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Winter temperature and Flavescence dorée vector ecology

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Abstract: The invasive leafhopper *Scaphoideus titanus* is spreading in Europe from ca. 35 to 50°N. According to the origin area of this insect, we hypothesized that its Southern limit of distribution could partly be the consequence of the lack of cold temperatures that are essential to break egg diapause. We investigated the effects of winter incubation temperature on hatching and post hatching development. Here, we conclude that winter temperatures could be one from other key factors explaining the weak colonization of Southern vineyards in Europe by *S. titanus*.

Key words: *Scaphoideus titanus*, diapause, winter temperatures, distribution area, invasive species

Introduction

The leafhopper *Scaphoideus titanus* (Homoptera: Cicadellidae) is a native of the Great Lakes region in North America (Vidano, 1966) and was reported for the first time in Europe in South Western France in 1958 (Bonfils & Schvester, 1960) but probably introduced much earlier if we accept that first observations usually occur at high population levels. Now, *S. titanus* is spreading in Europe from ca. 35 to 50°N (Chuche & Thiéry, 2009) and among abiotic factors, thermal conditions are supposed to explain this distribution. Considering its area of origin this insect is presumably well adapted to cold winter conditions and the Southern limit of the leafhopper distribution could be due to the lack of cold temperatures that are essential to break egg diapause (Caudwell & Larrue, 1979; Steffek *et al*., 2007). We test in this work the hypothesis that several fitness parameters are affected by varying egg temperature incubation in a leafhopper insect with winter diapausing eggs. We hypothesize that the lack of cold temperatures during winter incubation does not prevent hatchings but could explain the Southern limit of the distribution area by the disturbance of embryonic development that produces lower fitness in offspring and affects the operational sex-ratio.

In this study, we exposed a population of *S. titanus* eggs to a warm or a cold 3-months winter and we evaluated how exposure to warm temperatures during egg incubation may have affected the hatching of male and female eggs and the nymphal development.

Material and methods

Insects

An egg population was collected as previously described by Caudwell *et al*. (1970). Twenty-four kilograms of two-year-old grapevine woody canes (20-25cm long) were collected in October 2008 before the onset of winter and the exposure of the eggs to cold weather. The canes were from a vineyard without insecticide treatment in the Southern Bordeaux area where numerous *S. titanus* were observed every year. In order to ensure a similar egg number in each hatching cage, the woody canes were randomized by grouping all the collected canes which were further separated into 12 cages (50 × 38 × 36cm) with *ca.* 2kg in each. To avoid

egg desiccation a 1 cm layer of vermiculite was placed at the bottom of each cage, below the canes, and was moistened with distilled water every week.

Incubation
Three hatching cages were placed in each of 4 different temperature-regulated chambers. In two of them the eggs were submitted to a "warm winter" with a constant temperature of 20°C ± 1°C; in the other two to a "cold winter" of 5°C ± 1°C. The hatching cages were rotated within each climatic chamber once a week to minimize the effect of potential temperature gradients within the chamber. After a 3-month incubation, the hatching cages were placed in a climatic chamber under a 16:8 (L:D) photoperiod, at 23°C ± 1°C, and 65-70% relative humidity. In order to harvest neonate nymphs, six detached grapevine leaves (Cabernet-Sauvignon cultivar) kept in a glass tube with water were added to the cage *ca.* 20 days after the eggs were removed from the climatic chambers, and they were replaced when they began to wither (*ca.* every 15 days).

Hatching dynamics
In each hatching cage, nymphs were gently removed each day from beneath the leaves using a pooter and the number of nymphs found was taken to be the number of hatching eggs. Observations ended when no more hatching occurred during 7 consecutive days. The population dynamics of hatching was determined using the daily counts of nymphs while the hatching dynamics for each gender was based on weekly data.

Post hatching development
For life history trait measurements, every week we isolated all the nymphs that emerged on Monday and put them in a rearing cage with 2 Cabernet-Sauvignon cuttings. This was done from the first week for which cumulate number of hatching exceeded 100 and until this number dropped under this value (9 weeks for eggs incubated at 20°C and 7 weeks for those incubated at 5°C). Of each weekly collection, a sample of 40 insects was randomly selected and insects were measured, until they become all adults. Measurements were made to the nearest 0.01mm using a micrometer under a stereomicroscope, from the head extremity without antennae to the telson ending; we also weighed 4th instar or older nymphs to the nearest 0.01mg. We also checked the developmental instar and the gender of the 5th nymphal instar and adults. In order to compare the developmental state of *S. titanus* egg populations incubated at 20°C or 5°C, and between the hatching weeks for a same temperature, we calculated an Index of Development similar to a Developmental Index created for Psyllidae (Bird & Hodkinson, 2005):

$$I_D = \sum_{i=1}^{6} (n_i.i)/T \quad ,$$

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.

Results and discussion

Diapause breaking

Cold temperature was not required to trigger hatching in our studied egg populations: eggs hatched both at 5°C and 20°C (Figure 1) in similar amounts (mean ± SD; 5°C = 1309.2 ± 160.5, 20°C = 1184 ± 166.4). However, temperatures clearly affected egg hatching dynamics: after incubation at 20°C it began earlier and lasted longer than after incubation at 5°C (Log rank: $\chi^2 = 99.4$; Gehan-Wilcoxon: $\chi^2 = 255$; both: $P < 0.001$). We have already demonstrated the occurrence of temperature effect on hatching length (Chuche and Thiéry, 2009), but this is the first demonstration that cold is not required to break diapause in this species.

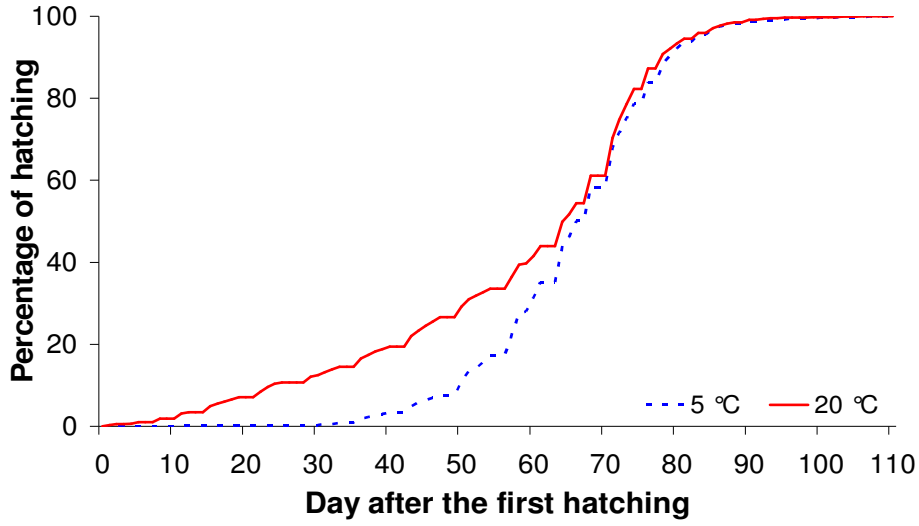

Figure 1. Cumulative percentage of hatchings after a 3-month incubation at 5°C and at 20°C.

Effects on sex-ratio

Incubation temperature did not affect the final sex-ratio of hatchings (0.35 at 5°C; 0.36 at 20°C) but it did change the hatching sex ratio (Figure 2). After 20°C incubation, the sex-ratio of hatching didn't vary a lot along the time while after a 5°C incubation, it increases exponentially. Thus, the degree of protandry was weak after 20°C-incubation and strong after incubation at 5°C.

The hatchings of males and females after warm or cold simulated winter were differentially affected. The male hatchings had a similar linear profile in both thermal conditions while female's profiles differed strongly (Figure 3). Female hatching dynamics after warm incubation was similar to that of males. On the other hand, after cold exposure, females hatched with an exponential pattern (Figure 3). Thus, the different patterns of sex-ratio of hatching after the two thermal treatments were mainly due to the very dissimilar hatchings dynamics in each gender. Hence, protandry varied with temperature incubation and the weaker degree of protandry observed after 20°C incubation was the consequence of similar hatching dynamics of males and females. However, protandry did not completely disappear after incubation at 20°C because developmental time for males was faster than for females and become adult earlier.

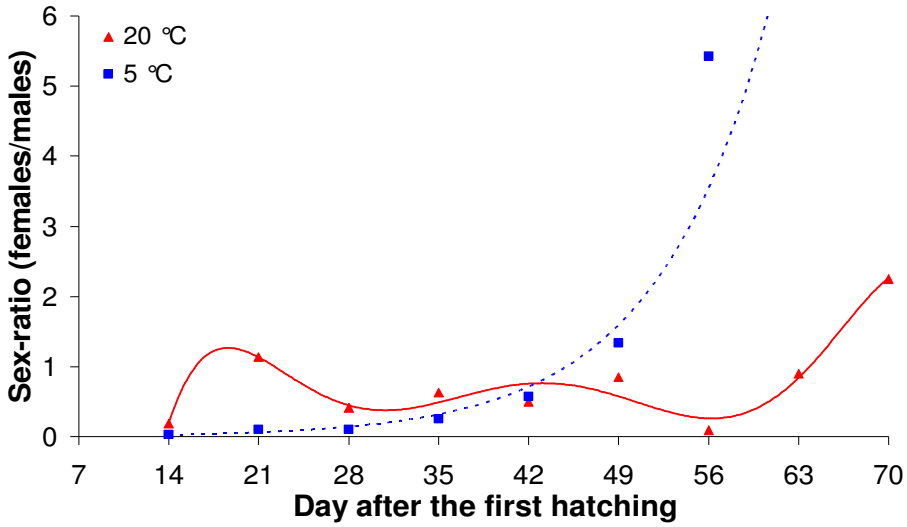

Figure 2. Sex-ratio dynamics of hatchings after incubation at 5°C and 20°C.

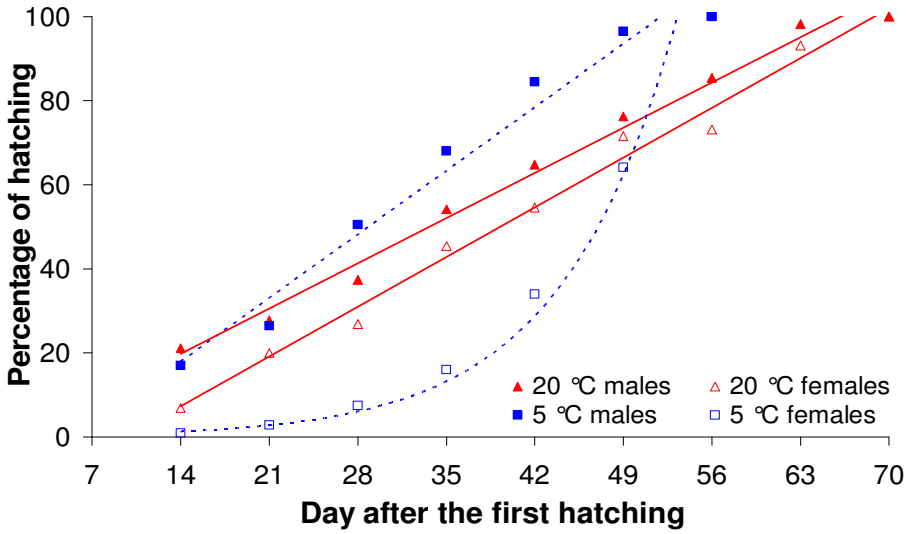

Figure 3. Cumulative percentages of males and females hatchings after incubation at 5°C and 20°C.

Life history traits

The post-hatching development was also affected by incubation temperatures. Temperature did not affect the body size of newly hatched nymphs, but the effect was significant from the third (size) and fourth (weight) nymphal instars: they were always larger and heavier, both for males and females, for insects resulting from 20°C-incubation than from 5°C-incubation (Table 1).

The incubation temperature also affected the developmental rate. Indeed, from one week after hatching and until the end of the experiment, the index of development was always superior for 20°C incubation temperature. The transition from one instar to the following one

is shorter after warm incubation of eggs. Thus, the time needed to become adult and to reproduce was shorter for 20°C incubation of the eggs and so the leafhoppers could be less exposed to natural enemies (predators and pathogens).

Table 1. Life history values for different instars *S. titanus* for two incubation temperatures and results of Wilcoxon test for the effects of temperature on life history traits. Significant P-values are given in bold. Nx: nymphal instar x; Ad: adult.

	5 °C		**20 °C**		**Wilcoxon test**	
	n	mean ± 1 SE	n	mean ± 1 SE	*W*	*P*
Size (mm)						
N1	476	1,63 ± 0,13	533	1,63 ± 0,15	127278	0,927
N2	367	2,12 ± 0,17	451	2,13 ± 0,22	93613	0,799
N3	418	2,73 ± 0,21	476	2,77 ± 0,25	113201,5	**< 0.001**
N4	470	3,49 ± 0,28	498	3,57 ± 0,31	1347,24	**< 0.001**
N5 males	190	4,67 ± 0,33	296	4,81 ± 0,31	83574	**<0.01**
N5 females	375	4,31 ± 0,31	398	4,37 ± 0,37	34464,5	**< 0.001**
Ad males	62	5,28 ± 0,22	174	5,43 ± 0,21	58050	**< 0.001**
Ad females	232	4,52 ± 0,17	398	4,58 ± 0,23	7556,5	**< 0.001**
weight (mg)						
L4	470	1,02 ± 0,22	495	1,10 ± 0,27	137549	**< 0.001**
L5 males	190	2,58 ± 0,57	296	2,80 ± 0,58	88342,50	**< 0.001**
L5 females	374	2,03 ± 0,43	398	2,19 ± 0,60	34284	**< 0.001**
Ad males	62	3,99 ± 0,57	174	4,29 ± 0,57	59000,5	**< 0.001**
Ad females	241	2,79 ± 0,28	396	2,89 ± 0,25	7133,5	**< 0.001**
Index of development						
week 1	360	1,39 ± 0,49	360	1,56 ± 0,51	60102	**< 0.001**
week 2	360	2,20 ± 0,55	360	2,29 ± 0,60	55737,5	**<0.01**
week 3	352	2,90 ± 0,66	339	3,02 ± 0,57	52545	**< 0.001**
week 4	350	3,49 ± 0,63	333	3,75 ± 0,61	56274	**< 0.001**
week 5	347	4,10 ± 0,67	331	4,37 ± 0,60	56634	**< 0.001**
week 6	344	4,58 ± 0,58	323	4,77 ± 0,54	54593	**< 0.001**
week 7	338	5,02 ± 0,64	320	5,26 ± 0,51	53658	**< 0.001**
week 8	310	5,38 ± 0,55	303	5,65 ± 0,50	45355	**< 0.001**
week 9	251	5,71 ± 0,45	238	5,91 ± 0,29	24812	**< 0.001**

Such effects of incubation temperature on post-hatching development were never reported in insects and were only described in reptiles for ectotherms (Shine 2004; Booth 2006), and in birds for endotherms (DuRant *et al.*, 2010).

As a conclusion, we have demonstrated that contrary to what is commonly accepted cold is not necessary to break the diapause. However, winter temperatures variation could be determinant of the geographic distribution of the leafhopper by disturbing the embryonic development and thus acting on others life history traits such as the degree of protandry. Indeed, we showed that an external factor like the temperature to which eggs are exposed can affect protandry by acting only on one sex which is to our knowledge have been observed only in birds (Bogdanova & Nager, 2008). This point is critical because protandry could be a

population strategy that reduces inbreeding by letting males disperse before the female emergence (Morbey & Ydenberg, 2001). That is often observed when males are more mobile than females (Clutton-Brock, 2007), which is the typical case in *S. titanus*.

To resume, winter incubation temperature could be one explaining factor for the lack or weak populations of *S. titanus* in vineyards of Southern Europe by acting on protandry (Chuche & Thiéry, 2011).

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References

Bird, J. M. & Hodkinson, I. D. 2005: What limits the altitudinal distribution of *Craspedolepta species* (Sternorrhyncha: Psylloidea) on fireweed? Ecol. Entomol. 30: 510-520.

Bogdanova, M. I., Nager, R. G. 2008: Sex-specific costs of hatching last: an experimental study on herring gulls (*Larus argentatus*). Behav. Ecol. Sociobiol. 62: 1533-1541.

Bonfils, J. & Schvester, D. 1960: Les cicadelles (Homoptera Auchenorrhyncha) dans leurs rapports avec la vigne dans le Sud-Ouest de la France. Ann. Epiphyt. 3: 325-336.

Booth, D. T. 2006: Influence of incubation temperature on hatchling phenotype in reptiles. Physiol. Biochem. Zool. 79: 274-281.

Caudwell, A., Kuszala, C., Bachelier, J. C., Larrue, J. 1970: Transmission de la Flavescence dorée de la vigne aux plantes herbacées par l'allongement du temps d'utilisation de la cicadelle *Scaphoideus littoralis* Ball et l'étude de sa survie sur un grand nombre d'espèces végétales. Ann. Phytopath. 2: 415-428.

Caudwell, A. & Larrue, J. 1979: Examen du problème de la Flavescence dorée dans le cadre de la sélection sanitaire des bois et plants de vigne. Progr. Agr. Viti. 96(6): 128-134.

Chuche, J. & Thiéry, D. 2009: Cold winter temperatures condition the egg-hatching dynamics of a grape disease vector. Naturwissenschaften. 96: 827-834.

Chuche, J. & Thiéry, D. 2011: Egg incubation temperature differentially affects female and male hatching dynamics and larval fitness in a leafhopper. Ecol. Evol. (in press).

Clutton-Brock, T. 2007: Sexual selection in males and females. Science 318: 1882-1885.

DuRant, S. E., Hepp, G. R., Moore, I. T., Hopkins, B. C., Hopkins, W. A. 2010: Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings. J. Exp. Biol. 213: 45-51.

Morbey, Y. E. & Ydenberg, R. C. 2001: Protandrous arrival timing to breeding areas: a review. Ecol. Lett. 4: 663-673.

Shine, R. 2004: Seasonal shifts in nest temperature can modify the phenotypes of hatchling lizards, regardless of overall mean incubation temperature. Funct. Ecol. 18: 43-49.

Steffek, R., Reisenzein, H., Zeisner, N. 2007: Analysis of the pest risk from Grapevine flavescence dorée phytoplasma to Austrian viticulture. EPPO Bull. 37: 191-203.

Vidano, C. 1966: Scoperta della ecologia ampelofila del Cicadellide *Scaphoideus littoralis* Ball nella regione neartica originaria. Ann. Fac. Sci. Agrar. Univ. Stud. Torino 3: 297-302.