary analysis, separate estimates of t_0 for constant and alternating temperatures did not differ significantly. A common value of t_0 was therefore estimated in the model. In a maximal model, factors for tree species (sp), alternating temperature (alt), sex of adults, initial larval mass

(lm) expressed as deviation from mean initial mass, and their interactions, were initially included. Backwards elimination of nonsignificant terms from a maximal model gave a final model for larvae of:

$$y_{ij} = [\text{temp}_{ij} - (t_0 + \mu_i)][b_0 + b_1 \times sp + b_2 \times \text{alt} + b_3 \\ \times (\text{lm} - 0.6)] + e_{ij} \qquad (1)$$

For pupae, the final model was identical to that used for eggs, and a similar weighting function was applied to the data. Prepupal development, unlike that of larvae and pupae, did not exhibit a linear relationship with temperature and a developmental threshold temperature could not be determined. The data on the length of time required to complete development at each temperature were modelled using methods similar to those used in survival analysis. The model was of the form $y = X\beta + \sigma\varepsilon$, where y is a vector of log-transformed prepupal development times, X is a matrix of covariates containing fixed effects for temperature (constant or alternating) and tree species (SP or SS), β is a vector of unknown regression parameters for temperature, σ is an unknown scale parameter, and ε is a vector of errors. Log-likelihood values were used to compare the goodness of fit of models to identify the most appropriate error distribution.

Larval and adult mass, growth rates and survival. Larval and adult mass and larval growth rate were analyzed using a mixed model analysis of variance. Fixed effects were estimated for temperature, its alternating form, tree species, sex and their two-way interactions, and random variance components were estimated for date and the residual insect to insect variability, using the restricted maximum likelihood algorithm. For survival, the fixed effects of developmental stage, constant and alternating temperatures, tree species and initial larval mass were analyzed by fitting a generalized linear mixed model (GLMM). A binomial error distribution and logit link function, $[\log(p/(1 - p))$ where p is the survival proportion, were selected as the most appropriate form of the GLMM considering the survival form of the data. Random date effects were also included in the model and, finally, any observed over-dispersion was taken into account when testing for significance of effects. All analyses were performed using SAS/STAT, version 9.2 statistical package (SAS Institute Inc., 2008).

Results

Development of eggs

Analysis of the relationship between egg development (from oviposition to hatching) and temperature (Fig. 1) showed that there was a significant linear effect of temperature on development rate ($t_{15} = 145.9$, $P < 0.0001$) with an estimated developmental threshold of $8.0 \pm 0.08\ °C$. There was no significant effect of oviposition date on egg development rate. Parameter estimates and significance levels for the analysis of egg development are shown in Table 1. DDs for development to hatching are given in Table 2.
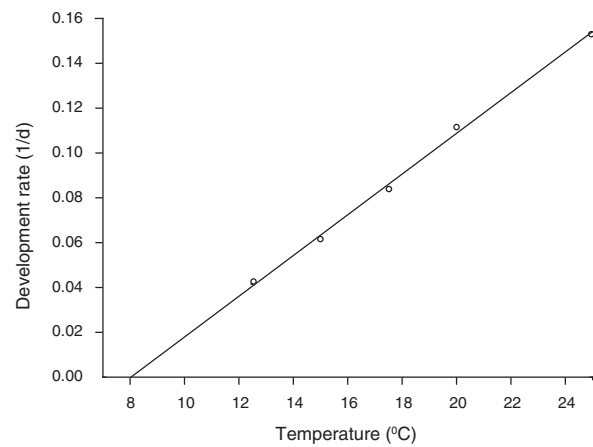
Figure 1 Development rate of eggs of *Hylobius abietis* (from oviposition to hatching) at different temperatures. Mean raw data are presented with the estimated regression line from the nonlinear mixed model. (d, days).

Table 1 Parameter estimates for fixed effects in models of egg, larval and pupal development

Parameter	Estimate	SE	d.f.	t	P
Eggs					
b_0	0.009086	0.000062	15	145.94	<0.0001
t_0	8.0303	0.07868	15	102.07	<0.0001
Larvae					
b_0	0.001304	0.000038	24	34.61	<0.0001
b_1	0.000210	0.000023	24	8.96	<0.0001
b_2	0.000118	0.000036	24	3.26	<0.01
b_3	0.000516	0.000138	24	3.75	0.001
t_0	4.449	0.3941	24	11.29	<0.0001
Pupae					
b_0	0.004569	0.000078	487	58.56	<0.0001
t_0	7.317	0.184	487	39.88	<0.0001

b_0, development rate; b_1, tree species; b_2, alternating temperature; b_3, initial mass of larva;
t_0, lower developmental threshold temperature.

Development of larvae, prepupae and pupae

Parameter estimates and significance levels for the analysis of larval development are shown in Table 1. Larval development rate increased linearly with temperature with a developmental threshold of $4.5 \pm 0.39\ °C$ (Fig. 2). Larvae developed more slowly on SS than on SP, and developed more slowly on pine at constant than at alternating temperature. The development rate increased with the initial mass of larvae and there was also a small effect related to the starting date of experiments. The predicted larval development rate at constant temperatures was approximately 16% faster on SP than on SS (Fig. 2). On SP, larval development was approximately 8% faster under alternating than constant temperature. The sex of the individual, determined at eclosion, had no significant effect on development rate and there were no significant interactions. For pupae, only temperature had a significant effect on development rate, with an estimated developmental threshold of $7.3 \pm 0.18\ °C$ (Fig. 2, Table 1). Estimated DDs for larval and pupal development are shown in Table 2.
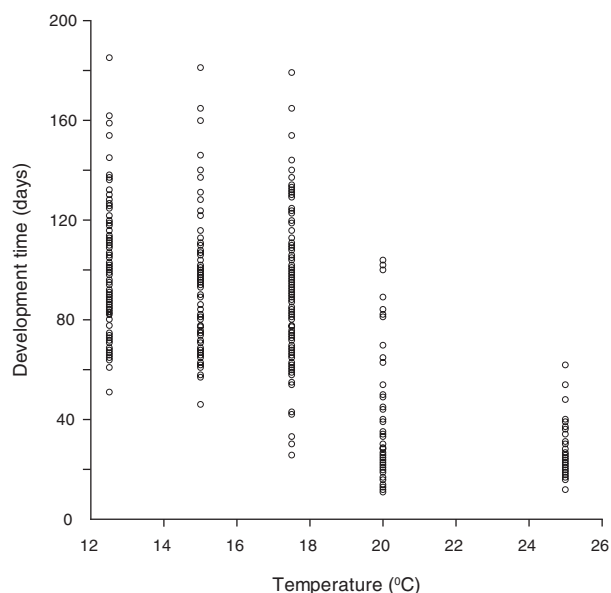
Table 2 Developmental threshold temperatures and estimated day degrees for completion of the egg, larval and pupal stage of *Hylobius abietis* under different experimental conditions

Life stage	Lower developmental threshold temperature (°C) (mean ± SE)	Tree species	Temperature	Day degrees (mean ± SE)
Egg	8.0 ± 0.08		Constant	110 ± 0.8
Larva	4.5 ± 0.39	Sitka spruce	Constant	767 ± 22.2
		Scots pine	Constant	660 ± 15.8
		Scots pine	Alternating	613 ± 22.2
Pupa	7.3 ± 0.18		Constant	219 ± 3.7

The time required for prepupal development is shown in Table 3.
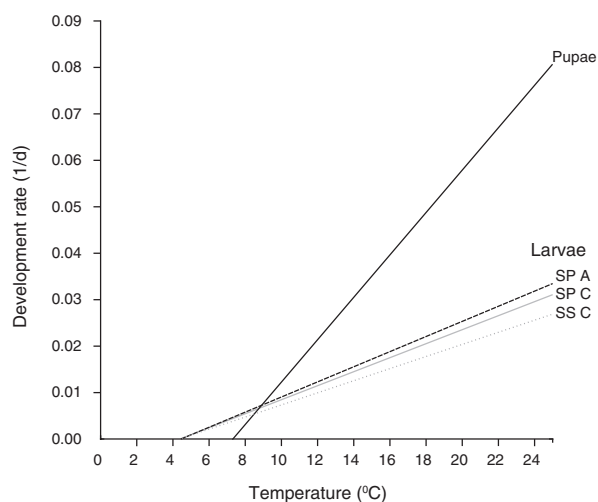
Figure 2 The development rates of larvae and pupae of *Hylobius abietis* in relation to temperature. Using parameters estimated from the nonlinear mixed model, regressions are shown for larvae developing at constant and alternating temperature on Scots pine, at constant temperature on Sitka spruce, and for pupae. (A, Alternating temperature; C, Constant temperature; SP, Scots Pine; SS, Sitka spruce; d, days)

Figure 3 Development time of prepupae of *Hylobius abietis*. Data are presented for individual weevils across all experimental treatments.

For prepupae there was considerable variation in development time, both between and within temperature treatments (Fig. 3). However, a step-like decrease in the time taken between the three lowest temperatures and the highest two of 20 and 25 °C was apparent. The analysis of prepupal development rates simplified to the single explanatory factor of temperature, with no significant effect of tree species, alternating or constant temperature, and with no interactions. The best fitting model used the log-logistic error distribution ($\chi^2 = 1496$, d.f. = 4; $P < 0.0001$). Estimated cumulative distribution functions (Fig. 4) showed three distribution responses corresponding to the temperatures 20 and 25 °C; 15 and 17.5 °C; and 12.5 °C. The predicted number of days to complete prepupal development for the 10%, 50% and 90% quantiles at each temperature are shown in Table 3. At 25 °C, 90% of prepupae were estimated to have completed development by 36 days, whereas, at 12.5 °C, only 10% had done so after 65 days. Estimated median development times (50% quantile) show an approximately four-fold difference in development time between the highest and lowest temperatures. Because the

weevils were essentially quiescent during the prepupal stage, and a developmental threshold cannot be estimated, the median time period (Table 3) was the most appropriate measure of the duration of this stage.

Larval and adult mass, growth rate and survival

In the analysis of larval and adult mass, nine outliers that had an undue influence on the analysis were excluded (three small and six large weevils). There was no indication that development of these individuals was abnormal and they were not removed from the dataset for any other analysis. Temperature had a significant influence on both larval and adult mass (Table 4), with a general increase in mass with temperature up to 20 °C but declining at 25 °C by approximately 10% in larvae ($t_{256} = 3.6$, $P < 0.001$) and approximately 14% in adults ($t_{120} = 5.6$, $P < 0.0001$) (Fig. 5). Tree species, alternating temperature and the sex of adults also affected larval and adult mass. Temperature had the largest effect on mass (Fig. 5) but weevils were 11–14 mg heavier if they either developed on SP rather
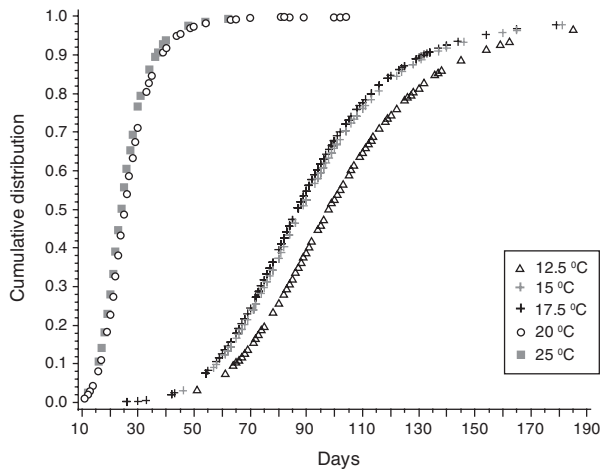
Figure 4 Estimated cumulative distribution functions for the duration of the prepupal stage at different temperatures.

Table 3 Selected quantile estimates of number of days to complete prepupal development (±SE)

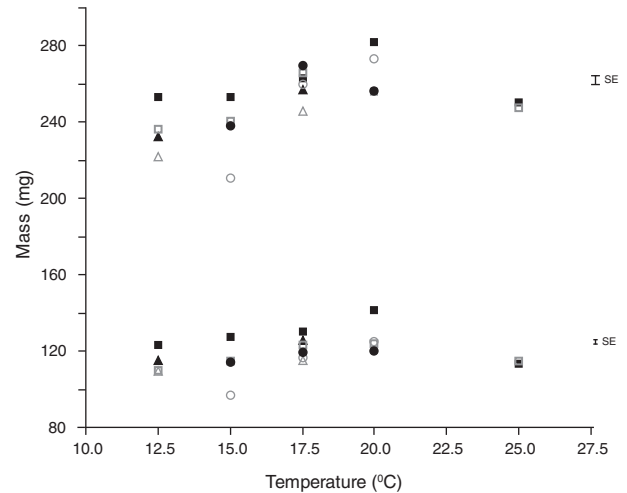| Temperature (°C)	Quantiles (days)		
	10%	50%	90%
12.5	65 ± 2.1	98 ± 2.8	149 ± 4.9
15.0	58 ± 2.2	88 ± 3.0	134 ± 5.0
17.5	57 ± 1.7	87 ± 2.2	132 ± 3.9
20.0	17 ± 0.8	25 ± 1.1	38 ± 1.8
25.0	16 ± 0.7	24 ± 1.0	36 ± 1.6

The median development time (50% quantile) was used as the estimate of stage duration.

Table 4 Effect of temperature, tree species, alternating temperature and sex of adults on larval and adult mass

|	Effect	d.f.	F	P
Larval mass	Temperature	4,314	18.5	<0.0001
	Tree species	1,466	9.3	0.0024
	Alternating temperature	1,312	8.0	0.0051
	Sex of adult	1,459	14.0	0.0002
Adult mass	Temperature	4,159	13.8	<0.0001
	Tree species	1,417	22.6	<0.0001
	Alternating temperature	1,177	3.8	0.0536
	Sex of adult	1,458	27.2	<0.0001

Figure 5 The effect of temperature, tree species and sex of adults on larval mass (upper) and adult mass (lower). ■/□ = female/male, constant temperature, Scots pine; ●/○ = female/male, constant temperature, Sitka spruce; ▲/△ = female/male, alternating temperature, Scots pine. Maximum standard error bars are shown for larval and adult data.
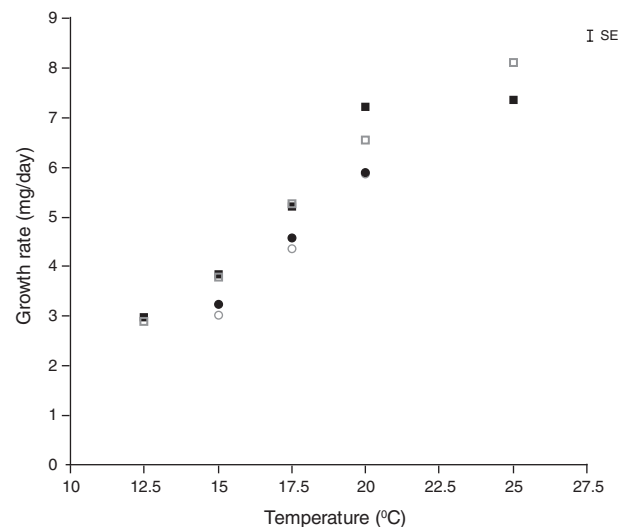
Figure 6 The effect of temperature, sex of adults and tree species on larval growth rate. ■/□ = female/male, constant temperature, Scots Pine; ●/○ = female/male, constant temperature, Sitka spruce. The maximum standard error bar is shown.

than SS, were kept in constant as opposed to alternating temperature or were female.

An initial analysis of larval growth rate showed no significant effect of alternating temperature and no significant interactions, and these were omitted from the final restricted maximum likelihood algorithm analysis. The growth rate of larvae increased over the whole range of temperatures ($F_{4,409} = 298.1$, $P < 0.0001$), levelling off at 25 °C (Fig. 6) but with no significant decline as observed for mass. Larvae grew more slowly on SS than on SP ($F_{1,464} = 95.0$, $P < 0.0001$), and females grew slightly faster than males ($F_{1,452} = 5.4$, $P = 0.02$) (Fig. 6).

In the analysis of mortality, there were no significant effects of temperature, tree species, alternating temperature or their interactions with larval initial mass. Mortality did, however, vary with developmental stage, with most mortality occurring in the larval stage ($F_{2,1723} = 37.4$, $P < 0.0001$). The initial mass of larvae had a significant effect on their mortality but also affected the mortality of prepupae and pupae ($F_{1,518} = 8.55$, $P < 0.01$). The predicted effects on percentage survival of the different stages were derived from the GLMM model, for larvae whose initial mass was 0.5, 0.6 or 0.7 mg, and are shown in Fig. 7.
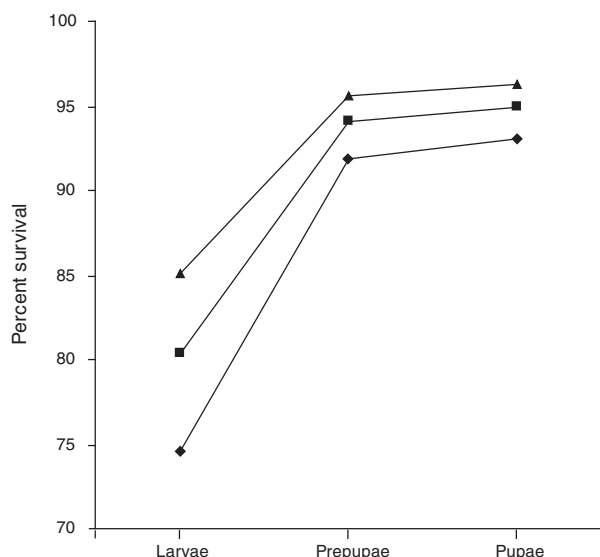
Figure 7 Relationship between initial mass of *Hylobius abietis* larvae and percentage survival at different life stages. Predictions are for percent survival of the number entering the stage. ▲, large larvae (0.7 mg); ■, average-sized larvae (0.6 mg); ◆, small larvae (0.5 mg).

Discussion

The estimated lower developmental threshold temperature for larval development was several degrees below that of eggs or pupae and probably reflects the fact that most larvae pass through at least one winter (Bejer-Petersen *et al.*, 1962; Moore *et al.*, 2004). Temperatures in the root-stump during the winter often exceed this threshold, at least in the U.K. (D. Inward, unpublished data), allowing for some development, although the influence on the life cycle is probably small in the current climate. A linear relationship between temperature and development rate of *H. abietis* larvae was evident across the temperature range studied. However, the faster development of larvae observed in alternating as opposed to constant temperature is a response usually attributed to the Kaufmann effect that results from a degree of nonlinearity in the relationship between temperature and development rate (Kaufmann, 1932; Worner, 1992; Bryant *et al.*, 1999). Our data were too limited to detect nonlinearity but the Kaufman effect appeared to be small. The requirement for larval development was reduced by approximately 7% (47 DDs), with no effect evident on the pupal and adult stages. We did, however, only use two alternating temperature regimes with a diurnal variation of 5 °C, and experiments over a wider temperature range and with greater diurnal fluctuations would be needed to adequately describe the developmental response to alternating temperature. Nevertheless, for larvae developing below ground buffered from seasonal temperature extremes and where diurnal summer temperature variation may be as little as 0.2–0.6 °C at a depth of 30 cm (D. Inward, unpublished data), there would be little effect on development rates. For larvae developing closer to the surface, especially in light soils, or in logs or fallen trees on the ground (Scott & King, 1974), temperature variations would be much larger, resulting in a greater acceleration in development rate.

Although temperature was the predominant factor affecting development rate, host plant species had the next greatest influence. Larvae feeding on SP developed faster than those on the introduced species, SS. This difference was assumed to arise from variation in the nutritional and defensive secondary chemical composition of the bark and reflects the findings of previous studies on host preference and relative suitability (Bakke & Lekander, 1965; Långström, 1982; Wainhouse *et al.*, 1998, 2001; Thorpe & Day, 2002).

The prepupal stage has no discernable developmental threshold temperature. The stage begins when larvae stop feeding, void their gut contents and construct a cell in which they will eventually pupate. The length of time spent in the prepupal stage was highly variable, with a nonlinear relationship between development time and temperature. The temperature range of 20–17.5 °C marked the transition between median development times of approximately 25 and 90 days. This extended prepupal development appears to be a facultative diapause (Tauber *et al.*, 1986; Saunders, 2002), and a prolonged period in the prepupal stage is only avoided when larvae develop at temperatures that are relatively high for a soil environment in temperate regions.

Temperature can play an important role in the regulation of dormancy in many wood and bark-inhabiting beetles (Tauber *et al.*, 1986) and subterranean insects (Leather *et al.*, 1993), in the absence of photoperiodic cues. However, buffered root-stump temperatures are unlikely to be a reliably precise predictor of season length or favourable conditions for the completion of uninterrupted prepupal development (3–4 weeks) and completion of the pupal stage (4 weeks at 15 °C). The fairly high temperature needed to avoid prepupal dormancy therefore represents a conservative response to a seasonal signal used to predict the likelihood of temperatures sufficiently high to ensure adult eclosion before the onset of winter. Prepupal dormancy may therefore function primarily to minimize the risk of overwintering in the pupal stage, which appears to be more vulnerable to mortality at low temperatures. It would also synchronize the life cycle (Hodek & Hodková, 1988; Danks, 1991) because younger larvae could 'catch up' with dormant prepupae as they continue development during the autumn period and, to a greater or lesser extent, during the winter period. A prepupal dormancy has also been reported in Scandinavian populations of *H. abietis*, with extended prepupal development occurring at temperatures less than approximately 21 °C (Eidmann, 1963, 1964; Christiansen, 1971). This somewhat higher temperature and apparently longer prepupal period may indicate an adaptation to the colder northern climate where autumnal temperatures decline more rapidly. Most prepupae that overwinter in the U.K. are likely to have completed their development before the end of winter. In the field, pupation usually begins around mid-June (Moore *et al.*, 2004), when the mean temperature in root-stumps is approximately 12.5 °C (D. Inward, unpublished data). This suggests that temperature, or perhaps rising temperatures, are important in determining the transition to the pupal stage in the field.

The closely-related North American species *Hylobius pales* Herbst appears to have a similar dormant period in the prepupal stage when development occurs at temperatures below 21 °C (Salom *et al.*, 1987). Although this suggests a common ancestry

of the effect of temperature on prepupal development and an important role in the life cycle, there was considerable phenotypic variation in the length of the prepupal period in *H. abietis*. This variability could be maintained by a significant fitness advantage for weevils of first emergence in the autumn. Adults are reproductively immature when they emerge, and may also have undeveloped wing muscles (Nordenhem, 1989; Tan *et al*., 2010). Emergence and feeding during the autumn can therefore be important for the completion of wing muscle development and the initiation of egg development. Autumn feeding may also increase overwintering survival, perhaps related to the size of the fat body (Guslits, 1969; Ohgushi, 1996), and weevils could re-emerge relatively early in spring to complete reproductive development.

All larvae exhibited lower growth rates on SS than SP and, despite an increased development time on spruce, final insect masses remained lower, supporting earlier findings that this is a sub-optimal host (Bakke & Lekander, 1965; Wainhouse *et al*., 2001; Thorpe & Day, 2002). Female *H. abietis* were heavier than males and had a higher growth rate, although the difference in mass was unaffected by tree species or alternating temperature and so there was no evidence of a sex difference in body size plasticity (Stillwell *et al*., 2010). For both sexes, there was an overall increase in mass with temperature, although there was a significant drop in mass (10–14%) at the highest temperature of 25 °C. There appeared to be some levelling off of the growth rate at 25 °C, although the development rate was unaffected. Because insect size is an exponential function of the ratio of growth/development (Gilbert & Raworth, 1996), the resultant small change in this ratio is a likely explanation of the observed effects on mass. Similarly, larvae developed slightly faster in alternating temperatures, whereas growth rate was unaffected and, as a consequence, both larval and adult mass was reduced in comparison to development at constant temperature.

Most insect species mature at a smaller size when developing at higher temperatures, which is a well known effect called the temperature-size rule (Atkinson, 1994; Angilletta & Dunham, 2003; Kingsolver & Huey, 2008). Some insects, however, are an exception to this rule (Atkinson, 1994; Walters & Hassall, 2006), including, as demonstrated in the present study, *H. abietis*. A suggested common characteristic of species that follow an inverse temperature-size rule is that their growth rate threshold temperature is higher than the developmental threshold (Walters & Hassall, 2006). Analysis of our pooled data on larval growth rate gave an estimated growth threshold of 5 °C on SP, and a significantly higher one of 7.4 °C on SS ($P < 0.0001$). These growth rate thresholds exceeded that for development, indicating that *H. abietis* conforms to this generalization. The positive relationship between rearing temperature and mass in *H. abietis* may be facilitated by particular aspects of the life cycle such as the absence of seasonal time constraints that can limit adult size (Nylin & Gotthard, 1998). *Hylobius abietis* has a predominantly semi-voltine life cycle, with food normally available throughout the year, and so completion of the flexible life cycle need not be limited by season length. The increase in mass with temperature may also be adaptive because there are potential fitness advantages in addition to that of increased fecundity.

For example, larger female *H. abietis* lay larger eggs, with consequent maternal effects on larval survival (Wainhouse *et al*., 2001). In the present study, the initial mass of larvae was positively related to development rate and was also correlated with mortality, with over half of the total number of larval deaths (55%) occurring in the smallest first instars.

Because of the variable length of the prepupal period, and the temperature dependent development of eggs, larvae and pupae, the length of the life cycle from egg to adult took an average of 99 days at 20 °C and 252 days at 12.5 °C. However, the length of the life cycle in *H. abietis* is best estimated using a combination of physiological time (DD) for all stages except prepupae, and calendar time for the duration of the prepupal stage. Prepupal dormancy lasts approximately 90 days so that, for an average root-stump temperature of 15 °C, development would take 1100 DD + 90 days on SS or 990 DD + 90 days on SP. Although development may vary somewhat under field conditions, our laboratory data are compatible with estimates based on existing field studies. For example in Ae forest, Dumfries, Scotland (Moore *et al*., 2004), the pupal development period lasts for approximately 30 days between mid-July and mid-August. With an estimated average root-stump temperature of 14.8 °C for this site (D. Inward, unpublished data), this indicates that pupal development in the field requires 225 DDs, which is very similar to the estimate from our laboratory study of 219 DDs.

The influence of temperature on voltinism in *H. abietis*, and how this might change as the climate warms, is the subject of a current study. Mean summer temperatures in the U.K. are predicted to rise by around 2.5 °C in Scotland, and 4 °C in southern England by the 2080s under a medium emissions scenario (Jenkins *et al*., 2009). In the temperate parts of its range, *H. abietis* usually has a 2-year generation time (egg–egg) (Bejer-Petersen *et al*., 1962; Bakke & Lekander, 1965; Lekander *et al*., 1985) but, in northern regions, this may extend to ≥4 years. With a flexible life cycle passed predominantly within an environment protected from climatic extremes, few seasonal constraints on food availability and the potential for larval development through the winter, it is likely to be influenced by both the warmer summers and winters predicted under climate change. Higher temperatures would increase the development rate and reduce the occurrence of prepupal dormancy, resulting in an increase in uni-voltine development. As a consequence of the inverse temperature-size rule, higher temperatures would result in larger weevils with increased fecundity and maternal effects. In addition, because the adults are large relative to seedling trees and large weevils have a higher feeding rate than smaller ones (Wainhouse *et al*., 2004), they may be more damaging to individual plants. As for some other insects, *H. abietis* appears to be pre-adapted to 'benefit' from predicted changes to the climate (Bale *et al*., 2002) and this has important implications for its management.

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