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### **Xylem-feeding Auchenorrhyncha potentially involved in Pierce's disease of grapevines in Europe**

**Abstract** - The bacterium *Xylella fastidiosa* Wells *et al.*, the causal agent of Pierce's disease of grapevines (PD), was isolated in Balkan Europe. Xylem-feeding Auchenorrhyncha are vectors of the bacterium. In this study the occurrence and phenology of European xylem-feeding Auchenorrhyncha in vineyard habitats, as well as their frequency within grapevine canopies, were investigated in north-eastern Italy. The leafhopper *Cicadella viridis* (L.) and the spittlebugs *Aphrophora alni* (Fallén), *Cercopis vulnerata* Rossi, *C. sanguinolenta* (Scopoli), *Lepyronia coleoptrata* (L.) and *Philaenus spumarius* (L.) were found. *Cercopis* spp. and *Ph. spumarius* are the cercopid species most likely involved in PD. In fact their adults emerge early in the season and are abundant even within grapevine canopies. Also the leafhopper *C. viridis* possibly plays a role in the bacterium transmission, particularly in vineyards where the ground is covered by herbaceous vegetation.

**Riassunto** - *Auchenorrhynchi xilemofagi potenzialmente implicati nella malattia di Pierce della vite in Europa.*

Il batterio *Xylella fastidiosa* Wells *et al.*, l'agente causale della Pierce's disease della vite (PD), è stato isolato in Europa. Auchenorrhynchi xilemofagi sono i vettori di questo batterio. In questa ricerca sono stati indagati sia la presenza e la fenologia degli adulti di auchenorrhynchi potenziali vettori in ecosistemi viticoli dell'Italia nord-orientale sia la loro frequenza nella chioma della vite. Sono stati rinvenuti il cicadellino *Cicadella viridis* (L.) e i cercopidi *Aphrophora alni* (Fallén), *Cercopis vulnerata* Rossi, *C. sanguinolenta* (Scopoli), *Lepyronia coleoptrata* (L.) e *Philaenus spumarius* (L.). *Cercopis* spp. e *Ph. spumarius* sembrano i cercopidi che più di altri potrebbero essere implicati con PD. I loro adulti compaiono, infatti, presto durante la stagione vegetativa e si rinvergono frequentemente sulle viti. Anche la cicalina *C. viridis* potrebbe avere un ruolo nella trasmissione del batterio soprattutto nei vigneti inerbati.

**Key words:** cercopids, *Cicadella viridis*, vector, *Xylella fastidiosa*, bacterial plant pathogen

## INTRODUCTION

Recently, the bacterium *Xylella fastidiosa* Wells *et al.*, the causal agent of Pierce's disease of grapevines (PD), was isolated from diseased grapevines in Kosova (Balkan Europe) (Berisha *et al.*, 1998). In southern USA the most prominent vectors of the pathogen are xylem-feeding Auchenorrhyncha such as leafhoppers (family Cicadellidae, subfamily Cicadellinae) and spittlebugs (family Cercopidae) (Severin, 1949, 1950; Purcell, 1980; Almeida & Purcell, 2003; Redak *et al.*, 2004).

Infections of *X. fastidiosa* are worse when they are precocious, since the bacterium has a better chance of surviving the winter (Purcell, 1981; Feil *et al.*, 2003). Therefore, potential vectors overwintering as adults should be emphasized since they can infect grapevines earlier in spring (Purcell, 1997). However, in Europe the potential vectors are not known to overwinter as adults. The leafhopper *Cicadella viridis* (L.) (Arzone, 1972; Nickel, 2003; Pavan & Gambon, 2004) and the spittlebugs *Aphrophora alni* (Fallén) (Goidanich, 1952; Nickel, 2003), *Lepyronia coleoptrata* (L.) (Barro & Pavan, 1999), *Philaenus spumarius* (L.) (Goidanich, 1957; Vidano *et al.*, 1989; Nickel, 2003) overwinter as eggs, whereas *Cercopis* spp. overwinter as nymphs (Paoli, 1933; Mauri, 1982; Nickel, 2003). Therefore, the most serious infections must be due to adults that emerge first in spring.

The aim of the present research was to investigate the occurrence and phenology of *C. viridis* and spittlebug adults in vineyard habitats of north-eastern Italy. Moreover, their frequency within grapevine canopies was also studied in several vineyards.

## MATERIAL AND METHODS

*Occurrence and phenology of xylem-feeding Auchenorrhyncha in vineyard habitats*

The phenology of adults was studied during 1987-1990, 1993 and 2004 in four vineyard habitats of north-eastern Italy: locality 1 (12° 40' longitude E, 45° 51' latitude N, 13 m altitude), locality 2 (12° 49' longitude E, 45° 59' latitude N, 50 m altitude), locality 3 (13° 26' longitude E, 45° 49' latitude N, 10 m altitude) and locality 4 (13° 08' longitude E, 46° 15' latitude N, 190 m altitude).

Adults of xylem-feeding Auchenorrhyncha were monitored on different types of vegetation (herbaceous, woody and grapevines) using three sampling methods, i.e. yellow sticky traps, sweep nets and beating trays. The samplings carried out in each locality are shown in Table 1.

Yellow sticky traps (23 x 11.5 cm) were smeared with glue (TEMPOCID, Kollant, Padova, Italy) and replaced weekly from April to November. The traps (n = 3) on herbaceous vegetation were placed in the midst of the ground cover under grapevines, at about 20 cm from ground level. The traps (n = 3) on grapevines were placed within the canopy at about 1.5 m from ground level. The traps (8 in locality 1 and 4 in locality 2) on woody vegetation (e.g. bramble, elm, maple, oak, willow) were placed on hedgerows around the vineyards at about 1.5 m from ground level.

Table 1 - Adults of xylem-feeding Auchenorrhyncha captured in four vineyard habitats of north-eastern Italy using yellow sticky traps (captures / three traps), sweep nets or beating trays. The monitoring period was April-November in all the years except 2004 (late May-late July).

Locality	Year	Sampling method	Type of vegetation	N. adults				
				<i>Cicadella viridis</i>	<i>Aphrophora alni</i>	<i>Cercopis vulnerata</i>	<i>Lepyronia coleoptrata</i>	<i>Philaenus spumarius</i>
1	1987	trap	herbaceous	276	0	0	4	48
	1988	trap	herbaceous	92	0	9	1	33
	"	trap	woody	2.2	6.4	25	0	33
	"	trap	grapevines	5	0	3	0	12
	1989	trap	herbaceous	87	0	2	0	19
	1990	trap	herbaceous	153	0	0	0	13
	1993	trap	herbaceous	457	0	1	1	7
		net	herbaceous	137	0	31	2	9
	2004	net	herbaceous	3	0	1	1	10
	"	tray	woody	0	0	2	0	9
	"	tray	grapevines	0	0	1	0	48
2	1987	trap	herbaceous	108	0	0	3	37
	"	trap	woody	0	0	0	0	26
	"	trap	grapevines	1	0	0	0	18
	1988	trap	herbaceous	107	0	0	11	114
	"	trap	woody	0	0	0	0	18
	"	trap	grapevines	4	0	1	0	50
	1989	trap	herbaceous	70	0	0	0	55
3	1987	trap	herbaceous	10	0	0	3	8
	1988	trap	herbaceous	180	0	0	14	10
4	1993	trap	herbaceous	12	0	0	4	4
	"	net	herbaceous	11	6	2	386	26

The adults captured on traps were counted in the laboratory.

In 1993 (loc. 1 and 4) for one hour in the morning permanent herbaceous vegetation was swept with an insect net. This sampling was repeated weekly from April to November. The specimens collected were brought to the laboratory in polyethylene bags for counting.

In 2004 (loc. 1) woody plants (i.e. grapevines and hedgerows) and herbaceous vegetation under grapevines were sampled respectively with a beating tray and with a sweep net. Every two weeks each type of vegetation was sampled for half an hour from late May to late July. The specimens collected were brought to the laboratory in polyethylene bags for counting.

Spittlebug species were identified using the dichotomous keys by Nast (1933), Ossiannilsson (1981) and Della Giustina (1983).

#### *Frequency of xylem-feeding Auchenorrhyncha within grapevine canopies*

During 1987-1989 adults of *C. viridis* and spittlebugs were monitored in 32 vineyards of north-eastern Italy (10 in hilly and 22 in flatland grape-growing areas). In hilly vineyards the ground between and along grapevine rows was not covered by herbaceous vegetation. Two yellow sticky traps per vineyard were placed within the canopy of grapevines and replaced every one or two weeks from the first (1988) or second (1987 and 1989) week of May to mid October. The adults captured on traps were counted in the laboratory.

## RESULTS

#### *Occurrence and phenology of xylem-feeding Auchenorrhyncha in vineyard habitats*

In the four vineyard habitats five xylem-feeding Auchenorrhyncha were captured: the leafhopper *C. viridis* and four cercopids (Table 1).

*C. viridis* was found in all the vineyard habitats considered (Table 1). Adult captures were far more abundant on ground cover (herbaceous vegetation) than on hedgerows (woody plants) and grapevines. However, on grapevines the leafhopper was captured in the both localities where monitoring was carried out. Both sticky traps and sweep nets were effective for monitoring *C. viridis*. Adults on herbaceous vegetation were captured from late May to November and three peaks of captures were observed respectively in June, late July-early September and October (Fig. 1; see also Pavan & Gambon (2004) for locality 1).

*A. alni* was observed only in two of the four vineyard habitats considered (Table 1). Using yellow sticky traps adults were captured only on hedgerows, whereas on ground cover only sweep nets were effective. In locality 1 on hedgerows 12 specimens out of 17 were captured on *Quercus* sp. Adults of *A. alni* were captured continuously from late May to early August and occasionally up to early October (Fig. 2). These data substantially agree with those reported for central Europe (Schiemenz, 1969; Nickel, 2003).

*Cercopis vulnerata* Rossi was captured in three of the four vineyard habitats (Table 1). Using yellow sticky traps the captures of adults were higher on hedgerows than on ground cover and grapevines. On herbaceous vegetation the sweep net was better than yellow sticky traps (Fig. 3). Adults of *C. vulnerata* were continuously captured

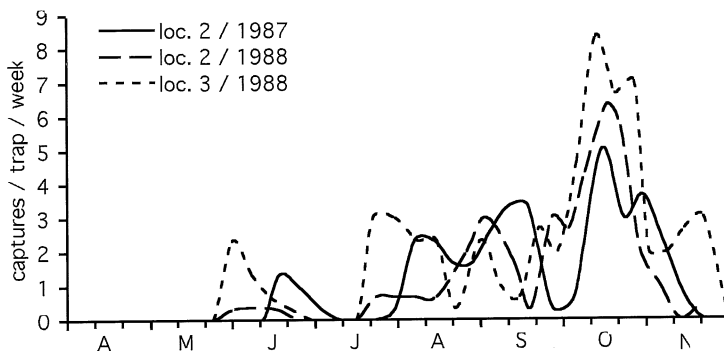


Fig. 1 - Adults of *Cicadella viridis* captured on herbaceous vegetation using yellow sticky traps. Data of locality 1 are reported in Pavan & Gambon (2004). For the other localities only the years with at least a total of 100 captures are considered.

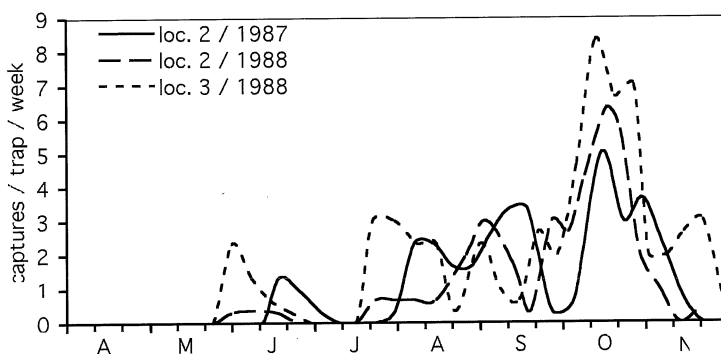


Fig. 2 - Adults of *Aphrophora alni* captured in locality 1 on woody plants using yellow sticky traps replaced weekly (captures / 8 trap) and in locality 4 on herbaceous vegetation using sweep nets (captures / hour). In the other localities and years no captures occurred.

for a short period from late April to late May with a peak in early May (Fig. 3). A few individuals were observed in June. The phenology substantially agrees with that reported for southern Switzerland (Mauri, 1982), whereas it was earlier than that observed in Germany (Nickel, 2003).

*L. coleoptrata* was found in all the vineyard habitats considered (Table 1). No captures were observed on grapevines and hedgerows using yellow sticky traps. Adults of *L. coleoptrata* were discontinuously captured from early June to the end of the monitoring period (early November) (Fig. 4) more or less in agreement with phenology reports for Italy by Barro & Pavan (1999) and for Germany by Nickel (2003). The periods of captures are consistent with the three generations that the spittlebug develops in northern Italy.

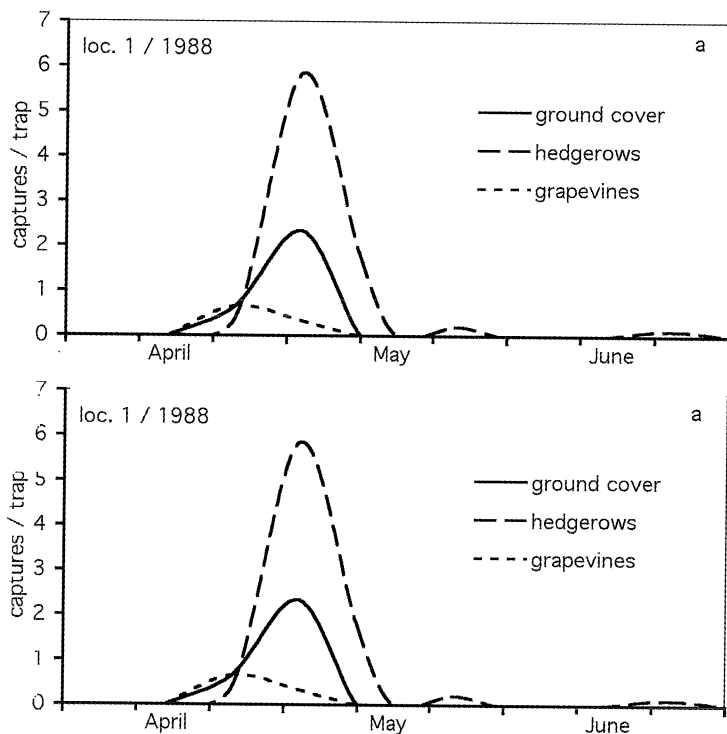


Fig. 3 - Adults of *Cercopis vulnerata* captured in a vineyard habitat using yellow sticky traps or sweep nets (captures / hour). In figure 3a three different types of vegetation and in figure 3b two sampling methods are compared. Only a few specimens were captured in the other localities and years.

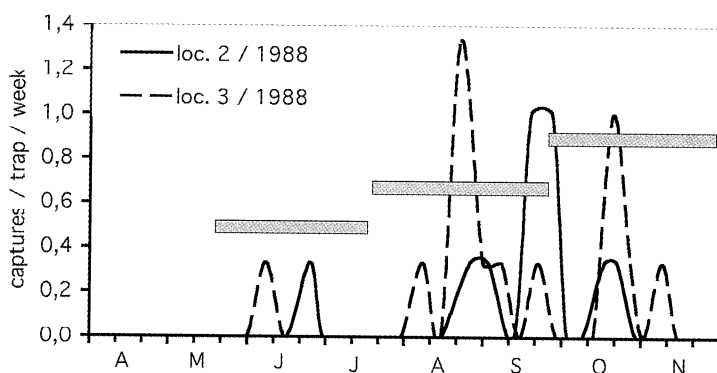


Fig. 4 - Adults of *Lepyrionia coleoprata* captured using yellow sticky traps. The horizontal bar indicates the presence of adults of the three generations observed in locality 4 as reported in Barro & Pavan (1994). Only the localities and the years with at least a total of 10 captures are considered.

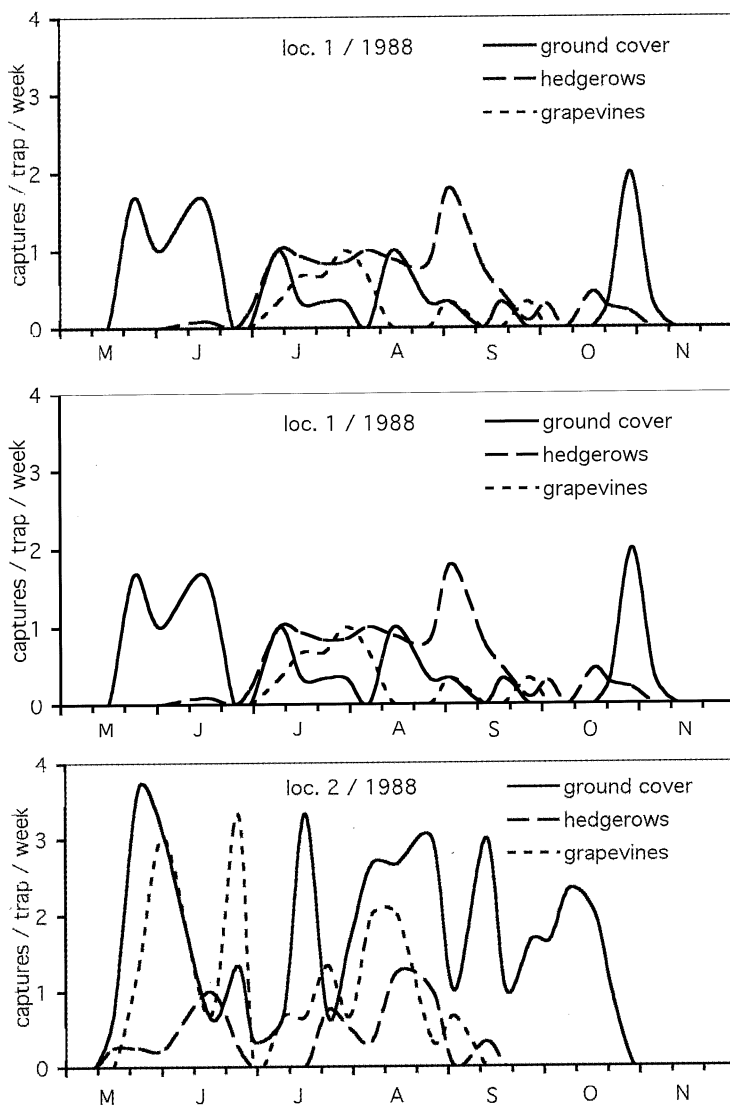


Fig. 5 - Adults of *Philaenus spumarius* captured using yellow sticky traps replaced weekly. Only the localities and years, where the three types of vegetation were sampled, are considered.

*Ph. spumarius* was abundantly captured in all the localities irrespectively of the sampling method and type of vegetation (Table 1). The captures on grapevines using yellow sticky traps were half that on ground cover. Adults of *Ph. spumarius* were

captured from mid or late May to late October or early November (Fig. 5). The phenologies previously observed in central Europe (Schiemenz, 1969; Nickel, 2003) and north-western Italy (Vidano *et al.*, 1989) are similar, except for the early emergence of adults in May. On herbaceous vegetation the captures were distributed over a period from mid May to October, whereas on woody plants of hedgerows and grapevines they were normally abundant only from June onwards. On grapevines in September only a few adults were captured. Using the beating tray a peak of adults was observed on grapevines in June during the flowering (Fig. 6).

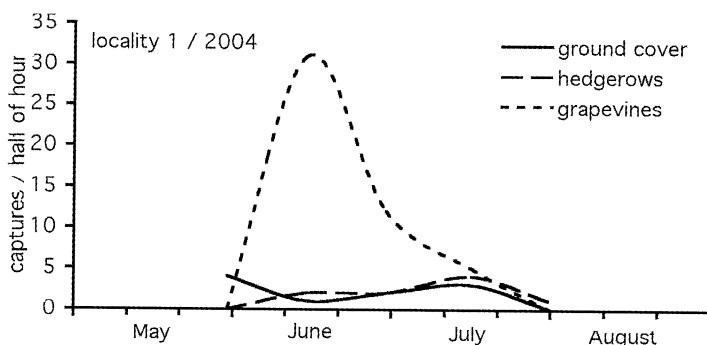


Fig. 6 - Adults of *Philaenus spumarius* captured on herbaceous vegetation using sweep nets and on woody plants using a beating tray.

#### *Frequency of xylem-feeding Auchenorrhyncha within grapevine canopies*

Adults of *C. viridis*, *A. alni*, two *Cercopis* species and *Ph. spumarius* were captured within grapevine canopies of the sampled vineyards (Table 2). *L. coleoptrata* specimens were not found.

*A. alni* was found only in hilly vineyards. More than half of these were interested by captures. The most part of these occurred in July and August with a slight delay in comparison to those observed on hedgerows and herbaceous vegetation.

*C. viridis* adults were captured only in flatland vineyards. In hilly vineyards the ground was not covered by the herbaceous vegetation leafhopper feeds on. About half the flatland vineyards showed captures. These were highest in early and late summer.

Captures of *Cercopis* spp. were observed in similar amounts both in hilly and flatland vineyards. *C. vulnerata* was the species mostly captured in the flatland vineyards, whereas in hilly vineyards *C. sanguinolenta* (Scopoli) was the most frequent. These spittlebugs were captured in half the flatland vineyards and in the majority of hilly ones. The captures mostly occurred in May.

*Ph. spumarius* adults were observed in all except one of the vineyards. The captures were very abundant and more than 70% of them occurred in June and July.

Table 2 - Adults of xylem-feeding Auchenorrhyncha captured in 10 hilly and 22 flatland vineyards during three years of sampling (1987-1989) with yellow sticky traps.

Species and area	Captures	Average captures	Vineyards with captures	Temporal distribution of captures (% on total)				
	N.	N.	%	May	June	July	Aug	Sept
<i>Aphrophora alni</i>								
hilly	11	1.1	60					
flatland	0	0	0					
total	11	0.3	19	0	17	45	38	0
<i>Cercopis</i> spp. (*)								
hilly	58	5.8	80					
flatland	41	1.9	52					
total	99	3.1	61	89	10	1	0	0
<i>Cicadella viridis</i>								
hilly	0	0	0					
flatland	27	1.2	45					
total	27	0.8	31	0	50	17	6	28
<i>Lepyronia coleoptrata</i>								
hilly	0	0	0					
flatland	0	0	0					
total	0	0	0					
<i>Philaenus spumarius</i>								
hilly	308	30.8	100					
flatland	443	20.1	95					
total	751	23.5	97	8	39	32	15	6

(\*) In 1987 and 1989 the monitoring began in the second week of May when the major part of emergence of adults had already occurred. Therefore, the greatest part of captures (N. 83) refers to 1988.

## DISCUSSION AND CONCLUSIONS

Phenological data show that *Cercopis* spp. are the potential vectors with adults present earlier in spring. The presence of adults of these spittlebugs already in late April, when grapevine budding has only just taken place, is due to their overwintering as nymphs. The other species overwinter as eggs and the first adults emerge in

mid May (*Ph. spumarius*) or late May-early June (other spittlebugs and *C. viridis*). Only adults of *Ph. spumarius* as well as those of *Cercopis* spp. were captured on grapevines in May.

*Ph. spumarius*, already reported to be the vector of *X. fastidiosa* (Purcell, 1981), and *Cercopis* spp. are also the spittlebugs most frequently observed in vineyards of north-eastern Italy. Grapevines appear to be good hosts for the adults of *Ph. spumarius*, since the amounts of captures are similar to those on herbaceous vegetation. In many vineyards of north-eastern Italy typical symptoms due to *Cercopis* spp. can be observed on leaves showing that these cercopids often feed on grapevines. Among other spittlebugs, *A. alni* was detected only in hilly vineyards and at low population levels, while *L. coleoptrata* was not observed on grapevines even when it was abundant on the ground cover. The absence of captures of *L. coleoptrata* on grapevines, unlike the other spittlebugs species, could be due to the fact that the former lays egg on herbaceous plants (Barro & Pavan, 1999), whereas the latter oviposit on woody plants. Also the adults of the leafhopper *C. viridis* visit grapevines, but during this investigation they were observed only in flatland vineyards where the ground was covered by herbaceous vegetation. Besides, considering that the leafhopper is hygrophilous (Arzone, 1972; Nickel, 2003), the highest risk of transmission of *X. fastidiosa* by *C. viridis* could occur for vineyards and nurseries placed near wet meadows. In any case the amount of adults of *C. viridis* on grapevines is much lower than on ground cover showing that grapevines are only occasionally visited. However, the possibility that *C. viridis* feeds on grapevines is supported by the fact that they can also be hosts of overwintering eggs (Dirimanov & Kharizanov, 1964; Linskii, 1979).

Therefore, this study suggests that the leafhopper *C. viridis* and the spittlebugs *Cercopis* spp. and *Ph. spumarius* are the species most likely involved in PD.

In the most southern European grape-growing areas higher temperatures and longer growing seasons increase the risk of severe infections of PD since the adults of vectors emerge earlier in spring and the bacterium has more time to multiply and move to the portion of the vine that will not be eliminated by winter pruning (Feil *et al.*, 2003).

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