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Evaluation of the effect of esca disease on bud break in *Vitis vinifera* L.: Possible relationship between cultivars and rootstocks

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Summary

The aim of this study was to investigate the effect of esca disease on bud break of grapevine in relation to different rootstock combinations. For this purpose under field conditions observations of flower bud phenological stages were carried out on three widespread grapevine cultivars 'Cabernet Sauvignon', 'Sangiovese' and 'Trebbiano toscano'. Further phenological observations were recorded under forcing conditions using 'Cabernet Sauvignon' as the most susceptible to esca disease. Each cultivar was grafted on K5BB, 1103P and own rooted. The plants were infected by esca due to the natural presence of fungi in the vineyard. Results showed that esca disease significantly delayed bud break of symptomatic vines for several years. Grapevines on different rootstocks showed different bud break changes, in particular those on K5BB were the most susceptible to esca.

K e y w o r d s : *Vitis vinifera* L., esca disease, phenology.

Introduction

Esca is a trunk complex disease that is one of the most serious grapevine illnesses of biotic origin around the world. The symptoms arise from structural and physiological changes that cannot be reduced to a simple scheme of cause and effect.

The esca symptoms are associated with a general decline, which includes deformed and chlorotic leaves, leaves with tiger stripes, precocious fading, lack of vigour, plugging of the xylem vessels and trunk dieback due to the formation of cankers in the vascular tissue (Martin and Cobos 2007). Apart from the occasional delayed sprouting of esca affected vines in the spring, external symptoms in the Northern Hemisphere develop on the crown between June and September, either through the entire vine or on single branches (Mugnai *et al.* 1999).

The year-to-year unpredictable discontinuity in symptom expression is characteristic of esca disease; specifically there is a long latency time of several years, the appearance of the symptoms may be chronic or acute, and there is high variability in symptom expression each year (Surico *et al*. 2000). As a consequence the diagnosis in vineyards and the detection of the internal symptoms are difficult. A vine that begins to show clear symptoms of esca in a given year may subsequently not display any symptoms for one or more years, during which time it will appear perfectly healthy (Mugnai *et al.* 1996, Surico *et al*. 2000, Calzarano *et al*. 2001). There is still no explication for this fluctuation in symptom expression, however it may be related to the variety, the interactions between the cultivars and management of the vineyard (Surico *et al*. 2000), namely the pruning (Scalabrelli and Ferroni 2010). The susceptibility to esca disease also appears to be related to the rootstock combination, in which case the evaluation of genotypic characteristics is useful in developing genetic improvement programmes to enhance the resistance to esca (Marchi 2001).

In temperate climates, the dormancy of grapevine buds is analogous to that of deciduous fruit trees (Pouget 1988, Guerriero and Scalabrelli 1991). It is commonly assumed that the rest period consists of an endodormancy phase, followed by an ecodormancy phase (Lang *et al*. 1987). Chilling temperatures, during autumn and winter, are perceived by the bud and cumulative chilling effects (chilling requirement) are generally considered to be the main factor in the endodormancy-breaking process (Tromp 2005). Following release from endodormancy, the ecodormancy phase is associated with unsuitable environmental conditions for active growth of the bud, due to low temperatures, nutrient deficiency, or water stress (Viti *et al.* 2008). Esca disease, as a biotic stress, could affect the dormancy process and the following bud break; however no evidence has actually been found.

In order to study the effect of esca disease on bud break in relation to only the genetic traits it is necessary to remove the influence of environmental factors (solar radiation, temperature variation, water availability and soil). For this purpose the forcing test (Guerriero *et al.* 2000, 2002) was carried out on 'Cabernet Sauvignon', a genotype considered to be very susceptible to esca (Christen *et al*. 2007, Borgo *et al*. 2008, Andreini *et al.* 2009).

This study aimed to verify the possibility of obtaining, through observation of bud development, an early phenological indication of disease occurrence which would allow to distinguish the infected plants to make a quick prevention (*i.e.* specific pruning, plants removal). The effect of esca infection on bud break in the year following the appearance of symptoms was studied taking into account different cultivar/rootstock combinations.

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This study was carried out in a context of a field and nursery experiment that was established in 2004 for the prevention and cure of esca disease (MESVIT, ARSIA-Toscana-Italy).

Material and Methods

Experimental site and plant material: The observations were made in the vineyard of an experimental station of the Department of Tree Science, Entomology and Plant Pathology (DTSEPP, University of Pisa) located in the Tuscan coastal area (altitude 6 m, 43°02' N, 10°36' E). The soil was uniform across the experimental plot (42% sand, 37 % silt and 21 % clay, pH 8). From 2004 to 2008 average minimum and maximum annual temperature of 9.4 °C ± 0.5 and 20.5 °C ± 0.3 respectively were recorded, and annual precipitation was 850 ± 193 mm (data from ARSIA). The vines were planted at 3x1m distance in 1981. The vineyard consisted of 9 longitudinal rows with 60 vines each. Excluding the two most external rows, a randomized complete block with four replicates situated transversally to the length of field was used. Each block included four cultivars 'Cabernet Sauvignon', 'Sangiovese', 'Trebbiano toscano' and 'Chardonnay' (30 plants/cultivar). Each cultivar was grafted on 1103 Paulsen (1103P), Kober 5BB (K5BB) rootstocks and own rooted (10 plants/rootstock). The main characteristics of the cultivars and rootstocks studied are summarised in Tab. 1. In the vineyard the vines were trained to a high free cordon, all agricultural practices (pruning, irrigation, fertilization and plant protection) were made at the same date and in the same way for all cultivars.

Observation of flower bud phenological stages - field conditions: The survey of bud break under field conditions was carried out in 2006 and 2008 on three cultivars: 'Cabernet Sauvignon', 'Sangiovese' and 'Trebbiano toscano'. The phenological bud stages according to the BBCH scale (Lorenz *et al.* 1994) were recorded by two inspections per week (Fig. 1). From dormant bud stage, bud break was monitored on different vines: control vines (CT) that have never shown symptoms during the experimental trial, and diseased vines (E) taking into account the number of times the symptoms have appeared during the previous years (E1, E2, E3). Due to mortality of vines affected by esca disease the total number of vines examined changed from 174 in 2006 to 162 in 2008 (Tab. 2). For each vine along the length of the cordon three spurs were selected on which the first two buds were monitored.

Forcing conditions: Bud break was also monitored by forcing test applied on 'Cabernet Sauvignon' during 2008. The phenological bud stages were recorded by two checks per week. Four types of vines were compared: control vines (CT) and symptomatic vines during previous years (E1, E2, E3). During the ecodormancy cuttings containing 10 nodes were collected (five replications/vine type). One-node segments were kept in water for 21 d (repeating the cut twice) in a heat chamber under the following environmental conditions insuring the best bud development: 25 °C (± 1), 60 % relative humidity, photoperiod 12 h of light at 300-400 µE $m^{-2} \cdot s^{-1}$. The heat requirement for growth after rest was calculated using the formula: GDH = 20 °C x n, where *n* is the number of hours during which the cuttings were forced and 20 °C is the most efficient temperature to stimulate budbreak (Richardson *et al*. 1977).

Results

Bud break under field conditions: Independent of the state of health of all vines (diseased and control) the effect of rootstock on bud break and shoot development was studied (Fig. 2). 'Sangiovese' own rooted showed a moderate trend of an early bud break compared to the vines grafted on K5BB and 1104P, however this effect was not significant during the shoot development phase. 'Cabernet Sauvignon' grafted on K5BB showed a significant delay only in the shoot development process. In 'Trebbiano toscano' vines the three different rootstock combinations did not have an evident influence on bud break and shoot development (Fig. 2).

Table 1

Main characteristics of cultivars and rootstocks

	Cultivar		
Characteristics	Sangiovese	Cabernet Sauvignon	Trebbiano toscano
Diffusion	Mainly in Italy and foreign countries	International	Italy and France
Bud break phenology	Early	Intermediate	Late
Vigour	Moderate	High	High
Susceptibility to esca	Moderate	High	Moderate
	[a]Rootstock		
	K5BB (*B.* x *Rip.*)	1103 P (*B.* x *Rup.*)	Own rooted
Drought tolerance	Fair	High	Medium
Vigour	High	Very high	Moderate
Tolerance to active limestone (%)	Good (20)	Good (20)	High

[a] *B., Vitis berlandieri; Rup., Vitis rupestris; Rip., Vitis riparia.*

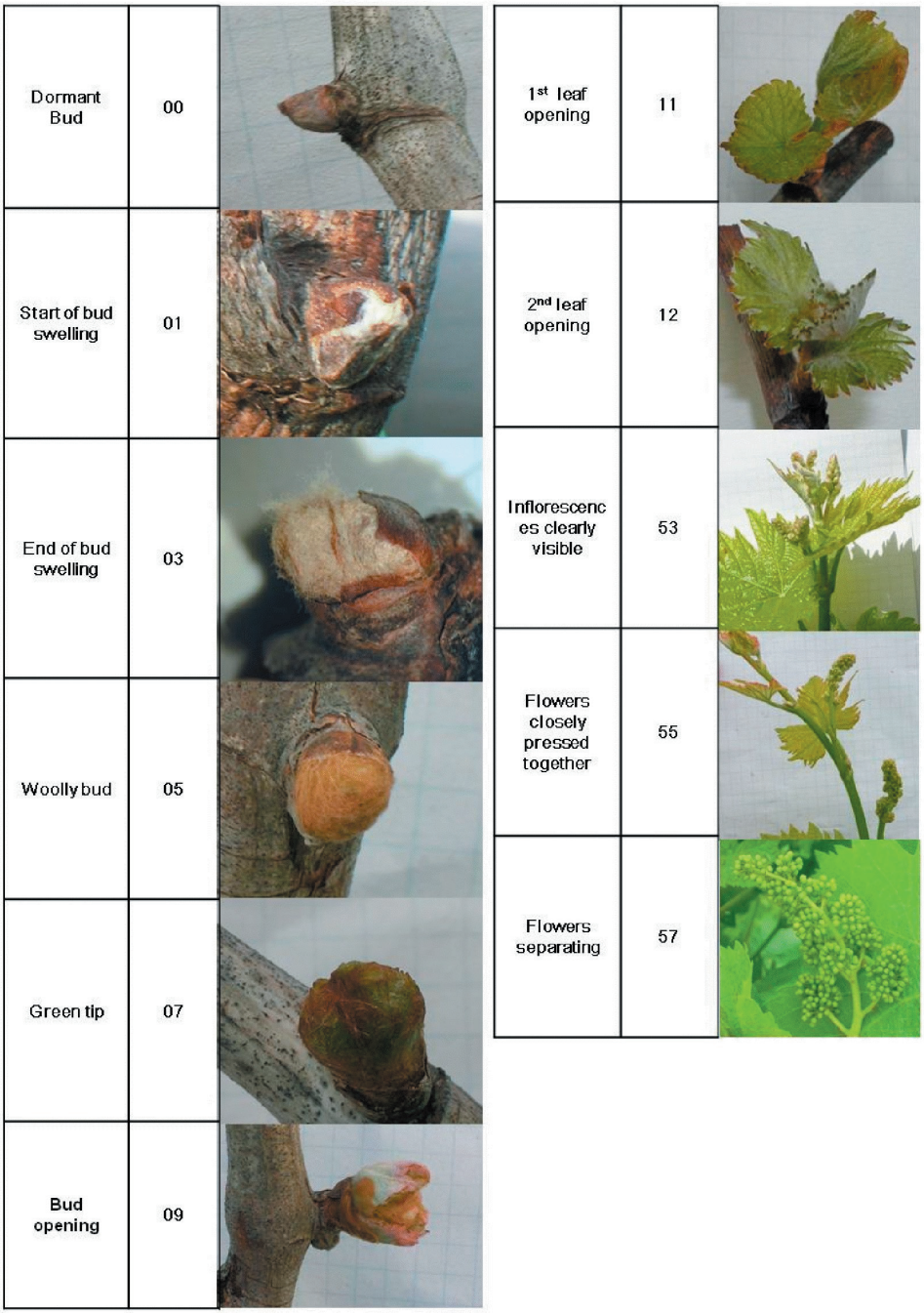

Fig. 1: Phenological stages of bud evolution according to BBCH scale: 00 dormant bud (brown bud scales strictly closed), 01 start of bud swelling (white whole tip), 03 end of bud swelling (bud scale opening), 05 woolly bud (brown wool), 07 green tip (appearance of leave tissues), 09 bud opening (new leaves clearly visible), 11 first leaf opening (start of shoot development), 12 second leaf opening, 53 inflorescences clearly visible, 55 inflorescences swelling, flowers closely pressed together, 57 inflorescences fully developed, flowers separating.

Table 2

Number of vines examined in 2006 and 2008 and mortality rate (%) calculated in 2008 with respect to the number of vines in 2006

	Cabernet Sauvignon	Trebbiano	Sangiovese	Total
2006	44	45	85	174
2008	39	42	81	162
Mortality (%)	11.3	6.7	4.7	6.9

The effect of esca on bud break was examined, monitoring phenological stages independent of rootstock combinations. At the beginning of bud break (April 2006) 'Trebbiano toscano' and 'Cabernet Sauvignon' were found to be at stage 03, corresponding to the bud scales opening. Whereas 'Sangiovese', as the earliest cultivar, reached stage 07, corresponding to the appearance of the new green leaf tissue (Tab. 3). The 'Sangiovese' vines that showed esca symptoms in 2005 (E1) were characterized by a significant delay in bud development with respect to the control vines. In contrast esca disease did not seem to affect the bud break of 'Trebbiano toscano'. 'Cabernet Sauvignon'

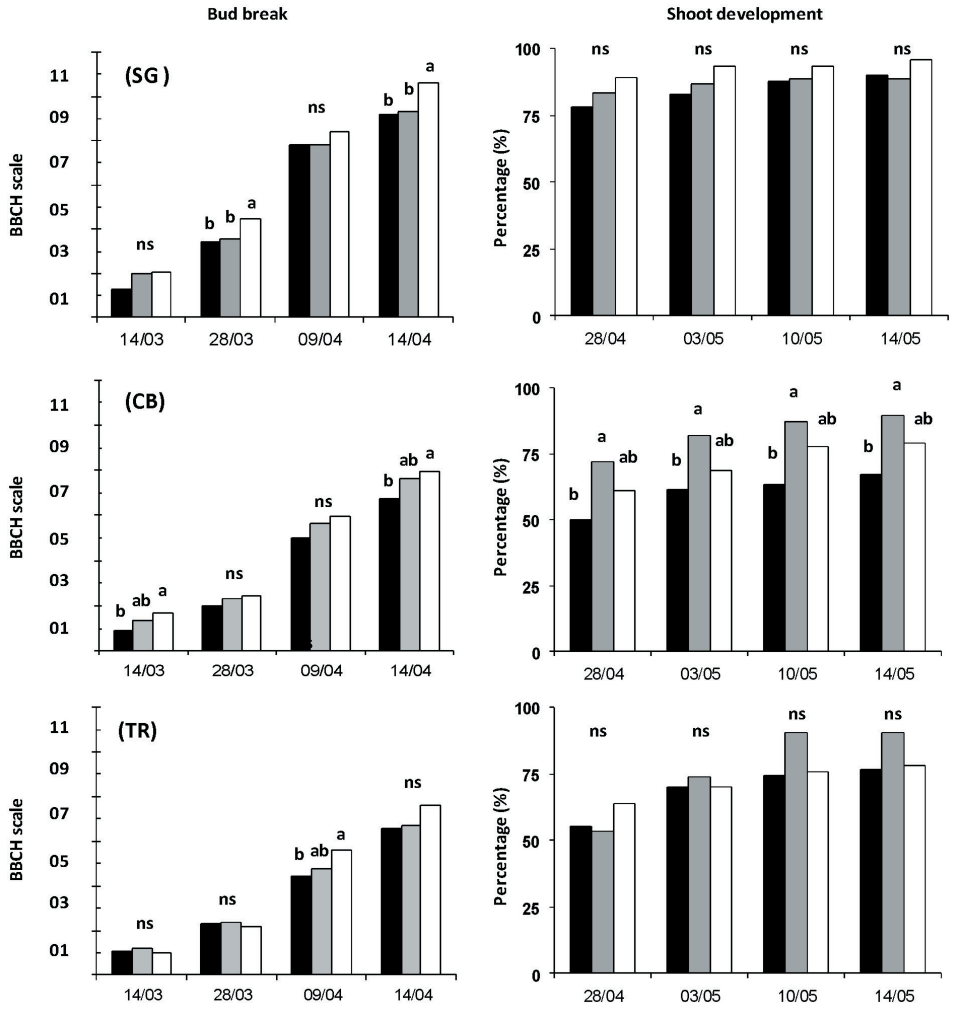

Fig 2: Effect of rootstock on bud evolution during the growth season of 2008**.** Bud break start: BBCH phenological stages (**00**: winter bud, **01**: start of swelling, **03**: bud scale opening, **05**: woolly bud, **07**: green tip, **09**: bud opening, **11**: first leaf opened). Shoot development: cumulative percentage of the first three floral phenological stages (53: inflorescences clearly visible, 55 inflorescences swelling, flowers closely pressed together, 57 inflorescences fully developed, flowers separating). Complementary value to reach 100 % represent the cumulative percentage of first phenological stages of bud break (from 01 to 11). Data recorded on 'Sangiovese' (SG), 'Cabernet Sauvignon' (CB) and 'Trebbiano toscano' (TR) each grafted on Kober 5BB (■), 1103 Paulsen (■) and own rooted (□). The stages are the mean of diseased and control vines. Within each date different letters correspond to statistically significant difference ($p < 0.05$ Tukey test).

Table 3

Representative phenological stages[a] according to BBCH scale observed at the start of bud break in 2006 (5th April) and 2008 (9th April) recorded on 'Sangiovese', 'Trebbiano toscano' and 'Cabernet Sauvignon' cultivars. E1: vines that showed esca disease symptoms in one previous year; CT: vines that never showed esca symptoms during the experimental years

Cultivar	Thesis	Phenological Stages	
		2006	2008
Sangiovese	CT	07	09
	E1	05*	09 *ns*
Trebbiano	CT	03	03/05
	E1	03 *ns*	05 *ns*
Cabernet	CT	03	05
Sauvignon	E1	01 *ns*	05 *ns*

* the asterisk denotes significant difference by Student's *t* test with $P \leq 0.05$.

[a] Stages are the mean of all rootstock combinations.

vines affected by esca showed a moderate, but not significant, trend towards late bud break. Results partially different were observed during the year 2008: at the start of bud break, none of the cultivars showed statistically significant differences in bud development between control vines and the vines symptomatic during the previous year (Tab. 3).

On the basis of a previous survey of symptom appearance carried out from 2004 to 2007, the vineyard in 2008 was characterised by several combinations of discontinuity in symptom expression. The effect of esca on bud break was also studied taking into account how many years individual vines had shown esca symptoms (Fig. 3). In the middle of May about 90 % of buds belonging to control vines showed emergence of inflorescence. At this time the percentage of control buds that did not show leaf development and that probably would not develop further, was on average 1% for all cultivars. In contrast diseased vines showed 10 % and 14 % in 'Sangiovese' and 'Trebbiano toscano' respectively, over three years, whereas in 'Cabernet

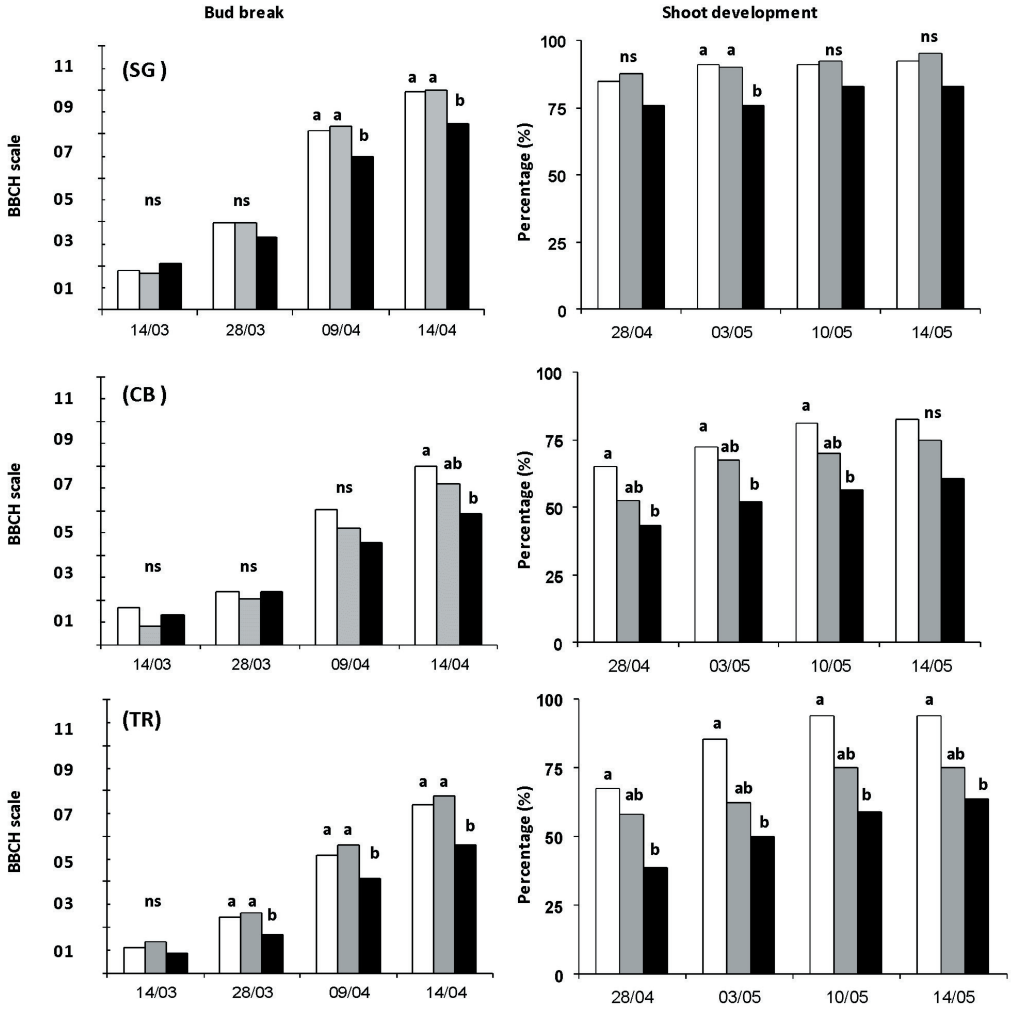

Fig. 3: Effect of esca disease on bud evolution during the growth season of 2008**.** Bud break start: BBCH phenological stages (**00**: winter bud, **01**: start of swelling, **03**: bud scale opening, **05**: woolly bud, **07**: green tip, **09**: bud opening, **11**: first leaf opened) Shoot development: cumulative percentage of the first three floral phenological stages (53: inflorescences clearly visible, 55 inflorescences swelling, flowers closely pressed together, 57 inflorescences fully developed, flowers separating). Complementary value to reach 100 % represent the cumulative percentage of first phenological stages of bud break (from 01 to 11). Data recorded on 'Sangiovese' (SG), 'Cabernet Sauvignon' (CB) and 'Trebbiano toscano' (TR) vines of control (□) and vines affected by esca for two (■) and three (■) years. The stages are the mean of all rootstock combinations. Within each date different letters denote statistically significant difference ($p < 0.05$ Tukey test).

Sauvignon' this value reached 26 % (data not shown). In all cultivars, vines symptomatic for three years showed a significant delay in bud break and in shoot development with respect to the control vines. However vines which had shown symptoms for two years showed a similar behaviour to the control vines, represented by a fast sprouting respect to the three years symptomatic vines (Fig. 3).

Combined effect of rootstock and esca on bud break: In all cultivars different rootstocks did not induce differences in the bud break process of control vines (Fig. 4). The observation of bud development of control vines (CT) under field conditions confirms 'Sangiovese' as the earliest cultivar. In the middle of April, when 'Sangiovese' showed the first expanded-fully leaf (stage 11), 'Cabernet Sauvignon' and 'Trebbiano toscano' displayed the green tip stage (07). All cultivars affected by esca for several consecutive years (E3) were characterised by a statistically significant delay of bud break when grafted on K5BB rootstock, as compared to the control vines. In contrast, the 'Trebbiano toscano' and 'Sangiovese' vines own rooted did not show differences in bud development whereas the Cabernet Sauvignon showed a delay (stage 05) respect to the control (stage 09). In diseased vines of 'Cabernet Sauvignon' rooted on 1103P a trend to early bud development was observed (Fig. 4). At the start of flowering phase all differences previously observed, both in control and diseased vines, were no longer evident. All cultivars then reached stage 53, corresponding to the appearance of inflorescence, at the same time showing a similar flowering stage (data not shown).

Bud break under forcing conditions: At the start of the experiment all buds of 'Cabernet Sauvignon' introduced to the forcing chamber were at the dormant stage (00). After 14 d, with 7200 GDH accumulated, significant differences in bud break between control and diseased vines were found (Fig. 5). At this time most of the buds, did not reach the phenological stage 03, corresponding to bud scale opening. After seven additional days

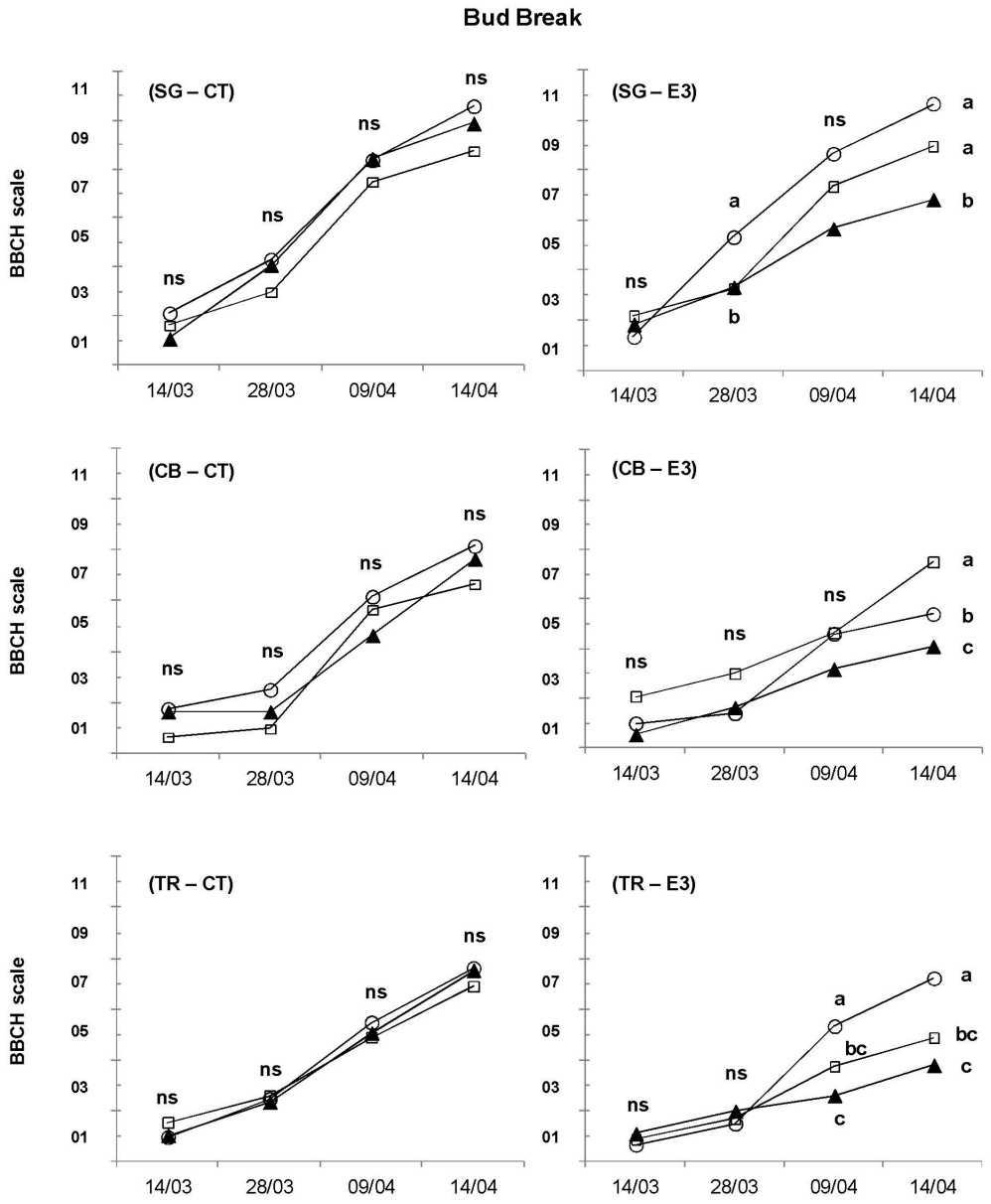

Fig. 4: BBCH phenological stages (**00**: winter bud, **01**: start of swelling, **03**: bud scale opening, **05**: woolly bud, **07**: green tip, **09**: bud opening, **11**: first leaf opened) of 'Sangiovese' (SG), 'Cabernet Sauvignon' (CB), and 'Trebbiano toscano' (TR) grafted on K5BB (▲), 1103 P (□) and own rooted (○). Data recorded during the growth season of 2008 on healthy vines (CT) and symptomatic vines for three consecutive years (E3). Different letters denotes statistically significant differences for $p < 0.05$ Tukey test.

in the forcing chamber (10560 GDH) the buds of control vines and of diseased vines which had been symptomatic for only one year (E1) were at phenological stage 11 (Figs 5 and 6). However, a significant delay in bud break was recorded in buds belonging to the symptomatic vines for consecutive years: E2 buds were at stage 05 while E3 buds at stage 03 (Figs 5 and 6).

Discussion

Our results suggest that esca disease mainly affected bud break of vines which had been symptomatic for several years. This effect was particularly evident on plants which exhibited symptoms for at least three consecutive years. The forcing test confirmed this change, which consisted of a delayed progression of bud break phenological stages but not complete inhibition of bud development. Under field conditions significant delay of bud break was limited to the early phases of the process, and the influence was no longer evident at flowering time.

During the spring – summer season appearance of the chronic form of esca, characterised by a deterioration in foliage, can cause perturbation of photosynthesis (BERTAMINI *et al*. 2002). As consequence, the reserve of carbohydrates can be not adequate, representing a limiting factor for growth reactivation of buds in the following year (PETIT *et al.* 2006). The reduction of reserve seems to be more severe with increasing numbers of symptomatic years, and this appears to cause a significant delay in bud break. When new leaf area becomes photosynthetically active, bud growth is recovered and bud development can

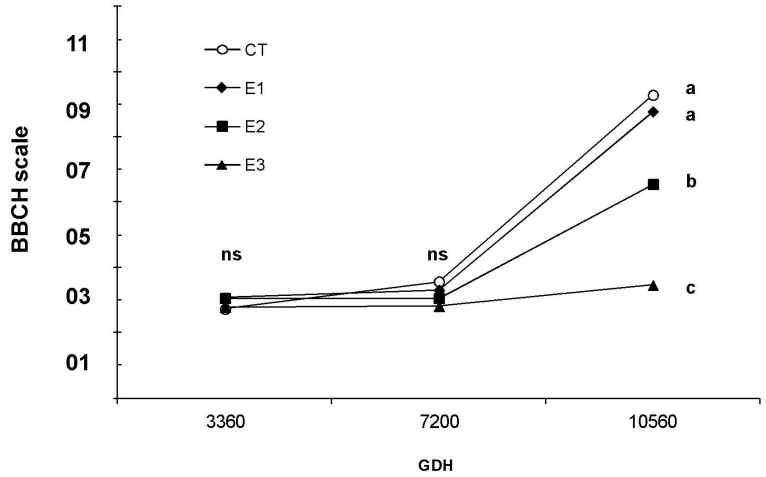

Fig. 5: Bud break phenological stages of one-node cuttings of 'Cabernet Sauvignon' recorded after 7, 14, 21 d under forcing conditions. GDH (Growing Degree Hours). Comparison between control vines (**CT**) and vines affected by esca for one (**E1**) two (**E2**) and three (**E3**) years. Values are the mean of five repetitions. Different letters denote statistically significant difference ($p < 0.05$ Tukey test).

Fig. 6: Phenological bud stages of 'Cabernet Sauvignon' at the end of forcing test. CT: buds of control vines, E1, E2, E3: buds of vines affected by esca for one, two and three years, respectively.

occur without limitations. In vines symptomatic for only one year, bud break was not significantly affected because the foliar damage probably was not critical for the sugar storage in roots and in perennial organs.

'Cabernet Sauvignon' and 'Trebbiano toscano'cultivars appear particularly susceptible to esca, indeed the delay of development was also observed during shoot growth. 'Sangiovese' was confirmed as the earliest cultivar also in diseased vines. This observation suggests that esca does not change the characteristics of vegetative growth of each cultivar even if the vine has shown symptoms for several years.

Our results show that the rootstock affects bud break in the presence of esca disease. In particular K5BB was observed to be the most susceptible to esca, provoking a significant delay in bud break in all cultivars examined. In general, the poor development of symptomatic vines could be related to the moderate vigour of K5BB, whereas combinations with the very vigorous 1103P showed a better bud break process, similar to that of control vines. Peterlunger *et al*. (1990), studied the hydraulic conductivity of several rootstocks showing that K5BB was characterised by an high transpiration rate, probably due to the high number of stomata. Contrarily, 1103P showed opposite hydraulic characteristics. In relation to these features it was possible hypothesize that the high and low susceptibility of K5BB and 1103 P to esca, respectively, could be related to their different hydraulic conductivity. The rootstock is an essential choice to make in relation to several factors (*i.e.* soil, climate, cultivars). In addition it appears that in the case of a new vineyard in an area with a significant presence of esca, it is possible that one should advise against the

use of K5BB rootstock. Results further confirm that during asymptomatic growing seasons diseased vines can regularly grow and produce. However when the symptoms of esca have occurred for several years, the vine could show significant delay, and in the worst case, a total lack of bud break in the spring time. Probably, the phenology is not an adequate marker to early identify the complex disease of esca and to obtain a diagnosis before symptoms appearance, biochemical studies may be most useful. Andolfi *et al.* (2009) reported that the identification of metabolic toxins produced by the fungi responsible of foliar symptoms could be represent a specific method to fast detect the esca disease also in asymptomatic vines.

Finally, it was not possible to correlate esca disease occurrence with an early phenological indication given the comparable behaviour of one year symptomatic vines and the healthy vines and the discontinuity of esca symptoms.

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