

# Phenological synchrony between *Scaphoideus titanus* (Hemiptera: Cicadellidae) hatchings and grapevine bud break: could this explain the insect's expansion?

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## Abstract

*Scaphoideus titanus* is the invasive vector of the phytoplasma causing the Flavescence dorée in European vineyards. This epidemic is a serious threat to viticulture that has been increasing for more than 60 years in Europe. We studied the effect of synchrony with the plant phenology and the effect of plant-sap quality on the individual fitness. Thus, we conducted laboratory experiments to determine if insect hatchings were synchronized with grapevine bud break. We used two natural populations: one from a cold winter vineyard and one from a mild winter vineyard. In both cases, egg hatching was synchronized with bud break and leaf appearance. The phloem quality of the young and old leaves as a food source was analysed by high-performance liquid chromatography, and the effects on *S. titanus* growth were evaluated. Phloem composition varied with the grapevine cutting's age but also varied between leaves of different ages from the same plant. The older leaves were less nutritious because they had the highest carbon-to-nitrogen ratio and the lowest content of essential amino acids. Despite diverse phloem qualities, no fitness difference was observed. We found that the synchronization of egg hatchings with bud break is well regulated. However, the nymphs are not affected by the phloem-sap quality, suggesting that *S. titanus* may accept different food qualities and that egg hatching synchrony could contribute to population expansion in vineyards.

**Keywords:** phloem quality, synchrony, grapevine, *Vitis vinifera*, Flavescence dorée

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## Introduction

In addition to directly affecting fitness through mortality, development, reproduction and/or offspring's performance

(Kroon & Veenendaal, 1998; Ellers & van Alphen, 2002; Wang *et al.*, 2006; Chuche & Thiéry, 2012), diapause may have an indirect influence by changing the synchrony of insect occurrence with host resources. In phytophagous insects, synchrony with the host plant's phenology can be defined as the time elapsed between the appearance of the insect and the most relevant host phenological stages to its development (Yukawa & Akimoto, 2006; van Asch & Visser, 2007). Variation in the degree of synchrony has important consequences on reproductive success. Indeed, larvae emerging

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when food is missing or not optimal for their development must escape to find more suitable habitats or develop on poor quality food, which has a fitness cost (Lawrence *et al.*, 1997; van Asch & Visser, 2007). In contrast, individuals leaving diapause when their host plant is in the most favourable condition for their development have a greater fitness (Dixon, 1976; Watt & McFarlane, 1991; Yukawa & Akimoto, 2006).

Depending on the host plant's phenology, the composition and texture of the targeted organs varies, resulting in great consequences on insect fitness (Mattson, 1980; Gould *et al.*, 2007; Marchi *et al.*, 2008). Thus, the poor food quality and/or quantity available for larvae generally leads to a smaller adult size with a lower energy reserve, which may hinder female fecundity and/or the reproductive success of male insects (Scriber & Slansky, 1981; Awmack & Leather, 2002; Moreau *et al.*, 2006, 2007).

Plant quality is mainly determined by its chemical composition, including the concentrations of secondary metabolites and the nitrogen content (Mattson, 1980; Awmack & Leather, 2002). Nitrogen content is a key factor for a wide range of phytophagous insects because of its importance in protein synthesis (Mattson, 1980; Karley *et al.*, 2002; Wilkinson & Douglas, 2003). Hence, a high availability of nitrogen often increases the growth and survival rates (Hunter & McNeil, 1997; Jonas & Joern, 2008). In the same way, the carbon-to-nitrogen (C:N) ratio is often crucial and was shown to limit the spatial distribution and growth of several sap-feeding insect populations (Mattson, 1980; Wilkinson & Douglas, 2003; Bi *et al.*, 2007). The chemical composition of plants varies within the season. This was specifically demonstrated for the sucrose and amino acid phloem contents (Douglas, 1993; Yao & Akimoto, 2002; Quental *et al.*, 2005). It was also demonstrated that the phloem composition varies among plants of the same species and within plants, particularly as a function of leaf age (Merriitt, 1996; Karley *et al.*, 2002).

*Scaphoideus titanus* (Hemiptera: Cicadellidae) feeds on phloem, which has a high sugar level and a low nitrogen content (Mattson, 1980; Douglas *et al.*, 2006). Free amino acids are the main available nitrogen form in the phloem and their composition, especially in essential amino acids, is a determining factor of the sieve quality (Wilkinson & Douglas, 2003; Hunt *et al.*, 2006). Sucrose is the most important mobile sugar in the phloem of many plants (Karley *et al.*, 2002) and, as the only carbon source for phloem-sap feeding insects, it acts as a phagostimulant, strongly impacting feeding behaviour (Douglas *et al.*, 2006; Pescod *et al.*, 2007).

Even though *S. titanus* was introduced from north-eastern America to France and the rest of Europe more than 70 years ago (Bonfils & Schvester, 1960; Chuche & Thiéry, 2014), it is subjected to surprisingly little predation or parasitism (Malausa & Sentenac, 2011). *Scaphoideus titanus* was first observed in southwestern France in 1958 (Bonfils & Schvester, 1960). In the 1960s, *S. titanus* individuals were discovered in many south French vineyards, southern Switzerland and in north-western Italy (Schvester *et al.*, 1962; Vidano, 1964). This species continued to expand quickly and was found in Slovenia in the 1980s, in Portugal in the 1990s, in Austria, Bulgaria, Bosnia-Herzegovina, Croatia and Romania in the 2000s (Chuche & Thiéry, 2014). *Scaphoideus titanus* is a specialist of *Vitis* and its distribution is restricted to the European vineyards between 35 and 50° north latitude (Chuche & Thiéry, 2014), which can partly be explained by climatic factors. The current hypothesis is that it adapts well to cold winters in accordance with its North American origin

(Caudwell & Larrue, 1979). Recent studies confirmed the role of cold winter conditions on hatching dynamics (Chuche & Thiéry, 2009) and on the synchrony between the two sexes (Chuche & Thiéry, 2012). *Scaphoideus titanus* is a univoltine species that lay their eggs at the end of the summer. Then, eggs pass the winter during a 6–8 month diapause stage (Chuche & Thiéry, 2014). Diapause does not require the exposure to cold temperatures to be broken but hatching dynamics appear to vary according to winter temperature (Chuche & Thiéry, 2009, 2012, 2014).

In this study, our goal was to assess if the success of *S. titanus* could be partly explained by good synchrony between hatchings and host plant bud break, and/or plasticity for maintaining a good fitness level despite the sub-optimal food quality. Specifically, we tested the following hypotheses: (1) *S. titanus* egg hatchings are synchronized with grapevine bud break, irrespective of the winter temperatures; (2) first and last hatching nymphs are not exposed to the same phloem quality; and (3) phloem quality has no significant effect on *S. titanus* nymph and adult fitness.

## Materials and methods

### Biological materials

Grapevine (*Vitis vinifera* cv. Cabernet Sauvignon) cuttings used for experiments were grown in a potting compost mix (Substrate 5; Klasmann-Deilmann, Geeste, Germany) and irrigated twice a week. No pesticide was used on these plants.

Leafhopper nymphs were obtained from two natural egg populations collected as previously described (Caudwell *et al.*, 1970; Chuche & Thiéry, 2009). Two-year-old grapevine woody canes carrying eggs were collected in mid-February 2010 in organic vineyards where sizable populations were observed over successive years. Egg hatchings were obtained by placing woody canes inside plastic hatching cages (50 × 38 × 36 cm) in a climate chamber under a 16:8 (L:D) photoperiod, at 23 ± 1°C, and 65–70% relative humidity (RH). To avoid egg desiccation, a 1-cm layer of vermiculite (Efisol, Strasbourg, France) was placed below the eggs and humidified with water every 7 days. To harvest neonatal nymphs, six cut leaves of a grapevine maintained in a glass tube with water, were added to the cage approximately 20 days after the eggs were placed at 23 ± 1°C. Leaves were replaced when they began to wither.

### Insect–plant synchrony

In this experiment, we used two *S. titanus* populations: one from the *S. titanus* introduction area, near Bordeaux (44°53' 54.41"N 0°2'0.30"W), and the other from the north-eastern limit of its spread in France, in Burgundy (46°45'27.31"N 4°41' 23.95"E). Woody canes were randomized by grouping all the collected canes from a same origin and splitting them into three samples with *ca.* 3 kg in each. Using this method, batches are expected to bear equivalent amounts of eggs. Because the egg populations came from two vineyards, the cultivars of origin also differed: in Bordeaux, the cultivar was Merlot and in Burgundy, it was Pinot Noir. Because the Burgundy vineyard is subjected to colder conditions than the Bordeaux vineyard, we assumed that these two geographical origins offered sufficiently different populations on which to conduct this experiment. In each hatching cage, nymphs were gently removed each day from the leaves using a pooter, and the number of nymphs found was taken to be the number of

hatching eggs. Additionally, the number of buds that broke on the Merlot or Pinot Noir woody canes carrying eggs were scored. Observations ended when no hatching occurred for 7 consecutive days.

#### *Leafhopper fitness*

When a high number (over 100) of eggs hatched on the same day, they were placed in rearing cages (same as hatching cages) on a different cultivar, Cabernet Sauvignon, with one of the grapevine age classes: young, at the 5–6 leaf phenological stage, or old, at the 20 leaf phenological stage. Young leaves of old cuttings were isolated from the leafhoppers by enclosing their apical shoots in a clear plastic box ventilated with a piece of insect-proof mesh. To avoid inducing physiological changes in the plants, they were not pruned. Rearing cages were then stored in a climate chamber under a 16:8 (L:D) photoperiod, at  $23 \pm 1^\circ\text{C}$ , and 65–70% RH, and cuttings were replaced every 3 weeks.

From the day of eggs hatching until all the individuals became adults, a weekly sample of 40 insects was taken, characterized and put back in the rearing cage. They were measured, to the nearest 0.01 mm, from the head extremity, without antenna, to the telson ending using a stereomicroscope with a micrometre, and the 4th instar or older nymphs were weighed to the nearest 0.01 mg. We also checked the developmental instar and the sex of 5th nymphal instar and adults (Della Giustina *et al.*, 1992). To compare the developmental state of *S. titanus* egg populations from Burgundy and Bordeaux, we treated all instars as equal development stages by averaging a population instar like the Developmental Index used by Bird & Hodkinson (2005) for Psyllidae:

$$ID = \sum_{i=1}^6 (n_i \cdot i) / T$$

where  $T$  = total number of *S. titanus*,  $i$  = instar code (1st nymphal instar = 1... 5th nymphal instar = 5, adult = 6) and  $n_i$  = number of individuals in instar  $i$ .

#### *Sampling leaf phloem*

Phloem, the food source of *S. titanus*, was analysed to compare the nutritional quality of leaves. Sap samples were collected following the exudation technique described by King & Zeevaert (1974) between the hours of 02.00 and 06.00 p.m. to avoid circadian composition fluctuations in the sap. Exudates were collected from three leaf classes of the Cabernet Sauvignon cultivar: mature leaves from the bottom of the old cuttings ( $N=30$ ), intermediate leaves from the top of the old stage cuttings ( $N=32$ ) and young leaves from the young cuttings ( $N=31$ ).

To ensure a convenient exudation before sap collection, cuttings were stored in a climate chamber at  $20 \pm 1^\circ\text{C}$  with a water vapour-saturated atmosphere using a saturated solution of  $\text{KH}_2\text{PO}_4$  for 24 h. Grapevine leaves were cut just above the petiole/stem junction with sharp scissors and directly re-cut with a razor blade in 20 mM  $\text{Na}_2\text{EDTA}$ , pH 7.0. The petioles were then placed into 1.5-ml Eppendorf tubes filled with 200  $\mu\text{L}$  of 20 mM  $\text{Na}_2\text{EDTA}$ , pH 7.0 solution. Exudation took place in a light proof-box at  $20^\circ\text{C}$  with close to 100% humidity owing to the saturated solution of  $\text{KH}_2\text{PO}_4$ . This eliminated evapotranspiration that would disturb exudation. After 20 h of exudation, 50  $\mu\text{L}$  of each sample were collected and stored in

Eppendorf tubes at  $-20^\circ\text{C}$  until analysed for sucrose contents. The remainder was evaporated with a vacuum concentrator (Speedvac RC1010; Jouan, Winchester, USA) and resuspended in 30  $\mu\text{L}$  of 0.1 N HCl until their use for amino acid analyses.

Because leaf area is highly correlated to the leaf's nitrogen content (van Wijk *et al.*, 2005), leaf areas were measured after exudation with a LI-3100 Area meter (Li-Cor; Nebraska, USA) to normalize samples by relating volume to leaf size.

#### *Amino acid compositions*

Exudates in the HCl solution were shaken by hand and centrifuged at 30,000g for 8 min. Free amino acids were analysed by reverse-phase high-performance liquid chromatography with pre-column derivatization using *o*-phthalaldehyde and 9-fluorenylmethyloxycarbonyl (Jones *et al.*, 1981). The amino acids were separated on a ZORBAX Eclipse AAA C18 column at  $40^\circ\text{C}$ , using an Agilent Technologies 1100 system, and fluorescence and ultraviolet detectors. Amino acids were then quantified by comparing sample peak areas to a reference Amino Acid Supplement (Agilent Technologies, Santa Clara, USA) containing glutamine, asparagine, tryptophan and norvaline using linear regression forced through the origin. All of the amino acids, except proline and cysteine, could be detected by this method, with a detection limit of approximately 0.5 pmol. The protocol followed the Agilent ZORBAX Eclipse AAA application with two mobile phases,  $\text{NaH}_2\text{PO}_4$  (40 mM, pH 7.8) and acetonitrile/methanol/water mix (45:45:10, v/v/v).

#### *Sucrose content*

Because the soluble phloem carbohydrate was mainly sucrose (Turgeon & Wolf, 2009), the sucrose:amino acid ratio was used as a measure of the C:N content of the phloem sap, on which leafhoppers feed. The sucrose concentration in the phloem exudates was analysed with a Sigma Glucose Assay Kit (procedure no. 510). This method required an enzymatic hydrolysis of sucrose by invertase at 10 u.i./ml dissolved in acetate buffer pH 4.5. A volume of 100  $\mu\text{L}$  of phloem exudates were mixed with 10  $\mu\text{L}$  of invertase and incubated for 30 min at  $37^\circ\text{C}$ . Then, 200  $\mu\text{L}$  of a reaction mixture containing glucose oxidase, peroxidase and *o*-dianisidine reagent was added. After a 30 min incubation at  $37^\circ\text{C}$ , the reaction was stopped by the addition of 200  $\mu\text{L}$  of 12 N  $\text{H}_2\text{SO}_4$ .

The sample absorbance was measured at 540 nm (UV-1605; Shimadzu, Kyoto, Japan) and compared with glucose standards.

#### *Statistics*

Effects of instar, insect origin and leaf age on nymph and adult size and weight were examined using an analysis of variance (ANOVA) test after a logarithmic transformation to improve the normality of the data. Before performing the ANOVA tests, data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using the Levene test.

The indexes of development ( $I_D$ ) were compared using an ANCOVA with a feeding category as a covariate (Garcia-Berthou, 2001).

The developmental rates of the leafhopper populations feeding on young or mature grapevine cuttings were determined using linear regressions calculated with  $I_D$  values.

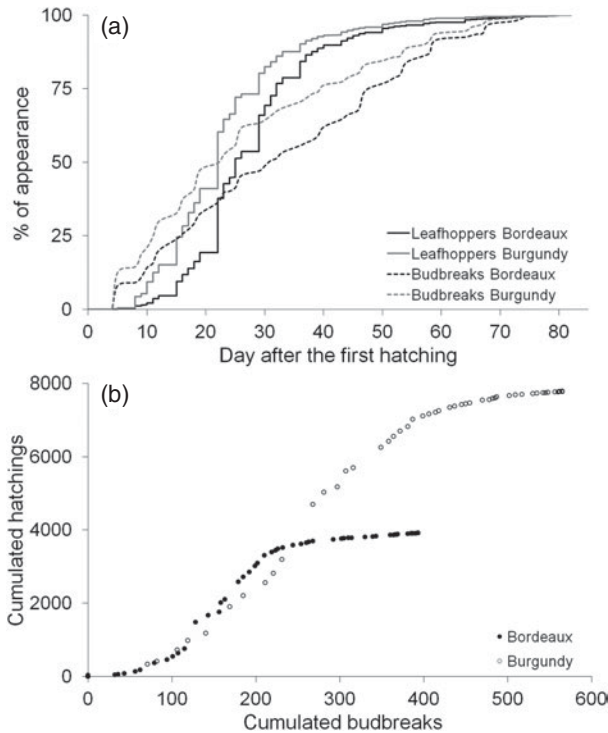


Fig. 1. (a) Cumulative percentage of *S. titanus* egg hatchings and grapevine wood bud breaks from Bordeaux and Burgundy. (b) Synchrony between hatching and bud break for the two vineyards.

Regressions were then compared using Fisher's test (Snedecor & Cochran, 1967).

Synchrony between hatching and bud break was analysed by Spearman's correlation test on the accumulated hatching and bud break dynamics.

In order to avoid any statistical bias in the comparison of phloem chemical composition, all exudates with no sucrose were discarded. The data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using the Levene test.

The amino acid compositions of phloem were compared using a non-parametric multiple ANOVA (NPMANOVA) for 1000 permutations (Anderson, 2001). Then, a principal component analysis (PCA) was performed to describe the influence of plant age on the phloem's amino acid composition after a logarithmic transformation of the data.

Total amino acid and sucrose contents, and the sucrose:amino acid (C:N) ratio, were compared with a Kruskal-Wallis test. Then, we performed post-hoc comparisons using Nemenyi's test (Zar, 2010).

All statistical analyses were performed using the software R 2.8.0 for Windows (R Development Core Team, 2007).

## Results

### *Insect-plant synchrony*

Eggs from Burgundy hatched in a shorter period than those from Bordeaux (log rank:  $\chi^2=371$ ; Gehan-Wilcoxon:  $\chi^2=636$ ,  $P<0.001$ , fig. 1a). After 15, 20 and 26 days, 25, 50 and 75%,

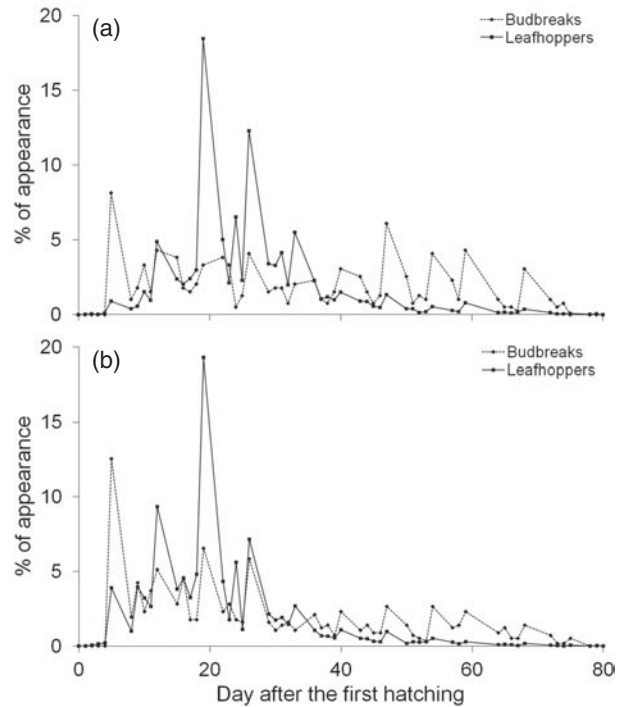


Fig. 2. Daily dynamics of *S. titanus* egg hatchings and grapevine wood bud breaks from (a) Bordeaux and (b) Burgundy.

respectively, of the eggs from Burgundy had hatched, whereas for the Bordeaux eggs to reach the same hatching proportions it took 20, 25 and 31 days, respectively. However, the major peak of hatching was similar, occurring 22 days after the first hatching for eggs that had been exposed to natural cold and mild winters before the experiments. Despite the difference between the two hatching dynamics, the daily variation of hatchings was significantly correlated ( $r_s=0.82$ ,  $P<0.001$ ). Hatching dynamics in the lab evolved similarly, irrespective of the winter conditions the eggs had been subjected to. The difference between the two hatching patterns was based on hatching levels before and after the major peak. Indeed, when the major peak of hatchings occurred, only 25% of the Bordeaux population had hatched, compared with 50% of the Burgundy population.

The number of hatchings were significantly correlated with grapevine bud break regardless of the origin of the eggs (Burgundy:  $r_s=0.99$ ,  $P<0.001$ ; Bordeaux:  $r_s=0.99$ ,  $P<0.001$ , fig. 1b).

For Bordeaux and Burgundy, the peak of bud break occurred 8 days after the first hatching, while the hatching peak was reached after 22 days (fig. 2). The degree of synchrony between food availability and hatchings is the same for both areas because the hatching dynamic exceeds the bud break dynamic after the hatching peak (fig. 2).

### *Leafhopper fitness*

Nymphal development was not affected by the leaf age in our experiments (table 1). Insects that fed on young or mature leaves had similar sizes ( $F=0.01$ ;  $P>0.05$ ) and weights ( $F=1.20$ ;  $P>0.05$ ). Moreover, size ( $F=0.81$ ;  $P>0.05$ ) and

Table 1. Body size and weight of *S. titanus* from Bordeaux and Burgundy vineyards when feeding on young or mature leaves. Nx, nymphal instar x; Ad, adult.

		Young leaves		Mature leaves		
		n	Mean $\pm$ $\sigma$	n	Mean $\pm$ $\sigma$	
Bordeaux	Size (mm)					
	L1	46	1.58 $\pm$ 0.1	40	1.59 $\pm$ 0.18	
	N2	43	2.12 $\pm$ 0.26	49	2.14 $\pm$ 0.49	
	N3	40	2.65 $\pm$ 0.17	40	2.31 $\pm$ 0.63	
	N4	55	3.48 $\pm$ 0.45	45	3.51 $\pm$ 1.09	
	N5 males	49	4.32 $\pm$ 0.25	65	4.44 $\pm$ 0.31	
	N5 females	11	4.66 $\pm$ 0.61	14	4.88 $\pm$ 0.32	
	Ad males	103	4.51 $\pm$ 0.49	88	4.62 $\pm$ 0.19	
	Ad females	13	5.08 $\pm$ 1.02	19	5.49 $\pm$ 0.29	
	Burgundy	N1	41	1.59 $\pm$ 0.09	42	1.55 $\pm$ 0.1
N2		45	2.00 $\pm$ 0.13	43	2.08 $\pm$ 0.13	
N3		47	2.77 $\pm$ 0.15	46	2.78 $\pm$ 0.41	
N4		45	3.51 $\pm$ 0.29	57	3.59 $\pm$ 0.71	
N5 males		65	4.54 $\pm$ 0.92	52	4.42 $\pm$ 0.29	
N5 females		6	4.85 $\pm$ 0.17	16	4.72 $\pm$ 0.33	
Ad males		98	4.67 $\pm$ 0.21	71	4.58 $\pm$ 0.16	
Ad females		12	5.52 $\pm$ 0.35	18	5.39 $\pm$ 0.29	
Bordeaux		Weight (mg)				
		N4	55	1.03 $\pm$ 0.26	45	0.97 $\pm$ 0.89
	N5 males	49	2.01 $\pm$ 0.34	65	2.07 $\pm$ 0.41	
	N5 females	11	2.66 $\pm$ 0.47	14	2.77 $\pm$ 0.57	
	Ad males	103	2.9 $\pm$ 0.38	88	2.85 $\pm$ 0.23	
Burgundy	N4	45	0.98 $\pm$ 0.27	57	0.98 $\pm$ 0.28	
	N5 males	65	2.07 $\pm$ 0.43	52	2.1 $\pm$ 0.45	
	N5 females	6	2.8 $\pm$ 0.37	16	2.67 $\pm$ 0.52	
	Ad males	98	2.84 $\pm$ 0.43	71	2.81 $\pm$ 0.27	
	Ad females	12	4.45 $\pm$ 0.94	18	4.28 $\pm$ 0.85	

weight ( $F=3.17$ ;  $P>0.05$ ) were not influenced by the origin of *S. titanus* populations.

The developmental rate at the different hatching dates could be presented by their linear regression (all  $r^2>0.95$ ,  $P<0.001$ ; fig. 3). The developmental rate did not depend on the leaf age (analysis of covariance; Burgundy:  $F_{1,16}=0.0458$ ;  $P>0.05$ ; Bordeaux:  $F_{1,16}=0.0098$ ;  $P>0.05$ ).

#### Effects of plant and leaf age on phloem quality

The amino acid composition of phloem exudates of young, intermediate and mature leaves were significantly different (NPMANOVA;  $F_{2,92}=5.17$ ;  $P<0.001$ , fig. 4). To explore the variation in phloem amino acid composition, a PCA was performed to compare the different leaf classes. The first two principal components (PC1 and PC2) accounted for 55.62% of the total variation in the dataset (fig. 4a). A plot of the amino acid attributes revealed that PC1 tended to separate essential from non-essential amino acids (fig. 4a). Non-essential amino acids were grouped under PC1, except asparagine. These two components achieved a good separation of young, intermediate and mature leaves (fig. 4b). There was a shift in the amino acid composition during plant development, with some essential amino acids that were present in young leaves, such as isoleucine, leucine and phenylalanine, disappearing during leaf maturation, while the non-essential amino acids content increased. The non-essential amino acids alanine and proline were at a higher concentration in intermediate

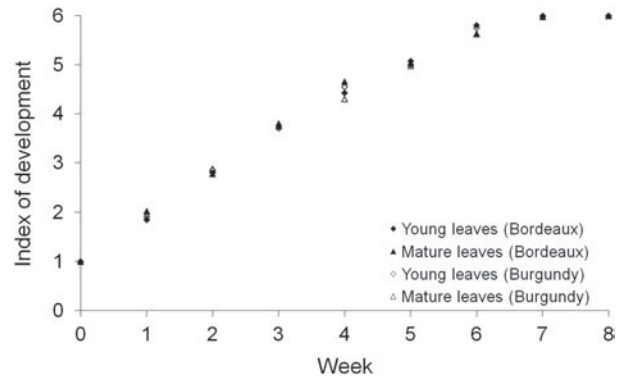


Fig. 3. Evolution of the developmental index of *S. titanus* from Bordeaux and Burgundy vineyards when reared on young or mature leaves.

and mature leaves, representing 29 and 26%, respectively, of the phloem composition, than in younger leaves (only 11% of the composition). Glutamine was detected in all leaves and represented approximately 40% of the concentration of amino acids in young leaves, as opposed to approximately 30% for intermediate and mature leaves. The essential amino acids leucine, isoleucine and phenylalanine represented 12% of the amino acid concentration of young leaves, whereas they are absent in intermediate and mature leaves (fig. 5).

There was a significant difference in the sucrose concentration between the three leaf classes (Kruskal–Wallis;  $\chi^2=7.71$ ;  $P<0.01$ ; fig. 6b). The sucrose:amino acid ratio in phloem exudates varied significantly depending on the age of the leaf (Kruskal–Wallis;  $\chi^2=15.39$ ;  $P<0.001$ ). Mature leaves had a higher ratio than intermediate and young leaves (fig. 6c). No differences of total amino acid concentrations were found between the leaves of different ages (Kruskal–Wallis;  $\chi^2=0.149$ ;  $P>0.05$ ; fig. 6a).

## Discussion

We examined if winter temperature affected the synchrony between a univoltine leafhopper and its host plant. We hypothesized that: (1) hatching would be synchronized with plant bud break, independent of the winter temperatures; (2) early and late hatching nymphs would encounter phloem of different chemical compositions; and (3) phloem quality would have no effect on *S. titanus* fitness.

Our results are in agreement with our hypotheses. We found that *S. titanus* hatching and grapevine bud break dynamics had similar degrees of synchrony after being exposed to either cold or mild winter in their natural environment. We showed that phloem quality varies during plant development without serious consequences on the fitness parameters measured. This minimizes the role of plant-sap quality and maximizes the role of food availability, as determined by hatching and bud break synchrony. We conclude that the persistence of good synchrony and the ability to develop on different qualities of food without a fitness cost can partly explain the invasive success of this species.

European vineyards have very different characteristics. A huge number of cultivars, mainly issued from hybridization within *V. vinifera*, but also across *V. vinifera* and other

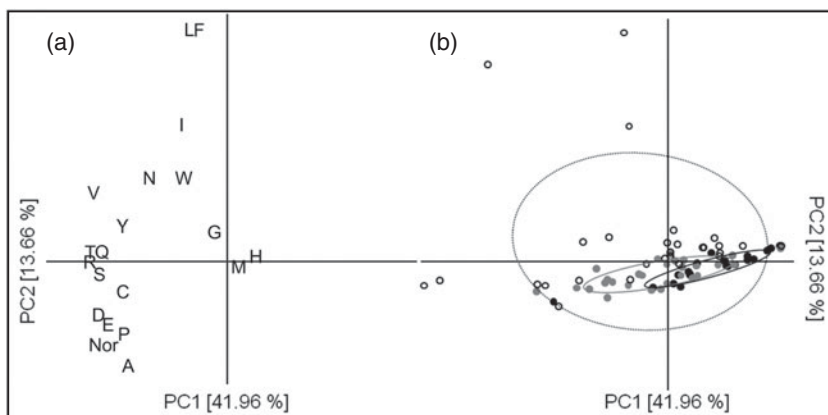


Fig. 4. PCA of the phloem profiles from *V. vinifera* leaves of different ages based on relative amounts of amino acids. (a) Plot of amino acids from leaves of different ages on a grid defined by principal components 1 and 2. For a better readability, amino acids are represented by their single letter code, excepted for norvaline that is a non-proteinogenous amino acid. A: alanine, C: cysteine, D: aspartic acid, E: glutamic acid, F: phenylalanine, G: glycine, H: histidine, I: isoleucine, L: leucine, M: methionine, N: asparagine, Nor: norvaline, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, V: valine, W: tryptophan, Y: tyrosine. (b) Plot of phloem samples on a grid defined by principal components 1 and 2. Open circle and broken ellipse: young leaves; grey circles and ellipse: intermediate leaves; black circles and ellipse: mature leaves.

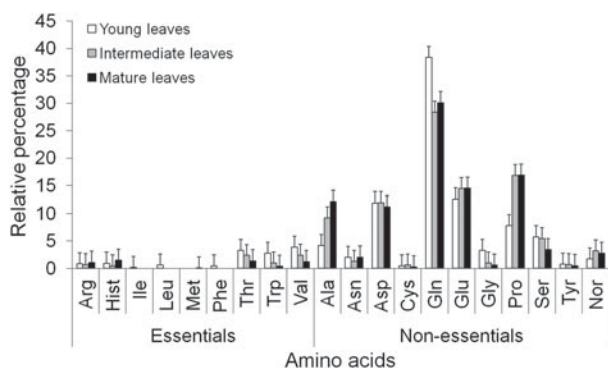


Fig. 5. Amino acid composition of phloem from grapevines of different ages. Ala: alanine, Arg: arginine, Asn: asparagine, Asp: aspartic acid, Cys: cysteine, Gln: glutamine, Glu: glutamic acid, Gly: glycine, His: histidine, Ile: isoleucine, Leu: leucine, Met: methionine, Nor: norvaline, Phe: phenylalanine, Pro: proline, Ser: serine, Thr: threonine, Trp: tryptophan, Tyr: tyrosine, Val: valine.

*Vitis* species, are planted under varying climates. These cultivars have very different anatomical and physiological characteristics, in particular in their phenology (van Leeuwen *et al.*, 2008). During its expansion in Europe, *S. tatanus* was confronted by varied ecological niches with different climates and host plants with different qualities and precocities. Despite this, *S. tatanus* is now widespread in most of the European vineyards (Chucho & Thiéry, 2014). The success of this invasive species is probably based, in part, on its ability to accept different phloem qualities, which are characteristic of the great number of different grape cultivars and their very different growing and training conditions, and on its capacity to maintain a good synchrony with its host plant whatever the climate and the cultivar. Another factor that may explain the invasive success of *S. tatanus* is that only a portion of the *S. tatanus* egg population is sensitive to winter temperatures, with the other part hatching independently of the temperature treatment (Chucho & Thiéry, 2009). This phenotypic

variance could be a bet-hedging strategy allowing for good synchronization in the temperature-sensitive population (Hopper, 1999), while the temperature-insensitive population will survive in an unfavourable environment. Thus, the risk of extinction caused by exceptional climatic events is limited (Rajon *et al.*, 2009).

Like most deciduous woody species of temperate zones, grapevines undergo vegetative dormancy. Bud dormancy is controlled genetically, naturally induced by photoperiod and temperature, and generally breaks after exposure to cold temperatures (Horvath *et al.*, 2003). The time required for initiating bud break, and the time between first and last bud to break, decrease with the duration of the grapevine's exposure to cold, while the number of breaking buds per grapevine increases (Kliewer & Soleimani, 1972). Winter temperatures are a signal inducing both the resumption of the development of the grapevine and of *S. tatanus*. Hence, one may expect the leafhopper and its host plant to be sensitive to the same climatic parameters, but not in the same manner. Indeed, cold temperatures are needed to have a good grapevine dormancy break (Lavee & May, 1997) but not to break *S. tatanus* diapause (Chucho & Thiéry, 2012). However, the different responses of grapevines and *S. tatanus* to temperatures allow the insect and the plant to be synchronized. Indeed, outbreaks of *S. tatanus* are correlated with bud break in *V. vinifera*, after exposure to a mild or a cold winter.

For univoltine phytophagous species, synchrony between hatchings and the occurrence of target plant organs insures optimal food, which reduces nymphal mortality (Rossi & Strong, 1991). To guarantee optimal nymphal survival and fitness, *S. tatanus* egg hatchings should be synchronized with grapevine bud break, not before leaf appearance, and when the young leaves have the highest nitrogen content (Mooney & Gulmon, 1982). This can be illustrated in *Drepanosiphum platanoidis* aphids (Homoptera: Callaphididae). Adults hatching at the same time as the bud of their host plant *Acer pseudoplatanus* (Sapindales: Aceraceae) are twice as large and have faster growing offspring than those hatched after bud break (Dixon, 1976).

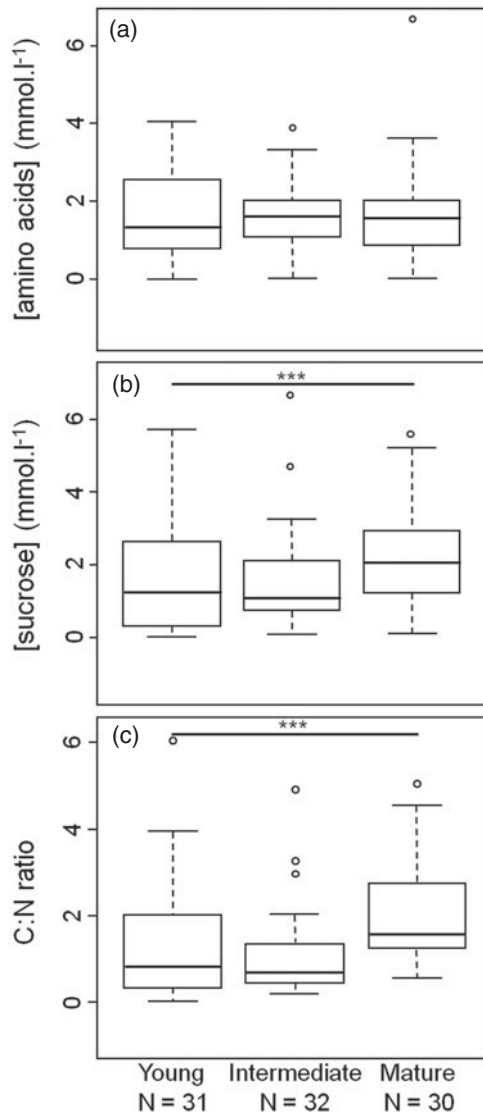


Fig. 6. (a) Amino acid, (b) sucrose content and (c) C:N ratio of phloem from plants of different ages.\*\*\*  $P < 0.001$  (Nemenyi post-hoc test).

The increase in average temperatures induces early bud break of vines (Duchêne & Schneider, 2005), and Chuche & Thiéry (2009) observed that a warmer winter induces a greater spread of hatchings in *S. titanus*. Therefore, an asynchrony between egg hatching and bud break can be associated with higher winter temperatures and have serious consequences on larval survival (Watt & McFarlane, 1991). For example, the synchrony of *Operophtera brumata* (Lepidoptera: Geometridae) with its host plant *Picea sitchensis* (Pinales: Pinaceae) in Scotland shows a lag of  $20 \pm 2.5$  days when the ambient temperature increases  $2^\circ\text{C}$  (Dewar & Watt, 1992). With global warming effecting warmer winter areas, one could expect an earlier but more prolonged hatching period (Chuche & Thiéry, 2009, 2012) when the bud occurrence was early. This could desynchronize the grapevine and *S. titanus*. The majority of nymphs would then appear on older leaves

with a lower nutritional quality but without consequences on *S. titanus* fitness, according to our results. Thus, in the context of climate warming, the expected asynchrony between *S. titanus* and *V. vinifera* would not affect leafhopper populations and *S. titanus* would not be expected to contract its distribution area in future years.

The synchrony of *S. titanus* with the grapevine is an important adaptive advantage, allowing them to receive optimal nutrition both quantitatively and qualitatively, because specialist insects often prefer to feed on young organs (Cates, 1980). The comparison of the phloem contents between leaves of different ages showed a quantitative variation of sucrose and the qualitative composition of amino acids. Phloem composition varies between leaf cuttings of different ages, even between leaves of different ages from the same plant. This can be explained by differences in metabolic activities. Young organs are sinks for compounds produced by other organs of the plant, and they use them to ensure their development. In contrast, mature leaves, by their intense photosynthetic activity, are sources, especially of carbon compounds such as sucrose (Araya *et al.*, 2006). The older leaves are thus less nutritious because of a higher C:N ratio (Wilkinson & Douglas, 2003; Jonas & Joern, 2008), and they have a lower content of essential amino acids (Hunt *et al.*, 2006; Bi *et al.*, 2007). This decrease in the resource quality exists in many plant species and stimulates phytophagous insects to feed on young bodies to increase their reproductive success (Karley *et al.*, 2002; Bi *et al.*, 2007).

Even when simulating asynchrony by feeding nymphs on mature leaves, the life traits measured were not affected by the age of the plant. Differences in measured qualities were not large enough to generate significant differences. The fact that sap quality and fitness were not related in this study suggests that *S. titanus* can accept food variation without a fitness cost, which could represent a strong advantage for the colonization process in different vineyards. Hemiptera, such as aphids, host many secondary symbionts, which provide many functions and allow the insects to expand their ecological niches (Oliver *et al.*, 2010). The role of hemipteran symbionts on plant host adaptation and nutrient supply are well known (Sandstrom *et al.*, 2000; Wu *et al.*, 2006; Chandler *et al.*, 2008; Frago *et al.*, 2012). These symbiotic microorganisms are capable of using low-quality food for the synthesis of vitamins and essential amino acids (Douglas, 1998, 2009; Chandler *et al.*, 2008). They may supply elements, such as sucrose, or amino acids, such as aspartate or glutamine (Sasaki & Ishikawa, 1995). The symbionts are particularly important for insects that are specialized to nutrient-deficient food resources, such as plant sap (Feldhaar & Gross, 2009). The microbial community associated with *S. titanus* was studied and revealed the existence of many symbionts, including the acetic acid bacteria *Asaia sp.* (Marzorati *et al.*, 2006; Sacchi *et al.*, 2008; Crotti *et al.*, 2009). Acetic acid bacteria are suspected to play a role in insect host metabolism by supplying nutrients, such as vitamins or cofactors, by oxidizing certain substrates, or indirectly by supplying metabolites to other microbes beneficial to the host (Crotti *et al.*, 2010). If the amino acid composition of old leaves is a limiting factor for the development of *S. titanus*, this deficiency could be filled via the synthesis of essential amino acids by symbionts, such as *Asaia sp.* For example glutamine could be produced from the phloem. This theory on the role of symbionts in *S. titanus*' invasive success, by providing food supplements when the host is unfavourable, requires further investigation.

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