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Life cycle and phenology of the green leafhopper, *Cicadella viridis* (L.) (Homoptera: Cicadellidae), in two different climatic areas of northeastern Italy

Abstract - During 1987-1998 research was carried out on the life cycle and phenology of the green leafhopper, *Cicadella viridis* (L.) (Homoptera: Cicadellidae), in a mountain and in two flatland localities of northeastern Italy. Field samplings of nymphs and adults were made using sweet-net and yellow-sticky traps. In flatland localities nymphs were observed from mid April to mid October and adults from mid-late May to mid-late November. Three peaks were observed both for nymphs and for adults. In the mountain locality nymphs were observed from early May to mid October and adults from early June to late October. Two peaks occurred both for nymphs and for adults. The data collected on adult and nymph dynamics, adult sex-ratio and females with eggs in ovaries show that *C. viridis* in northeastern Italy has three generations a year in flatland localities and two in the mountain locality. The day-degrees were calculated for the most important phenological steps. Results of this research were compared with those reported in the literature and are discussed in relation to the fact that the leafhopper could be a vector of the bacterium *Xylella fastidiosa* Wells *et al.*, the causal agent of Pierce's disease of grapevine.

Riassunto - *Ciclo biologico e fenologia di* Cicadella viridis *(L.)* (Homoptera: Cicadellidae) *in due aree climatiche dell'Italia nord-orientale.*

Nel periodo 1987-1998 sono state condotte ricerche sul ciclo biologico e sulla fenologia di *Cicadella viridis* (L.) (Homoptera: Cicadellidae) in due località di pianura e in una località montana dell'Italia nord-orientale. Campionamenti di forme giovanili e adulti sono stati effettuati con retino da sfalcio e trappole cromotropiche gialle invischiate. Nelle due località di pianura le forme giovanili e gli adulti sono stati osservati rispettivamente da metà aprile a metà ottobre e da metà-fine maggio a metà-fine novembre ed hanno presentato entrambi tre picchi di cattura. Nella località montana i giovani e gli adulti sono stati osservati rispettivamente da inizio maggio a metà ottobre e da inizio giugno a fine ottobre ed hanno presentato entrambi due picchi di cattura. I dati raccolti sulla dinamica di popolazione delle diverse età giovanili e degli adulti, come pure sul rapporto fra i sessi e sulla presenza di femmine con uova negli ovari, mostrano che *C. viridis* completa tre generazioni nelle località di pianura e due nella località montana. Sono anche state calcolate le somme termiche per le più importanti fasi fenologiche. I risultati ottenuti sono stati confrontati con quelli riportati in letteratura e discussi anche alla luce del fatto che *C. viridis* potrebbe essere un vettore del batterio *Xylella fastidiosa* Wells *et al.*, agente causale della malattia di Pierce della vite.

Key words: *Cicadella viridis*, Cicadellidae, life cycle, phenology, day-degrees.

INTRODUCTION

The green leafhopper, *Cicadella viridis* (L.) (Homoptera: Cicadellidae), is the most common and widespread sharpshooter leafhopper (subfamily Cicadellinae) in the Palaearctic region (Nast, 1972).

C. viridis overwinters as eggs laid into shoot bark of woody plants or into stems of herbaceous plants (Balachowsky, 1941; Servadei e Grasso, 1948; Frediani, 1955; Arzone, 1972). In the zone of the Palaearctic region between latitudes 37° and 47° N, this leafhopper is reported to be bivoltine or trivoltine (Table 1). Two generations were observed in northwestern Italy (Arzone, 1972), in Bulgaria (Dirimanov & Kharizanov, 1964) and in Kazakhstan (Linskii, 1979). Three generations are reported for central Italy (Frediani, 1955) and northern China (Chu & Teng, 1950; Yang, 1994)

The harmfulness of *C. viridis* is due mostly to overwintering-eggs laid in the bark of different woody plants (Balachowsky, 1941; Servadei e Grasso, 1948; Linskii, 1979). The leafhopper is also reported to be a vector of phytoplasma (Vlasov *et al.*, 1992), but this needs to be confirmed because *C. viridis* is a xylem-feeder.

Recently, interest in the biology of this leafhopper has increased as the bacterium *Xylella fastidiosa* Wells *et al.*, the causal agent of Pierce's disease of grapes (PD), was isolated from diseased grapevines in Kosova (Berisha *et al.*, 1998). In southern USA the most prominent vectors of the bacteria are xylem sap-feeding Auchenorrhyncha belonging to sharpshooter leafhoppers (family Cicadellidae, subfamily Cicadellinae) and spittlebugs (family Cercopidae) (Purcell, 1979, 1981a, 1989).

Infections of *X. fastidiosa* are worse when they are precocious since the bacterium has a better chance of surviving the winter (Purcell, 1981b, 1989). *C. viridis* overwinters as eggs and therefore the most serious infections could be due to adults that emerge early in spring.

In Italy *C. viridis* was captured in vineyards in many localities (Braccini & Pavan, 2000). In northeastern Italy abundant adults were captured using yellow sticky traps on herbaceous vegetation under grapevine plants and occasionally on the high part of the grapevine canopy at about 1.5 m from the ground (Pavan, unpublished data). It has also reported that eggs overwinter on grapevines (Dirimanov & Kharizanov, 1964; Linskii, 1979). Therefore, this sharpshooter leafhopper can be considered a potential vector of the PD agent.

The aim of this research was to study the life cycle and phenology of *C. viridis* in two different climatic areas of northeastern Italy.

MATERIAL AND METHODS

From 1987 to 1998 research was carried out in the Friuli Venezia Giulia region (northeastern Italy) by means of field samplings. Two localities (loc. 1 and 2) were in the flatland area and one (loc. 3) in the bottom of a mountain valley.

SAMPLINGS

In 1987, 1988, 1989, 1990 and 1993 adults of *C. viridis* were monitored in the permanent herbaceous vegetation under grapevine plants of locality 1 (Pasiano di Pordenone, 12° 40' longitude E, 45° 51' latitude N, 13 m altitude). Sampling was carried out from early May to late November. Each year three yellow sticky traps were placed on herbaceous vegetation under grapevine plants at about 20 cm from the ground. The traps (20 x 10 cm) were smeared with glue (Temoocid, Kollant, Padova, Italy) and replaced weekly. The adults captured on traps were counted in the laboratory. Only in 1993 for one hour in the morning a person swept the herbaceous vegetation with an insect net. The specimens collected were brought to the laboratory in polyethylene bags for counting.

In 1997 periodical samplings of adults and nymphs were carried out in locality 2 (Feletto Umberto, 13° 13' longitude E, 46° 7' latitude N, 132 m altitude) from mid May to mid October and in locality 3 (Enemonzo, 12° 55' longitude E, 46° 25' latitude N, 367 m altitude) from mid May to early November. In the two localities the sampling interval was two weeks and one week respectively. During each sampling for one hour in the morning two people swept the permanent herbaceous vegetation with an insect net. The specimens captured were brought to the laboratory in polyethylene bags for counting. The nymphs were sorted according to instar (Frediani, 1955) and the adults to sex. The females were further separated into females with or without eggs in their ovaries.

In 1998 samplings of adults and nymphs were carried out as in 1997 in localities 2 and 3, but only in specific periods so as to complete information collected the previous year. Observations were carried out in locality 2 during April to detect the earliest presence of the 1st-instar nymphs.

PHENOLOGY

The phenology of eggs, nymphs and adults of different generations was established on the basis of data collected during the samplings. The criteria adopted are reported below.

1st generation

The last presence of overwintering eggs was established on the basis of the last of the 1st-instar nymphs collected during the 1st-generation samplings.

The appearance of 1st-genearation nymphs and adults were fixed on the basis of first recording in the spring. The last nymphs of the 1st generation were established when during the following samplings no more individuals of the 5th instar were collected. The last adults were established on the basis of their absence in captures before emergence of 2nd-generation adults.

Following generations

The beginning of egg laying was fixed for the 2nd generation on the basis of first females with eggs in ovaries, for the other summer-generations on the basis of new increases in the proportion of the youngest instar nymphs. The beginning of overwintering-egg laying was supposed when no more 1st- and 2nd-instar nymphs and a new increase in females with eggs in ovaries were observed. The last 2nd- and 3rd-generation eggs were indirectly established on the basis of the last of the 1st-instar nymphs collected during the 2nd- and 3rd-generation samplings.

The beginning of egg hatching in summer generations was established when 1st-instar individuals were again collected after a period of absence. The last nymphs of the last generation were established on the basis of last captures during the season. In the case of more than two generations a year occurring the last nymphs of the penultimate generation were indirectly supposed on the basis of the proportion of different instar nymphs.

The beginning of adult emergence of summer generations was fixed indirectly on the basis of a new increase in adult captures and the proportion of males on total adults and of females with eggs in ovaries on total females.

Day-degrees

Day-degrees (DD) were calculated for the most important phenological steps obtained during this investigation or reported in papers concerning central Italy (Tuscany; Frediani, 1955) and northwestern Italy (Piedmont; Arzone, 1972).

Meteorological data (min and max daily temperatures were obtained from Centro Servizi Agrometeorologici per il Friuli-Venezia Giulia (localities 2 and 3), the Agriculture Service of Pordenone province (locality 1) and Annali Idrologici (Pisa and Turin).

The DD were calculated using the equation DD = [(Tmin + T max)/2] – developmental threshold. This was fixed at 9 °C (ShanChun *et al.*, 1996).

The phenological steps were fixed at an intermediate day of sampling interval (week or decade) adopted in this investigation or in those concerning central and northwestern Italy.

Results

Population dynamics

Flatland area

In locality 1 adults were found from mid-late May to late November using both yellow sticky traps and sweet net. Three peaks of captures were observed respectively in early-mid June, early August and mid October (Fig. 1). Only in 1989 a fourth peak occurred in mid November.

In locality 2 nymphs were found from mid April to mid October. They showed three peaks i.e. in mid May, mid July and mid September (fig. 2). Three periods (early May, late June and mid-late August) were clearly visible in which samplings with a high presence of the youngest nymphal instars were followed by samplings with a progressively higher proportion of the oldest instars (fig. 2). Adults were found from late May to early November and peaks of captures were observed respectively in early June, mid August and mid October (Fig. 2).

Mountain area

In locality 3 nymphs were found from early May to mid-late October. They showed two peaks i.e. in late June and late August-early September (Fig. 3). Two periods (early-mid May and mid-late July) were clearly visible in which samplings with a high presence of the youngest nymphal instars were followed by samplings with a progressively higher proportion of the oldest instars (Fig. 3).

Adults were found from early June to early November and two peaks of captures were observed, one in mid-late June and the other in early-mid September (Fig. 3).

Sex-ratio and proportion of females with eggs in ovaries

Flatland area

In locality 2 three samplings (late May-early June, late July and early October) with a higher proportion of males were followed by an increase in the proportion of females (Fig. 4). The percentage of females with eggs in their ovaries was low in late May-early June, late July-early August and early October and near 100% in mid-late June, mid September and late October (Fig. 4).

Mountain area

In locality 3 two short periods (early-mid June and late August - early September) with a higher proportion of males were followed by a progressive increase in the proportion of females that reached more than 70% of the total adults (July and August) (Fig. 5). The percentage of females with eggs in their ovaries was low in June and in late August (1998) or early September (1997) and near 100% in July and October (Fig. 5).

Life cycle and phenology

All the above data showed that in the flatland localities of northeastern Italy (loc. 1 and 2) the leafhopper completes three generations a year, whereas in the mountain area considered it has two generations a year. The phenologies are reported in figures 6 and 7.

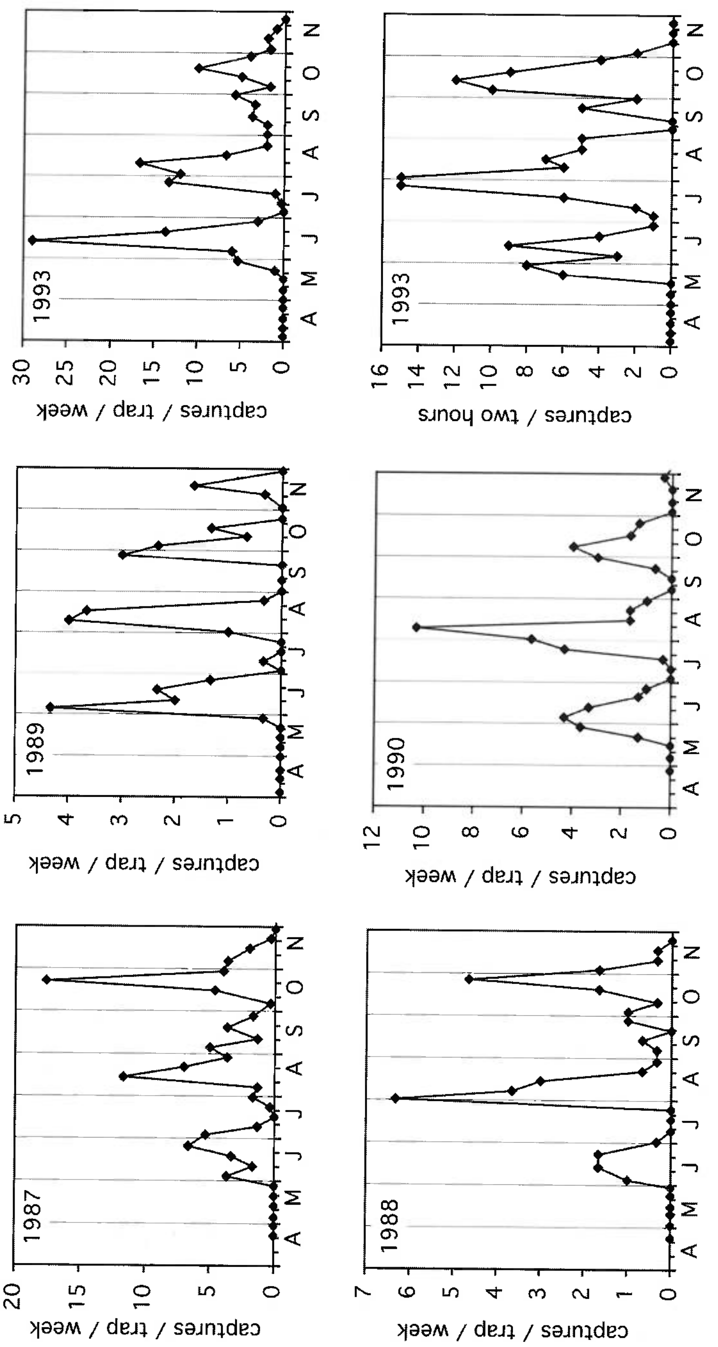

Fig. 1- Captures of *C. viridis* adults in locality 1 by yellow sticky traps and sweet net (only 1993).

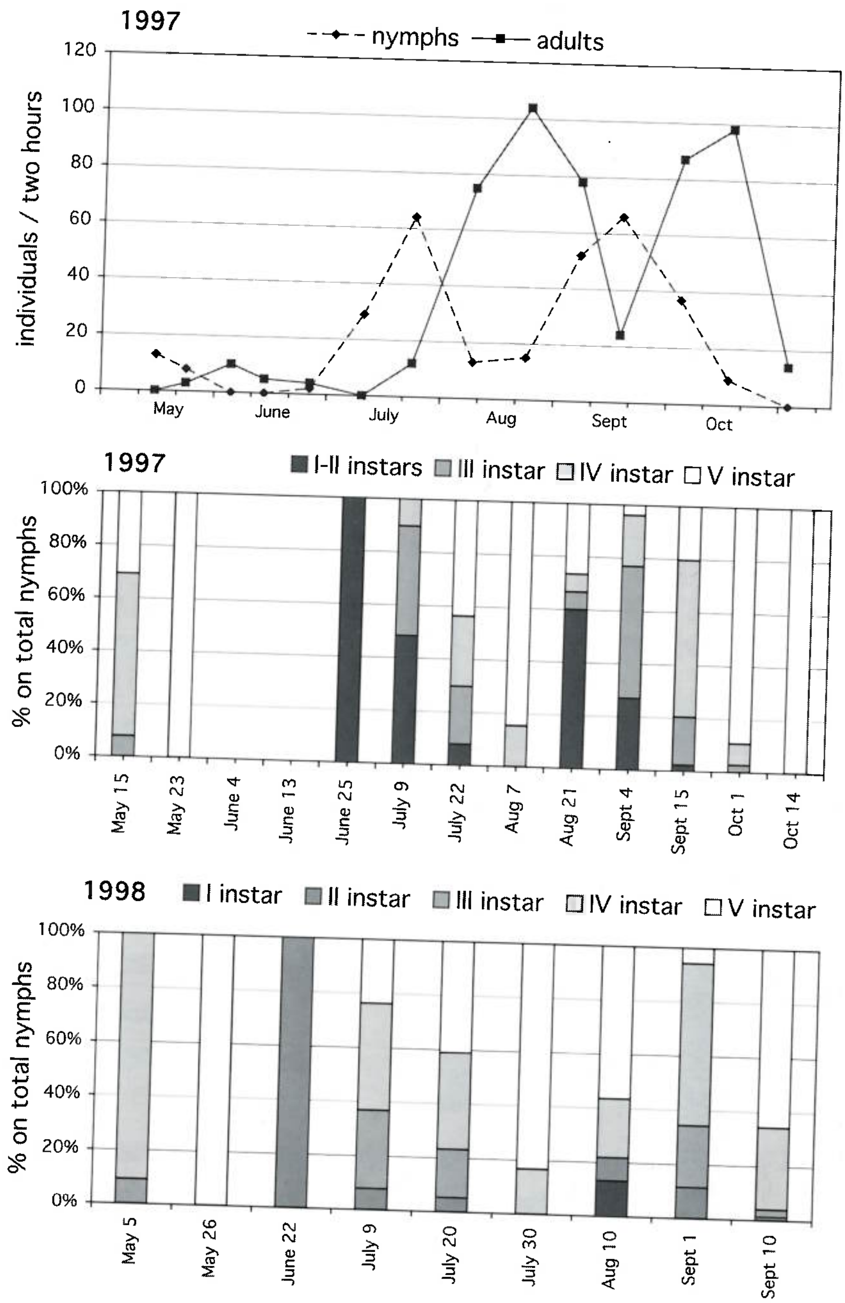

Fig. 2 - Captures of *C. viridis* in locality 2 by sweet net.

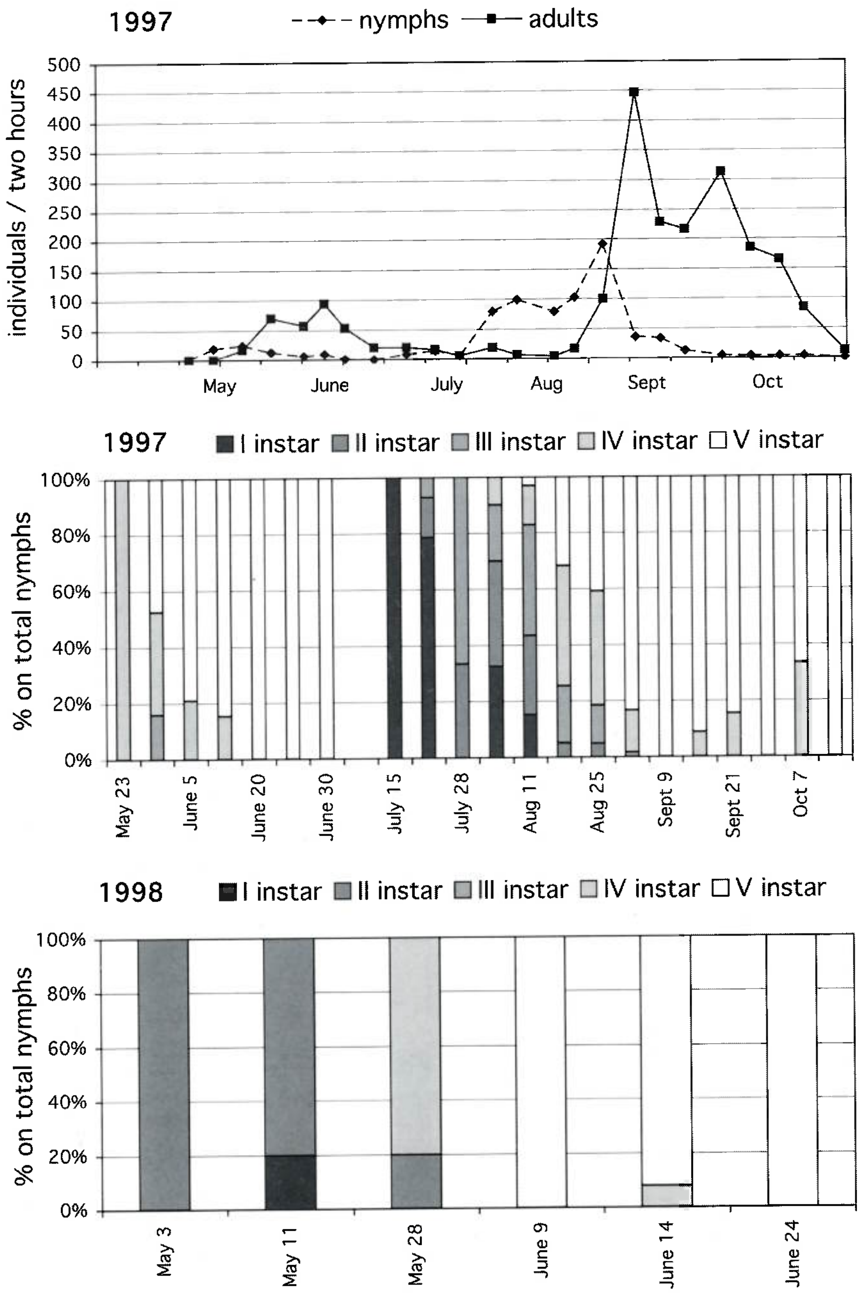

Fig. 3 - Captures of *C. viridis* in locality 3 by sweet net.

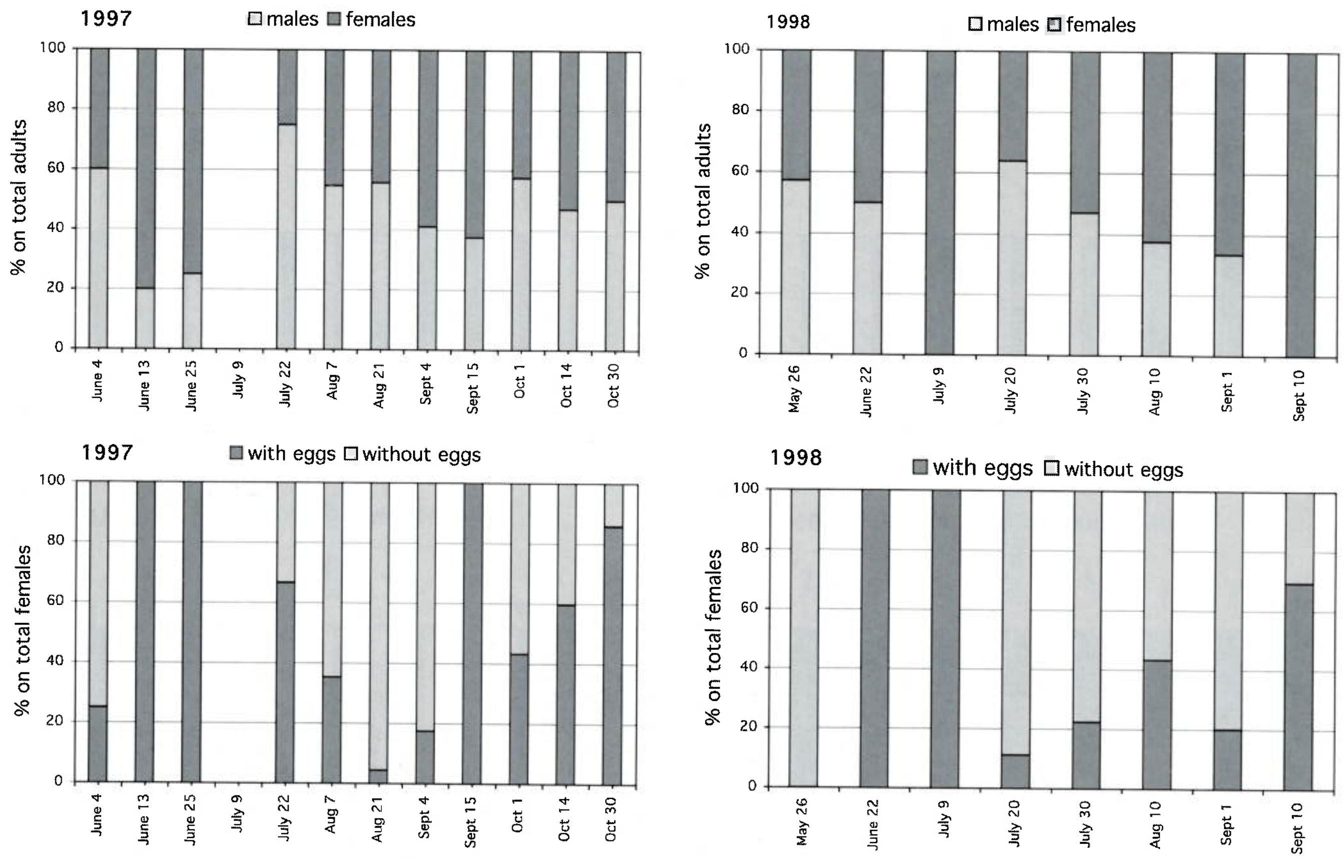

Fig. 4 - Captures of *C. viridis* adults in locality 2 by sweet net.

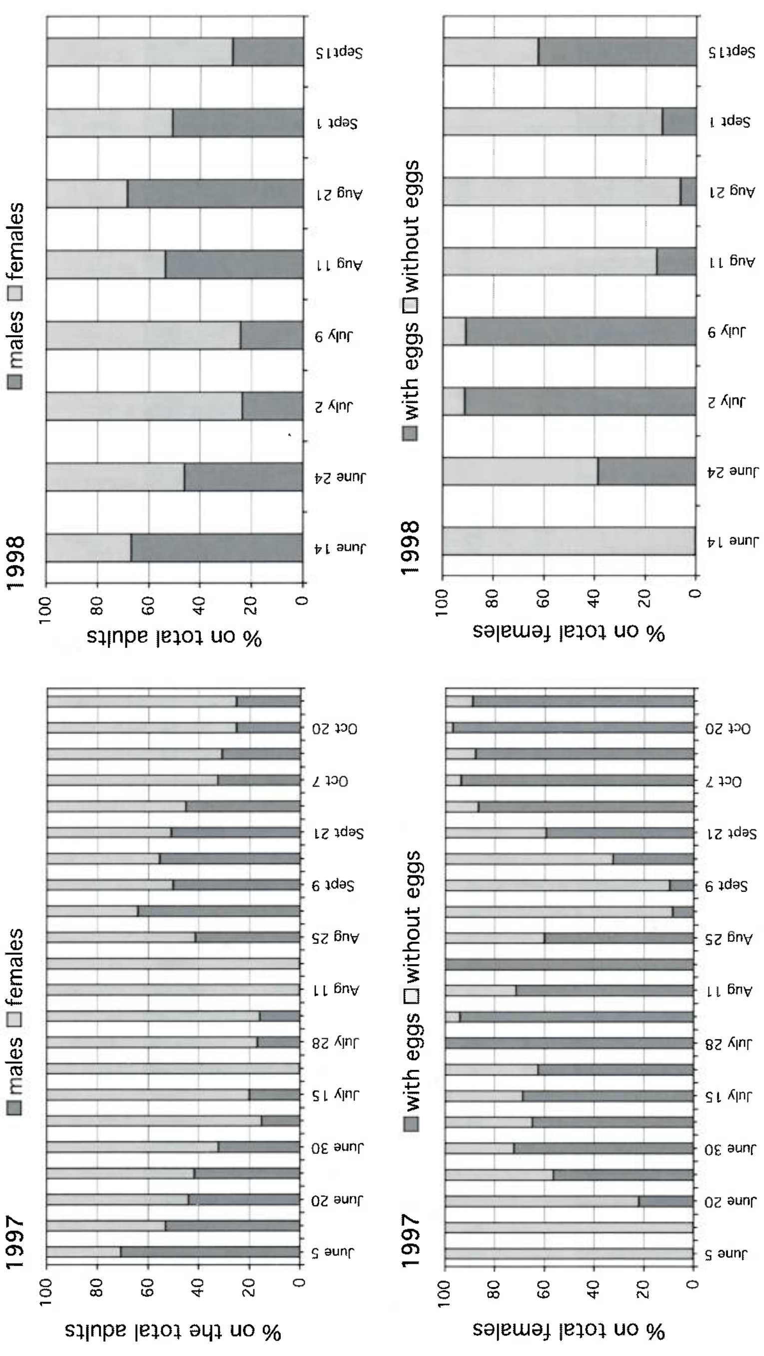

Fig. 5 - Captures of *C. viridis* adults in locality 3 by sweet net.

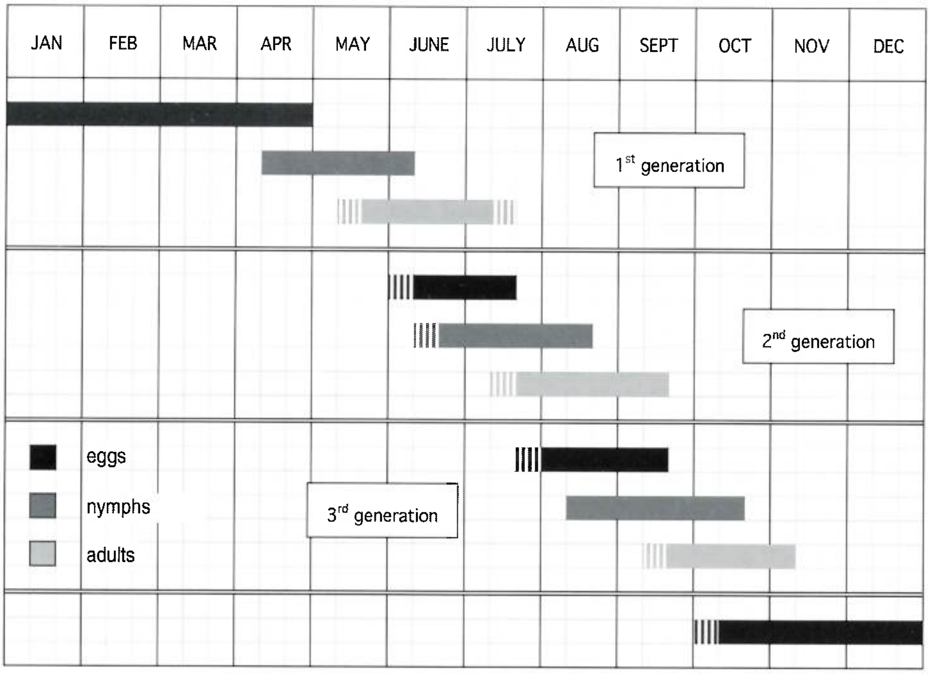

Fig. 6 - Life history of *C. viridis* in the flatland area of northeastern Italy.

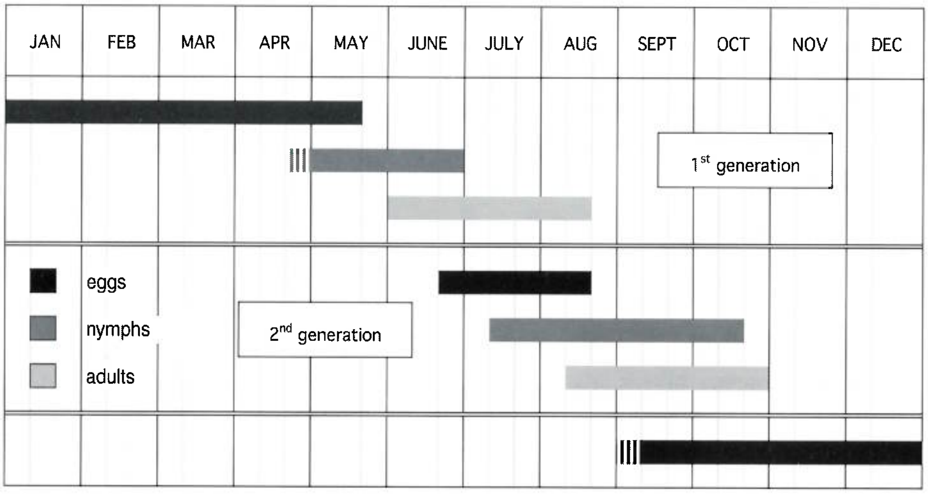

Fig. 7 - Life history of *C. viridis* in a mountain area of northeastern Italy.

The northeastern flatland areas (loc. 1 and 2) differed from the mountain locality (loc. 3) both in regard to number of generations and phenology of 1^{st} and 2^{nd} generations (Table 1).

The most important steps in phenology observed in flatland localities of northeastern Italy (loc. 1 and 2) were similar to those reported for flatland areas of central Italy (Frediani, 1955) and northern China (Chu and Teng, 1950) (Table 1). Phenology concerning 1^{st} and 2^{nd} generations accorded also with that reported for northwestern Italy (Arzone, 1972) and Bulgaria (Dirimanov & Kharizanov, 1964), even if in these latter areas the 3^{rd} generation does not develop.

The phenology of the 1^{st} and 2^{nd} generations observed in the mountain locality (loc. 3) of northeastern Italy was delayed compared to that reported in the literature for central Italy, northwestern Italy, Bulgaria and China. Therefore the differences are similar irrespective of whether areas with two or three annual generations are considered (Table 1). In the contrast the 1^{st}-generation phenology of this mountain locality agreed with that reported for Kazakhstan (Linskii, 1979) where two generations occur also.

Day-degrees

The emergence of 1^{st}-generation adults needed similar DD in the three localities of northeastern Italy (Table 2). For locality 1 differences of about 80 DD were observed between 1987 and 1988 corresponding at about seven days.

The DD calculated for locality 2 and 3 is similar for the principal steps of phenology of the 1^{st} and 2^{nd} generations (Table 2). Differences greater than 50 DD were observed for 2^{nd}-generation nymphs and adults. However, they correspond at less than five days.

The DD calculated for each phenological step of the 1^{st} and 2^{nd} generations were also similar to those obtained for the other localities in Italy, even if the differences were even greater than 100 DD (Table 2). Localities in northwestern and central Italy needed more DD for almost all steps. However, there were already large differences from the first step (1^{st}-generation nymphs) and in the following period they did not significantly increase.

DISCUSSION AND CONCLUSION

Number of generations in northeastern Italy

C. viridis in the investigated areas in northeastern Italy complete two or three generations. Only one piece of information could even suggest the development of a fourth generation. By comparing DD, calculated for the most important phenological steps, it appears that temperature is a good parameter to explain the differences in biological cycle.

Table 1 - Period of occurrence of phenological steps observed in this study (loc. 1, 2, 3) or reported in literature.

locality	year	latitude North	altitude (m)	number generation	1st generation		2nd generation			3rd generation			overwintering eggs
					nymphs	adults	eggs	nymphs	adults	eggs	nymphs	adults	
ITALY northeastern													
Locality 1	1987	45° 51'	13	3		l-May							
	1988			3		l-May							
	1989			3(4)		l-May							
	1990			3		m-May							
	1993			3		m-May							
Locality 2	1997-98	46° 7'	132	3	m-Apr	m-May	e-June	m/l-June	m/l-July	e-Aug	m-Aug	m-S	e-Oct
Locality 3	1997-98	46° 25'	367	2	l-Apr	e-June	l-June	m-July	m/l-Aug				e/m-Sept
north-western Turin (Piedmont)	1972	45° 10'	239	2	m-Apr	l-May	m-June	l-June	l-July				l-Sept
central Pisa (Tuscany)	1953	43° 45'	4	3	m-Apr	l-May	m-June	l-June	l-July	m-Aug	l-Aug	l-Sept	m-Oct
OTHER COUNTRIES													
Bulgaria (Plovdiv)	1960-62	42° 10'	161	2	e-Apr	m/l-May	e/m-June	m-June	m-July				l-Sept
China (Peking)	1947-49	40°	43	3	Apr	May	June			July			
China (Taiyuan)	1994	37° 8'	plain	3									
Kazakhstan (Alma Ata)	1979	43° 14'	848	2	lA-eM	June							l-Sept

Table 2 - Day-degrees calculated for the Italian localities investigated in this study (loc. 1, 2, 3) or reported in literature.

locality	year	latitude North	altitude (m)	number generation	1st generation		2nd generation			3rd generation			overwintering eggs
					nymphs	adults	eggs	nymphs	adults	eggs	nymphs	adults	
ITALY northeastern Locality 1 (*)	1987	45° 51'	13	3		293							
	1988			3		376							
	1989			3(4)		299							
	1990			3		332							
	1993			3		322							
Locality 2	1997-98	46° 7'	132	3	124	320	516	621	966	1187	1332	1726	1871
Locality 3	1997-98	46° 25'	367	2	115	320	491	674	1023				1314
north-western Turin (Piedmont)	1972	45° 10'	239	2	158	349	566	669	1085				1700
central Pisa (Tuscany)	1953	43° 45'	4	3	183	382	602	693	1044	1360	1440	1786	1927

(*) Day-degrees were calculated only for 1st-generation adults because on the basis of captures by yellow sticky traps it was not possible to know when adults of other generations started to emerge.

Comparison with the literature

The phenology observed in the flatland area during this research concurs with that previously reported for central Italy.

The same was true for the 1st and 2nd generations observed in northwestern Italy and Bulgaria, however in these latter areas the 3rd generation does not develop. The 2nd-generation adults appear in same period both in northeastern Italy and northwestern Italy/Bulgaria, but then in these latter areas they remain for two months without laying eggs. The biological significance of this behavior is difficult to understand since the risk of adult mortality during this period is high. In contrast in the mountain area of Friuli Venezia Giulia the 2nd-generation adults lay overwintering eggs a few days after emergence as observed for 3rd-generation adults in the event of a trivoltine cycle. In central Italy the 2nd generation adults do not survive for even two months, since they live at the most 35-40 days.

The variations in DD between the northeastern and other Italian localities are not so great as to be considered biologically significant. They already appeared at the first step. The different tools used to measure the temperature could explain the dissimilarities in early spring when temperatures lower than zero occurred. Other problems could also depend on the difficulty of establishing exactly the moment at which each step takes place since samplings were periodical.

Critical period for X. fastidiosa *transmission*

According to the literature concerning the southern regions of the USA, the heaviest infections of PD are the precocious ones. For this region the emergence period of 1st-generation adults is important. If in northern Italy adults emerge from mid-late May, then in southern Italy they could emerge in early May, when the risk of severe infections due to PD is higher (Purcell, pers. com.).

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