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Larval instars determination for the European Grapevine Moth (Lepidoptera: Tortricidae) based on the frequency distribution of head-capsule widths

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ABSTRACT

Morphological measurements such as head-capsule (HC) width can be very useful and accessible tools that may be employed for classifying Lepidopteran larval instars according to stage of life history. The availability of such measures is crucial in the management of larval pests, because their control relies upon making accurate assessment of the life history stage at which larvae has reached in various environmental conditions. Such forecasts are then used in order to estimate the timing of emergence for future adult populations. Previous studies investigated the use of head-capsule widths from field larvae of European Grapevine Moth (EGVM), *Lobesia botrana* Den. and Schiff., to describe the distributions of the five instars during three generations of the insect. The observations were performed in 1998 and 2002 in a vineyard near Bordeaux. The results presented here increase the scope of earlier methods by providing statistical confidence. Our method was calibrated on a large number of individuals ($N = 552$) issued from our insect culture and uses a nonlinear least-squares parameter estimation to describe the distribution of each larval instar inside each generation. The model was tested on a wild larval population ($n = 3007$) occurring in our experimental vineyard during two complete years. The instar class ranges and boundaries were characterized with the associated probabilities of misclassification. A final classification statistical model is developed for each instar and each generation. From this study, we conclude that larval HC sizes increase statistically according to the generation of the year, and thus is influenced by grape phenology.

The statistical tool may be easily used either by technicians or scientists to determine the larval phenological development of wild populations of the EGVM. The extension of the model to other moths is discussed.

1. Introduction

The European Grapevine Moth (EGVM), *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), is a polyphagous moth (Bovey, 1966; Thiéry and Moreau, 2005) developing on plants from various families. This tortricid undergoes two to four generations in Europe, and usually three in Bordeaux area (Stockel, 2000; Thiéry, 2005). To date, EGVM is one of the most noxious vineyard-pests in the European and Mediterranean vine producer Countries (Bovey, 1966; Gabel and Roehrich, 1995). Damages are focussed on bunches and allow the initiation of several fungi infections, *e.g.* *Botrytis cinerea* (Persoon: Fries) (Sclerotiniaceae) rot on grapes in mid-season and increases grey mold severity at harvest (Fermaud and Le Menn, 1989) or black aspergilli's rot (*Aspergilus niger* and *Aspergilus carbonarius*) producers of ochratoxin A (Cozzi et al., 2006). These infections are quite often related to the larval feeding activity of *L. botrana*.

The control of EGVM populations is more efficiently achieved either by mating disruption or egg/young larvae treatments using chemicals or *Bt* toxin (Stockel et al., 1994; Stockel, 2000), or regulation by natural enemies (Xuéreb and Thiéry, 2006). The efficient control of injuries by winegrowers relies on population dynamics forecasting, and especially oviposition, currently based upon adults traps monitoring (Thiéry, 2005; Thiéry et al., 2006). Against EGVM, the grape protection is currently the most efficient using ovicide techniques applied before or during the ovipositing period of the cycle. However, the oviposition dynamics is difficult to forecast using male sexual pheromone traps and often request the time consuming direct counting on eggs on bunches. Alternative techniques are currently under development like the female trapping and the evaluation of larval age distribution at the previous generation in order to predict the distribution of female emergence.

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Large amounts of fresh eggs can occur and were for example regularly found during one month in summer 2007 in Médoc (Thiéry, unpublished data). In this typical case, almost all larval instars were present in the vineyard at the same time. In such a situation, instar identification is crucial to understand the structure of the larval population.

For some species, the successive larval development steps are clearly defined and visually identifiable. On the contrary, for most worm-like larvae, the use of morphometric parameters is needed to distinguish the different instars.

As the larval head-capsule (HC) is sclerotized (Andersen, 2003), it has a discontinuous growth and thus its size does not increase within one instar. Its width is thus an valuable criteria of identification (Daly, 1985) already used for many species of Lepidoptera and Coleoptera (Caltagirone et al., 1983; Daly, 1985; Got, 1988; Beaver and Sanderson, 1989; Jobin et al., 1992; Mc Clellan and Logan, 1994; Goldson et al., 2001; Hammack et al., 2003). The distribution of this measure allows to quantify the proportion of each instar in a larval population and then to build larval population age pyramids.

This study was intended to provide accurate measures of larval HC in natural populations of *L. botrana* and to propose a statistical tool to determine to which instar larvae collected in the field belong, and thus their age. This was done by sampling large number of specimens in a vineyard and comparing their distribution to a strain laboratory population to validate the results.

2. Materials and methods

2.1. Experimental vineyard

Experiments were conducted in our experimental vineyard [INRA Bordeaux Research centre, Villenave d'Ornon (France)]. This naturally infested plot was surrounded by cultivated vine with conventional management. The vineyard of 1250 m^2 was planted in 1991 with 672 vine stocks of "Merlot noir" cultivar grafted on "101-14 Couderc" with intervals of 1.7 m between rows and 1.1 m between vine stocks. No insecticide was applied to this plot, and a classical fungicide programme was used to protect the bunches (Savary et al., 2009) (copper, cymoxanil with mancozeb, fosetyl with folpel, demethylation inhibitors, quinoxyfen, wettable sulphur and pyriméthanyl applied until the berry maturation stage at mid August). This last fungicide was necessary to control Botrytis mold because Savopoulou-Soultani and Tzanakakis (1990) showed that feeding on botrytis may increase the larval size and HC width. The effect of fungicides on larval growth was not tested in this study, though, to our knowledge only one study on the Coleoptera *Atomaria* spp. related differences in HC width in one from five experiments (Reddersen, 2001).

2.2. Field population larval sampling

Larvae collected in the field under study came from natural infestation. The observations were carried out after egg hatching and before the larval pupation. Insects were collected throughout the growing season, *i.e.*, from April to September in 1998 and 2002 (Table 1). It covered three generations of the moth (G1) occurring in spring on the flower bud period, (G2) during the beginning of summer on green berries and (G3) at the end of summer on ripe berries and beginning of the harvest period. Bunches were collected and dissected under binocular microscope and in total 3007 HC widths were measured as described below.

2.3. Control larvae from insect stock culture

We used the strain of *L. botrana* raised in stock culture conditions as described in Stockel et al. (1989). It originated from larvae collected in different vineyards close to Bordeaux, France. The stock colony is maintained without diapause. Larvae were fed on a semi-artificial diet in a plastic box ($18 \times 12 \times 6$ cm) with the following composition: 220 ml water, 4 g agar, 15 g maize floor, 15.6 g wheat germ, 15 g yeast, 1.28 g ascorbic acid, 0.4 g benzoic acid, 0.4 ml maize oil, 0.4 g Nipagine, and 0.2 g benomyl. The laboratory colony was bred under controlled conditions: 22 ± 1 °C, 60 ± 10% RH and a light (15 h + 1 h): dark (8 h) photoperiod. The first 15 h of the photophase was set at a 1000-lux luminosity and the last one (dusk) at 25 lux.

552 larvae were sampled five times between 7 and 33 days after hatching to have a sufficient sample size for each instar (Tables 1 and 2). They were then collected using a fine brush and their HC were measured as described below.

2.4. Head-capsule measurement

Collected larvae were placed in 1.5 ml of 70% ethanol to stop their growth. Widths were measured as the distance between the most distant lateral sides of HC margins (Fig. 1). These measurements were made with a binocular microscope equipped with a calibrated eyepiece micrometer of 10 μm accuracy at a 50 times fold magnification.

2.5. Statistical analysis

We performed the analysis using the method developed by Mc Clellan and Logan (1994). The overall capsule-size distribution is made under the assumption that the distribution associate to each instar is assimilated to a normal distribution. This approach assumes that no sexual dimorphism exists for the cephalic capsule as shown in the European corn borer (Got, 1988) and in the EGVM (Savopoulou-Soultani and Tzanakakis, 1990). We kept an adjustment method of the equation (1) by nonlinear regression where $b_i = 1/2\delta_i^2$, $c_i = \mu_i$ and a_i depend upon the number of individuals:

$$h_i = \sum_{i=1}^{5}(y_i) \quad \text{with } y_i = a_i e^{-b_i(x-c_i)^2}, \ i = 1,\ldots,5 \tag{1}$$

The calculation function used was performed using SAS® for Windows® and the NLIN procedure (SAS Institute Inc., 2004). We considered a model by sample and by generation. The model was fitted using a *pseudo-*R^2 value computed as $1 - (SSR/SST)$, where SSR is the sum of squares for residuals and SST is the total of sum of squares (Schabenberger and Pierce, 2002). The discrimination

Table 1
Larval sampling periods and counts.

Samples origin	Field						Laboratory
Year	1998			2002			–
Generation	G1	G2	G3	G1	G2	G3	–
Period	May 19th–June 6th	July 9th–24th	September 8th–23rd	May 27th–June 7th	July 11th–31th	September 2nd–27th	7–33 days after hatching
Total number	396	1524	581	242	84	180	552

G1–G3: first to third generation.

Table 2
Larval instar distribution for field and laboratory samples.

Samples origin	Field						Laboratory
Year	1998			2002			–
Instar, *i*	G1	G2	G3	G1	G2	G3	–
1	56	244	57	7	3	13	110
2	66	364	87	19	8	21	70
3	80	312	162	93	41	68	72
4	119	301	160	87	19	62	91
5	75	303	115	36	13	16	209
Total	396	1524	581	242	84	180	552

i: instar number; G1–G3: first to third generation.

criteria between two consecutive instars are based on the misclassifying-error probabilities. The separation point is determined by computing the boundary between the two distributions. It was done looking for the root of $f_i = f_i + 1$ (equation (2)), integrating the averages (m_i and m_{i+1}) and the standard deviations (s_i^2 and s_{i+1}^2) estimations for the considered instar.

$$f_i = \left[1/\delta_i\sqrt{\left(2\pi e^{-1/2(x-\mu_i/\delta_i)^2}\right)}\right] \quad (2)$$

By using the quantiles $q_{i,(i+1)}$ and defining the limits between the instars, we determined the misclassifying-error probabilities of the instar i in $i-1$, $P(X_i < q_{(i-1),i})$, and of the instar i in $i+1$, $P(X_i > q_{i,(i+1)})$ as well as their sum.

In moth species with no supernumerary instar, the HC width follows a geometrical function expressed with the growth rate

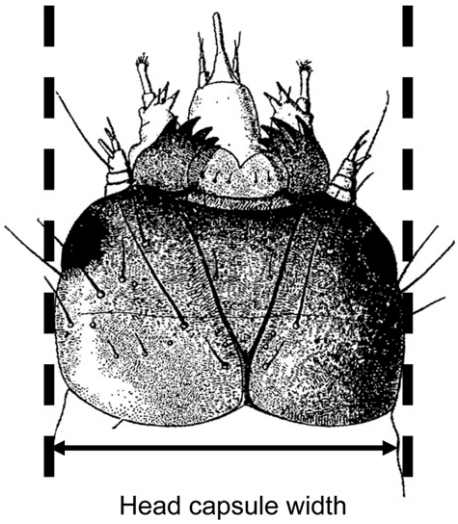

Fig. 1. Principle of head-capsule measurement (adapted from Marchal, 1912).

(Dyar, 1890). It was computed as the ratio of average estimations for two consecutive instars i and $i+1$ (equation (3)):

$$D_{i,i+1} = m_{i+1}/m_i \quad (3)$$

If the assumption is true, the ratio is constant between the successive instars.

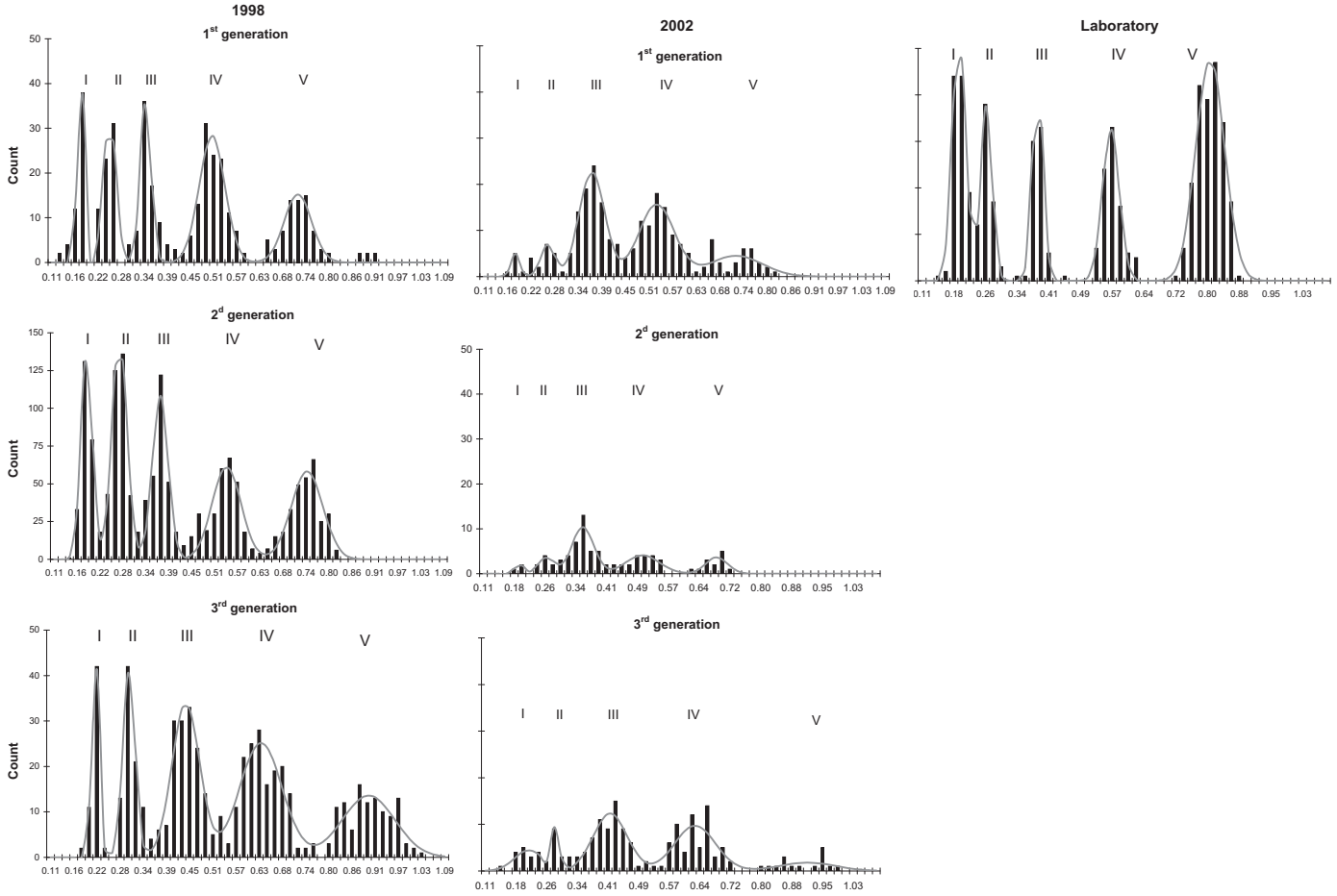

Fig. 2. Distribution of head-capsule widths during each field generation in 1998 and 2002, and for Laboratory. The bars represent the observed data. The line represents combined distribution of the 15 parameter function. The Latin numeral represent the instar larvae.

Table 3
Parameters estimations by instar and generation obtained by mean of nonlinear square from equation (1).

Samples origin	Year	Generation	Instar, i	a_i	b_i	c_i (mm)	R^2
Field	1998	G1	1	59.76	9399.9	0.176	0.9373
			2	33.00	2010.1	0.250	
			3	36.08	2659.4	0.340	
			4	28.44	482.5	0.506	
			5	15.17	421.0	0.721	
		G2	1	135.60	2379.2	0.187	0.9594
			2	147.40	1405.4	0.270	
			3	108.60	1331.8	0.373	
			4	61.19	378.6	0.540	
			5	58.35	319.6	0.743	
		G3	1	45.59	6055.3	0.217	0.9207
			2	41.27	2186.6	0.301	
			3	33.89	419.5	0.442	
			4	25.15	179.0	0.629	
			5	13.58	116.0	0.897	
	2002	G1	1	4.97	4032.9	0.183	0.9629
			2	6.81	1610.4	0.262	
			3	22.65	521.1	0.370	
			4	15.54	262.0	0.528	
			5	4.51	119.8	0.722	
		G2	1	11.23	22980.2	0.193	0.9236
			2	3.57	1142.5	0.263	
			3	10.38	691.1	0.354	
			4	4.10	284.3	0.503	
			5	3.64	670.2	0.688	
		G3	1	4.42	374.4	0.215	0.8776
			2	16.67	13959.1	0.286	
			3	12.35	278.7	0.419	
			4	9.69	190.2	0.632	
			5	1.79	103.1	0.914	
Laboratory	–	–	1	52.02	1840.5	0.195	0.9778
			2	37.90	2558.2	0.262	
			3	41.07	2931.2	0.386	
			4	32.69	1194.3	0.564	
			5	47.65	412.4	0.805	

G1–G3: first to third generation; i: instar number; a_i: constant; b_i: $1/2\delta^2{}_i$ with δ: standard deviation; c_i: μ_i with μ: mean; R^2: obtained from final nonlinear least square fit of equation (1) to the data set.

Table 4
Head-capsule width mean (mm) and growth ratios for field and laboratory samples.

Samples origin	Year	Generation	Instar, i	Mean(i) ± SD(i)	Growth ratio
Field	1998	G1	1	0.176 ± 0.007	–
			2	0.250 ± 0.016	1.42
			3	0.340 ± 0.014	1.36
			4	0.506 ± 0.032	1.49
			5	0.721 ± 0.034	1.43
		G2	1	0.187 ± 0.015	–
			2	0.270 ± 0.019	1.44
			3	0.373 ± 0.019	1.38
			4	0.540 ± 0.036	1.45
			5	0.743 ± 0.040	1.38
		G3	1	0.217 ± 0.009	–
			2	0.301 ± 0.015	1.39
			3	0.442 ± 0.035	1.47
			4	0.629 ± 0.053	1.42
			5	0.897 ± 0.066	1.43
	2002	G1	1	0.183 ± 0.011	–
			2	0.262 ± 0.018	1.43
			3	0.370 ± 0.031	1.41
			4	0.528 ± 0.044	1.43
			5	0.722 ± 0.065	1.37
		G2	1	0.193 ± 0.005	–
			2	0.263 ± 0.021	1.36
			3	0.354 ± 0.027	1.35
			4	0.503 ± 0.042	1.42
			5	0.688 ± 0.027	1.37
		G3	1	0.215 ± 0.037	–
			2	0.286 ± 0.006	1.33
			3	0.419 ± 0.042	1.46
			4	0.632 ± 0.051	1.51
			5	0.914 ± 0.070	1.45
Laboratory	–	–	1	0.195 ± 0.016	–
			2	0.262 ± 0.014	1.34
			3	0.386 ± 0.013	1.47
			4	0.564 ± 0.020	1.46
			5	0.805 ± 0.035	1.43

G1–G3: first to third generation; i: instar number; Mean(i): mean size for instar i; SD(i): standard deviation for instar i.

To test if there is a difference in observed data of the HC widths between the samples origin and the three generations, we used a mixed-model analysis of variance (mixed-ANOVA) on the five instar numbers. The mixed-ANOVA was followed, when necessary, by pairwise comparisons of means. All tests were done by using a 0.05 type I error rate and the MIXED procedure of SAS® (SAS Institute Inc., 2004).

3. Results and discussion

3.1. Control larvae

The widths ranged from 0.144 mm to 0.875 mm. The distribution plots of the head-capsule size show five distinct modes. Each peak corresponds to one instar of EGVM as was observed by Savopoulou-Soultani and Tzanakakis (1990), and Javier saenz-de-cabezon Irigaray et al. (2006). Overlapping observed between first and second instars can be explained by large sample size, like Mc Clellan and Logan (1994). These results however differ from those published by Javier saenz-de-cabezon Irigaray et al. (2006) which present five distinct peaks, without any overlapping. One main difference between this work and our study relies on the sample sizes, much bigger in our study which necessarily promotes overlapping. Also their work used lab individually reared larvae.

The fitting of the 15 parameter function used and our data is presented in Fig. 2. Overlapping corresponds to none equal zero regions on the graph. Equation (1) fitted the overall data set well ($R^2 = 0.978$). In Table 3, we list the value of parameters determined on the course of fitting equation (1) to the data. The data follow Dyar's (1890) hypothesis of a geometrical growth pattern (Table 4). This growth ratio and mean head-capsule size measured for each instar are quite similar to those observed by Javier saenz-de-cabezon Irigaray et al. (2006) on larvae observed before moult.

The boundary values and their associated probabilities of misclassifying are given in Table 5. It illustrates a very low probability of error ($p \leq 0.014$), the maximum value is obtained for the overlapping I–II region.

We can assume that this instar classification method is suitable for EGVM and can be tested in field sampling.

3.2. Larvae collected in the vineyard

The HC widths in 1998 ranged from 0.125 mm to 0.913 mm in G1, 0.144 mm to 0.817 mm in G2 and 0.183 mm to 1.029 mm in G3. In 2002, the range was from 0.163 mm to 0.817 mm in G1, 0.183 mm to 0.721 mm in G2 and 0.144 mm to 0.990 mm in G3 (Fig. 2).

In 2002, level of population was low (one larva per ten bunches), and a small quantity of individual could be sampled especially in G2 (Table 1). The samples in the first instar for the two initial generations were thus very small in 2002 as compared to 1998.

The distribution plots clearly show five distinct peaks for each generation and each year. Modes correspond to instar likes in our stock insect culture. Depending on the generation, we observed some degrees of overlapping between instars. The important

Table 5
Head-capsule width limits estimations and instar misclassification probabilities based on equation (2).

Samples origin	Year	Generation	Instar, *i*	Head-capsule width limits (mm)		misclassification probability		
				Lower	Upper	*i* as *i* − 1	*i* as *i* + 1	*i* as another instar
Field	1998	G1	1	–	0.201	–	0.000	0.000
			2	0.201	0.298	0.001	0.001	0.002
			3	0.298	0.392	0.001	0.000	0.001
			4	0.392	0.610	0.000	0.001	0.001
			5	0.610	–	0.001	–	0.001
		G2	1	–	0.224	–	0.005	0.005
			2	0.224	0.321	0.007	0.003	0.011
			3	0.321	0.434	0.003	0.001	0.004
			4	0.434	0.638	0.002	0.003	0.005
			5	0.638	–	0.004	–	0.004
		G3	1	–	0.250	–	0.000	0.000
			2	0.250	0.347	0.000	0.001	0.002
			3	0.347	0.520	0.003	0.011	0.015
			4	0.520	0.751	0.020	0.010	0.030
			5	0.751	–	0.013	–	0.013
	2002	G1	1	–	0.215	–	0.002	0.002
			2	0.215	0.304	0.004	0.008	0.012
			3	0.304	0.439	0.017	0.013	0.030
			4	0.439	0.612	0.021	0.027	0.048
			5	0.612	–	0.044	–	0.044
		G2	1	–	0.208	–	0.001	0.001
			2	0.208	0.304	0.005	0.024	0.028
			3	0.304	0.416	0.030	0.011	0.042
			4	0.416	0.612	0.019	0.005	0.024
			5	0.612	–	0.003	–	0.003
		G3	1	–	0.271	–	0.063	0.063
			2	0.271	0.306	0.005	0.000	0.006
			3	0.306	0.517	0.004	0.010	0.014
			4	0.517	0.756	0.012	0.008	0.020
			5	0.756	–	0.012	–	0.012
Laboratory	–	–	1	–	0.231	–	0.014	0.014
			2	0.231	0.326	0.014	0.000	0.014
			3	0.326	0.456	0.000	0.000	0.000
			4	0.456	0.655	0.000	0.000	0.000
			5	0.655	–	0.000	–	0.000

G1–G3: first to third generation; *i*: instar number.

numbers of larvae, especially in 1998, may however account for that result (Muggli and Miller, 1980). As the number of insects per bunch never exceed in average two EGVM per bunch, we supposed that competition for food may not have occurred, and was therefore neglected, a bunch of Merlot can easily feed up to 5–6 larvae with no consequence on their individual fitness (our unpublished data).

The measurements of HC sizes and the fitting functions allow separating the five larval instar classes (Fig. 2). In each generation, equation (1) fitted the overall data set with good correlation coefficients ($R^2 = 0.937$, 0.959 and 0.921 respectively for G1, G2 and G3 in 1998; $R^2 = 0.963$, 0.924 and 0.878 respectively for G1, G2 and G3 in 2002). In Table 3, we list the value of parameters determined on the course of fitting equation (1) to the data.

Statistical determination of boundary values between instars is crucial in order to distribute individuals among the classes. Table 5 shows the boundary values and the probabilities of misclassification in each instar. The misclassification probabilities of larval instars were lowest in 1998 (0.000–0.030) than in 2002 (0.001–0.063). The risks of overlapping found are however lower or similar in this study to that observed in other insect pests (Mc Clellan and Logan, 1994). However, this varied and increased for instars three to five from G1 to G3 (Fig. 3), but the maximum probability is still acceptable (less than 0.07). One could thus accept the lower and upper boundaries found in this study. The fact that the risk of misclassifying slightly increased according to the generation in the year could not be attributed to the amount of larvae, but may be attributed to variations in resource quality (Davidowitz et al., 2004) which may have occurred in our field sampling. A previous study showed that bunches used as food by the larvae provides different qualities and thus produced adults with an increasing reproductive success among the year (Torres-Vila et al., 1999); this phenomenon being also observed when offering different grape cultivars or different host plants to the larvae (Thiéry and Moreau, 2005; Moreau et al., 2006a).

According to the Dyar's rule (Dyar, 1890), the mean growth ratio is near 1.4 (Table 4) as we found on laboratory larvae. This matches

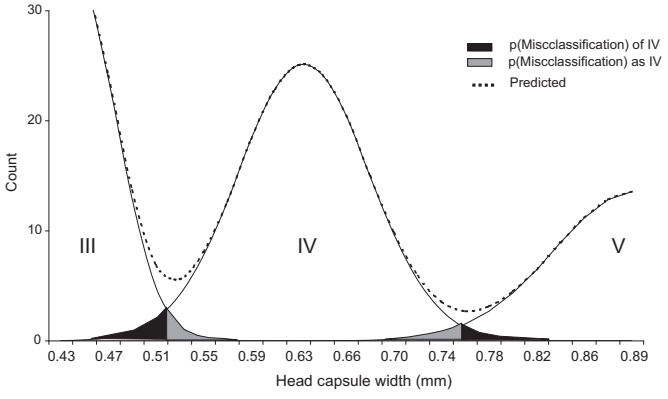

Fig. 3. Fitted functions for instars III–V showing point of intersection and region of overlap for instar IV of the third generation (G3) 1998.

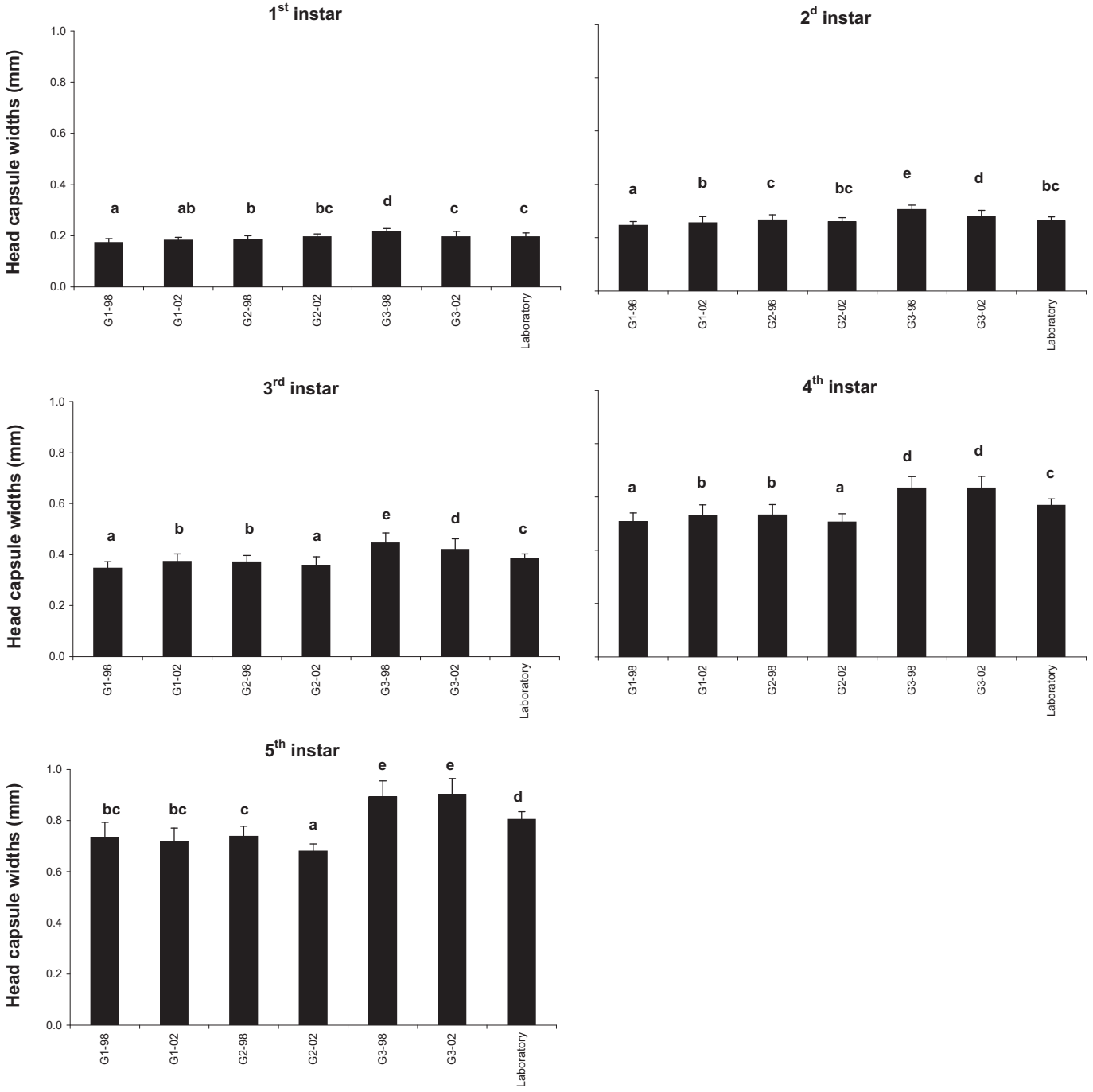

Fig. 4. Mean head-capsule widths per instar larvae on observed data during each field generation (G1: first generation; G2: second generation; G3: third generation) in 1998 (98) and 2002 (02) and for Laboratory. Letters represent statistical grouping of the data defined by the Analysis of variance of the effects of origin vs generation and followed by pairwise difference of least-squares means at the 0.05 level of confidence.

with other studies (Savopoulou-Soultani and Tzanakakis, 1990; Javier saenz-de-cabezon Irigaray et al., 2006). The geometrical growth of the HC width in the field is widely accepted: the mean size of the first instar larvae determined the mean size of the following instars.

3.3. Comparison between samples origin, years and generations

The HC size range has varied widely according to generations (Fig. 4). We observed a statistical difference between generations for a given instar (mixed-ANOVA, $p < 0.0001$ for instars 1–4, and $p = 0.0005$ for the instar 5). In 1998, the first generation larvae were smaller than those of the second one, themselves smaller than the third ones. This result is consistent, being also observed in 2002, but different for the last three instars with the larvae of the second generation statistically smaller than the first generation ($p = 0.0092$, 0.0113 and 0.0098 respectively for the instars 3–5). The third generation was always the largest one. Between the samples, the year 1998 gave taller size larvae than in 2002. The smallest size of the samples collected for this last year can explain this difference (Tables 1 and 2).

The HC widths of laboratory strain are always higher than the first field generation and intermediate with the last two

generations for each year, statistically different from the third generation except for instar 1 in 2002 ($p = 0.9233$). This result is consistent to that obtained by Moreau et al. (2007) who observed that eggs sizes obtained from female reared in our insect stock culture since their immature stage is bigger than those from wild female collected during first generation of EGVM. Also, Savopoulou-Soultani and Tzanakakis (1990) showed that an artificial diet leads to smaller HC widths than ripening berries.

In our laboratory, control larvae were raise with high density (1 larva per 1.1 g of diet) while, during our study, in the last field generation, the density don't exceed 1 larva per 110 g of berries. We can thus assume that diet were not liming in the vineyard samples. The quality of the larval host is a key determinant of fitness (Moreau et al., 2006b) and it suspected for the grapevine to increase during the season. Grape phenology is an important cue to explain oviposition (Thiéry and Gabel, 2000; Maher and Thiéry, 2003; Masante-Roca et al., 2007). After ripening, berries contain higher concentrations of glucose and fructose and oviposition is greater (Maher et al., 2006). This point was confirmed by Savopoulou-Soultani et al. (1999) who had observed an effect of berry stage maturity (sugar and organic acids concentration's) on female pupal weight. The female size depends on of the food quality: larvae that fed on inflorescence (G1) are smaller than those that fed on unripe berries (G2), these last one are smaller than those fed on ripe berries (G3) (Torres-Vila et al., 1999, 2005). The grape phenological stage of the wine and also the cultivar affect the size of the egg (Torres-Vila and Rodriguez-Molina, 2002; Moreau et al., 2006a) and also the size of neonate larvae (Torres-Vila and Rodriguez-Molina, 2002): the larger the size of eggs, the larger the size of emerging larvae.

The morphometric difference was assumed by Daly (1985). The growth of immature insects is strongly influenced by food quality (Berg and Merritt, 2003; Björkman and Petterson, 2003). Such variations had been already observed in Coleoptera (Ali and Hazarika, 1994; Goldson et al., 2001), in Diptera (Easton and Lysyk, 1986) but also on pupal weight on Lepidoptera (Asaro and Berisford, 2001). The larval diet affects the HC size of *L. botrana* as shown by comparing the three generations of larvae within each instar (Savopoulou-Soultani and Tzanakakis, 1990; Mondy and Corio-Costet, 2000), but the variation is less important than for the body size. Temperature was shown to be of minor importance on insect morphometric (Lindroth et al., 1997) except by modifying the growth rate and further reproductive success. This seems to be contradicted, in *L. botrana*, larvae reared under variable temperature were bigger than those collected at constant temperature (Javier saenz-de-cabezon Irigaray et al., 2006), which has however to be confirmed. This effect attributed to temperature may also be a consequence of the feeding behaviour. Inversely than what was suspected by Savopoulou-Soultani and Tzanakakis (1990), larvae growing in the vineyard present bigger HC than laboratory-reared larvae on an artificial diet and under uniform climatic conditions, and the fact that differences exist among the different generations allows us to conclude that HC size is influenced by the quality of the resource.

The present study based on important field samples population demonstrates that *L. botrana* HC width offers a convenient and reliable tool to estimate larval age structure and growth in different field or laboratory conditions. This tool may turn out to be useful in grape protection by offering the possibility to build accurate larval age pyramid within one generation. For example, samples followed by HC measures allowed to describe the wild population in contingency table taking into account a define error level. This can be also be used to feed EGVM mathematical models especially the age structured ones. With a good knowledge of growth timetables as function of temperature and food quality, one could easily predict the dynamics of emergence of the adults, being a key factor to understand the mating success and its possible variation and also the subsequent oviposition dynamics which determine the control strategy to be applied against this pest. Additional data are however needed in order to improve the model of larval instar determination for a more practical use.

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