

Seasonal changes in leaf nutritional quality influence grass miner performance

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Abstract. 1. The plant phenological age hypothesis predicts that phytophagous insects should prefer and perform better on phenologically young plants than on old plants because plant nutritional quality decreases with plant phenological age. This hypothesis was tested under field and laboratory conditions with the grass miner *Chromatomyia milii* on the free-growth species *Holcus lanatus*.

2. The field experiment was conducted at four sample sites in Belgium where nutritional quality of *H. lanatus* leaves and performance of *C. milii* were monitored throughout the growth season. Foliar nutritional quality was highest early in the season due to high levels of proteins and soluble carbohydrates and low levels of lignin. Offspring performance (pupal size) decreased with plant phenological age, due at least partially to the decreasing foliar nutritional quality.

3. In the laboratory experiment, preference and performance of *C. milii* were determined on three different age classes of *H. lanatus*. Multiple-choice experiments demonstrated that oviposition preference did not differ among age classes. Offspring survival decreased with plant phenological age, while pupal size did not differ among age classes. The relationship between foliar nutritional quality and plant phenological age was equivocal and did not correspond to the predictions of the plant phenological age hypothesis.

4. The results of the field experiment were consistent with the idea that the phenological age hypothesis holds in free-growth species. The laboratory experiment gave only little support to the plant phenological age hypothesis. Possible causes for the differences between field and laboratory experiments are discussed.

Key words. *Chromatomyia milii*, host plant preference, leaf miner, offspring performance, plant-insect interactions, plant phenological age.

Introduction

The nutritional quality of plants to herbivorous insects varies temporally and spatially (Whitham, 1981; Schoonhoven *et al.*, 1998). Seasonal changes in plant nutritional quality are a major source of variation on the temporal scale (Raupp & Denno, 1983; Bemays & Chapman, 1994) and have been shown to affect the population dynamics of a broad range of phytophagous insects (e.g. Feeny, 1970; Schweitzer, 1979; Raupp & Denno, 1983; Schroeder, 1986; Kearsley & Whitham, 1989). The plant phenological age or developmental age hypothesis (Kearsley & Whitham, 1989; Karban, 1990) predicts that

herbivores prefer and perform better on phenologically young plants than on old plants because plant nutritional quality decreases with phenological age. As a general rule, the nutrient level, i.e. nitrogen and water, decreases while the non-nutrient content and leaf toughness increase with plant phenological age (e.g. Feeny, 1970; Schweitzer, 1979; Mattson, 1980; Scriber & Slansky, 1981; Raupp & Denno, 1983; Schroeder, 1986). Three types of process influence the seasonal changes in plant nutritional quality: ontogenetic processes like maturation (Raupp & Denno, 1983; Kearsley & Whitham, 1989), physiological processes like senescence (see Kearsley & Whitham, 1989), and a broad range of abiotic (temperature, rainfall) or biotic (herbivores, diseases) environmental variables (Van Soest, 1982; Karban, 1990; Bowers & Stamp, 1993; Larcher, 1995).

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Several studies have postulated that plant growth form may affect the seasonal changes in plant nutritional quality (Schultz *et al.*, 1982; Meyer & Montgomery, 1987; Stamp & Bowers, 1990a; Bowers *et al.*, 1992; Jordano & Gomariz, 1994). Two growth forms are distinguished: fixed-growth (= determinate) species with leaves flushing over a short period in the season and free-growth (= indeterminate) species with leaves flushing continuously (see Meyer & Montgomery, 1987). It has been hypothesised that the nutritional quality of fixed-growth species should decrease more rapidly and more severely with plant phenological age than in free-growth species because of a different age structure of the leaves (Schultz *et al.*, 1982; Bowers *et al.*, 1992). As foliar nutritional quality decreases with leaf age (reviewed by Raupp & Denno, 1983) due to maturation and senescing processes (Kearsley & Whitham, 1989), free-growth species are believed to maintain foliar nutritional quality at higher levels because of the continuous flush of young, nutritious leaves, contrary to fixed-growth species where all leaves age simultaneously from the start of the season with no rejuvenation.

Herbivores are also assumed to respond differently to fixed and free-growth species. The limited availability of high-quality leaves in fixed-growth species has been shown to constrain many phytophagous insects to a single generation in a season, synchronised with the flush of young leaves (e.g. Feeny, 1970; Schroeder, 1986; Rossiter *et al.*, 1988; Potter & Kimmerer, 1989). In contrast, temporal constraints are believed to be reduced in free-growth species because of the constant availability of high quality leaves (Niemelä *et al.*, 1982; Jordano & Gomariz, 1994).

Most studies addressing the plant phenological age hypothesis have focused on fixed-growth species. The predictions made by this hypothesis are therefore based mainly on observations of this growth form. Remarkably few empirical studies have investigated the plant phenological age hypothesis in free-growth species. Exceptions are the large number of studies on *Plantago lanceolata* L. (Stamp & Bowers, 1990a; Bowers *et al.*, 1992; Bowers & Stamp, 1993) and one study on *Aristolochia longa* L. and *A. baetica* L. (Jordano & Gomariz, 1994).

The plant phenological age hypothesis was tested in this study with the grass miner *Chromatomyia milii* (Kaltenbach) (Agromyzidae: Diptera) on a free-growth species, *Holcus lanatus* L. *Chromatomyia milii* is an oligophagous miner that occurs commonly in shaded plots occupied by members of the grass genera *Holcus*, *Poa*, and *Milium* throughout Europe (Griffiths, 1980). It is a multivoltine species that can be found during most parts of the growing season of its hosts. *Holcus lanatus* is its most common host plant in the study area and, like all grasses, is a free-growth species. The plant phenological age hypothesis was studied by evaluating the relationships between plant phenological age, host nutritional quality, and miner performance under both field and laboratory conditions, which is an original approach to testing the hypothesis. The following questions were addressed: Does foliar nutritional quality decrease with plant phenological age in *H. lanatus*? Is the performance of the larvae of *C. milii* altered by changes in nutritional quality? Does *C. milii*

discriminate between plants of different phenological age? Are the relationships between plant phenological age, nutritional quality, and miner performance comparable under field and controlled conditions?

Materials and methods

Study system

Chromatomyia milii is a multivoltine species occurring from early March to the end of July in Belgium (De Bruyn, 1990). Females insert their eggs directly in the mesophyll layer between two veins of a grass leaf. Larvae emerge within 1 week and immediately eat their way through internal leaf tissues. They consume parts of the available mesophyll cells and parts of sclerenchyma strands and bundle sheath cells when crossing the numerous adjoining veins (Scheirs *et al.*, 2001a). Larvae complete four instars (three larval and one prepupal stadia) then pupate within their mines. Adults emerge through the leaf surface. Females feed on leaf sap exuding from feeding punctures made by their ovipositors in the leaf blade of host grasses (Scheirs *et al.*, 2001b).

Holcus lanatus is the most common host plant of *C. milii* in the study area. The growth period of this small-tufted perennial ranges from April to August in Belgium. In the study area, *H. lanatus* has two generations of shoots: a reproductive generation followed by a vegetative generation. In winter, *H. lanatus* remains wintergreen with little winter growth (Grime *et al.*, 1988; J. Scheirs, pers. obs.). *Holcus lanatus* is distributed widely because of its unusually wide ecological range; its highest vigour is reached in relative fertile, moist habitats (Grime *et al.*, 1988).

Field experiment

The field experiment was conducted in 1995 at four study sites near Antwerp, Belgium (51°09'51.23"N, 4°21'43.37"E): Antwerp, Hoboken, Wilrijk, and Wuustwezel. All sites are open woodlands with the grass *Holcus lanatus* dominating the understorey. The dominant tree species were: *Quercus robur* L. and *Fagus sylvatica* L. at Antwerp; *Salix alba* L. and *Sambucus nigra* L. at Hoboken; *Q. robur* at Wilrijk; *Betula pubescens* Ehrh., *Prunus serotina* Ehrh., and *Q. robur* at Wuustwezel. The four sampling sites were monitored every 2 weeks from April to mid July, resulting in eight sampling events per site. This sampling campaign covered most of the growth season of *H. lanatus*, starting with the end of the winter growth period and ending when plants had largely died back.

Plant growth was monitored by counting the number of green, fully expanded leaves of 30 stems per sample site and by recording whether the stem belonged to the first or second generation of shoots.

Pupal size was determined as a measure of offspring performance because pupal size is related positively to host nutritional quality (Minkenberg & Ottenheim, 1990) and later adult fitness in Agromyzidae (Quiring & McNeil, 1984b; Via,

1984; Minkenberg & Ottenheim, 1990). Other performance measures such as offspring survival and development time were not determined because these parameters cannot be measured accurately enough under field conditions for *C. milii*. Leaves containing pupae were collected and transported to the laboratory, where the number of pupae, larvae, and feeding punctures per leaf were recorded. Pupae were then dissected from their mines and put in individual rearing containers under controlled laboratory conditions (LD 16:8h, 20 °C, 70% RH in the light, 100% RH in the dark) until they emerged. The positions of the rearing containers were randomised daily within the environmental chamber to avoid position effects. Adult flies were sexed and pupal size was assessed by measuring the length of the empty puparium (to the nearest 0.05 mm) using an ocular micrometer fitted to a dissecting microscope. Dead or parasitised pupae were not included in the analysis. Because inter- and intra-specific competition decreases offspring performance of Agromyzidae (Parrella, 1983; Quiring & McNeil, 1984a,b; Pettit & Wietlisbach, 1992), only pupae that completed their development in the absence of competition were considered in the analyses of offspring performance. Competition was considered to be present when the mine of the respective larva was crossed by other mining larvae or the mine crossed a batch of feeding punctures.

Finally, 100 leaves without mines were collected randomly from different plants at each study site for nutritional quality analysis. Leaf age and position of the leaf on the stem were not controlled for. Leaves were always sampled between 12.00 and 14.00 hours to reduce variation due to time of day (see Feeny, 1970; Raupp & Denno, 1983; Bernays & Chapman, 1994). Immediately after collection, 50 leaves were put into airtight, plastic bags and stored on ice for transport. On arrival at the laboratory, these leaves were used to determine leaf water content. The other 50 leaves were placed immediately in liquid nitrogen in the field and stored at -80 °C on arrival in the laboratory until they were used for nutrient analyses.

Laboratory experiment

Three age classes of *H. lanatus* were reared (15, 30, and 45 weeks old) by sowing plants at intervals of 15 weeks. *Holcus lanatus* was grown in pots (10 X 10 X 11 cm) containing a soil mixture of 50% white sand, 50% vermiculite. Plants were watered weekly and nutrient availability was kept constant equivalent to a half strength Hoagland solution (Hoagland & Arnon, 1950). Plants were reared and the experiments were performed under controlled conditions (LD 16:8h, 20 °C, 70% RH in the light, 100% RH in the dark).

Oviposition preference of *C. milii* was determined by multiple-choice experiments. One pot of every age class was offered simultaneously to five gravid females of *C. milii* during one trial. Pots were positioned randomly in a triangle in a flight cage (50 cm long X 75 cm wide X 75 cm tall). Twelve trials lasting 48 h were conducted. After the trials, the number of eggs was recorded as a measure of adult oviposition preference. Eggs were counted by examining each individual leaf under a microscope with light projecting through the leaf.

Flies used in these experiments came from a laboratory stock living on *H. lanatus* L. for less than three generations (2 months). The laboratory stock originated from adults collected from a field population near Antwerp (Hoboken), Belgium. Flies and grasses were only used once in an experiment.

Offspring performance was determined by raising the eggs that were laid in the multiple-choice experiments. Pots were censused daily for pupated and dead larvae. The pupae were dissected from the leaves and put in individual rearing containers. Whether the larvae had completed development or <lied in the presence or absence of intraspecific competition was recorded using the above-mentioned criteria. Rearing containers were checked daily for emerging adults and emergence data were recorded. After emergence, pupal size was measured as in the field experiment. In this way, three measures of offspring performance were assessed: offspring survival, development time, and pupal size. Only larvae that completed their development in the absence of competition were considered in the analysis.

Before the pots were offered to the flies in the choice trials, 10 leaves of each pot were collected randomly for later nutritional quality analyses. Seven leaves were stored at -80 °C for later nutrient analyses, three leaves were used immediately to determine their water content.

Nutritional quality

Foliar nutritional quality was determined by assessing the concentration of nutrients (water, proteins, amino acids, soluble carbohydrates), and lignin content was determined as a measure of the amount of structural defences. Whole leaf samples were used instead of the mesophyll as suggested for selective feeders (Kimmerer & Potter, 1987). Whole leaf samples are a good estimate of larval food quality because the mesophyll layer of grasses consists of one homogeneous layer (Vincent, 1982) and the larvae feed on the mesophyll as well as on the veins (Scheirs *et al.*, 2001a).

Foliar water content was analysed by determining the fresh mass of *H. lanatus* leaves immediately after collection and their dry mass after drying at 105 °C for 48 h. Water content was then calculated as $\{[(\text{fresh mass} - \text{dry mass}) / \text{fresh mass}] \times 100\}$. The lignin content of these leaves was then determined using the acetyl bromide technique of Morrison (1972a) modified for small quantities (Morrison, 1972b; Lin & Dence, 1992).

For analysis of nutrient content, leaves stored at -80 °C were lyophilised, ground, and extracted on ice for 30 min with a Tris-EDTA buffer (pH 7.6). Extracts were centrifuged at 5500 r.p.m. The supernatants were collected and stored at -80 °C until further analysis. Protein levels were analysed using the Bradford assay (Sigma, B6916) (Bradford, 1976; Jones *et al.* 1989), with bovine serum albumin as a standard. Soluble carbohydrate content was determined using the anthrone method (Yemm & Willis, 1954). Glucose was used as a standard. Prior to amino acid analysis, proteins were precipitated using trichloroacetic acid (Bollag *et al.* 1996).

The supernatants were collected after centrifugation at 5500 r.p.m. Amino acid content was determined using the ninhydrin method (Sigma, N1632) (Moore, 1968; Allen, 1989). Leucine was used as a standard.

Statistical analysis

For the field experiment, mixed model regression analyses were used to investigate the relationship between foliar nutritional quality and plant phenological age. Regression rather than ANOVA was used in this and later analyses in order to test whether there was a relationship between plant phenological age and the dependent variable, i.e. nutritional quality or insect performance. Analysis of variance would have been less informative as it only tests for differences among (age) classes. Phenological age was therefore considered to be a continuous rather than a categorical variable in all analyses; for this purpose, the eight sampling events were numbered sequentially. Phenological age was treated as a fixed effect and sample site as a random effect. Amino acid and carbohydrate content were Box-Cox transformed, protein and lignin were log transformed, and water was arcsin transformed in order to meet the assumptions of normality. The relationship between plant phenological age and pupal size was analysed in the same way, but sex was added to the list of independent variables as an additional fixed effect. The presence of quadratic age effects in all previous analyses was tested by the inclusion of a squared plant age variable in the list of independent variables. All non-significant interactions were excluded from the models. The relationship between foliar nutritional quality and offspring performance was investigated using mixed model regression analysis with normally distributed errors. The mean pupal size of males and females per sampling date and per site was included as a dependent variable. The mean content of all food quality parameters and sex were considered as fixed effects. Sampling site was treated as a random effect.

For the laboratory experiment, mixed model regression analyses were used to investigate the relationship between foliar nutritional quality parameters and plant phenological age. Plant phenological age was considered as a fixed effect and pot was treated as a random effect. Protein, carbohydrate, and amino acid content were square-root transformed and water and lignin content were arcsin transformed in order to meet the assumptions of normality. Offspring survival was analysed using mixed model regression analysis with logit link function and binomially distributed data. Choice trial was included as a random effect and plant age as a continuous, fixed effect. Total development time and pupal size were analysed using mixed model regression analysis. Pot nested within choice trial was treated as a random effect, plant age (continuous) and sex as fixed effects. The interaction between plant age and sex was never significant and was excluded from the models. Pupal size was log transformed in order to meet the assumptions of normality. The results of the multiple-choice experiments (oviposition preference) were analysed using an exact Quade test (Quade, 1979; Conover, 1980) calculated using StatXact 3 (Mehta & Patel, 1995). This non-parametric

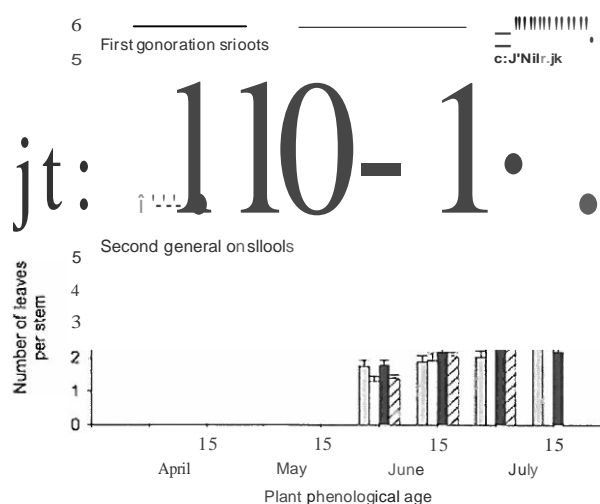


Fig.1. Growth of *Holcus lanatus* (mean \pm I SE) in the field experiment for tillers of the (a) first and (b) second generation.

test takes the Jack of independence among the simultaneously offered food types into account (Roa, 1992).

All previous mixed model analyses were calculated using the PROC MIXED module in SAS (Littell *et al.*, 1996) in the case of normally distributed data, and with the GLIMMIX macro in the case of binomially distributed data (Littell *et al.*, 1996). The degrees of freedom of the fixed effects F-test were adjusted for statistical dependence using Satterthwaite formulas (Littell *et al.*, 1996). Variance components were estimated by the restricted maximum likelihood method (REML) (Littell *et al.*, 1996).

Results

Field experiment

New shoots of *H. lanatus* were formed from the beginning of April. These shoots grew and flushed leaves continuously until the end of May when they started flowering (Fig. 1). After flowering, the first generation of shoots largely died back and was replaced by a second generation of vegetative shoots that grew and flushed leaves until the end of the growth season (mid July).

All food quality parameters changed during the growth season of *H. lanatus* (Table 1, Fig. 2). Water content was low early in the season (April), reached its highest concentration in May, then decreased with increasing plant phenological age. Protein content decreased with plant phenological age, except in Hoboken where it was more or less constant. There was also a peak in the protein content at the end of the season (early July) at two sample sites: Wilrijk and Wuustwezel. Carbohydrate content was very high early in April and showed a sharp decrease in the first half of May, after which concentrations were constant or showed only a small decrease depending on the sample site. The changes in amino acid content were very difficult to interpret because all sites were

Table 1. Mixed model analyses of the seasonal changes in food quality in the field experiment.

Effect	Water	Protein	Carbohydrate	Amino acid	Lignin
Plant age	F1,15ss = 129.66**	F1,624 = 0.79	F1,623 = 60.69***	Ft,567 = 1.07	F1,154 = 30.20***
Plant age ²	F1,15ss = 19596***	F1,624 = 2.21	F1,623 = 15.80**	Ft,567 = 1.07	F1,154 = 16.01***
Plant age X place	F3,641 = 20.08**	F3,511 = 4.89**	F3,623 = 18.59***	F3,309 = 12.48***	
Plant age ² X place	F3s14 = 24.62***		F3,623 = 9.92***	F3,374 = 11.83***	

P<0.01, *P<0.001.

affected differently and there was no clear relationship with plant phenological age. The lignin content of *H. lanatus* leaves increased from the beginning of the season until it reached its highest point early in June, after which it showed a slight decrease or remained constant according to the sample site. There was also a marked interaction for most food quality characteristics (water, protein, amino acid, carbohydrate content) between plant phenological age and sample site (Table 1), indicating that food quality patterns differ among sample sites. Only lignin content showed the same consistent pattern at all sites.

Pupal size was highest early in the season (Table 2, Fig. 3), decreased gradually during the first 2 months (April and May), and levelled off to reach a plateau during June and July. There was an interaction between plant phenological age and place, indicating that the changes in pupal size differed among sites. Pupal size was related positively to all nutrient components except protein content (Table 3). The amount of lignin was related strongly and negatively to pupal size. The puparium of females was significantly longer than that of males.

Laboratory experiment

Only two food quality parameters were related to plant phenological age (Table 4). Water content was highest in the youngest age class and decreased with plant age. The opposite pattern was found for protein content, which increased with plant age. The other food quality parameters (carbohydrates, amino acids, lignin content) were unrelated to plant phenological age.

Oviposition preference did not differ among age classes ($Q = 0.17$, $P = NS$; Fig. 4). Offspring survival decreased with plant age (Table 5, Fig. 5). The other measures of offspring performance, total development time and pupal size, did not differ among treatments, although pupal size tended to be smaller on the oldest age class.

Discussion

The field experiment illustrated that most nutrient components (water, carbohydrates, proteins) peaked early in the season and decreased with plant phenological age, and that lignin content increased clearly with plant phenological age. This is consistent with the pattern observed in fixed-growth species

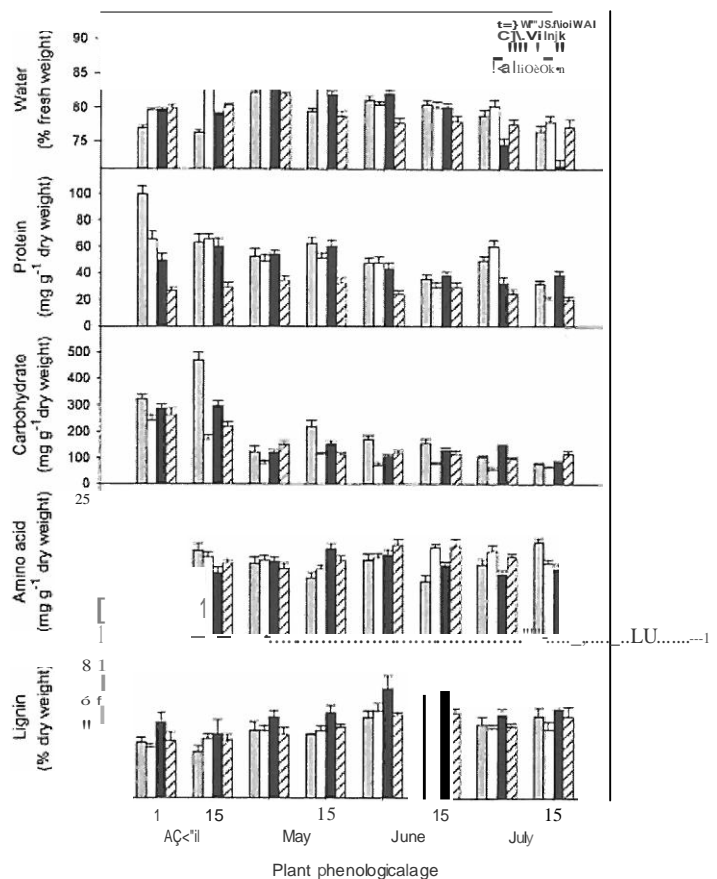


Fig. 2. Seasonal changes in leaf nutritional quality (mean \pm 1 SE) in the field experiment.

Table 2. Mixed model analysis of the seasonal changes in pupal size in the field experiment.

Effect	d.f.	F	p
Sex	1,377	285.29	0.0001
Plant age	1,380	117.52	0.0001
Plant age ²	1,380	64.36	0.0001
Plant age X place	3,114	6.57	0.0004

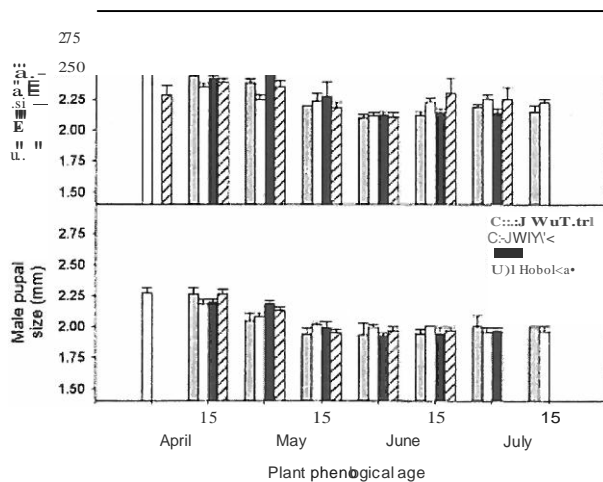


Fig. 3. Seasonal changes in pupal size (mean \pm 1 SE) in the field experiment.

although some variations were observed. For instance, the period of high foliar water content early in the season (May) was preceded by a period of low water content due to the presence of many winter-growth and expanding leaves. Secondly, the protein content decreased with plant phenological age at most sample sites, as observed in other grasses (Bernays & Barbehenn, 1987; Buxton & Marten, 1989; Bernays & Chapman, 1994) and fixed-growth species (e.g. Feeny, 1970; McNeil & Prestidge, 1982; Potter & Kimmerer, 1989), however the pattern was less straightforward compared with the Jatter group. Firstly, the general decrease in protein content was not observed at one site, i.e. Hoboken. Different growth conditions, namely a higher light exposure at Hoboken, may explain this difference (Russel & Grace, 1979; Van Soest, 1982; Kephart *et al.* 1992; Kephart & Buxton, 1993). Secondly, protein concentrations peaked for a second time at the end of the season at Wuustwezel and Wilrijk. This second peak was most probably caused by the large flush of protein-rich leaves produced by the second generation of shoots (see also Tscharrntke & Greiler, 1995).

Few studies testing the plant phenological age hypothesis have focused on the seasonal variation in carbohydrate or amino acid content despite the potential influence of these components on herbivore preference and performance (Schoonhoven *et al.* 1998; Bernays & Chapman, 1994). The Jatter is confirmed by the positive effects of carbohydrate and amino acid content on the pupal size of *C. milii* in the field experiment. Extremely high carbohydrate concentrations were observed early in the season (April), with a steep decrease in the first half of May, after which they remained more or less constant. Studies on fixed-growth species have shown a more gradual decrease in carbohydrate content (Hili, 1982; Schroeder, 1986). The unusually high carbohydrate concentrations found in April are typical for winter growth species, and are assumed to be an adaptation to overcome cold periods because they provide some freezing tolerance (Bernays &

Table 3. Mixed model analysis of the relationship between pupal size of *Chromatomyia milii* and the seasonal changes in food quality in the field experiment.

Effect	Estimate	SE est.	d.f.		<i>p</i>
Intercept	1.0433	0.3886	45.2	2.68	0.0101
Sex: males	-0.2166	0.0177	43.7	-12.24	0.0001
females	0.0000				
Water	0.0168	0.0046	45.7	3.63	0.0007
Protein	-0.0017	0.0012	43.8	-1.48	0.1453
Carbohydrate	0.0004	0.0002	46.8	2.52	0.0151
Amino acid	0.0229	0.0053	47.0	4.33	0.0001
Lignin	-0.1003	0.0184	41.1	-5.44	0.0001

Chapman, 1994; Larcher, 1995). The only nutrient component that showed no obvious relationship with plant phenological age in the field was amino acid content. This is to be expected because amino acid content is altered continuously throughout the growth season by a broad range of processes that involve transport needs such as growth, senescing, flowering, and responses to stress conditions (Bernays & Barbehenn, 1987; Bernays & Chapman, 1994).

In the laboratory experiment, most food quality parameters showed a different relationship with plant phenological age compared with the field experiment. The soluble carbohydrate, amino acid, and lignin content were not related to plant phenological age in the laboratory, while carbohydrate content decreased and lignin content increased in the field. Protein content was related positively to plant phenological age, which is at variance with the literature and the field experiment. The water content decreased with plant phenological age, which is consistent with the field experiment.

The differences between field and laboratory conditions may be explained by the different experimental approach between the experiments. Firstly, environmental influences were held constant (i.e. temperature, humidity, nutrient availability) or were excluded (i.e. herbivory) during the laboratory experiment. As environmental variables influence the seasonal changes in foliar nutritional quality (Van Soest, 1982; Karban, 1990; Stamp & Bowers, 1990b; Bowers & Stamp, 1993; Larcher, 1995), it is likely that the lack of environmental influences in the laboratory experiment partly determines the difference from the field experiment. Secondly, the field experiment was performed with perennial plants subjected to winter conditions before the start of the experiment. Winter growth may result in extremely high foliar carbohydrate concentrations, elevated protein content, and lowered water content in winter growth and early season leaves (Bernays & Chapman, 1994; Larcher, 1995), as illustrated by the foliar nutrient content of leaves collected in April in the field experiment. The fact that the plants in the laboratory experiment were reared from seedlings and therefore not from perennial tufts subjected to winter conditions before the experiment is most likely an additional reason for the relatively small variation in nutritional quality observed among age classes in the laboratory experiment. These results point out that great care should be taken with the extrapolation of laboratory results to field conditions in the context of the plant

Table 4. Mixed model analyses of the relationship between plant age and food quality in the laboratory experiment.

Age class	Water (mg g ⁻¹ dry mass)	Protein (mg g ⁻¹ dry mass)	Carbohydrate (mg g ⁻¹ dry mass)	Amino acid (mg g ⁻¹ dry mass)	Lignin (% dry mass)
15 weeks	88.15 ± 0.29	10.23 ± 0.41	81.01 ± 4.50	26.08 ± 1.12	4.98 ± 0.13
30 weeks	85.99 ± 0.24	10.86 ± 0.50	77.87 ± 2.50	22.51 ± 0.99	4.70 ± 0.11
45 weeks	85.66 ± 0.23	14.88 ± 0.92	75.63 ± 4.47	28.71 ± 1.11	4.70 ± 0.08
Results mixed model regression analysis:					
Effect: plant age	F _{134,0} =24.27 P=0.0001	F _{1,3} •2= 13.35 P=0.0009	F _{1,3} •9=0.68 P=0.4161	F _{1,33,2} =1.33 P=0.2579	F _{1,30} =2.39 P=0.1313

phenological age hypothesis, and suggest that environmental influences play a greater role in determining the changes in nutritional quality with plant phenological age of *H. lanatus* than do ontogenetic (maturation) and physiological (senescence) processes.

Despite the continuous flushing of young leaves, the nutritional quality of *H. lanatus* leaves in the field experiment decreased with plant phenological age. This has been observed in other free-growth species (Schultz *et al.*, 1982; Stamp & Bowers, 1990a; Bowers *et al.*, 1992). Foliar nutritional quality of free-growth species is equally likely to decrease with plant phenological age as in fixed-growth species, for the following reasons: (1) The continuous flushing of leaves in free-growth species does not imply that leaf ageing processes will not influence the seasonal changes in nutritional quality. Mean leaf age varies throughout the season, and the largest proportion of young leaves will always be found at the start of the growth season (Stamp & Bowers, 1990a; Bowers *et al.*, 1992; this study). (2) Several environmental conditions such as temperature and rainfall show seasonal cycles. At least in temperate regions, growth conditions become more unfavourable from spring towards the end of the summer because moisture availability and consequently nutrient availability generally decrease during the growth season (Mattson, 1980; Van Soest, 1982). Because plant vigour is related positively to plant nutritional quality in many plant species (Larsson, 1989; Koricheva *et al.* 1998), this process may also account for the general decrease in nutritional quality with plant phenological age in free-growth species. The observation of Stamp and Bowers (1990a) that the nutritional quality of newly flushed leaves decreases with plant phenological age in free-growth species is consistent with this idea. (3) In temperate regions, early season leaves of free-growth species may protect themselves from freezing by high concentrations of carbohydrates and proteins (Bemays & Chapman, 1994; this study). These high levels of nutrients are reduced later in the season as frost becomes less frequent and protection unnecessary.

Offspring performance decreased with plant phenological age in the field and the laboratory experiments. The positive relationship between pupal size and foliar nutritional quality in the field experiment suggests that the changes in food quality are at least partially responsible for the decrease of pupal size with plant phenological age. In the laboratory experiment, food quality differences were much smaller relative to the field

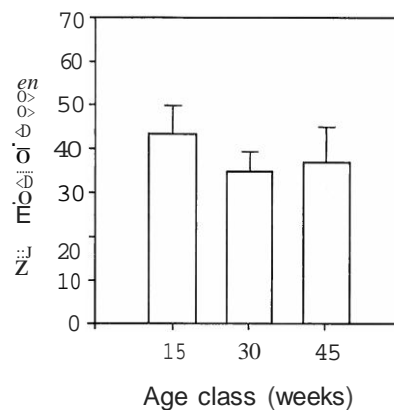


Fig. 4. Oviposition preference of *Chromaromyia milii* (mean ± 1SE) on the three age classes in the laboratory experiment.

experiment, which may explain why pupal size was unrelated to plant phenological age under controlled conditions. Yet, offspring survival was related negatively to plant age at least under laboratory conditions, but offspring performance parameters do not necessarily have to be affected to the same degree (see Thompson, 1988).

The plant phenological age hypothesis predicts that phytophagous insects should prefer young plants to old plants. This is not supported by the laboratory experiment because *C. milii* showed no oviposition preference for younger plants despite the negative fitness consequences of ovipositing on older plants. A similar pattern has been observed for the agromyzid *Phytomyza ilicicola* Loew (Marino & Comell, 1993). Poor correlations between oviposition preference and offspring performance are frequently observed (Thompson, 1988; Courtney & Kibota, 1989). As a consequence, a large number of hypotheses have been proposed to explain this seemingly non-adaptive oviposition behaviour of many phytophagous insects (Thompson, 1988; Courtney & Kibota, 1989). A popular hypothesis, for instance, is that top-down forces such as parasitoids, predators, and diseases may shape host preference patterns, rather than bottom-up forces such as host nutritional quality (Bemays & Graham, 1988); however this does not seem to be the case in *C. milii*. Recent results suggest that the optimisation of adult performance (adult

Table 5. Larval performance of *Chromatomyia mili* on the different age classes in the laboratory experiment.

Effect	d.f.	F	p
Offspring survival			
Plant age	1,23	16.52	0.0005
Total development time			
Plant age	1,30.2	0.71	0.4062
Sex	1,361	0.21	0.6466
Pupal length			
Plant age	1,27.6	2.92	0.0988
Sex	1,401	396.30	0.0001

performance = realised fecundity) rather than the optimisation of offspring performance (offspring performance = survival, development time, and size of offspring) shapes feeding and oviposition preference patterns of *Chromatomyia* miners (Scheirs, 2000; Scheirs *et al.*, 2000). This means that females of *C. mili* feed and oviposit on the host that best enhances adult performance not offspring performance. The influence of plant phenological age on adult performance of *C. mili* should therefore be investigated in order to explain the oviposition preference of this miner. As the optimisation of adult performance is most probably an important, but largely neglected, mechanism that shapes host preference patterns of phytophagous insects (Scheirs *et al.*, 2000; Mayhew, 2001), future studies testing the plant phenological age hypothesis should also take this mechanism into account when focusing on host preference.

Variation in food quality and availability is believed to constrain the number of generations of phytophagous insects in fixed-growth species (e.g. Feeny, 1970; Schroeder, 1986; Rossiter *et al.*, 1988; Potter & Kimmerer, 1989; Reavey & Lawton, 1991). It has been postulated that temporal constraints should be reduced for insects that attack free-growth species (Niemelä *et al.*, 1982; Jordano & Gomariz, 1994). The results of this study are consistent with this hypothesis because *C. mili* occurs throughout the whole growth season of its host despite the seasonal variation in offspring performance. The effect of host nutritional quality on insect phenology should not be overemphasised, however, as the number of generations is also determined by constraints other than food, such as the degree of parasitisation and the length of the life cycle (see Reavey & Lawton, 1991).

Foliar nutritional quality varied not only temporally but also spatially in the field experiment, as indicated by the many significant interactions between sampling site and the different food quality parameters (water, proteins, amino acids, carbohydrates). This may be caused by inter-site variation in environmental conditions but also by a different phenotypic or genotypic response of the grasses to changing environmental conditions at the different sites (Bowers & Stamp, 1993; Wainhouse & Ashburner, 1996). There was also considerable spatial variation in pupal size. Because of the positive relationship between pupal size and foliar nutritional quality, it is most likely that at least some of the inter-site variation in

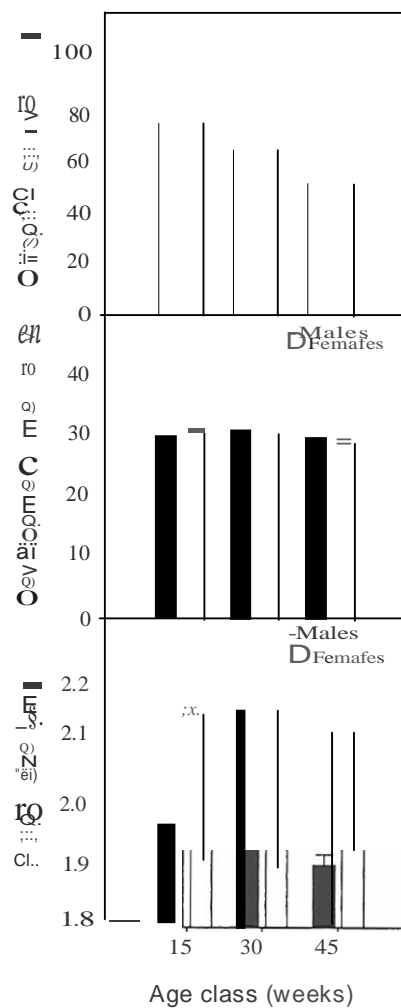


Fig. 5. Larval performance of *Chromatomyia mili* (mean \pm 1 SE) on the three age classes in the laboratory experiment: (a) offspring survival, (b) offspring development time, (c) pupal size.

pupal size is due to the spatial variation in food quality. Therefore, spatial and temporal variation in food quality plays an important role in the population dynamics of *C. mili*, as observed in other plant-insect systems (see also Bowers *et al.*, 1992; Floater, 1997).

In conclusion, the field experiment supported the plant phenological age hypothesis clearly while the laboratory experiment gave only little support. Therefore, only the data from the field experiment are consistent with the idea that this hypothesis holds for free-growth species. It is argued that leaf ageing processes and environmental conditions influence the seasonal changes in food quality under field conditions, and that the constant environmental conditions in the laboratory experiment account at least partially for the differences from the field study. Future studies should test whether the plant phenological age hypothesis holds in a broad range of free-growth species. Direct comparisons of closely related free- and fixed-growth species should point out whether the food quality

patterns differ between these growth forms and whether food quality can constrain the number of generations of phytophagous insects in free-growth species.

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