Host-plant quality adaptively affects the diapause threshold: evidence from leaf beetles in willow plantations

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Abstract

Voltinism (the number of generations produced per year) of herbivorous insects can vary depending on environmental conditions. The leaf beetle *Phratora vulgatissima* is commonly univoltine in central Sweden but will sometimes initiate a second generation on coppiced willows (*Salix viminalis*) grown in plantations for bioenergy purposes. The study investigated whether increased voltinism by *P. vulgatissima* can be explained by (1) rapid life-cycle development in plantations allowing two generations, or (2) postponed diapause induction on willows grown in plantations. In the field, no difference was found in the phenology or development of first-generation broods between plantations (*S. viminalis*) and natural willow habitats (*S. cinerea*). On re-sprouting shoots of recently coppiced *S. viminalis*, however, the induction of diapause occurred 1-2 weeks later than on mature (un-coppiced) plants. A laboratory experiment indicated no genetic difference in the critical day-length for diapause induction (CDL) between beetles from plantations and natural habitats. Although development time was unaffected by host-plant quality, CDL was prolonged by almost an hour when the beetles were reared on a sub-optimal willow species (*S. phylicifolia*). A subsequent experiment found that diapause incidence, when the beetles were reared on new leaves from re-sprouting shoots of previously coppiced *S. viminalis* and *S. cinerea* plants, was significantly less than when the beetles were reared on mature leaves from un-coppiced plants. The study suggests that *P. vulgatissima* has a plastic diapause threshold influenced by host-plant quality. The use of host-plant quality as a diapause-inducing stimulus is likely to be adaptive in cases where food resources are unpredictable, such as when new host-plant tissue is produced after a disturbance. Coppiced willows may allow two beetle generations because re-sprouting willows continue to produce new leaves throughout the season.
The number of generations that insects produce per year (i.e. voltinism) is an important life-history trait that can strongly affect population growth, fitness and potential rate of adaptability (Kurota and Shimada 2002, Steinbauer et al. 2004, Yamanaka et al. 2008). Voltinism in herbivorous insects often varies across latitude and altitude (Tauber et al. 1986, Masaki 1999). Insects with a wide distribution range may therefore produce one generation per year (univoltine life-cycle) at northern latitudes where the growing season is relatively short, but produce two (bivoltine) or even more (multivoltine) generations further south. Such latitudinal clines in voltinism partly reflect local adaptations to seasonal environments, with a genetic basis, but plasticity in life-history traits is also a crucial component of the insects’ seasonal adaptations (Nylin and Gotthard 1998). Plasticity in voltinism may allow the insect to immediately adjust the number of generations in response to prevailing environmental conditions, without need for selection to operate, and there is indeed evidence that recent climate change has led to extra generations in response to warmer temperature conditions, through plasticity (Altermatt 2010, Poyry et al. 2011). For most insects, however, we have too limited knowledge about what processes influence such life-history plasticity to be able to predict under what circumstances voltinism may change.

In temperate climate zones, insect voltinism is determined by the number of generations produced before the seasonal timing of winter diapause (Tauber et al. 1986, Danks, 2007). Diapause is a dormant stage characterized by lowered metabolic rates, cold hardening and cessation of reproductive development in insects that overwinters as adults (Kostal 2006). Many insects have a facultative diapause, i.e. individuals make a “decision” during development to
either enter diapause (thus, to wait with reproduction until the following year) or to exhibit direct
development and to become reproductively active as adults and produce another generation
within the same year (Gotthard 2008). This decision-making is determined by seasonal cues to
which the insects respond for the induction of diapause, with day-length (photoperiod) being the
most important cue (Nelson et al. 2010, Saunders 2010). Hence, in insects with facultative
diapause voltinism is in a sense always a plastic trait, responding primarily to day-length. On the
other hand, the critical day-length (CDL), defined as the day-length when 50% of a population
enters diapause (Tauber et al. 1986, Saunders 2010) is a genetically determined property that
varies adaptively among insect populations (Bradshaw 1976, Solbreck and Sillen-Tullberg 1981;
Masaki 1999, Dalin et al. 2010). This critical day-length sets the timing for when diapause
induction occurs over the course of the year and can therefore severely limit the possibilities for
altered voltinism (Tobin et al. 2008).

Importantly, however, other factors that - in contrast to day-length - can vary from year to year
as well as seasonally, such as temperature and (in the case of herbivorous insects) host-plant
quality, may also plastically affect the incidence of diapause and hence voltinism (Tauber et al.
1986). These effects can be indirect or direct, and it is seldom clear whether they are adaptations
per se, i.e. have been selected for rather than just having incidental positive effects on fitness
(Gotthard and Nylin 1995). Both temperature and host-plant conditions can strongly influence
growth and development during the season and will in the field therefore indirectly influence
voltinism by affecting the timing of when the insect reaches the critical stage for diapause
induction. For example, slow growth on a poor host-plant or in response to low temperature
conditions will delay the critical stage for diapause induction. If the critical stage is reached after
day-length has declined below CDL; the insect will choose the developmental pathway leading to diapause. Furthermore, laboratory studies suggest that temperature and host-plant quality can modify the insects’ photoperiodic responses (Masaki 1999, Ishihara and Ohgushi 2006, Dolezal and Sehnal 2007, Dalin et al. 2010), and such more direct effects on voltinism are stronger candidates for being true adaptations to environmental variation (in the sense of Gotthard and Nylin 1995). Since temperature - for physical and chemical reasons - affects so many processes in the insect, it may be almost impossible to disentangle adaptive responses to temperature from spurious indirect effects, but effects of host plant quality provides an interesting opportunity for a deeper understanding of voltinism plasticity.

For example, Hunter and McNeil (1997) showed that the generalist lepidopteran Choristoneura rosaceana (Lepidoptera: Tortricidae) was more likely to enter diapause when reared on a poor quality food than when reared on high-quality food under controlled laboratory conditions, and similar results were found in the polyphagous comma butterfly Polygonia c-album (Wedell et al. 1997). These studies suggest that food quality can influence the induction of diapause and voltinism of herbivorous insects, and such a plastic diapause threshold could prevent the insects from producing maladaptive generations on host-plants of low or declining quality. A poor host-plant may indicate that the plant cannot support rapid-enough growth and development for another generation to develop within the same year and, thus, that it is better to wait with reproduction until the following year. Even in these laboratory experiments, however, it is still not clear whether the insects can use the chemical properties of the host-plant as a direct signal or cue – similar to photoperiod – influencing the induction of diapause, or whether the potentially adaptive response is rather to growth rate, as determined by host quality, or indeed
even simply constitutes a spurious physiological side-effect of the host plant (Wedell et al. 1997).

The leaf beetle *Phratora vulgatissima* is an important pest in willow plantations grown for bioenergy in northern Europe (Sage and Tucker 1998, Björkman et al. 2000, Dalin et al. 2009). It is commonly univoltine in northern Europe and bivoltine in central Europe. The species sometimes initiate a partial second generation also in northern Europe (Dalin 2011). This has particularly been observed in short-rotation coppiced willow (*Salix viminalis*) plantations grown for biomass productions in Sweden (P. Dalin, pers. obs.). The leaf beetle overwinters in the adult stage and emerges from overwintering sites in the spring. The phenology of adult emergence is usually well synchronized with willow bud-break in the spring. Adults feed on newly developed leaves and oviposit on the ventral side of the leaves. Larvae of the first generation continue to feed on leaves during the summer before they pupate in the soil. The next generation of adult beetles (first-generation adults) emerges in late July or beginning of August in central Sweden (Dalin 2011). These adults are normally in reproductive diapause and become the overwintering generation. However, those individuals that complete development to adulthood before August may become reproductively active and initiate a second generation (Dalin 2011).

The purpose of this study was to investigate whether the partial second generation of *P. vulgatissima* in willow plantations could be explained by (1.) advanced phenology and accelerated development of first-generation broods in willow plantations, or (2) postponed diapause induction of beetles in willow plantations. The central Swedish population that we study has previously been shown to have a facultative diapause induced by day-length with a
CDL estimated to be 18 hours 10 minutes (Dalin 2011). A second purpose of our study was to investigate whether CDL may differ between *P. vulgatissima* populations from willow plantations (*S. viminalis*) and natural willow stands (*S. cinerea*). This was tested by rearing the insects under controlled conditions in the laboratory. A genetic difference in CDL between host-populations, with an expected shorter CDL in beetles from plantations, could explain why the species is more likely to produce a second generation in plantations. We also tested the hypothesis that *P. vulgatissima* has a plastic diapause threshold that is influenced by host-plant quality. First, we predicted CDL to be prolonged when the species was reared on a sub-optimal willow; in this case *S. phylicifolia* that contains relatively high concentrations of phenolic glycosides. Second, if the beetles are able to postpone diapause in response to vigorous host-plant growth on previously coppiced willows, we predicted diapause incidence to be reduced if the species was reared on newly produced leaves from coppiced willows (*S. viminalis* and *S. cinerea*) than when reared on older leaves from mature and un-coppiced plants.

**Materials and Methods**

*Life-cycle development and diapause induction of field populations*

During the summer 2009, we studied when natural populations of *P. vulgatissima* enter diapause in the field on *S. viminalis* in plantations and on *S. cinerea* in natural willow habitats. A postponed diapause induction in plantations could explain why the species is more likely to initiate a second generation in this habitat. In the following year (2010), we studied the phenology and life-cycle development of first-generation broods of naturally occurring populations of *P. vulgatissima* in one willow plantation and one natural willow habitat in the field. If the beetles are able to complete development of the first generation faster in willow
plantations, this could explain why *P. vulgatissima* is more prone to produce a second generation in this habitat.

From mid-July in 2009 (Julian date: 196), when the first-generation adults started to emerge in the field, we collected adult beetles once every week to estimate the proportion of females in diapause over time. The study was done at two willow plantations (*S. viminalis*) and two natural willow habitats (*S. cinerea*) located within 20 km from the Ultuna campus of the Swedish University of Agricultural Sciences in Uppsala (59°49’N, 17°40’E). The first willow plantation consisted of newly coppiced *S. viminalis* plants (first-year shoots) growing in an experimental bioenergy plantations near the campus (Weih and Nordh 2005). The second plantation consisted of more mature *S. viminalis* plants that had been left to grow for five years since the last coppice. The two natural habitats consisted of mainly mature *S. cinerea* plants growing in a mixed conifer forest, but also a few coppiced plants with re-sprouting shoots that had been cut back by a harvester machine during the previous year to prevent the trees from interfering with traffic on a nearby road. The four sites were chosen because they were easy to access and harbored similar and moderate densities of *P. vulgatissima*. Female beetles were collected from plants by the hand and beetles were brought to the laboratory and dissected under a microscope to confirm reproductive status (Dalin 2011). Collections were made on July 15, July 22, July 29, August 7, and the last collection was made on August 14 (Julian date: 226) when all (100%) females were found to be in diapause at all four study sites. The proportion of beetles in diapause was plotted over time. Due to poor emergence of adult beetles at one of the natural sites, data from the two natural habitats were pooled together in figure 1.
From mid April to October (Julian dates 102-285) in 2010, we monitored the phenology and life-cycle development of *P. vulgatissima* in one willow plantation (*S. viminalis*) and one natural willow habitat (*S. cinerea*) near Uppsala (59°53’N, 17°38’N). The *S. viminalis* plantation consisted of two-year old shoots (coppiced during the winter 2008/2009) whereas the natural habitat consisted on mature (un-coppiced) *S. cinerea* plants. The two sites were visited at least once, but most often twice, per week to estimate the number of adults, eggs and larvae of *P. vulgatissima* on the plants in the two habitats. The number of individuals in different developmental stages was counted during five-minute observation periods. One five-minute period was devoted to search for adult beetles on the dorsal side of leaves. Another five-minute period was devoted to search for eggs and larvae on the ventral side of leaves. The two sites were visited on the same days and observations were mainly done during days with no precipitation and minimal wind. The number of counted individuals in the different life-stages was plotted over time.

**Critical day-length response for diapause induction— genetic difference between populations or phenotypic plasticity to host-plant quality?**

The aim of this experiment was to: (1.) investigate if photoperiodic responses differ between *P. vulgatissima* beetles originating from plantations and natural willow habitats, and (2.) study if photoperiodic responses can be plastic in response to host-plant quality. From a previous study we know that *P. vulgatissima* respond to photoperiod for the induction of diapause. The critical day-length for the induction of diapause was estimated to be 18 hrs 10 min when the beetles were reared on greenhouse grown *S. viminalis* at 20°C in the laboratory (Dalin 2011).
Life history theory predicts that univoltine populations should have a longer CDL than bivoltine population at the same latitude and altitude (Roff 1980, Tauber et al. 1986). This is because univoltine populations need to enter diapause earlier in the season, at a time-point when day-lengths are longer, to avoid producing additional generations that may be unable to complete development to the diapausing stage before the onset of winter. Consequently, based on the observation that *P. vulgatissima* sometimes produce a second generation in plantations, we predicted that CDL should be longer in univoltine populations from natural habitats than in partially bivoltine populations from plantations.

An alternative hypothesis was that the induction of diapause can be influenced by host-plant quality. This phenotypic plasticity hypothesis predicts that CDL can be modified by host-plant quality. More specifically, we predicted that the propensity of diapause should increase when the insects were reared on a sub-optimal host-plant. To test this hypothesis, we reared the insects on two different willow species: *S. viminalis* which is frequently fed upon by *P. vulgatissima* in plantations, and *S. phylicifolia* which is a native willow growing along creeks and rivers in central Sweden but which is avoided by *P. vulgatissima* due to high concentrations of phenolic glycosides in the leaves (Kendall et al. 1996).

Stem cuttings were collected in January 2010 from *S. viminalis* (clone 78021, used in Dalin 2011) growing in experimental plantations at the Ultuna campus, and from wild *S. phylicifolia* growing along the river Fyrisån near the campus. Stem cutting were planted in individual pots and placed in a greenhouse for shoot growth before the start of the experiment. When the plants had started to produce foliage (in February), we collected overwintering beetles from two
populations, one originating from a *S. viminalis* plantation (59°56’N, 17°28’E), and one from a natural *S. cinerea* stand located about 17 km east of the willow plantation. Beetles from the willow plantation originated from the same population that was used in Dalin (2011). The two *P. vulgatissima* populations were first reared for one generation under controlled conditions in a greenhouse (20:4 L.D; 15-20°C) to reduce potential influence of maternal effects on diapause incidence. The two populations were then reared for another generation in the experiment (from eggs to adults) on leaves of greenhouse grown *S. viminalis* and *S. phylicifolia* under controlled conditions inside climate chambers (AB Ninolab, Upplands-Väsby, Sweden, Termaks Model KB8400L). We used a similar experimental procedure as in Dalin (2011), including four climate chambers with separate photoperiods (20:4, 19:5, 18:6 and 17:7 light:dark cycles) and constant 20°C temperature. In the climate chambers, we reared the beetles in groups of 50-100 larvae inside transparent plastic containers (19x19x11cm). We used two replicate containers per photoperiod, host-plant and population treatments (16 containers in total). The containers were sealed with a mesh net over the open top to provide ventilation. The number of emerging adult beetles was counted every 2-3 days when fresh leaves were provided to ensure that larvae always had a surplus of food. Pieces of wet oasis were placed at the base of leaf petioles to provide moisture to the leaves. A layer of potting soil mixed with sand was added to the bottom of the containers to be used as pupation substrate by larvae.

Emerging adult beetles were removed and kept in separate containers provided with fresh leaves under the same experimental conditions as the beetles had been raised from eggs. The adults were allowed to feed and mate for approximately 14 days. Female beetles were then dissected under a microscope to confirm reproductive status (diapause or reproductively active).
The propensity of diapause in *P. vulgatissima* was analyzed using logistic regression (PROC GENMOD, binomial, logit; SAS Institute, 2008). Reproductive status (diapause or reproductively active) of individual female beetles was the dependent, binary response variable (1 for diapause, 0 for reproductively active). Thus, we pooled the results from the two replicate containers and treated each female as an individual observation in the analyses (Dalin, 2011). Photophase (hours of light), population origin and host-plant species were used as independent categorical variables. Logistic regressions with inverse predictions (PROC PROBIT INVERSECL, SAS Institute 2008) were used to calculate critical day-lengths (±95% confidence interval) (Dalin et al., 2010). Development time (i.e. the number of days it took for development from eggs to adult eclosion) was compared among treatments using two-way ANOVA and Tukey test for post-hoc treatment comparisons (PROC GLM, SAS Institute, 2008). The mean number of days to adult eclosion was calculated for each replicate container to be used as individual observations in the analysis.

*Diapause incidence on coppiced versus mature willow plants*

A second laboratory experiment was conducted in 2011 to further investigate the effect of host-plant quality on diapause incidence in *P. vulgatissima*. The purpose of this experiment was to test whether diapause in adult females is reduced when the beetles are reared on leaves from previously coppiced and vigorously growing willow plants. For this experiment, we used a mixture of beetles collected from willow plantations and natural willow habitats. The beetles were exposed to three host-plant treatments: (1.) leaves from re-sprouting shoots of previously
coppiced *S. viminalis*, (2.) leaves from re-sprouting shoots of experimentally coppiced *S. cinerea*, and (3.) leaves from mature (un-coppiced) *S. cinerea* trees.

The beetles were collected in the field as eggs in May 2010. The proportion of eggs collected from plantations and natural habitats was approximately 50:50. Larvae were reared to adulthood under controlled conditions in a greenhouse to reduce maternal effects. Eggs from the second generation were then distributed between nine (3x3) rearing containers (see above) inside a climate chamber with constant 18.50 hours of light (photophase) and 20°C. One container per host-plant treatment was placed on three separate shelves (top, middle and bottom shelf) inside the chamber. The groups of containers located on different shelves were treated as blocks in the statistical analysis (described below). The specific photoperiod condition was chosen based on the previous experiment indicating that diapause incidence will vary among individuals when reared under this condition. Thus, we wanted to avoid all individuals becoming either reproductively active or in diapause.

The beetles were fed fresh leaves collected from plants in the field every 2-3 days. The coppiced plants used in the experiment had been coppiced (complete removal of shoots and branches) in the previous year. The coppiced *S. cinerea* plants were located less than five meters away from the mature *S. cinerea* to receive similar growth conditions of the two *S. cinerea* treatments. The *S. viminalis* plants were growing in experimental plantations near the Ultuna campus (see description of site above). Leaves collected in the field were immediately transported to the laboratory and fed to larvae. We used similar methods described above for the rearing and testing of reproductive status in female beetles.
The propensity of diapause in relation to host-plant treatments was analyzed using logistic regression (PROC GENMOD, binominal, logit; SAS Institute 2008). Reproductive status of individual female beetles was again used as the dependent, binary response variable (1 for diapause, 0 for reproductively active) and host-plant treatment and block the independent categorical factors. We also scored the amount of fat-bodies in the abdomen of diapausing females as either small or large amounts. Chi-square tests were used to compare fat-bodies among host-plant treatments. In the analyses of fat-bodies, we pooled results from the three blocks. The total number of females included in the analysis of fat-bodies was 15 for S. *viminalis*, 50 for coppiced S. *cinerea* and 71 for mature S. *cinerea*. The size of adult females was also estimated by measuring the width of the thorax using a scale in a microscope (9x magnification lens). Data from 19-31 females per treatment were included in a one-way ANOVA (PROC GLM; SAS Institute 2008) with host-plant treatment the independent factor.

**Results**

*Life-cycle development and diapause induction of field populations*

In the field, we found that first-generation adults of *P. vulgatissima* became reproductively active if they enclosed from pupation before August in 2009 (before Julian date 205 in Fig. 1).

Although most beetles emerged later (around mid-August) and were in diapause, we observed mating by first-generation adults in July on recently coppiced plants of both *S. viminalis* and *S. cinerea* in the two habitats. However, the natural habitat mainly consisted of mature (uncoppiced) *S. cinerea* plants on which we did not observe any mating. Overall, diapause induction occurred earlier in the willow plantations with un-coppiced plants (five year-old shoots) and in
the natural habitat (late July), whereas first-generation adults remained reproductively active at least until early August in the recently coppiced *S. viminalis* plantation (Fig. 1).

In the subsequent year (2010), we did not find any differences in the phenology of adult emergence from overwintering in the spring, or in the development of the first-generation broods during the summer, or in the phenology of when first-generation adults enclosed from pupation, between the willow plantation and the natural habitat studied (Fig. 2). However, we found that first-generation adults produced a small partial second generation in the willow plantation by late July-August in 2010 (Julian date 225-240), which was not observed in the natural habitat (Fig. 2).

Critical day-length responses for diapause induction—genetic difference between populations or phenotypic plasticity to host-plant quality?

The laboratory experiment showed significant effects of photophase (hours of light) and host-plant species on diapause incidence in *P. vulgatissima* (Table 1). Fig. 3 shows that the proportion of females in diapause decreased with increasing day-length, and that a higher proportion of females entered diapause on *S. phylicifolia*. Diapause incidence was also marginally affected by population origin (*P* = 0.049), with beetles originating from the natural willow habitat having a higher diapause incidence than beetles from willow plantations. The non-significant Population x Photophase interaction suggests however that the two populations responded similarly to day-length for the induction of diapause (Table 1). We found a significant Host-plant species x Photophase interaction, indicating different day-length responses of *P. vulgatissima* on the two willow species (Table 1, Fig. 3). CDL was estimated to be 18.08 hours (95% confidence interval:
17.94-18.22 hrs) or 18.20 hrs (18.06-18.33) on *S. viminalis* (estimations for beetles originating from willow plantation and natural willow habitat, respectively); and 18.77 hrs (18.53-19.03) or 19.03 hrs (18.66-19.53) on *S. phylicifolia*.

Developmental time was significantly affected by day-length, but not by host-plant species or population origin (Table 2). Fig. 4 shows a significant reduction in developmental time for beetles reared under the shortest day-length treatment (17 hours of light).

Diapause incidence on coppiced versus mature willow plants

Diapause incidence in female *P. vulgatissima* was significantly affected by host-plant treatments ($\chi^2 = 58.88, d.f. = 2, P < 0.001$; Fig. 5). The proportion of females in diapause was 95 ± 5% ($n = 75$) on leaves from mature *S. cinerea* plants (means ± standard errors calculated from three replicate rearing containers), 60 ± 11% ($n = 83$) on coppiced *S. cinerea*, and 39 ± 9% ($n = 72$) on coppiced *S. viminalis*. Diapause incidence was unaffected by the placement of containers within the climate chamber, as shown be the non-significant block effect ($\chi^2 = 3.87, d.f. = 2, P = 0.15$).

The amount of fat-bodies stored in the abdomen of diapausing females was higher in beetles reared on the two coppiced treatments: coppiced *S. viminalis* vs. mature *S. cinerea* ($\chi^2 = 19.77, d.f. = 1, P < 0.001$), coppiced vs. mature *S. cinerea* ($\chi^2 = 25.94, d.f. = 1, P < 0.001$). No difference was found between the two coppiced treatments of *S. viminalis* and *S. cinerea* ($\chi^2 = 1.60, d.f. = 1, P > 0.20$). Host-plant treatments did not affect the size of adult females, as measured by the width of the thorax ($F_{2,69} = 0.69, d.f. = 2, P = 0.50$).
**Discussion**

The leaf beetle *P. vulgatissima* sometimes initiate a second generation in short-rotation coppiced willow plantations in central Sweden. During 2010, we found that the beetles produced a partial second generation in a *S. viminalis* plantation but not in a nearby natural *S. cinerea* habitat. This second generation could not be explained by different phenology or development of first-generation broods between the two habitats. However, the seasonal timing of diapause was found to differ among leaf beetle populations in the field with diapause occurring 1-2 weeks later in coppiced willow plantation than in mature (un-coppiced) willow stands. A postponed (later) diapause induction could explain why the beetles sometimes initiate a second generation in plantations.

Using climate chamber experiments, we did not detect any difference in the critical day-length (CDL) response for diapause induction between beetles originating from plantations and natural habitats. However, the propensity to enter diapause was significantly affected by host-plant quality and was reduced when the beetles were reared on leaves from re-sprouting shoots of previously coppiced willow plants than when reared on leaves from mature plants. Moreover, diapause incidence was significantly reduced on the willow *S. phylicifolia* compared with *S. viminalis*. The results suggest that host-plant quality influenced diapause induction in *P. vulgatissima*. Willow plantations are coppiced for woody biomass every 3-4 years which stimulates compensatory plant growth. The shoots of re-sprouting willows continue to elongate and produce new leaves over the course of the season whereas mature plants cease leaf production around mid-summer (Nakamura et al. 2005, P. Dalin pers. obs.). This implies that coppiced willow may provide new leaves during an extended period of time, which may support
the development of a second beetle generation. Although we lack information about the performance of second-generation larvae, the results suggest that the current harvesting regime, where willow plantations are coppiced every 3-4 years, can induce postponed diapause of \textit{P. vulgatissima} resulting in a second generation.

The fact that host-plant conditions can affect voltinism of herbivorous insects is not new and has been documented in a number of insect species (Tauber et al. 1986, Hunter and McNeil 1997, Wedell et al. 1997, Ishihara and Ohgushi 2006, Takagi and Miyashita 2008). However, host-plant quality may influence insect voltinism both directly and indirectly (Wedell et al. 1997), although few studies have been able to separate these effects experimentally. First, host-plant quality can have a “trivial” indirect effect on insect voltinism in the field by affecting the timing of when the insects reach the critical stage for diapause induction during development. In the current study, we did not detect any difference in the development or seasonal occurrence of naturally occurring leaf beetle populations between plantations and natural habitats, although the beetles produced a second generation in the plantation. We therefore believe that we can reject the “trivial-effect hypothesis” as an explanation to why the beetles sometimes produce a second generation in plantations.

Larval host-plants may also affect the propensity of insects to enter diapause. This may either occur as a direct response to cues from the host-plant or more indirectly via altered insect growth (Wedell et al. 1997). Such plasticity in diapause threshold could prevent insects from producing extra generations on a host-plant of poor or declining quality, a situation where their offspring may fail to complete the extra generation. To our knowledge, no study has been able to confirm
that insects respond directly to host-plant traits for the induction of diapause. This is because diapause propensity often co-varies with insect development, such as growth rates, which also may influence the choice of developmental pathway (Hunter and McNeil 1997, Wedell et al. 1997). Several studies show that insects are more likely to exhibit direct development (e.g. produce another generation) when reared on host-plants that support rapid larval development (Hunter and McNeil 1997, Wedell et al. 1997, Ishihara and Ohgushi 2006). This “growth-rate hypothesis” predicts that insects can make use of their own growth rate as a cue to predict future conditions and for choosing developmental pathways (Wedell et al. 1997). Feeding on a high quality host-plant may, for example, indicate that the focal host-plant can support rapid development not only in the present, but also in the future, which then may allow another generation to develop within the same year.

We believe that our study reveals evidence that *P. vulgatissima* responded directly to cues signaling host-plant quality for the induction of diapause. This was because we did not detect any difference in developmental rate (time to adult eclosion) between beetles reared on *S. viminalis* and *S. phylicifolia* in the laboratory experiment, although the beetles were more likely to enter diapause on the latter plant species. The study is also one of the first to describe how the critical photoperiodic response changes in response to host-plant conditions. We found that CDL was prolonged by almost an hour when the beetles were reared on the willow *S. phylicifolia*. A population CDL that is 19 hours or longer will certainly decrease the likelihood for a second generation in central Sweden. Although these results in combination suggest that we can reject the “growth-rate hypothesis” as an explanation for longer CDL on *S. phylicifolia*, it cannot be ruled out that the insects may have responded to some other internal physiological process.
(rather than an external cue from the plant) when “choosing” developmental pathway in the experiments. We found that adult beetles contained larger amounts of fat-bodies stored in the abdomen when they had been reared on new leaves from vigorously growing willow plants than when reared on old leaves from mature plants. This suggests that the beetles gained extra resources when developing on new leaves. This is speculative, but if the beetles are unable to gain enough resources during larval development, they may choose the developmental pathway leading to diapause. However, until this is investigated more rigorously, we will reject the original “growth-rate hypothesis” in its current form because the beetles would otherwise be expected to develop faster on S. viminalis than on S. phylicifolia.

For insects that develop on the leaves of woody plants, the quality of their food often decline over the course of the summer, which may reduce the growth and survival of individuals in subsequent generations (Ishihara and Ohgushi 2006, Nylin et al. 2009). The leaves often becomes tougher and accumulate higher concentrations of quantitative defense compounds after expansion (Feeny 1970, Strong et al. 1994). Many herbivorous insects have therefore synchronized egg hatch and the occurrence of young larval stages with the seasonal timing of bud break to be able to feed on the tender new leaves in the spring that also often are more nutritious than later in the season (Feeny 1970, van Asch and Visser 2007). In fact, many herbivorous insect species feeding on woody plants are always univoltine with an obligatory diapause that prevents them from producing additional generations (Tauber et al. 1986, Tammaru et al. 2001). Although the leaf beetle P. vulgatissima has a facultative diapause, the species is also normally univoltine in central Sweden (Dalin 2011). The first-generation completes development to adulthood before mid August when day-degree models predict that they should
be able to produce another generation in central Sweden (P. Dalin unpubl. data). Thus, it seems that the seasonal climate could allow two generations in Sweden. As far as we know, the species is univoltine at least down to central Europe where they may switch to a bivoltine life-cycle. One possible reason why bivoltinism is restricted to central and southern Europe could be because the quality of willow leaves declines over the course of the summer and, thus, can only support the development of one generation per year further north. One may therefore wonder why the species has a facultative diapause that can result in additional generations as far north as in Sweden. Willow plants may, however, sometimes provide high-quality food also later in the season that may allow a second generation. Willows are known to respond to disturbances, such as wind breaks and mammalian herbivory, by producing many lateral shoots that grow vigorously. These re-sprouting plants continue to produce new leaves throughout the summer that may be of high-quality for leaf beetles also when a potential second generation is developing.

In summary, the results reveal that the leaf beetle *P. vulgatissima* has a facultative diapause that is influenced by both photoperiod and host-plant quality. We believe that this is the first study to confirm that herbivorous insects can respond directly to host-plant quality for the induction of diapause. This can allow the insects to produce extra insect generations under certain circumstances, such as in response to a sudden but unpredictable availability of high-quality food sources. It remains however to be investigated precisely what type of plant signal or cue the insects respond to for the induction of diapause.

Acknowledgements
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References


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Table 1. Results of logistic regression (binominal, logit, type 3) investigating the effects of host-plant species, population origin and hours of light (photophase) on diapause incidence in two populations of *Phratora vulgatissima* originating from willow plantations and natural willow habitats in central Sweden (59°56′N latitude).

<table>
<thead>
<tr>
<th>Effect</th>
<th>$\chi^2$</th>
<th>d.f.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host-plant species (Host)</td>
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<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Population origin (Pop)</td>
<td>3.9</td>
<td>1</td>
<td>0.049</td>
</tr>
<tr>
<td>Hours of light (Photophase)</td>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td>Host x Photophase</td>
<td>31.2</td>
<td>3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pop x Photophase</td>
<td>3.2</td>
<td>3</td>
<td>0.359</td>
</tr>
<tr>
<td>Host x Pop</td>
<td>0.4</td>
<td>1</td>
<td>0.511</td>
</tr>
</tbody>
</table>
Table 2. Results of ANOVA investigating the effects of host-plant species, population origin and hours of light (photophase) on development time (days) from eggs to adult in two populations of *Phratora vulgatissima* origination from willow plantations and natural willow habitat in central Sweden (59°56’N latitude).

<table>
<thead>
<tr>
<th>Effect</th>
<th>MS</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.2</td>
<td>0.699</td>
</tr>
<tr>
<td>Population origin (Pop)</td>
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<td>1</td>
<td>1.4</td>
<td>0.255</td>
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<tr>
<td>Hours of light (Photophase)</td>
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<td>46.2</td>
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<tr>
<td>Host x Photophase.</td>
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<td>0.926</td>
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<tr>
<td>Pop x Photophase</td>
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<td>3</td>
<td>0.6</td>
<td>0.647</td>
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<tr>
<td>Host x Pop</td>
<td>0.5</td>
<td>1</td>
<td>0.6</td>
<td>0.443</td>
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</table>
Fig. 1. Field diapause induction of first-generation adult females of *Phratora vulgatissima* on *S. cinerea* in natural habitats and on *S. viminalis* in willow plantations in 2009.

- Willow plantation (*Salix viminalis*, mature 5 year shoots)
- Natural willows (*Salix cinerea*)
- Willow plantation (*S. viminalis*, coppiced 1st year shoots)
Fig. 2. Phenology and life-cycle development of the leaf beetle *Phratora vulgatissima* in a willow plantation (*Salix viminalis*) and a natural willow habitat (*S. cinerea*) during 2010. The beetles produced a partial second generation in the plantation (Julian dates 225-240).
Fig. 3. Diapause incidence of two populations of *Phratora vulgatissima* originating from the same latitude (59°56’N), reared on greenhouse-grown plants of *Salix viminalis* (circles) and *S. phylicifolia* (triangles), under four day-length (hours of light) treatments and constant 20°C. Pop. A (filled symbols) represent beetles originating from a willow plantation (*S. viminalis*), whereas Pop. B (open symbols) represents beetles from a natural willow habitat (*S. cinerea*).
Fig. 4. Developmental time from egg to adult of *Phratora vulgarissima* in relation to day-length (hours of light) when reared on *Salix viminalis* and *S. phylicifolia* at 20°C. Different superscripts represent significant different means among day-length treatments, as revealed by Tukey tests.
Fig. 5. Diapause incidence of *Phratora vulgatissima* when reared on leaves from three host-plant treatments: previously coppiced *Salix viminalis*, previously coppiced *S. cinerea*, and mature (uncoppiced) *S. cinerea* trees in the field. The figure presents the results from three replicate rearing containers per treatment with groups of larvae reared under controlled conditions (18.5 hours of light, 20°C) in a climate chamber.