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

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Abstract. 1. Voltinism of herbivorous insects can vary depending on environmental conditions. The leaf beetle *Phratora vulgatissima* L. is univoltine in Sweden but will sometimes initiate a second generation in short-rotation coppice (SRC) willow plantations.

2. The study investigated whether increased voltinism by *P. vulgatissima* in plantations can be explained by (i) rapid life-cycle development allowing two generations, or (ii) postponed diapause induction on coppiced willows.

3. 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*). However, the induction of diapause occurred 1–2 weeks later in SRC willow plantations.

4. Laboratory experiments indicated no genetic difference in the critical day-length for diapause induction between beetles originating from plantations and natural habitats. Development time was unaffected by host-plant quality but critical day-length was prolonged by almost an hour when the beetles were reared on a non-preferred willow species (*S. phylicifolia*). When reared on new leaves from re-sprouting shoots of recently coppiced willow plants, diapause incidence was significantly less than when the beetles were reared on mature leaves from uncoppiced plants.

5. 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. SRC willows may allow two beetle generations due to longer growing seasons of coppiced plants that grow vigorously.

Key words. Critical day-length, diapause, host-plant quality, seasonal adaptations, voltinism.

Introduction

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 & Shimada, 2002; Steinbauer *et al.*, 2004; Yamanaka *et al.*, 2008). Voltinism in herbivorous insects often varies across latitude and altitude (Tauber *et al.*, 1986; Ishihara, 1998; Masaki, 1999). Insects with a wide distribution range can for example produce one generation per year (univoltine life cycle) in 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 & Gotthard, 1998). Plasticity in voltinism may allow the insect to adjust immediately the number of generations in response to prevailing environmental conditions, without need for selection to operate. There is indeed evidence that recent climate change has led to extra generations in response to warmer temperature conditions,

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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 diapause for overwintering (Tauber *et al*., 1986; Danks, 2007). Diapause is a dormant stage characterised by lowered metabolic rates, cold hardening, and cessation of reproductive development in insects that overwinter 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 (Tauber *et al*., 1986; 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) for the induction of diapause (Tauber *et al*., 1986; Saunders, 2010) is a genetically determined property that varies adaptively among insect populations (Solbreck & Sillen-Tullberg, 1981; Masaki, 1999; Dalin *et al*., 2010). The CDL 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 & 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 & Ohgushi, 2006; Dolezal & Sehnal, 2007; Dalin *et al*., 2010), and as such more direct effects on voltinism are stronger candidates for being true adaptations to environmental variation (sensu Gotthard & Nylin, 1995). As 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.

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 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; Friberg & Wiklund, 2010).

The leaf beetle *Phratora vulgatissima* L. (Coleoptera: Chrysomelidae) is an important pest in willow plantations grown for bioenergy in northern Europe (Sage & Tucker, 1998; Björkman *et al*., 2000; Dalin *et al*., 2009). It is commonly univoltine in northern Europe and bivoltine in central Europe. In addition, the species sometimes initiate a partial second generation in northern Europe, e.g. in short-rotation coppiced (SRC) willow plantations grown for biomass production (Dalin, 2011). The leaf beetle overwinters in the adult stage and emerges from overwintering sites in the spring. The phenology of adult emergence is usually well synchronised with willow bud-break in the spring. Adults feed on newly developed leaves and oviposit on the ventral side of 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 SRC plantations could be explained by (i) advanced phenology and accelerated development of first-generation broods in willow plantations, or (ii) postponed diapause induction of beetles in willow plantations. The central Swedish population in this study has previously been shown to have a facultative diapause induced by day-length with a CDL estimated to be 18 h 10 min (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 beetles are more likely to produce a second generation in plantations. The hypothesis that *P. vulgatissima* has a plastic diapause threshold that is influenced by host-plant quality was tested. Coppiced SRC willows grow vigorously and may provide high-quality leaves for a longer period of time than mature (uncoppiced) plants in natural habitats. If the beetles are able to postpone diapause in response to vigorous plant growth, this could explain why the beetles sometimes initiate a second generation in plantations. First, we predicted CDL to be prolonged when beetles were reared on a non-preferred host-plant; in this case it was *S. phylicifolia*, which is rarely fed upon by *P. vulgatissima* in the field (Pasteels & Rowell-Rahier, 1992; Kendall *et al*., 1996). Second, if the beetles are able to postpone diapause in response to vigorous growth of coppiced willows, we predicted diapause incidence to be reduced if the species was reared on leaves from coppiced willows (*S. viminalis* and *S. cinerea*) than when reared on leaves from uncoppiced plants.

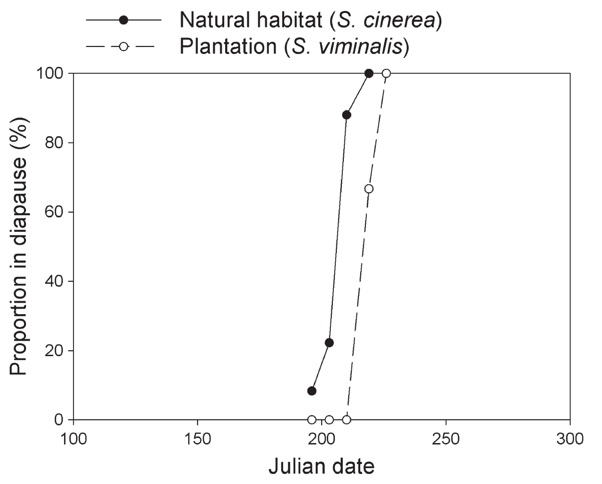

Fig. 1. Field diapause induction of first-generation adult females of *Phratora vulgatissima* on *Salix viminalis* in plantations and on *S. cinerea* in natural habitats during 2009.

Materials and methods

Life-cycle development and diapause induction of field populations

During the summer of 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, adult beetles were collected once every week to estimate the proportion of females in diapause over time. Beetles were collected from two willow plantations (*S. viminalis*) and two natural willow habitats (*S. cinerea*) located within 20 km distance from the Ultuna campus, Swedish University of Agricultural Sciences in Uppsala (59°49′N, 17°40′E). The four sites were chosen because they were easy to access and harboured similar and moderate densities of *P. vulgatissima*. Female beetles were collected from plants by hand and were brought to the laboratory and dissected under a microscope to confirm reproductive status using methods described in Dalin (2011). Collections were made on 15 July, 22 July, 29 July, 7 August, and the last collection was made on 14 August (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. Owing to poor emergence of first-generation adults, data were pooled together for plantations and natural habitats, respectively, in Fig. 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 2-year-old shoots (coppiced during the winter 2008/2009) whereas the natural habitat consisted of mature (uncoppiced) *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 plants in the two habitats. The number of individuals in different developmental stages was counted during 5-min observation periods. One 5-min period was devoted to searching for adult beetles on the dorsal side of leaves. Another 5-min period was devoted to searching 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. We also estimated leaf nitrogen (N) concentrations to follow how host-plant quality changes over the season in the two habitats. A non-destructive SPAD method using an optical leaf chlorophyll meter (Model SPAD-502, Konica Minolta Sensing, Japan) was used. This method has been applied to assess leaf N concentrations in tree species (Loh *et al*., 2002), including willows (Weih & Rönnberg-Wästljung, 2007). Fifteen individual leaves on three haphazardly selected plants per site were marked with a filter pen in the spring. All leaves were measured with the chlorophyll meter at each site visit. SPAD-values were then recalculated to mass-based leaf N concentration (%) using the following regression models: $N = 0.064 \times (SPAD) - 0.29$ for *S. viminalis*; and $N = 2.597 \times e^{0.078 \times (SPAD)}$ for *S. cinerea*. The first model was developed to estimate leaf N concentrations in commercial willow genotypes, including *S. viminalis* (Weih & Rönnberg-Wästljung, 2007), whereas the second model was developed specifically for *S. cinerea* (A.-S. Liman *et al*., unpublished).

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: (i) investigate if photoperiodic responses differ between *P. vulgatissima* beetles originating from plantations and natural willow habitats, and (ii) 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 CDL for the induction of diapause was 18 h 10 min when 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 diapause 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 could 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 non-preferred 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 is avoided by *P. vulgatissima* possibly due to phytochemical quality of the leaves (Julkunen-Tiitto, 1986; 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 cuttings 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 the greenhouse (by mid-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 (LD 20 : 4; 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 (LD cycles 20 : 4, 19 : 5, 18 : 6 and 17 : 7) and constant 20 °C temperature. In the climate chambers, we reared the beetles in groups of 50–100 larvae inside transparent plastic containers (19 × 19 × 11 cm). 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 analysed using logistic regression (PROC GENMOD, binominal, 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 CDLs (± 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 investigate the effect of host-plant growth on diapause incidence in *P. vulgatissima*. The purpose was to test whether diapause in adult females is reduced when 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: (i) leaves from re-sprouting shoots of previously coppiced *S. viminalis*; (ii) leaves from re-sprouting shoots of experimentally coppiced *S. cinerea*; and (iii) leaves from mature (uncoppiced) *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 (3 × 3) rearing containers (see above) inside a climate chamber with constant 18.50 h 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 5 m 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 analysed 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. Least squares means were used for *post-hoc* comparisons among host-plant treatments (LSMEANS; SAS Institute, 2008). We also scored the amount of fat bodies in the abdomen of diapausing females as either small or large amounts. χ^2-tests were used to compare fat bodies among host-plant treatments. In 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 the microscope (9 × 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 *P. vulgatissima* entered diapause 1–2 weeks earlier in the natural habitats than in the willow plantations studied (Fig. 1). We observed mating by first-generation adults in July (before Julian date 205 in Fig. 1) on both *S. viminalis* and *S. cinerea* in the two habitats.

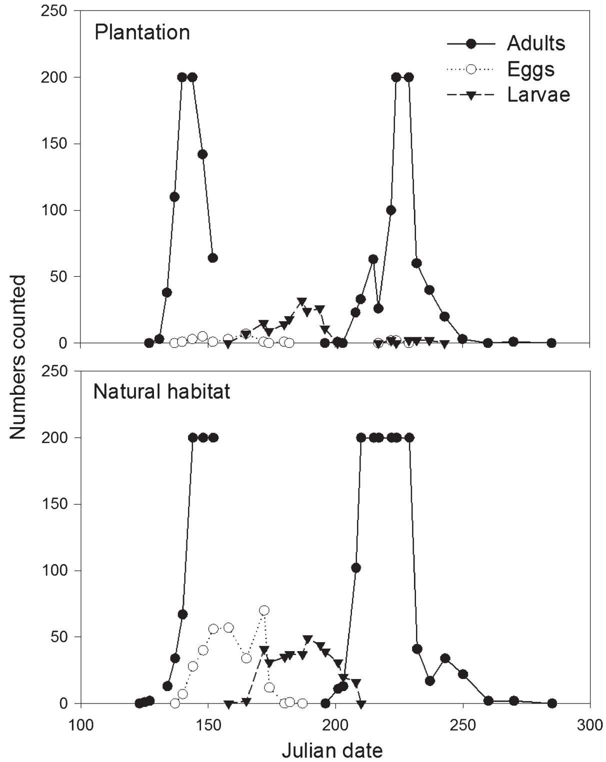

Fig. 2. Phenology and life-cycle development of *P. vulgatissima* in a willow plantation (*S. viminalis*) and a natural willow habitat (*S. cinerea*) during 2010. The beetles produced a partial second generation in the plantation (Julian dates 225–240).

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). Leaf N concentrations were overall higher in *S. viminalis* grown in the SRC plantation (Fig. 3). The leaves of *S. cinerea* in the natural habitat started to senesce earlier than the leaves of *S. viminalis* in plantations, which is shown by the decline in leaf N concentrations in *S. cinerea* after Julian date 235 (late August) in Fig. 3.

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). Figure 4 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

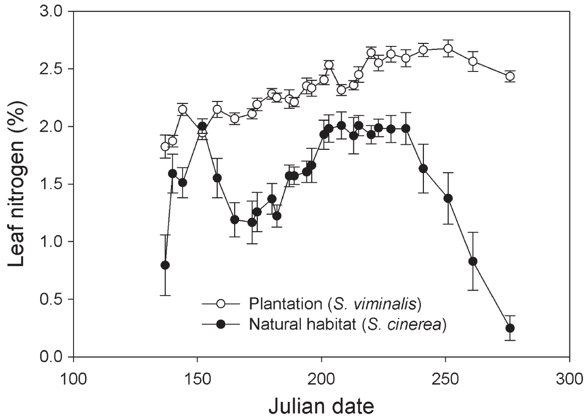

Fig. 3. Seasonal variation in mass-based leaf nitrogen concentration in leaves of *S. viminalis* plants in plantations and *S. cinerea* plants in natural habitats.

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).

Effect	χ^2	d.f.	P
Host-plant species (host)	35.1	1	<0.001
Population origin (Pop)	3.9	1	0.049
Hours of light (Photophase)	425.2	3	<0.001
Host × Photophase	31.2	3	<0.001
Pop × Photophase	3.2	3	0.359
Host × Pop	0.4	1	0.511

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 × 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 × Photophase interaction, indicating different day-length responses of *P. vulgatissima* on the two willow species (Table 1; Fig. 4). CDL was estimated to be 18.08 h (95% confidence interval: 17.94–18.22 h) or 18.20 h (18.06–18.33) on *S. viminalis* (estimations for beetles originating from willow plantation and natural willow habitat, respectively); and 18.77 h (18.53–19.03) or 19.03 h (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). Figure 5 shows a significant reduction in developmental time for beetles reared under the shortest day-length treatment (17 h of light).

Diapause incidence on coppiced versus mature willow plants

Diapause incidence in female *P. vulgatissima* was significantly affected by host-plant treatments ($\chi^2 = 58.88$,

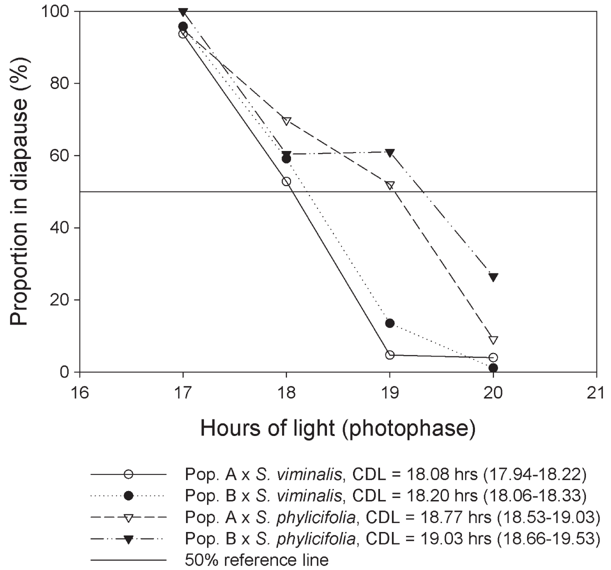

Fig. 4. Diapause incidence of two populations of *P. vulgatissima* originating from the same latitude (59°56′N), reared on greenhouse-grown plants of *S. viminalis* (circles) and *S. phylicifolia* (triangles), under four day-length (hours of light) treatments and constant 20 °C. Logistic regressions were used to estimate the critical day-length (CDL) for diapaus induction; here defined as the day-length when there is a 50% probability for diapause. Pop. A (open symbols) represent beetles originating from a willow plantation, whereas Pop. B (filled symbols) represents beetles from a natural willow habitat.

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).

Effect	MS	d.f.	F	P
Host-plant species (host)	0.1	1	0.2	0.699
Population origin (Pop)	1.1	1	1.4	0.255
Hours of light (Photophase)	37.7	3	46.2	<0.001
Host × Photophase	0.2	3	0.2	0.926
Pop × Photophase	0.5	3	0.6	0.647
Host × Pop	0.5	1	0.6	0.443

d.f. = 2, $P < 0.001$; Fig. 6), but 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, $P = 0.15$). *Post-hoc* comparisons among host-plant treatments revealed that diapause incidence was highest on mature leaves from *S. cinerea* ($z = 4.40$ and 5.84, $P < 0.001$; mature *S. cinerea* vs. coppiced *S. cinerea* and coppiced *S. viminalis*, respectively), intermediate on new leaves from coppiced *S. cinerea* ($z = 2.61$, $P = 0.009$; coppiced *S. cinerea* vs. coppiced *S. viminalis*) and lowest on new leaves from coppiced *S. viminalis*.

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

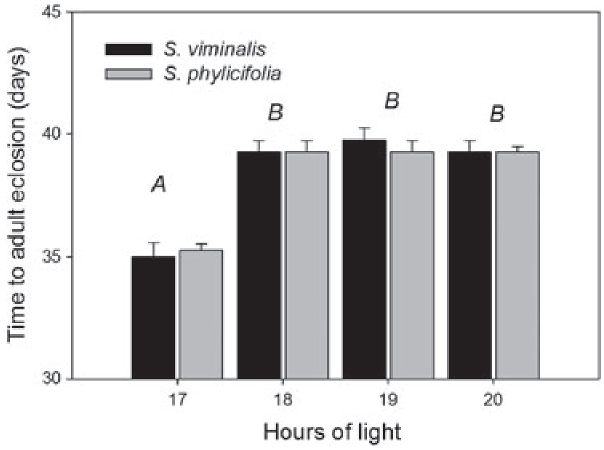

Fig. 5. Developmental time from egg to adult of *P. vulgatissima* in relation to day-length (hours of light) when reared on *S. viminalis* and *S. phylicifolia* at 20 °C. Different superscripts represent significant different means among day-length treatments, as revealed by Tukey tests.

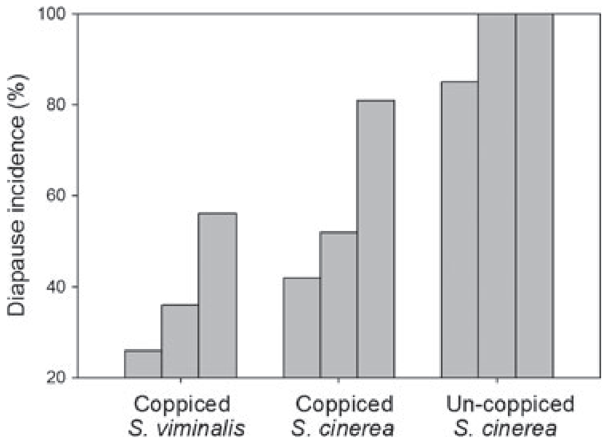

Fig. 6. Diapause incidence of *P. vulgatissima* when reared on leaves from three host-plant treatments: previously coppiced *S. viminalis*, previously coppiced *S. cinerea*, and mature (uncoppiced) *S. cinerea* trees in the field. The figure presents the results from three replicate rearing containers per treatment with groups of larvae reared under controlled conditions (18.5 h of light, 20 °C) in a climate chamber.

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 initiates a second generation in SRC 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 vary under field conditions with diapause occurring 1–2 weeks later in coppiced SRC plantations than in mature (uncoppiced) natural 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 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 treatment 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 enhanced 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). 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. We also found that already expanded leaves of SRC willows (*S. viminalis*) contained higher concentrations of leaf N than mature leaves in natural habitats (*S. cinerea*). The leaves also started to senesce later in plantations. Although we lack information about the performance of second-generation larvae, the results suggest that the enhanced quality and longer growing season of SRC willows can induce postponed diapause of *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 & McNeil, 1997; Ishihara & Ohgushi, 2006; Takagi & Miyashita, 2008; Friberg & Wiklund, 2010; Friberg *et al*., 2012). 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 (but see Friberg *et al*., 2012). 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 & 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 & McNeil, 1997; Wedell *et al*., 1997; Ishihara & 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; Friberg *et al*., 2012). 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 signalling 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 h 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' the developmental pathway in 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 & Ohgushi, 2006; Nylin *et al*., 2009). The leaves often become tougher and accumulate higher concentrations of quantitative defence compounds after expansion (Feeny, 1970; Strong *et al*., 1994). Many herbivorous insects have therefore synchronised egg hatch and occurrence of young larval stages with the seasonal timing of bud break to be able to feed on tender new leaves in spring that also often are more nutritious than later in the season (Feeny, 1970; van Asch & 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, unpublished). 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. In the current study, we found that leaf N concentrations remained relatively high throughout the growing season in coppiced SRC willows whereas N concentrations started to decline around late August in the leaves of mature (uncoppiced) willows. 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 one of the first studies to confirm that herbivorous insects can respond directly to host-plant conditions for the induction of diapause (see also Friberg *et al*., 2012). This can allow insects to produce an extra generation 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.

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References

Altermatt, F. (2010) Climatic warming increases voltinism in European butterflies and moths. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1281–1287.

van Asch, M. & Visser, M.E. (2007) Phenology of forest caterpillars and their host trees: the importance of synchrony. *Annual Review of Entomology*, **52**, 37–55.

Björkman, C., Höglund, S., Eklund, K. & Larsson, S. (2000) Effects of leaf beetle damage on stem wood production in coppicing willow. *Agricultural and Forest Entomology*, **2**, 131–139.

Dalin, P. (2011) Diapause induction and termination in a commonly univoltine leaf beetle (*Phratora vulgatissima*). *Insect Science*, **18,** 443–450.

Dalin, P., Kindvall, O. & Björkman, C. (2009) Reduced population control of an insect pest in managed willow monocultures. *PLoS ONE*, **4**, e5487.

Dalin, P., Bean, D.W., Dudley, T.L., Carney, V.A., Eberts, D., Gardner, K.T. *et al*. (2010) Seasonal adaptations to day length in ecotypes of *Diorhabda* spp. (Coleoptera: Chrysomelidae) inform selection of agents against saltcedars (*Tamarix* spp.). *Environmental Entomology*, **39**, 1666–1675.

Danks, H.V. (2007) The elements of seasonal adaptations in insects. *Canadian Entomologist*, **139**, 1–44.

Dolezal, P. & Sehnal, F. (2007) Effects of photoperiod and temperature on the development and diapause of the bark beetle *Ips typographus*. *Journal of Applied Entomology*, **131**, 165–173.

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.

Friberg, M. & Wiklund, C. (2010) Host-plant induced larval decision-making in a habitat/host-plant generalist butterfly. *Ecology*, **91**, 15–21.

Friberg, M., Dahlerus, J. & Wiklund, C. (2012) Strategic larval decision-making in a bivoltine butterfly. *Oecologia*, **169**, 623–635.

Gotthard, K. (2008) Adaptive growth decisions in butterflies. *BioScience*, **58**, 222–230.

Gotthard, K. & Nylin, S. (1995) Adaptive plasticity and plasticity as adaptation – a selective review of plasticity in animal morphology and life-history. *Oikos*, **74**, 3–17.

Hunter, M.D. & McNeil, J.N. (1997) Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology*, **78**, 977–986.

Ishihara, M. (1998) Geographical variation in insect developmental period: effect of host plant phenology on the life cycle of the bruchid seed feeder *Kytorhinus sharpianus*. *Entomologia Experimentalis et Applicata*, **87**, 311–319.

Ishihara, M. & Ohgushi, T. (2006) Reproductive inactivity and prolonged developmental time induced by seasonal decline in host plant quality in the willow leaf beetle *Plagiodera versicolora* (Coleoptera : Chrysomelidae). *Environmental Entomology*, **35**, 524–530.

Julkunen-Tiitto, R. (1986) A chemotaxonomic survey of phenolics in leaves of northern Salicaceae species. *Phytochemistry*, **25**, 663–667.

Kendall, D.A., Hunter, T., Arnold, G.M., Liggitt, J., Morris, T. & Wiltshire, C.W. (1996) Susceptibility of willow clones (*Salix* spp) to herbivory by *Phyllodecta vulgatissima* (L) and *Galerucella lineola* (Fab) (Coleoptera, Chrysomelidae). *Annals Applied Biology*, **129**, 379–390.

Kostal, V. (2006) Eco-physiological phases of insect diapause. *Journal of Insect Physiology*, **52**, 113–127.

Kurota, H. & Shimada, M. (2002) Geographical variation in the seasonal population dynamics of *Bruchidius dorsalis* (Coleoptera: Bruchidae): constraints of temperature and host plant phenology. *Environmental Entomology*, **31**, 469–475.

Loh, F.C.W., Grabosky, J.C. & Bassuk, N.L. (2002) Using the SPAD-502 meter to assess chlorophyll and nitrogen content of Benjamin fig and Cottonwood leaves. *HortTechnology*, **12**, 682–686.

Masaki, S. (1999) Seasonal adaptations of insects as revealed by latitudinal diapause clines. *Entomological Science*, **2**, 539–549.

Nakamura, M., Utsumi, S., Miki, T. & Ohgushi, T. (2005) Flood initiates bottom-up cascades in a tri-trophic system: host plant regrowth increases densities of a leaf beetle and its predators. *Journal of Animal Ecology*, **74**, 683–691.

Nylin, S. & Gotthard, K. (1998) Plasticity in life-history traits. *Annual Review of Entomology*, **43**, 63–83.

Nylin, S., Nygren, G., Söderlind, L. & Stefanescu, C. (2009) Geographical variation in host plant utilization in the comma butterfly: the roles of time constraints and plant phenology. *Evolutionary Ecology*, **23**, 807–825.

Pasteels, J.M. & Rowell-Rahier, M. (1992) The chemical ecology of herbivory on willows. *Proceedings of the Royal Society Edinburgh. Section B: Biological Sciences*, **98**, 63–73.

Poyry, J., Leinonen, R., Soderman, G., Nieminen, M., Heikkinen, R.K. & Carter, T.R. (2011) Climate-induced increase of moth multivoltinism in boreal regions. *Global Ecology and Biogeography*, **20**, 289–298.

Roff, D. (1980) Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. *Oecologia*, **45**, 202–208.

Sage, R.B. & Tucker, K. (1998) The distribution of *Phratora vulgatissima* (Coleoptera : Chrysomelidae) on cultivated willows in Britain and Ireland. *European Journal of Forest Pathology*, **28**, 289–296.

SAS Institute (2008) *SAS/STAT User's Guide, Version 9.1.* SAS Insitute, Cary, North Carolina.

Saunders, D.S. (2010) Photoperiodism in insects: migration and diapause responses. *Photoperiodism: The Biological Calendar* (ed. by R. J. Nelson, D. L. Denlinger and D. E. Somers), pp. 218–257. Oxford University Press, New York, New York.

Solbreck, C. & Sillen-Tullberg, B. (1981) Control of diapause in a monovoltine insect, *Lygaeus equestris* (Heteroptera). *Oikos*, **36**, 68–74.

Steinbauer, M.J., Kriticos, D.J., Lukacs, Z. & Clarke, A.R. (2004) Modelling a forest lepidopteran: phenological plasticity determines voltinism which influences population dynamics. *Forest Ecology and Management*, **198**, 117–131.

Strong, D.R., Lawton, J.H. & Southwood, T.R.E. (1994) *Insects on Plants.* Blackwell Publishing, Oxford, U.K.

Takagi, S. & Miyashita, T. (2008) Host plant quality influences diapause induction of *Byasa alcinous* (Lepidoptera : Papilionidae). *Annals of the Entomological Society of America*, **101**, 392–396.

Tammaru, T., Tanhuanpää, M., Ruohomäki, K. & Vanatoa, A. (2001) Autumnal moth – why autumnal? *Ecological Entomology*, **26**, 646–654.

Tauber, M.J., Tauber, C.A. & Masaki, S. (1986) *Seasonal Adaptations of Insects.* Oxford University Press, New York, New York.

Tobin, P.C., Nagarkatti, S., Loeb, G. & Saunders, M.C. (2008) Historical and projected interactions between climate change and insect voltinism in a multivoltine species. *Global Change Biology*, **14,** 951–957.

Wedell, N., Nylin, S. & Janz, N. (1997) Effects of larval host plant and sex on the propensity to enter diapause in the comma butterfly. *Oikos*, **78**, 569–575.

Weih, M. & Rönnberg-Wästljung, A.-C. (2007) Shoot biomass growth is related to the vertical leaf nitrogen gradient in *Salix* canopies. *Tree Physiology*, **27**, 1551–1559.

Yamanaka, T., Tatsuki, S. & Shimada, M. (2008) Adaptation to the new land or effect of global warming? An age-structured model for rapid voltinism change in an alien lepidopteran pest. *Journal of Animal Ecology*, **77**, 585–596.

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